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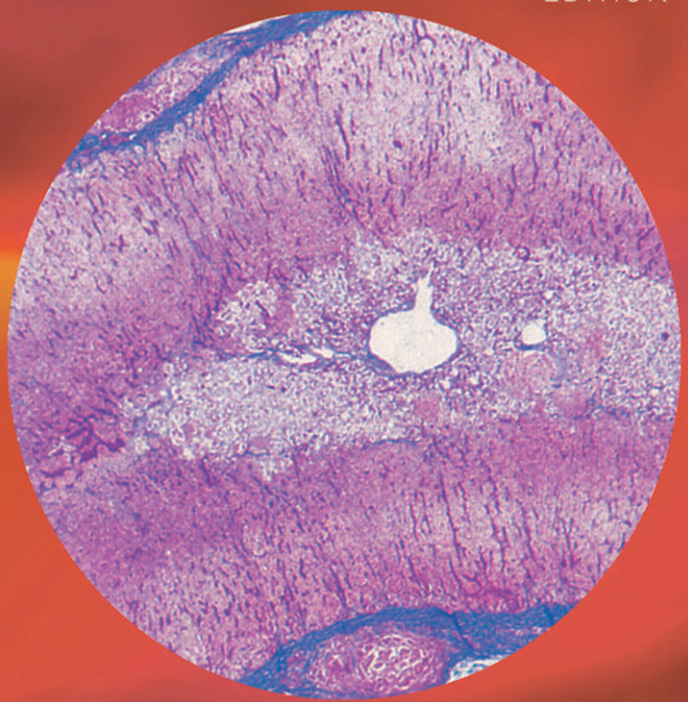
4th EDITION



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Endocrine and Reproductive Physiology

FOURTH EDITION

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PREFACE

This 4th edition, *Endocrine and Reproductive Physiology*, has been updated and, to some extent, reorganized. The most substantive change is Chapter 3. In fact, Chapter 3 grew to an untenable length for this monograph. Nevertheless, the worldwide type 2 diabetes epidemic emphasizes the need for comprehensive understanding of the role of hormones in regulating energy metabolism. To retain background information, we placed a significant amount of Chapter 3 material online in Student Consult. We think it provides an adequate background for the student to understand the important points of hormonal regulation of energy metabolism.

Also in this 4th edition, Key Words and Concepts has been moved to Student Consult, along with Abbreviations and Symbols, and Suggested Readings. The student is encouraged to define the key words, stating their importance, function, and interactive molecules, using the text as reference when necessary.

This edition has been reorganized in that the life history of the reproductive systems has been allocated its own chapter. This brings together embryonic/fetal development of the male and female reproductive systems, the changes that occur at puberty in boys and girls, and the decline of reproductive function with age (especially in women).

I wish to thank my two colleagues at UConn Health Center, Drs. John Harrison and Lisa Mehlmann, who wrote significant parts of Chapters 4 and 11, respectively. I also want to thank Rebecca Persky (UConn School of Medicine, Class of 2014), who read several chapters and whose comments/suggestions led to significant improvement of those chapters.

I also want to thank Elyse O'Grady and Barbara Cicalese at Elsevier for their patience and assistance in developing the 4th Edition.

Bruce A. White

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CONTENTS

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1 INTRODUCTION TO THE ENDOCRINE SYSTEM

OBJECTIVES

1. Identify the chemical nature of the major hormones.
2. Describe how the chemical nature influences hormone synthesis, storage, secretion, transport, clearance, mechanism of action, and appropriate route of exogenous hormone administration.
3. Explain the significance of hormone binding to plasma proteins.
4. Describe the major signal transduction pathways, and their mechanism for termination, for different classes of hormones and provide a specific example of each.

Endocrine glands secrete chemical messengers, called **hormones** (Table 1-1), into the extracellular fluid. Secreted hormones gain access to the circulation, often via fenestrated capillaries, and regulate **target organs** throughout the body. The endocrine system is composed of the **pituitary gland**, the **thyroid gland**, **parathyroid glands**, and **adrenal glands** (Fig. 1-1). The endocrine system also includes the **ovary** and **testis**, which carry out a gametogenic function that is absolutely dependent on their endogenous endocrine function. In addition to dedicated endocrine glands, endocrine cells reside as a minor component (in terms of mass) in other organs, either as groups of cells (the **islets of Langerhans** in the pancreas) or as individual cells spread throughout several glands, including the **gastrointestinal (GI) tract, kidney, heart, adipose tissue**, and **liver**. In addition there are several types of **hypothalamic neuroendocrine neurons** that produce hormones. The **placenta** serves as a transitory exchange organ, but also functions as an important endocrine structure of pregnancy.

The endocrine system also encompasses a range of specific enzymes, either cell associated or circulating, that perform the function of **peripheral conversion of hormonal precursors** (see Table 1-1). For example, angiotensinogen from the liver is converted in the circulation to angiotensin I by the renal-derived enzyme renin, followed by conversion to the active hormone angiotensin II by the transmembrane ectoenzyme angiotensin I–converting enzyme (ACE) that is enriched in the endothelia of the lungs (see Chapter 7). Another example of peripheral conversion of a precursor to an active hormone involves the two sequential hydroxylations of vitamin D in hepatocytes and renal tubular cells.

Numerous extracellular messengers, including prostaglandins, growth factors, neurotransmitters, and cytokines, also regulate cellular function. However, these messengers act predominantly within the context of a microenvironment in an autocrine or paracrine manner, and thus are discussed only to a limited extent where needed.

TABLE 1-1
Hormones and Their Sites of Production

Hormones Synthesized and Secreted by Dedicated Endocrine Glands

Pituitary Gland
- Growth hormone (GH)
- Prolactin
- Adrenocorticotropic hormone (ACTH)
- Thyroid-stimulating hormone (TSH)
- Follicle-stimulating hormone (FSH)
- Luteinizing hormone (LH)

Thyroid Gland
- Tetraiodothyronine (T_4; thyroxine)
- Triiodothyronine (T_3)
- Calcitonin

Parathyroid Glands
- Parathyroid hormone (PTH)

Islets of Langerhans (Endocrine Pancreas)
- Insulin
- Glucagon
- Somatostatin

Adrenal Gland
- Epinephrine
- Norepinephrine
- Cortisol
- Aldosterone
- Dehydroepiandrosterone sulfate (DHEAS)

Hormones Synthesized by Gonads

Ovaries
- Estradiol-17β
- Progesterone
- Inhibin

Testes
- Testosterone
- Antimüllerian hormone (AMH)
- Inhibin

Hormones Synthesized in Organs with a Primary Function Other Than Endocrine

Brain (Hypothalamus)
- Antidiuretic hormone (ADH; vasopressin)
- Oxytocin
- Corticotropin-releasing hormone (CRH)
- Thyrotropin-releasing hormone
- Gonadotropin-releasing hormone (GnRH)
- Growth hormone–releasing hormone (GHRH)
- Somatostatin
- Dopamine

Brain (Pineal Gland)
- Melatonin

Heart
- Atrial natriuretic peptide (ANP)

Kidney
- Erythropoietin

Adipose Tissue
- Leptin
- Adiponectin

Stomach
- Gastrin
- Somatostatin
- Ghrelin

Intestines
- Secretin
- Cholecystokinin
- Glucagon-like peptide-1 (GLP-1)
- Glucagon-like peptide-2 (GLP-2)
- Glucose-dependent insulinotropic peptide (GIP; gastrin inhibitory peptide)
- Motilin

Liver
- Insulin-like growth factor-1 (IGF-I)

Hormones Produced to a Significant Degree by Peripheral Conversion

Lungs
- Angiotensin II

Kidney
- 1α,25-dihydroxyvitamin D

Adipose, Mammary Glands, Other Organs
- Estradiol-17β

Liver, Sebaceous Gland, Other Organs
- Testosterone

Genital Skin, Prostate, Other Organs
- 5-Dihydrotestosterone (DHT)

Many Organs
- T_3

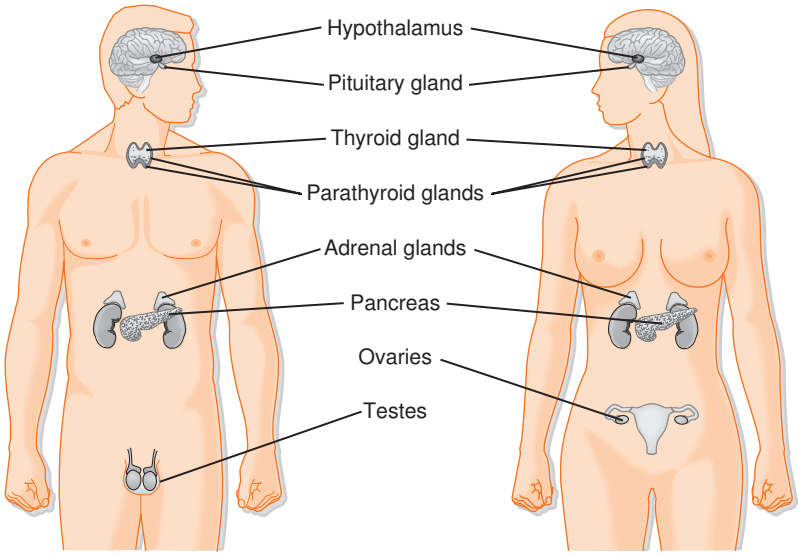


FIGURE 1-1 ■ Major glands of the endocrine system. *(From Koeppen BM, Stanton BA, editors:* Berne and Levy Physiology*, 6th ed., Philadelphia, 2010, Mosby.)*

To function, hormones must bind to specific **receptors** expressed by specific **target cell types** within **target organs**. Hormones are also referred to as **ligands**, in the context of ligand receptor binding, and as **agonists**, in that their binding to the receptor is transduced into a cellular response. Receptor **antagonists** typically bind to a receptor and lock it in an inactive state, unable to induce a cellular response. Loss or inactivation of a receptor leads to **hormonal resistance**. **Constitutive activation** of a receptor leads to unregulated, hormone-independent activation of cellular processes.

The widespread delivery of hormones in the blood makes the endocrine system ideal for the functional coordination of multiple organs and cell types in the following contexts:

1. Allowing normal development and growth of the organism
2. Maintaining internal homeostasis
3. Regulating the onset of reproductive maturity at puberty and the function of the reproductive system in the adult

In the adult, endocrine organs produce and secrete their hormones in response to **feedback control systems** that are tuned to **set-points**, or set ranges, of the levels of circulating hormones. These set-points are genetically determined but may be altered by age, circadian rhythms (24-hour cycles or diurnal rhythms), seasonal cycles, the environment, stress, inflammation, and other influences.

The material in this chapter covers generalizations common to all hormones or to specific groups of hormones. The chemical nature of the hormones and their mechanisms of action are discussed. This presentation provides the generalized information necessary to categorize the hormones and to make predictions about the most likely characteristics of a given hormone. Some of the exceptions to these generalizations are discussed later.

CHEMICAL NATURE OF HORMONES

Hormones are classified biochemically as **proteins/peptides, catecholamines, steroid hormones**, and **iodothyronines**. The chemical nature of a hormone determines the following:

1. How it is synthesized, stored, and released
2. How it is carried in the blood
3. Its biologic half-life ($t_{1/2}$) and mode of clearance
4. Its cellular mechanism of action

Proteins/Peptides

The **protein and peptide hormones** can be grouped into structurally related molecules that are encoded by gene families (Box 1-1). Protein/peptide hormones gain their specificity from their primary amino acid

BOX 1-1

CHARACTERISTICS OF PROTEIN/PEPTIDE HORMONES

- Synthesized as prehormones or preprohormones
- Stored in membrane-bound secretory vesicles (sometimes called *secretory granules*)
- Regulated at the level of secretion (regulated exocytosis) and synthesis
- Often circulate in blood unbound
- Usually administered by injection
- Hydrophilic and signal through transmembrane receptors

sequence, which confers specific higher-order structures, and from posttranslational modifications, such as glycosylation.

Protein/peptide hormones are synthesized on the polyribosome as larger **preprohormones** or **prehormones** (remove). The nascent peptides have at their N terminus a group of 15 to 30 amino acids called the **signal peptide**, which directs the growing polypeptide through the endoplasmic reticular membrane into the cisternae. The signal peptide is enzymatically removed, and the protein is then transported from the cisternae to the Golgi apparatus, where it is packaged into a membrane-bound secretory vesicle that buds off into the cytoplasm. Posttranslational modification occurs in the endoplasmic reticulum, Golgi apparatus, and secretory vesicle.

The original gene transcript is called either a **prehormone** or a **preprohormone** (Fig. 1-2). Removing the signal peptide produces either a hormone or a prohormone. A **prohormone** is a polypeptide that requires further cleavage before the mature hormone is produced. Often this final cleavage occurs while the prohormone is within the Golgi apparatus or the secretory vesicle. Sometimes prohormones contain the sequence of multiple hormones. For example, the protein, proopiomelanocortin (POMC), contains the amino acid sequences of adrenocorticotropic hormone (ACTH) and α-melanocyte-stimulating hormone (αMSH). However, the pituitary corticotrope produces ACTH only, whereas keratinocytes and specific hypothalamic neurons produce αMSH, but not ACTH. The ability of cells to process the same prohormone into different peptides is due to cell type expression of **prohormone** (also called proprotein) **convertases**, resulting in cell-specific processing of the prohormone.

Protein/peptide hormones are stored in the gland as membrane-bound **secretory vesicles** and are released by exocytosis through the **regulated secretory pathway**. This means that hormones are not continually

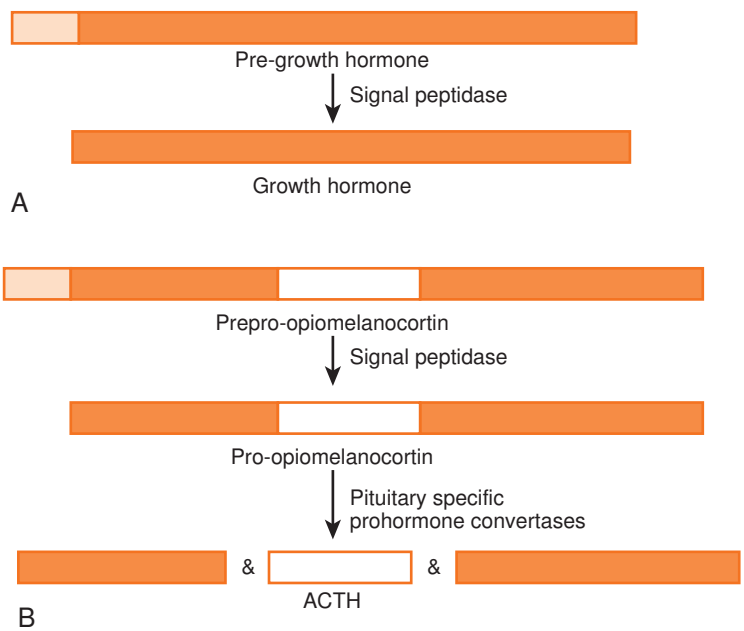


FIGURE 1-2 ■ Prehormone and preprohormone processing.

secreted, but rather that they are secreted in response to a stimulus, through a mechanism of **stimulus-secretion coupling**. Exocytosis involves the coupling of transmembrane Snare proteins that reside in the secretory vesicular membrane (*V-Snares*) and in the cell membrane (*target* or *T-Snares*). Regulated exocytosis is induced by an elevation of **intracellular Ca^{2+}** along with activation of other components (e.g., small G proteins), which interact with Snares and Snare-associated proteins (e.g., a Ca^{2+}-binding protein called synaptotagmin). This ultimately leads to the fusion of the secretory vesicular membrane with the cell membrane and exocytosis of the vesicular contents.

Protein/peptide hormones are soluble in aqueous solvents and, with the notable exceptions of the insulin-like growth factors (IGFs) and growth hormone (GH), circulate in the blood predominantly in an unbound form; therefore, they tend to have short biologic half-lives ($t_{1/2}$). Protein hormones are removed by endocytosis and lysosomal turnover of hormone receptor complexes (see later). Many protein hormones are small enough to appear in the urine in a physiologically active form. For example, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) are present in urine. Pregnancy tests using human urine are based on the presence of the placental LH-like hormone, human chorionic gonadotropin (hCG).

Proteins/peptides are readily digested if administered orally. Hence, they must be administered by injection or, in the case of small peptides, through a mucous membrane (sublingually or intranasally). Because proteins/peptides do not cross cell membranes readily, they signal through **transmembrane receptors**.

Catecholamines

Catecholamines are synthesized by the **adrenal medulla** and neurons and include norepinephrine, epinephrine, and dopamine (Fig. 1-3; Box 1-2). The primary hormonal product of the adrenal medulla is **epinephrine**, and to a lesser extent, norepinephrine. Epinephrine is produced by enzymatic modifications of the amino acid **tyrosine**. Epinephrine and other catecholamines are ultimately stored in secretory vesicles that are part of the regulated secretory pathway. Epinephrine is hydrophilic and circulates either unbound or loosely bound to albumin. Epinephrine and norepinephrine are similar to protein/peptide hormones in that they signal through membrane receptors, called **adrenergic receptors**. Catecholamines have short biologic half-lives (a few minutes) and are inactivated by intracellular enzymes. Inactivated forms diffuse out of cells and are excreted in the urine.

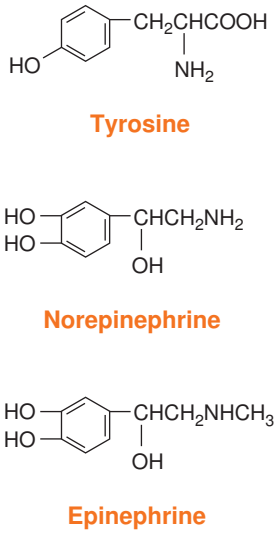


FIGURE 1-3 ■ Structure of the catecholamines, norepinephrine and epinephrine, and their precursor, tyrosine.

BOX 1-2
CHARACTERISTICS OF CATECHOLAMINES

- Derived from enzymatic modification of tyrosine
- Stored in membrane-bound secretory vesicles
- Regulated at the level of secretion (regulated exocytosis) and through the regulation of the enzymatic pathway required for their synthesis
- Transported in blood free or only loosely associated with proteins
- Often administered as an aerosol puff for opening bronchioles, and several specific analogs (agonists and antagonists) can be taken orally
- Hydrophilic and signal through transmembrane G-protein-coupled receptors called *adrenergic receptors*

BOX 1-3

CHARACTERISTICS OF STEROID HORMONES

- Derived from enzymatic modification of cholesterol
- Cannot be stored in secretory vesicles because of lipophilic nature
- Regulated at the level of the enzymatic pathway required for their synthesis
- Transported in the blood bound to transport proteins (binding globulins)
- Signal through intracellular receptors (nuclear hormone receptor family)
- Can be administered orally

Steroid Hormones

Steroid hormones are made by the **adrenal cortex, ovaries, testes**, and **placenta** (Box 1-3). Steroid hormones from these glands fall into five categories: **progestins, mineralocorticoids, glucocorticoids, androgens**, and **estrogens** (Table 1-2). Progestins and the corticoids are 21-carbon steroids, whereas androgens are 19-carbon steroids and estrogens are 18-carbon steroids. Steroid hormones also include the active metabolite of **vitamin D**, which is a secosteroid (see Chapter 4).

Steroid hormones are synthesized by a series of enzymatic modifications of **cholesterol** (Fig. 1-4). The enzymatic modifications of cholesterol are of three general types: hydroxylations, dehydrogenations/hydrogenations, and breakage of carbon-carbon bonds. The purpose of these modifications is to produce a cholesterol derivative that is sufficiently unique to be recognized by a specific receptor. Thus, progestins bind to the **progesterone receptor (PR)**, mineralocorticoids bind to the **mineralocorticoid receptor (MR)**, glucocorticoids bind to the **glucocorticoid receptor (GR)**, androgens bind to the **androgen receptor (AR)**, estrogens bind to the **estrogen receptor (ER)**, and the active vitamin D metabolite binds to the **vitamin D receptor (VDR)**.

The complexity of steroid hormone action is increased by the expression of multiple forms of each receptor. Additionally, there is some degree of nonspecificity between steroid hormones and the receptors they bind to. For example, glucocorticoids bind to the MR with high affinity, and progestins, glucocorticoids, and androgens can all interact with the PR, GR, and AR to some degree. An appreciation of this "cross-talk" is important to the physician who is prescribing synthetic steroids. For example, medroxyprogesterone acetate (a synthetic progesterone given for hormone replacement therapy in postmenopausal women) binds well to the AR as well as the PR. As discussed subsequently, steroid hormones are lipophilic and pass through cell membranes easily. Accordingly, classic steroid hormone receptors are localized intracellularly and act by regulating gene expression. More recently, membrane and juxtamembrane receptors have been discovered that mediate rapid, nongenomic actions of steroid hormones.

Steroidogenic cell types are defined as cells that can convert **cholesterol** to **pregnenolone**, which is the first reaction common to all steroidogenic pathways. Steroidogenic cells have some capacity for cholesterol synthesis but often obtain cholesterol from circulating **cholesterol-rich lipoproteins** (low-density lipoproteins and high-density lipoproteins; see Chapter 3). Pregnenolone is then further modified by six or fewer enzymatic reactions. Because of their hydrophobic nature, steroid hormones and precursors can leave the steroidogenic cell easily and so are not stored. Thus, steroidogenesis is regulated at the level of uptake,

TABLE 1-2

Steroid Hormones

FAMILY	NO. OF CARBONS	SPECIFIC HORMONE	PRIMARY SITE OF SYNTHESIS	PRIMARY RECEPTOR
Progestin	21	Progesterone	Ovary placenta	Progesterone receptor (PR)
Glucocorticoid	21	Cortisol, corticosterone	Adrenal cortex	Glucocorticoid receptor (GR)
Mineralocorticoid	21	Aldosterone, 11-Deoxycorticosterone	Adrenal cortex	Mineralocorticoid receptor (MR)
Androgen	19	Testosterone, Dihydrotestosterone	Testis	Androgen receptor (AR)
Estrogen	18	Estradiol-17β, Estriol	Ovary placenta	Estrogen receptor (ER)

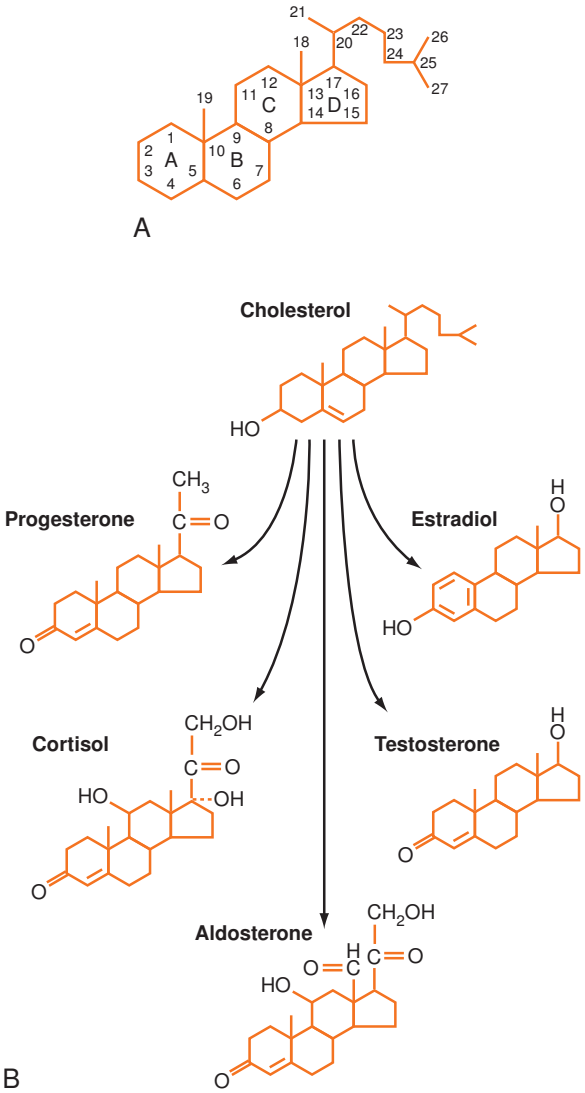


FIGURE 1-4 ■ Cholesterol and steroid hormone derivatives. *(From Koeppen BM, Stanton BA, editors:* Berne and Levy Physiology, *6th ed., Philadelphia, 2010, Mosby.)*

storage, and mobilization of cholesterol and at the level of steroidogenic enzyme gene expression and activity. Steroids are *not* regulated at the level of secretion of the preformed hormone. A clinical implication of this mode of secretion is that high levels of **steroid hormone precursors** are easily released into the blood when a *downstream* steroidogenic enzyme within a given pathway is inactive or absent (Fig. 1-5). In comparing the ultrastructure of a protein hormone–producing cell to that of a steroidogenic cell, protein hormone–producing cells store the product in secretory granules and have extensive rough endoplasmic reticulum. In contrast, steroidogenic cells store precursor (cholesterol esters) in the form of lipid droplets, but do not store product. Steroidogenic enzymes are localized to smooth endoplasmic reticulum membrane and within mitochondria, and these two organelles are numerous in steroidogenic cells.

An important feature of steroidogenesis is that steroid hormones often undergo further modifications (apart from those involved in deactivation and excretion) after their release from the original steroidogenic cell. This is referred to as **peripheral conversion**. For example, estrogen synthesis by the ovary and placenta requires at least two cell types to complete the pathway of cholesterol to estrogen (see Chapters 10 and 11). This means that one cell secretes a precursor, and a second cell converts the precursor to estrogen. There is also considerable peripheral conversion of active steroid hormones. For example, the testis secretes sparingly little estrogen. However, adipose, muscle, and other tissues express the enzyme for converting testosterone (a potent androgen) to estradiol-17β. Peripheral conversion of steroids plays an important role in several endocrine disorders (e.g., see Fig. 1-5).

Steroid hormones are hydrophobic, and a significant fraction circulates in the blood bound to transport proteins (see later). These include albumin, but also the specific transport proteins, **sex hormone–binding globulin (SHBG)** and **corticosteroid-binding globulin (CBG)** (see later). Excretion of hormones typically involves inactivating modifications followed by glucuronide or sulfate conjugation in the liver. These modifications increase the water solubility of the steroid and decrease its affinity for transport proteins, allowing the inactivated steroid hormone to be excreted by the kidney. Steroid compounds are absorbed fairly readily in the gastrointestinal tract and therefore often may be administered orally.

Thyroid Hormones

Thyroid hormones are classified as **iodothyronines** (Fig. 1-6) that are made by the coupling of iodinated tyrosine residues through an ether linkage (Box 1-4; see Chapter 6). Their specificity is determined by

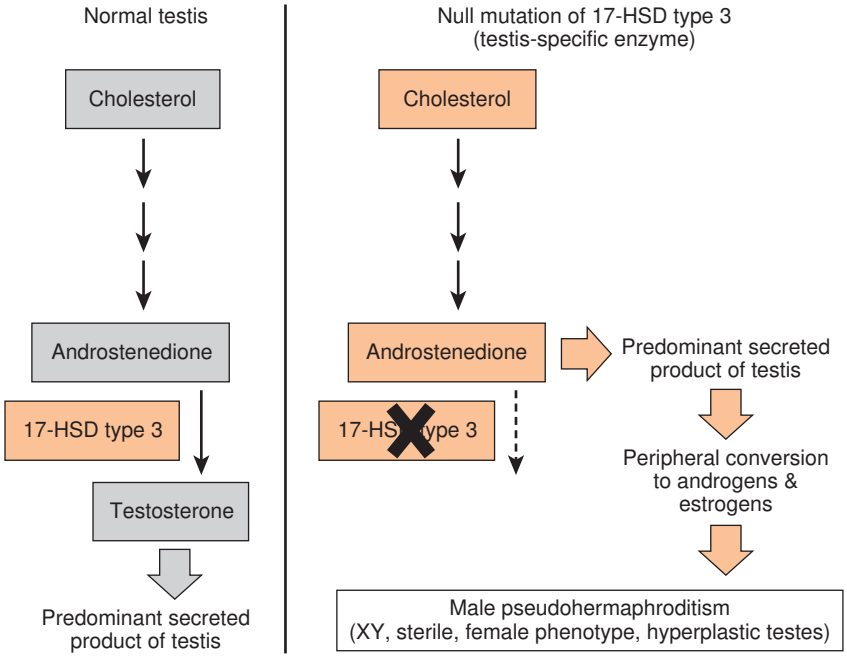


FIGURE 1-5 ■ Example of the effect of an enzyme defect on steroid hormone precursors in blood.

the thyronine structure, but also by exactly where the thyronine is iodinated. Normally, the predominant iodothyronine released by the thyroid is **T_4 (3,5,3′,5,-tetraiodothyronine**, also called **thyroxine**), which acts as a circulating precursor of the active form, **T_3 (3,5,3′-triiodothyronine**). Thus, peripheral conversion through specific 5′-deiodination plays an important role in thyroid function (see Chapter 6). Thyroid hormones cross cell membranes by both diffusion and transport systems. They are stored extracellularly in the thyroid as an integral part of the glycoprotein molecule thyroglobulin (see Chapter 6). Thyroid hormones are sparingly soluble in blood and are transported in blood bound to **thyroid hormone–binding globulin (TBG)**. T_4 and T_3 have long half-lives of 7 days and 24 hours, respectively. Thyroid hormones are similar to steroid hormones in that the **thyroid hormone receptor (TR)** is intracellular

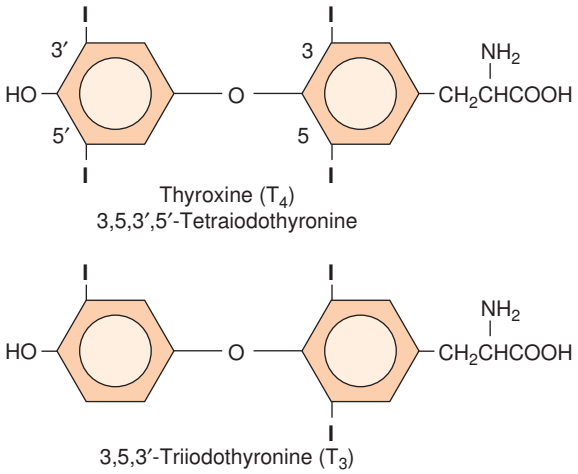


FIGURE 1-6 ■ Structure of thyroid hormones, which are iodinated thyronines.

BOX 1-4

CHARACTERISTICS OF THYROID HORMONES

- Derived from the iodination of thyronines
- Lipophilic, but stored in thyroid follicle by covalent attachment to thyroglobulin
- Regulated at the level of synthesis, iodination, and secretion
- Transported in blood tightly bound to proteins
- Signal through intracellular receptors (nuclear hormone receptor family)
- Can be administered orally

and acts as a transcription factor. In fact, the TR belongs to the same gene family that includes steroid hormone receptors and vitamin D receptors. Thyroid hormones can be administered orally and sufficient hormone is absorbed intact to make this an effective mode of therapy.

TRANSPORT OF HORMONES IN THE CIRCULATION

A significant amount of steroid and thyroid hormones is transported in the blood bound to plasma proteins that are produced in a regulated manner by the liver. Protein and polypeptide hormones are generally transported free in the blood. There exists an equilibrium among the concentrations of bound hormone (HP), free hormone (H), and plasma transport protein (P); if free hormone levels drop, hormone will be released from the transport proteins. This relationship may be expressed as follows:

$$[H] \times [P] = [HP] \text{ or } K = [H] \times [P]/[HP]$$

where K = the dissociation constant.

The **free hormone** is the biologically active form for target organ action, feedback control, and clearance by uptake and metabolism. Consequently, in evaluating hormonal status, one must sometimes determine free hormone levels rather than total hormone levels alone. This is particularly important because hormone transport proteins themselves are regulated by altered endocrine and disease states.

Protein binding serves several purposes. It prolongs the circulating $t_{1/2}$ of the hormone. The bound hormone represents a "reservoir" of hormone and as such can serve to *buffer* acute changes in hormone secretion. In addition, steroid and thyroid hormones are lipophilic and hydrophobic. Binding to transport proteins prevents these hormones from simply partitioning into the cells near their secretion and allows them to be transported throughout the circulation.

CELLULAR RESPONSES TO HORMONES

Hormones regulate essentially every major aspect of cellular function in every organ system. Hormones control the growth of cells, ultimately determining their size and competency for cell division. Hormones regulate the differentiation of cells through genetic and epigenetic changes and their ability to survive or undergo programmed cell death. Hormones influence cellular metabolism, ionic composition, and transmembrane potential. Hormones orchestrate several complex cytoskeletal-associated events, including cell shape, migration, division, exocytosis, recycling/endocytosis, and cell-cell and cell-matrix adhesion. Hormones regulate the expression and function of cytosolic and membrane proteins, and a specific hormone may determine the level of its own receptor, or the receptors for other hormones.

Although hormones can exert coordinated, pleiotropic control on multiple aspects of cell function, any given hormone does not regulate every function in every cell type. Rather, a single hormone controls a subset of cellular functions in only the cell types that express **receptors** for that hormone (i.e., the **target cell**). Thus, selective receptor expression determines which cells will respond to a given hormone. Moreover, the differentiated epigenetic state of a specific cell will determine how it will respond to a hormone. Thus, the specificity of hormonal responses resides in the structure of the hormone itself, the receptor for the hormone, and the cell type in which the receptor is expressed. Serum hormone concentrations are extremely low (10^{-11} to 10^{-9} M). Therefore, a receptor must have a **high affinity**, as well as **specificity**, for its cognate hormone.

Hormone receptors fall into two general classes: **transmembrane receptors** and **intracellular receptors** that belong to the **nuclear hormone receptor** family.

Transmembrane Receptors

Most hormones are proteins, peptides, or catecholamines that cannot pass through the cell membrane. Thus, these hormones must interact with **transmembrane protein receptors**. Transmembrane receptors are proteins that contain three domains (proceeding from outside to inside the cell): (1) an extracellular domain that harbors a high-affinity binding site for a specific hormone; (2) one to seven hydrophobic, transmembrane domains that span the cell membrane; and (3) a cytosolic domain that is linked to signaling proteins.

Hormone binding to a transmembrane receptor induces a conformational shift in all three domains of the receptor protein. This hormone receptor binding–induced conformational change is referred to as a **signal**. The signal is **transduced** into the activation of one or more **intracellular signaling molecules**. Signaling molecules then act on **effector proteins**, which, in turn, modify specific cellular functions. The combination of hormone receptor binding (signal), activation of signaling molecules (transduction), and the regulation of one or more effector proteins is referred to as a **signal transduction pathway** (also called simply a **signaling pathway**), and the final integrated outcome is referred to as the **cellular response**.

Signaling pathways linked to transmembrane receptors are usually characterized by the following:

A. **Receptor binding** followed by a **conformational shift** that extends to the cytosolic domain. The conformational shift may result in one or more of the following:
   1. Activation of a guanine exchange function of a receptor (see later).
   2. Homodimerization and/or heterodimerization of receptors to other receptors or co-receptors within the membrane.
   3. Recruitment and activation of signaling proteins by the cytosolic domain.

B. **Multiple, hierarchal steps** in which *downstream* effector proteins are dependent on and driven by *upstream* receptors and signaling molecules and effector proteins. This means that loss or inactivation of one or more components within the pathway leads to **hormonal resistance**, whereas constitutive activation or overexpression of components can provoke a cellular response in a **hormone-independent**, unregulated manner.

C. **Amplification** of the initial hormone receptor binding–induced signal, usually by inclusion of an enzymatic step within a signaling pathway. Amplification can be so great that maximal response to a hormone is achieved upon hormone binding to a fraction of available receptors.

D. **Activation of multiple divergent or convergent pathways** from one hormone receptor–binding event. For example, binding of insulin to its receptor activates three separate signaling pathways.

E. **Antagonism by constitutive and regulated negative feedback reactions**. This means that a signal is dampened or terminated by opposing pathways. Gain of function of opposing pathways can result in **hormonal resistance**.

Signaling pathways use several common modes of informational transfer (i.e., intracellular messengers and signaling events). These include the following:

1. **Conformational shifts**. Many signaling components are proteins and have the ability to toggle between two (or more) conformational states that alter their activity, stability, or intracellular location. As discussed previously, signaling begins with hormone receptor binding that induces a conformational change in the receptor (Fig. 1-7). The other modes of informational transfer discussed later either regulate or are regulated by conformational shifts in transmembrane receptors and in downstream signaling proteins.
2. **Covalent phosphorylation of proteins and lipids** (Fig. 1-8). Enzymes that phosphorylate proteins or lipids are called **kinases**, whereas those

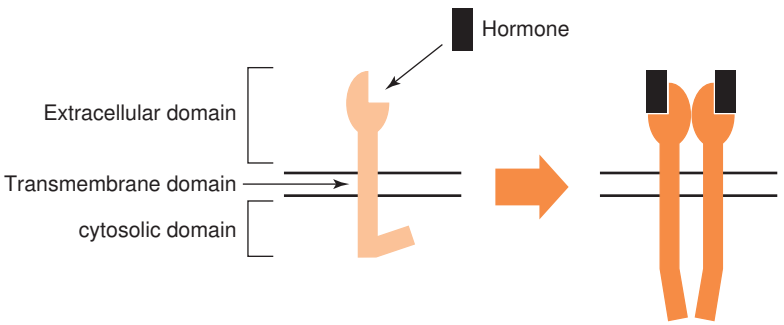


FIGURE 1-7 ■ Example of hormone-induced conformational change in transmembrane receptor. This often promotes dimerization of receptors as well as conformational changes in the cytosolic domain that unmasks a specific activity (e.g., guanine nucleotide exchange factor activity, tyrosine kinase activity).

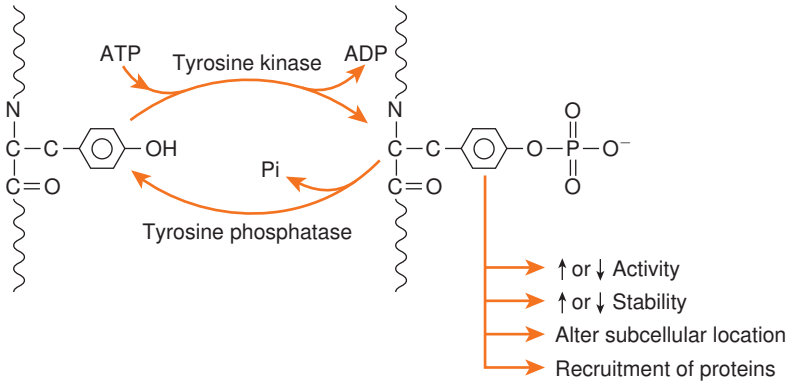


FIGURE 1-8 ■ Phosphorylation/dephosphorylation in signal transduction pathways. In this case, phosphotyrosine is shown.

that catalyze dephosphorylation are called **phosphatases**. Protein kinases and phosphatases can be classified as either tyrosine-specific kinases and phosphatases or serine/threonine-specific kinases and phosphatases. There are also *mixed function* kinases and phosphatases that recognize all three residues. An important lipid kinase is phosphatidylinositol-3-kinase (PI3K; see later).

The phosphorylated state of a signaling component can alter the following:

a. **Activity**. Phosphorylation can activate or deactivate a substrate, and proteins often have multiple sites of phosphorylation that induce quantitative and/or qualitative changes in the protein's activity.
b. **Stability**. For example, phosphorylation of proteins can induce their subsequent ubiquitination and proteasomal degradation.
c. **Subcellular location**. For example, the phosphorylation of some nuclear transcription factors induces their translocation to and retention in the cytoplasm.
d. **Recruitment and clustering of other signaling proteins**. For example, phosphorylation of the cytosolic domain of a transmem-brane receptor often induces the recruitment of signaling proteins to the receptor where they are phosphorylated. Recruitment happens because the recruited protein harbors a domain that specifically recognizes and binds to the phosphorylated residue. Another important example of recruitment by phosphorylation is the recruitment of the protein kinase Akt/PKB to the cell membrane, where it is phosphorylated and activated by the protein kinase, PDK1. In this case, Akt/PKB and PDK1 are recruited to the cell membrane by the phosphorylated membrane lipid, phosphatidylinositol 3,4,5-triphosphate (PIP_3).

3. **Noncovalent guanosine nucleotide triphosphate (GTP) binding to GTP-binding proteins (G proteins)**. **G proteins** represent a large family of molecular switches, which are latent and inactive when bound to GDP, and active when bound to GTP (Fig. 1-9). G proteins are activated by **guanine nucleotide exchange factors (GEFs)**, which promote the dissociation of GDP and binding of GTP. G proteins have intrinsic **GTPase** activity. GTP is normally hydrolyzed to GDP within seconds by the G protein, thereby terminating the transducing activity of the G protein. Another G-protein termination mechanism (which represents a target for drug development to treat certain endocrine diseases) is the family of proteins called **regulators of G-protein signaling (RGS proteins)**, which bind to active G proteins and increase their intrinsic GTPase activity.
4. **Noncovalent binding of cyclic nucleotide monophosphates to their specific effector proteins** (Fig. 1-10). **Cyclic adenosine monophosphate (cAMP)** is generated from adenosine triphosphate (ATP) by **adenylyl cyclase**, which is primarily a membrane protein. Adenylyl cyclase is activated and inhibited by the G proteins, Gs-α and Gi-α,

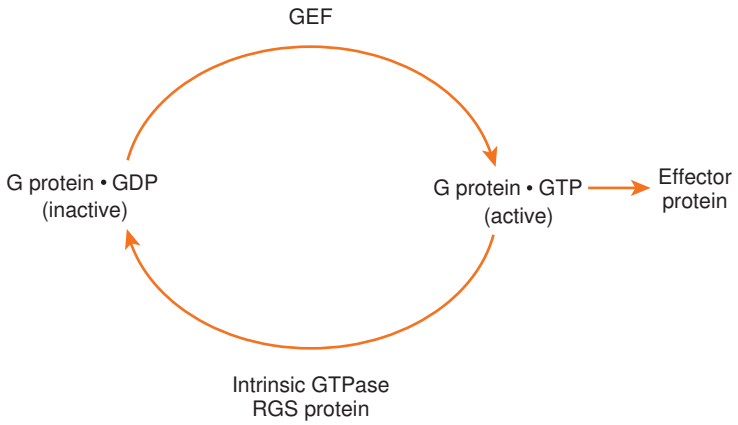


FIGURE 1-9 ■ G proteins in signal transduction pathways. GEF, guanine nucleotide exchange factor; RGS, regulator of G-protein signaling.

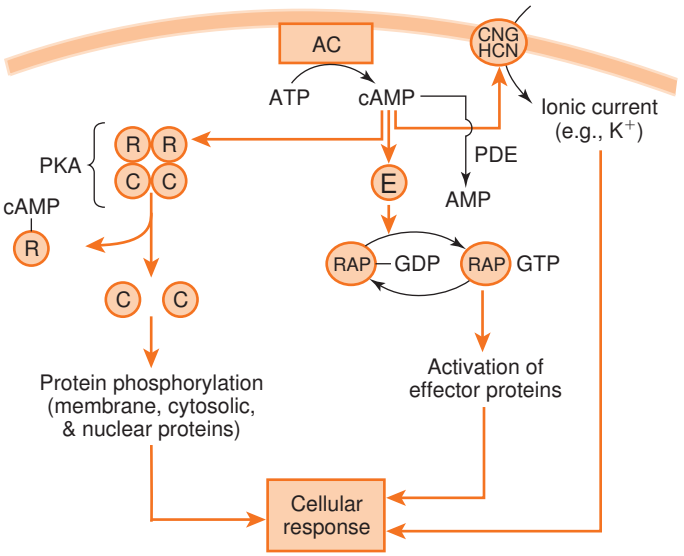


FIGURE 1-10 ■ Cyclic AMP/PKA in signal transduction pathways. AC, adenylyl cyclase; PDE, phosphodiesterase; R & C, regulatory and catalytic subunits, respectively, of protein kinase A (PKA); E, EPAC (exchange protein activated by cAMP); CNG, cyclic nucleotide–gated channel; HCN, hyperpolarization-induced cyclic nucleotide–modulated channel.

respectively (see later). There are three general intracellular effectors of cyclic AMP (cAMP):

a. cAMP binds to the regulatory subunit of **protein kinase A** (**PKA**; also called **cAMP-dependent protein kinase**). Inactive PKA is a heterotetramer composed of two catalytic subunits and two regulatory subunits. cAMP binding causes the regulatory subunits to dissociate from the catalytic subunits, thereby generating two molecules of active catalytic PKA subunits (PKA_c). PKA_c phosphorylates numerous proteins on serine and threonine residues. Substrates of PKA_c include numerous cytosolic proteins as well as transcription factors, most notably **cAMP-responsive element–binding protein** (**CREB protein**).

b. A second effector of cAMP is **Epac** (***ex*change *p*rotein *a*ctivated by *c*AMP**), which has two isoforms. Epac proteins act as GEFs (see earlier) for small G proteins (called Raps). Raps in turn control a wide array of cell functions, including formation of cell-cell junctional complexes and cell-matrix adhesion, Ca^{2+} release from intracellular stores (especially in cardiac muscle) and in the augmentation

of glucose-dependent insulin secretion by glucagon-like peptide-1 in pancreatic islet β cells (see Chapter 3).

c. cAMP (and cyclic guanosine monophosphate [cGMP], discussed later) also binds directly to and regulates **ion channels**. These are of two types: **cyclic nucleotide gated (CNG)** channels and **hyperpolarization-activated cyclic nucleotide modulated (HCN)** channels. For example, norepinephrine, which acts through a Gs-coupled receptor, increases heart rate in part through increasing a depolarizing inward K^+ and Na^+ current via an HCN at the sinoatrial node.

cGMP is produced from GTP by **guanylyl cyclase**, which exists in both transmembrane and soluble forms (Fig. 1-11). The transmembrane form of guanylyl cyclase is a hormone receptor, **natriuretic peptide receptor (NPR-A** and **NPR-B)**, for the **natriuretic peptides (atrial = ANP; brain = BNP; C-type = CNP)**. The soluble form of guanylyl cyclase is activated by another messenger, **nitric oxide (NO)**. Nitric oxide is produced from molecular oxygen and arginine by the enzyme **nitric oxide synthase (NOS)**. In vascular endothelial cells, endothelial NOS (eNOS) activity is the target of vasodilatory neuronal signals (e.g., acetylcholine) and certain hormones (estrogen). NO then diffuses into vascular smooth muscle and activates soluble guanylyl cyclase to produce cGMP. cGMP activates **protein kinase G (PKG)**, which phosphorylates and regulates numerous proteins. In vascular smooth muscle, this leads to relaxation and vasodilation. As discussed earlier, cGMP also regulates ion channels.

cAMP and cGMP are degraded to AMP and GMP, respectively, by **phosphodiesterases** (see Figs. 1-10 and 1-11), thereby terminating their signaling function. Phosphodiesterases represent a large family of proteins and display cell-specific expression. cAMP phosphodiesterases are inhibited by caffeine and other methylxanthines. cGMP is degraded cGMP phosphodiesterases, of

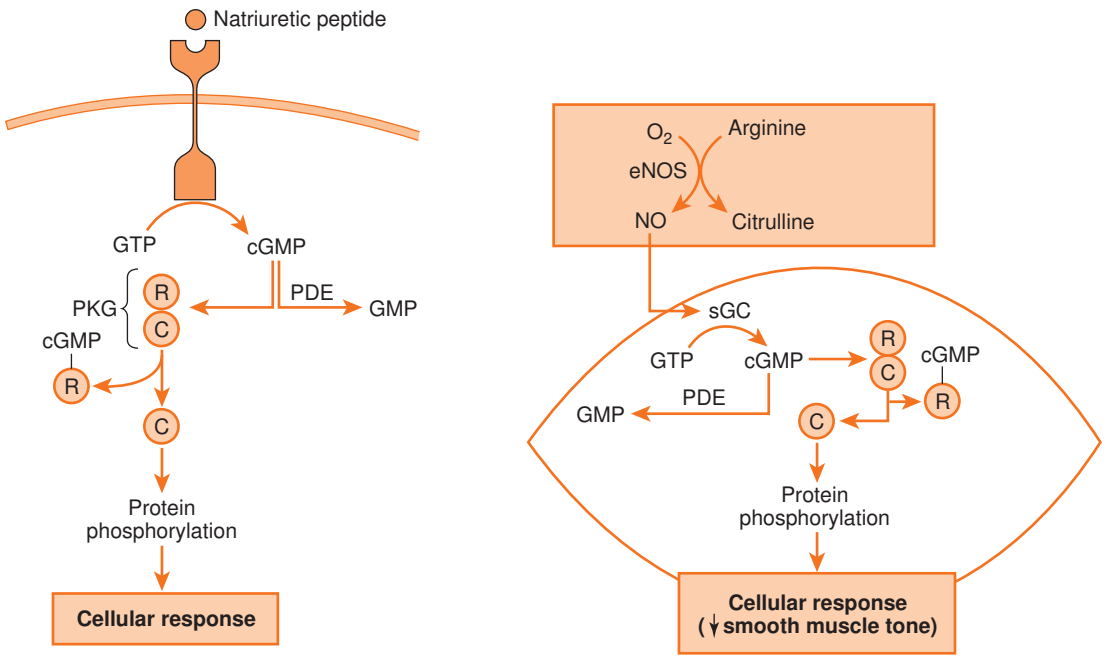


FIGURE 1-11 ■ Membrane-bound and soluble guanylyl cyclases. R and C, regulatory and catalytic subunits, respectively, of protein kinase G (PKG). eNOS, endothelial nitric oxide synthase; NO, nitric oxide; sGC, soluble guanylyl cyclase.

which one isoform is inhibited by sildenafil (Viagra). In some contexts, cAMP and cGMP can modulate each other (a phenomenon called **cross-talk**) through the regulation of phosphodiesterases. For example, oocyte arrest is maintained by high levels of cAMP. The LH surge decreases cGMP in surrounding follicle cells by decreasing the local production of a natriuretic peptide. This results in lowered oocyte cyclic GMP. Because cGMP inhibits the oocyte cAMP-specific phosphodiesterase, lowered cGMP leads to decreased cAMP, thereby allowing the oocyte to complete the first meiotic division (see Chapter 10).

5. **Generation of lipid informational molecules, which act as intracellular messengers. These include diacylglycerol (DAG) and inositol 1,4,5-triphosphate (IP_3)**, which are cleaved from phosphatidylinositol 4,5-bisphosphate (PIP_2) by **membrane-bound phospholipase C (PLC)**. DAG activates certain isoforms of protein kinase C (Fig. 1-12). IP_3 binds to the IP_3 receptor, which is a large complex forming a Ca^{2+} channel, on the endoplasmic reticulum membrane, and promotes Ca^{2+} efflux (see later) from the endoplasmic reticulum into the cytoplasm. Some isoforms of DAG-activated PKC are also Ca^{2+} dependent, so the actions of IP_3 converge on and reinforce those of DAG. The DAG signal is terminated by lipases, whereas IP_3 is rapidly inactivated by dephosphorylation.
6. **Noncovalent Ca^{2+} binding** (see Fig. 1-12). Cytosolic levels of Ca^{2+} are maintained at very low levels (i.e., 10^{-7} to 10^{-8} M), by either active transport of Ca^{2+} out of the cell, or into intracellular compartments (e.g., endoplasmic reticulum). As discussed earlier, IP_3 binding to the IP_3 receptor increases the flow of Ca^{2+} into the cytoplasm from the endoplasmic reticulum. Ca^{2+} can also enter the cytoplasm through the regulated opening of Ca^{2+} channels in the cell membrane. This leads to an increase in Ca^{2+} binding directly to numerous specific effector proteins, which leads to a change in their activities. Additionally, Ca^{2+} regulates several effector proteins indirectly, through binding to the messenger protein, calmodulin. Several of the Ca^{2+}/calmodulin targets are enzymes, which amplify the initial signal of increased cytosolic Ca^{2+}. The Ca^{2+}-dependent message is terminated by the lowering of cytosolic Ca^{2+} by cell membrane and endoplasmic reticular Ca^{2+} ATPases (i.e., Ca^{2+} pumps).

Transmembrane Receptors Using G Proteins

The largest family of hormone receptors is the **G-protein-coupled receptor (GPCR)** family. These receptors span the cell membrane seven times and are referred to as 7-helix transmembrane receptors. The G proteins that directly interact with GPCRs are termed **heterotrimeric G proteins** and are composed of an **α subunit (Gα)**, and a **β/γ subunit dimer** (Gβ/γ). The Gα subunit binds GTP and functions as

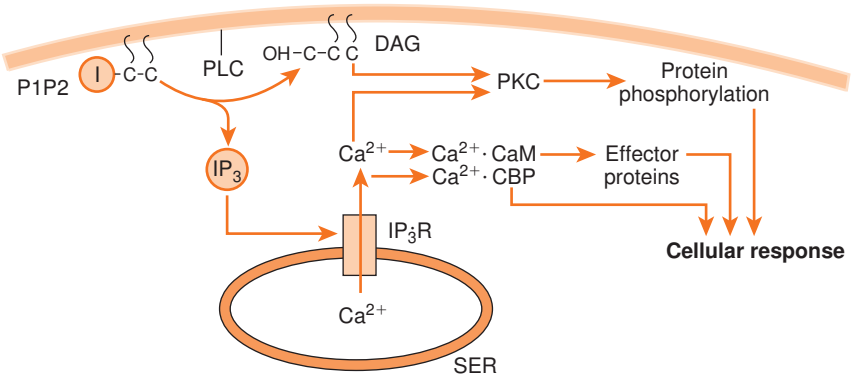


FIGURE 1-12 ■ IP_3 (inositol 1,4,5-triphosphate) and DAG (diacylglycerol) in signaling pathways. PLC, phospholipase C; PIP_2, phosphatidylinositol 4,5-bisphosphate; IP_3R,IP_3 receptor; SER smooth endoplasmic reticulum; CaM, calmodulin; CBP, calcium-binding proteins.

the primary G-protein signal transducer. GPCRs are, in fact, ligand-activated GEFs (see earlier). This means that on hormone binding, the conformation of the receptor shifts to the active state. Once active, the GPCR induces the exchange of GDP for GTP, thereby activating Gα. One hormone-bound receptor activates 100 or more G proteins. GTP-bound Gα then dissociates from Gβ/γ and binds to and activates one or more effector proteins (Fig. 1-13).

How do G proteins link specific hormone receptor–binding events with specific downstream effector proteins? There are at least 16 Gα proteins that show specificity with respect to cell-type expression, GPCR binding, and effector protein activation. A rather ubiquitous Gα protein is called **Gs-α**, which stimulates the membrane enzyme, adenylyl cyclase, and increases the levels of another messenger, cAMP (see earlier). Some GPCRs couple to **Gi-α**, which inhibits adenylyl cyclase. A third major hormonal signaling pathway is through **Gq-α**, which activates **phospholipase C** (**PLC**). As discussed previously, PLC generates two lipid messengers, **DAG** and **IP_3**, from PIP_2. Defects in G-protein structure and expression are linked to endocrine diseases such as pseudohypoparathyroidism (loss of Gs activity) or pituitary tumors (loss of intrinsic GTPase activity in Gs, thereby extending its time in the active state).

GPCR-dependent signaling pathways regulate a broad range of cellular responses. For example, the pancreatic hormone, glucagon, regulates numerous aspects of hepatic metabolism (see Chapter 3). The glucagon receptor is linked to the Gs-cAMP-PKA pathway, which diverges to regulate enzyme activity at both posttranslational and transcriptional levels. PKA phosphorylates and thereby activates phosphorylase kinase. Phosphorylase kinase phosphorylates and activates glycogen phosphorylase, which catalyzes the release of glucose molecules from glycogen. Catalytic subunits of PKA also enter the nucleus, where they phosphorylate and activate the transcription factor, CREB protein. Phospho-CREB then increases the transcriptional rate of genes encoding specific enzymes (e.g., phosphoenolpyruvate carboxykinase).

In summary, *signaling from one GPCR can regulate a number of targets in different cellular compartments with different kinetics* (Fig. 1-14).

As mentioned, G-protein signaling is terminated by intrinsic GTPase activity, converting GTP to GDP. This returns the G protein to an inactive state (bound to GDP). Another termination mechanism involves **desensitization** and **endocytosis** of the GPCR (Fig. 1-15). Hormone binding to the receptor increases the ability of GPCR kinases (GRKs) to phosphorylate the intracellular domain of GPCRs. This phosphorylation recruits

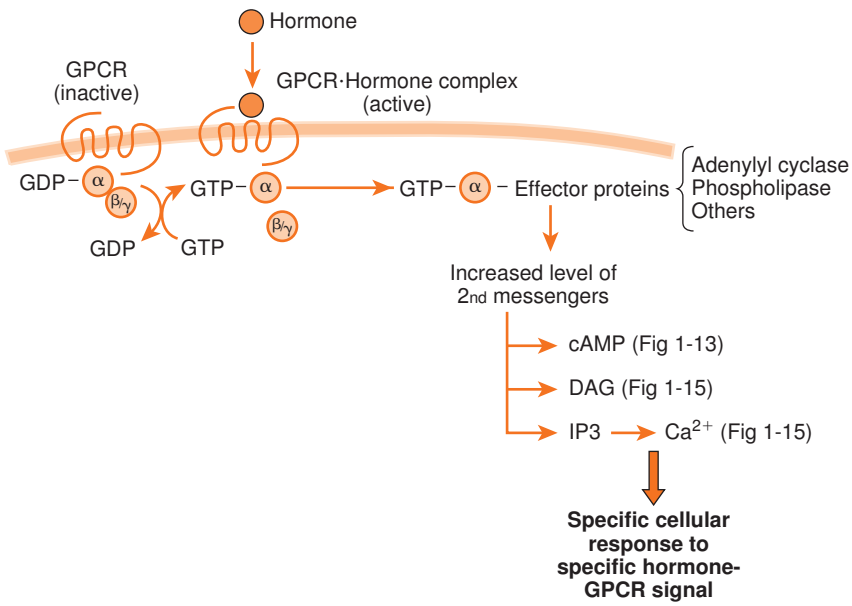


FIGURE 1-13 ■ Signaling pathway for hormones that bind to GPCRs.

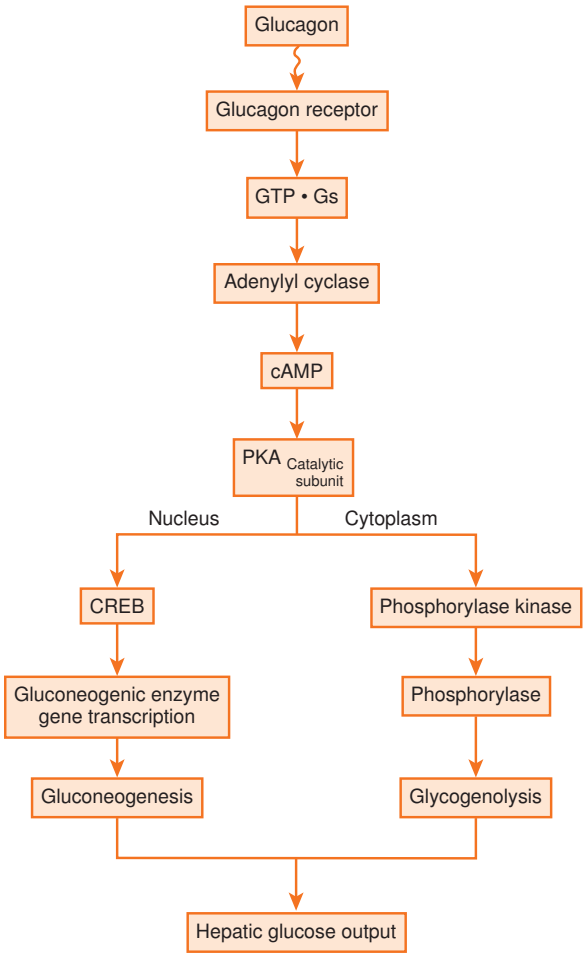


FIGURE 1-14 ■ Coordinated regulation of cytoplasmic and nuclear events by PKA to produce a general cellular response.

proteins called **β-arrestins**. GRK-induced phosphorylation and β-arrestin binding inactivate the receptor, and β-arrestin couples the receptor to clathrin-mediated endocytotic machinery. Some GPCRs are dephosphorylated and rapidly recycled back to the cell membrane (without hormone), whereas others are degraded in lysosomes. GRK/β-arrestin-dependent inactivation and endocytosis is an important mechanism for hormonal desensitization of a cell after exposure to excessive hormone. Hormone receptor endocytosis (also called *receptor-mediated endocytosis*) is also an

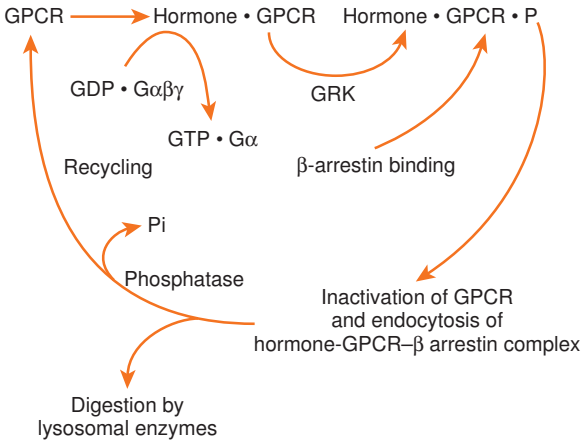


FIGURE 1-15 ■ GPCR inactivation and endocytosis to lysosomes (desensitization) and/or recycling back to the cell membrane in a dephosphorylated form (resensitization).

important mechanism for clearing protein and peptide hormones from the blood.

Receptor Tyrosine Kinases

Receptor tyrosine kinases (RTKs) can be classified into two groups: the first acting as receptors for several growth factors (e.g., epidermal growth factor, platelet-derived growth factor), and the second group for insulin and insulin-like growth factors (IGFs). The former group of RTKs comprises transmembrane glycoproteins with an intracellular domain containing intrinsic tyrosine kinase activity. Growth factor binding induces dimerization of the RTK within the cell membrane, followed by transphosphorylation of tyrosine residues, generating phosphotyrosine (pY). The phosphotyrosines function to recruit proteins. One recruited protein is phospholipase C, which is then activated by phosphorylation and generates the messengers DAG and IP_3 from PIP_2 (see earlier). A second critically important protein that is recruited to pY residues is the adapter protein, Grb2, which is complexed with a GEF named SOS. Recruitment of SOS to the membrane allows it to activate a small, membrane-bound monomeric G protein called Ras. Ras then binds to its effector protein, Raf. Raf is a serine-specific kinase that phosphorylates and activates the dual-function kinase, MEK. MEK then phosphorylates and activates a

mitogen-activated protein kinase (MAP kinase, also called ERK). Activated MAP kinases then enter the nucleus and phosphorylate and activate several transcription factors. This signaling pathway is referred to as the *MAP kinase cascade*, and it transduces and amplifies a growth factor–RTK signal into a cellular response involving a change in the expression of genes encoding proteins involved in proliferation and survival.

The insulin receptor (IR) differs from growth factor RTKs in several respects. First, the latent IR is already dimerized by Cys-Cys bonds, and insulin binding induces a conformational change that leads to transphosphorylation of the cytoplasmic domains (Fig. 1-16). A major recruited protein to pY residues is the insulin receptor substrate (IRS), which is then phosphorylated on tyrosine residues by the IR. The pY residues on IRS recruit the Grb-2/SOS complex, thereby activating growth responses to insulin through the MAP kinase pathway (see Fig. 1-16). The pY residues on the IRS also recruit the lipid kinase, PI3K, activating and concentrating the kinase near its substrate, PIP_2, in the cell membrane. As discussed earlier, this ultimately leads to activation of Akt/PKB, which is required for the metabolic responses to insulin (Fig. 1-17). The IR also activates a pathway involving the small G protein, TC-10 (see Fig. 1-17). The small G-protein-dependent pathway and the Akt/PKB pathway are both required for the actions of insulin on glucose uptake (see Chapter 3).

RTKs are down regulated by ligand-induced endocytosis. Additionally, the signaling pathways from RTKs, including IR and IRS, are inhibited by serine/threonine phosphorylation, tyrosine dephosphorylation, and the suppressor of cytokine signaling proteins (see next section).

Receptors Associated with Cytoplasmic Tyrosine Kinases

Another class of membrane receptor falls into the **cytokine receptor family** and includes receptors for growth hormone, prolactin, erythropoietin, and leptin. These

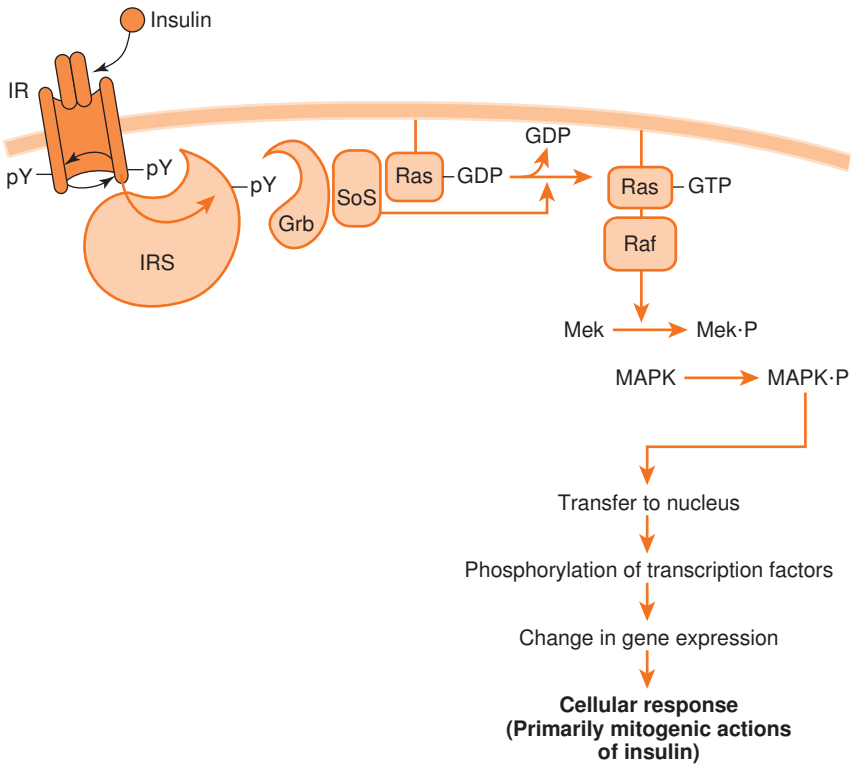


FIGURE 1-16 ■ Signaling from the insulin receptor (a receptor tyrosine kinase) through the MAPK pathway. pY, phosphorylated tyrosine residue in protein.

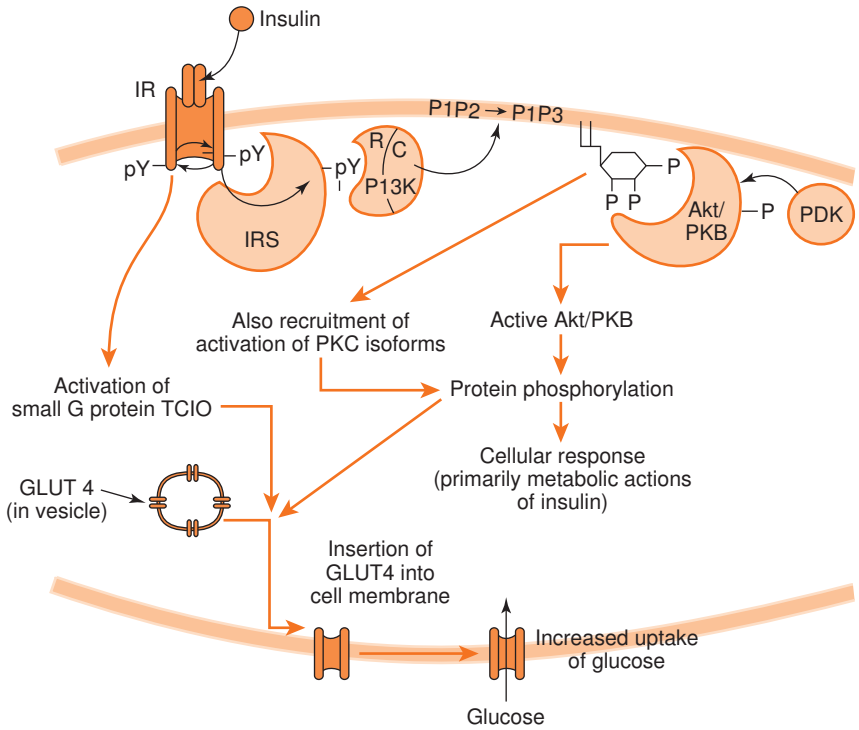


FIGURE 1-17 ■ Signaling from the insulin receptor through the phosphatidylinositol-3-kinase (PI3K)/Akt/PKB pathway. R and C; regulatory and catalytic subunits, respectively, of PI3K. PIP_2, phosphatidylinositol 4,5-bisphosphate; PIP_3, phosphatidylinositol 3,4,5 trisphosphate. PKC, protein kinase C; pY, phosphorylated tyrosine residue in protein.

receptors, which exist as dimers, do not have intrinsic protein kinase activity. Instead, the cytoplasmic domains are stably associated with members of the **JAK kinase family** (Fig. 1-18). Hormone binding induces a conformational change, bringing the two JAKs associated with the dimerized receptor closer together and causing their transphosphorylation and activation. JAKs then phosphorylate tyrosine residues on the cytoplasmic domains of the receptor. The pY residues recruit latent transcription factors called **STAT** (**signal transducers and activators of transcription**) proteins. STATs become phosphorylated by JAKs, which causes them to dissociate from the receptor, dimerize, and translocate into the nucleus, where they regulate gene expression.

A negative feedback loop has been identified for JAK/STAT signaling. STATs stimulate expression of one or more **suppressors of cytokine signaling (SOCS) proteins**. SOCS proteins compete with STATS for binding to the pY residues on cytokine receptors (Fig. 1-19). This terminates the signaling pathway at the step of STAT activation. Recent studies show that a SOCS protein is induced by insulin signaling. SOCS 3 protein plays a role in terminating the signal from the IR, but also in reducing insulin sensitivity in hyperinsulinemic patients.

Receptor Serine/Threonine Kinase Receptors

One group of transmembrane receptors are bound and activated by members of the **transforming growth factor (TGF)-β family**, which includes the hormones **antimüllerian hormone** and **inhibin**. Unbound receptors exist as dissociated heterodimers, called **RI** and **RII** (Fig. 1-20). Hormone binding to RII induces dimerization of RII with RI, and RII activates RI by phosphorylation. RI then activates latent transcription factors called **Smads**. Activated Smads heterodimerize with a **Co-Smad**, enter the nucleus, and regulate specific gene expression.

Membrane Guanylyl Cyclase Receptors

As discussed previously, the membrane-bound forms of guanylyl cyclase constitute a family of a receptors for natriuretic peptides (see Fig. 1-11). The hormonal role

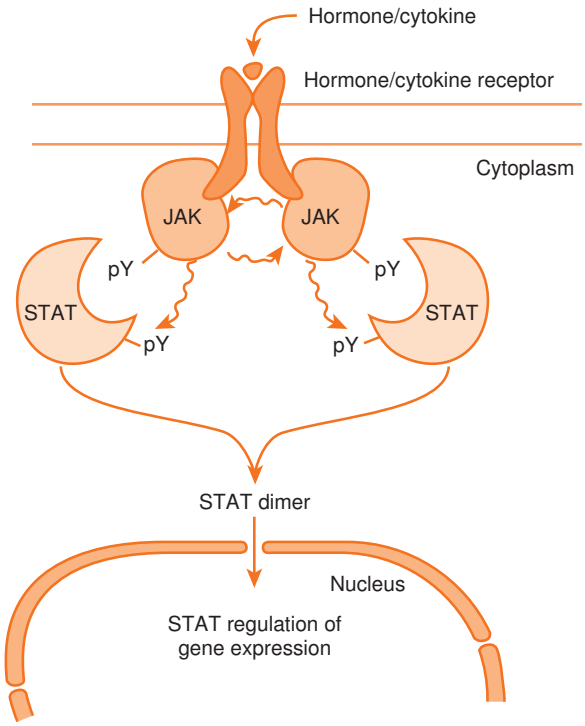


FIGURE 1-18 ■ Signaling from cytokine receptor family.

of atrial natriuretic peptide (ANP) will be discussed in Chapter 7.

Signaling from Intracellular Receptors

Steroid hormones, thyroid hormones, and 1,25-dihydroxyvitamin D act primarily through intracellular receptors. These receptors are structurally similar and are members of the **nuclear hormone receptor superfamily** that includes receptors for **steroid hormones**, **thyroid hormone**, **lipid-soluble vitamins**, **peroxisome proliferator–activated receptors (PPARs)**, and other *metabolic* receptors (liver X receptor, farnesyl X receptor).

Nuclear hormone receptors act as transcriptional regulators. This means that the signal of hormone receptor binding is transduced ultimately into a change in the transcriptional rate of a subset of the genes that are expressed within a differentiated cell type. One receptor binds to a specific DNA sequence, called a **hormone response element**, often close to the promoter of one gene, and influences the rate of transcription of that gene in a hormone-dependent manner (see later). However, multiple hormone receptor–binding events

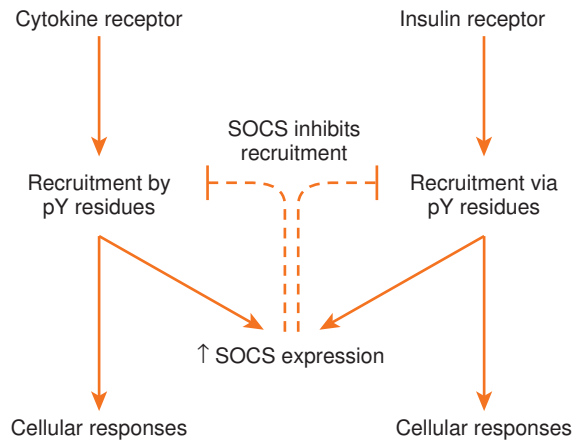


FIGURE 1-19 ■ Role of suppressor of cytokine signaling SOCS protein in terminating signals from cytokine family and insulin receptors.

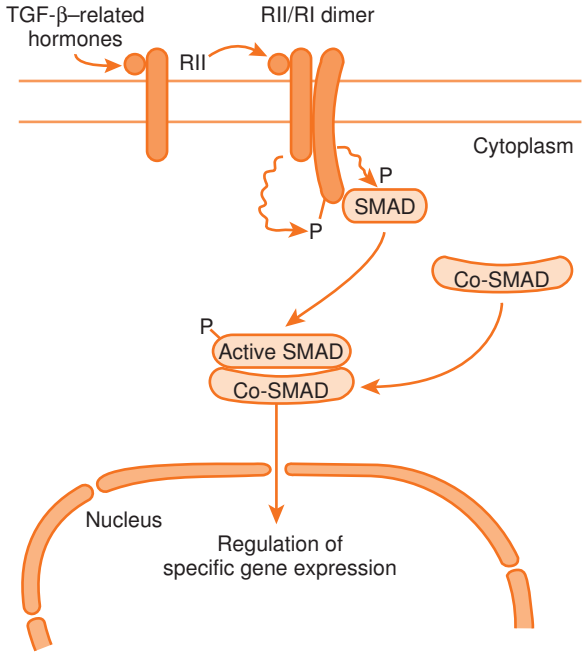


FIGURE 1-20 ■ Signaling from TGF-β-related hormones.

TABLE 1-3

Mechanisms by Which Hormones Regulate Gene Expression

HORMONE TYPE	STEROID HORMONES	THYROID HORMONES	CATECHOLAMINES, PEPTIDES, PROTEINS	CATECHOLAMINES PEPTIDES, PROTEINS
Cell membrane	Passes through cell membrane	Passes through cell membrane, possibly use transporter	Binds to extracellular domain of transmembrane receptor	Binds to extracellular domain of transmembrane receptor
Cytoplasm	Binds to receptor, HRC translocates to nucleus	Moves through cytoplasm directly to nucleus to bind receptor	Ultimately activates cytoplasmic protein kinase, translocates to the nucleus	Activates a latent transcription factor in cytoplasm, TF translocates to the nucleus
Nucleus	HRC binds to response elements (often as dimer), recruits co-regulatory proteins and alters gene expression	Hormone binds to receptor already bound to response elements, HRC induces exchange of co-regulatory proteins, alters gene expression	Phosphorylates TF, which binds to DNA and recruits co-regulatory proteins, alters gene expression	TF binds to DNA and recruits co-regulatory proteins, alters gene expression
Examples	Cortisol	T_3	Glucagon	Growth hormone

HRC, hormone-receptor complex; TF, transcription factor.

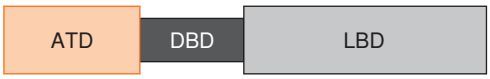
are collectively transduced into the regulation of several genes. Moreover, regulation by one hormone usually includes activation and repression of the transcription of many genes in a given cell type. Note that we have already discussed examples of signaling to transcription factors by transmembrane receptors. Table 1-3 summarizes the four general modes of hormonal regulation of gene transcription.

Nuclear hormone receptors have three major structural domains: an **amino terminus domain (ABD), a middle DNA-binding domain (DBD), and a carboxyl terminus ligand-binding domain (LBD)** (Fig. 1-21). The amino terminus domain contains a hormone-independent transcriptional activation domain. The DNA-binding domain contains two zinc finger motifs, which represent small loops organized by Zn^{2+} binding to four cysteine residues at the base of each loop. The two zinc fingers and neighboring amino acids confer the ability to recognize and bind to specific DNA sequences, which are called **hormone-response elements (HREs)**. The **carboxyl terminal ligand-binding domain** contains several subdomains:

1. Site of hormone recognition and binding
2. Hormone-dependent transcriptional activation domain
3. Nuclear translocation signal
4. Binding domain for heat-shock proteins
5. Dimerization subdomain

There are numerous variations in the details of nuclear receptor mechanisms of action. Two generalized pathways by which nuclear hormone receptors increase gene transcription are the following (Fig. 1-22):

Pathway 1: Unactivated receptor is cytoplasmic or nuclear and binds DNA and recruits co-activator



ATD (Amino Terminus Domain)
- Ligand-independent association with co-regulatory proteins
- Ligand-independent phosphorylation sites

DBD (DNA Binding Domain)
- DNA binding via zinc finger domains
- Dimerization

LGB (Ligand Binding Domain)
- Ligand-binding
- Ligand-dependent association with co-regulatory proteins
- Dimerization
- Nuclear translocation
- Association with chaperone proteins

FIGURE 1-21 ■ Domains of nuclear hormone receptor.

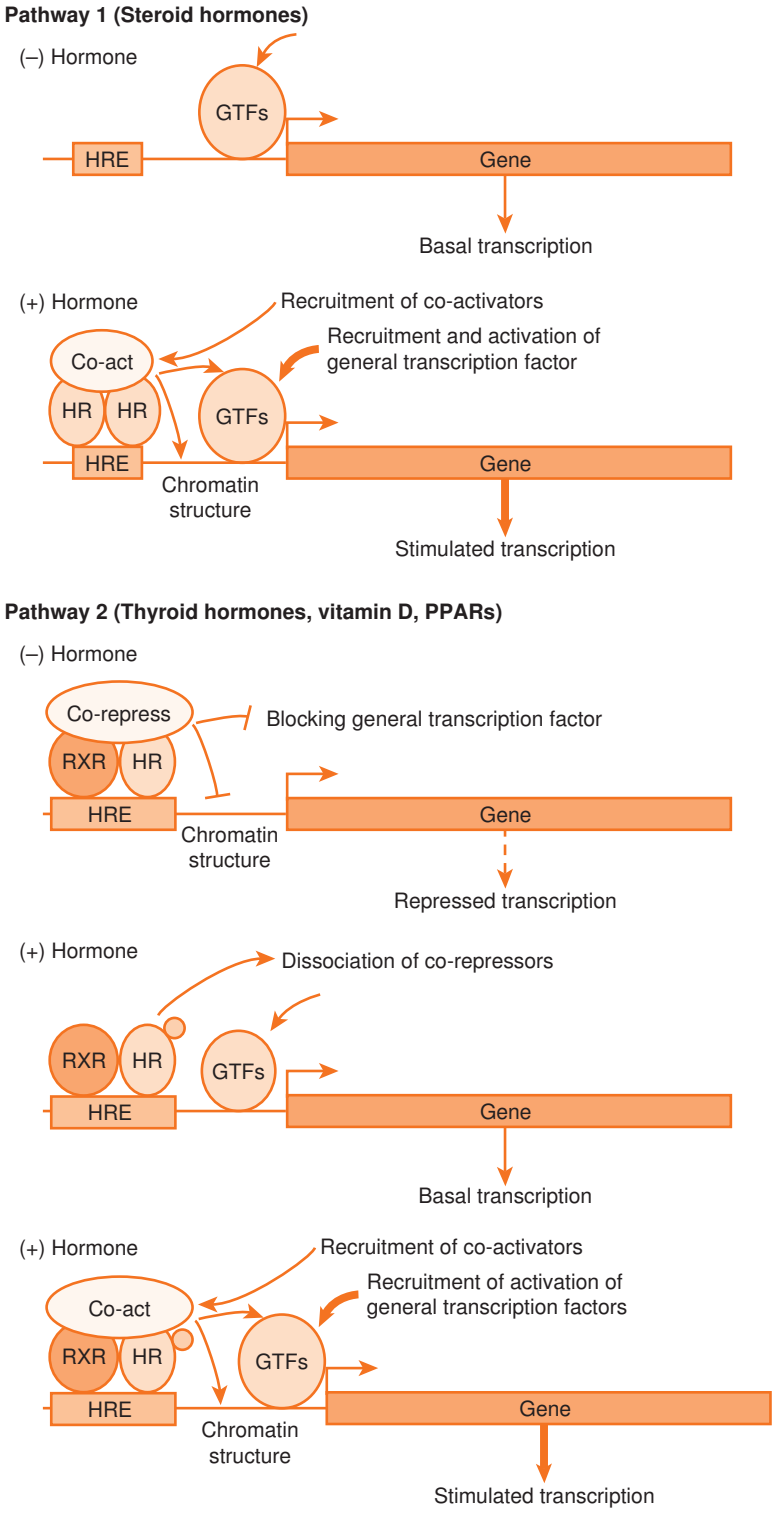


FIGURE 1-22 ■ Two general mechanisms by which nuclear receptor and hormone complexes increase gene transcription. HRE, hormone response element; co-repress, co-repressor proteins; GTFs, general transcription factors; HR, hormone receptor; RXR, retinoid X receptor; Co-act, co-activator proteins.

proteins on hormone binding. This mode is observed for the ER, PR, GR, MR, and AR (i.e., steroid hormone receptors). In the absence of hormone, some of these receptors are held in the cytoplasm through an interaction with **chaperone proteins** (so-called **heat-shock proteins** because their levels increase in response to elevated temperatures and other stresses). Chaperone proteins maintain the stability of the nuclear receptor in an inactive configuration. Hormone binding induces a conformational change in the receptor, causing its dissociation from heat-shock proteins. This exposes the nuclear localization signal and dimerization domains, so receptors dimerize and enter the nucleus. Once in the nucleus, these receptors bind to their respective HREs. The HREs for the PR, GR, MR, and AR are inverted repeats with the recognition sequence, AGAACANNNTGTTCT. Specificity is conferred by neighboring base sequences and possibly by receptor interaction with other transcriptional factors in the context of a specific gene promoter. The ER usually binds to an inverted repeat with the recognition sequence, AGGTCANNNTGACCT. The specific HREs are also referred to as an **estrogen-response element (ERE), progesterone-response element (PRE), glucocorticoid-response element (GRE), mineralocorticoid-response element (MRE), and androgen-response element (ARE)**. Once bound to their respective HREs, these receptors recruit other proteins, called **co-regulatory proteins**, which are either **co-activators** or **co-repressors**. Co-activators act to recruit other components of the transcriptional machinery and probably activate some of these components. Co-activators also possess intrinsic histone acetyltransferase (HAT) activity, which acetylates histones in the region of the promoter. Histone acetylation relaxes chromatin coiling, making that region more accessible to transcriptional machinery. Although the mechanistic details are beyond the scope of this chapter, the student should appreciate that steroid receptors can also repress gene transcription through recruitment of co-repressors that possess **histone deacetylase (HDAC)** activity and that transcriptional activation and repression pathways are induced concomitantly in the same cell. HDAC inhibitors are being studied in the context of treating some cancers because they restart the expression of silenced tumor suppressor genes.

Pathway 2: Receptor is always in nucleus and exchanges co-repressors with co-activators on hormone binding. This pathway is used by the **thyroid hormone receptors (THRs), vitamin D receptors, PPARs, and retinoic acid receptors**. For example, the THR is bound, usually as a heterodimer, with the retinoic acid X receptor (RXR). In the absence of thyroid hormone, the THR/RXR recruits co-repressors. As stated earlier, co-repressors recruit proteins with histone deacetylase (HDAC) activity. In contrast to histone acetylation, histone deacetylation allows tighter coiling of chromatin, which makes promoters in that region less accessible to the transcriptional machinery. Thus, THR/RXR heterodimers are bound to **thyroid hormone response elements (TREs)** in the absence of hormone and maintain the expression of neighboring genes at a "repressed" level. Thyroid hormone (and other ligands of this class) readily move into the nucleus and bind to their receptors. Thyroid hormone binding induces dissociation of co-repressor proteins, thereby increasing gene expression to a *basal* level. The hormone receptor complex subsequently recruits co-activator proteins, which further increase transcriptional activity to the "stimulated" level.

Termination of steroid hormone receptor signaling is poorly understood but appears to involve phosphorylation, ubiquitination, and proteasomal degradation. Circulating steroid and thyroid hormones are cleared as described previously.

In summary, hormones signal to cells through membrane or intracellular receptors. Membrane receptors have rapid effects on cellular processes (e.g., enzyme activity, cytoskeletal arrangement) that are independent of new protein synthesis. Membrane receptors can also rapidly regulate gene expression through either mobile kinases (e.g., PKA, MAPKs) or mobile transcription factors (e.g., STATs, Smads). Steroid hormones have slower, longer-term effects that involve chromatin remodeling and changes in gene expression. Increasing evidence points to rapid, nongenomic effects of steroid hormones as well, but these pathways are still being elucidated.

The presence of a functional receptor is an absolute requirement for hormone action, and loss of a

receptor produces essentially the same symptoms as loss of hormone. In addition to the receptor, there are fairly complex pathways involving numerous intracellular messengers and effector proteins. Accordingly, endocrine diseases can arise from abnormal expression or activity of any of these signal transduction pathway components.

Overview of the Termination Signals

Most of what has been discussed in this chapter describes the stimulatory arm of signal transduction. As noted earlier, all signal transduction of hormonal signals must have termination mechanisms to avoid sustained and uncontrolled stimulation of target cells. Part of this stems from the cessation of the original stimulus for increasing a hormone's level, and mechanisms to clear the hormone (i.e., removal of signal). However, there exist a wide array of intracellular mechanisms that terminate the signaling pathway within the target cells. Some of these are listed in Table 1-4. Note that overactivity of terminating mechanisms can lead to hormonal resistance.

TABLE 1-4
Some Modes of Signal Transduction Termination

MECHANISM OF SIGNAL TRANSDUCTION TERMINATION	EXAMPLE
Receptor-mediated endocytosis linked to lysosomal degradation	Many transmembrane receptors
Phosphorylation/dephosphorylation of receptor or "downstream" components of signaling pathway	Serine phosphorylation of insulin receptor and insulin receptor substrate by other signaling pathways
Ubiquitination/proteasomal degradation	Steroid hormone receptors
Binding of an inhibitory regulatory factor	Regulatory subunit of PKA
Intrinsic terminating enzymatic activity	GTPase activity of G proteins

SUMMARY

1. The **endocrine system** is composed of:
   - **Dedicated hormone-producing glands (pituitary, thyroid, parathyroid, and adrenal)**
   - **Testes** and **ovaries**, whose intrinsic endocrine function is absolutely necessary for gametogenesis
   - **Hypothalamic neuroendocrine neurons**
   - Scattered endocrine cells that exist as clusters of endocrine-only cells (**islets of Langerhans**) or as **cells** within organs that are have a nonendocrine primary function (pancreas, GI tract, kidney)
2. Endocrine signaling involves the secretion of a chemical messenger, called a **hormone**, that circulates in the blood and reaches an equilibrium with the extracellular fluid. Hormones alter many functions of their target cells, tissues, and organs through specific, high-affinity interactions with their **receptors**.
3. Protein/peptide hormones:
   - Are produced on ribosomes, become inserted into the cisternae of the endoplasmic reticulum, transit the Golgi apparatus, and finally are stored in **membrane-bound secretory vesicles**. The release of these vesicles represents a **regulated mode of exocytosis**. Each hormone is first made as a **prehormone**, containing a signal peptide that guides the elongating polypeptide into the cisternae of the endoplasmic reticulum.
   - Are frequently synthesized as **preprohormones**. After removal of the signal peptide, the **prohormone** is processed by **prohormone convertases**.
   - Typically do not cross cell membranes and act through **transmembrane receptors** (see later).
   - Mostly circulate as **free hormones**, and are excreted in the urine or cleared by **receptor-mediated endocytosis** and lysosomal degradation.
4. Catecholamine hormones:
   - Include the hormones, **epinephrine (Epi)** and **norepinephrine (Norepi)**. Epi and Norepi are derivatives of tyrosine, which is enzymatically modified by several reactions. Ultimately, Epi and Norepi are stored in a secretory vesicle and are released in through regulated exocytosis.
   - Act through transmembrane GPCRs receptors called **adrenergic receptors**.

5. Steroid hormones:
   - Include **cortisol (glucocorticoid), aldosterone (mineralocorticoid), testosterone, and dihydrotestosterone (androgens), estradiol (estrogen), progesterone (progestin), and 1,25 dihydroxyvitamin D_3 (secosteroid)**.
   - Are derivatives of **cholesterol**, which is modified by a series of cell-specific enzymatic reactions.
   - Are **lipophilic** and cross membranes readily. Thus, steroid hormones cannot be stored in secretory vesicles. Steroid production is regulated at the level of synthesis. Several steroid hormones are produced to a significant extent by peripheral conversion of precursors.
   - Circulate bound to **transport proteins**. Steroid hormones are cleared by enzymatic modifications that increase their solubility in blood and decrease their affinity for transport proteins. Steroid hormones and their inactive metabolites are excreted in the urine.
   - Act through intracellular receptors, which are members of the **nuclear hormone receptor family**. Most steroid hormone receptors reside in the cytoplasm and are translocated to the nucleus after ligand (hormone) binding. Each steroid hormone regulates the expression of numerous genes in their target cells.
6. Thyroid hormones are:
   - **Iodinated derivatives of thyronine**. The term *thyroid hormone* typically refers to **3,5,3′,5′-tetraiodothyronine (T_4 or thyroxine)** and **3,5,3′-triiodothyronine (T_3)**. T_4 is an inactive precursor of T_3, which is produced by 5′-deiodination of T_4.
   - Synthesized and released by the **thyroid epithelium** (see Chapter 6 for more detail)
   - Circulate tightly bound to **transport proteins**
   - Lipophilic and cross cell membranes. T_3 binds to one of several isoforms of **thyroid hormone receptors (THRs)**, which form heterodimers with retinoid X receptor (RXR) and reside bound to their response elements in the nucleus in the absence of hormone. Hormone binding induces an exchange in the co-regulatory proteins that interact with the THRs.
7. Protein, peptide, and catecholamine hormones signal through transmembrane receptors and use several common forms of informational transfer:
   - **Conformational change**
   - **Binding by activated G proteins**
   - **Binding by Ca^{2+} or Ca^{2+}-calmodulin**. **IP_3** is a major lipid messenger that increases cytosolic Ca^{2+} levels through binding to the **IP_3 receptor**.
   - **Phosphorylation** and **dephosphorylation**, using **kinases** and **phosphatases**, respectively. The phosphorylation state of a protein affects **activity, stability, subcellular localization**, and **recruitment binding** of other proteins. Note that **phosphorylated lipids** such as **PIP_3** also play a role in signaling.
8. **Transmembrane receptor families:**
   - **G-protein-coupled receptors (GPCRs)** act as **guanine nucleotide exchange factors (GEFs)** to activate the Gα subunit of the heterotrimeric α/β/γ G-protein complex. Depending on the type of Gα subunit that is activated, this will **increase cAMP levels, decrease cAMP levels**, or **increase protein kinase C activity and Ca^{2+} levels**. All catecholamine receptors (adrenergic receptors) are **GPCRs**. GPCRs are internalized by a receptor-mediated endocytosis that involves **GRK** and **β-arrestin**. Endocytosis results in the lysosomal clearance of the hormone. The receptor may be digested in the lysosome or may be recycled to the cell membrane.
   - The **insulin receptor** is a **tyrosine kinase receptor** that activates the **Akt/PKB pathway, the G-protein TC10-related pathway, and the MAPK pathway**. The insulin receptor uses the scaffolding protein **insulin receptor substrate (IRS; four isoforms)** as part of its signaling to these three pathways.
   - Some protein hormones (e.g., growth hormone, prolactin) bind to transmembrane receptors that belong to the **cytokine receptor family**. This are constitutively dimerized receptors that are bound by **janus kinases (JAKs)**. Hormone binding interacts with both extracellular domains and induces JAK-JAK cross-phosphorylation, followed by recruitment and binding of **STAT proteins**. Phosphorylation of STATs activates them and induces their translocation to the nucleus, where they act as transcription factors.

- Hormones that are related to **transforming growth factor-β (TGF-β), such as antimüllerian hormone**, signal through a **co-receptor (receptor I and receptor II) complex** that ultimately signals to the nucleus through activated **Smad** proteins.
- **Atrial natriuretic peptide (and related peptides)** bind to a transmembrane receptor that contains a **guanylyl cyclase** domain within the cytosolic domain. These receptors signal by **increasing cGMP**, which activates **protein kinase G (PKG)** and **cyclic nucleotide-gated channels**. cGMP also regulates selective **phosphodiesterases**.
- **Steroid hormones** bind to members of the **nuclear hormone transcription factor family**. Steroid hormone receptors usually reside in the cytoplasm. Hormone binding induces **nuclear translocation, dimerization**, and **DNA binding**. Steroid hormone receptor complexes regulate many genes in a target cell.

9. **Thyroid hormone (T_3) receptors (THRs)** are related to steroid hormone receptor, but they constitutively remain in the nucleus bound to thyroid hormone response DNA elements. T_3 binding typically induces an exchange of co-regulatory proteins and altered gene expression.

SELF-STUDY PROBLEMS

1. How do protein hormones differ from steroid hormones in terms of their storage within an endocrine cell?
2. How does binding to serum transport proteins influence hormone metabolism and hormone action?
3. How would a large increase in the GTPase activity of Gs-α affect signaling through GPCRs linked to Gs-α?
4. What role does the IRS protein play in transducing insulin receptor signaling into a growth response? a metabolic response?
5. Name an example of a transmembrane receptor–associated transcription factor that translocates to the nucleus.
6. Explain the mechanism of receptor-mediated endocytosis of a hormone that binds to a GPCR.
7. What is the importance of the GEF activity of a GPCR to its ability to signal?
8. Explain how PLC generates two second messengers.

KEYWORDS AND CONCEPTS

- 7-Helix transmembrane receptors
- Adenylyl cyclase
- Adrenal cortex

For full list of keywords and concepts see Student Consult

SUGGESTED READINGS

Huang CC, Tesmer JJ: Recognition in the face of diversity: Interactions of heterotrimeric G proteins and G protein-coupled receptor (GPCR) kinases with activated GPCRs, *J Biol Chem* 286:7715–7721, 2011.

Jean-Alphonse F, Hanyaloglu AC: Regulation of GPCR signal networks via membrane trafficking, *Mol Cell Endocrinol* 331:205–214, 2011.

Rose RA, Giles WR: Natriuretic peptide C receptor signalling in the heart and vasculature, *J Physiol* 586:353–366, 2008.

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KEYWORDS AND CONCEPTS

- Agonist
- Androgen
- Androgen receptor
- Androgen response element (ARE)
- Antagonist
- β-Arrestins
- Ca^{2+}
- Ca^{2+} ATPases
- Ca^{2+} channels
- Calmodulin
- cAMP phosphodiesterase
- cAMP response element–binding protein (CREB)
- Catecholamine
- Cellular response
- cGMP phosphodiesterase
- Circadian (diurnal) rhythms
- Co-activator proteins
- Co-repressors
- Corticosteroid-binding globulin
- Covalent phosphorylation of proteins and lipids
- Cyclic AMP
- Cyclic GMP
- Cyclic nucleotide monophosphates
- Cycloperhydrophenanthrene ring
- Cytokine receptor family
- Diacylglycerol (DAG)
- Docking protein
- Effector proteins
- Eicosanoids
- Endocrine gland
- Endocrine system
- Epinephrine
- Estrogen
- Estrogen receptor
- Estrogen response element (ERE)
- Exocrine gland
- Exocytosis
- G-protein exchange factor (GEF)
- Ga
- Gi-α
- Glucocorticoid
- Glucocorticoid receptor
- Glucocorticoid response element (GRE)
- Glucuronide conjugation
- GPCR kinase (GRK)
- G-protein-coupled receptor (GPCR)
- Gq-α
- Grb2/SOS
- Gs-α
- GTP-binding proteins (G proteins)
- Guanylyl cyclase
- Gβ/γ
- Heterotrimeric G proteins
- High-affinity receptor
- Histone acetyltransferase (HAT)
- Histone deacetylase (HDAC)
- Hormonal desensitization
- Hormonal resistance
- Hormone
- Hormone response elements (HREs)
- Inositol 1,4,5-triphosphate (IP_3)
- Insulin receptor (IR)
- Insulin receptor substrate (IRS)
- Intracellular messengers
- Intrinsic GTPase activity
- Iodothyronine
- JAK kinase family
- Leukotrienes
- Ligand
- Ligand-activated GEF
- Ligand-induced endocytosis
- MEK
- Mineralocorticoid
- Mineralocorticoid receptor
- Mineralocorticoid response element (MRE)
- Mitogen-activated protein kinase (MAPK)
- Mixed-function kinases and phosphatases
- Nitric oxide (NO)
- Norepinephrine
- Nuclear receptor superfamily
- Ovary
- Peripheral conversion
- Phosphatidylinositol 3,4,5-triphosphate (PIP_3)
- Phosphatidylinositol-3-kinase (PI3K)
- Phospholipase C
- Phosphotyrosine (pY)
- PKA catalytic subunit
- PKA regulatory subunit
- Placenta

- Prehormone
- Preprohormone
- Progesterone receptor
- Progesterone response element (PRE)
- Progestin
- Prohormone convertase
- Prostacyclin
- Prostaglandins
- Protein kinase A (PKA)
- Protein kinase B (PKB/Akt)
- Protein kinase G (PKG)
- Protein/peptide hormone
- Raf
- Ras
- Receptor
- Receptor serine/threonine kinases
- Receptor tyrosine kinases (RTKs)
- Regulated secretory pathway
- Regulators of G-protein signaling (RGS proteins)
- Second messenger hypothesis
- Serine/threonine-specific kinases and phosphatases
- Set-point
- Sex hormone–binding globulin
- Signal peptidase
- Signal peptide
- Signal recognition complex
- Signal transduction pathway
- Smads
- STAT
- Steroid hormone
- Steroidogenic cells
- Stimulus-secretion coupling
- Sulfate conjugation
- Suppressors of cytokine signaling (SOCS) proteins
- Target cell
- Target organ
- Testis
- Thromboxanes
- Thyroid hormone receptor
- Thyroid hormone–binding globulin
- Thyroid hormone–response element (TRE)
- Transforming growth factor (TGF)-β family
- Transport proteins
- Tyrosine kinases and phosphatases
- Ultradian rhythms
- Vitamin D
- Vitamin D receptor
- Vitamin D response element (VRE)

2 ENDOCRINE FUNCTION OF THE GASTROINTESTINAL TRACT

OBJECTIVES

1. Understand the role of well-established GI hormones associated with the following four major aspects of GI physiology:
   - The regulation of gastric acid secretion and gastric motility
   - The regulation of secretion from the exocrine pancreas and the gallbladder and their associated ducts
   - The stimulation of GI tract growth (an enterotropic action)
   - The enhancement of nutrient-induced insulin secretion by the endocrine pancreas (incretin action)

Note: A fifth general function of GI hormones, the effect on appetite, is discussed in the context of energy homeostasis in Chapter 3.

We begin our discussion of endocrine physiology with the hormonal function and regulation of the gastrointestinal (GI) tract. The discovery of secretin in 1902 by Bayliss and Starling represented the first characterization of a *hormone* as a blood-borne chemical messenger, released at one site and acting at multiple other sites. Indeed, the epithelial layer of the mucosa of the GI tract harbors numerous **enteroendocrine cell types**, which collectively represent the largest endocrine cell mass in the body.

The **diffuse enteroendocrine system** is perhaps the most basic example of endocrine tissue in that it is composed of unicellular *glands* situated within a simple epithelium. Most enteroendocrine cells, called **open cells**, extend from the basal lamina of this epithelium to the apical surface (Fig. 2-1), although there are also **closed enteroendocrine cells**, which do not extend to the luminal surface. The apical membranes of open enteroendocrine cells express either receptors or transporters that allow the cell to *sample* the contents of the lumen. Luminal contents, called **secretogogues**, stimulate specific enteroendocrine cell types to secrete their hormones. This *sampling* or *nutrient tasting* is independent of osmotic and mechanical forces. The secretogogue mechanisms involved are poorly understood, but some appear to require the absorption of the nutrient. There is also evidence for the luminal secretion of **paracrine peptide factors** from the surrounding absorptive epithelial cells that stimulate hormonal release from enteroendocrine cells. As part of their response to luminal contents, specific enteroendocrine cell types display distinct localizations along the GI tract (Table 2-1). We will see that these localizations are central to the regulation and function of each cell type.

In the simplest model of enteroendocrine cell function, a hormone is released from the basolateral membrane in response to the presence of a secretogogue at

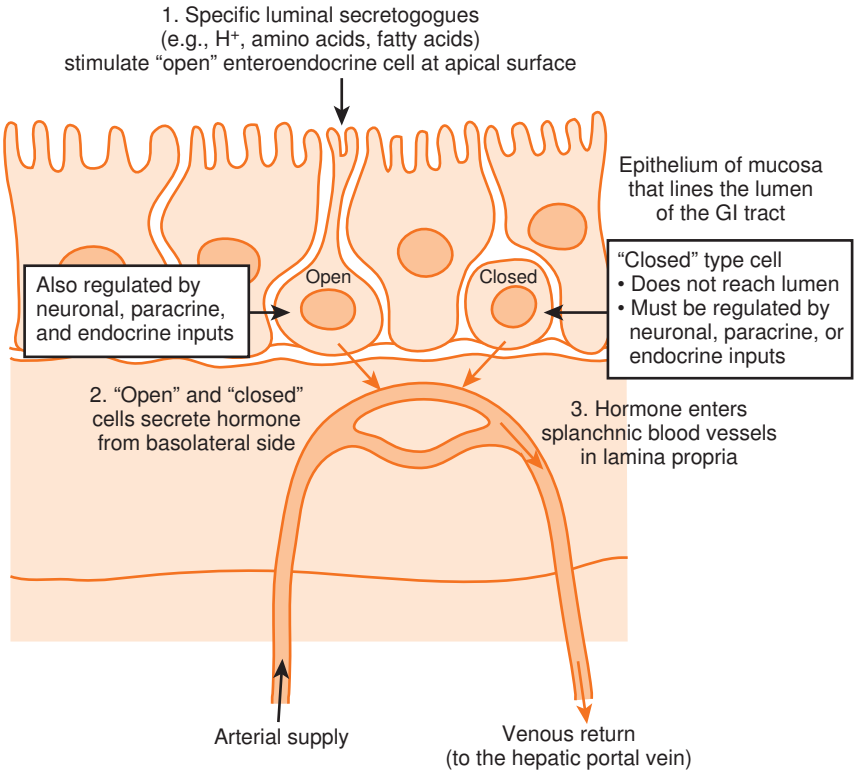

FIGURE 2-1 ■ Closed and open enteroendocrine cells. Enteroendocrine cells sit within the simple epithelium of the GI tract. "Open" cells extend from the basal lamina to the lumen. "Closed" cells do not reach the lumen. Both cells secrete hormones that enter capillaries in the lamina propria beneath the epithelium.

the luminal side of the cell. The secreted hormone diffuses into blood vessels in the underlying lamina propria, thereby gaining access to the general circulation. Circulating GI hormones regulate GI tract functions by binding to specific receptors at one or more sites within the GI tract and its extramural glands. In the classic model, the secretion of the hormone by an enteroendocrine cell is subsequently terminated when the luminal concentration of its secretogogue diminishes, thereby terminating the secretion of the hormone.

This simple model of the enteroendocrine system does not fully account for the integration with other systemic responses to a meal. Both *open* and *closed* enteroendocrine cells are regulated by the **enteric nervous system (ENS)** and paracrine factors secreted by neighboring epithelial cells (**intrinsic regulators of**

TABLE 2-1

Distribution of Enteroendocrine Cells Along the GI Tract

	STOMACH	DUODENUM	JEJUNUM	ILEUM	COLON
G cell (gastrin)	x	(x)			
S cell (secretin)		x	x		
I cell (CCK)		x	x	(x)	
K cell (GIP)		x	x		
L cell (GLP-1)				x	x
L cell (GLP-2)				x	x
M cell (motilin)		x	x		
Ghrelin-secreting cell	x	(x)	(x)	(x)	(x)

x, Primary location of concentration; (x), less concentrated.

enteroendocrine cell function). Additionally, there are **extrinsic regulators of enteroendocrine cells**, most notably the **autonomic nervous system** and endocrine glands that reside outside of the GI tract. Conversely, GI hormones can have local (i.e., paracrine) actions on the **afferent nerves of autonomic or enteric reflexes**, so the response to a GI hormone can be mediated by a neurotransmitter. Thus, GI tract function is orchestrated through a complex interplay of neural and endocrine responses and actions. It is not surprising, therefore, that GI function is often perturbed in patients with psychiatric disorders (e.g., depression) and endocrine disorders (e.g., hyperthyroidism).

The hormones secreted by the enteroendocrine system function to maintain the health of the GI tract and its extramural glands and provide an integrated response to the acquisition of nutrients. This integrated response to GI hormones is due, in part, to their ability to regulate multiple functions of the GI tract.

ENTEROENDOCRINE HORMONE FAMILIES AND THEIR RECEPTORS

All established GI hormones are **peptides** and bind to **G-protein-coupled receptors** (**GPCRs**; see Chapter 1) located on the plasma membrane of target cells. GI hormones, as well as their cognate GPCRs, can be organized into gene families based on structural homologies. In this chapter, we discuss members of **three enteroendocrine hormone families**: **gastrin, secretin**, and **motilin** (Table 2-2).

The **gastrin family** includes **gastrin** and **cholecystokinin** (**CCK**), which share a common stretch of 5 amino acids at the C-terminus. Gastrin binds with high affinity to the **CCK-2 receptor** (previously called the CCK-B/gastrin receptor). CCK binds with high affinity to the **CCK-1 receptor**.

The **secretin family** includes the hormones **secretin, glucagon, and glucagon-like peptides** (**including GLP-1 and GLP-2**) **and gastric inhibitory polypeptide**

TABLE 2-2

Enteroendocrine Hormone Families and Their Receptors

HORMONE FAMILY	MEMBERS OF FAMILY	RECEPTOR AND PRIMARY SIGNALING PATHWAY	PRIMARY DISTRIBUTION OF THE RECEPTOR (RELATED TO GI FUNCTION)
Gastrin	Gastrin (G cell)	CCK2 receptor Gq - ↑ in Ca^{2+} and PKC	Gastric ECL cell and parietal cell
	CCK (I cell)	CCK1 receptor Gq - ↑ in Ca^{2+} and PKC	Gallbladder muscularis and sphincter of hepatopancreatic ampulla Pancreatic acinar cells Pancreatic ducts Vagal afferents and enteric neurons Stomach muscularis and pyloric sphincter Gastric D cells
Secretin	Secretin (S cell)	Secretin receptor Gs - ↑ in cAMP	Pancreatic ducts and biliary ducts Pancreatic acinar cells G cells and pancreatic cells
	GLP-1 (L cell)	GLP-1 receptor Gs - ↑ in cAMP	β Cells of pancreatic islets
	GLP-2 (L cell)	GLP-2 receptor Gs - ↑ in cAMP	GI tract, especially small intestine
	GIP (K cell)	GIP receptor Gs - ↑ in cAMP	β Cells of pancreatic islets Gastric mucosa and muscularis
Motilin	Motilin (M cell)	Motilin receptor Gq - ↑ in Ca^{2+} and PKC (also binds erythromycin)	Stomach and small intestines, especially in smooth muscle cells and enteric neurons
	Ghrelin (P/D1 cell)	GHS receptor type 1a (GHS-RIa) Gq - ↑ in Ca^{2+} and PKC	Pituitary and hypothalamus

(**GIP;** more recently referred to as **glucose-dependent insulinotropic peptide**—see later). This family also includes the neurocrine factor, **vasoactive intestinal peptide (VIP)**. The corresponding GPCRs for each member of the secretin family of peptides are also structurally related. These receptors are all primarily coupled to **Gs signaling pathways** that increase intracellular cyclic adenosine monophosphate (cAMP) in target cells.

The **motilin family** includes the hormones **motilin** and **ghrelin**. Ghrelin was originally identified as a growth hormone secretogogue (GHS) but is most abundant in the fundus of the stomach. The receptors for motilin and ghrelin are GPCRs that are linked to **Gα-q/phospholipase/IP_3 pathways**, which, in turn, stimulate p**rotein kinase C**- and **Ca^{2+}-dependent signaling pathways** (see Chapter 1).

Many GI peptides are also expressed by tissues outside of the GI tract. Pathophysiologically, GI peptides can be secreted in an uncontrolled manner from tumors. Other physiologic sites of production include other endocrine glands (e.g., the pituitary gland) and reproductive structures. Several peptides are produced by the central (CNS) and peripheral (PNS) nervous systems, where they are used as neurotransmitters or neuromodulatory factors. For example, cholecystokinin (CCK) is expressed in the neocortical region of the CNS and the genitourinary-associated nerves of the PNS. As for its role in the CNS, CCK has been linked to anxiety and panic disorders. This also means that receptors for these peptides also reside within the CNS, the PNS, and probably other non-neural tissues. Thus, a pharmacologic agent (agonist or antagonist) related to a specific GI peptide can potentially have a wide range of effects, depending on its stability and whether it can cross the blood-brain barrier. The possibility also exists that extra-GI sites of synthesis can "spill over" into the general circulation and affect GI function.

GASTRIN AND THE REGULATION OF GASTRIC FUNCTION

The **stomach** acts as a food reservoir. People eat discontinuously and typically eat more at one sitting than their GI tract can process immediately. Thus, the stomach holds the ingested food and gradually releases partially digested food (chyme) into the first part of the small intestine, the duodenum. The layers of the stomach wall carry out two basic functions: secretion and contraction/relaxation.

Overview of Regulation of Gastric Secretion and Motility

The innermost layer of the stomach wall, the **gastric mucosa**, contains glandular and surface mucus-producing epithelia and can be divided into proximal and distal segments. Two of the proximal portions of the stomach (**fundus** and **body**) contain the main gastric mucosal glands (Fig. 2-2). Within these glands, the **parietal cells** secrete **HCl**, which is important for hydrolysis of macromolecules, activation of proenzymes, and the sterilization of ingested food. Parietal cells also secrete **intrinsic factor**, which is a glycoprotein required for the efficient absorption of vitamin B_{12}.

The glands of the fundus and body also contain the **chief cells**, which secrete digestive enzymes (e.g., pepsinogen, gastric lipase). A third cell type, the **mucous cell**, is found in the neck of the gastric glands and on the surface throughout the stomach. Mucous cells secrete mucigens, which buffer and protect the lining of the stomach, particularly in the vicinity of the main gastric glands. Because gastric enzyme and mucus production is primarily under nervous control, with little endocrine input, we focus here on gastric acid secretion and motility.

The distal part of the gastric mucosa, the **pyloric antrum**, has an important enteroendocrine function. This part of the stomach contains two types of "open" enteroendocrine cells. The **G cells** secrete **gastrin**, a hormone, and the **D cells** secrete **somatostatin**, a paracrine factor. These two peptides act antagonistically in a negative feedback loop to regulate gastric blood flow, cell growth, secretion, and motility (see later). D cells are also found within the fundus and body region, where they directly inhibit parietal cell secretion.

An outer layer of the stomach wall, the muscularis externa, is composed of smooth muscle. The relaxation of this muscle allows distention and storage, and its contractions ultimately move the partially digested food (**chyme**) into the duodenum. There are two gateways into and out of the stomach. These are the lower esophageal sphincter (LES) and the

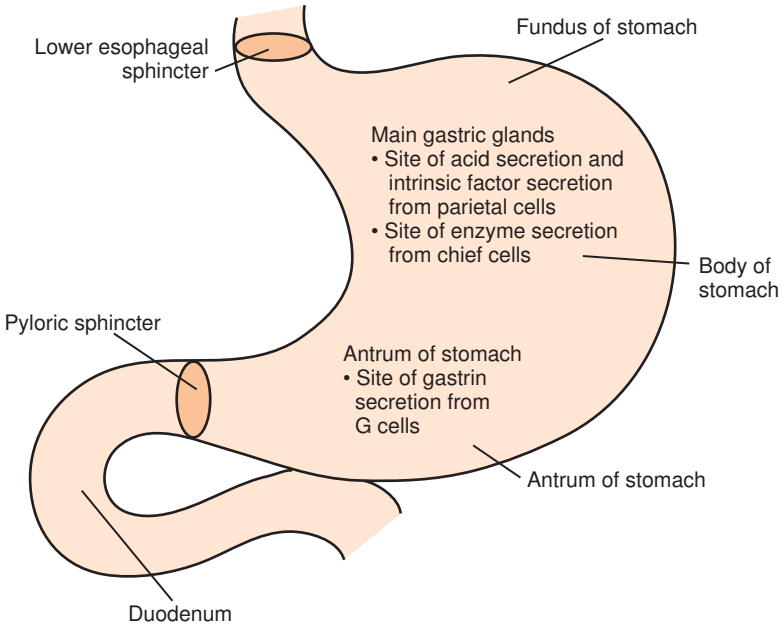

FIGURE 2-2 ■ Anatomy of the stomach.

pyloric sphincter, respectively. The LES allows swallowed food particles to enter the stomach and protects the esophagus from the reflux of acidic chyme. The pyloric sphincter operates in conjunction with the muscularis externa to allow only small particles of digested chyme to escape the stomach and enter the duodenum. The pyloric sphincter also prevents backflow of chyme into the stomach.

In general, regulation of gastric function involves the stimulation of secretion and motility as needed (i.e., in the presence of food), and the inhibition of gastric secretion and motility as acidic chyme reduces the pH of the stomach, or as chyme moves into the small intestine and colon. In this way, the stomach avoids excessive acid secretion in the absence of buffering foodstuffs. Further, the portion of the GI tract below the stomach protects itself from exposure to excessive amounts of acid, which is both damaging to the intestinal lining and inhibitory to the activity of intestinal enzymes. Additionally, the small intestine, in which the majority of digestion and absorption occurs, controls the flow rate of food into and through the small intestines in order to optimize digestion and absorption of nutrients, salts, and water. The inability to properly regulate acid secretion and its flow into the intestine usually gives rise to duodenal ulcers, although patients with a **gastrin-producing tumor (Zollinger-Ellison syndrome)** can present with ulceration of the esophagus, stomach, and duodenum.

The general model of gastric control in response to a meal can be organized into three phases. The **cephalic phase**, which accounts for about 20% of the response to a meal during the digestive period, is activated by the actual or imagined smell and sight of food, or by the presence of food in the mouth. The cephalic phase is associated with increased gastric secretion but decreased motility, in anticipation of the need to store and start digesting food. The **gastric phase**, which accounts for about 10% of the postprandial response, is activated by the presence of food in, and mechanical distention of, the stomach. During the gastric phase, secretion is strongly stimulated, and this is accompanied by an increase in peristaltic contractions and gastric emptying. The third phase is the **intestinal phase**, during which an acidic mixture of partially digested food (chyme) moves in a regulated manner through the pyloric sphincter into the small intestine and ultimately into the colon. The processes of enzymatic digestion and absorption that occur during the digestive phase account for 70% of the digestive period. The movement of food into the lower GI tract generally moderates both gastric secretion and emptying.

Gastrin and the Stimulation of Gastric Function

Gastric HCl secretion from parietal cells is stimulated by three pathways:

- **Paracrine stimulation by histamine, which is secreted by neighboring enterochromaffin-like (ECL) cells**
- **Enteric nervous system and vagal parasympathetic nervous system stimulation via gastrin-releasing peptide (GRP) and acetylcholine**
- Direct and indirect hormonal stimulation by the peptide hormone **gastrin**

Gastrin is produced by the G cells of the stomach antrum and proximal duodenum. In humans, the term *gastrin* refers to a 17-amino acid peptide that has modifications at both termini (**G-17**). In fact, the production of G-17 is an excellent example of how a peptide-encoding gene gives rise to multiple, larger precursors, which are also secreted into the blood. G-17 is the product of sequential posttranslational processing of **preprogastrin**, which can be generally characterized in three phases (Fig. 2-3). In the first phase, sulfation and proteolysis generate a mixture of gastrin precursors, called *progastrins.* The second phase involves proteolysis within secretory granules that generates C-termini peptides. Processing of these intermediates also includes the cyclization of the glutaminyl to a pyroglutamyl residue. The third stage involves the amidation of the C-terminus to produce amidated gastrins. The primary secreted bioactive product of human G cells is G-17 (i.e., 17 amino acids). The pyroglutamyl residue at the amino terminus and the amidation of the C-terminus protect G-17 from digestion by circulating aminopeptidases and carboxypeptidases. G-17 binds with high affinity to the CCK2 receptor and is responsible for all of the gastrin effects on the stomach. The last four amino acids assign gastrin-like biologic activity to G-17. A synthetic, clinically used form of gastrin, **pentagastrin**, contains the last four amino acids, plus an alanine at the amino terminus that confers increased stability.

During the cephalic phase, gastric HCl secretion is stimulated by **vagal (parasympathetic) inputs. Preganglionic vagal efferents** activate **enteric neurons** that directly stimulate the **parietal cells** and stimulate the release of **histamine** from **ECL cells** (Fig. 2-4). These actions are mediated by **acetylcholine**, which binds to the **muscarinic receptor**. Vagal stimulation of gastrin is mediated by the neurocrine factor, **GRP,** released from **enteric neurons**.

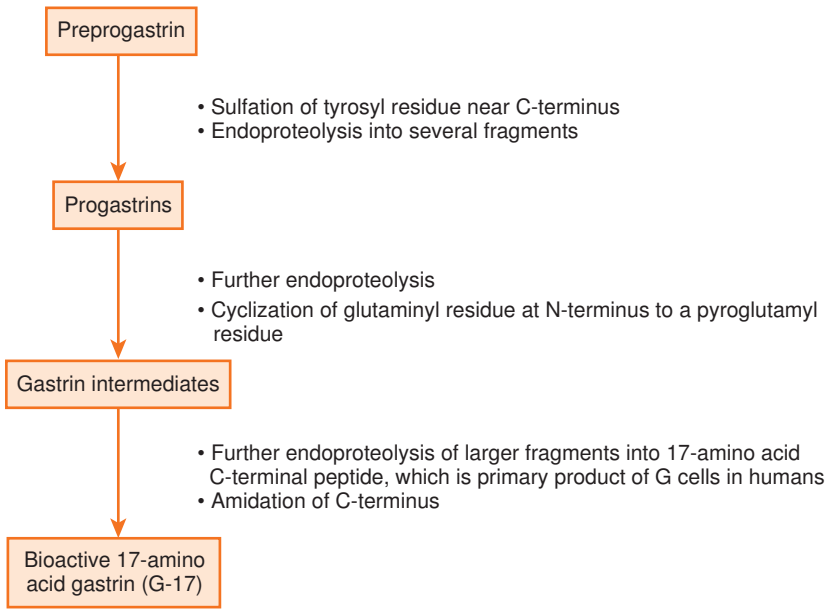

FIGURE 2-3 ■ Processing of preprogastrin.

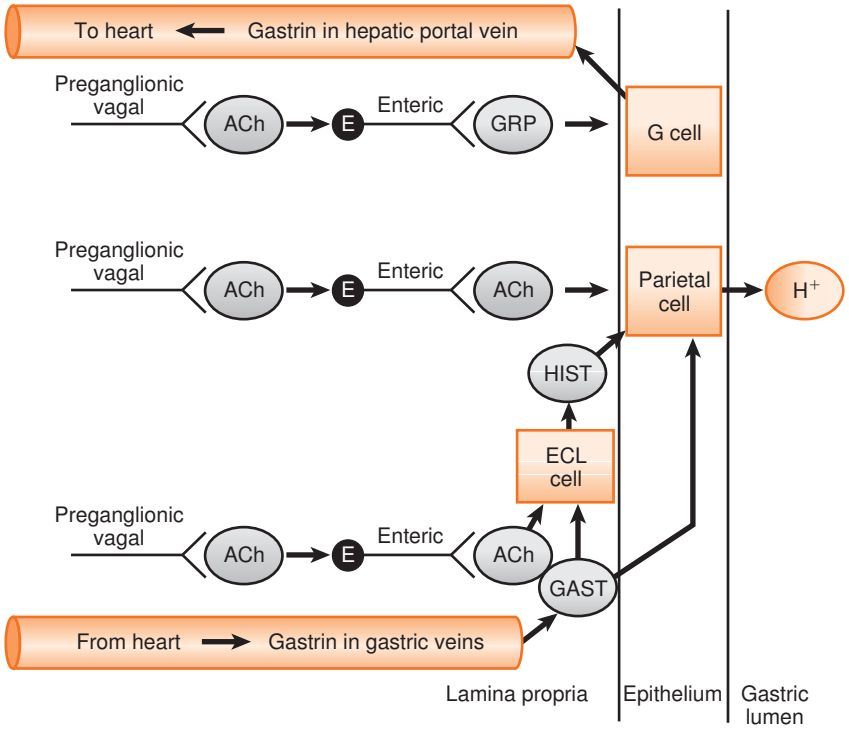

FIGURE 2-4 ■ Regulation of gastric HCl secretion during the cephalic phase of a meal. The thought, sight, or smell of food, or the presence of food in the mouth, stimulates acid secretion through the vagal preganglionic parasympathetic nerves, which stimulate the release of acetylcholine (ACh) from postganglionic enteric nerves. Enteric nerve fibers secreting ACh stimulate parietal cells directly and through the release of histamine from enterochromaffin-like (ECL) cells. Gastrin is also stimulated by enteric neuronal fibers that release gastrin-releasing peptide (GRP). As a hormone, gastrin levels increase in the general circulation. Gastrin stimulates gastric HCl secretion by binding to CCK2 receptors on ECL cells (and, to a lesser extent, on parietal cells).

During the gastric phase, gastrin secretion from G cells is primarily stimulated by the presence of peptides and amino acids in the lumen of the antrum (Fig. 2-5). Gastrin secretion can also be stimulated by **stomach distention** as detected by **mechanosensors** during the gastric phase, acting through local neuronal pathways, and through a **vagovagal reflex**. Circulating gastrin levels increase by several-fold within 30 to 60 minutes after ingestion of a meal.

The primary action of gastrin is the stimulation of HCl secretion by the parietal cells of the gastric glands within the fundus and body of the stomach. To accomplish this, gastrin must enter and circulate through the general circulation and then exit capillaries and venules within the lamina propria of the gastric mucosa in the body and fundus (i.e., *upstream* of where gastrin is released within the stomach).

Gastrin evokes HCl secretion primarily through binding to the **CCK2 receptor** on **ECL cells**. ECL cells, which reside in the lamina propria of the gastric mucosa, produce **histamine** in response to gastrin (see Fig. 2-5). Gastrin binding to the Gq-coupled CCK2 receptor on ECL cells increases intracellular Ca^{2+}, which leads to exocytosis of histamine-containing secretory vesicles. Gastrin also increases histamine synthesis and storage by increasing the expression of histidine decarboxylase, which generates histamine from histidine, and type 2 vesicular monoamine transporter (VMAT-2), which transports and concentrates histamine into the secretory vesicles. Thus, gastrin coordinates both the secretion and synthesis of histamine in ECL cells. Histamine, in turn, stimulates HCl secretion in a paracrine manner by binding to the **H_2 receptor** on nearby epithelial parietal cells. Gastrin also has a direct, although less important, effect on parietal cells.

During the **intestinal phase** of a meal, the decrease in gastric contents relieves the stimulation of G cells by amino acids and peptides, and by distention-induced vagovagal pathways. The decrease in gastric contents also reduces the buffering capacity of the gastric lumen. Thus, during the intestinal phase and the interdigestive period, the acidity of the stomach decreases. When the pH falls below 3, acid stimulates the **D cells** to secrete the paracrine peptide, **somatostatin**. Somatostatin acts through its receptors (SS-R) to inhibit gastrin secretion from neighboring G cells (Fig. 2-6).

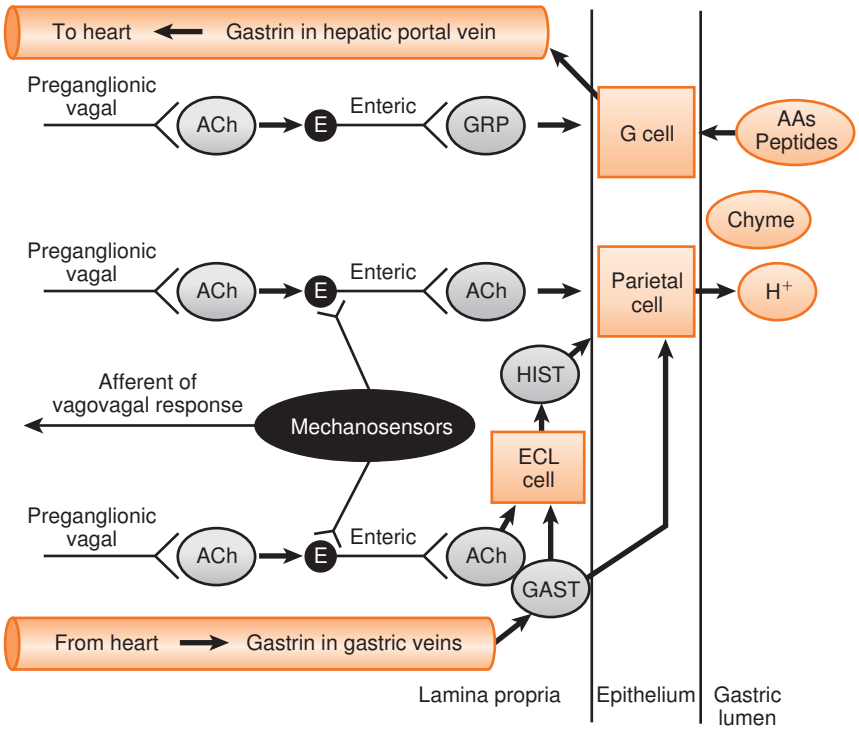

FIGURE 2-5 ■ Regulation of gastrin secretion during the gastric phase of a meal. Luminal amino acids and peptides strongly stimulate G cells in the antrum to secrete gastrin. Gastrin secretion and HCl secretion are also stimulated by stomach distention through local and autonomic (vagovagal) reflexes.

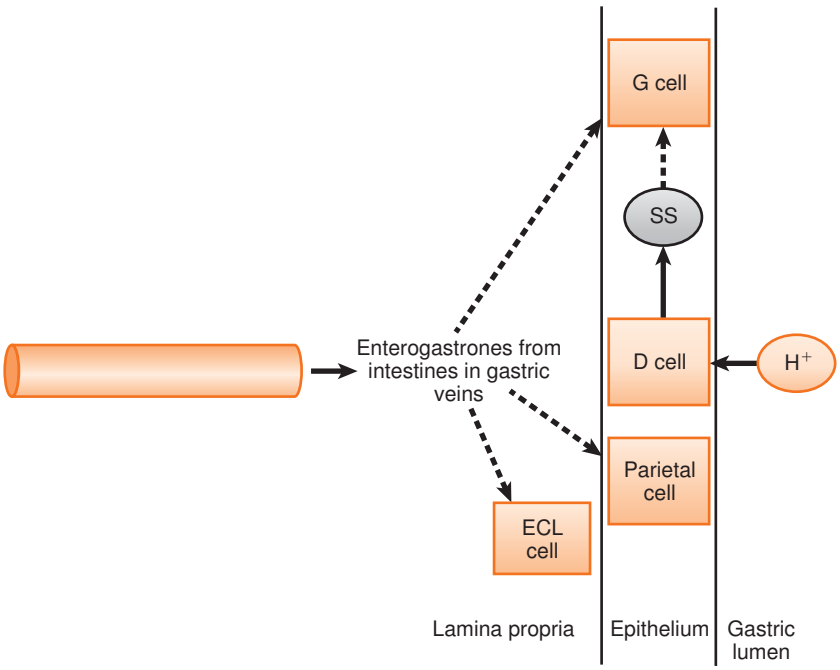

FIGURE 2-6 ■ Regulation of gastrin secretion during the intestinal phase of a meal. The exit of food (chyme) from the stomach lumen reduces buffering of HCl. A low pH stimulates D cells to release the paracrine factor, somatostatin (SS), which inhibits gastrin secretion from neighboring G cells. The exact nature of physiologic enterogastrones in humans is not well established. Candidates include secretin and gastric-inhibitory peptide (gip) from the small intestine, and peptide yy from the ileum and colon.

Gastrin release and gastric emptying are also inhibited during the intestinal phase by the release of hormones and neural signals from the small intestine and colon in response to acidity, hypertonicity, distention, and specific molecules (e.g., fatty acids). These hormones are collectively referred to as **enterogastrones**. The identity of the physiologic enterogastrones in humans that inhibit gastric acid secretion remains uncertain but includes candidates such as **secretin** and **GIP** from the duodenum and jejunum and **peptide YY** and **GLP-1** from the distal ileum and colon. **CCK** is a well-established **inhibitor of gastric motility and emptying**. CCK is released from the duodenum and jejunum in response to the presence of luminal fatty acids (see Fig. 2-6).

ENTEROENDOCRINE REGULATION OF THE EXOCRINE PANCREAS AND GALLBLADDER

The **exocrine pancreas** is an extramural gland that empties its secretory products through a main excretory duct into the GI tract at the **duodenum** (Fig. 2-7). The **acini** of the **exocrine pancreas** produce enzymes necessary to digest macromolecules in the small intestine. Pancreatic enzymes have optimal activities at a neutral pH. Accordingly, the cells that line the **pancreatic ducts** secrete a **bicarbonate-rich fluid**, which serves to neutralize acidic chyme in the duodenum. The **gallbladder** is also an extramural organ. It receives **bile** that is secreted by the **liver**. Bile is both stored and concentrated in the gallbladder. Bile is

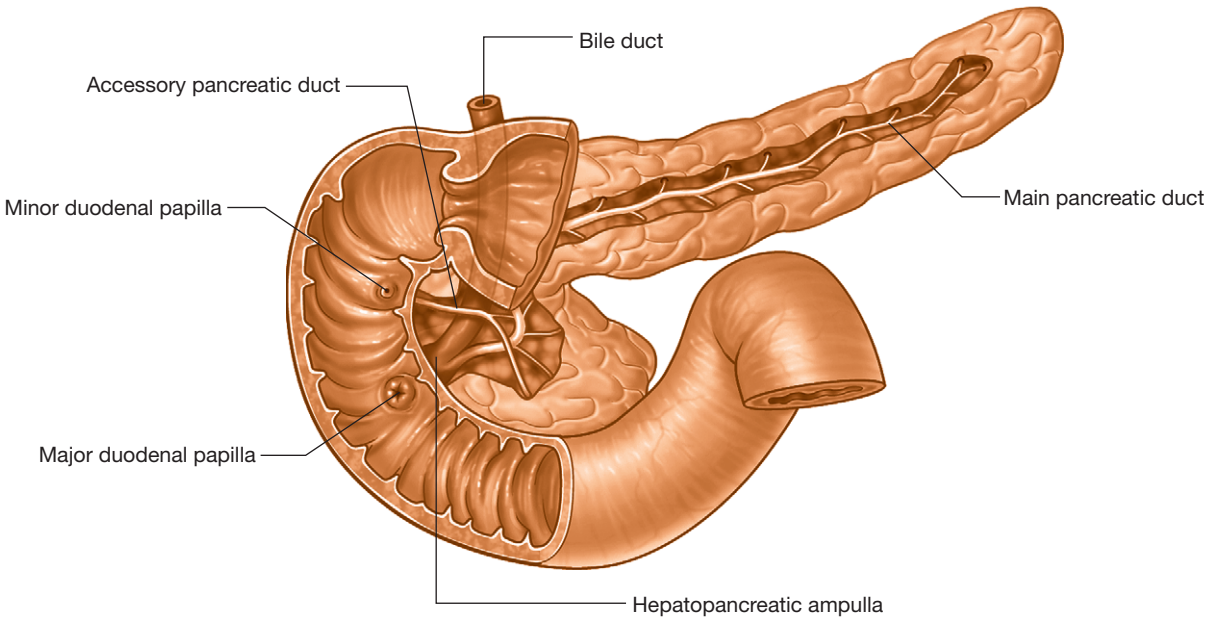

FIGURE 2-7 ■ Anatomy of the common bile duct, pancreas, pancreatic duct, and duodenum. The gallbladder (not shown) stores and concentrates bile from the liver. Contraction of the gallbladder and relaxation of the sphincter of Oddi (surrounds the hepatopancreatic ampulla) allows bile to flow down the common bile duct into the duodenum. Pancreatic enzymes and bicarbonate reach the duodenum via larger and larger ducts that eventually form the main pancreatic duct. This duct joins the common bile duct just before it reaches the duodenum, to form the hepatopancreatic ampulla. *Inset* shows a higher magnification of the exocrine pancreas. The termini of the secretory units are the pancreatic acini, which secrete enzymes. The ductal epithelium secretes a bicarbonate-rich fluid. Note that the ductal epithelium of the common bile duct also secretes a bicarbonate-rich fluid. *(© Elsevier. Drake et al:* Gray's Anatomy for Students, *www.studentconsult.com.)*

released into small intestine through the **common bile duct**, which usually joins the **main pancreatic duct** to form the **hepatopancreatic ampulla** just before opening into the duodenum (see Fig. 2-7). A major function of bile is the **emulsification of triglycerides** to increase their accessibility to pancreatic lipase. In order to perform this function, aggregates (called *micelles*) of bile acids and other lipids are required. Micelle formation requires neutral or slightly alkaline conditions. Accordingly, the **epithelial cells of the common bile duct** secrete a **bicarbonate-rich fluid**.

Pancreatic and gallbladder functions are primarily regulated by the autonomic nervous system during the interdigestive period (pancreatic secretion occurs in phase with the migrating myoelectric complex [MMC] in humans), and during the cephalic and gastric phases of the digestive period. However, during the **intestinal phase**, when these glands are most active, they are predominantly under endocrine control by two GI hormones, **secretin** and **CCK**. Secretin primarily regulates ductal secretion of a bicarbonate-rich fluid from both pancreatic and bile ducts. CCK primarily stimulates enzyme secretion from pancreatic acinar cells and gallbladder contraction. This dual regulation allows for fine-tuning of the qualitative nature of the product (e.g., in terms of the percentage of bicarbonate and protein in pancreatic juice) that is finally secreted into the duodenum.

The classic model for secretin and CCK action on the pancreas is that the appearance of acid, long-chain fatty acids, and glycine-containing dipeptides and tripeptides in the duodenum stimulates the *open* enteroendocrine cells to secrete the two hormones. Secretin and CCK then circulate in the blood and bind to their specific receptors on either ductal or acinar cells, respectively (Fig. 2-8).

However, there is evidence that secretin has **permissive effects** on CCK actions, and vice versa. Moreover, it is also clear that the autonomic and enteric nervous systems have a permissive effect on the secretin and CCK actions. The neurotransmitter, **ACh**, and a **secretin-related enteric neurocrine peptide, VIP**, stimulate pancreatic ductal and acinar cells and synergize with secretin and CCK. Patients who have a **VIPoma** (i.e., a tumor producing high levels of VIP) suffer from pancreatic diarrhea because of a constant high level of pancreatic secretion into the gut.

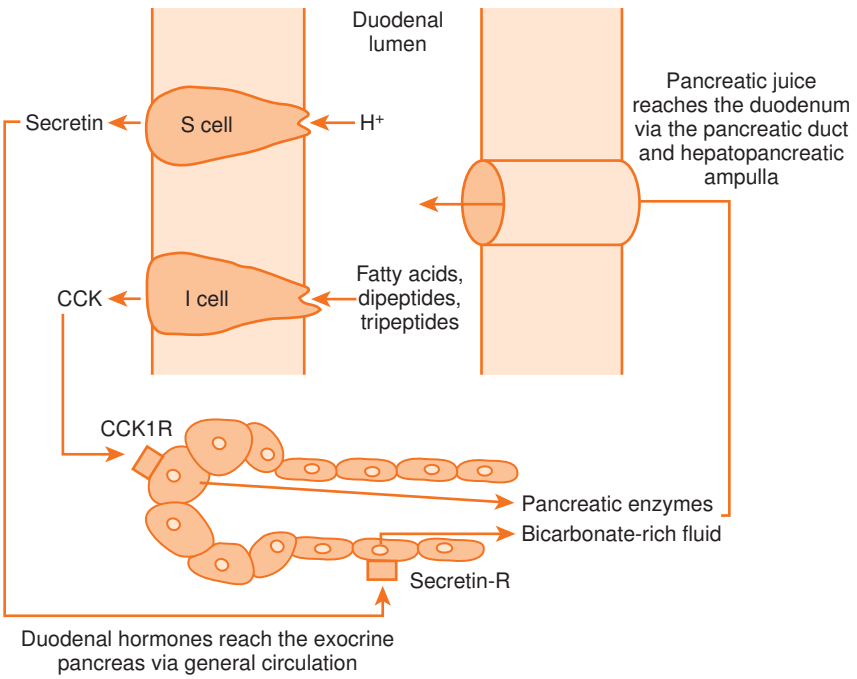

FIGURE 2-8 ■ Hormonal regulation of pancreatic secretion by secretin and CCK.

Secretin

Secretin is produced by **S cells** in the duodenum and jejunum. Similar to gastrin, secretin is produced by posttranslational processing of a larger **preprosecretin** molecule. Most secretin is a carboxyl amidated 27-amino acid peptide.

The primary stimulus for secretin release is a decrease in **duodenal pH**. The threshold pH value for secretin release is 4.5. Circulating secretin levels increase rapidly (approximately 10 minutes) after acidified chyme passes through the pyloric sphincter into the duodenum. The exact mechanism by which H^+ induces secretin release from S cells is unclear. There is evidence for a direct action of H^+ on S cells as well as evidence for indirect actions through enteric neurons and through a phospholipase A_2–like **secretin-releasing factor**.

The primary short-term action of secretin is the stimulation of the secretion of a **bicarbonate-rich fluid** from the **pancreatic** and **biliary ducts** during the **intestinal phase** of the digestive period (see Fig. 2-8). Secretin acts through the **secretin receptor**, which is linked to cAMP-dependent pathways. Signaling from the secretin receptor opens apical Cl^- channels (**cystic fibrosis transmembrane conductance regulator** or **CFTR**) thereby increases the flow of transport Cl^- (and, through paracellular osmotic drag, water) into the lumen. Cl^- is then exchanged for HCO_3^-. Upregulation of this process by secretin can occur through the opening of preexisting CFTR transporters in the apical membrane and through the exocytotic insertion of transporter-containing vesicles into the membrane. The importance of the CFTR channel to pancreatic function underlies the dysfunction of pancreatic secretion observed in patients with **cystic fibrosis**.

Secretin also binds to its receptor on the pancreatic acinar cells. Although secretin has a minimal effect on acinar cells by itself, secretin synergizes with the hormone CCK to further enhance pancreatic enzyme secretion over that achieved by CCK alone. Secretin may also function as an **enterogastrone** by inhibiting stomach acid secretion.

Cholecystokinin

CCK is a 33-amino acid peptide produced by the **I cells** of the duodenum and jejunum. CCK is structurally similar to gastrin, with the 5 amino acids at the carboxyl terminus identical to both hormones. CCK is also sulfated on a tyrosine that is the seventh amino acid from the carboxy terminus. CCK binds primarily to the **CCK1 receptor** (formerly called the *CCKA receptor*), whereas gastrin preferentially binds to the CCK2 receptor. Both hormones can weakly interact with the other's receptor, and desulfation of CCK increases its affinity for the CCK2 receptor. The CCK1 receptor is linked to protein kinase C–dependent and Ca^{2+}-dependent pathways.

The primary stimulus for CCK secretion is the presence of **long-chain fatty acids** or **monoglycerides** in the small intestine (see Fig. 2-8). CCK secretion is also induced by glycine-containing dipeptides and tripeptides. The mechanism by which any of these act to stimulate CCK release is obscure, although there is some evidence for a postabsorptive effect of lipids after their assembly into chylomicrons. There is also evidence for a CCK-releasing peptide (CCK-RP) that is released luminally from enterocytes and stimulates CCK release through binding to a CCK-RP receptor on the apical membrane of I cells. Like secretin, CCK primarily regulates **pancreatic** and **biliary function**. In the pancreas, CCK stimulates enzyme secretion from the acinar cells (see Fig. 2-8). The CCK1 receptor increases intracellular DAG and Ca^{2+}, which results in the exocytosis of enzyme-containing secretion granules. CCK also has a permissive effect on the ability of secretin to stimulate bicarbonate secretion.

CCK is a strong stimulator of **gallbladder contraction,** and CCK deficiency disorders have been linked to impairment of gallbladder contraction and **cholelithiasis** (**gallstones**). CCK induces gallbladder contraction both directly and indirectly through activation of vagal afferent neurons. CCK also stimulates bile secretion into the duodenum through promoting **relaxation of the sphincter of the hepatopancreatic ampulla** (**sphincter of Oddi**). This latter action on hepatobiliary function is likely due to the **CCK-dependent release of inhibitory neurotransmitters**, such as nitric oxide, from enteric neurons. As mentioned, CCK also **inhibits gastric emptying**, which reduces duodenal acidity and allows emulsification, digestion, and absorption of lipids.

Motilin and Stimulation of Gastric and Small Intestinal Contractions During the Interdigestive Period

Motilin is a 22-amino acid peptide produced from a 114-amino acid **prepromotilin** and secreted by the **M cells** of the small intestine. Motilin secretion is **inhibited by the presence of food or acid** in the small intestine and is **stimulated by alkalinization** of the small intestine.

Circulating motilin levels peak every 1 to 2 hours in fasting individuals, in phase with the **MMC**. The MMC is a set of organized contractions that move aborally from the stomach to the ileum and clean the stomach and small intestines of indigestible particles. The MMC may also prevent the colonic bacteria from migrating into the small intestine. Motilin may function to either initiate or integrate the MMC.

The **motilin receptor** is a GPCR that activates the phospholipase C signaling pathway. The motilin receptor also binds and is activated by the macrolide antibiotic, **erythromycin** (see Table 2-2). Erythromycin and other motilin receptor agonists are used in the treatment of delayed gastric emptying (**gastroparesis**), which is common in patients with diabetes mellitus and in some postsurgical patients.

INSULINOTROPIC ACTIONS OF GASTROINTESTINAL PEPTIDES (INCRETIN ACTION)

Elevated circulating levels of nutrients, particularly blood glucose, are strong stimuli of insulin secretion from the pancreatic β cells (see Chapter 3). The possibility that GI hormones also regulate the secretion of insulin was revealed by observations that oral administration of glucose caused a greater rise in insulin than did glucose administered by an intravenous route. This **enteroinsular response** gave rise to the concept of **incretins**. In this model, an enteroendocrine cell type senses nutrients in the GI tract and releases a hormone (an incretin), which, in turn, prepares the pancreatic β cells for the impending rise in blood nutrients (primarily blood glucose). There are two incretins in humans, **gastric inhibitory peptide** (**GIP**; also referred to as ***g*lucose-dependent *i*nsulinotropic *p*eptide**), and **glucagon-like peptide-1** (**GLP-1**). These peptides (or analogs thereof) are currently being investigated for the treatment of type 2 diabetes mellitus (see Chapter 3). An important feature of incretins is that their ability to increase insulin secretion is strongly dependent on glucose levels. This means that incretin analogs pose a low risk for inducing severe hypoglycemia (low blood sugar) because once blood glucose falls, the effect of incretins is terminated.

In general, GIP and GLP-1 act through Gs-coupled receptors on β cells, which increase cAMP. This acts in a permissive or synergistic manner with the main glucose/adenosine triphosphate (ATP)-dependent pathway that leads to an increased intracellular Ca^{2+} and the release of insulin. For example, cAMP-EPAC signaling (see Chapter 1) may promote the docking and regulated exocytosis of secretory vesicles in β cells. Incretins also enhance the synthesis of insulin and of proteins that sensitize the β cells to glucose levels, such as the glucose transporter, GLUT-2, and hexokinase.

Gastric Inhibitory Peptide/Glucose-Dependent Insulinotropic Peptide

GIP is a 42-amino acid peptide secreted by the **K cells** of the small intestine and is a member of the secretin gene family. The **primary stimulus** for GIP release is the presence of **long-chain fatty acids, triglycerides, glucose, and amino acids** in the lumen of the small intestine.

GIP was first discovered as an enterogastrone in animal models, in which it inhibited gastric acid secretion and intestinal motility. However, physiologic levels of GIP have only a modest effect on stomach function in humans. In contrast, GIP has an important physiologic role as an incretin. GIP knockout mice display a reduced ability to maintain normal blood glucose levels after an oral glucose load (impaired glucose tolerance).

In rare cases, the **GIP receptor** is inappropriately expressed on cells of the zona fasciculata of the adrenal cortex (see Chapter 7). These patients display enlarged adrenals and **food-induced hypercortisolism**. In these patients, food in the small intestine stimulates the release of GIP, which then stimulates cortisol production by the adrenal cortex (see Chapter 7).

Glucagon-like Peptide-1

The **glucagon gene** is an example of a gene that encodes a large precursor protein (**preproglucagon**), which is proteolytically processed to form active and inactive peptides (Fig. 2-9). Furthermore, the **prohormone convertases** that digest preproglucagon display cell-specific expression, so different products are released from different cell types. In the α cells of the endocrine pancreas, the active product is **glucagon** (see Chapter 3). In contrast, **intestinal L cells** express preproglucagon but secrete **GLP-1** and **GLP-2** as biologically active peptides. GLP-1 is stimulated by the presence of **free fatty acids and glucose** in the lumen of the ileum and colon. GLP-1 secretion is also increased by neuronal pathways stimulated by free fatty acids and glucose in the upper small intestine. GLP-1 is co-secreted with the other glucagon-derived peptide, **GLP-2,** and **peptide YY** (which is not structurally related to glucagon). The tropic effect of GLP-2 is discussed later.

Like GIP, GLP-1 acts as an incretin. GLP-1 knockout mice have impaired glucose tolerance. GLP-1, along with peptide YY, also appears to be a component of the **ileal brake**, in which free fatty acids and carbohydrates in the ileum inhibit gastric emptying through increased secretion of GLP-1 and peptide YY. This enterogastrone action of GLP-1 further enhances the ability of the organism to control excessive blood glucose excursions. A problem with the therapeutic use of native GLP-1 is the fact that it is rapidly degraded. The use of more stable analogs, called **exendins**, and inhibitors of enzymatic degradation are currently under investigation for enhancing pancreatic β-cell function in type 2 diabetic patients.

ENTEROTROPIC ACTIONS OF GASTROINTESTINAL HORMONES

An important characteristic of many hormones is their ability to promote the growth of their target tissues. This tropic effect helps to maintain the health and integrity of the target tissues and optimizes the ability of target tissues to perform their differentiated functions. In addition to the actions of GI hormones on the maintenance of healthy GI structure and physiology, the tropic actions of GI hormones are of current clinical interest for several reasons, including the following:

- The promotion of hypertrophy and hyperplasia of GI tissues, which sometimes progress to cancer, by the excessive secretion of a GI hormone (usually from a tumor)
- The ability of the GI tract to adapt to a diseased portion of the tract, and/or corrective surgery that involves resection or bypass of a GI segment
- The ability to grow new pieces of GI tissue in vitro (i.e., tissue-engineered neointestine) from pluripotential or stem cells, which can be used for replacement of diseased or resected portions
- The ability to promote pancreatic islet growth and neogenesis in diabetic patients

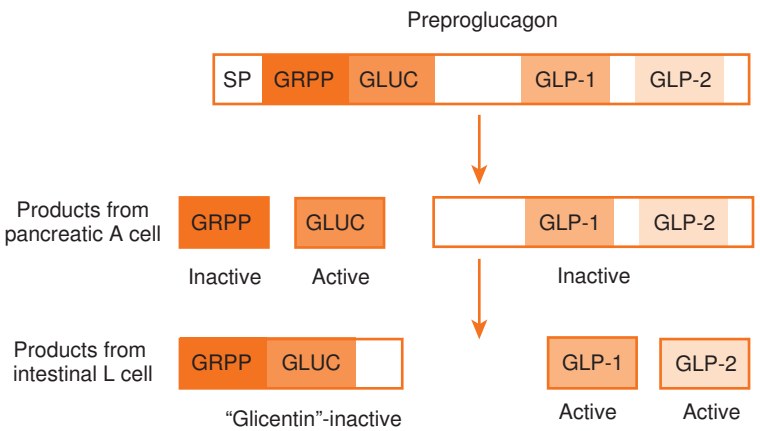

FIGURE 2-9 ■ Cell-specific processing of preproglucagon.

Gastrin

In addition to its well-established role in the regulation of gastric acid secretion, gastrin exerts several other effects on the stomach and GI tract. The second most important action of gastrin is its **developmental and trophic effect** on the **gastric mucosa**. Gastrin knockout mice display poorly differentiated gastric mucosa, with a reduced number of ECL and parietal cells. In contrast, patients suffering from Zollinger-Ellison syndrome (see earlier) exhibit hypertrophy and hyperplasia of the gastric mucosa, as well as enlarged submucosal rugal folds. Overgrowth is particularly true for the ECL cell population. Although ECL cell proliferation can progress to **carcinoid tumor** formation, this is rare and usually requires other abnormalities. As discussed earlier, progastrin and glycine-extended gastrin (G-Gly) appear to promote the proliferation of colonic mucosa.

Gastric acid, through its effects on D cells and somatostatin release, inhibits the growth of G cells. Thus, long-term inhibition of gastric acid production (e.g., with pharmacologic proton pump inhibitors or H_2 receptor blockers) can lead to an overgrowth of antral G cells.

Secretin and Cholecystokinin

CCK has a direct effect on **pancreatic acinar cells** that **promotes their maintenance and growth**. **Secretin inhibits pancreatic ductal cell growth** through binding to the secretin receptor. In contrast, the secretin-related neurotransmitter, **VIP**, **stimulates ductal growth** through the **VIP receptor** (called **$VPAC_1$ receptor**). In some **ductal pancreatic adenocarcinomas**, the secretin receptor is defective, but the $VPAC_1$ receptor is intact. Thus, loss of secretin receptor function may shift the cell toward net proliferation.

Glucagon-like Peptide-1

One of the most exciting and promising aspects of enterotropic actions of GI hormones is the **tropic effect that GLP-1 has on pancreatic islet development and growth**, particularly with respect to the β cells. GLP-1 has been shown to induce differentiation of human islet stem cells into β cells in vitro. In mice and rats, GLP-1 and exendin-4 have protected against surgically and chemically induced diabetes, increased β-cell mass and neogenesis, and inhibited β-cell apoptosis. Further, GLP-1 receptor knockout mice do not display exendin-4-induced regeneration of islets after partial pancreatectomy. Thus, GLP-1 or analogs may become valuable reagents in the treatment of diabetic patients whose β-cell mass has been compromised.

Glucagon-like Peptide-2

GLP-2 is **co-secreted** with **GLP-1** by the **intestinal L cells**. Unlike GLP-1, GLP-2 does not have an insulinotropic action. GLP-2 binds to its own receptor (the GLP-2 receptor) and has **potent trophic effects on the intestines**. In fact, evidence of this effect was first discovered in a patient who presented with a massive overgrowth of the small intestine. The patient was also found to have a tumor in the kidney that was producing large amounts of glucagon-related peptides. **GLP-2** has been used to **prevent mucosal atrophy** in patients receiving total parenteral nutrition, and it **promotes intestinal growth and adaptation** in patients undergoing resection of bowel. GLP-2 also has positive effects on hexose transport and **may enhance other absorptive functions of intestinal villi**.

SUMMARY

1. Gastrointestinal (GI) hormones are produced by enteroendocrine cells. GI hormones are peptides or proteins and bind to G-protein-coupled receptors on their target cells. GI hormones are produced by specific cell types that reside in specific regions of the GI tract. The secretion of GI hormones is stimulated primarily by luminal secretogogues and by neuronal (enteric and autonomic) and paracrine signals.
2. Gastrin plays a major role in the stimulation of gastric acid secretion. Gastrin is secreted by G cells in the stomach antrum in response to amino acids and peptides in the antral lumen and in response to neuronal stimulation. The primary secreted form of gastrin by the stomach is the 17-amino acid G-17 form. G-17 has a cyclized glutaminyl residue at its N-terminus and an amidated glycine at its C-terminus, which increase the biologic half-life of secreted gastrin. Gastrin binds to the CCK2 receptor and acts primarily by stimulating enterochromaffin-like cells (ECL cells) to secrete histamine. Histamine then stimulates the parietal cells of the stomach to secrete HCl.
3. The major enteroendocrine cells of the duodenum and jejunum are the S cells and I cells, which secrete secretin and cholecystokinin (CCK), respectively. Secretin is released primarily during the *intestinal* phase of a meal in response to increased acidity in the duodenum. Secretin promotes the secretion of a bicarbonate-rich fluid from the bile duct and pancreatic ducts, which empty into the duodenum. CCK promotes the contraction of the gallbladder and relaxation of the sphincter of the hepatopancreatic ampulla, thus promoting the emptying of bile into the duodenum. CCK also stimulates enzyme secretion from pancreatic acinar cells.
4. Motilin is secreted by the M cells of the small intestine during the interdigestive phase (i.e., in between meals), in phase with the migrating myoelectric complex. Motilin promotes emptying of the stomach and small intestines. The motilin receptor is activated by erythromycin, which can be used to treat delayed gastric emptying (gastroparesis).
5. GI hormones called incretins are secreted in response to luminal nutrients (especially glucose) and increase the ability of blood glucose to stimulate insulin secretion from the pancreatic islets of Langerhans. Incretins include gastric inhibitory peptide (GIP), which has been named more recently for its incretin effect as glucose-dependent insulinotropic peptide. GIP is secreted from the K cells of the small intestine. Another important incretin is glucagon-like peptide-1 (GLP-1), which is secreted by the intestinal L cells. Because of their ability to sensitize insulin-producing β cells to glucose, incretins are being tested for the treatment of type 2 diabetes mellitus (T2DM; see Chapter 3).
6. GI hormones also have important trophic effects. Gastrin stimulates the growth of the gastric mucosa, especially the ECL cells and submucosa. Secretin and CCK promote the growth of exocrine pancreas tissue. GLP-1 promotes β-cell proliferation, which may prove an important function of GLP-1 in the treatment of T2DM. GLP-2, which is related to, but is a separate hormone from, GLP-1, promotes GI mucosal growth and is used to treat patients at risk for GI mucosal atrophy.
7. Zollinger-Ellinger syndrome is caused by a gastrin-producing tumor. Patients have ulcerations of the esophagus, stomach, and duodenum and overgrowth of the stomach mucosa and rugal submucosal folds.

SELF-STUDY PROBLEMS

1. What are the three phases of the digestive period? Which one has the greatest release of gastrin? Why?
2. When administered during the interdigestive period, what are the predicted effects on gastrin secretion of the following experimental agents?
   a. A somatostatin antagonist
   b. A mix of amino acids in the antral lumen
   c. Increased acidity in the antral lumen
   d. A muscarinic agonist
   e. Gastrin-releasing peptide
3. What is the relation between gastric emptying and gastrin secretion from duodenal S and I cells?
4. What are the effects of CCK on the following?
   a. Pancreatic bicarbonate secretion
   b. Pancreatic enzyme secretion
   c. Biliary bicarbonate secretion
   d. Contraction of the gallbladder muscularis
   e. Contraction of the sphincter of Oddi
5. What is the relation between GLP-1 and glucagon?
6. Define *incretin*. Name two incretins.
7. What enterotropic effect is observed in patients with Zollinger-Ellison syndrome?
8. Why does erythromycin promote gastric emptying?

KEYWORDS AND CONCEPTS

- Acetylcholine
- Amidated gastrins
- Autonomic nervous system

For full list of keywords and concepts see Student Consult

SUGGESTED READINGS

Fourmy D, Gigoux V, Reubi JC: Gastrin in gastrointestinal diseases, *Gastroenterology* 141:814–818, e811–e813, 2011.

Ishii H, Sato Y, Takei M, et al: Glucose-incretin interaction revisited, *Endocr J* 58:519–525, 2011.

Poitras P, Peeters TL: Motilin, *Curr Opin Endocrinol Diabetes Obes* 15:54–57, 2008.

Rehfeld JF: Incretin physiology beyond glucagon-like peptide 1 and glucose-dependent insulinotropic polypeptide: Cholecystokinin and gastrin peptides, *Acta Physiol* 201:405–411, 2011.

KEYWORDS AND CONCEPTS

- CCK
- CCK1 receptor
- Cephalic phase
- Chief cells
- Cholecystokinin (CCK)
- Chyme
- Duodenum
- Endocrine glands
- Enteric nervous system
- Enteroendocrine cell
- Enterogastrones
- Enterochromaffin-like (ECL) cells
- Enterotropic action
- Erythromycin
- Exendins
- Exocrine pancreas
- Extrinsic regulators
- Food-induced hypercortisolism
- Fundus and body
- G-17
- Gallbladder
- Gastric phase
- Gastrin
- Gastrin-releasing peptide (GRP)
- Gastroparesis
- Ghrelin
- Glucose-dependent/insulinotropic peptide
- G-protein-coupled receptors
- Growth hormone secretogogue
- HCl
- Hormone
- I cells
- Impaired glucose tolerance
- Incretins
- Incretion action
- Intestinal phase
- Intrinsic factor
- Intrinsic regulators
- Migrating myoelectric complex (MMC)
- Motilin
- Mucigens
- Oxyntic cells
- Paracrine
- Parietal cells
- Pentagastrin
- Peptide YY
- Preprogastrin
- Pyloric antrum
- S cells
- Secretin
- Secretin-releasing factor
- Secretogogues
- Somatostatin
- Stomach
- Vagal parasympathetic nervous system
- Vagovagal reflex
- Vasoactive intestinal peptide
- Vitamin B_{12}
- Zollinger-Ellison syndrome

3 ENERGY METABOLISM

OBJECTIVES

1. Provide an overview of energy metabolism with emphasis on maintaining blood glucose within the normal range.
2. Introduce the primary hormones involved in the regulation of energy metabolic homeostasis.
3. Cover the hormonal regulation of specific enzymatic pathways.
4. Discuss the role of adipose tissue as an endocrine organ.
5. Discuss imbalances in energy metabolism and their consequences in type 1 and type 2 diabetes mellitus.

Note: See Key Pathways Involved in Energy Metabolism on Student Consult.

OVERVIEW OF ENERGY METABOLISM

Cells continually perform work to grow, proliferate, and migrate; to maintain their structural integrity and internal environment; to respond to stimuli; and to perform their differentiated functions (Fig. 3-1). The resting metabolic rate of humans constitutes about 60% to 70% of total potential energy expenditure. Cells derive their energy to perform this work from the universal energy carrier, **adenosine triphosphate (ATP)**. The enzymatic hydrolysis of the terminal phosphate group (thereby generating adenosine diphosphate, or ADP) releases a significant amount of energy that is coupled to and drives many other energetically unfavorable reactions. Cells need a continual supply of ATP. This is achieved by oxidizing carbon-based fuels (also referred to as *nutrients* or *energy substrates*). The primary fuels are **monosaccharides; free fatty acids (FFAs**; also called *nonesterified fatty acids*); **amino acids (AAs)**, and **ketone bodies**.

The regulation of energy metabolism serves to maintain adequate levels of intracellular ATP in all cell types (especially the brain) at all times, while keeping intracellular and circulating energy substrates within normal ranges. Thus, energy metabolism needs to be viewed from both the single cell and the organismal points of view, and with the appreciation that the details of energy metabolism and its regulation vary among different cell types.

Nutrient Partitioning

During a time of **caloric excess** (i.e., during the digestive period shortly after a meal, referred to as the **fed state**), a fraction of nutrients are used for ATP production, and excess fuel is partitioned into various **storage depots** (see Fig. 3-1). **Insulin** is the primary hormone that orchestrates fuel use and storage during the fed state, thereby preventing blood glucose and lipids from exceeding certain thresholds. Insulin also promotes protein synthesis.

SUPPLEMENT TO CHAPTER 3: OVERVIEW OF KEY PATHWAYS INVOLVED IN ENERGY METABOLISM

Use, storage, and de novo synthesis of fuels are performed by **metabolic pathways**, that is, a series of enzymatic reactions. Various **cell membrane** and **intracellular transporters** are closely integrated into the enzymatic pathways to ensure adequate flow of substrates and products. Metabolic pathways display the following characteristics:

1. *Cell specificity.* Some enzymatic pathways and transporters exist in all cells, but not all cells can use all types of nutrients.
2. *Subcellular localization.* Enzymes and transporters have specific subcellular locations associated with different intracellular compartments or organelles.
3. *A mix of reversible and irreversible reactions.* The term ***reversible*** means that one enzyme catalyzes the forward and reverse reactions, depending on the relative amounts of substrate and product. The term ***irreversible*** means that, given the physiologic concentrations of substrate and product, one enzyme catalyzes the forward reaction, while a second enzyme catalyzes the reverse reaction. Irreversible reactions are important targets of hormonal control.
4. *Multiple layers of regulation.* Many enzymes (especially those that catalyze irreversible reactions) are controlled by allosteric regulators, including immediate or distant substrates and products. Intracellular sensors of nutrients and energy status (e.g., mTORC1 and AMPK) help to orchestrate anabolic and catabolic pathways within the cell. Hormones and neurotransmitters, which are themselves regulated by nutrient status in the blood, impose additional regulation on the expression, localization, and/or activity of metabolic enzymes at the transcriptional, posttranscriptional, and posttranslational levels.

ATP Synthesis by Glycolysis

ATP is generated from the oxidation of carbohydrates through the process of **glycolysis**, or the splitting (lysis) of glucose. For a cell to initiate glycolysis, it must facilitate transport of glucose into the cytoplasm and phosphorylate glucose to **glucose-6-phosphate**. Circulating glucose is transported across the cell membrane by **bidirectional facilitative glucose transporters, called *GLUTs*.** The phosphorylation of glucose to glucose-6-phosphate is catalyzed by **hexokinases.** The hexokinase found in **hepatocytes** and **pancreatic β cells** has a low affinity for glucose (i.e., it transports glucose only when glucose is available at elevated concentrations) and is designated glucokinase. In these two cell types, expression of **glucokinase** is paired with the expression of a low-affinity, high-capacity glucose transporter, **GLUT2**.

Glycolysis is an oxidative, catabolic process that can be used by **all cell types** to generate ATP. Glycolysis has two general stages:

1. *The energy investment phase* (Fig. S3-1; left panel). This involves the consumption of two ATPs to generate a 6-carbon structure with 2 phosphorylated carbons (**fructose-1,6-bisphosphate**).
2. *The energy capture phase* (see Fig. S3-1; right panel). This involves the splitting of the 6-carbon intermediate into two 3-carbon intermediates, each with 1 phosphate, followed by an oxidation and reduction step that uses inorganic phosphate (Pi) and NAD^+ to phosphorylate a second carbon. This generates a total of 4 Pi groups per molecule of glucose, each of which is used to convert ADP to ATP.

Glycolysis yields a net of 2 ATP molecules per glucose oxidized. It also consumes NAD^+ by reducing it to NADH and H^+. NAD^+ must be continually replenished from NADH for glycolysis to continue. During **anaerobic glycolysis**, the conversion of pyruvate to **lactate** in the cytoplasm regenerates NAD^+ from NADH (see Fig. S3-1; *gray arrows*). Anaerobic glycolysis does not require oxygen or mitochondria and therefore can be used by mitochondria-poor cells (erythrocytes, white fast-twitch skeletal muscle fibers, lens cells of the eye) to generate ATP. It is also used by certain rapidly proliferating cancer cells, which may find themselves in a hypoxic environment, and which use TCA cycle intermediates for macromolecular (especially phospholipid) synthesis as opposed to oxidative phosphorylation.

In **aerobic glycolysis** (see Fig. S3-1; *orange arrows*), the final product of glycolysis, pyruvate, is transported

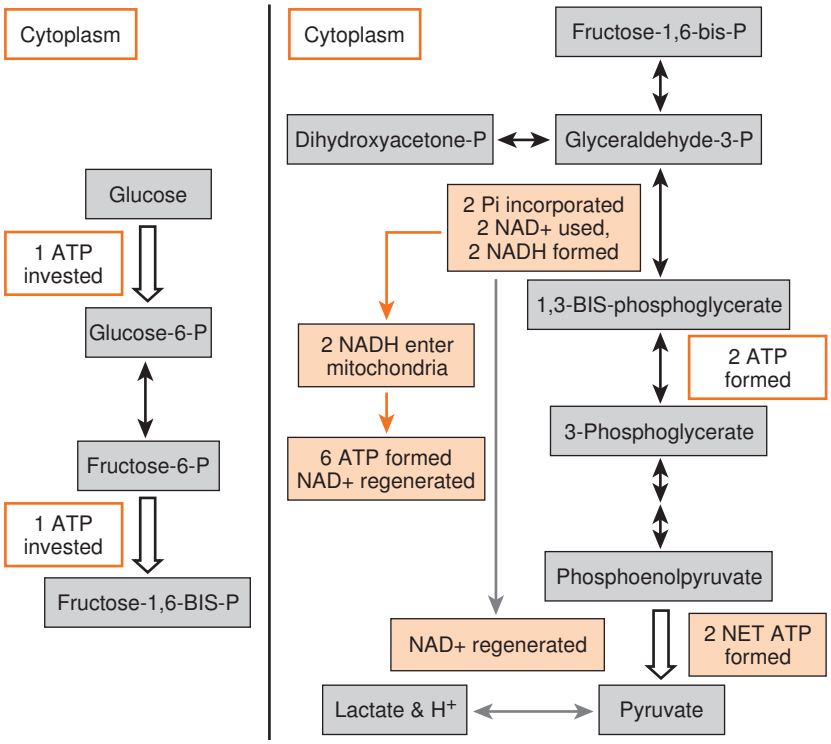

FIGURE S3-1 ■ The two phases of glycolysis: the left panel shows investment of ATP, and the right panel shows capture of ATP. *Open arrows* indicate hormonally regulated irreversible reactions. In the right panel, the *gray arrow* indicates anaerobic glycolysis, and the *orange arrow* indicates aerobic glycolysis.

into mitochondria and oxidatively decarboxylated to acetyl CoA. The 2 moles of NADH formed during glycolysis will also be transported into the inner mitochondrial matrix through shuttles and will be oxidized back to NAD^+ by **oxidative phosphorylation** (see later). This process will yield 3 more moles of ATP/NADH, or 6 moles of ATP per 2 NADH molecules generated from 1 molecule of glucose. Thus, aerobic glycolysis potentially yields 8 moles of ATP per mole of glucose.

The glycolytic pathway includes mostly reversible, bidirectional reactions. However, there are two irreversible reactions that occur during the energy investment phase, each at the point of ATP use, and one irreversible reaction during the energy capture phase at a point of ATP production (see Fig. S3-1; *white arrows*). For these reactions to be reversed, a second enzyme is required for each step. In the liver, these three irreversible reactions of glycolysis are key targets of allosteric and hormonal regulation. Therefore, we return to these reactions in more detail later.

ATP Synthesis by Oxidative Phosphorylation

In most cell types, the product of glycolysis, **pyruvate** (3 carbons), enters the mitochondria and is converted into the acetyl coenzyme A (acetyl CoA; 2 carbons) by oxidative decarboxylation, generating 1 mole of CO_2 per mole of acetyl CoA (Fig. S3-2). **Acetyl CoA** enters the **TCA cycle** by condensation with **oxaloacetate** (4 carbons) to form **citrate** (6 carbons). During the cycling of citrate back to oxaloacetate, the two carbons of acetyl CoA are lost as CO_2, and 3 moles of NADH and 1 mole of $FADH_2$ are generated through the reduction of NAD^+ and FAD, respectively. NADH and $FADH_2$ are oxidized by the process of **oxidative phosphorylation**, using the electron transport chain and ATP synthase complex, yielding more than 20 moles

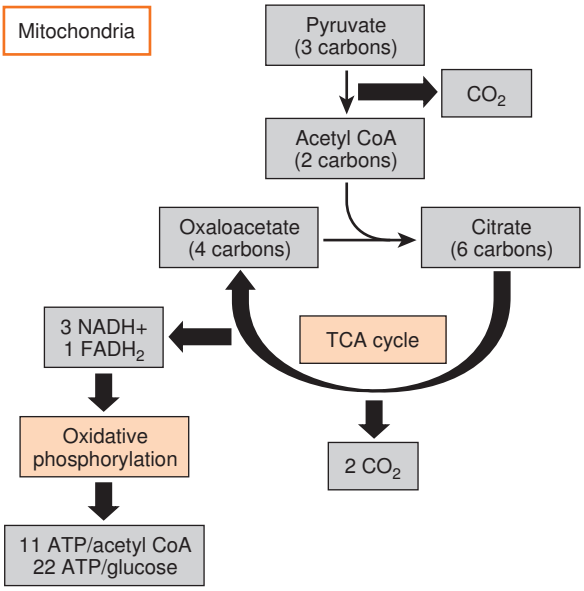

FIGURE S3-2 ■ Overview of the TCA cycle and oxidative phosphorylation.

of ATP per mole of glucose (see Fig. S3-2). Thus, the TCA cycle and oxidative phosphorylation, which are in close proximity to each other in the mitochondria, are very efficient methods of generating ATP from glucose. However, this requires molecular oxygen (O_2) and generates CO_2. This is why humans need to breathe air and exhale (i.e., perform respiration), and why oxidative phosphorylation can proceed only as fast as the respiratory and cardiovascular systems can deliver O_2 to, and remove CO_2 from, tissues. Therefore, even tissues with mitochondria rely on anaerobic glycolysis for some energy needs (which, by the way, still needs adequate vascular supply to deliver glucose and remove lactate and H^+). As discussed subsequently, the process of oxidative phosphorylation is also a major contributor to the generation of **reactive oxygen species (ROS)**, which impose oxidative stress that is harmful to cells.

An important feature of oxidative phosphorylation is the existence of some variability in the efficiency of coupling proton flow to ATP synthesis. For example, certain hormonal and energy states promote the expression of **uncoupling proteins (UCPs)** that allow protons to circumvent the ATP synthase as they flow from the outer to the inner mitochondrial matrix. This results in the release of energy as **heat**, instead of incorporation into the high-energy phosphate bonds in ATP, and induces an overall decrease in the efficiency of oxidative phosphorylation.

Glycolytic and TCA Cycle Intermediates Are Also Used for Biosynthetic Pathways Unrelated to ATP Synthesis

It should be emphasized that numerous pathways converge on the TCA cycle, either removing carbons from (**cataplerosis**) or introducing carbons into (**anaplerosis**) the cycle. For example, citrate can be shuttled to the cytoplasm and be used for de novo lipid biosynthesis (cataplerosis), whereas glutamine and glutamate can be converted to α-ketoglutarate that enters the TCA cycle (anaplerosis). Another example is the exit of malate for the production of glucose (gluconeogenesis; see later) from pyruvate in the liver. Accordingly, the TCA cycle is not a closed pathway, but one that is in dynamic equilibrium with other biosynthetic and metabolic requirements of the cell.

The same is true for glycolysis as glucose-6-phosphate is shuttled into several pathways other than glycolysis (e.g., pentose phosphate pathway), and other glycolytic intermediates can exit the glycolytic pathway (e.g., dihydroxyacetone phosphate for glycerol production; 3-phosphoglycerate for serine biosynthesis). Thus, the exit of carbons from ATP-producing pathways has to be balanced with the biosynthetic needs of cells, their nutrient status, and their AMP/ATP ratio. mTORC1 and AMPK contribute significantly to the ability of the cells to balance the myriad fates of carbons with the need for energy.

Making ATP from Glucose

As discussed earlier, glucose enters glycolysis and generates a net of 2 ATPs per glucose. If the cell contains mitochondria, the pyruvate generated by glycolysis can enter the TCA cycle and produce significantly more ATPs through oxidative phosphorylation. The accessibility of glucose for glycolytic metabolism depends on GLUT transporters and the hexokinases. As shown in Table S3-1, most GLUT isoforms in most cells are not regulated by hormones. However, skeletal muscle and adipose tissue rely heavily on **GLUT4** and

TABLE S3-1
Abridged List of Glucose Transporters

TRANSPORTERS	DISTRIBUTION	CHARACTERISTICS
GLUT1	Ubiquitous	High affinity; transports basal levels of glucose
GLUT2	Pancreatic β cell	Low affinity; important during fasting-to-fed transition
GLUT3	Ubiquitous	High affinity; primary GLUT in neuronal tissue
GLUT4	Skeletal muscle and adipose tissue	Dependent on insulin signaling for translocation to cell membrane from intracytoplasmic site
GLUT5	Small intestine and spermatozoa	Fructose transporter

GLUT12 transporters but only express these transporters at the cell membrane during the fed state in response to insulin. Insulin acts primarily by inducing the exocytosis and membrane fusion of intracellular vesicles (called **GLUT storage vesicles**) containing GLUT4 and GLUT12 transporters within their membranes.

Making ATP from FFAs

A clear advantage of the TCA cycle and mitochondrial respiration is the ability of cells to use energy substrates other than glucose that can form 2-carbon acetyl CoA molecules. The most densely caloric fuel is FFAs, of which long-chain fatty acids are most commonly stored as triglyceride. **Long-chain fatty acids (12 to 22 carbons**; referred to simply as FFAs herein) are released by lipolysis from triglyceride storage depots that reside primarily in adipose tissue (see later). FFAs circulate almost entirely bound to albumin (Fig. S3-3) and enter cells either **passively** (owing to their lipophilic nature) or through a process of facilitated diffusion involving **FFA transport proteins** (e.g., CD36, also called *fatty acid translocase,* or FAT). In the cytoplasm, FFAs are bound to fatty acid–binding proteins and become activated by conversion to **fatty acyl CoA (FACoA)**. For long-chain FACoAs to be oxidized for ATP production, they need to be transported to the inner mitochondrial matrix by **the carnitine-palmitoyl transferase-I (CPTI; outer mitochondrial membrane [OMM]) and CPTII (inner mitochondrial membrane [IMM]) transport system** (see Fig. S3-3). As discussed later, CPTI is tightly regulated by an intermediate **(malonyl CoA)** in fatty acid

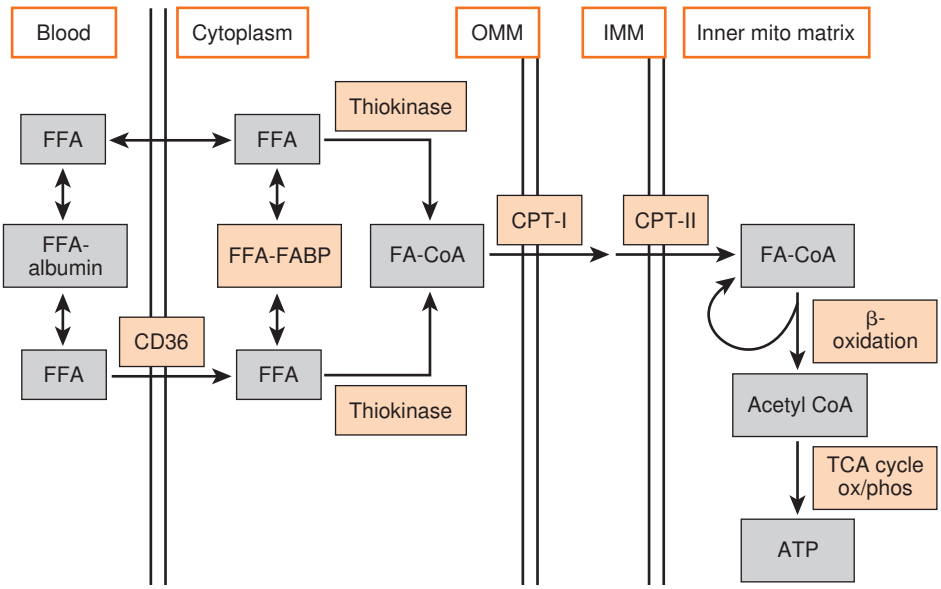

FIGURE S3-3 ■ ATP synthesis from FFAs.

synthesis. FACoA is catabolized by the repetitive, cyclical process of **β-oxidation**, during which 2 carbons are removed per cycle as acetyl CoA and enter the TCA cycle. More than 120 moles of ATP are generated from the oxidation of 1 mole of palmitoyl CoA (palmitate is a 16-carbon long-chain saturated fatty acid). One molecule of triglyceride with three palmitate acyl chains stores the potential of more than 350 molecules of ATP. This fact underscores the challenge for overweight or obese patients with insulin resistance or T2DM to improve insulin sensitivity and reduce cardiovascular risk by losing weight.

Making ATP from Amino Acids

The presence of mitochondrial respiration and the TCA cycle also allows cells to use AAs for energy. AAs enter the circulation during the digestion of a meal. AAs are also released from functional proteins (e.g., skeletal muscle α-actin) during fasting. There are multiple selective AA transporters in cell membranes. AAs can be oxidized for energy only after removal of their amino group. This occurs through **transamination** (transfer of their amino group to another molecule) or **oxidative deamination**. The use of amino acids for energy requires the conversion of toxic **ammonia (NH_3) to urea** by the urea cycle in the liver and excretion of urea by the kidneys. Most cells transfer the amino group of amino acids to **glutamic acid (Glu)**, thereby generating **glutamine (Gln)**, which is sent to the liver for detoxification through the urea cycle (Fig. S3-4). Skeletal muscle also uses pyruvate as a receptor of amino groups, thereby generating alanine. Alanine is converted back to pyruvate at the liver (see Fig. S3-4). The flow of carbons from glucose to pyruvate to alanine in muscle, followed by alanine to pyruvate to glucose in liver, is referred to as the **alanine-glucose cycle**. The carbon skeletons (ketoacids) of deaminated AAs converge on the TCA cycle by conversion to multiple intermediates. In the liver, some AAs can be used for the production of glucose during gluconeogenesis (see later), some AAs can be used for production of ketone bodies (KBs; see later), and some can be used for either gluconeogenesis or ketogenesis.

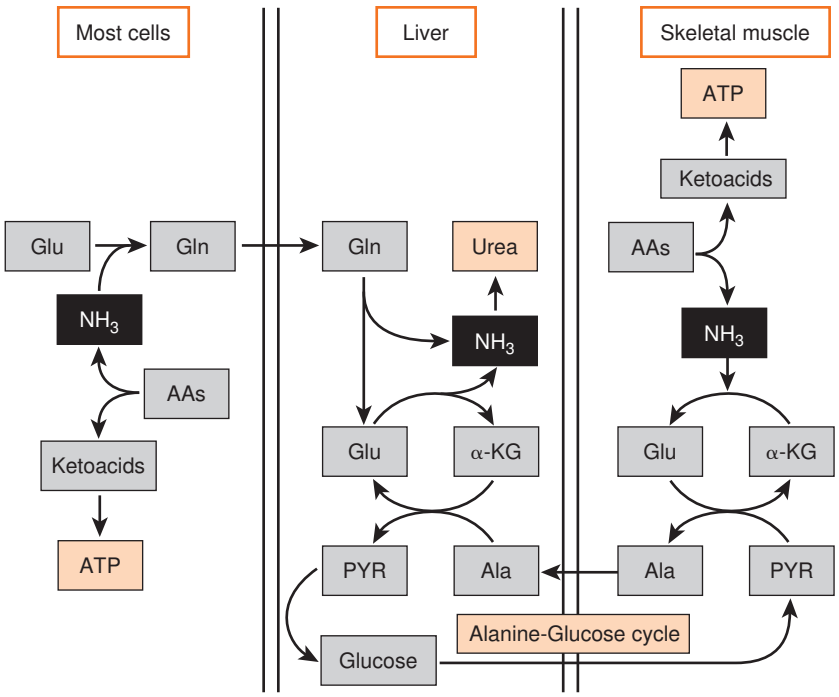

FIGURE S3-4 ■ Making ATP from amino acids.

Making ATP from Ketone Bodies

Acetoacetate and **3-hydroxybutyrate** represent the energetically useful **KBs** (acetone is a third KB, but does not contribute to ATP synthesis). KBs do not exist in significant levels in the diet. Rather, KBs represent a fourth class of fuels that are synthesized de novo from acetyl CoA (primarily from FFAs and ketogenic AAs) in the liver and exported into the bloodstream for other organs to use. Extrahepatic tissues convert KBs back to acetyl CoA using enzymes not expressed in hepatocytes (Fig. S3-5). KBs are sufficiently hydrophilic to not require albumin or lipoproteins for transport in the blood and can be readily used by all extrahepatic cells with mitochondria. Even the brain can use KBs at the high levels reached during an extended fast or starvation for up to 50% of its ATP production. However, KBs are acids, and the unchecked production of KBs in untreated T1DM leads to the potentially fatal condition of **ketoacidosis**.

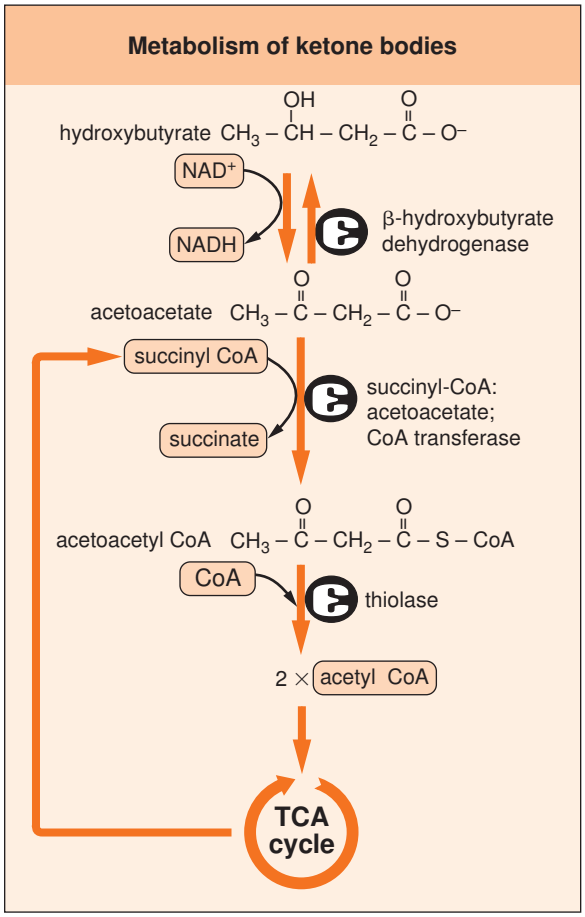

FIGURE S3-5 ■ Metabolism of ketone bodies. *(© Elsevier. From Baynes JW, Dominiczak MH:* Medical Biochemistry, *2nd ed., Philadelphia, 2005, Mosby. See http://www.studentconsult.com.)*

Storage Forms of Energy

In general, a significant fraction of nutrients are stored during the fed state. The enzymes involved in both synthesis of energy storage molecules during the fed state and their catabolism and release of energy substrates during the fasting state are key targets of hormonal regulation of energy metabolism.

Glycogen Glucose can be stored as glycogen, which is a large, branched polymer of glucose molecules (Fig. S3-6). For **glycogen synthesis**, glucose-6-phosphate must be converted to **glucose-1-phosphate** by the enzyme, **phosphoglucomutase**. Glucose-1-phosphate is then added to glycogen chains by two repetitive reactions. Two metabolically important depots of glycogen reside in the **liver** and in **skeletal muscle.** Glycogen particles are not enclosed by membranes but are associated with numerous proteins, including **glycogen synthase** and **branching enzyme** (i.e., enzymes that build glycogen polymers) and **glycogen phosphorylase** and **debranching enzyme** (i.e., enzymes that break down glycogen polymers and release glucose-1-phosphate) and interact with intracellular membranes.

During the fasting period, individual glucose-1-phosphate moieties can be cleaved from glycogen and metabolized back to glucose-6-phosphate. In the liver, glucose-6-phosphate is transported into the smooth endoplasmic reticulum (sER) and converted to **free glucose** by **glucose-6-phosphatase** (see Fig. S3-6). Glucose exits the sER through a poorly defined transporter and exits the hepatocyte through bidirectional **GLUT2 transporters** and probably other glucose transporters (e.g., GLUT1, GLUT9). Thus, liver glycogen can directly contribute to blood glucose levels. Skeletal muscle does not express glucose-6-phosphatase, so glycogenolysis is linked to intramyocellular glycolysis in response to increased muscle contraction. Lactate produced by anaerobic glycolysis

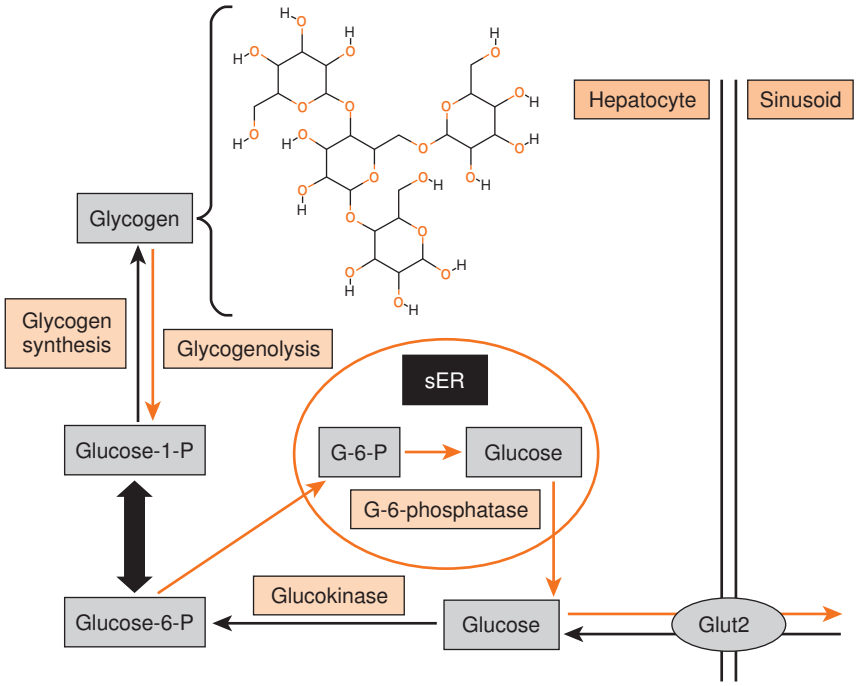

FIGURE S3-6 ■ Storage of glucose as glycogen *(black arrows)* and production of glucose from glycogen *(orange arrows)*.

in skeletal muscle can be converted to glucose in the liver by gluconeogenesis (see later). In this manner, muscle glycogen potentially contributes indirectly to blood glucose levels.

Triglyceride **Triglyceride (TG)** represents the storage form of **fatty acids** (Fig. S3-7). Each molecule of TG contains three long-chain fatty acids (saturated or unsaturated) esterified to each of the 3 carbons of glycerol (which must be in the form of glycerol-3-phosphate to initiate this sequence). **TG** can be synthesized from **FFAs** and **glycerol-3-phosphate** in multiple tissues, but only adipose tissue has evolved as a relatively safe storage depot for TG. Significant TG accumulation in nonadipose tissue and organs, such as skeletal muscle and liver (called *ectopic lipid*), can seriously compromise their physiologic functions and render them resistant to insulin.

During fasting and exercise, FFAs and glycerol are released from the adipocyte TG store through the action of three lipases. Adipocyte triglyceride lipase (ATGL) and **hormone-sensitive lipase (HSL)** digest TG to diacylglyceride (DAG) and 2-monoacylglyceride (MAG). MAGs are catabolized to a fatty acyl chain and glycerol by MAG lipase. As discussed later, catecholamines (epinephrine and norepinephrine) constitute the primary signal for intracellular lipolysis through their activation of HSL activity and the accessibility of HSL to the TG droplet–cytoplasm interface. Thus, in general, the hormonal milieu associated with the fed state favors a net flux of FFAs into TG, whereas the fasting and exercise states favor a net release of FFAs and glycerol from adipose tissue.

Dietary Triglyceride As discussed earlier, FFAs are a rich source of energy and can be extracted from the blood and metabolized through oxidation by many cell types. During the digestion of a mixed meal (fed state), the uptake and metabolism of glucose by liver, skeletal muscle, and adipose tissue serve to prevent prolonged elevated levels of circulating glucose. Excessively high levels of FFAs during this time would compete with glucose for metabolic pathways. The inability to metabolize glucose would lead to impaired glucose uptake **(glucose intolerance),** longer and higher excursions of glucose levels after a meal, and **reactive hyperinsulinemia**, which would stress pancreatic β cells (see later).

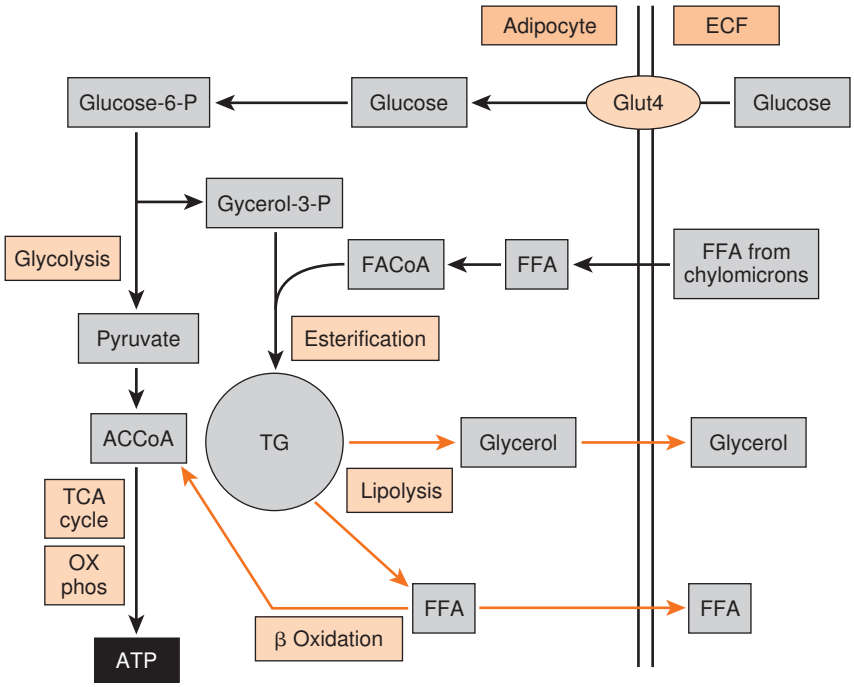

FIGURE S3-7 ■ Reesterification and storage of FFAs as TG in adipocytes *(black arrows)* and lipolysis of TG to release glycerol and FFAs *(orange arrows)*.

The competition between glucose and FFAs during the fed state is avoided by the fact that FFAs that are absorbed by the intestine do not enter the blood as FFAs. Instead, absorbed FFAs are reesterified with 2-monoglycerol to form TGs within the intestinal enterocytes. Because TGs are highly hydrophobic, they must be incorporated into lipoprotein particles called **chylomicrons** before entering the circulation (Fig. S3-8). Chylomicrons have a hydrophobic core of TG and cholesterol esters and an amphipathic shell of phospholipids, free cholesterol, and several associated proteins (called *apoproteins*). The primary apoprotein of nascent chylomicrons is called **ApoB48**. Chylomicrons first enter **abdominal lymphatic vessels**, which empty into the thoracic duct, which in turn empties into the peripheral circulation at the bifurcation of the left subclavian and left jugular veins. This means that chylomicrons, unlike glucose, bypass the hepatic portal vein and the liver during their first pass through the circulation. Within the circulation, chylomicrons receive additional apoproteins that are transferred from circulating **high-density lipoprotein (HDL) particles**. In particular, **ApoE** and **ApoCII** are acquired by chylomicrons in this way (see Fig. S3-8). Note that one of the main functions of HDL is to provide a circulating pool of apoproteins for other lipoprotein particles.

During the fed state, insulin induces the expression of **lipoprotein lipase (LPL)** specifically within adipocytes. LPL is secreted by the adipocytes, binds to heparan sulfate proteoglycans, undergoes transcellular transport across the capillary endothelial cells, and adheres to the external side of the apical (luminal) membrane of the endothelia in association with a scaffolding protein (called GPIHBP1). LPL binds to chylomicrons and digests their TG core. The apoprotein, **ApoCII**, is an absolutely required cofactor for LPL. FFAs that are released by this extracellular lipolysis are transported out of the capillary bed and into the adipocytes by several fatty acid–binding proteins and transporters (Box S3-1; see Fig. S3-8).

For FFAs to be reesterified into TG within adipocytes, there needs to be a supply of glycerol-3-phosphate (see Fig. S3-8). During the fed state, adipocytes also actively take up glucose through insulin-dependent GLUT4 transporters, metabolize it by glycolysis to dihydroxyacetone phosphate (DHAP), and then convert it to

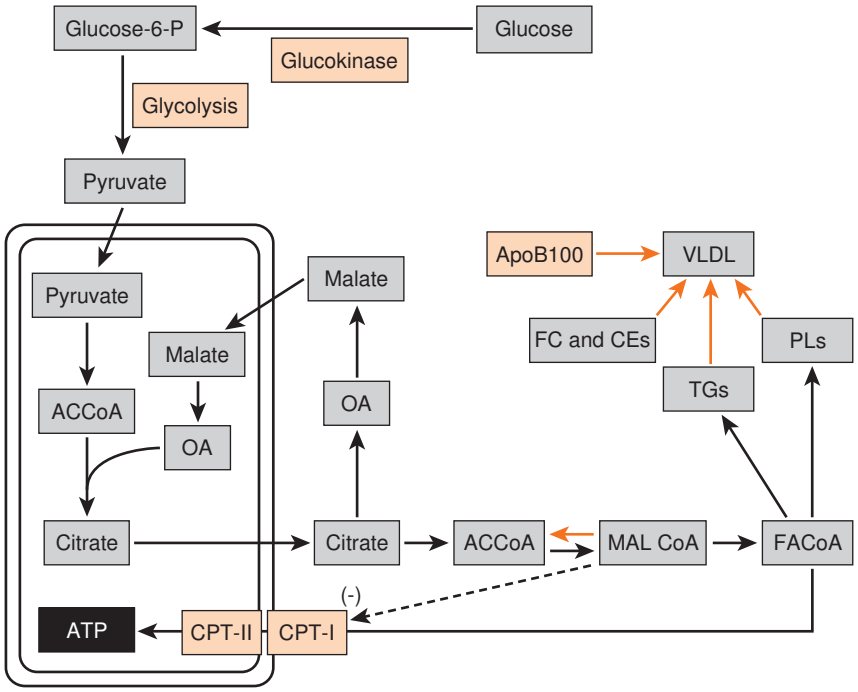

FIGURE S3-8 ■ Conversion of glucose into lipid in the liver. In the face of excess nutrients and high levels of ATP and NADH, the TCA slows down, and citrate accumulates and leaves the mitochondria. Cytosolic citrate is converted to fatty acyl CoA (FACoA) through the process of de novo lipogenesis. Malonyl CoA levels block the flow of FACoAs into the mitochondria through the carnitine-palmitoyl transferase-I transporter. This effectively blocks the use of newly synthesized FACoAs for oxidation and ATP production and for ketogenesis. During the fast state, lipids are assembled with ApoB100 into VLDL and exported *(orange arrows)*.

BOX S3-1
TYPE 1 HYPERLIPOPROTEINEMIA

Several human mutations in proteins that are involved in chylomicron catabolism lead to a condition called **type 1 hyperlipoproteinemia or chylomicronemia** (excessive levels of chylomicrons in the blood). These mutations affect several gene products involved in the ability of LPL to digest chylomicrons. These include mutations in **lipoprotein lipase, ApoCII, lipase maturation factor** (allows proper folding assembly of LPL in the endoplasmic reticulum), and **GPIHBP1** (tethers LPL on the apical side of capillaries). Chylomicronemia presents in childhood with **extreme abdominal pain** caused by **acute pancreatitis. Retinal blood vessels appear milky (lipemia retinalis). The treatment for chylomicronemia involves rigid dietary fat restriction**.

glycerol-3-phosphate by glycerol-3-phosphate dehydrogenase. This provides the needed substrate for reesterification of FFAs and their intracellular storage as TG. TG is stored as lipid droplets within the cytoplasm. Again, because of the extreme hydrophobicity of TGs, these intracytoplasmic droplets are coated and stabilized by several proteins, including the **perilipins**.

Endogenous Triglyceride What happens to the chylomicrons after they leave the capillary beds of the adipose tissue? Most chylomicrons are not completely divested of their TG content during their pass through adipose tissue, and these partially digested, TG-depleted chylomicrons are called *chylomicron remnants*. Chylomicron remnants represents the overflow fraction of dietary TG, and their abundance is determined, in part, by the amount of dietary TG ingested and the relative sensitivity (or resistance) of adipose tissue to insulin, which determines the levels of lipoprotein lipase.

Chylomicron remnants are atherogenic and need to be effectively cleared from the circulation. This is performed by the liver through the process of receptor-mediated endocytosis, using the **low-density lipoprotein (LDL) receptor** and the **LDL receptor–related protein-1 (LRP1)**. Chylomicron remnants bind to heparan sulfate proteoglycans (HSPG) in the liver extracellular space (called the *space of Disse*), which increase clearance by increasing the affinity of remnants to LRP1 and by binding to **HSPG receptors,** which also

internalize the remnants through receptor-mediated endocytosis. LDL receptor and LRP1 recognize the apoprotein, Apo E, which is transferred to nascent chylomicrons from HDL particles (see Fig. 3-8). Endocytosed chylomicron remnants are completely digested by lysosomal lipases and proteases, thereby releasing FFAs, glycerol, cholesterol, and phospholipids, along with amino acids from the apoproteins, into the hepatocyte cytoplasm. Depending on the fed or fast state and the relative abundance of nutrients to the liver, the released FFAs from chylomicron remnants can be used for the following:

1. ATP production through β-oxidation
2. KB synthesis
3. TG synthesis through reesterification with glycerol-3-phosphate to form TG (Fig. S3-9)

Unlike adipocytes, the liver expresses the enzyme, glycerol kinase, which directly converts glycerol to glycerol-3-phosphate. If nutrients and ATP are abundant, the TG, cholesterol esters, free cholesterol, and phospholipids will be reassembled into lipoprotein particles called *very-low-density lipoproteins* (VLDLs).

Another important source of intrahepatic fatty acyl CoAs and TG formation is **monosaccharides**. These include **glucose, galactose** (which is converted to glucose), and **fructose**. Monosaccharides are most abundant during the fed state, and insulin and other factors (see later) promote their conversion to fatty acyl CoA by **de novo lipid synthesis** (Fig. S3-10). This involves the generation of pyruvate through glycolysis, followed by the entry of pyruvate into the mitochondria and decarboxylation to acetyl CoA. Acetyl CoA enters the TCA cycle by condensing with oxaloacetate to form **citrate.** The abundance of carbons during the fed state leads to an inhibition of subsequent steps within the TCA cycle, allowing citrate to accumulate and exit the mitochondria through a transport system. **Cytoplasmic citrate** is metabolized to cytoplasmic **acetyl CoA, malonyl CoA,** and finally **long-chain fatty acyl CoA. Malonyl CoA also inhibits the CPT1 transporter**, thereby avoiding the **futile cycle** of fatty acyl CoA synthesis coupled to β-oxidation of fatty acyl CoA.

Other sources of intrahepatic TG include circulating FFA-albumin complexes and receptor-mediated endocytosis or lipolysis by hepatic lipase (HL) of intermediate-density lipoprotein (IDL) particles (see Fig. S3-9). These two sources are more likely to provide fatty acyl CoA chains during fasting or exercise, at a time when they are more likely to be used for ATP production and KB synthesis than for TG and VLDL synthesis.

The primary apoprotein associated with VLDL is **ApoB100** (see Figs. S3-9 and S3-10). In a manner similar to chylomicrons, nascent **VLDL** particles accept other apoproteins, including ApoE and ApoCII, from circulating HDL (see Fig. S3-9). The formation and secretion of VLDL by hepatocytes occurs primarily during the fasting and exercise states. During this metabolic state, LPL activity is low in adipose tissue. Conversely, LPL activity in the capillary beds of skeletal muscle is not dependent on insulin and is enhanced by physical activity probably through an AMPK-dependent mechanism. Thus, VLDL provides high-energy substrates (i.e., FFAs) to working skeletal muscle between meals. Excess VLDL can also eventually return TG to adipose tissue.

Low-Density Lipoprotein What happens to partially digested VLDL? Most VLDL particles lose a significant amount of TG, giving rise to **VLDL remnants**, also called *IDL particles* (see Fig. S3-9). Like chylomicron remnants, some IDL binds to LDLR or LRP1 and is endocytosed by the liver. A major difference between chylomicron remnants and IDL is that a significant fraction of IDL escapes endocytosis and is further processed by the ectoenzyme, hepatic lipase, into **LDL particles** (see Fig. S3-9). LDL particles have lost most of their TG and are rich in cholesterol, primarily cholesterol esters. Like chylomicrons, VLDL particles receive cholesterol esters from HDL particles by the action of **cholesterol ester transfer protein (CETP),** which is associated with HDL (see Fig. S3-9). This further increases the cholesterol in LDL (as the descendant of VLDL).

Even though cholesterol is never metabolized for ATP, **the fact that VLDL particles represent a means to remove excess FFAs** from the liver ties circulating LDL to energy balance. Essentially all cells synthesize cholesterol from cytoplasmic acetyl CoA. Most cholesterol is synthesized by hepatocytes, and the liver represents the largest pool of intracellular cholesterol in the body. LDL particles serve to deliver supplemental cholesterol to peripheral cells, especially proliferating cells, in which cholesterol is a required component of the cell membrane. LDL particles also provide

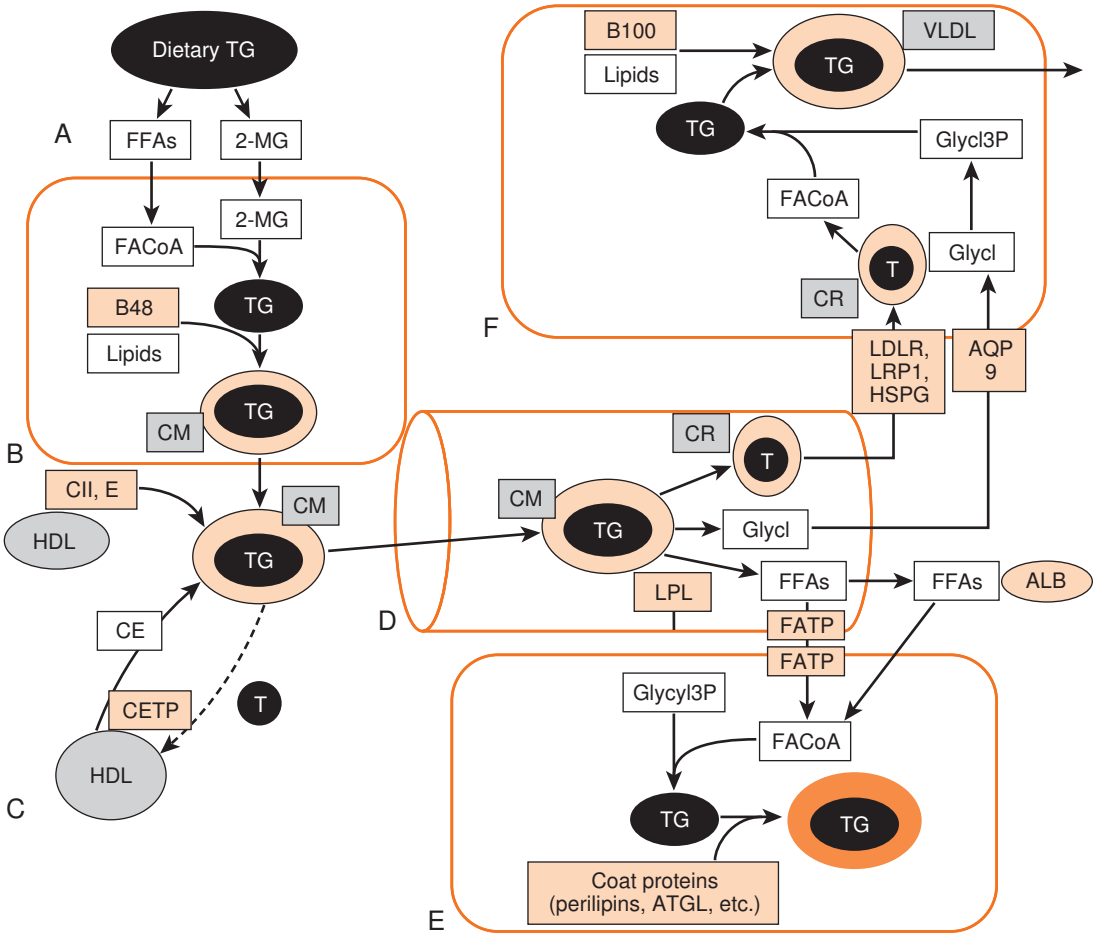

FIGURE S3-9 ■ Delivery of dietary TG to adipocytes through chylomicrons (CM) and the overflow of chylomicron remnants (CR) to the liver. **A,** Dietary TGs are enzymatically digested to FFAs and 2-monoglycerides (2-MG). **B,** Absorbed FFAs are "activated" by conversion to fatty acyl CoAs (FACoA) and reesterified with 2-MG to reform TG. TG, ApoB48 (B48), and other lipids (phospholipids, free cholesterol, cholesterol esters) are assembled into CMs and secreted at the basolateral side of the enterocytes. CMs enter lymphatics and gain access to the peripheral circulation. **C,** CMs obtain additional apoproteins, including ApoCII (CII) and ApoE (E) from HDL. CMs also receive cholesterol esters (CE) from HDL as mediated by the cholesterol ester transfer protein (CETP). In the presence of large, TG-rich CMs, some TGs (T) are exchanged for the CE, so that HDLs accept some TGs. **D,** The TGs within CM are digested by lipoprotein lipase (LPL) in the capillary beds of adipocytes. FFAs may diffuse or are transported by fatty acid transport proteins (FATP) across both endothelial cell membranes and the adipocyte cell membrane and enter the adipocyte. Glycerol is taken up by several cell types, including hepatocytes, through the glycerol transporter, aquaporin-9 (AQP9). Note that hepatocytes express glycerol kinase, which converted glycerol to glycerol-3-phosphate. **E,** FFAs are converted to fatty acid CoAs within the adipocyte and esterified with glycerol-3-phosphate to form TG. Because of their extreme hydrophobicity, TG droplets within the adipocyte cytoplasm are associated with amphipathic coat proteins, including perilipins and adipose TG lipase (ATGL). **F,** Partially digested CMs are called *chylomicron remnants* (CRs). They are effectively removed from the circulation by the liver by receptor-mediated endocytosis involving the LDL receptor (LDLR), LDLR-related protein-1 (LRP1), and heparan sulfate proteoglycan (HSPG) receptors. CRs are completely digested within lysosomes, and the released FFAs are converted to FACoAs and are reesterified with glycerol-3-phosphate to form TGs. TGs, ApoB100 (B100), and other lipids will be assembled into VLDLs and exported.

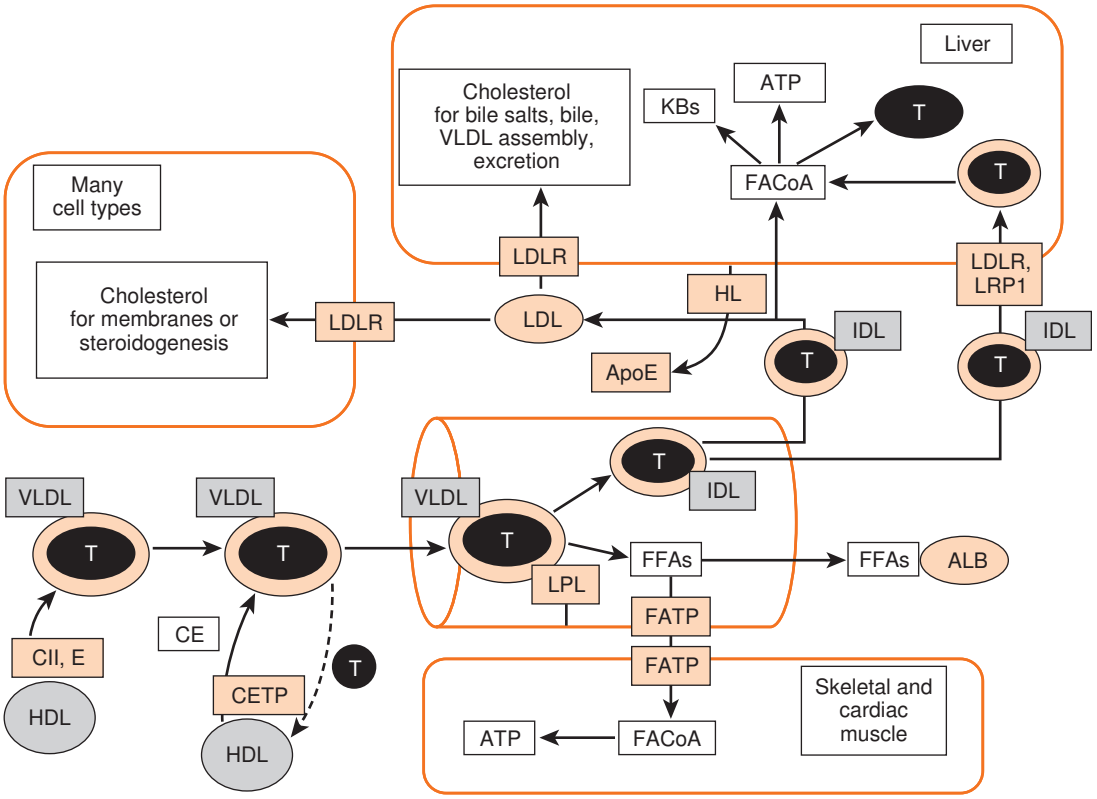

FIGURE S3-10 ■ Fates of VLDL. VLDL receives several apoproteins from circulating HDL, including ApoCII (CII) and ApoE (E). Cholesterol ester transfer protein (CETP) transfers cholesterol esters (CE) from HDL to VLDL. Normally, very little TG (T) is exchanged for the CE, but this increases in the face of large, very TG-laden VLDL. VLDL TG is used by skeletal and cardiac muscle, which produces lipoprotein lipase (LPL) in an insulin-independent manner. Partial digestion of VLDL generates intermediate-density lipoproteins (IDLs; essentially VLDL remnants). About 50% of IDLs are cleared from the blood by the liver by receptor-mediated endocytosis. The remaining IDLs undergo further digestion of TG by hepatic lipase (HL). This generates cholesterol-rich, TG-poor low-density lipoprotein (LDL) particles. LDL particles are endocytosed by the LDL receptor (LDLR) and provide cholesterol for dividing cells and for steroidogenic cells. LDL is cleared through LDLR-mediated endocytosis at the liver. Note that LDL particles lose ApoE and retain only ApoB100, which is recognized by the LDLR only.

supplemental cholesterol to cells of the adrenal cortex (see Chapter 7), testis (see Chapter 9), ovary (see Chapter 10), and placenta (see Chapter 11) for the synthesis of steroid hormones. Cholesterol is also used in keratinocytes for the production of vitamin D_3 (see Chapter 4). Cholesterol is delivered to the liver by LDL, where it is used for the synthesis of **bile acids** or secreted as free cholesterol within bile, and to the liver and intestine for the assembly of VLDL and chylomicrons, respectively. A small amount of cholesterol (about 500 mg/day) is excreted from the liver through the gastrointestinal tract, and the conjugated metabolites of steroid hormones and vitamin D–related molecules are primarily excreted by the kidney (about 25 mg/day).

Cholesterol imposes **negative feedback** on **cholesterol synthesis and uptake**, thereby helping to keep intracellular cholesterol within a normal range (Fig. S3-11). This negative feedback involves the binding of sterols to a protein complex that ultimately

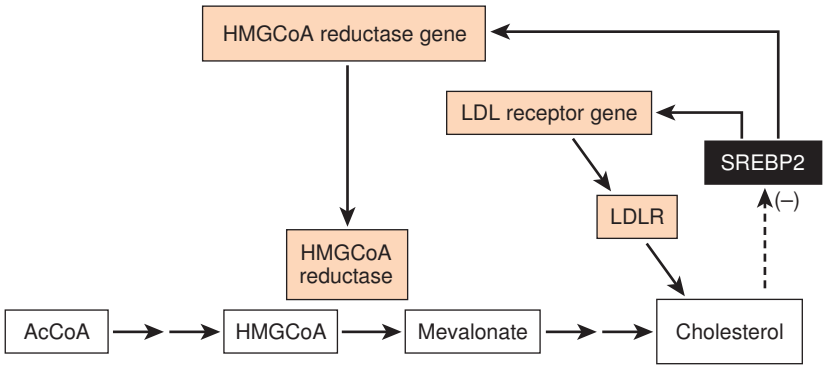

FIGURE S3-11 ■ Regulation of cellular cholesterol levels by negative feedback on SREBP2.

inhibits the nuclear localization of a transcription factor, **sterol-regulatory element–binding protein-2 (SREBP2)**. SREBP2 promotes the transcription of cholesterol synthetic enzymes, especially the rate-limiting enzyme, **HMG CoA reductase**. SREBP2 also promotes the transcription of the gene encoding the **LDL receptor**.

LDL particles lose ApoE, retaining only ApoB100 for receptor binding (see Fig. S3-10). This means that LDL particles can only undergo receptor-mediated endocytosis through binding to the LDL receptor; they are not recognized by LRP1. Thus, mutations in the LDL receptor (or associated proteins involved in endocytosis of this receptor) cause **severe hypercholesterolemia** owing to the failure of tissues (primarily the liver) to remove LDL from the blood. The ensuing severe hypercholesterolemia leads to cardiovascular disease and death.

High-Density Lipoprotein The last category of lipoproteins that needs to be discussed briefly is **HDL** (Fig. S3-12). HDL particles begin as **ApoA1**, secreted by the intestine and liver, which quickly acquires **lecithin (phosphatidylcholine)**. This discoidal form of HDL accepts excess cholesterol from peripheral cells, especially macrophages that have engulfed dead cells or oxidized lipoproteins. The efflux of cholesterol is carried out by the **ABCA1** and **ABCG1 transporters**. As cholesterol loads onto HDL, it is immediately esterified by the HDL-associated enzyme, **lecithin-cholesterol acyltransferase (LCAT),** which is produced by the liver, binds to nascent HDL, and is activated by ApoA1. The hydrophobic cholesterol esters cluster to the center of the growing HDL particle. As mentioned earlier, cholesterol esters can be transferred to the TG-rich lipoproteins, chylomicrons, and VLDL (see Figs. S3-8 and S3-10), a process mediated by the protein CETP. In the presence of large, highly TG-rich VLDL, transfer of cholesterol esters involves the reciprocal transfer of TG from VLDL to HDL. HDL can deliver supplemental cholesterol to peripheral cells (e.g., steroidogenic cells), but most HDLs return cholesterol to the liver (see Fig. S3-12). HDL unloads cholesterol esters on binding to the **HDL receptor**, called *scavenger receptor class B1* **(SR-B1)**. Lipid-rich HDL particles (called *HDL2*) can also be digested by hepatic lipase, regenerating small HDL particles called *HDL3*. This entire process is referred to as *reverse cholesterol transport*, which is especially important in removing cholesterol from macrophages within the intima of blood vessels.

In summary, FFAs are a rich source of energy and are stored as TG, primarily within adipocytes. TG from the diet is carried as TG to the adipocytes by chylomicrons. Overflow of this pathway, as represented by chylomicron remnants, transfers residual TG, as well as some cholesterol esters, free cholesterol, and phospholipids, to the liver. The liver cannot safely store significant amounts of TG and assembles TG into VLDL particles. VLDL particles are secreted by the liver primarily during fasting and exercise and supply skeletal and cardiac muscle (and some other tissues) with FFAs or return the TGs to adipose tissue. The metabolism of VLDL generates the cholesterol-rich LDL. HDL particles are generated by the liver and small intestine, and accept

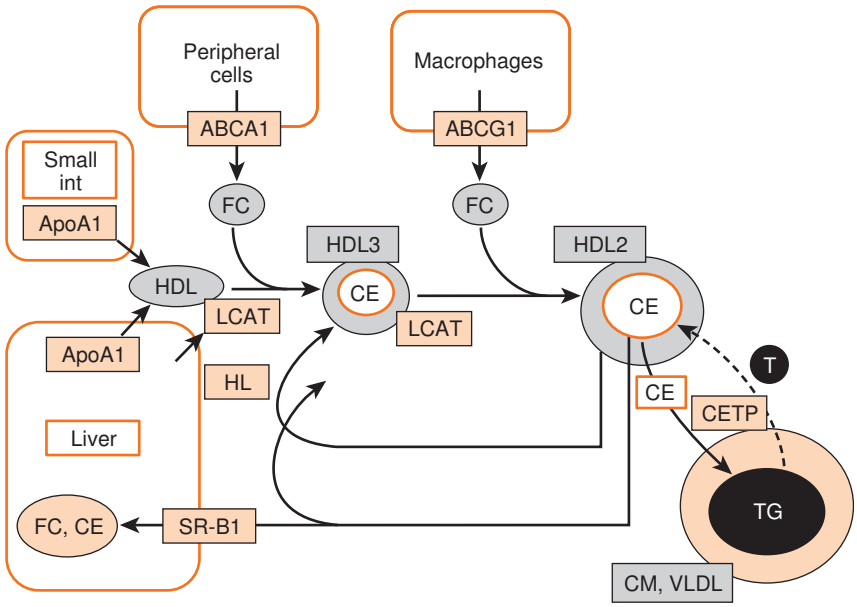

FIGURE S3-12 ■ HDL formation and reverse cholesterol transport. ApoA1 is secreted by the liver and small intestine and rapidly acquires lecithin (phosphatidylcholine). HDLs receive excess cholesterol from peripheral cells, especially macrophages, and rapidly convert cholesterol to cholesterol esters (CE) through the HDL-associated protein, lecithin-cholesterol acyltransferase (LCAT). Some CE will be transferred to TG-rich lipoprotein particles through CETP. HDL particles grow from HDL3 to HDL2 as more CE is loaded. HDL2 unloads free cholesterol (FC) and CEs at the liver through interaction with scavenger receptor B1 (SR-B1). HDL particles can also be digested by hepatic lipase (HL).

free cholesterol from peripheral cells, esterify it, and transfer free cholesterol and cholesterol esters back to the liver for excretion.

Protein Unlike glucose stored as glycogen or TG stored in depot fat, proteins perform many dynamic functions other than storage of energy. Nevertheless, proteins are hydrolyzed when needed to produce amino acids. Proteins are catabolized primarily through the **proteosome**. Protein breakdown occurs during autophagy, a process by which cells catabolize their intracellular macromolecules and organelles to provide a carbon source for energy. Not surprisingly, mTORC1 inhibits and AMPK promotes protein breakdown and autophagy.

Gluconeogenesis: Making Glucose from Glycerol, Lactate, and Amino Acids

The breakdown of glycogen is a transient way by which the liver can contribute directly to blood glucose levels during a short fast (i.e., 8 to 12 hours). The liver, and to a lesser extent the kidney, can also produce glucose for a much longer period by converting circulating energy substrates such as lactate, glycerol, and some amino acids into glucose through **gluconeogenesis**. This is accomplished by the carboxylation of pyruvate to oxaloacetate by **pyruvate carboxylase** as opposed to the oxidative decarboxylation of pyruvate by the pyruvate dehydrogenase complex (Fig. S3-13). **Oxaloacetate** exits the mitochondria as malate, which is then reoxidized to oxaloacetate. Oxaloacetate is then converted to **phosphoenolpyruvate (PEP)** by the enzyme, **PEP carboxykinase (PEPCK)**. As discussed later, PEP is essentially processed through a reverse glycolysis back to glucose-6-phosphate. As discussed earlier, glucose-6-phosphate is transported into the smooth endoplasmic reticulum and is dephosphorylated by glucose-6-phosphatase. Glucose exits the mitochondria and is released into the blood through the bidirectional GLUT2 transporter.

Importantly, **acetyl CoA cannot be used to make net glucose**. This means that FFAs, KBs, and certain amino acids cannot directly contribute to blood glucose

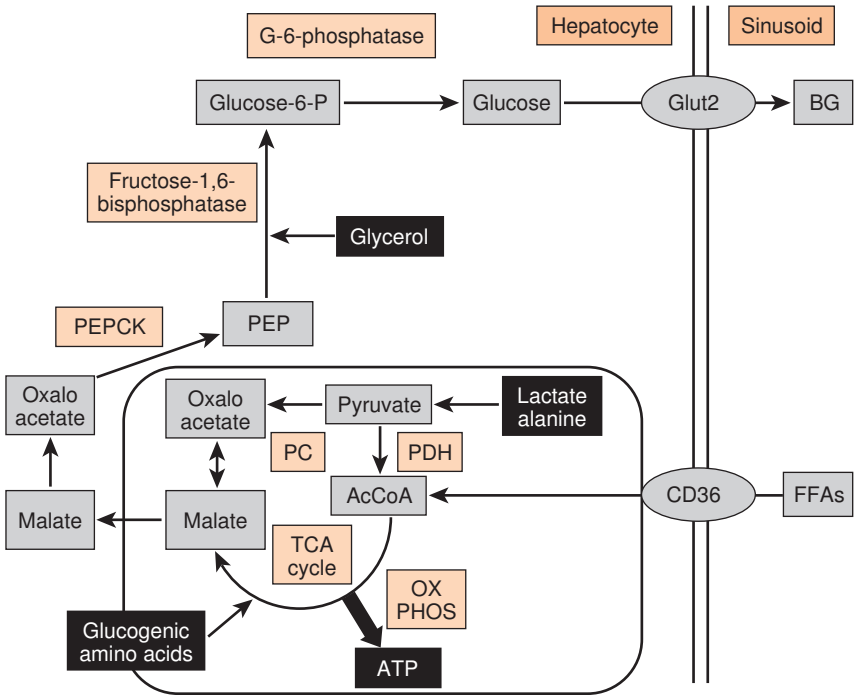

FIGURE S3-13 ■ Gluconeogenesis in the liver. FFAs become the source of ATP during the fast state. The large amounts of acetyl CoA (AcCoA) produced inhibit pyruvate dehydrogenase (PDH) and activate pyruvate carboxylase (PC), driving carbons toward oxaloacetate that enters the cytoplasm through the malate shuttle. Oxaloacetate is then converted to glucose through the actions of gluconeogenic enzymes, pyruvate carboxykinase (PEPCK), fructose-1,6-bisphosphatase, and glucose-6-phosphatase (G-6-phosphatase). Carbons for gluconeogenesis come from lactate, alanine, and other glucogenic amino acids and glycerol *(black boxes)*. Note that acetyl CoA by itself cannot yield a net increase in glucose.

levels. However, the use of FFAs by tissues spares blood glucose (i.e., it has a **glucose-sparing effect**) for use by the brain and cells without mitochondria. Additionally, KBs eventually reach levels sufficient to be used by the brain, thereby reducing glucose use by the brain.

Summary of Key Metabolic Pathways

ATP is the primary source of energy in all cells. The body can make ATP from carbohydrates (glucose, galactose, and fructose), FFAs, amino acids, and KBs. However, the brain is exclusively dependent on glucose, except after days of fasting, when it can use KBs. Humans can store excess calories as glycogen, TGs, and protein during a meal and release stored energy substrates as needed during a fast or for physical activity. The liver releases glucose into the blood, which is called *hepatic glucose production*. Hepatic glucose production involves glycogenolysis in the short term and gluconeogenesis in the long term, using lactate, glycerol, and some amino acids for the de novo synthesis of glucose. Additionally, in times of an extended fast, the liver can convert energy substrates to KBs for use by other organs (especially the brain). The enzymatic pathways that coordinate the partitioning of energy stores during a meal, and their use between meals, are regulated by both nutrient status and by key hormones. Before discussing how hormones regulate these pathways, we first need to learn about the hormones themselves; return to Chapter 3.

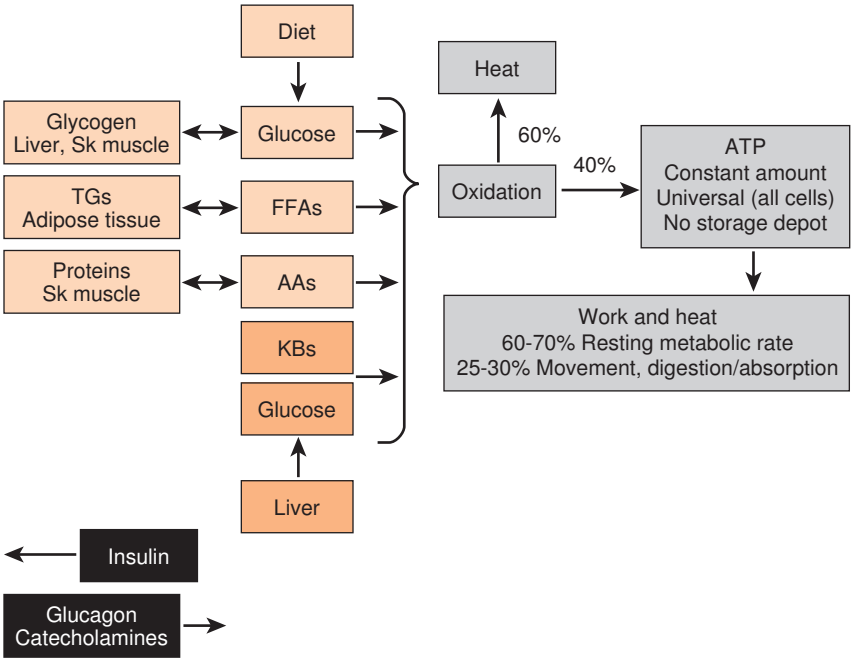

FIGURE 3-1 ■ Energy sources that can be used for ATP synthesis. FFAs, free fatty acids; AAs, amino acids; KBs, ketone bodies. Note that KBs do not exist in the diet, but are made by the liver. Insulin drives storage, whereas glucagon and catecholamines drive use for ATP.

During a time of **caloric deficit** (i.e., interdigestive period between meals, sleeping, and fasting, referred to as the **fasting state**; or during physical work or exercise, referred to as **exercise**), the stored depots of fuel are mobilized and burned. Also, during the fasting state, the liver synthesizes two fuels (**glucose and ketone bodies**) from various carbon sources and exports these fuels for use by extrahepatic tissues (see Fig. 3-1). Glucagon and catecholamines represent the primary hormones that induce the mobilization of energy stores and new synthesis of glucose and ketone bodies during the fasting state, thereby preventing blood glucose from declining to dangerously low levels (see later). **Glucagon and catecholamines** also promote proteolysis and the release of amino acids.

Several other hormones, including **cortisol** and **growth hormone**, also play important roles in the mobilization of energy stores, the control of circulating glucose and lipids, and the balance between protein synthesis and degradation; these are discussed in Chapters 5 and 7.

Importantly, all cells must balance energy needs with other uses of carbon, especially the synthesis of macromolecules (lipids, nucleic acids, proteins) and the assembly of organelles. There are several **master regulatory nutrient and energy sensors** that monitor intracellular nutrient levels and the relative amount of ATP to balance anabolic pathways with catabolic ones. Two of these regulatory factors are **mammalian target of rapamycin complex-1 (mTORC1)** and **adenosine monophosphate (AMP)-activated kinase (AMPK**; also a multimeric complex). In the presence of insulin and high levels of intracellular nutrients (amino acids, glucose) that signal an abundance of carbons, mTORC1 promotes energy-consuming anabolic pathways, such as ribosomal RNA production and ribosome assembly, protein synthesis, and lipogenesis (Fig. 3-2). Also, activation of mTORC1 inhibits the consumption of intracellular macromolecules and organelles for energy and survival, a process termed **autophagy**. In contrast, a drain on energy supply associated with an elevation of AMP levels, or an indication of high energy use such as elevated intramuscular Ca^{2+} levels and or reduced O_2, activates AMPK (see Fig. 3-2). Hormones associated with a fasting state (ghrelin, adiponectin; discussed later) also activate AMPK. Active AMPK inhibits anabolic pathways (in part by inhibiting mTORC1 activity)

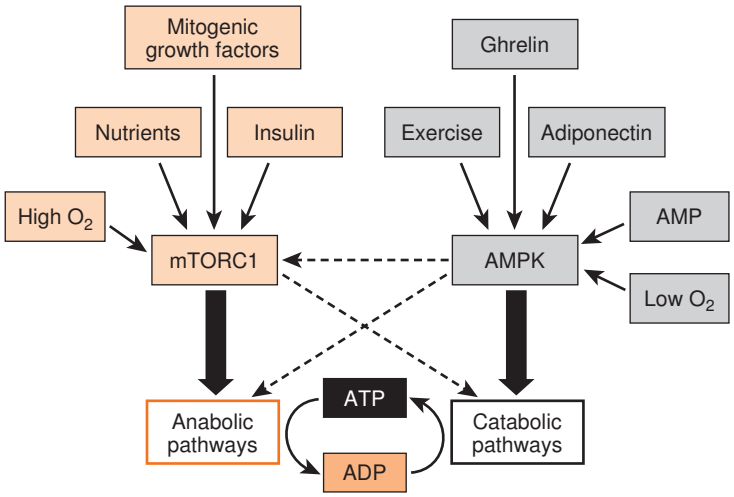

FIGURE 3-2 ■ mTORC1 and AMPK act as nutrient and energy sensors and regulate metabolism in conjunction with hormones.

and promotes energy-generating catabolic pathways, including glycolysis and β-oxidation of FFAs (see later). Although other important energy-sensing factors exist in cells, mTORC1 and AMPK have emerged as two centrally important factors involved in cellular energy balance, and the interaction of these two complexes with hormonal signaling is discussed later.

In thinking about the regulation of intracellular energy metabolism in different metabolic states (fed vs. fast), one should always keep in mind that the **brain** normally relies exclusively on glucose for energy. As an **obligate glucose user**, the function of the brain is critically dependent on circulating levels of blood glucose, much as it is dependent on a continuous supply of oxygen. A fall in blood glucose levels below 60 mg/100 mL (i.e., **acute hypoglycemia**) first leads to a response by the **autonomic nervous system** (sweating, nausea, heart racing). Further decline in blood glucose causes **neuroglycopenia,** which is associated with **impaired central nervous system functions**, including the loss of vision, cognition, and muscle coordination, as well as lethargy and weakness. **Severe hypoglycemia** can ultimately lead to coma, convulsions, and death. Thus, a major role of the fasting-related hormones (glucagon, catecholamines) is to maintain blood glucose levels above 60 mg/100 mL.

Conversely, it is important that fasting blood glucose levels remain below 100 mg/100 mL. Chronic elevation of glucose, due to **glucose intolerance** or, worse, **diabetes mellitus (DM)**, imposes a broad range of stresses on cells that ultimately are manifested by the compromised function or failure of specific organs. As discussed later, DM also causes serious metabolic and osmotic derangements as well as cardiovascular complications. Thus, the fed-related hormone, insulin, maintains blood glucose levels below the upper normal limit (i.e., below 100 mg/dL for an overnight fasting glucose).

Circulating levels of lipids, notably FFAs, triglycerides (TGs), and cholesterol, are closely linked to glucose metabolism and need to be kept below specific thresholds. As a fed-related hormone, insulin also plays an important role in the maintenance of blood lipids below these thresholds. Abnormally high blood lipid levels can lead to the accumulation of TG and other lipids in nonadipose tissue organs (called **ectopic lipid**; especially in liver and skeletal muscle). This **ectopic TG and lipid** compromise the ability of insulin to regulate glucose and lipid levels (termed *insulin resistance*) and increases the risk for type 2 diabetes (T2DM) and cardiovascular disease.

GENERAL PATHWAYS INVOLVED IN ENERGY METABOLISM

A discussion of the metabolic pathways involved in energy metabolism is beyond the scope of this book. An overview of the pathways involved in ATP production, fuel storage and use, the production of new fuels

(glucose and ketone bodies) by the liver, and the production and metabolism of lipoprotein particles is provided in the Supplement to Chapter 3 on the Student Consult site. For a more in-depth discussion of these pathways, the student is encouraged to consult a biochemistry text (e.g., Baynes JW, Dominiczak MH: *Medical Biochemistry,* 2nd ed., Philadelphia, 2005, Mosby.)

KEY HORMONES INVOLVED IN METABOLIC HOMEOSTASIS

Endocrine Pancreas

The **islets of Langerhans** constitute the endocrine portion of the pancreas (Fig. 3-3). About 1 million islets, making up about 1% to 2% of total pancreatic mass, are spread throughout the pancreas. The islets are composed of several cell types, each producing a different hormone. In islets situated in the body, tail, and anterior portion of the head of the pancreas (all of which have a common embryologic origin), the most abundant cell type is the **β cell**. The β cells make up about three fourths of these cells of the islets and produce the hormone **insulin**. The **α cells** make up about 10% of these islets and secrete the hormone **glucagon**. The third major cell type of the islets within these regions is the δ (D) cells, which make up about 5% of the cells and produce the peptide somatostatin (gastric somatostatin was discussed in Chapter 2, as an inhibitor of gastrin secretion). A fourth cell type, the F cell, represents about 80% of the cells in the islets situated within the posterior portion of the head of the pancreas (including the uncinate process) and secretes the peptide pancreatic polypeptide. Because the physiologic function of pancreatic polypeptide in humans remains obscure, it is not further discussed here.

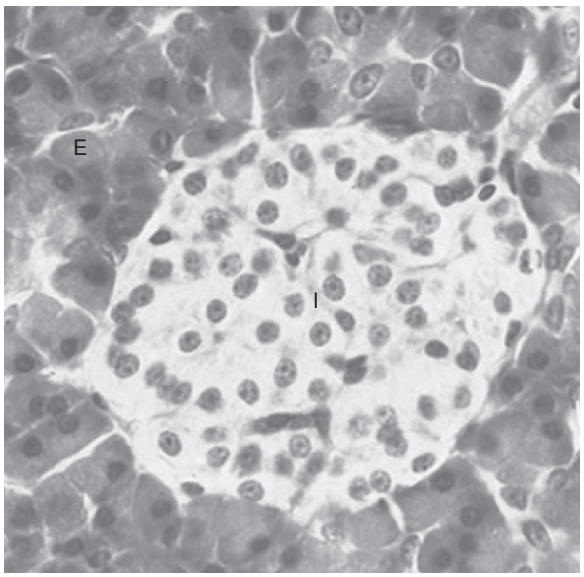

FIGURE 3-3 ■ Islet of Langerhans (I) surrounded by exocrine pancreatic tissue (E), but separated by a connective tissue capsule (C).

Blood flow through the islets is somewhat autonomous from the blood flow to the surrounding exocrine tissue. Insulin secreted from the inner β cells reaches the outer α cells. Consequently, the first cells affected by circulating insulin are the α cells, in which insulin inhibits glucagon secretion.

Insulin

Insulin is the primary anabolic hormone that is responsible for maintaining the upper limit of blood glucose and FFA levels. Insulin achieves this by the following mechanisms:

1. Promoting glucose uptake and use by skeletal muscle and adipose tissue
2. Increasing glycogen storage in liver and skeletal muscle
3. Suppressing glucose output by the liver
4. Promoting TG synthesis and storage in the liver and adipose tissue
5. Promoting the clearance of chylomicrons from the blood
6. Suppressing lipolysis of adipose TG stores

Insulin also promotes protein synthesis from amino acids and inhibits protein degradation in peripheral tissues. Finally, insulin regulates metabolic homeostasis through effects on satiety.

Insulin Structure, Synthesis, and Secretion **Insulin** is a protein hormone that belongs to a gene family that also includes insulin-like growth factors I and II (IGF-I, IGF-II), relaxin, and several insulin-like peptides. Organized, functional islets appear in the human pancreas at the beginning of the third trimester of gestation. Insulin gene expression and islet cell biogenesis

BOX 3-1

OVERVIEW OF INSULIN ACTIONS

Insulin is an anabolic hormone secreted in times of excess nutrient availability. It allows the body to use carbohydrates as an energy source and to store nutrients.

are dependent on several transcription factors (e.g., hepatocyte nuclear factor-4 [HNF-4α], HNF-1α, or HNF-1β; pancreatic and intestinal homeobox-1 [PDX1]; neuroD1) (Box 3-1).

The insulin gene encodes **preproinsulin**. Preproinsulin is converted to **proinsulin** as microsomal enzymes as the peptide enters the endoplasmic reticulum. Proinsulin is packaged in the Golgi apparatus into membrane-bound secretory granules. **Proinsulin** contains the amino acid sequence of **insulin** plus the 31-amino acid C (connecting) peptide and four linking amino acids. The proteases that cleave proinsulin, prohormone (or proprotein) convertase-2 and -3, are packaged with proinsulin within the secretory vesicle. The mature hormone consists of two chains, **an α chain and a β chain**, connected by two disulfide bridges (Fig. 3-4). Insulin is stored in secretory vesicles in zinc-bound crystals. Because the entire contents of the granule are released, equimolar amounts of insulin and C peptide are secreted, as are small amounts of proinsulin. C peptide has no known biologic activity, and proinsulin has about 7% to 8% of the biologic activity of insulin. Measurements of C peptide in the blood are used to quantify endogenous insulin production in patients receiving exogenous insulin, which has been purified from C peptide.

Insulin has about a 5-minute half-life and is cleared rapidly from the circulation by receptor-mediated endocytosis. It is degraded by lysosomal insulinase in the liver, kidney, and other tissues. Because insulin is secreted into the hepatic portal vein, almost one half of the insulin is degraded before leaving the liver. Recombinant human insulin and insulin analogs are now available, with different characteristics of onset and duration of action and peak activity.

Serum insulin levels normally begin to rise within 10 minutes after food ingestion and reach a peak in 30 to 45 minutes. The higher serum insulin level rapidly lowers blood glucose to baseline values. When insulin secretion is stimulated, insulin is released rapidly (within minutes), and this is called the early phase of insulin secretion. If the stimulus is maintained, insulin secretion falls within 10 minutes and then slowly rises over a period of about 1 hour (Fig. 3-5). The second phase is referred to as the *late phase of insulin release.* The early phase of insulin release probably involves release of preformed insulin, whereas the late phase represents the release of newly formed insulin.

Glucose is the primary stimulus of insulin secretion. Glucose entry into β cells is facilitated by the **GLUT-2 transporter** (see the Supplement to Chapter 3 on the

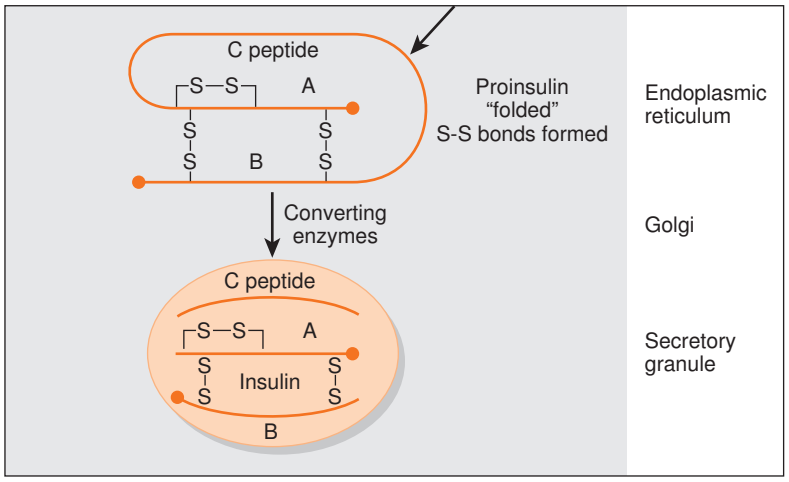

FIGURE 3-4 ■ Structure of insulin. *(Modified from Koeppen BM, Stanton BA, editors:* Berne & Levy Physiology, *6th ed., Philadelphia, 2010, Mosby.)*

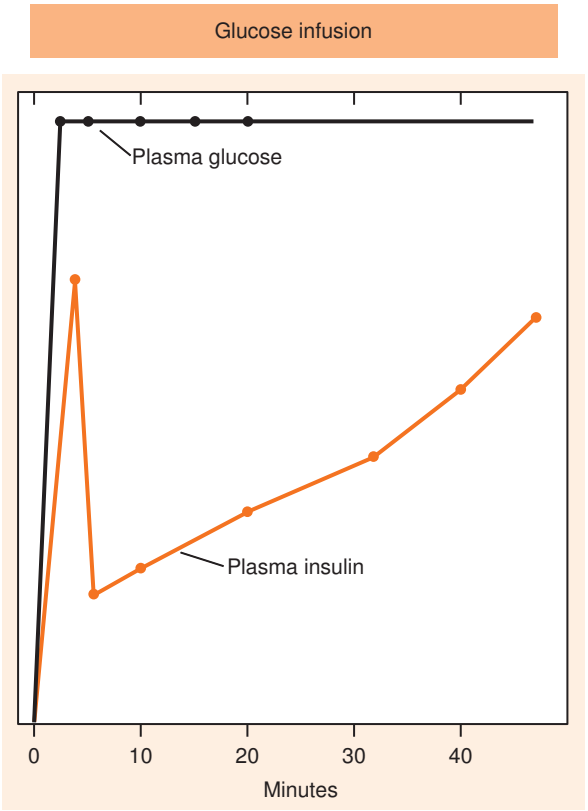

FIGURE 3-5 ■ Biphasic response of insulin secretion to glucose infusion. *(Modified from Koeppen BM, Stanton BA, editors:* Berne & Levy Physiology, *6th ed., Philadelphia, 2010, Mosby.)*

Student Consult site for a list of GLUT isoforms). Once glucose enters the β cell, it is phosphorylated to **glucose-6-phosphate** by the low-affinity hexokinase, **glucokinase**. Glucokinase is referred to as the glucose sensor of the β cell because the rate of glucose entry is correlated to the rate of glucose phosphorylation, which, in turn, is directly related to insulin secretion. Heterozygous mutations in glucokinase are one defect that leads to inadequate insulin release in patients with maturity-onset diabetes of youth (MODY). Glucose-6-phosphate is metabolized by β cells, increasing the intracellular ATP/ADP ratio and closing an **ATP-sensitive K^+ channel** (Fig. 3-6). This results in depolarization of the β-cell membrane, which opens **voltage-gated Ca^{2+} channels.** Increased intracellular Ca^{2+} levels activate **exocytosis** of secretory vesicles.

CLINICAL BOX 3-1

Heterozygous mutations in any one of the islet transcription factors, as well as glucokinase (see later), result in progressively inadequate production of insulin. This leads to MODY, which typically manifests before 25 years of age.

CLINICAL BOX 3-2

The ATP-sensitive K^+ channel is a protein complex that contains an **ATP-binding subunit called SUR1**. This subunit is also activated by **sulfonylurea** and **meglitinide** drugs, which are used as oral agents to treat **hyperglycemia** in patients with partially impaired β-cell function. **Rare mutations in the ATP-sensitive K^+ channel** that keep it in the *open* conformation, thereby blocking glucose-induced insulin secretion, result in early-onset diabetes.

Glucose is the primary stimulus for insulin secretion. When serum glucose levels rise, insulin secretion is stimulated; when the levels fall, insulin secretion decreases to baseline. Certain **amino acids** (leucine) and **vagal (parasympathetic) cholinergic innervation** (i.e., in response to a meal) also stimulate insulin through increasing **intracellular Ca^{2+} levels** (see Fig. 3-6). **Long-chain FFAs** also increase insulin secretion, although to a lesser extent than glucose and amino acids. FFAs may act through a G-protein-coupled receptor **(GPR40)** on the β-cell membrane or as a nutrient that increases ATP through β-oxidation (see Fig. 3-6).

As discussed in Chapter 2, nutrient-dependent stimulation of insulin release is enhanced by the **incretin hormones glucagon-like peptide-1 (GLP1)** and **gastric inhibitory peptide (GIP)** and possibly other gastrointestinal (GI) hormones (e.g., cholecystokinin [CCK], secretin, and gastrin). These act primarily by **raising intracellular cyclic AMP (cAMP)**, which amplifies the intracellular Ca^{2+} effects of glucose (see Fig. 3-6). Intracellular cAMP acts both through **phosphokinase A (PKA)-dependent and EPAC (*e*xchange *p*rotein *a*ctivated by *c*AMP)–dependent pathways**

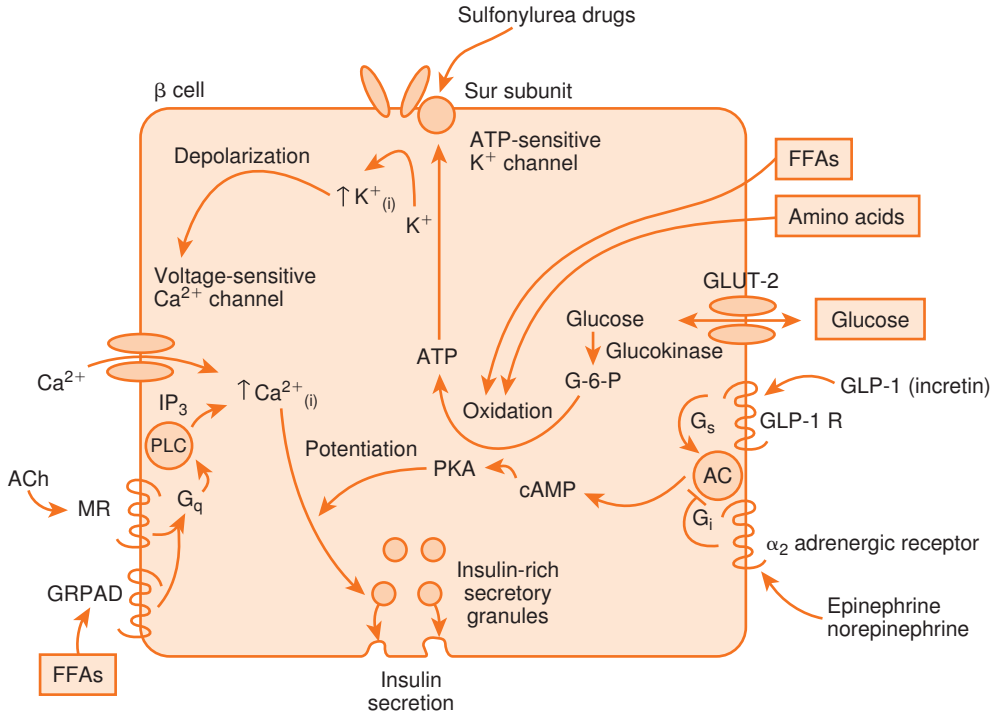

FIGURE 3-6 ■ Regulation of insulin secretion from β cells by nutrients (glucose, amino acids, FFAs) and the hormones/neurotransmitters, glucagon-like peptide-1 (GLP1), epinephrine, norepinephrine, and acetylcholine (ACh).

(see Chapter 1) in β cells. Incretin hormones do not increase insulin secretion in the absence of glucose.

Insulin secretion is inhibited by **α_2-adrenergic receptors**, which are activated by **epinephrine** (from the **adrenal medulla**) and norepinephrine (from **postganglionic sympathetic fibers**). The α_2-adrenergic receptors are coupled to a **Gi-containing trimeric G-protein complex** that inhibits adenylyl cyclase and decreases cAMP levels (see Fig. 3-6). Adrenergic inhibition of insulin serves to protect against hypoglycemia, especially during exercise. Although somatostatin from D cells inhibits both insulin and glucagon, its physiologic role in pancreatic islet function is unclear.

Insulin Receptor The **insulin receptor** is a member of the **receptor tyrosine kinase (RTK) family** (see Chapter 1), which includes receptors for several other growth factors, such as IGFs, platelet-derived growth factor (PDGF), and epidermal growth factor (EGF). The insulin receptor is expressed on the cell membrane as a homodimer composed of α/β monomers (Fig. 3-7). The α/β monomer is synthesized as one protein, which is then proteolytically cleaved, with the two fragments connected by a disulfide bond. The two α/β monomers are also held together by a disulfide bond between the α subunits. The α subunits are external to the cell membrane and contain the hormone-binding site. The β subunits span the membrane and contain tyrosine kinase on the cytosolic surface.

Insulin binding to the insulin receptor induces the β subunits to cross-phosphorylate each other on three tyrosine residues. These phosphotyrosine residues recruit three classes of adaptor proteins: the **insulin-receptor substrates (IRS1-4)**, **Shc protein,** and **APS protein**. The IRS proteins are phosphorylated by the tyrosine kinase activity of the insulin receptor. The phosphotyrosine residues on IRS recruit **PI3 kinase** to the membrane, where it is phosphorylated and activated by the insulin receptor. PI3 kinase converts **phosphoinositide-4,5-bisphosphate (PIP2)** to

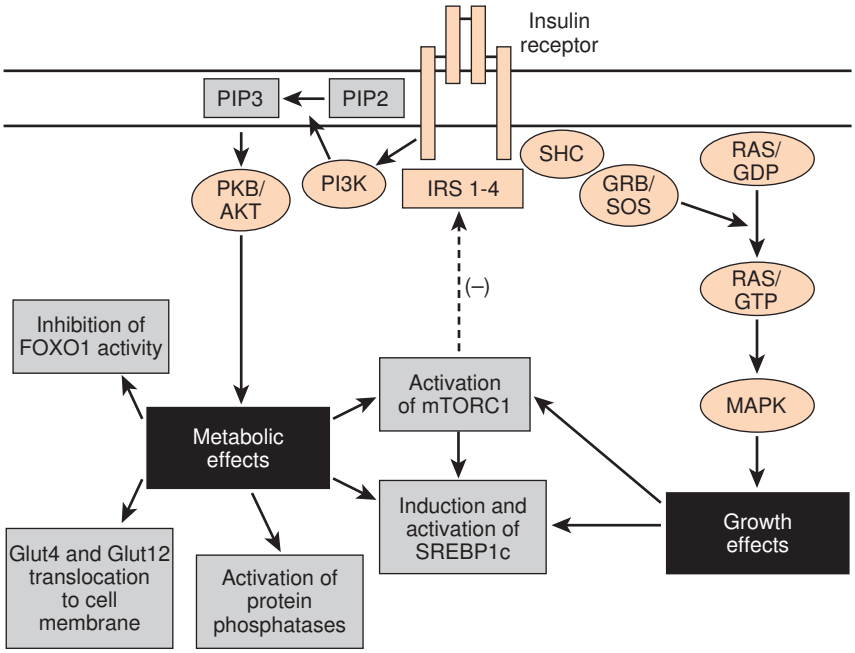

FIGURE 3-7 ■ Insulin receptor and postreceptor signaling pathways.

phosphoinositide-3,4,5-triphosphate (PIP3). PIP3 recruits to the membrane and leads to the activation of a pleiotropic protein kinase, called **protein kinase B (PKB)** or **AKT** (see Fig. 3-7).

PKB/AKT regulates numerous enzymes and transcription factors that mediate the metabolic actions of insulin. PKB/AKT acts in five general ways that largely account for the metabolic effects of insulin:

1. Phosphorylation of exocytotic components that induce the insertion of GLUT4 glucose transporters into the cell membranes of muscle and adipose tissue (see later). This action requires combined IRS/PI3K-dependent signaling and an additional APS adaptor protein-dependent pathway that activates a small G-protein pathway (not shown).
2. Activation of **protein phosphatases** that, in turn, regulate metabolic enzymes through dephosphorylation.
3. Induction of synthesis and activation of the lipogenic transcription factor, the sterol-regulatory element–binding protein-1 C (SREBP1C). **SREBP1C** stimulates the expression of enzymes involved in **glycolysis, lipogenesis**, and the **pentose phosphate pathway**.
4. Activation of **mTORC1** (indirectly through inactivation of upstream inhibitors). mTORC1 is a kinase complex that promotes ribosomal RNA synthesis, ribosome assembly, and protein synthesis. mTORC1 also increases SREBP1C activity.
5. Phosphorylation and inactivation of the transcription factor, FOXO1.

The Shc protein is linked to the mitogen-activated protein kinase (MAPK) pathway (see Fig. 3-7), which mediates the growth and mitogenic actions of insulin (in conjunction with the activation of mTORC1).

The termination of insulin receptor signaling is a topic of high interest because these mechanisms potentially play a role in **insulin resistance**. Insulin induces the down regulation of its own receptor by receptor-mediated endocytosis. Additionally, several serine and threonine protein kinases are indirectly activated by insulin and by other molecules (such as inflammatory cytokines) and subsequently inactivate the insulin receptor or IRS proteins. mTORC1 negatively feeds back on IRS proteins (see Fig. 3-7). A third mechanism appears to involve the activation of the **suppressor of cytokine signaling (SOCS) family of proteins,** which

reduces activity or levels of the insulin receptor and IRS proteins.

Glucagon

Glucagon is an important **counter-regulatory hormone** that increases blood glucose levels through its effects on liver glucose output. Glucagon promotes the production of glucose through elevated glycogenolysis and gluconeogenesis and through decreased glycolysis and glycogen synthesis. Glucagon also inhibits hepatic FFA synthesis from glucose. Glucagon also maintains blood glucose indirectly through stimulation of ketogenesis, which provides an alternative energy source that leads to glucose sparing in many tissues.

Glucagon Structure, Synthesis, and Secretion As discussed in Chapter 2, glucagon is a member of the secretin gene family. Preproglucagon is proteolytically cleaved in the **pancreatic islet α cell** in a cell-specific manner to produce the 29-amino acid glucagon (refer to Fig. 2-10 in Chapter 2). Glucagon is highly conserved among mammals.

Like insulin, glucagon circulates in an unbound form and has a short half-life (about 6 minutes). The predominant site of glucagon degradation is the liver, which degrades as much as 80% of the circulating glucagon in one pass. Because glucagon (either from the pancreas or the gut) enters the hepatic portal vein and is carried to the liver before reaching the systemic circulation, a large portion of the hormone never reaches the systemic circulation. The liver is the primary target organ of glucagon, with lesser effects on adipose tissue.

As discussed later, glucagon opposes the actions of insulin. Thus, several factors that stimulate insulin inhibit glucagon. Indeed, it is the insulin-to-glucagon ratio that determines the net flow of hepatic metabolic pathways. A major stimulus for glucagon secretion is a drop in blood glucose, which is primarily an indirect effect of the removal of inhibition by insulin (Fig. 3-8). Circulating catecholamines, which inhibit insulin secretion through α_2-adrenergic receptors, stimulate glucagon secretion through β_2-adrenergic receptors (see Fig. 3-8). Serum amino acids also stimulate glucagon secretion. This means that a protein meal will increase postprandial levels of glucagon along with insulin, thereby protecting against hypoglycemia. In contrast, a carbohydrate-only meal stimulates insulin secretion and inhibits glucagon secretion.

Glucagon Receptor The glucagon receptor is a 7-transmembrane receptor primarily linked to Gs-containing heterotrimeric G-protein complex (see Chapter 1). Consequently, glucagon increases intracellular cAMP levels in the liver. The increase in cAMP initiates the cascade of metabolic changes associated with enzyme phosphorylation. As discussed earlier, activation of opposing protein phosphatases is one of the general pathways of insulin signaling.

Epinephrine and Norepinephrine

The other major counter-regulatory factors are the **catecholamines epinephrine** and **norepinephrine.** Epinephrine and norepinephrine are secreted by the **adrenal medulla** (see Chapter 7), whereas only norepinephrine is released from **postganglionic sympathetic nerve endings**. The direct metabolic actions of catecholamines are mediated primarily by **β_2- and β_3-adrenergic receptors** located on **muscle, adipose**, and **liver.** Like the glucagon receptor, β-adrenergic receptors are linked to a Gs signaling pathway that increases intracellular cAMP. Epinephrine also promotes glycogenolysis and gluconeogenesis through the α_1 adrenergic receptor, which is coupled to the Gq/IP3/DAG signaling pathway.

Catecholamines are released from sympathetic nerve endings and the adrenal medulla in response to decreased glucose concentrations, stress or alarm, and exercise. Decreased glucose levels (i.e., hypoglycemia) are primarily sensed by hypothalamic neurons, which initiate a sympathetic response to release catecholamines.

Catecholamines circulate in the blood as free hormones, and both circulating and tissue catecholamines are rapidly enzymatically inactivated (see Chapter 7).

METABOLIC HOMEOSTASIS: THE INTEGRATED OUTCOME OF HORMONAL AND SUBSTRATE/PRODUCT REGULATION OF METABOLIC PATHWAYS

The hormonal regulation of the major metabolic pathways, with emphasis on key regulated enzymes and transporters, is presented in this section.Three organs

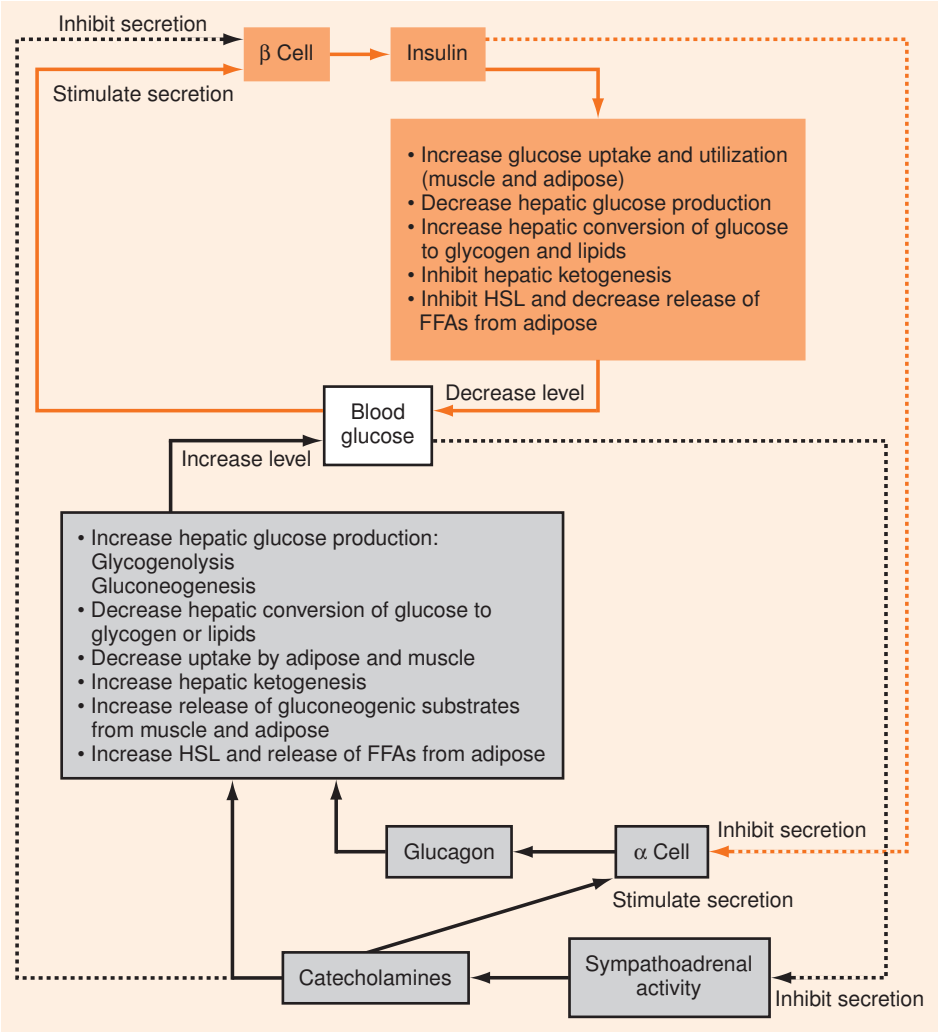

FIGURE 3-8 ■ Interaction of insulin, glucagon, and catecholamines in the regulation of each other and of blood glucose (the primary secretogogue for insulin). *(Modified from Koeppen BM, Stanton BA, editors:* Berne & Levy Physiology, *6th ed., Philadelphia, 2010, Mosby.)*

play predominant roles in energy use and storage: the **liver, skeletal muscle**, and **adipose tissue**. A fourth organ, the **hypothalamus**, through its own metabolic pathways and in response to hormones and nutrients, also plays a key role in the acquisition, use, and storage of fuels. We will also introduce additional hormones that originate from the **GI tract** and **adipose tissue** and modulate energy metabolism. As stated earlier, an overview of key metabolic pathways and lipoprotein metabolism is available on Student Consult (see the Supplement to Chapter 3 on the Student Consult site).

Energy Metabolism During the Fed State

During the fed state, insulin drives the storage of excess calories and the new synthesis of cellular macromolecules and organelles.

Hepatic Metabolism of Nutrients in the Fed State

Anatomic Considerations The liver is anatomically and functionally situated between the GI tract and the heart. The main veins that drain the GI tract of its ingested nutrients all converge to form the **hepatic portal vein**. A portal vein is one that ends in a capillary bed before reaching the heart. In the

case of the hepatic portal vein, it receives the venous drainage of the GI tract and then ends at the hepatic sinusoids (a sinusoid is a discontinuous capillary). This means that the liver is the first organ to receive the ingested carbohydrates and amino acids before they reach the general circulation (TGs bypass the liver at first because they enter lymphatics as chylomicron particles). The liver is also the first organ to respond to hormones and cytokines from the pancreas and intra-abdominal adipose tissue.

The Big Picture **Glucose** enters the **liver** and is stored as **glycogen (glycogen synthesis)**; the normal maximal amount of glycogen in the liver is about 80 g (Fig. 3-9). Glucose is then diverted into **glycolysis**, generating pyruvate. Pyruvate enters the **tricarboxylic acid (TCA)** cycle to produce reducing equivalents (NADH, $FADH_2$) for use in ATP synthesis through **oxidative phosphorylation**, but excess ATP and NADH will allow the diversion of **citrate** into the cytoplasm, where it is converted to malonyl coenzyme A (CoA) and ultimately fatty acyl CoA (**de novo lipogenesis**). Citrate can also provide carbons for cholesterol synthesis (not shown). Some glucose also enters the **pentose phosphate pathway (PPP)**, thereby generating NADPH, which is required for lipogenesis and defense against oxidative damage. Fatty acyl CoAs are modified (e.g., desaturated) and ultimately esterified to glycerol-3-P to produce TG. However, the export of TG and other lipids as **very-low-density lipoproteins (VLDLs)** is suppressed during the fed state. The breakdown of glycogen (**glycogenolysis**) to glucose and the production of glucose from 3-carbon glucogenic molecules (**gluconeogenesis**) are both suppressed by insulin, thereby reducing hepatic glucose production (see Fig. 3-9). Fatty acyl oxidation and **ketogenesis** are also suppressed (see Fig. 3-9).

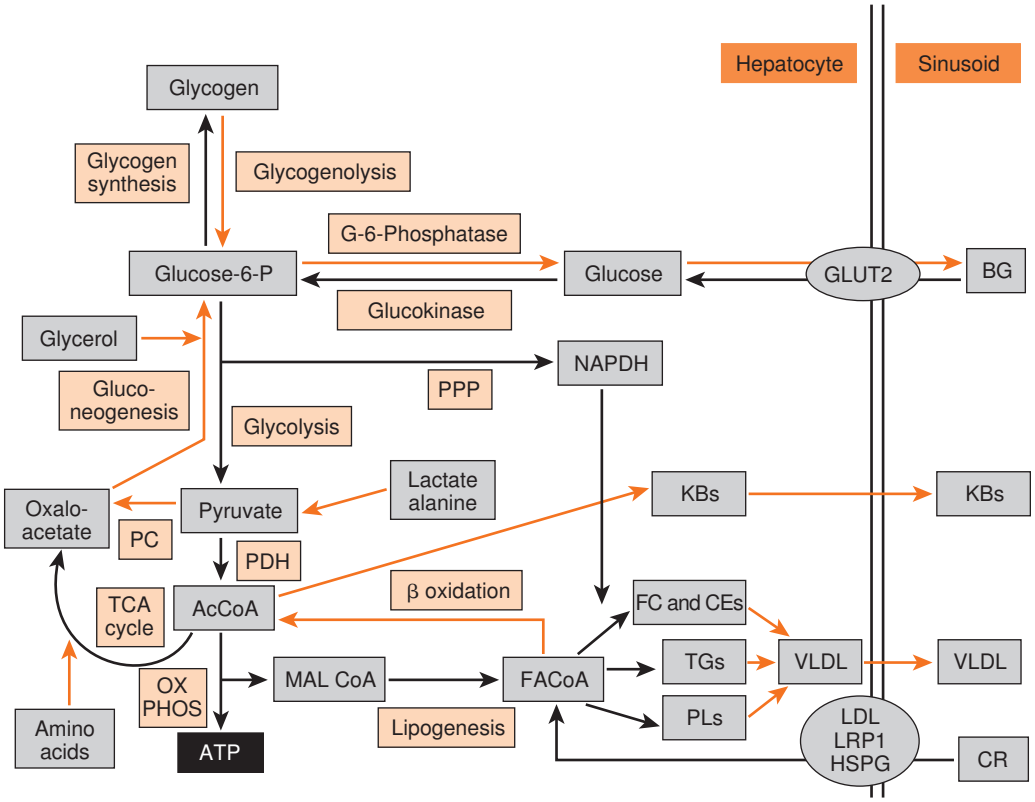

FIGURE 3-9 ■ Overview of metabolic pathways in the liver during the fed stage *(black arrows)* and the fast stage *(orange arrows)*.

Amino acids are assembled into proteins through activation of mTORC1 by both amino acids and insulin. Amino acids are also used for the synthesis of other macromolecules (e.g., heme, nucleotides). Amino acids, in particular glutamine and glutamic acid, also provide carbons (as α-ketoglutarate) to the TCA cycle **(anaplerosis)** as a replacement for carbons exiting the TCA cycle as citrate **(cataplerosis)**.

As the fed state progresses, **chylomicron remnants** enter the hepatocyte through receptor-mediated endocytosis, releasing TG to be packaged into VLDL later (see Fig. 3-9). Circulating FFAs (as FFA-albumin complexes) are diminished during the fed state (see adipose tissue below), so the uptake of FFAs by the liver is minimal.

All of these metabolic pathways are orchestrated by insulin. As discussed earlier, a major insulin-activated transcription factor is SREBP1C. The genes and enzymes stimulated by SREBP1C are listed in Table 3-1.

TABLE 3-1
Hepatic Genes Stimulated by SREBP1C

GENE	ENZYME	PATHWAY
GCKR	Glucokinase (hexokinase-4)	Glycolysis
PKLR	Pyruvate kinase (liver and red blood cells)	Glycolysis
ACLY	Adenosine triphosphate and citrate lyase	De novo lipogenesis
ACACA	Acetyl coenzyme A (CoA) carboxylase-1(acetyl CoA carboxylase-α)	De novo lipogenesis
ACACB	Acetyl CoA carboxylase-2 (acetyl CoA carboxylase-β)	De novo lipogenesis
FASN	Fatty acid synthase	De novo lipogenesis
G6PD	Glucose-6-phosphate dehydrogenase	Pentose phosphate pathway (hexose monophosphate shunt)

Hormonal Regulation of Key Reactions in the Liver During the Fed State

See the Supplement to Chapter 3 on the Student Consult site for additional figures.

Intracellular Transport and Trapping of Monosaccharides Ingested monosaccharides (glucose, fructose, and galactose) reach the liver sinusoids and flow down their concentration gradient into hepatocytes through a bidirectional, facilitative transporter, called **GLUT-2** (Fig. 3-10). **GLUT2** is a **low-affinity, high-capacity facilitative transporter** well suited to

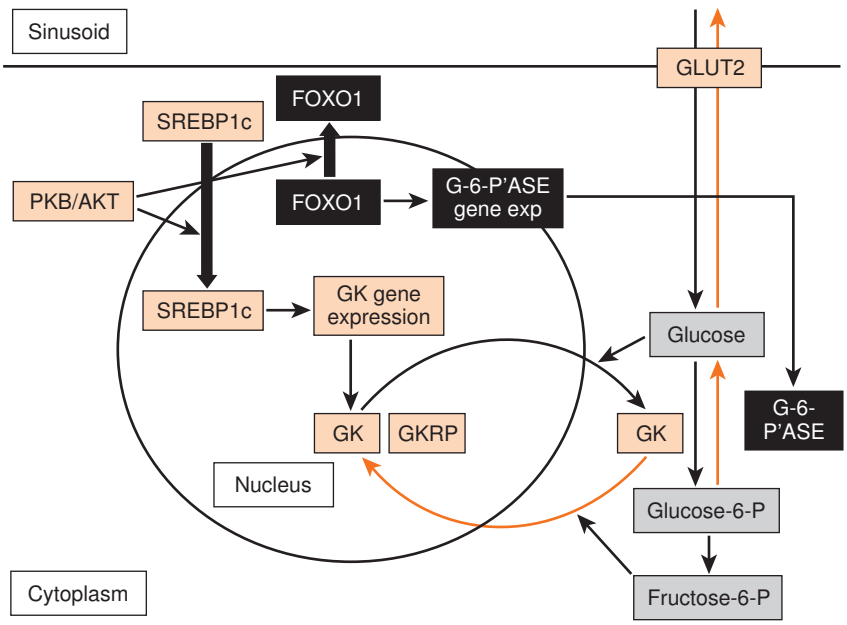

FIGURE 3-10 ■ Regulation of glucokinase (GK) and glucose-6-phosphatase (G-6-P'ASE) during fed *(black arrows)* and fast *(orange arrows)* stages.

moving high amounts of absorbed glucose during and soon after a meal. GLUT-2 expression or localization at the cell membrane is *not* regulated.

Intrahepatic glucose (galactose is efficiently converted to glucose) is trapped by phosphorylation to glucose-6-phosphate by the enzyme, **glucokinase (GK).** As discussed previously for the pancreatic β cells, glucokinase has a low affinity and high V_{max}. Thus, glucokinase, which is a member of the **hexokinase family of enzymes**, is paired with GLUT-2 in the β cell and hepatocyte. The glucokinase reaction is irreversible and is opposed by the enzyme **glucose-6-phosphatase** (see Fig. 3-10).

Hepatic glucokinase is tightly regulated by metabolites and by insulin (see Fig. 3-10). In the absence of glucose, glucokinase is sequestered in the nucleus by binding to **glucokinase regulatory protein (GKRP).** Increased glucose promotes the dissociation of glucokinase from its regulatory protein and translocation into the cytoplasm, where it converts glucose to glucose-6-phosphate. The downstream product, fructose-6-phosphate, inhibits glucokinase by promoting its sequestration.

Insulin, acting through the PKB/AKT signaling pathway, stimulates the new synthesis of glucokinase through activation of SREBP1C. Insulin drives the nuclear localization of SREBP1C, which in turn drives glucokinase gene expression (see Fig. 3-10). Insulin also inhibits expression of glucose-6-phosphatase through the inactivation of FOXO1. Nuclear FOXO1 stimulates glucose-6-phosphatase gene expression. PKB/AKT phosphorylates FOXO1, thereby sequestering the transcription factor in the cytoplasm (see Fig. 3-10).

Storage of Glucose as Glycogen Insulin increases the activity of **glycogen synthase** in several ways (Fig. 3-11). Insulin indirectly increases glycogen synthase through increased expression of glucokinase because high levels of glucose-6-phosphate allosterically increase glycogen synthase activity.

Insulin also increases the activity of **protein phosphatase-1** (see Fig. 3-11), which dephosphorylates and thereby activates glycogen synthase. Insulin also inactivates **glycogen synthase kinase-3**, which, in turn, promotes the accumulation of dephosphorylated

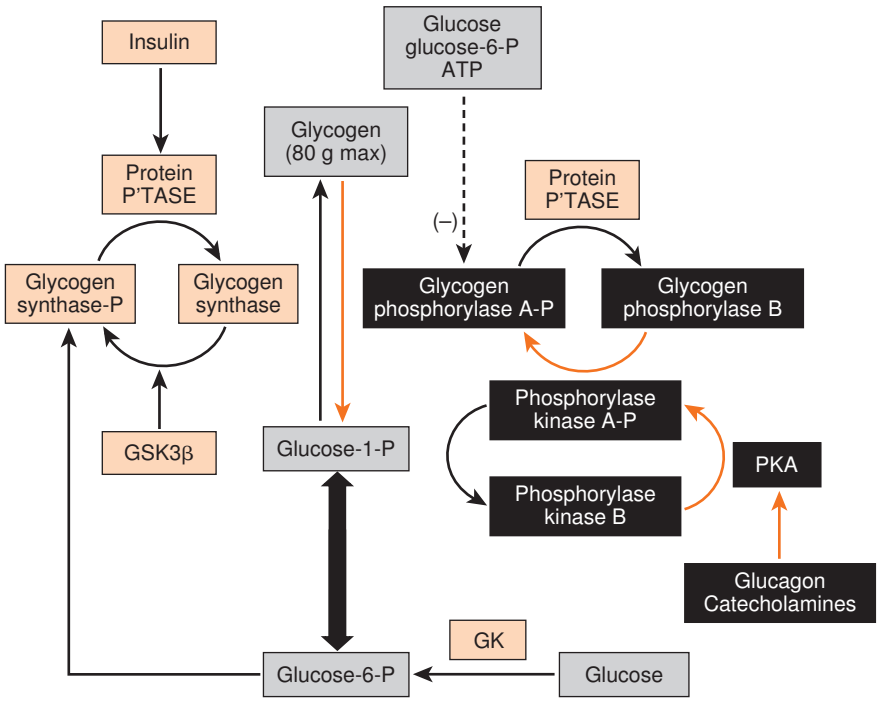

FIGURE 3-11 ■ Regulation of glycogen synthesis (*black arrows,* fed) and glycogenolysis (*orange arrows,* fast).

(active) glycogen synthase. Insulin may also prevent the futile cycle of glycogen synthesis and glycogenolysis through inhibition of **glycogen phosphorylase** (see Fig. 3-11). Insulin-activated protein phosphatase-1 dephosphorylates and inactivates **glycogen phosphorylase** as well as **phosphorylase kinase**. Glycogen phosphorylase is also inactivated by glucose, glucose-6-phosphate, and ATP.

Glycolysis Glucose is also metabolized through the glycolytic pathway. Once glycogen stores are filled, glycogen synthesis ceases, further increasing the flux of glucose through glycolysis. There are two hormonally regulated cytoplasmic enzymes within glycolysis (after glucokinase): phosphofructokinase-1 (PFK1) and liver-specific pyruvate kinase (PK-L).

Phosphofructokinase-1 The **PFK1** reaction converts fructose-6-phosphate to fructose-1,6-bisphosphate (Fig. 3-12). This reaction is irreversible, requiring a separate enzyme, fructose-1,6-bisphosphatase, to catalyze the reverse reaction. The PFK1 reaction is one of the most tightly regulated reactions in metabolism, and it is also one that does not involve SREBP1C. During the fed state, PFK1 activity is moderated by high levels of ATP and cytoplasmic citrate (as discussed earlier, citrate moves from the mitochondria to the cytoplasm at times when the TCA cycle is slowed down by abundant ATP and NADH during the fed state). In contrast, AMP allosterically activates PFK-1.

Another major allosteric activator of PFK1 is the glycolytic side product, **fructose-2,6-bisphosphate** (see Fig. 3-12). Fructose-2,6-bisphosphate is produced from fructose-6-phosphate by the **phosphofructose kinase-2 (PFK2) function of the bifunctional enzyme, PFK2/fructose-2,6-bisphosphatase**. The **PFK2 function** of the enzyme is active when the enzyme is **dephosphorylated** by an insulin-activated protein phosphatase-1 (see Fig. 3-12). The **fructose-2,6-bisphosphatase function** is active when the enzyme is **phosphorylated** by a glucagon- and catecholamine-activated protein kinase A (see Fig. 3-12). Through the generation of fructose-2,6-bisphosphate, insulin partially offsets the inhibitory actions of ATP and cytoplasmic citrate on PFK1. This allows glucose to continue through the glycolytic pathway during the fed state.

The opposing reaction that converts fructose-1,6-bisphosphate to fructose-6-phosphate is catalyzed by the gluconeogenic enzyme, **fructose-1,6-bisphosphatase**. Fructose-2,6-bisphosphate is an allosteric inhibitor of fructose-1,6-bisphosphatase (see Fig. 3-12).

Pyruvate Kinase The **pyruvate kinase** reaction is also irreversible and converts **phosphoenol pyruvate** to **pyruvate** (Fig. 3-13). This reaction is coupled to ATP synthesis and accounts for the net two ATPs produced by anaerobic glycolysis. Pyruvate kinase is allosterically activated by the upstream intermediate, fructose-1,6-bisphosphate (see previous section). In this way, insulin activates pyruvate kinase indirectly

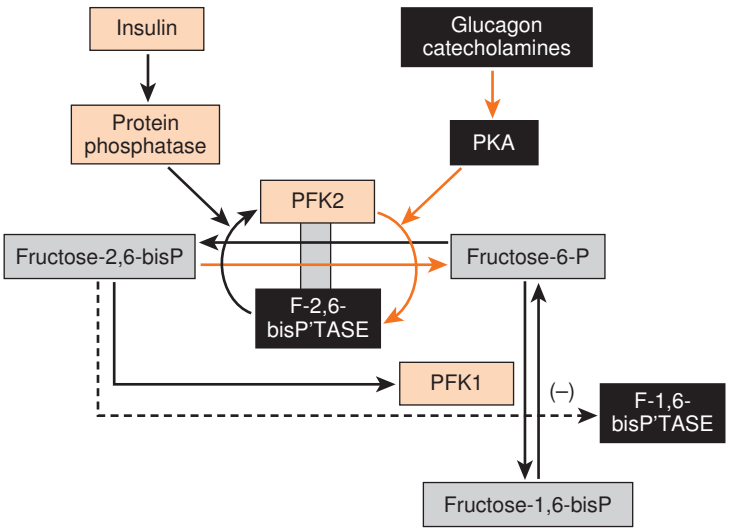

FIGURE 3-12 ■ Hormonal regulation of phosphofructose kinase-1 (PFK1) and fructose-1,6-bisphosphatase (F-1,6-bisP'TASE) by levels of fructose-2,6-bisphosphate. The dual activity enzyme, PFK2/F-2,6-bisP'TASE, acts as the kinase when dephosphorylated and as a phosphatase when phosphorylated. *Dashed lines* indicate allosteric inhibition.

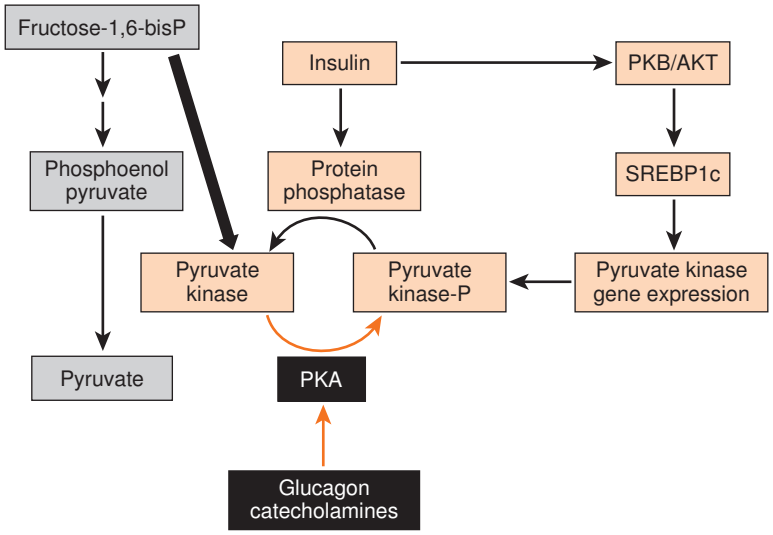

FIGURE 3-13 ■ Regulation of pyruvate kinase during the fed *(black arrows)* and fast *(orange arrows)* states. Fructose-1,6-bisphosphate is an allosteric activator of pyruvate kinase.

through a feed-forward mechanism that is initiated by the insulin-induced production of fructose-2,6-bisphosphate (see Fig. 3-13). Pyruvate kinase is also regulated by phosphorylation. Again, insulin activates pyruvate kinase through insulin-dependent dephosphorylation by protein phosphatase. Insulin also has a longer-term effect on pyruvate kinase gene transcription through the PKB/AKT-SREBP1C pathway (see Fig. 3-13).

Entry of Pyruvate into the Tricarboxylic Acid Cycle **Glycolysis** generates **pyruvate,** which can be converted to **lactate** and H^+ by **lactate dehydrogenase**. This replenishes **NAD^+** levels required for glycolysis to continue, and lactate dehydrogenase is activated by a low NAD^+/NADH ratio. In hepatocytes with active mitochondria, NAD^+ is continually regenerated from NADH through the electron transport chain. This promotes the entry of pyruvate into the mitochondria, where it enters the **TCA cycle**. This requires the conversion of **pyruvate** into **acetyl CoA**, as catalyzed by the **pyruvate dehydrogenase (PDH) complex. PDH kinase** is associated with the PDH complex, and phosphorylation of the PDH complex **inhibits** activity. PDH kinase is **activated by ATP, acetyl CoA,** and **NADH**. In addition, the PDH complex itself is **inhibited by NADH** and **acetyl CoA.** However, in the face of **high glycolytic flux** during the fed state, abundant **pyruvate** acts as a potent **inhibitor of PDH kinase**, thereby activating PDH and promoting the conversion of pyruvate to acetyl CoA (Fig. 3-14). In addition, insulin stimulates PDH phosphatase, which dephosphorylates and activates PDH.

Tricarboxylic Acid Cycle **Acetyl CoA** (2 carbons) is condensed with **oxaloacetate** (4 carbons) to form **citrate**, as catalyzed by **citrate synthase**. In the absence of abundant energy, citrate is cycled through seven reactions back to oxaloacetate, generating 3 NADH and 1 $FADH_2$, which will generate 11 ATPs through processing by the electron transport chain and oxidative phosphorylation.

In the fed state, however, **ATP and NADH** are abundant and allosterically inhibit two TCA reactions after citrate is formed. The slowing of the TCA cycle allows citrate to accumulate and ultimately flow through citrate transporters into the cytoplasm. Cytoplasmic citrate is the first intermediate in **de novo lipogenesis** (see Fig. 3-14).

De Novo Lipogenesis **De novo lipogenesis** normally occurs only in the liver, adipose tissue, and mammary glands. De novo synthesis of fatty acyl CoAs involves three hormonally regulated enzymes: **ATP-citrate lyase (ACLY), acetyl CoA carboxylase (ACC1 and ACC2)**, and **fatty acid synthase (FASN)**. Cytoplasmic citrate is converted to acetyl CoA, and acetyl CoA can be used for fatty acyl CoA synthesis (see Fig. 3-14) or cholesterol synthesis (not shown).

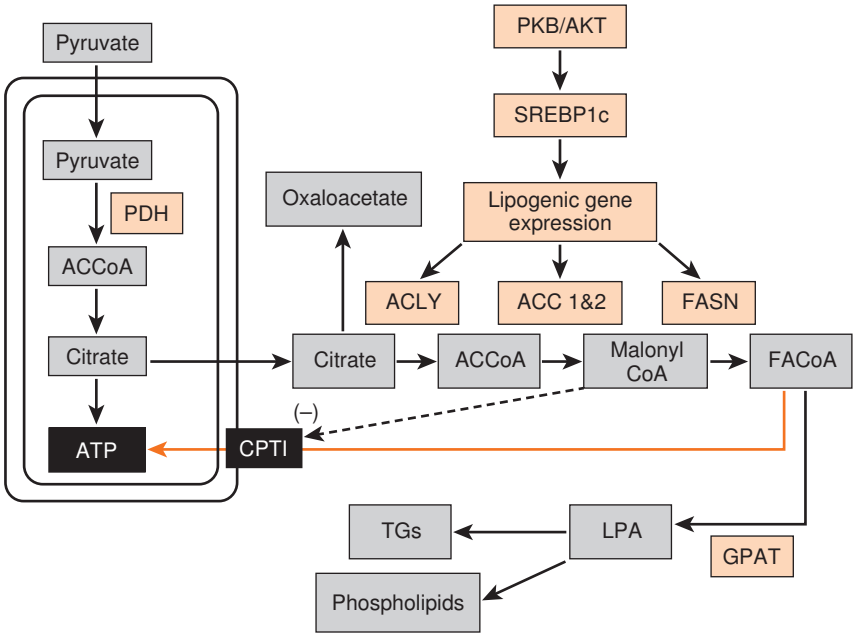

FIGURE 3-14 ■ De novo lipogenesis from pyruvate and TG/phospholipid production. *ACC*, acetyl CoA carboxylase; *ACCoA, acetyl CoA*; *ACLY*, ATP-citrate lyase; *CPT1, carnitine-palmitoyl transferase; FACoA, fatty acyl CoA; FASN,* fatty acid synthase; GPAT, glycerol phosphate acyltransferase; *LPA, lysophosphatidic acid; PDH,* pyruvate dehydrogenase; *TG,* triglyceride

ACC converts acetyl CoA to malonyl CoA, which is the key substrate for production of fatty acyl CoAs by FASN (see Fig. 3-14).

The expression of all four enzymes (ACLY, ACC1, ACC2, FASN) is stimulated by insulin-activated **SREBP1C** (see Fig. 3-14). In addition, **ACC1 activity** is increased by insulin-activated dephosphorylation (Fig. 3-15). Conversely, ACC1 and ACC2 are phosphorylated and inactivated by AMPK during the fasted state. The production of malonyl CoA by ACC1 is also antagonized by AMPK-activated **malonyl CoA decarboxylase. ACC2** is localized to the outer mitochondrial membrane. The malonyl CoA generated by ACC2 directly inhibits **carnitine-palmitoyl transferase-I (CPTI)** on the outer mitochondrial membrane. Thus, ACC1 promotes lipogenesis, whereas ACC2 inhibits fatty acyl oxidation and ketogenesis (see Fig. 3-15).

Fatty acyl CoAs are used for the synthesis of phospholipids and TG. These lipids, along with cholesterol and cholesterol esters, are packaged into VLDL. Insulin acutely promotes the degradation of the VLDL apoprotein, apoB100. More importantly, insulin inhibits FOXO1, which induces the synthesis of ApoB100 and the microsomal transport protein (MTP) involved in VLDL assembly. This keeps the liver from secreting VLDL during a meal, when the blood is rich with chylomicrons. Thus, the lipid made in response to insulin during a meal is released as VLDL during the interdigestive (fasting) period and provides an important source of energy to skeletal and cardiac muscle.

NADPH Production Through the Pentose Phosphate Pathway De novo lipogenesis is dependent on abundant **NADPH** as a cofactor. NADPH is derived primarily from the **PPP**. Insulin promotes the flux of glucose-6-phosphate into the PPP through the stimulation of **glucose-6-phosphate dehydrogenase** (see Fig. 3-9) gene transcription by SREBP1C.

In summary, in the liver, insulin **promotes** the following:

- Glycogen synthesis
- Glycolysis
- De novo lipid synthesis, leading to production of cholesterol, phospholipids, and TG
- NADPH production through the PPP
- Protein and organelle synthesis, in part through mTORC1 (not discussed)

In the liver, insulin **inhibits** the following:

- Hepatic glucose production by inhibiting glycogenolysis and gluconeogenesis

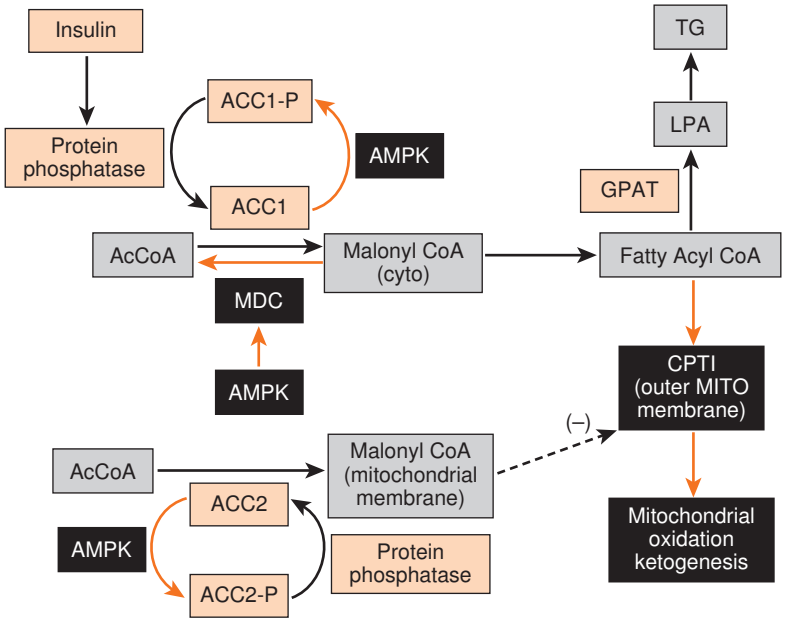

FIGURE 3-15 ■ Regulation of malonyl CoA synthesis as the critical regulator of lipogenesis versus oxidation of lipid. AcCoA, cytoplasmic acetyl CoA; ACC, acetyl CoA carboxylase; AMPK, AMP-activated kinase; CPTI, carnitine-palmitoyl transferase-I; GPAT, glycerol phosphate acyltransferase; LPA, lyso-phosphatidic acid; MCD, malonyl CoA decarboxylase.

- Fatty acid oxidation and ketogenesis
- Proteolysis

In terms of the intracellular nutrient and energy sensors:

1. mTORC1 is activated in the fed state by a combination of increased amino acids and insulin-PKB/AKT signaling. mTORC1 stimulates protein synthesis. mTORC1 also stimulates de novo lipid synthesis through stimulating SREBP1C activity.
2. In the fed state, with a surfeit of ATP, AMPK is inactive.

Skeletal Muscle The glucose that is not metabolized by the liver contributes to the postprandial rise in glucose levels in the peripheral circulation. **Glucose tolerance** refers to the ability to minimize the degree and duration of excursions of blood glucose concentrations. A primary way in which insulin promotes glucose tolerance is through promoting the uptake of glucose into skeletal muscle (Fig. 3-16). Insulin stimulates the translocation of preexisting **GLUT-4** (and GLUT-12) **transporters** to the cell membrane. In the absence of insulin, GLUT transporters reside in **GLUT storage vesicles**, which are largely retained in the cytoplasm. Insulin signaling, both by PKB/AKT and a small G-protein pathway, blocks the retention of the vesicles, thereby significantly increasing their insertion into the cell membrane.

As in the liver, insulin also promotes the storage of intramyocellular glucose by stimulating **glycogen synthesis** in **muscle**, and the use of glucose for ATP synthesis through glycolysis and the TCA cycle. Insulin stimulates muscle-associated isoforms of hexokinases (converting glucose to glucose-6-phosphate) and glycolytic enzymes. Importantly, in the presence of excessive carbohydrate intake, insulin increases TG synthesis from glucose in skeletal muscle (through activation of SREBP1C), which can lead to **ectopic intramyocellular TG and associated lipids**. The intramyocellular lipid load may lead to lipotoxicity and insulin resistance (see later). Note that exercise increases intracellular Ca^{2+} and the AMP/ATP ratio, both of which stimulate AMPK, which inhibits lipogenesis and stimulates oxidation of FFAs.

Skeletal muscle contains a large amount of protein. During the fed state, insulin and amino acids stimulate mTORC1, which in turn stimulates protein synthesis.

Adipose Tissue Insulin also stimulates **GLUT4-** and GLUT-12-**dependent uptake** of glucose and subsequent glycolysis in adipose tissue (Fig. 3-17).

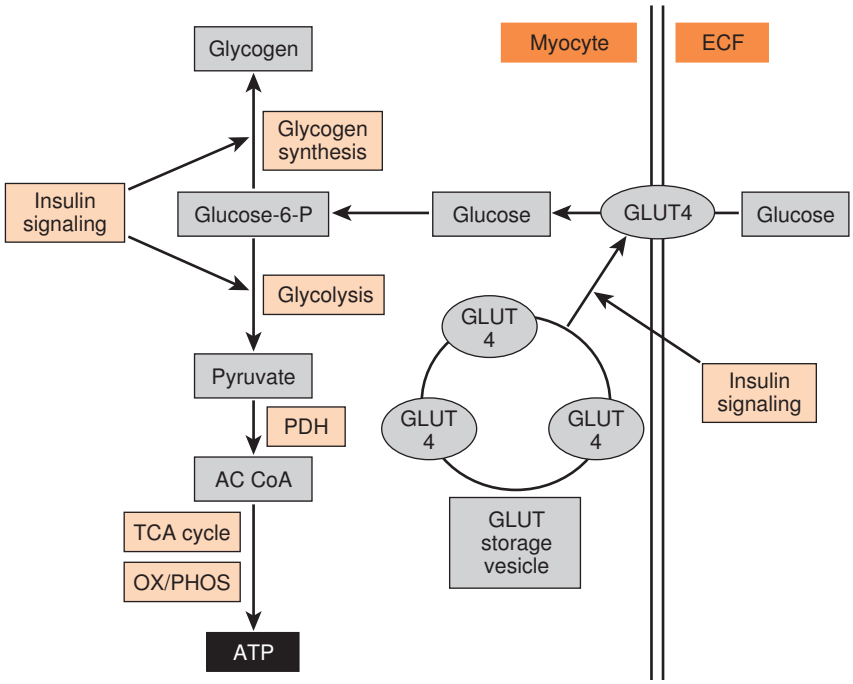

FIGURE 3-16 ■ Regulation of glucose uptake and use in skeletal muscle during the fed stage.

Adipose tissue uses glycolysis for energy needs, but also for the generation of **glycerol-3-phosphate**, which is required for the reesterification of FFAs into TGs (see Fig. 3-17).

Dietary FFAs reach the adipocytes in the form of TGs within chylomicrons (refer to the Supplement to Chapter 3 on the Student Consult site for a description of lipoproteins and their functions). The TGs within the chylomicrons are digested by the extracellular lipase **lipoprotein lipase**. Insulin stimulates the expression of lipoprotein lipase within adipocytes and its exocytosis and migration to the apical side of endothelia of adipose capillary beds (Fig. 3-18). This action of insulin thereby promotes the release of FFAs from chylomicrons within adipose tissue. Insulin also stimulates translocation of **fatty acid transport proteins** (e.g., CD36) into the cell membrane. Fatty acid transport proteins facilitate the movement of FFAs into the adipocyte and the activation of FFAs by their conversion to **fatty acyl CoAs**. Insulin stimulates glycolysis in adipocytes, which generates the **glycerol-3-phosphate** required for reesterification of FFAs into TGs (see Fig. 3-17). Insulin stimulates protein phosphatases, which, in turn, dephosphorylate and inactivate hormone-sensitive lipase (HSL) and dephosphorylate and stabilize TG droplet coat proteins (e.g., perilipins) (see Fig. 3-18).

Insulin signaling also activates a nuclear receptor, called **peroxisome proliferator-activated receptor-γ (PPARγ)**. PPARγ promotes the differentiation of **preadipocytes** into **adipocytes**. Expansion of the mature adipocyte population can alleviate insulin resistance by expanding the cellular compartment where TG can be stored safely.

As in liver and skeletal muscle, insulin and amino acids promote the protein synthesis through activation of mTORC1.

Overview of Energy Metabolism During the Fasting State

Several hours after a meal, nutrient levels fall, leading to lower levels of insulin secretion. Consequently, the actions of insulin on hepatic, muscle, and adipose tissue are attenuated. The decrease in insulin also relieves inhibition of glucagon secretion (see

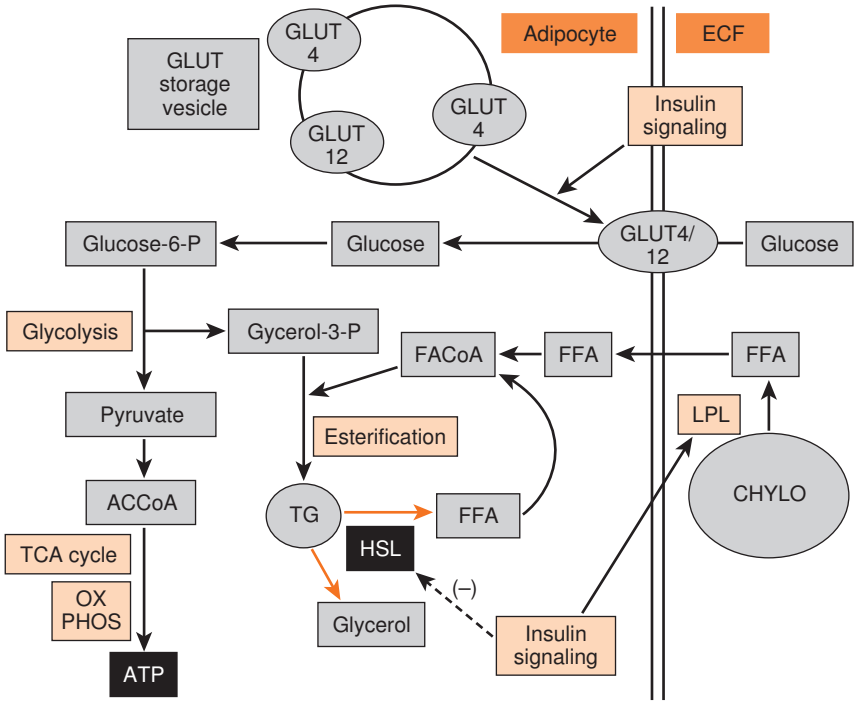

FIGURE 3-17 ■ Regulation of glucose uptake, lipolysis of chylomicrons, and reesterification of FFAs in the adipocyte during the fed stage. HSL, hormone-sensitive lipase; LPL, lipoprotein lipase.

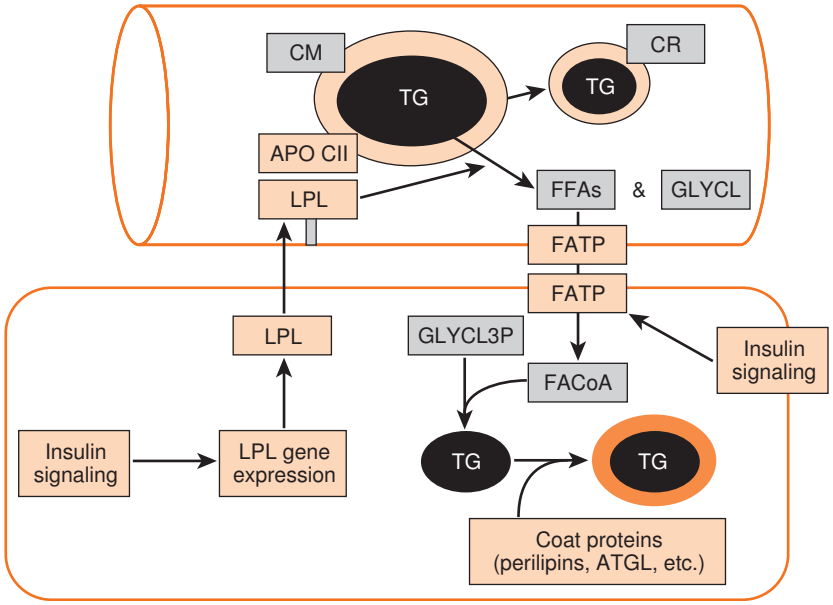

FIGURE 3-18 ■ Regulation of lipoprotein lipase (LPL) synthesis secretion and transcellular transport to the apical membrane of endothelial cells. CM, chylomicron; CR, chylomicron remnant; GLYCL3P, glycerol-3-phosphate.

Fig. 3-8). During the fast state, **glucagon** and **catecholamines** promote **hepatic glucose production, VLDL assembly and export, FFA oxidation** and **ketone body synthesis and release** (Fig. 3-19). Another major consequence of low insulin is the release of Akt-induced inhibition of the transcription factor FOXO1. FOXO1 promotes gluconeogenesis and VLDL assembly in the liver.

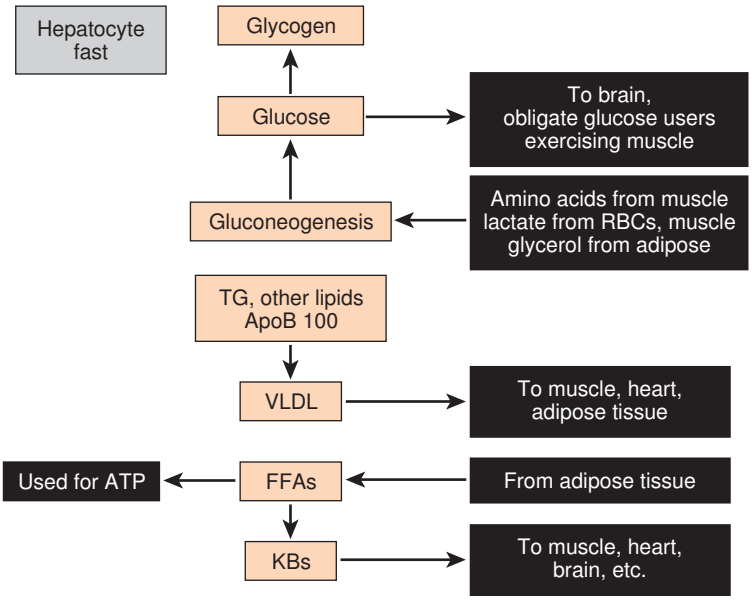

FIGURE 3-19 ■ Overview of hepatocyte function during the fasting stage.

Muscle fibers import minimal amounts of glucose because of basal levels of GLUT4 in their plasma membrane. Muscle fibers switch from using glucose to using **abundant FFAs**, as well as **ketone bodies** in a longer fast, for ATP synthesis (Fig. 3-20). Skeletal muscle also releases **lactate** and **amino acids**. During exercise or in response to a fight-or-flight situation, muscle mobilizes its **glycogen stores** for ATP production (see Fig. 3-20).

The adipocytes have low GLUT-4 levels at their cell membrane and consequently cannot generate glycerol-3-phosphate for TG synthesis. The low insulin and high catecholamine levels promote lipolysis of TG and the release of FFAs into the blood (Fig. 3-21). Adipocytes will also switch to FFAs and ketone bodies for ATP synthesis.

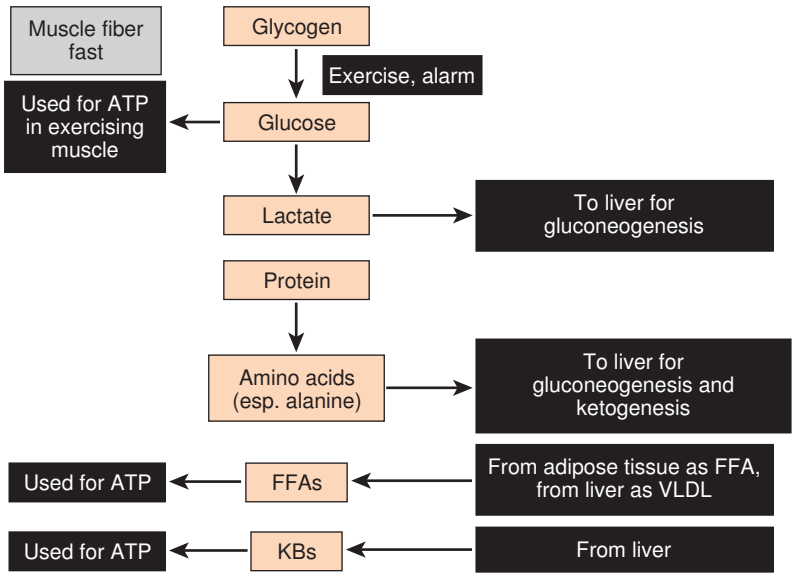

FIGURE 3-20 ■ Overview of muscle fiber function during the fasting stage.

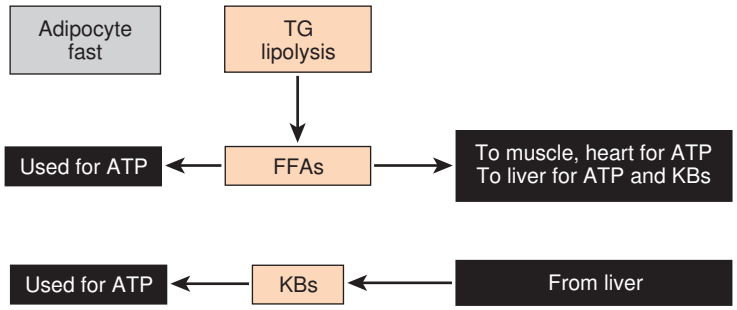

FIGURE 3-21 ■ Overview of adipocyte function during the fasting stage.

LIVER

Hormonal Regulation of Key Reactions in the Liver During the Fasting State

See Figure 3-19 for an overview.

Hepatic Glucose Production

Glycogenolysis **Glucagon** and **catecholamines**, acting through Gs-coupled receptors (**glucagon receptor** and **β_2-adrenergic receptor**, respectively) stimulate PKA, leading to the phosphorylation and activation of **phosphorylase kinase** and **glycogen phosphorylase** (Fig. 3-11). Additionally, the loss of insulin signaling prevents the dephosphorylation and activation of glycogen synthase. Collectively, these actions lead to the increase in **hepatic glucose-6-phosphate levels**.

For glucose-6-phosphate to leave the liver through the GLUT-2 transporter (and contribute to hepatic glucose production), it needs to be dephosphorylated to **glucose**. This reaction is catalyzed by **glucose-6-phosphatase** within the smooth endoplasmic reticulum (see Fig. 3-10). In the absence of insulin-PKB/AKT signaling, FOXO1 remains in the nucleus and stimulates glucose-6-phosphatase gene expression (see Fig. 3-22).

Glycogenolysis supports hepatic glucose production for about 12-16 hours at the beginning of a fast.

Gluconeogenesis **Gluconeogenic enzymes** include **pyruvate carboxylase, phosphoenolpyruvate carboxykinase (PEPCK),** and **fructose-1,6-bisphosphatase,** as well as **glucose-6-phosphatase** (discussed earlier). All of these reactions are irreversible (i.e., opposed by a separate glycolytic enzyme). Gluconeogenesis can continue for days and weeks, as long as glucogenic substrates are delivered to the liver.

PYRUVATE CARBOXYLASE During the fast state, hepatic pyruvate is primarily generated from **lactate alanine, and glycerol** as opposed to glucose (Fig. 3-22). Furthermore, little pyruvate is decarboxylated to acetyl CoA by the PDH complex. Rather, pyruvate is converted to the 4-carbon oxaloacetate by **pyruvate carboxylase (PC)**. This switch occurs primarily in response to an increase in FFA oxidation. As discussed later, adipose tissue releases FFAs during the fast state, and FFAs are used by the liver, skeletal muscle, and adipose tissue for ATP production during the fast state. FFAs are readily oxidized in the liver. Loss of insulin signaling decreases malonyl CoA levels, removing the inhibition on CPTI and allowing FFAs to enter the mitochondria. As FFAs undergo β-oxidation, the massive amounts of acetyl CoA released inhibit the PDH complex, both directly and indirectly through activation of PDH kinase and allosterically activate pyruvate carboxylase. PDH is further inhibited by the low activity of PDH phosphatase in the absence of insulin. The oxaloacetate produced exits the mitochondria through the malate shuttle (see Fig. 3-22).

PEPCK Cytoplasmic oxaloacetate is converted to **phosphoenolpyruvate (PEP)** by **PEPCK** (see Fig. 3-22). PEPCK expression is increased by glucagon- and epinephrine-PKA-**CREB** signaling (see Chapter 1) and by **FOXO1**, which remains active in the nucleus in the absence of insulin. Importantly, phosphoenolpyruvate is not efficiently converted back to pyruvate (a futile cycle) because glucagon and epinephrine phosphorylate and inactivate pyruvate kinase.

FRUCTOSE-1,6-BISPHOSPHATASE In the absence of insulin signaling and increased glucagon/PKA signaling, levels of fructose-2,6-bisphosphate decrease. This leads to a reduction in PFK1 activity, but a release of inhibition

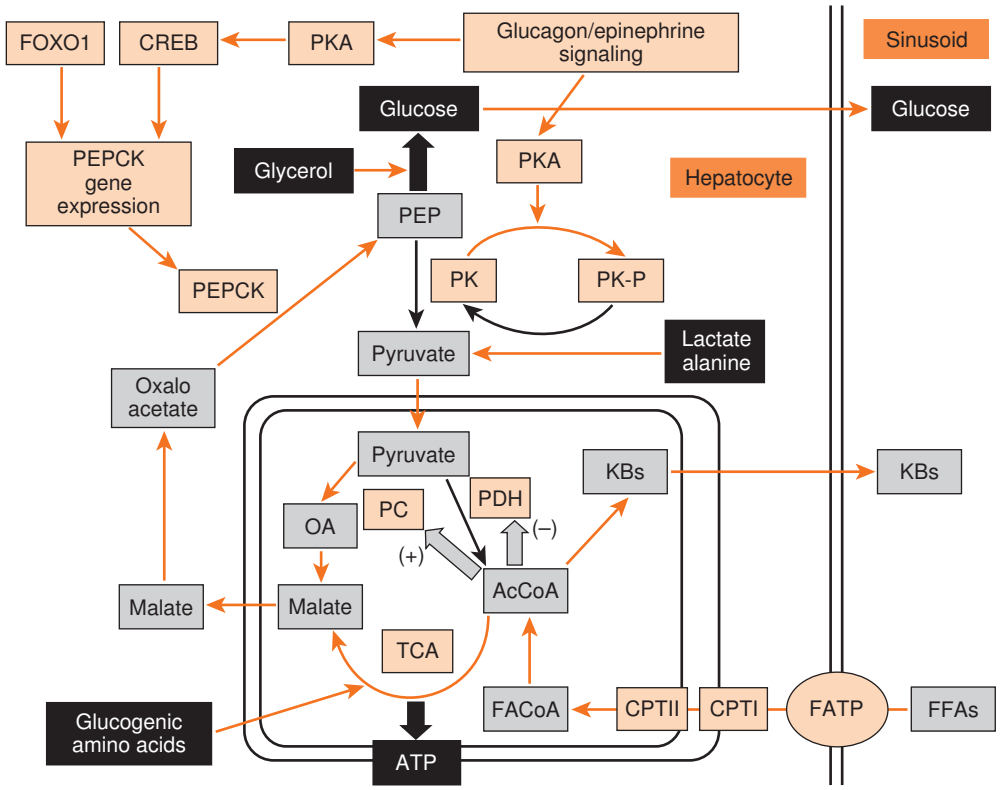

FIGURE 3-22 ■ Regulation of the gluconeogenic enzymes, pyruvate carboxylase (PC) and phosphoenolpyruvate carboxykinase (PEPCK) during the fast stage. CREB, cyclic AMP response element binding protein; CPT, carnitine-palmitoyl transferase (CPTI is in outer mitochondrial membrane; CPTII is in inner mitochondrial membrane; FATP, fatty acid transport protein; KBs, ketone bodies (acetoacetate and hydroxybutyrate); OA, oxaloacetate; PDH, pyruvate dehydrogenase; PK, pyruvate kinase; PKA protein kinase A.

(i.e., stimulation) of **fructose-2,6-bisphosphatase** (see Fig. 3-12). As this enzyme becomes activated, its substrate, fructose-2,6-bisphosphate, reaches an equilibrium at a lower level. This further inhibits **pyruvate kinase,** which is allosterically activated by fructose-1,6-bisphosphate (see Fig. 3-13).

Switch to Use of FFAs for ATP and Ketogenesis As discussed earlier, adipose tissue releases FFAs during the fast state. These will be used by the hepatocytes for ATP production through β-oxidation (see Fig. 3-22). In the face of low insulin, malonyl CoA is not produced by acetyl CoA carboxylase-2, thereby allowing fatty acyl CoAs to enter the mitochondria through the CPT transporters. **Intrahepatic fatty acids** and **fatty acid derivatives** act as **ligands** and activate a member of the nuclear receptor family (see Chapter 1), called **PPARα**. Activated PPARα stimulates the expression of CPTI and the enzymes involved in β-oxidation, thereby promoting fatty acid oxidation. PPARα is also activated by glucagon- and catecholamine-PKA signaling and by AMPK.

The oxidation of fatty acyl CoAs requires their conversion to acetyl CoA, and the continuation of fatty acyl oxidation requires the regeneration of free CoA. This is accomplished in the liver by the formation of **ketone bodies, acetoacetate** (which degrades spontaneously to **acetone**), and **β-hydroxybutyrate**. The liver does not possess the enzymes for ketone body catabolism. Consequently, ketone bodies are released into the blood (see Fig. 3-22) and, because of their hydrophilic nature, easily circulate unbound to carrier

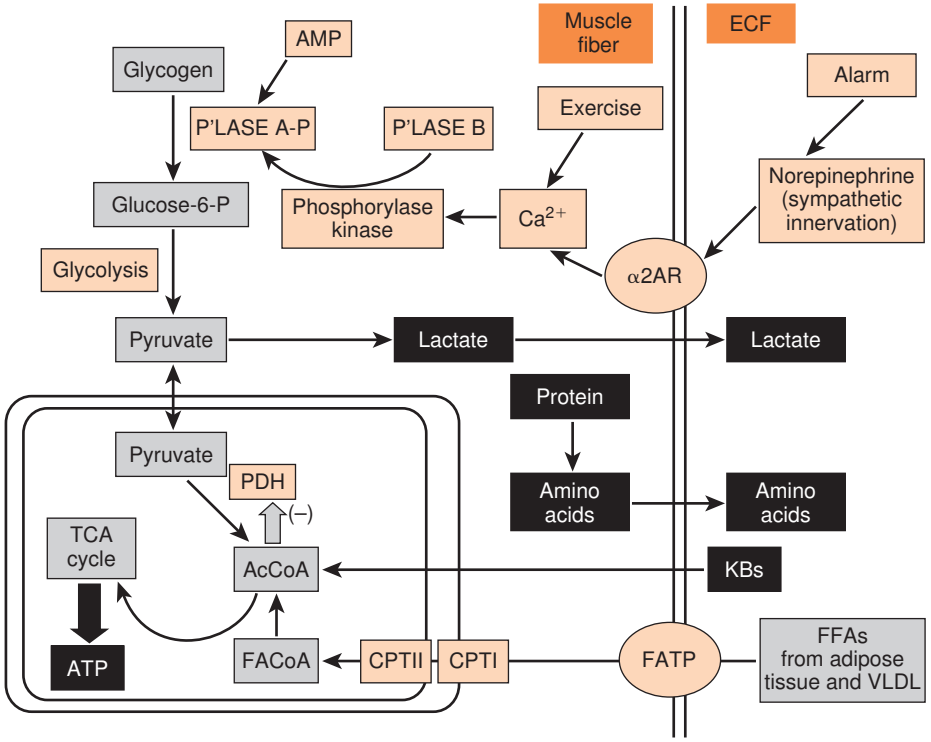

FIGURE 3-23 ■ Regulation of muscle metabolism during fast stage and during exercise of a fight-or-flight alarm response. α2AR, α_2-adrenergic receptor.

proteins or particles. Ketone bodies can be used by all cells with mitochondria and are used by the brain during an extended fast or starvation.

Metformin is first-line therapy to treat patients with T2DM. Metformin acts primarily at the liver, which effectively transports it into the hepatocyte cytoplasm. Metformin appears to target AMPK indirectly and promotes fatty acid oxidation, while suppressing the anabolic pathways, gluconeogenesis, and de novo lipogenesis.

SKELETAL MUSCLE

In the skeletal muscle, a high catecholamine-to-insulin ratio promotes increased proteolysis and decreased protein synthesis. This results in the release of gluconeogenic and ketogenic amino acids for use by the liver.

Skeletal muscle is very capable of using FFAs for energy. FFAs enter myocytes and generate high levels of intramitochondrial acetyl CoA (see Fig. 3-23). As in the liver, pyruvate dehydrogenase is inhibited by the relatively abundant acetyl CoA generated by β-oxidation. Thus, more pyruvate is converted to **lactate** (there is no pyruvate carboxylase or gluconeogenesis in muscle), and the released lactate is used by the liver for gluconeogenesis. Skeletal muscle also expresses **lipoprotein lipase (LPL)** in an **insulin**-independent manner, and skeletal muscle LPL increases during the fast state and exercise. This allows skeletal muscle to import FFAs from two sources: circulating FFA-albumin complexes and VLDL.

As in the liver, PKA and fatty acids activate **PPARα**, which drives the process of lipid oxidation. The switch from glucose to FFAs as a source of energy, along with low levels of GLUT4 in the cell membrane (due to low insulin), allows the skeletal muscle to **spare glucose** for the brain and obligate glucose users.

During exercise, muscle glycogen is mobilized for ATP production (see Fig. 3-23). **Glycogenolysis** in muscle is largely driven by **intracellular Ca^{2+}**, which activates phosphorylase kinase as a Ca^{2+}-calmodulin complex. In response to **acute stress** or **alarm, norepinephrine** is released as a fight-or-flight reaction. Norepinephrine signals through the **α_1-adrenergic**

receptor that is coupled to a **Gq/phospholipase C signaling pathway**. This ultimately causes a rapid release of **intracellular Ca^{2+}** (see Chapter 1). **AMP** is also an allosteric activator of **muscle-specific glycogen phosphorylase**. During exercise in a fasted individual, the depletion of ATP also leads to activation of **AMPK**, which also activates **PPARα** and promotes **lipid oxidation**. However, AMPK also increases GLUT4 transporters at the cell membrane, thereby increasing glucose uptake, and activates glycolysis. Thus, exercise has the potential of causing **hypoglycemia**.

Adipose Tissue

In adipose tissue, **catecholamines** (and to a much lesser extent, glucagon) stimulate phosphorylation of **HSL** and **perilipin proteins** that surround and stabilize fat droplets (Fig. 3-24). Phosphorylated perilipins dissociate from the triglyceride-cytoplasm interface and allow access to **adipose triglyceride lipase (ATGL)** and **hormone-sensitive lipase**, which is activated by phosphorylation. Complete deesterification of TGs results in the production of FFAs and glycerol. FFAs circulate in the blood as **FFA-albumin complexes**, and, as discussed previously, become a very important source of energy substrate in muscle and liver. This use of FFAs, especially by skeletal muscle, plays an essential **glucose-sparing role**.

The adipocyte uses FFAs (and ketone bodies) for ATP synthesis because it has very little access to carbohydrates in the fast state. The ability of adipocytes to import glucose is very low because only basal levels of insulin-dependent GLUT-4 transporters are localized at the cell membrane, and the adipocyte does not store glycogen. The low abundance of glucose also results in very low levels of glycerol-3-phosphate. This results in minimal reesterification of released FFAs back to acylglycerides, avoiding a futile cycle.

In summary, both skeletal muscle and adipose tissue contribute to circulating blood glucose through the release of gluconeogenic substrates (lactate, amino acids, glycerol) and indirectly through the release of FFAs, which allow skeletal muscle and other tissues to consume less glucose (glucose-sparing). Finally, release of FFAs and ketogenic amino acids supports ketogenesis by the liver.

ADIPOSE TISSUE–DERIVED HORMONES AND ADIPOKINES

White adipose tissue (WAT) contributes to the regulation of energy metabolism in the adult through the production of hormones and **adipokines**. Adipose tissue is composed of several cell types. The TG-storing

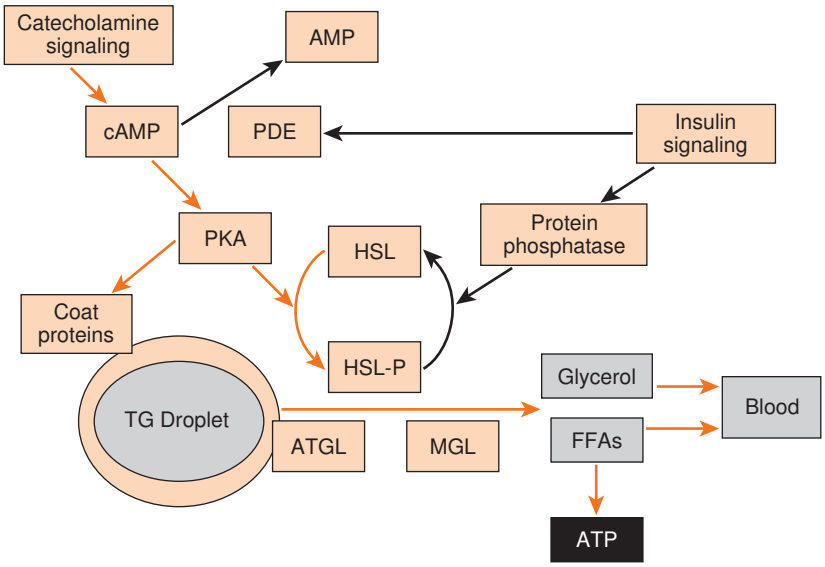

FIGURE 3-24 ■ Regulation of lipolysis in the adipocyte during the fast stage. ATGL, adipocyte triglyceride lipase; HSL, hormone-sensitive lipase; MGL, monoglyceride lipase; PDE, cyclic AMP phosphodiesterase; PKA, protein kinase A.

cell is called the adipocyte. These cells develop during gestation in humans from preadipocytes. This process of adipocyte differentiation, which may continue throughout life, is promoted by several transcription factors. One of these factors is SREBP1C, which regulates genes involved in FFA and TG synthesis. SREBP-1 C is activated by lipids as well as insulin and several growth factors and cytokines. Another important transcription factor in adipose tissue is PPARγ. PPARγ is a member of the nuclear receptor superfamily and the natural ligands for PPARγ are FFAs and their derivatives. Activated PPARγ promotes expression of genes involved in TG storage. Thus, an increase in food consumption leads to SREBP1C and PPARγ activation, which increases preadipocyte differentiation into small adipocytes, and an up regulation of enzymes within these cells to allow storage of the excess fat. The thiazolidinediones (TZDs) are pharmacologic activators of PPARγ that are used to treat insulin resistance and T2DM because they increase the adipose storage depot, but some forms of TZDs have several serious side effects and contraindications.

In addition to adipocytes, about 50% of WAT is composed of nonadipocyte cells, including resident connective tissue cells (e.g., fibroblasts, macrophages) and a connective tissue matrix, cells associated with blood vessels, and cells associated with inflammatory and immune responses. WAT also receives a rich autonomic innervation. Several cell types contribute to the integrated endocrine function of WAT.

WAT is divided into **subcutaneous** and **intra-abdominal (visceral)** depots. Intra-abdominal WAT refers primarily to omental and mesenteric fat and is the smaller of the two depots. These depots receive different blood supplies that are drained in a fundamentally different way, in that the venous return from the intra-abdominal fat leads into the hepatic portal system. Thus, intra-abdominally derived FFAs are mostly cleared by the liver, whereas subcutaneous fat is the primary site for providing FFAs to muscle during exercise or fasting. The regulation of intra-abdominal and subcutaneous adipose tissue also differs. These depots are innervated by distinct sets of neurons within autonomic nuclei in the spinal cord and brainstem and are influenced differently by sex steroids. Men tend to gain fat in the intra-abdominal depot (**android [apple-shaped] adiposity**), whereas women tend to gain fat in the subcutaneous depot, particularly in the thighs and buttocks (**gynecoid [pear-shaped] adiposity**). Finally, these two depots display differences in hormone production and enzyme activities (Table 3-2).

Visceral adipose tissue secretes less **adiponectin,** which has beneficial effects on insulin resistance through activation of PPARα and AMPK. Adiponectin also has a strong beneficial action on cardiovascular vascular tissue, including exerting anti-inflammatory effects, opposing oxidative stress, and increasing cell survival in response to disease or stress (see Table 3-2). Visceral tissue also secretes less **leptin**. Leptin has an important role in liporegulation in peripheral tissues. Leptin protects peripheral tissues (i.e., the liver, skeletal muscle, cardiac muscle, β cells) from the accumulation of too much lipid, directing storage of excess caloric intake into adipose tissue. This action of leptin, while opposing the lipogenic actions of insulin, contributes significantly to the maintenance of insulin sensitivity (as defined by insulin-dependent glucose uptake) in peripheral tissues. Leptin also acts as a signal that the body has sufficient energy stores to allow for reproduction and to enhance erythropoiesis, lymphopoiesis, and myelopoiesis. For example, in women suffering from anorexia nervosa, leptin levels are extremely low, resulting in low ovarian steroids, amenorrhea (lack of menstrual bleeding), anemia due to low red blood cell production, and immune dysfunction, which promotes lipid oxidation and glucose uptake and use in nonadipose tissue.

Visceral adipose tissue secretes more **inflammatory cytokines than subcutaneous adipose tissue**, including **tumor necrosis factor-α (TNF-α)** and **interleukin-6 (IL-6)**. These cytokines oppose insulin signaling at the receptor and post-receptor levels.

APPETITE CONTROL AND OBESITY

The amount of energy stored by an individual is determined by calorie intake and calories expended as energy/day. In many individuals, input and output are in balance, so the weight of that individual remains relatively constant. However, the abundance of inexpensive high-fat, high-carbohydrate food, along with more sedentary lifestyles, is contributing

TABLE 3-2

Adipose Tissue– and Stomach-Derived Hormones Involved in Metabolism, Appetite, and Insulin Resistance and Sensitivity

HORMONE/ CYTOKINE	CELL OF ORIGIN	SC VS. IA	STIMULUS FOR SECRETION	PRIMARY TARGET	ACTIONS
Leptin	Adipocyte	SC > IA	Increased adiposity	Hypothalamus	Decreases appetite Increases energy expenditure in adipose and nonadipose tissue Improves insulin sensitivity Allows for reproductive maturity
Adiponectin	Adipocyte	SC > IA	Weight loss (including surgical)	Muscle Liver Blood vessels and heart Macrophages	Activates oxidation of free fatty acids Anti-inflammatory/antioxidant Improves insulin sensitivity Improves health of cardiovascular tissues and protects against cell death
Tumor necrosis factor-α	Adipocyte White adipose tissue Macrophage	IA > SC	Engorgement of adipocytes	Liver Muscle Adipocytes Other organs	Reduces adipocyte mass Proinflammatory Opposes insulin signaling Increases insulin resistance Atherogenic
Interleukin-6	White adipose tissue Macrophage	IA > SC	Other inflammatory cytokines	Liver Muscle Adipocytes Other organs	Opposes insulin signaling Increases insulin resistance Increases acute-phase protein production by liver Proinflammatory systemically
Ghrelin	P/D1 cells of stomach ε cells of islets	Not applicable	Empty stomach	Pituitary gland Hypothalamus	Increases growth hormone secretion (Opposes glucoregulatory and liporegulatory actions of insulin) Increases insulin resistance Increases appetite

to a pandemic of obesity and the pathologic sequelae of obesity, including T2DM and cardiovascular disease.

The preponderance of stored energy consists of fat, and individuals vary greatly in the amounts and percentages of body weight that are accounted for by adipose tissue. About 25% of the variance in total body fat appears to be accounted for by genetic factors. A genetic influence on fat mass is supported by the following:

- Tendency to correlate better with that of their biologic parents than with that of their adoptive parents
- Greater similarity of adipose stores in identical (monozygotic) twins, whether reared together or apart, than in fraternal (dizygotic) twins
- Greater correlation between the gains in body weight and in abdominal fat in identical twins than in fraternal twins when they are fed a caloric excess
- Discovery of several genes that cause obesity

In addition, the gestational environment has a profound effect on the body mass of the adult. The effect of maternal diet on the weight and body composition of offspring is called **fetal programming**. Low birthweight correlates with increased risk for obesity, cardiovascular disease, and diabetes. These findings suggest that the efficiency of fetal metabolism has plasticity and can be altered by the environment in utero. The development of a *thrifty* metabolism would be advantageous to an individual born to a mother who received poor nutrition and into a life that meant chronic undernourishment. However, a thrifty

metabolism increases the risk for obesity in the face of the caloric excess often confronting today's individuals.

Body Mass Index

A measure of adiposity is the **body mass index (BMI)**. The BMI of an individual is calculated as follows:

$$\text{BMI} = \text{weight (kg)}/\text{height}\,(\text{m}^2)$$

The BMI of healthy lean individuals ranges from 20 to 25. A BMI greater than 25 indicates that the individual is overweight, whereas a BMI over 30 indicates obesity. The condition of being overweight or obese is a risk factor for multiple pathologies, including insulin resistance, dyslipidemia, diabetes, cardiovascular disease, and hypertension.

Clearly, an excess of abdominal fat poses a greater risk factor for the pathologies mentioned previously. Thus, another indicator of body composition is circumference of the waist (measured in inches around the narrowest point between ribs and hips when viewed from the front after exhaling) divided by the circumference of the hips (measured at the point where the buttocks are largest when viewed from the side). This **waist-to-hip ratio** may be a better indicator than BMI of body fat, especially as it relates to the risk for developing diseases. A waist-to-hip ratio of greater than 0.95 in men, or 0.85 in women, is linked to a significantly higher risk for developing diabetes and cardiovascular disease.

Hypothalamic Neurons and Appetite Control

The **arcuate (ARC) nucleus** (a nucleus is a collection of neuronal cell bodies within the central nervous system) in the hypothalamus is the key regulator of fuel sensing and food intake. One group of ARC neurons synthesizes **pro-opiomelanocortin (POMC)** and **cocaine and amphetamine-regulated transcript (CART)**. As discussed more in Chapter 5, POMC is proteolytically processed in a cell specific manner: in the ARC POMC/CART neurons, POMC is processed to form **α-MSH**. POMC/CART neurons project to second-order neurons located in several areas of the brain, including the ventromedial hypothalamus, the lateral hypothalamus, and the brainstem. α-MSH binds to the **melanocortin receptors, MC3R** and **MC4R**, on second-order neurons, which innervate other areas of the central nervous system and orchestrate the cessation of eating (**anorexigenic** effect) and an increase in energy output. These circuits also coordinate autonomic nervous system activity, with diverse endocrine actions on thyroid gland function, reproduction, and growth. CART has a similar anorexigenic action. A second group of neurons expresses the peptides, **NPY** and **agouti-related peptide (AgRP)**. AgRP competes for α-MSH at MC4R receptors and inhibits their activation. NPY acts at NPY receptors and is a potent orexigen. Thus, the NPY/AgRP neurons increase eating (orexigenic effect) and diminish energy use.

Leptin represses signaling from the NPY/AgRP and stimulates the production of POMC-derived α-MSH and the production of CART, both of which inhibit food intake. Thus, leptin acts as a satiety signal that decreases food consumption and increases energy expenditure (Fig. 3-25).

To maintain overall energy homeostasis, the system must also balance specific nutrient intake and expenditure, for example, CHO intake with CHO oxidation. This may account for some specificity in neuropeptide

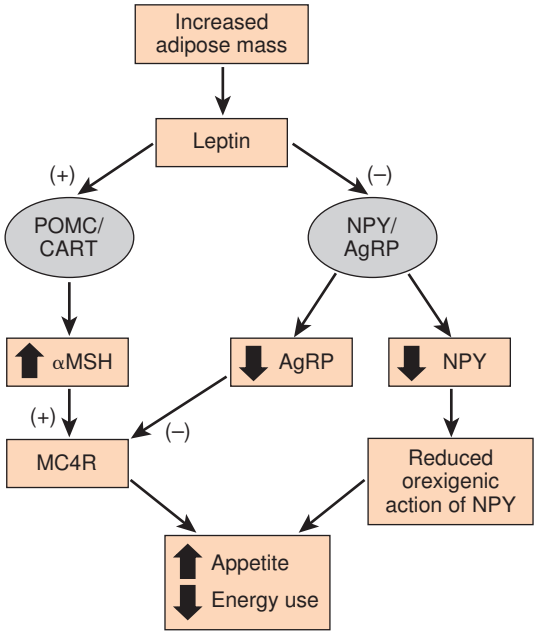

FIGURE 3-25 ■ Regulation of appetite by the action of leptin on two groups of neurons in the arcuate nucleus of the hypothalamus. See text for explanation. *Thin arrows* denote inhibited action.

and neurotransmitter responses to meals. Serotonin produces satiety after glucose ingestion. Gastrointestinal hormones, such as CCK and GLP-1, produce satiety by humoral effects, but their local production in the brain may participate in nutrient and caloric regulation. Insulin is also an important regulator of appetite. The recently discovered hormone **ghrelin** (see Table 3-2) is an acylated peptide with potent orexigenic activity that arises in endocrine cells in the mucosa of the stomach. Plasma levels of ghrelin rise in humans in the 1 to 2 hours that precede their normal meals. The plasma levels of ghrelin fall drastically to minimal values about 1 hour after eating. Ghrelin appears to stimulate food intake by reacting with its receptor in hypothalamic neurons that express NPY.

DIABETES MELLITUS

DM is a disease in which insulin levels and responsiveness of tissues to insulin are insufficient to maintain normal levels of plasma glucose. Although the diagnosis of DM is based primarily on plasma glucose, DM also causes dyslipidemia (high TG-rich lipoproteins, low high-density lipoprotein [HDL]). Normal fasting (i.e., no caloric intake for at least 8 hours) plasma glucose levels should be below 100 mg/dL. A patient is considered to have impaired glucose control if the fasting plasma glucose is between 110 and 126 mg/dL, and the diagnosis of DM is made if the fasting plasma glucose exceeds 126 mg/dL on two successive days. Another approach to the diagnosis of diabetes is the oral glucose tolerance test. After overnight fasting, the patient is given a bolus amount of glucose (usually 75 g) orally, and blood glucose levels are measured at 2 hours. The glucose is administered orally rather than intravenously (IV) because the insulin response to an oral glucose load is faster and greater than the response to an IV load (i.e., the incretin effect; see Chapter 2). A 2-hour plasma glucose greater than 200 mg/dL on 2 consecutive days is sufficient to make the diagnosis of DM. The diagnosis of diabetes is also indicated if the patient presents with symptoms associated with diabetes (see later) and has a nonfasting plasma glucose value of greater than 200 mg/dL.

T1DM and **T2DM** represent the two major forms of DM. T1DM accounts for about 10% of newly diagnosed cases. T1DM usually, but not always, occurs in the early teenage years. T1DM involves an autoimmune-mediated destruction of the islet β cells. T1DM represents an absolute deficit in insulin. Untreated T1DM results in runaway catabolic and starvation-associated metabolism, in which FFAs released from adipose tissue flood the liver and are converted into ketone bodies, while hepatic glucose production is increased and glucose uptake by muscle is minimal. The rampant ketogenesis leads to **diabetic ketoacidosis**, which is a form of metabolic acidosis. Uncorrected ketoacidosis can lead to cardiovascular collapse and coma. Diabetic ketoacidosis also results in the loss of K^+ from the intracellular compartment and ultimately from the body through increased renal excretion. Thus, K^+ replacement needs to be one component of the treatment of diabetic ketoacidosis. Hyperglycemia causes osmotic diuresis and dehydration but ultimately can lead to systemic hyperosmolality and neurologic dysfunction and coma. T1DM is usually not associated with obesity. Instead, muscle wasting and dehydration promote weight loss, muscle pain, and weakness. Patients experience frequent urination matched with excessive thirst and frequent drinking (**polydipsia**). Patients also experience hunger due to imbalances in the hypothalamic signaling, leading to frequent eating (**polyphagia**). Patients with T1DM require insulin replacement therapy.

T2DM is by far the more common form, accounting for 90% of diagnosed cases. However, T2DM is usually a progressive and insidious disease that remains undiagnosed in a significant percentage of patients for several years. T2DM is often associated with visceral obesity and lack of exercise; indeed, obesity-related T2DM is reaching epidemic proportions worldwide. Usually, there are multiple causes for the development of T2DM in a given individual that are associated with defects in the ability of target organs to respond to insulin (i.e., **insulin resistance**), along with some degree of β-cell damage and deficiency. Insulin sensitivity can be compromised at the level of the insulin receptor (IR) or, more commonly, at the level of postreceptor signaling. T2DM appears to be the consequence of insulin resistance, followed by **reactive hyperinsulinemia**, but ultimately by **relative hypoinsulinemia** (i.e., inadequate release of

insulin to compensate for the end-organ resistance). Although *insulin resistance* specifically refers to an inability of insulin to maintain blood glucose levels below normal upper limits, the underlying causes of insulin resistance differ among patients. Three major underlying causes of obesity-induced insulin resistance are as follows:

1. *A decreased ability of insulin to increase GLUT4-mediated uptake of glucose, especially by skeletal muscle.* This function, which is specifically a part of the glucometabolic regulation by insulin, may be due to the excessive accumulation of TG in the muscle of obese individuals. Excessive caloric intake induces hyperinsulinemia. Initially, this leads to excessive glucose uptake into skeletal muscle. Just as in the liver (see Fig. 3-13), excessive calories in the form of glucose promote lipogenesis and, through the generation of malonyl CoA, repression of fatty acyl CoA oxidation. By-products of fatty acid and TG synthesis, such as diacylglycerol and ceramide, may accumulate and stimulate signaling pathways (e.g., protein kinase C–dependent pathways) that antagonize signaling from the insulin receptor or IRS proteins. Thus, insulin resistance in the skeletal muscle of obese individuals may be due to **lipotoxicity**. High caloric intake is also associated with abundant circulating amino acids, which stimulate mTORC1. mTORC1 negatively feeds back on the insulin receptor and IRS proteins.

CLINICAL BOX 3-3

Exercise and weight loss are effective treatments for obesity-related insulin resistance and T2DM. The beneficial effects from exercise are due, in part, to the activation of AMPK. AMPK activates PPARγ and lipid oxidation and inhibits de novo lipogenesis. The oral hypoglycemic agent, metformin, is a front-line therapy for T2DM and appears to act, in part, through activation of AMPK. Metformin is readily transported by hepatocytes and inhibits hepatic glucose production and increases lipid oxidation. Metformin also uncouples oxidative phosphorylation, allowing more calories to be lost as heat. Fibrate drugs target PPARα, which stimulates FFA oxidation. Fibrates are used to lower circulating lipids in T2DM patients with dyslipidemia.

2. *A decreased ability of insulin to repress hepatic glucose production.* The liver makes glucose by glycogenolysis in the short term and by gluconeogenesis in the long term. The ability of insulin to repress key hepatic enzymes in both of these pathways is attenuated in insulin-resistant individuals. Insulin resistance in the liver may also be due to lipotoxicity in obese individuals (e.g., **fatty liver** or **hepatic steatosis**). The degree of insulin resistance is correlated to degree of visceral (e.g., abdominal) obesity. Note that secreted products of visceral adipose tissue enter the hepatic portal system, conveying these products directly to hepatocytes. Visceral adipose tissue is likely to affect insulin signaling at the liver in several ways, in addition to the effects of lipotoxicity. For example, visceral adipose tissue releases the cytokine, TNF-α, which has been shown to antagonize insulin signaling pathways. Also, TG in visceral adipose tissue has a high rate of turnover (possibly owing to a rich sympathetic innervation) so that the liver is exposed to high levels of FFAs, which further exacerbate hepatic lipotoxicity.
3. *An inability of insulin to repress hormone-sensitive lipase or increase LPL in adipose tissue.* High HSL and low LPL are major factors in the **dyslipidemia** associated with insulin resistance and diabetes. The dyslipidemia is characterized as **hypertriglyceridemia** and large TG-rich VLDL particles produced by the liver. Because of their high TG content, large VLDLs give rise to **small, dense low-density lipoprotein (LDL) particles**, which are very atherogenic, and low levels of HDL particles, which normally play a protective role against vascular disease. Insulin resistance in adipose tissue is likely due to the production of anti-insulin local factors, such as TNF-α and other inflammatory cytokines. Note that reduction of LPL in adipose tissue results in the import of more TG by the liver upon endocytosis of TG-rich chylomicron remnants.

CLINICAL BOX 3-4

The ability of hepatocytes to export TG is critical to their viability and normal function. TGs are not normally stored in the liver to a large extent. However, a sedentary lifestyle and overeating can result in intrahepatocyte TG levels that are out of balance with VLDL synthesis and export as well as FFA oxidation. This leads to the development of **hepatic steatosis (fatty liver)** and insulin resistance at the liver. Hepatic steatosis predisposes the liver to more serious disease, such as **hepatocellular carcinoma** and **fibrotic changes.** Hepatic steatosis represents the inability of the liver to form and export VLDL at a pace that equals the influx of TG (via chylomicron remnants), FFAs, and carbohydrates and is closely associated with **diet-induced obesity**.

Numerous other factors promote insulin resistance and may act at skeletal muscle, liver, and adipose tissue. Hyperinsulinemia per se causes down regulation of the insulin receptor and components of the insulin receptor signaling pathway (especially IRS proteins) and activates intracellular negative feedback pathways such as the **suppressor of cytokine signaling-3** (SOCS3) pathway and **mTORC1.** Inflammatory cytokines (e.g., IL-6) similarly increase SOCS3, thereby inducing a **crossover negative feedback** loop in which insulin signaling is inhibited. Glucocorticoids, which are released in response to stress and acute hypoglycemia, are diabetogenic. Sex steroids also antagonize insulin signaling. The growth hormone prolactin and its homolog human placental lactogen (which is also high during pregnancy) also induce insulin resistance. Finally, the ARC region of the hypothalamus, acting through the autonomic nervous system, can induce insulin resistance.

As insulin resistance worsens, reactive hyperinsulinemia progressively increases in an attempt to regulate glucose. This often leads to some degree of compromised β-cell function; patients with T2DM may require insulin therapy at some point in their life. Patients with T2DM can also benefit from agents that optimize β-cell function, such as sulfonylurea drugs or GLP-1 analogs.

Long-Term Sequelae of Diabetes Mellitus

Hyperglycemia leads to elevated intracellular glucose in specific cell types, especially endothelial cells in the retina, kidney, and capillaries associated with peripheral nerves. This glucotoxicity alters cell function in several ways that may contribute to pathologic changes. These include increased synthesis of **polyols, hexosamines,** and **diacylglycerol** (which activates protein kinase C). Although the exact mechanisms by which intracellular accumulation of these molecules causes abnormal cell function remain unclear, current thinking indicates that these changes lead to increased oxidative stress within the cell. Additionally, intracellular nonenzymatic glycation of proteins gives rise to **advanced glycation end products (AGEs)**. Intracellular AGEs have altered function, whereas secreted AGEs in the extracellular matrix interact abnormally with other matrix components and matrix receptors on cells. Finally, some secreted AGEs interact with receptors on macrophages and endothelial cells. Endothelial **receptors for AGEs (RAGEs)** lead to proinflammatory gene expression.

An important circulating product of glycation is **hemoglobin A_{1c} (HbA_{1c})**, which is a useful marker for long-term glucose regulation. A red blood cell has a 120-day life span; once glycation occurs, the hemoglobin remains glycated for the remainder of the red blood cell's life span. The proportion of HbA_{1c} present in a nondiabetic person is low. However, a diabetic patient who has had prolonged periods of hyperglycemia over the past 8 to 12 weeks will have elevated levels. HbA_{1c} measurements are clinically useful for checking treatment compliance.

Retinopathies are various forms of retinal abnormalities that develop in diabetic patients. Retinopathies are the major cause of new-onset blindness in preretirement adults in the United States. Hyperglycemia results in high intracellular glucose concentrations in retinal endothelial cells and pericytes (capillary supportive cells). This is due to the inability of these cells specifically to adapt to hyperglycemia by decreasing GLUT expression. As discussed earlier, elevated intracellular glucose probably initiates multiple mechanisms that ultimately lead to endothelial cell dysfunction, leading to increased resistance, hypertensive-induced changes, and cell death. These microvascular changes lead to microaneurysms, increased capillary permeability, small retinal hemorrhages, and excessive microvascular proliferation. Proliferative retinopathy is caused by impaired blood flow to the retina and subsequent tissue hypoxia. Subsequent vascular degeneration can produce vitreal

hemorrhage, retinal detachment, and neovascular glaucoma, all of which can lead to severe visual loss. As blood glucose and therefore blood osmolarity rise, the volume of the lens changes, distorting vision. Diabetic patients commonly have cataracts, and sorbitol and glycosylated protein accumulation have been proposed as mechanisms for inducing cataract formation.

Peripheral nerve damage (neuropathy) can occur as a result of metabolic, oxidative, or immune-related damage to neurons or Schwann cells. Additionally, the microvasculature of peripheral nerves undergoes changes similar to those seen in retinopathies and may represent an event that is concurrent with, or causal to, peripheral neuropathy. Schwann cells (supportive cells involved in myelination) are among those shown to accumulate sorbitol as a result of hyperglycemia. Diabetic patients can exhibit sensory loss, paresthesias, and even pain as a result of the neurologic damage. Neuropathies of the autonomic nerves also develop in diabetic patients, which can lead to numerous symptoms in multiple organ systems, including erectile dysfunction, postural hypotension, and heat intolerance. The sensory loss is more apparent in the extremities, particularly the lower portions of the legs and feet. This poses particular problems because, as diabetic patients lose cutaneous sensation in the feet, they become unaware of poorly fitting shoes and are more prone to injuries. Poor peripheral circulation aggravates this problem. Because diabetic patients have impaired wound healing, foot ulcerations can become a serious threat.

Diabetes is a common cause of impairment of renal function **(nephropathy)** and is the greatest cause of end-stage renal disease in North America. Clinical or overt diabetic nephropathy is characterized by the loss of greater than 300 mg of albumin in the urine over a 24-hour period (microalbuminuria) and progressive decline of renal function. Nephropathies develop from microvascular changes that occur in the glomerular capillaries. The glomerular capillary basement membrane thickens, resulting in thicker walls and narrower lumina (glomerulosclerosis) and expansion of the supportive mesangial cells. Podocytes detach and undergo apoptosis. Poor renal filtration also leads to activation of the renin-angiotensin system (see Chapter 7), inducing hypertension.

Atherosclerosis develops in diabetic patients at an accelerated rate **(macroangiopathies)**. Diabetic patients are more likely to have coronary artery disease and myocardial infarction than are nondiabetic individuals. Macrovascular disease is also associated with necrosis of lower extremities and the need for amputation. Many diabetic patients with coronary artery disease have the additional risk factors of **hypertension, abdominal obesity, insulin resistance,** and **dyslipidemia.** This cluster of factors has been identified as the **metabolic** syndrome (also called *syndrome X, insulin resistance syndrome,* and *cardiovascular dysmetabolic syndrome*). Some of the consequences of visceral obesity, insulin resistance, and dyslipidemia were discussed earlier.

SUMMARY

1. Cells make ATP to meet their energy needs. ATP is made by glycolysis and by the TCA cycle coupled to oxidative phosphorylation.
2. Cells express intracellular sensors of nutrients and energy. Two of these are mTORC1, which senses amino acids and growth factor/insulin signaling and promotes anabolic pathways; and AMPK, which senses a low level of energy (AMP/ATP ratio) and inhibits anabolic pathways, while driving catabolic pathways.
3. Cells can oxidize carbohydrate (primarily in the form of glucose), amino acids, and FFAs to make ATP. Additionally, the liver makes ketone bodies (as well as glucose) for other tissues to oxidize for energy in times of fasting.
4. Some cell types are limited in what energy substrates they can oxidize for energy. The brain is normally exclusively dependent on glucose for energy. Thus, blood glucose must be maintained above 60 mg/dL for normal autonomic and central nervous system function. Conversely, inappropriately high levels of glucose (i.e., fasting glucose above 100 mg/dL) promote glucotoxicity in specific cell types, leading to the long-term complications of diabetes.
5. The endocrine pancreas produces the hormones insulin, glucagon, somatostatin, gastrin, ghrelin, and pancreatic polypeptide.
6. Insulin is produced from the β cells and is an anabolic hormone that is secreted in times of excess

nutrient availability. It allows the body to use carbohydrates as energy sources and store nutrients.

7. Major stimuli for insulin secretion include increased serum glucose and certain amino acids. Cholinergic (muscarinic) receptor activation also increases insulin secretion, whereas α_2-adrenergic receptors inhibit insulin secretion. The GI tract releases incretin hormones that stimulate pancreatic insulin secretion. GLP-1 and GIP are particularly potent in augmenting glucose-dependent stimulation of insulin secretion.
8. Insulin binds to the insulin receptor, which is linked to multiple pathways that mediate metabolic and growth effects of insulin.
9. During the digestive period, insulin acts on the liver to promote conversion of glucose to glucose-6-phosphate. Insulin also increases glycogenesis, glycolysis, and fatty acid synthesis in the liver. Insulin inhibits hepatic glucose production (glycogenolysis and gluconeogenesis) and ketogenesis. Insulin regulates hepatic metabolism by both regulating gene expression and posttranslational dephosphorylation events.
10. Insulin increases GLUT-4-mediated glucose uptake in muscle and adipose tissue. Insulin increases glycogenesis, glycolysis, and, in the presence of caloric excess, lipogenesis in muscle and adipose tissue. Insulin increases muscle amino acid uptake and protein synthesis. Insulin also increases fatty acid esterification and lipoprotein lipase activity and decreases HSL activity in the adipocyte.
11. Glucagon is a catabolic hormone. Its secretion increases during periods of food deprivation, and it acts to mobilize nutrient reserves. It also mobilizes glycogen, fat, and protein.
12. Glucagon is released in response to decreased serum glucose (and therefore insulin) and increased serum amino acid levels and β-adrenergic signaling.
13. Glucagon binds to the glucagon receptor, which is linked to Gs/PKA-dependent pathways.
14. The primary target organ for glucagon is the liver. Glucagon increases liver glucose output by increasing glycogenolysis and gluconeogenesis. It also increases oxidation of fatty acids and ketogenesis.
15. Glucagon regulates hepatic metabolism by both regulating gene expression, and through posttranslational PKA-dependent phosphorylation events.
16. The major counter-regulatory factors in muscle and adipose is the adrenal hormone epinephrine, and the sympathetic neurotransmitter norepinephrine. These two factors act through β_2- and β_3-adrenergic receptors to increase cAMP levels. Epinephrine and norepinephrine increase glycogenolysis and fatty acyl oxidation in muscle and increase hormone-sensitive lipase in adipose tissue.
17. It is increasingly well established that adipose tissue has an endocrine function, especially in terms of energy homeostasis. Hormones produced by adipose tissue include leptin, adiponectin, IL-6, and TNF-α.
18. The ARC of the hypothalamus is the central regulator of appetite and energy use. The POMC/CART neurons secrete α-MSH, which acts on second-order neurons to promote satiety and increase energy expenditure. NPY/AgRP neurons act to promote eating and reduce energy expenditure. Several hormones act on these neurons to control nutrient and energy balance, including leptin, insulin, CCK, and ghrelin.
19. The more common forms of DM are classified as type 1 (T1DM) and type 2 (T2DM). T1DM is characterized by the destruction of pancreatic β cells and requires exogenous insulin for treatment. T2DM can be due to numerous factors but usually is characterized as insulin resistance coupled to some degree of β-cell deficiency. Patients with T2DM may require exogenous insulin at some point to maintain blood glucose levels.
20. Obesity-associated T2DM is currently at epidemic proportions worldwide. Obesity-associated T2DM is characterized by insulin resistance due to lipotoxicity, hyperinsulinemia, and inflammatory cytokines produced by adipose tissue. T2DM is often associated with obesity, insulin resistance, hypertension, and coronary artery disease. This constellation of risk factors is referred to as *metabolic syndrome.*
21. Major symptoms of DM include hyperglycemia, polyuria, polydipsia, polyphagia, muscle wasting, electrolyte depletion, and ketoacidosis (in T1DM).
22. Long-term complications of poorly controlled diabetes are due to excess intracellular glucose (glucotoxicity) in specific cells, especially in the retina, kidney, and peripheral nerves. This leads to retinopathies, nephropathies, and neuropathies.
23. Diabetes also increases the risk for cardiovascular disease and loss of adequate blood flow in the lower extremities.

CLINICAL BOX 3-5

Statin drugs are used to treat individuals with hypercholesterolemia due to excessive LDL cholesterol. Statins target 3-hydroxy-3-methylglutaryl (HMG) CoA reductase, so one effect of these drugs is to simply lower cholesterol synthesis. Additionally, lowering cholesterol synthesis in the liver leads to an activation of SREBP2 and an increase in LDL receptor expression. This allows the liver to increase the clearance of LDL cholesterol form the blood.

Why is LDL cholesterol a risk factor for cardiovascular disease? LDL particles are relatively small and make their way into the lamina propria of the tunica intima (the layer just below the endothelial lining) of blood vessels at regions of endothelial damage and death due to hypertension, cigarette smoke, or other factors. Oxidation of LDL components leads to engulfment by macrophages that ultimately become engorged with cholesterol. At this point, macrophages are called *foam cells*. Foam cells become participants in a series of events that lead to the development of an atherosclerotic plaque. Plaques are dangerous because they can become unstable and rupture. Once this happens, the blood is exposed to prothrombic molecules. This induces the formation of blood clots (thrombi) that can occlude the arterial lumen. In the coronary arteries, a thrombus potentially leads to myocardial infarction, and in arteries in the brain, a thrombus causes a stroke.

SELF-STUDY PROBLEMS

1. During the fed state, how does the role of glycolysis in the liver differ from the role of glycolysis in adipose tissue?
2. How does the function of glycogen differ in the liver versus the skeletal muscle?
3. Normally, the brain is dependent on glucose. What other energy substrate is used by the brain during a prolonged fast? What is the origin of this substrate?
4. What is the relation between mitochondrial citrate levels and lipogenesis?
5. What two enzymes in adipocytes are dysregulated in DM that contribute to high levels of circulating TGs?
6. Why does loss of the LDL receptor give rise to high blood cholesterol?
7. What futile cycle do high levels of malonyl CoA prevent?
8. How would a mutant glucokinase with decreased transport activity affect insulin secretion?
9. How does insulin regulate the following hepatic enzymes: glucokinase, fructose-1,6-bisphosphatase, pyruvate kinase, acetyl CoA carboxylase, PEPCK? Be specific.
10. What is the basis for ketoacidosis in patients with poorly managed type 1 diabetes mellitus?
11. How is obesity related to insulin resistance?

KEYWORDS AND CONCEPTS

- Acetoacetate
- Acetyl CoA carboxylase
- Adenosine triphosphate (ATP)

For full list of keywords and concepts see Student Consult

SUGGESTED READINGS

Asher G, Schibler U: Crosstalk between components of circadian and metabolic cycles in mammals, *Cell Metab* 13:125–137, 2011.

Baker RG, Hayden MS, Ghosh S: NF-kappaB, inflammation, and metabolic disease, *Cell Metab* 13:11–22, 2011.

Benito M: Tissue specificity on insulin action and resistance: Past to recent mechanisms, *Acta Physiol* 201:297–312, 2011.

Jitrapakdee S: Transcription factors and coactivators controlling nutrient and hormonal regulation of hepatic gluconeogenesis, *Int J Biochem Cell Biol* 44:33–45, 2010.

Kim MS, Wang Y, Rodrigues B: Lipoprotein lipase mediated fatty acid delivery and its impact in diabetic cardiomyopathy, *Biochim Biophys Acta* 1821:800–808, 2012.

Lin HV, Accili D: Hormonal regulation of hepatic glucose production in health and disease, *Cell Metab* 14:9–19, 2011.

Mitra S, Goyal T, Mehta JL: Oxidized LDL, LOX-1 and atherosclerosis, *Cardiovasc Drugs Ther* 25:419–429, 2011.

Rowland AF, Fazakerley DJ, James DE: Mapping insulin/GLUT4 circuitry, *Traffic* 12:672–681, 2011.

KEYWORDS AND CONCEPTS

- Adipocyte
- Adipocytokine
- Adiponectin
- AdipoR1
- AdipoR2
- Adipose tissue
- Adrenal hormone epinephrine
- Albuminuria
- Amino acid (AA)
- AMP kinase
- Android (apple-shaped) adiposity
- ApoB48
- ApoCII
- ApoE
- ApoB100
- Apoprotein (Apo)
- APS protein
- Atherogenic
- ATP-citrate lyase
- ATP-sensitive K^+ channel
- β-hydroxybutyrate
- β-oxidation
- Brain
- Brown adipose tissue (BAT)
- Cachexia
- Cachexin
- Carnitine palmitoyl-transferase (CPTI and CPTII)
- Cholesterol ester transfer protein (ETP)
- Chylomicron
- Chylomicron remnants
- Dawn phenomenon
- Diacylglycerol
- Diet-induced obesity
- Early phase of insulin release
- Electron transport chain
- Endothelial cell dysfunction
- Energy metabolism
- Epinephrine
- Fatty acid synthase (FAS) complex
- Fatty liver
- Fibrate
- Free fatty acid (FFA)
- Fructose-1,6-bisphosphate
- Fructose-2,6-bisphosphate
- Fructose-6-phosphatase
- Fructose-bisphosphate-2
- Glucagon
- Glucokinase
- Glucometabolic
- Glucose
- Glucose sensor
- Glucose tolerance
- Glucose-6-phosphatase
- Glucose-6-phosphate dehydrogenase
- Glucotoxicity
- Glycerol kinase
- Glycogen
- Glycogen phosphorylase
- Glycogen synthase
- Glycogen synthase kinase-3
- Glycolysis
- Gynecoid (pear-shaped) adiposity
- Hemoglobin A_{1c} (HbA_{1c})
- Hepatic lipase
- Hepatic steatosis
- Hexosamines
- High-density lipoprotein (HDL)
- Hormone-sensitive lipase
- Hypoglycemia
- Insulin
- Insulin resistance
- Insulin receptor substrate (IRS)
- Intermediate-density lipoprotein (IDL)
- Ketone bodies
- Late phase of insulin release
- Leptin
- Leptin receptor (LR); long form (LRb)
- Leptin resistance
- Lipoprotein lipase (LPL)
- Lipoprotein particle
- Liporegulation
- Lipotoxicity
- Liver
- Low-density lipoprotein (LDL)
- Metformin
- Nephropathy
- Neuropathy
- Nonenzymatic glycation
- Nonesterified fatty acids
- Non-insulin-dependent diabetes mellitus

- Norepinephrine
- Oxidative phosphorylation
- Oxidized LDL
- Palmitoyl CoA desaturase
- Pancreatic islet
- Pancreatic polypeptide
- Pentose phosphate shunt
- PEP carboxykinase (PEPCK)
- Peroxisome proliferation–activated receptor-γ (PPARγ)
- Phosphoenolpyruvate carboxylase (PEPCK)
- Phosphofructokinase-1 (PFK1)
- Phosphofructokinase-2 (PFK2)
- Phosphorylase kinase
- Polydipsia
- Polyols
- Polyphagia
- Polyuria
- Proinsulin (connecting) peptide
- Protein kinase B (PKB)–dependent pathway
- Protein phosphatase-1
- Pyruvate carboxylase
- Pyruvate dehydrogenase (PDH)
- Pyruvate kinase
- Reactive oxygen species (ROS)
- Scavenger receptors
- Shc protein
- Skeletal muscle
- Somogyi effect
- Sterol-regulatory element–binding protein-1C (SREBP1C)
- Sulfonylurea drugs
- Suppressor of cytokine signaling (SOCS)
- SUR
- Sympathetic neurotransmitter norepinephrine
- Thiazolidinedione (TZD)
- Thiophorase
- Transamination
- Tricarboxylic acid (TCA) cycle
- Triglyceride (TG)
- Tumor necrosis factor-α (TNF-α)
- Type 1 diabetes mellitus (T1DM)
- Type 2 diabetes mellitus (T2DM)
- Urea cycle
- Very-low-density lipoprotein (VLDL)
- VLDL remnants
- Voltage-gated Ca^{2+} channels
- White adipose tissue (WAT)

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4 CALCIUM AND PHOSPHATE HOMEOSTASIS

OBJECTIVES

1. Describe the structure and synthesis of PTH, the regulation of PTH secretion, and the nature of the PTH receptor.
2. Describe the structure and synthesis of 1,25-dihydroxyvitamin D, the regulation of 1,25-dihydroxyvitamin D production, and the receptor for 1,25-dihydroxyvitamin D.
3. Discuss the roles of the GI tract, bone, and kidneys in Ca^{2+}/Pi homeostasis.
4. Discuss the actions of calcitonin, PTHrP, FGF23, and gonadal and steroid hormones on Ca^{2+}/Pi metabolism.
5. Discuss the pathophysiology associated with imbalances in PTH and 1,25-dihydroxyvitamin D.

Calcium ($\mathbf{Ca^{2+}}$) and phosphate (**Pi**) are essential to human life, playing important structural roles in hard tissues (i.e., bones and teeth) and important regulatory roles in metabolic and signaling pathways. The two primary sources of circulating Ca^{2+} and Pi are the diet and the skeleton (Fig. 4-1).

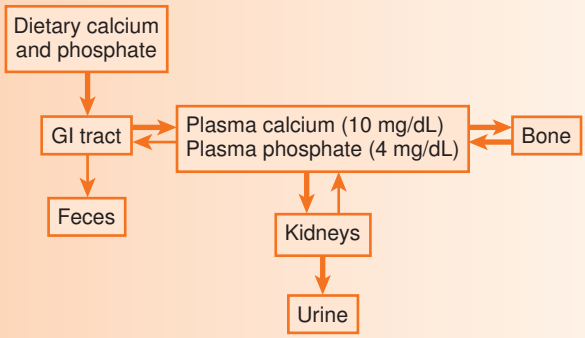


FIGURE 4-1 ■ Daily Ca^{2+} and Pi fluxes.

Two hormones, **1,25-dihydroxyvitamin D** (also called **calcitriol**) and **parathyroid hormone** (**PTH**), regulate intestinal absorption of Ca^{2+} and Pi and the release of Ca^{2+} and Pi into the circulation after bone resorption. The primary processes for removal of Ca^{2+} and Pi from the blood are renal excretion and bone formation (see Fig. 4-1), and 1,25-dihydroxyvitamin D and PTH regulate these processes as well. Other hormones and paracrine growth factors also have clinical relevance to Ca^{2+} and Pi homeostasis.

CALCIUM AND PHOSPHORUS ARE IMPORTANT DIETARY ELEMENTS THAT PLAY MANY CRUCIAL ROLES IN CELLULAR PHYSIOLOGY

Calcium is an essential dietary element. In addition to getting calcium from the diet, humans contain a vast store (i.e., >1 kg) of calcium in their bones, which can be called on to maintain normal circulating levels of

TABLE 4-1
Forms of Ca^{2+} and Pi in Plasma

ION	SIZE (MG/DL)	IONIZED (%)	PROTEIN BOUND (%)	COMPLEXED (%)
Ca^{2+}*	10	50	45	5
Pi	4	84	10	6

*Ca^{2+} is bound (i.e., complexed) to various anions in the plasma, including HCO_3^-, citrate, Pi, and SO_4^-. Pi is complexed to various cations, including Na^+ and K^+.
From Koeppen BM, Stanton BA: *Renal Physiology,* 4th ed., Philadelphia, 2007, Mosby.

calcium in times of dietary restriction and during the increased demands of pregnancy and nursing. Circulating calcium exists in three forms (Table 4-1): free ionized calcium (Ca^{2+}), protein-bound calcium, and calcium complexed with anions (e.g., phosphates, bicarbonate, citrate). The ionized form represents about 50% of circulating calcium, and because this form is so critical to many cellular functions, Ca^{2+} levels in both extracellular and intracellular compartments are tightly controlled (see Chapter 1 for discussion of Ca^{2+}-dependent signaling pathways). Circulating Ca^{2+} is under direct hormonal control and is normally maintained in a relatively narrow range. Either too little of Ca^{2+} (**hypocalcemia**; total serum $Ca^{2+} < 8.5$ mg/dL [2.1 mM]) or too much Ca^{2+} (**hypercalcemia**; total serum $Ca^{2+} > 10.5$ mg/dL [2.6 mM]) in the blood can lead to a broad range of pathophysiologic changes, including neuromuscular dysfunction, central nervous system dysfunction, renal insufficiency, calcification of soft tissue, and skeletal pathologies.

Phosphorus is also an essential dietary element and is stored in large quantities in bone complexed with calcium. In the blood, most phosphorus exists in the ionized form of phosphoric acid, which is called **inorganic phosphate** (**Pi**). Most circulating Pi is in the free ionized form, but some Pi (<20%) circulates as a protein-bound form or complexed with cations (see Table 4-1). Phosphorus also exists as pyrophosphate (two Pi groups in a covalent linkage). Unlike Ca^{2+}, phosphate is incorporated covalently as single or multiple phosphate groups into many molecules, and consequently, soft tissues contain about 10-fold more phosphate than Ca^{2+}. This means that significant tissue damage (e.g., crush injury with massive muscle cell death) can result in **hyperphosphatemia**, which can then complex with Ca^{2+} to cause acute hypocalcemia. Phosphate represents a key intracellular component. Indeed, it is the high-energy phosphate bonds of adenosine triphosphate (ATP) that maintain life. Phosphorylation and dephosphorylation of proteins, lipids, second messengers, and cofactors represent key regulatory steps in numerous metabolic and signaling pathways, and phosphate also serves as the backbone for nucleic acids.

PHYSIOLOGIC REGULATION OF CALCIUM AND PHOSPHATE: PARATHYROID HORMONE AND 1,25-DIHYDROXYVITAMIN D

PTH and **1,25-dihydroxyvitamin D** represent the two physiologically important hormones that are dedicated to the maintenance of normal blood Ca^{2+} and Pi levels in humans. As such, they are referred to as a **calciotropic hormones**. The structure, synthesis, and secretion of these two hormones and their receptors will be discussed here. In the following section, the detailed actions of PTH and 1,25-dihydroxyvitamin D on the three key sites of Ca^{2+}/Pi homeostasis (i.e., gut, bone, and kidney) will be discussed.

Parathyroid Hormone

PTH is a key hormone that protects against a hypocalcemic challenge. The primary targets of PTH are bone and kidneys. PTH also functions in a positive feed-forward loop by stimulating 1,25-dihydroxyvitamin D production.

Parathyroid Glands The **parathyroid glands** develop from the endodermal lining of the third and fourth branchial pouches. They usually develop into four loosely organized glands: two superior and two inferior parathyroid glands. The embryonic anlage of the parathyroids become associated with the caudal migration of the thyroglossal duct, so the parathyroid glands usually become situated on the dorsal side of the right and left lobes of the thyroid gland (Fig. 4-2). The exact positions of the parathyroid glands are variable, and more than 10% of humans harbor a fifth parathyroid gland. The predominant parenchymal cell type in the parathyroid gland is the

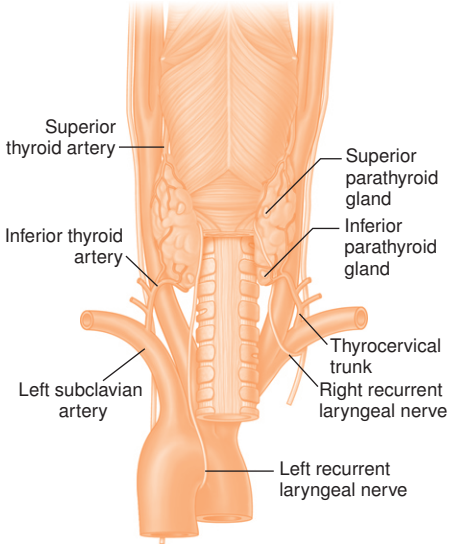


FIGURE 4-2 ■ Anatomic position of the parathyroid glands. *(Redrawn from Drake RL, Vogl W, Mitchell AWM:* Gray's Anatomy for Students, *Philadelphia, 2005, Elsevier.)*

principal (also called **chief**) **cell**. These cells are the primary endocrine cell of the gland. With age, a larger mitochondria-rich, eosinophilic cell type, the **oxyphil cell**, appears. Although the oxyphil cell is not normally important to PTH secretion, PTH-overproducing tumors (i.e., primary hyperparathyroidism) can be derived from both principal and oxyphil cells.

Structure, Synthesis, and Secretion of Parathyroid Hormone Secreted PTH is an 84-amino acid polypeptide. PTH is synthesized as a **preproPTH**, which is proteolytically processed to **proPTH** at the endoplasmic reticulum, and then to PTH in the Golgi and secretory vesicles. Unlike proinsulin, all intracellular proPTH is normally converted to PTH before secretion. PTH has a short half-life (<5 minutes) because it is proteolytically cleaved into biologically inactive N-terminal and C-terminal fragments that are excreted by the kidney.

The primary signal that stimulates PTH secretion is low circulating Ca^{2+} levels (Fig. 4-3). The extracellular Ca^{2+} concentration is sensed by the parathyroid principal cells through a **Ca^{2+}-sensing receptor** (**CaSR**). The CaSR is a member of the seven-transmembrane G-protein-coupled receptor superfamily, which forms disulfide-linked dimers in the membrane of chief cells of the parathyroid glands. The CaSR is also expressed in calcitonin-producing C cells, renal tubules, and several other tissues. In the parathyroid gland, increasing amounts of extracellular Ca^{2+} bind to the CaSR and activate incompletely understood downstream signaling pathways that repress PTH secretion. Thus, basal PTH secretion (i.e., PTH secretion in the absence of

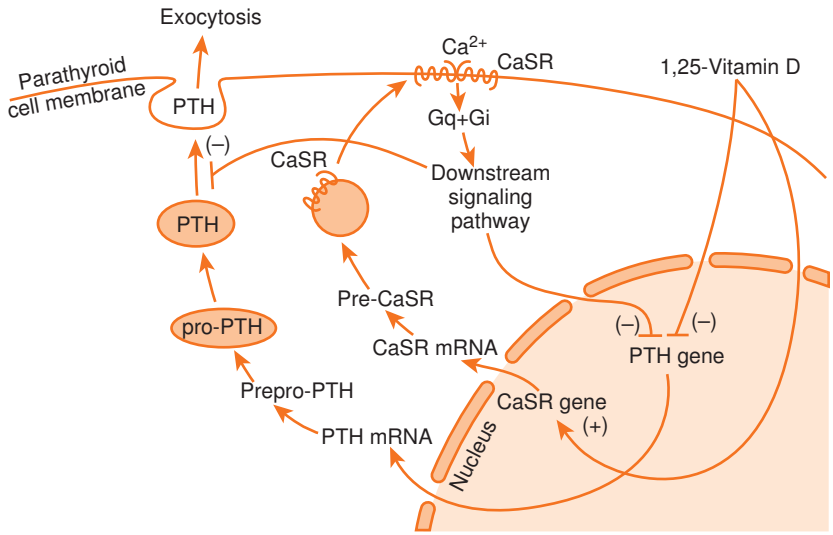


FIGURE 4-3 ■ Regulation of PTH gene expression and PTH secretion. The primary regulator of PTH is extracellular Ca^{2+}, which is sensed by the Ca^{2+}-sensing receptor (CaSR). The CaSR is a G-protein-coupled receptor (GPCR) linked to Gq and Gi, but the downstream signaling that inhibits PTH secretion and PTH gene expression is poorly understood. 1,25-Dihydroxyvitamin D inhibits PTH gene expression directly and indirectly by stimulating CaSR gene expression.

CaSR signaling) is high but is inhibited by high extracellular Ca^{2+}-CaSR binding and signaling.

Although the CaSR binds to extracellular Ca^{2+} with relatively low affinity, the CaSR is extremely sensitive to *changes* in extracellular Ca^{2+}. A 0.1-mM drop in blood Ca^{2+} produces an increase in circulating PTH levels from basal (5% of maximum) to maximal levels (Fig. 4-4). Thus, the CaSR regulates PTH output in response to subtle fluctuations in Ca^{2+} on a minute-to-minute basis. It should be noted that the CaSR is also stimulated by high levels of magnesium, so hypermagnesemia also inhibits PTH secretion.

CLINICAL BOX 4-1

Patients with **familial benign hypocalciuric hypercalcemia** (**FBHH**) or **neonatal severe hyperparathyroidism** are heterozygous or homozygous, respectively, for inactivating mutations of the CaSR. In these patients, the CaSR fails to appropriately inhibit PTH secretion in response to high levels of blood calcium. The CaSR also plays a direct role in Ca^{2+} reabsorption at the kidney. The **hypocalciuria** (i.e., inappropriately low Ca^{2+} excretion in the face of high circulating Ca^{2+} levels) in patients with FBHH is due to the lowered ability of the CaSR to monitor blood calcium and respond by increasing urinary Ca^{2+} excretion.

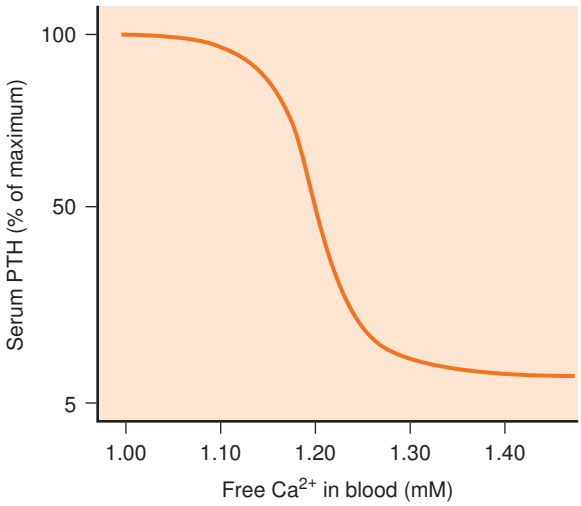


FIGURE 4-4 ■ Ca^{2+}/PTH secretion dose-response curve.

PTH production is also regulated at the level of gene transcription (see Fig. 4-3). The preproPTH gene is repressed by a **calcium-response element** within the promoter of this gene. Thus, the signaling pathway that is activated by Ca^{2+} binding to the CaSR ultimately leads to repression of preproPTH gene expression and PTH synthesis. The preproPTH gene is also repressed by 1,25-dihydroxyvitamin D (acting through vitamin D–responsive elements, discussed later). The ability of 1,25-dihydroxyvitamin D to hold PTH gene expression in check is reinforced by the coordinated up-regulation of CaSR gene expression by positive vitamin D–responsive elements in the promoter of the CaSR gene (see Fig. 4-3).

Parathyroid Hormone Receptor The PTH receptor is a seven-transmembrane, G-protein-linked membrane receptor. Because this receptor also binds PTHrP (see later), it is usually referred to as the **PTH/PTHrP receptor**. The PTH/PTHrP receptor is primarily coupled to a Gαs signaling pathway that leads to increased cyclic adenosine monophosphate (cAMP), although it also is coupled to Gαq/11-phospholipase C–dependent pathways. The PTH/PTHrP receptor is expressed on osteoblasts in bone, and in the proximal and distal tubules of the kidney, as the receptor for the systemic actions of PTH. However, the PTH/PTHrP receptor is also expressed in many developing structures in which PTHrP has an important paracrine function.

Vitamin D

Vitamin D is actually a prohormone that must undergo two successive hydroxylations to become the active form, **1,25-dihydroxyvitamin D** (Fig. 4-5). Vitamin D plays a critical role in Ca^{2+} absorption, and to a lesser extent Pi absorption, by the small intestine. Vitamin D also regulates aspects of bone remodeling and renal reabsorption of Ca^{2+} and Pi.

Structure, Synthesis, and Transport of Active Vitamin D Metabolites **Vitamin D_3** (D_3; also called **cholecalciferol**) is synthesized by ultraviolet light (UV B) conversion of 7-dehydrocholesterol in the more basal layers of the skin (Fig. 4-6). UV radiation opens up

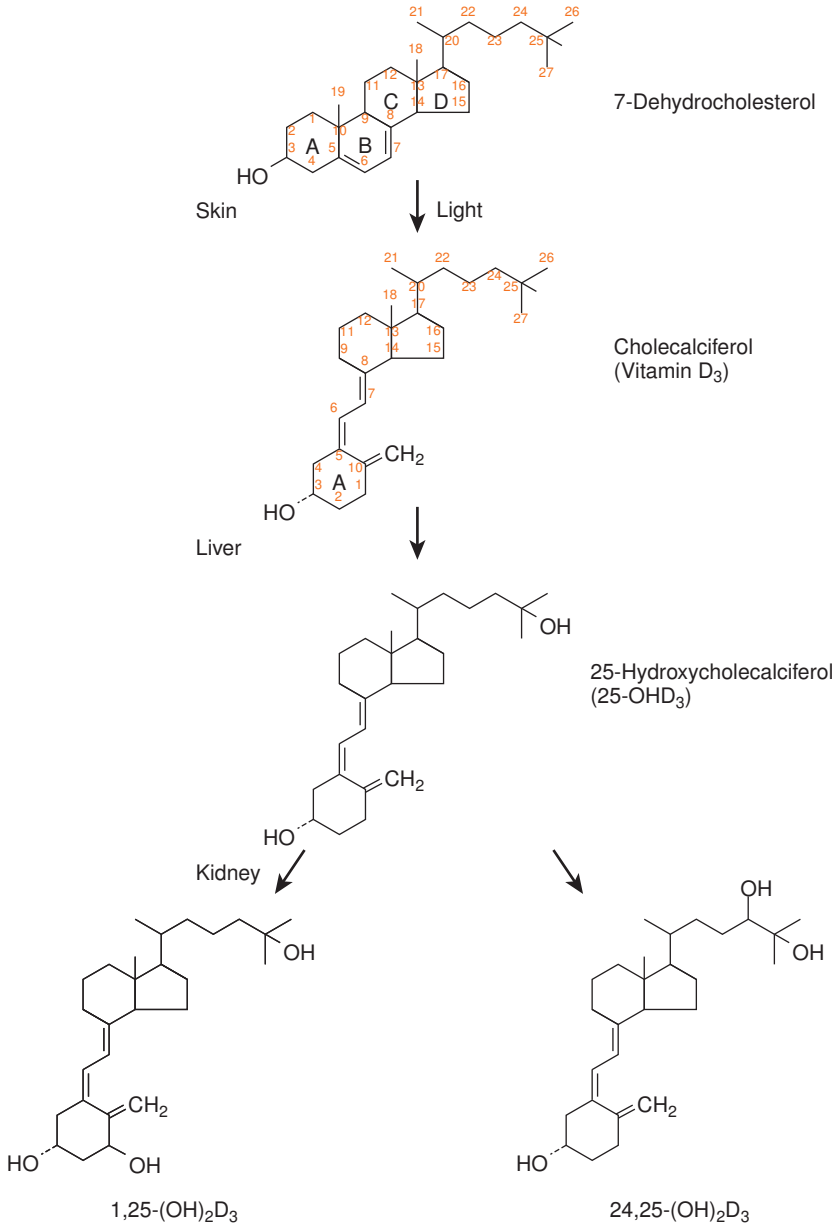


FIGURE 4-5 ■ Biosynthesis of 1,25-dihydroxyvitamin D dihydroxycholecalciferol.

the B ring of cholesterol, generating pre–vitamin D_3, which then undergoes a temperature-dependent isomerization into D_3. Vitamin D_3 is therefore referred to as a **secosteroid**, which is a class of steroids in which one of the cholesterol rings is opened. **Vitamin D_2** (D_2, also called **ergocalciferol** and also a secosteroid) is the form produced in plants. Vitamins D_3 and to a lesser extent D_2 are absorbed from the diet and are equally effective after conversion into active hydroxylated forms.

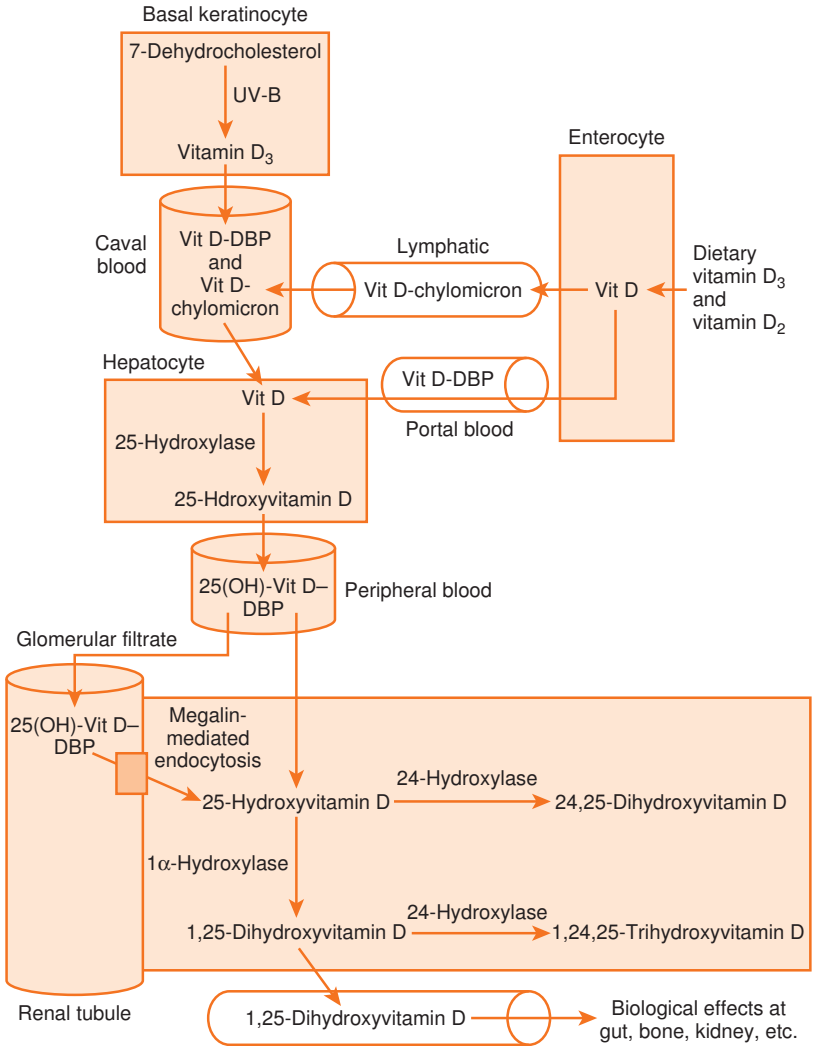


FIGURE 4-6 ■ Vitamin D metabolism. Vitamin D can be synthesized in the skin keratinocyte or absorbed from the gastrointestinal tract enterocyte. Vitamin D is transported to the liver hepatocyte, where it is hydroxylated at the 25 carbon. 25-Hydroxyvitamin D is carried in the blood by vitamin D–binding protein (DBP) to the renal proximal tubules, where it is hydroxylated at either the 1α position (activating) or the 24 position (inactivating). Note that 25-hydroxyvitamin D that exits into the glomerular filtrate can be recaptured by the apical membrane protein, megalin, which binds and internalizes DBP.

CLINICAL BOX 4-2

The balance between UV-dependent, endogenously synthesized vitamin D_3 and the absorption of dietary forms of vitamin D becomes important in certain situations. Individuals with higher epidermal melanin content and those who live in higher latitudes convert less 7-dehydrocholesterol into vitamin D_3 and thus are more dependent on dietary sources of vitamin D. Dairy products are enriched in vitamin D_3, but not all individuals tolerate or enjoy dairy products. Institutionalized, sedentary elderly patients who stay indoors and avoid dairy products are particularly at risk for developing **vitamin D deficiency**.

D_3 is transported in the blood from the skin to the liver. Dietary D_3 and D_2 reach the liver directly through transport in the portal circulation and indirectly through chylomicrons (see Fig. 4-6). In the liver, D_2 and D_3 are hydroxylated at the 25 carbon position to yield **25-hydroxyvitamin D** (at this juncture, no distinction will be made between D_3 and D_2 metabolites because they are equipotent). Hepatic vitamin D 25-hydroxylase is expressed at a relatively constant and high level, so circulating levels of 25-hydroxyvitamin D largely reflect the amount of precursor available for 25-hydroxylation. Because the hydroxyl group at the 25 carbon position represents the second hydroxyl group on the molecule,

25-hydroxyvitamin D is also referred to as **calcife*diol*** (see Fig. 4-6).

25-Hydroxyvitamin D is further hydroxylated in the mitochondria of the proximal tubules of the kidney at either the 1α carbon or 24 carbon position (see Figs. 4-5 and 4-6). The 1α-hydroxylase (also called **CYP1α** in humans) generates **1,25-dihydroxyvitamin D** (also called **calci*triol***), which is the most active form of vitamin D. Hydroxylation at the 24 position, generating **24,25-dihydroxyvitamin D** and **1,24,25-trihydroxyvitamin D**, represents an inactivation pathway.

Vitamin D and its metabolites circulate in the blood primarily bound to **vitamin D–binding protein** (**DBP**). DBP is a serum glycoprotein of about 60 kDa that is related to the albumin gene family and is synthesized by the liver. DBP binds more than 85% of 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D. As a result of binding to other proteins, only 0.4% of the active metabolite, 1,25-dihydroxyvitamin D, circulates as free steroid. DBP allows for the movement of the highly lipophilic molecules within the aqueous environment of the blood. Studies using experimental mouse genetics indicate that DBP provides a reservoir of vitamin D metabolites that protects against vitamin D deficiency. The bound fractions of vitamin D metabolites have a circulating half-life of several hours.

DBP may assist in the reuptake of the fraction of 25-hydroxyvitamin D that passes through the glomerular filter. DBP binds to 25-hydroxyvitamin D with high affinity, so a significant amount of 25-hydroxyvitamin D that enters the glomerular lumen is complexed to DBP. The apical membranes of the proximal tubule cells express the protein, **megalin**, which is a member of the low-density lipoprotein (LDL) receptor superfamily. Megalin functions to recapture a broad range of proteins from the glomerular filtrate. Megalin binds DBP, some of which is complexed with 25-hydroxyvitamin D, and internalizes the complex through receptor-mediated endocytosis (see Fig. 4-6).

The renal 1α-hydroxylase represents a key target of regulation of vitamin D action (Fig. 4-7). First, there exists a product feedback loop, in which 1,25-dihydroxyvitamin D inhibits 1α -hydroxylase expression and stimulates 24-hydroxylase expression. Ca^{2+} is also an important regulator of the renal 1α-hydroxylase. Low circulating levels of Ca^{2+} indirectly stimulate renal 1α-hydroxylase expression through increased PTH levels, whereas elevated Ca^{2+} inhibits 1α-hydroxylase activity directly through the CaSR in the proximal tubule. A low-phosphate diet also stimulates renal 1α-hydroxylase activity in a PTH-independent manner. Some of the effect of a low-phosphate diet on renal 1α-hydroxylase activity is dependent on a functional pituitary gland, which may respond to hypophosphatemia by increased growth hormone secretion (see Chapter 5).

Vitamin D Receptor 1,25-Dihydroxyvitamin D exerts its actions primarily through binding to the **nuclear vitamin D receptor** (**VDR**). The VDR is a 50-kDa protein and is a member of the nuclear hormone receptor superfamily, which also includes steroid and thyroid hormone receptors and metabolic receptors such as the PPARs (see Chapter 1). The VDR is a transcription factor and binds to DNA sequences (**vitamin D–responsive elements**) as a heterodimer with the retinoid X receptor (RXR). Thus, a primary action of 1,25-dihydroxyvitamin D is to regulate gene expression in its target tissues, including the small intestine, bone, kidneys, and parathyroid gland.

SMALL INTESTINE, BONE, AND KIDNEY DETERMINE Ca^{2+} AND PI LEVELS

The general effects of PTH and 1,25-dihydroxyvitamin D on Ca^{2+} and Pi levels at the small intestine, bone, kidneys, and parathyroid glands is summarized in Table 4-2.

Handling of Ca^{2+} and Pi by the Small Intestine

(Mosby Physiology Monograph Series cross reference: Chapter 12 in Gastrointestinal Physiology, Sixth Ed., LR Johnson)

Dietary levels of calcium can vary, but in general, North Americans consume about 1.5 kg of calcium per day. Of this, about 200 g is absorbed by the proximal small intestine. Importantly, fractional absorption of calcium is stimulated by 1,25-dihydroxyvitamin D, so absorption can be made more efficient in the face of declining dietary calcium.

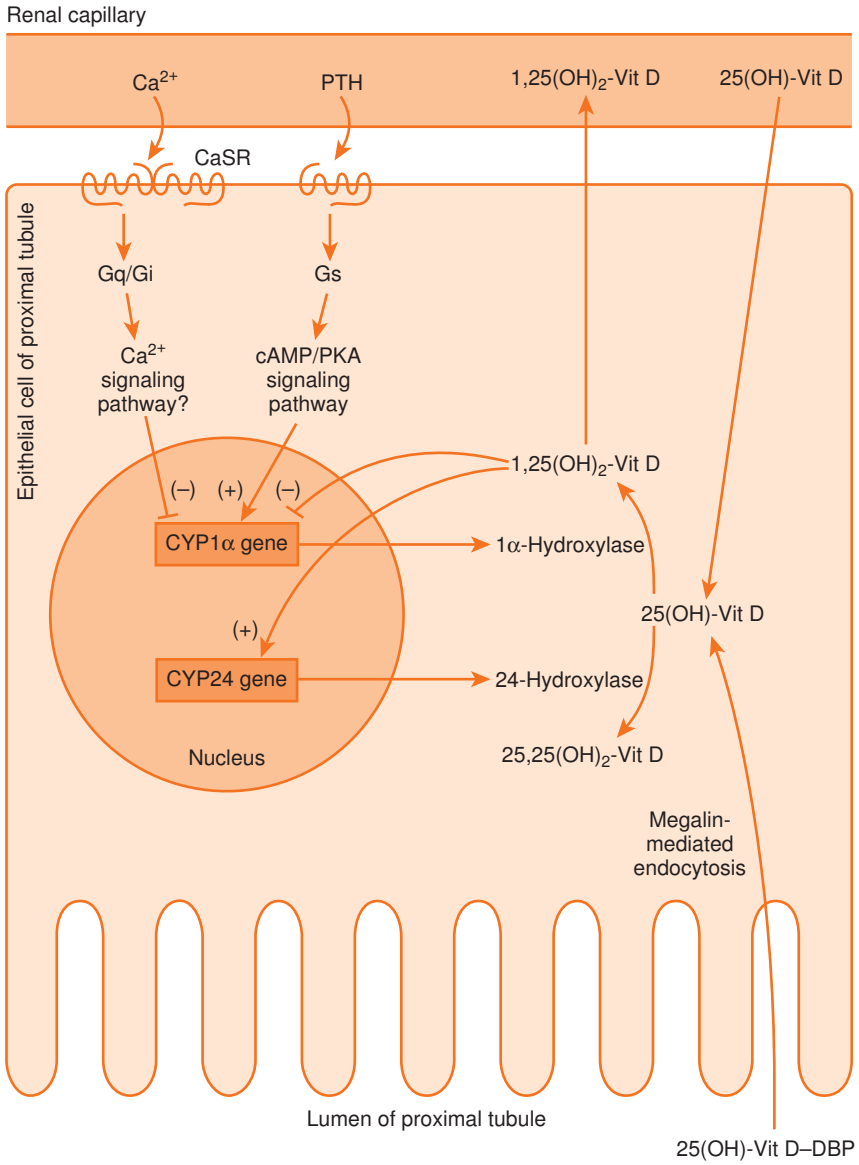


FIGURE 4-7 ■ Regulation of the renal CYP1α gene expression by Ca^{2+} and hormones.

Ca^{2+} is absorbed from the duodenum and jejunum both by a Ca^{2+}- and hormone-regulated, active-transport transcellular route and by a passive, bulk-flow paracellular route. Little is known about whether the paracellular route is regulated. However, significant progress has been made in our understanding of the transcellular route (Fig. 4-8). Ca^{2+} enters the transcellular route by gaining access to the intestinal enterocytes through the apical membrane. The movement of Ca^{2+} from the lumen of the gastrointestinal (GI) tract into the enterocyte, which is favored both by concentration and electrochemical gradients, is facilitated by apical **epithelial calcium channels**, called **TrpV5** and **TrpV6**. Once inside, Ca^{2+} ions bind to abundant cytoplasmic proteins called **calbindin-D**, specifically **calbindin-D_{9K}**, in the human intestine. Calbindin-D_{9K} serves to maintain the low cytoplasmic free Ca^{2+} concentrations, thus preserving the favorable lumen-to-enterocyte

TABLE 4-2

Actions of Parathyroid Hormone and 1,25-Dihydroxyvitamin D on Ca^{2+}/Pi Homeostasis

	SMALL INTESTINE	BONE	KIDNEY	PARATHYROID GLAND
PTH	No direct action	Promotes osteoblastic growth and survival	Stimulates 1a-hydroxylase activity	No direct action
		Regulates M-CSF, RANKL, and OPG production by osteoblast	Stimulates Ca^{2+} reabsorption by distal nephron by increasing	
		Chronic high levels promote net Ca^{2+} and Pi release from bone	Inhibits Pi reabsorption by proximal nephron (represses NPT2a expression)	
1,25-Dihydroxyvitamin D	Increases Ca^{2+} absorption by increasing TrpV channels, calbindin-D, and PMCA expression	Sensitizes osteoblasts to PTH	Minimal actions on Ca^{2+} reabsorption	Directly inhibits PTH gene expression
	Marginally increases Pi absorption	Regulates osteoid production and calcification	Promotes Pi reabsorption by proximal nephron (stimulates NPT2a expression)	Directly stimulates CaSR gene expression

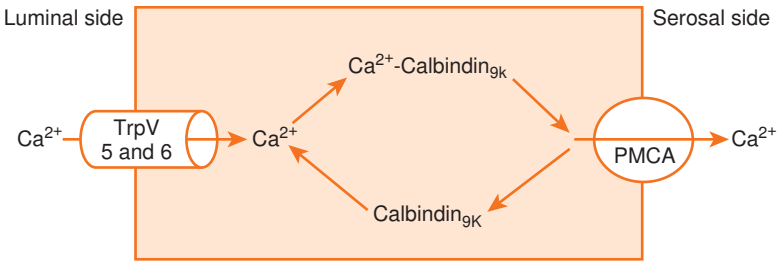


FIGURE 4-8 ■ Intestinal absorption of Ca^{2+} through the transcellular route. Ca^{2+} enters through the Ca^{2+} channel, TrpV 5 or TrpV 6, in the luminal membrane of the enterocyte. Ca^{2+} is then shuttled from the apical side of the cell to the basal side by the carrier protein, calbindin-D_{9k}. Ca^{2+} is then actively transported out of the basolateral side by the plasma membrane Ca^{2+} ATPase (PMCA), and calbindin-D_{9K} recycles. 1,25-Dihydroxyvitamin D increases the expression of all these proteins in the gastrointestinal tract.

concentration gradient during a meal. Calbindin-D_{9K} may also play a role in apical-to-basolateral shuttling of Ca^{2+}. Ca^{2+} is actively transported across the basolateral membrane, against an electrochemical and concentration gradient, by the **plasma membrane calcium ATPase** (**PMCA**). The **sodium-calcium exchanger** (**NCX**) may also contribute to the active transport of Ca^{2+} out of the enterocytes. 1,25-Dihydroxyvitamin D stimulates the expression of all of the components (i.e., TrpV channels, calbindin-D_{9K}, and PMCA) involved in Ca^{2+} uptake by the small intestine. PTH affects Ca^{2+} absorption at the gut indirectly by stimulating renal 1α-hydroxylase activity.

The fraction of phosphate absorbed by the jejunum remains relatively constant at about 70% and is under minor hormonal control by 1,25-dihydroxyvitamin D. The limiting process in transcellular Pi absorption is transport across the apical brush border, which is carried out by an isoform (NPT2b) of the **sodium-Pi cotransporter**, **NPT2**.

Handling of Ca^{2+} and Pi by Bone

Bone represents a massive and dynamic extracellular deposit of proteins and minerals (mainly Ca^{2+} and Pi). Once maximal bone mass has been achieved in the adult, the skeleton is constantly remodeled through the concerted activities of the resident bone cell types. The processes of **bone accretion** and **bone resorption** are in balance in a healthy, physically active, and appropriately nourished individual. Of the about 1 kg of calcium immobilized in bone, about 500 mg of Ca^{2+} (i.e., 0.05% of skeletal calcium) is mobilized from and deposited into bone each day. However, the process of bone remodeling can be modulated to provide a net gain or loss of Ca^{2+} and Pi to the blood and is responsive to physical activity (or lack thereof), diet, age, and hormonal regulation. Because the integrity of bone is absolutely dependent on Ca^{2+} and Pi, chronic dysregulation of Ca^{2+} and Pi levels, or of the hormones that regulate Ca^{2+} and Pi, leads to pathologic changes in bone.

Histophysiology of Adult Bone

The biogenesis, growth, and remodeling of bone is a complex process and will not be fully explained here. The key features required to understand the role of adult bone in the hormonal regulation of calcium-phosphate metabolism are discussed next.

Most of the bone (about 75%) is **compact, cortical bone** that makes up the outer surfaces of long and flat bones (Fig. 4-9). The inner core of bones is composed of interconnecting spicules whose orientation becomes organized by stress forces. This bone is called **cancellous** (or **trabecular**) bone, and although it makes up only 25% of total bone mass, its surface area is several-fold greater than that of cortical bone. The greater surface area means that trabecular bone is much more accessible to bone cells and thus more dynamic in its turnover.

In the adult, bone remodeling involves the destruction of preformed bone, with the release of Ca^{2+}, Pi, and hydrolyzed fragments of the proteinaceous matrix (called **osteoid**) into the blood; and new synthesis of osteoid at the site of resorption, with subsequent calcification of the osteoid, primarily with Ca^{2+} and Pi, from the blood. Bone remodeling occurs continually in about 2 million discrete sites involving subpopulations of bone cells, called **basic multicellular units**.

The cells involved in bone remodeling fall into two major classes: cells that promote the formation of bone (**osteoblasts**) and cells that promote the resorption of bone (**osteoclasts**). However, it should be emphasized that bone remodeling is a highly integrated process, and osteoblasts also play a primary role in the initiation and regulation of bone resorption (Fig. 4-10).

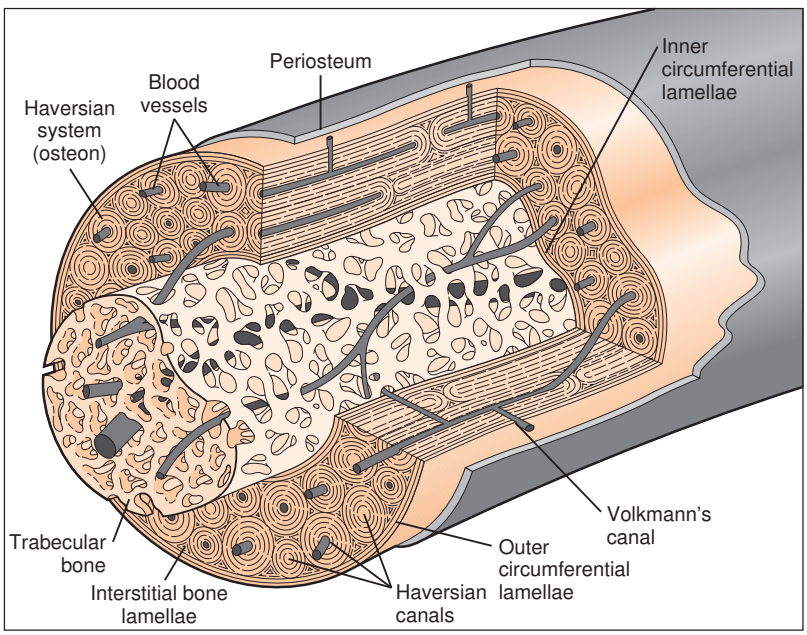


FIGURE 4-9 ■ Diagram of a typical long bone shaft showing compact cortical bone around the perimeter and cancellous trabecular bone in the center. *(From Stevens A, Lowe J:* Human Histology, *3rd ed., Philadelphia, 2005, Mosby.)*

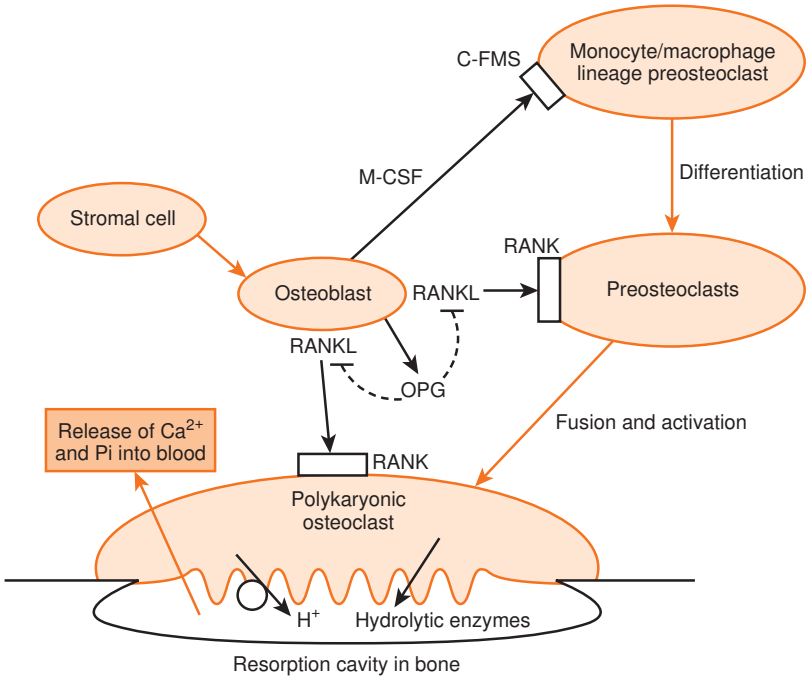


FIGURE 4-10 ■ Osteoblast regulation of osteoclast differentiation and function.

Osteoblasts develop from mesodermally derived stromal cells that have the potential to differentiate into muscle, adipose, cartilage, and bone (i.e., osteoblasts) cells. Several paracrine and endocrine factors modulate the osteoblast differentiation program, which is dependent on the expression of bone-specific transcription factors. For example, the transcription factor Runx2 is essential for osteoblast differentiation and is mutated in patients with **cleidocranial dysplasia**, which is a congenital syndrome characterized by multiple defects in bone formation.

Osteoblasts express factors that induce osteoclast differentiation from cells of the monocyte-macrophage lineage and fully activate osteoclast function (see Fig. 4-10). Osteoblasts release **monocyte colony-stimulating factor** (**M-CSF**), which is a secreted cytokine that binds to its receptor, **c-Fms**, on osteoclast precursor cells. M-CSF induces the earliest differentiating processes that lead to osteoclast precursors. M-CSF also acts in concert with another factor, **RANKL** (named for ***r*eceptor *a*ctivator of *N*F-*κ*B *l*igand**), to promote osteoclastogenesis. RANKL can exist as a 40- to 45-kDa protein on the cell membrane of osteoblasts and as a soluble 31-kDa form. RANKL binds to its receptor, **RANK**, on osteoclast precursor membranes. RANK is structurally related to the receptor for **tumor necrosis factor-α** (**TNF-α**) and signals through NF-κB-related pathways to induce osteoclastogenesis. This process involves the clustering and fusion of several osteoclast precursors, giving rise to a fused, polykaryonic osteoclast. The perimeter of the osteoclast membrane facing the bone adheres tightly to the bone, essentially sealing off the area of osteoclast-bone contact (see Fig. 4-10). The osteoclast cell membrane facing the bone transforms into a **ruffled border**, from which enzymes (e.g., cathepsin K) and HCl are secreted. The acidic enzyme-rich microenvironment proceeds to dissolve the calcified crystals and ultimately hydrolyzes type I collagen and other osteoid components. After about 2 weeks, osteoclasts receive a different signal from neighboring osteoblasts. This signal is **osteoprotegerin** (**OPG**), which acts as a soluble decoy receptor for RANKL (see Fig. 4-10). Consequently, the proosteoclastic signal from osteoblasts is terminated.

During a reversal phase, osteoclasts are then recruited to adjacent sites of bone, which extends the resorption cavity, also called the *cutting cone,* further into the bone. Alternatively, some osteoclasts may undergo apoptosis. Adjacent osteoblasts migrate into the resorbed area (now vacated by osteoclasts) and begin to lay down **osteoid**. Some of the components in osteoid (e.g., osteocalcin and alkaline phosphatase) promote its calcification. This process removes Ca^{2+} and Pi from the blood and deposits them first as calcium phosphate crystals. Later, bicarbonate and hydroxide ions are incorporated into the calcium phosphate to form **hydroxyapatite crystals**. The bone is laid down in organized layers, called *lamellae,* starting from the perimeter of the resorption cavity and progressing inward. In the fully repaired region, multiple concentric lamellae surround a central **haversian canal** or groove housing a nutritive capillary (see Fig. 4-9). This area of bone accretion is also called the *closing cone.* As the osteoblasts become surrounded by and entrapped within bone, they become **osteocytes** that sit within small spaces, called **haversian lacunae**. **Osteocytes** remain interconnected through cell processes that run within canaliculi and form communicating junctions with adjacent cell processes. The new concentric layers of bone, along with the interconnected osteocytes and the central canal, are referred to collectively as an **osteon**. The exact function of osteocytes is presently unclear, although evidence exists for a role of osteocytes in the sensing of mechanical stress in bones.

CLINICAL BOX 4-3

The importance of the RANK/RANKL/osteoprotegerin system is made evident by mutations in the human genes for RANK and osteoprotegerin that are associated with bone deformities. Loss of RANKL in mice causes **osteopetrosis** (i.e., excessive bone density) because of the loss of osteoclasts. Conversely, loss of osteoprotegerin causes **osteoporosis** (reduced bone density) because of a high number of overly active osteoclasts. Furthermore, our current understanding of bone regulation is based on how hormones, cytokines, and other factors alter the balance between RANKL and osteoprotegerin and on how they regulate the differentiation, survival, and apoptosis of osteoblasts versus osteoclasts.

As a calciotropic hormone, PTH is a primary endocrine regulator of bone remodeling in adults. The PTH/PTHrP receptor is expressed on osteoblasts, but not on osteoclasts. Therefore, PTH directly stimulates osteoblastic activity and indirectly stimulates osteoclastic activity through osteoblast-derived paracrine factors (i.e., M-CSF, RANKL). Sustained elevated levels of PTH shift the balance to a relative increase in osteoclast activity, thereby increasing bone turnover and reducing bone density. In contrast, intermittent administration of low doses of PTH promotes osteoblast survival and bone anabolic functions, increases bone density, and reduces the risk for fracture in humans. This is mediated, at least in part, by decreased production of **sclerostin (SOST)** by osteocytes. SOST is an inhibitor of bone formation that suppresses osteoblast differentiation by inhibiting Wnt signaling in osteoblast progenitor cells. Loss-of-function mutations of the SOST gene in humans cause **sclerosteosis**, a disease characterized by excessive bone mass.

CLINICAL BOX 4-4

Regulation of bone remodeling by PTH requires normal levels of 1,25-dihydroxyvitamin D. In vitamin D–deficient individuals, the Ca^{2+}-PTH secretion curve is shifted to the right. Thus, normal Ca^{2+} levels are less effective in suppressing PTH secretion, and elevated PTH levels and increased bone turnover result. The vitamin D receptor is expressed in osteoblasts, and normal 1,25-dihydroxyvitamin D levels are also required for coordination of osteoid production with its calcification. In vitamin D–deficient individuals, osteoid is not properly calcified, and the bone is weak. In children, this leads to **rickets**, in which growth of long bones is abnormal, and the weakened bones lead to bowing of extremities and collapse of the rib cage (see later). In adults, vitamin D deficiency leads to **osteomalacia**, which is characterized by poorly calcified osteoid associated with pain, increased risk for fracture, and vertebral collapse (see later).

Handling of Ca^{2+} and Pi by the Kidneys

(Mosby Physiology Monograph Series cross reference: Chapter 9 in Renal Physiology, Third Ed., BM Koeppen and BA Stanton)

The kidneys filter a large amount of Ca^{2+} (about 10 g) each day, but most of the filtered Ca^{2+} is reabsorbed by the nephron. Renal excretion typically accounts for the loss of about 200 mg of Ca^{2+} per day, which is counterbalanced by net intestinal absorption of about 200 mg/day. In the proximal tubule, most of the Ca^{2+} is reabsorbed by a passive, paracellular pathway. As in the duodenum, transcellular Ca^{2+} transport also exists and involves the constitutive expression of apical epithelial calcium channels (Trp-V5 and Trp-V6), intracellular Ca^{2+}-binding proteins (calbindins), and active Ca^{2+} extrusion (by PMCA and NCX) at the basolateral membrane. Ca^{2+} reabsorption in the **thick ascending limb (TAL) of the loop of Henle** uses both paracellular and transcellular transport mechanisms. **Paracellular transport** in the TAL is driven by a lumen-positive electrical gradient established by the Na-K-2Cl transporter in the luminal membrane following K^+ leakage back into the lumen. The CaSR is located in the basolateral membrane of TAL cells, and its activation by high serum calcium inhibits the Na-K-2Cl symporter and reduces paracellular Ca^{2+} transport (Fig. 4-11). Clinically, inhibition of this transporter by **loop diuretics** such as furosemide has been used to treat hypercalcemia. Transcellular Ca^{2+} transport in the **cortical portion of the TAL** and the **distal convoluted tubule** occurs by an active transport process that is stimulated by PTH (see Fig. 4-11). Inhibition of the thiazide-sensitive Na^+-Cl^- symporter in the luminal membrane of distal tubule cells enhances Ca^{2+} reabsorption. **Thiazide diuretics** are therefore used to prevent renal calcium wasting in idiopathic hypercalciuria.

As discussed earlier, intestinal absorption of phosphate is largely proportional to the amount of phosphate in the diet and is only slightly regulated by 1,25-dihydroxyvitamin D. This leaves the kidney with an important role in the regulation of circulating phosphate levels. Phosphate is mostly reabsorbed by the proximal convoluted tubule through a hormonally regulated transcellular route. As in the small intestine, phosphate enters the apical surface of the proximal tubules in a rate-limiting manner through a **sodium-phosphate cotransporter (NPT)**. In contrast to the NPT isoform expressed in the intestine (NPT2b), the kidney expresses an additional isoform, **NPT2a**, which is under strong hormonal regulation. PTH down regulates NPT2a expression on the apical membranes of proximal renal tubule cells, thereby increasing phosphate excretion (see Fig. 4-11). In contrast, 1,25-dihydroxyvitamin D increases NPT2 gene expression in the proximal tubules.

Integrated Physiologic Regulation of Ca^{2+}/Pi Metabolism: Response of PTH and 1,25-Dihydroxyvitamin D to a Hypocalcemic Challenge

The integrated response of PTH and 1,25-dihydroxyvitamin D to a hypocalcemic challenge is shown in Figure 4-12. Low blood Ca^{2+}, as detected by the CaSR on the parathyroid chief cells, stimulates PTH secretion. In the kidney, PTH rapidly increases Ca^{2+} levels by increasing fractional reabsorption of Ca^{2+} in the distal renal tubules. The renal effects of PTH on Ca^{2+} reabsorption are reinforced by Ca^{2+} levels as sensed by the CaSR and, to a lesser extent, by 1,25-dihydroxyvitamin D. PTH also inhibits the activity of the sodium-dependent phosphate transporter (NPT2), thereby increasing Pi excretion. The relative loss of phosphate serves to increase free, ionized Ca^{2+} in the blood. At the bone, PTH stimulates osteoblasts to secrete RANKL, which, in turn, rapidly increases osteoclast activity, leading to increased bone resorption and the release of Ca^{2+} and Pi into the blood.

In a slower phase of the response to hypocalcemia, PTH and low Ca^{2+} directly stimulate 1α-hydroxylase (CYP1α) expression in the proximal renal tubule, thereby increasing 1,25-dihydroxyvitamin D levels. In the small intestine, 1,25-dihydroxyvitamin D supports adequate Ca^{2+} levels in the long term by stimulating Ca^{2+} absorption. These effects occur over hours and days and involve increasing the expression of TrpV5 and TrpV6 calcium channels, calbindin-D_{9k}, and PMCA. 1,25-Dihydroxyvitamin D also stimulates osteoblast release of RANKL, thereby amplifying the effect of PTH.

1,25-Dihydroxyvitamin D and the CaSR play key roles in negative feedback. Thus, elevated PTH stimulates 1,25-dihydroxyvitamin D production, which then inhibits PTH gene expression directly, and indirectly by up regulating the CaSR. 1,25-Dihydroxyvitamin D also represses renal 1α-hydroxylase activity while increasing 24-hydroxylase activity. As blood Ca^{2+} levels rise back to normal levels, they shut off PTH secretion and 1α-hydroxylase.

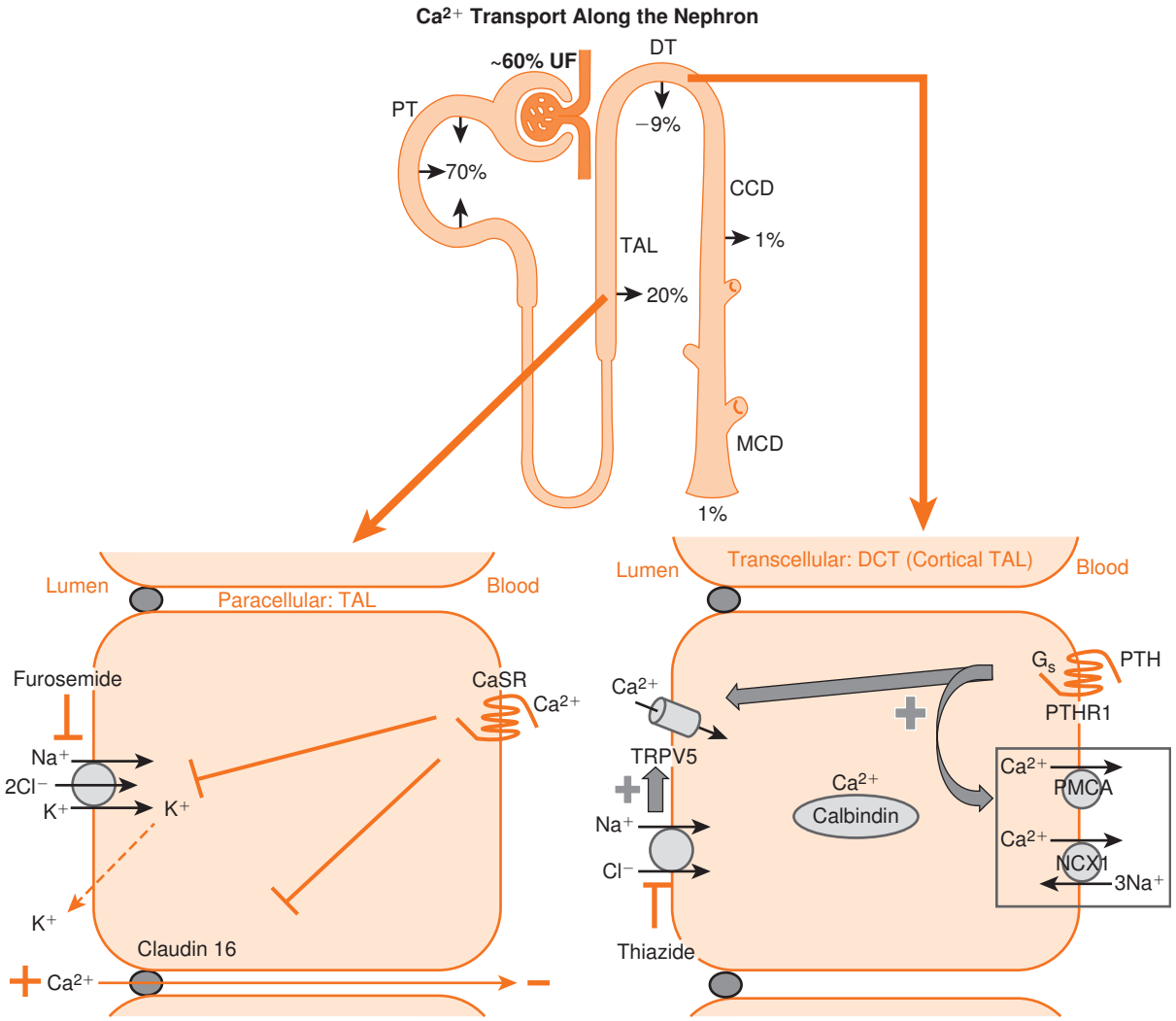


FIGURE 4-11 ■ Handling of Ca^{2+} by the distal nephron and proximal nephron (see text). Diagram of the nephron and the transport pattern of Ca^{2+} along the nephron. *(From Koeppen BM, Stanton BA:* Renal Physiology, *3rd ed., St. Louis, 2001, Mosby. Details of the Ca^{2+} reabsorption by the TAL and DCT were drawn by Dr. John Harrison, University of Connecticut Health Center, Farmington, Conn.)*

Hormonal Regulation of Calcium and Phosphate: Pharmacologic Regulators

Calcitonin The primary actions of **calcitonin** are on bone and kidney. Calcitonin lowers serum calcium and phosphate levels, primarily by inhibiting bone resorption, in many species of animals. However, although human calcitonin can lower serum calcium and phosphate levels in humans, it takes high doses to show this effect. There are no definitive complications from calcitonin deficiency or excess in humans. For this reason, it is unlikely that calcitonin has an important physiologic role in humans. Medical interest in calcitonin stems from the fact that potent forms of calcitonin can be used therapeutically in the treatment of bone disorders. Calcitonin is also a useful histochemical marker of medullary thyroid cancer.

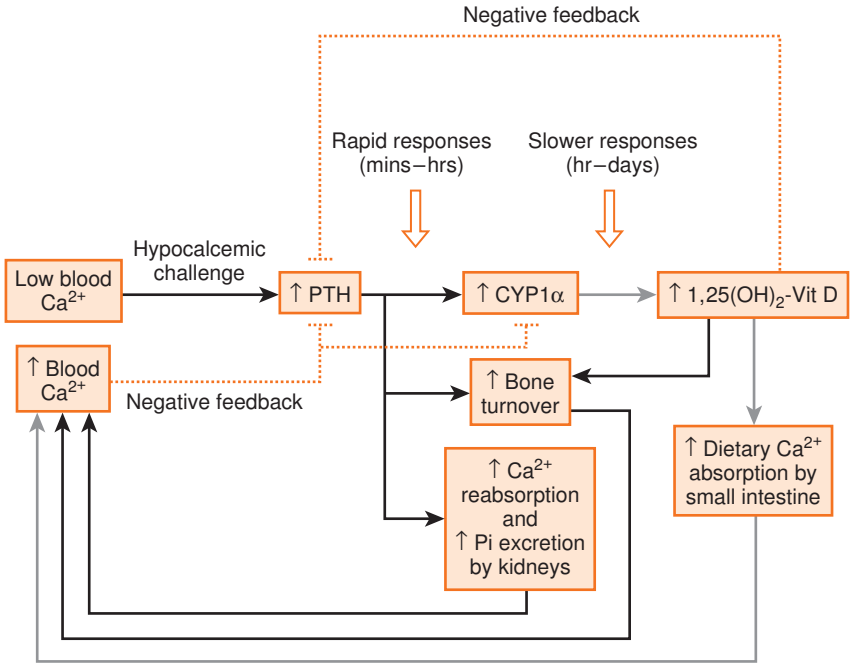


FIGURE 4-12 ■ Integrated response to a hypocalcemic challenge.

Parafollicular C Cells The cells that produce calcitonin are called the **parafollicular C cells**. These cells are derived from the ultimobranchial bodies and become incorporated and interspersed among the thyroid follicles as the thyroglossal duct migrates caudally. Parafollicular C cells do not invade the thyroid epithelium and thus are not in contact with the follicular colloid.

Structure, Synthesis, and Secretion of Calcitonin Calcitonin is a 32-amino acid polypeptide. Because there is minimal species variation, calcitonins from other species are biologically active in humans. In fact, salmon calcitonin is about 20 times more potent in humans than human calcitonin. Normal serum calcitonin levels are about 10 to 50 pg/mL, and its half-life in circulation is less than 1 hour. Because the primary site of inactivation is the kidney, serum calcitonin levels are often elevated in renal failure. Alternative splicing of the calcitonin gene in other tissues can produce **calcitonin gene-related peptide** (**CGRP**), which is a potent vasodilator and positive cardiac inotrope.

The secretion of calcitonin is primarily regulated by the same CaSR that regulates PTH secretion. However, elevated extracellular Ca^{2+} levels stimulate the synthesis and secretion of calcitonin.

Calcitonin Receptor The **calcitonin receptor** is closely related to the secretin and PTH/PTHrP receptors. It is a seven-transmembrane Gαs-coupled receptor that acts primarily through cAMP-dependent signaling pathways. In contrast to the PTH/PTHrP receptor, the calcitonin receptor is expressed in osteoclasts. Calcitonin acts rapidly and directly on osteoclasts to suppress bone resorption. **Paget disease** is characterized by excessive bone turnover that is driven by large, bizarre osteoclasts (see later). Because these osteoclasts retain the calcitonin receptor, active forms of calcitonin can be used to suppress aberrant osteoclastic activity in patients with this disease.

The calcitonin receptor is also expressed in the nephron, where calcitonin inhibits phosphate and calcium reabsorption.

Hormonal Regulation of Calcium and Phosphate: Regulators Overexpressed by Cancers

Parathyroid Hormone–Related Peptide **Parathyroid hormone–related peptide (PTHrP)** is a peptide paracrine factor that shows limited structural similarity to PTH but nevertheless binds to and signals through the PTH/PTHrP receptor. PTHrP is expressed in several

developing tissues, including the growth plate of bones and the mammary glands. PTHrP is not regulated by circulating calcium and normally does not play a role in Ca^{2+}/Pi homeostasis in the adult. However, certain neoplasias can secrete high levels of PTHrP, which then produces symptoms of hyperparathyroidism (see later).

CLINICAL BOX 4-5

Fibroblast growth factor-23 (FGF23) is an approximately 30-kDa peptide that is normally expressed by osteocytes. It acts on proximal tubule cells of the kidney to inhibit Pi reabsorption and promote phosphate excretion. FGF23 is inactivated by a protease that cleaves FGF23 into N-terminal and C-terminal peptides. One protease involved in FGF23 processing, although it is not a direct substrate, is **PHEX** (for phosphate-regulating gene with homologies to endopeptidases on the X chromosome). PHEX is mutated in **X-linked hypophosphatemia**, characterized by renal phosphate wasting, rickets, osteomalacia, and inappropriate low-normal levels of 1,25-dihydroxyvitamin D.

Current evidence indicates that when PHEX is mutated, FGF23 levels increase and inhibit both phosphate reabsorption and 1α-hydroxylase in the proximal renal tubules. Increased expression of FGF23 has been linked to **autosomal recessive hypophosphatemic rickets** and **tumor-induced rickets/osteomalacia**.

Regulation of Ca^{2+}/Pi Metabolism by Immune and Inflammatory Cells

It is interesting to note that the RANKL/RANK/osteoprotegerin signaling system is similar to the TNF receptor/NF-κB signaling pathways used in cells involved in the immune system and in inflammation. This link is further stressed by the fact that activated T cells express high levels of RANKL in response to stimulation by the cytokines, TNF-α, and several interleukins. Thus, inflammatory bone diseases (e.g., rheumatoid arthritis) are associated with increased RANKL-to-osteoprotegerin ratios in the vicinity of the inflammatory site, with subsequent erosions of bone and osteoporosis.

RANKL is also overproduced by cells associated with several malignant bone diseases (e.g., multiple myeloma, skeletal metastatic breast cancer). As noted earlier, some malignant cells also overexpress PTHrP, which induces RANKL expression in neighboring osteoblasts. Thus, several malignancies are associated with bone damage and hypercalcemia.

The 1α-hydroxylase enzyme is expressed by monocytes and peripheral macrophages. In the autoimmune disease of sarcoidosis, overactive macrophages produce high levels of 1,25-dihydroxyvitamin D, resulting in hypercalcemia.

Regulation of Ca^{2+}/Pi Metabolism by Gonadal and Adrenal Steroid Hormones

Gonadal and **adrenal steroid hormones** have profound effects on calcium and phosphate metabolism and skeletal health. **Estradiol-17β** (**E_2**; see Chapter 10) has a bone anabolic and calciotropic effect at several sites. E_2 stimulates intestinal calcium absorption and renal tubular calcium reabsorption. E_2 is also one of the most potent regulators of osteoblast and osteoclast function. Estrogen promotes survival of osteoblasts and apoptosis of osteoclasts, thereby favoring bone formation over resorption. In postmenopausal women, estrogen deficiency results in an initial phase of rapid bone loss that lasts about 5 years, followed by a second phase of slower bone loss. During the second phase, the individual is chronically challenged with hypocalcemia because of inefficient calcium absorption and renal calcium wasting. This can result in secondary hyperparathyroidism, which further exacerbates bone loss. Exercise, high levels of dietary calcium with supplemental vitamin D, and hormonal replacement therapy can prevent postmenopausal osteoporosis. **Androgens** also have bone anabolic and calciotropic effects, although some of these effects are due to the peripheral conversion of testosterone to E_2 (see Chapter 9).

In contrast to gonadal steroids, the **adrenal glucocorticoids** (e.g., **cortisol**) promote bone resorption and renal calcium wasting and inhibit intestinal calcium absorption. Patients treated with high levels of a glucocorticoid (e.g., as an anti-inflammatory and immunosuppressive drug) can develop glucocorticoid-induced osteoporosis.

PATHOLOGIC DISORDERS OF CALCIUM AND PHOSPHATE BALANCE

Hyperparathyroidism (Primary)

Primary hyperparathyroidism is caused by excessive production of PTH by the parathyroid glands. It is frequently caused by a single adenoma confined to one of

the parathyroid glands. A common cause of parathyroid adenoma is the overexpression of the *PRAD1* gene (parathyroid adenomatosis gene), which encodes the cell cycle regulator, cyclin D1.

Patients with primary hyperparathyroidism have high serum calcium levels and, in most cases, low serum phosphate levels. Hypercalcemia is a result of bone demineralization, increased GI calcium absorption (mediated by 1,25-dihydroxyvitamin D), and increased renal calcium reabsorption. The major symptoms of the disorder are directly related to increased bone resorption, hypercalcemia, and hypercalciuria (Fig. 4-13). High serum calcium levels decrease neuromuscular excitability. People with hyperparathyroidism often show psychological disorders, particularly depression, that may be associated with increased serum calcium levels (Box 4-1). Other neurologic symptoms include fatigue, mental confusion, and at very high levels (> 15 mg/dL), coma. Hypercalcemia can cause cardiac arrest. Hypercalcemia can result in peptic ulcer formation because calcium increases gastrin secretion (see Chapter 2). Kidney stones (nephrolithiasis) are common because hypercalcemia eventually leads to hypercalciuria and increased phosphate clearance leads to phosphaturia. The high urinary calcium and phosphate concentrations increase the tendency for precipitation of calcium-phosphate salts in the soft tissues of the kidney. When serum calcium levels exceed about 13 mg/dL with a normal phosphate level, the calcium-phosphate **solubility product** is exceeded. At this level, insoluble calcium-phosphate salts form, which results in calcification of soft tissues such as blood vessels, skin, lungs, and joints.

People with hyperparathyroidism have evidence of increased bone turnover, such as elevated levels of serum alkaline phosphatase and osteocalcin, which indicate high osteoblastic activity, and increased urinary hydroxyproline levels, which indicates high bone resorptive activity. Hydroxyproline is an amino acid characteristically found in type I collagen. When the collagen is degraded, urinary hydroxyproline excretion increases. Although hyperparathyroidism will eventually cause **osteoporosis** (bone loss involving both

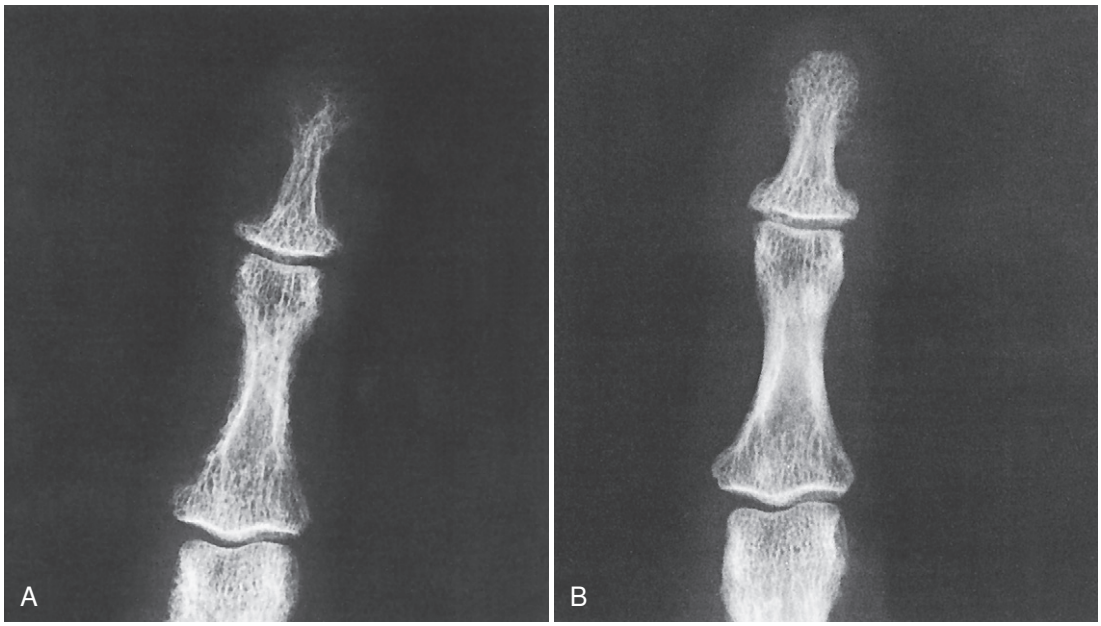


FIGURE 4-13 ■ Primary hyperparathyroidism. **A,** Radiographs of middle and distal phalanges of index finger show subperiosteal bone resorption of shafts and tip of distal phalanx. **B,** Second radiograph taken after bone had healed after treatment by removal of parathyroid hematoma. *(From Besser GM, Thorner MO:* Clinical Endocrinology, *London, 1994, Mosby-Wolfe.)*

BOX 4-1
SYMPTOMS OF HYPERPARATHYROIDISM

- Kidney stones
- Osteoporosis
- Gastrointestinal disturbances, peptic ulcers, nausea, constipation
- Muscle weakness, decreased muscle tone
- Depression, lethargy, fatigue, mental confusion
- Polyuria
- High serum phosphate concentration; low serum calcium concentration

osteoid and mineral), it is not necessarily the presenting symptom. However, bone demineralization is apparent. These individuals frequently exhibit **hyperchloremic acidosis**. Some people with hyperparathyroidism have the bone disorder **osteitis fibrosa cystica,** which is characterized by bone pain, multiple bone cysts, a tendency for pathologic fractures of long bones, and histologic abnormalities of the bone.

Pseudohypoparathyroidism

Pseudohypoparathyroidism is a rare familial disorder characterized by tissue resistance to PTH. In many instances, the problem is thought to originate with the PTH receptor. Often there is a decrease in levels of the guanine nucleotide–binding protein, Gs. Individuals with pseudohypoparathyroidism demonstrate increased PTH secretion and low serum calcium levels, sometimes associated with congenital defects of the skeleton, including shortened metacarpal and metatarsal bones.

Hypoparathyroidism

Hypoparathyroidism is associated with low serum calcium levels and high serum phosphate levels. The hypocalcemia results from both a PTH and a 1,25-dihydroxyvitamin D deficiency. Consequently, there is a decrease in bone calcium mobilization by both osteoclastic resorption and osteocytic osteolysis. Because 1,25-dihydroxyvitamin D is deficient, GI absorption of calcium is impaired. The PTH deficiency decreases renal calcium reabsorption, thereby decreasing fractional calcium reabsorption. Although fractional calcium reabsorption decreases, the urinary calcium level is generally low. Alkalosis occurs because bicarbonate excretion decreases; this further lowers the free calcium level in serum. Although the serum calcium level is low, bone demineralization is usually not a problem because of the high serum phosphate level. Hypocalcemia increases neuromuscular excitability, increasing the possibility of tetany and even convulsions. Hypocalcemia alters cardiac function. It can produce a first-degree heart block. The low serum calcium level decreases myocardial contractility.

The most prominent symptom of hypoparathyroidism is increased neuromuscular excitability (Box 4-2). Low serum calcium concentrations decrease the neuromuscular threshold. This can be manifested as repetitive responses to a single stimulus and as spontaneous neuromuscular discharge. The increased neuromuscular excitability can result in tingling in the fingers or toes (paresthesia), muscle cramps, or even tetany. Laryngeal spasms can be fatal. Sometimes the serum calcium level is not low enough to produce overt tetany, but latent tetany can be demonstrated by inflating a blood pressure cuff on the arm to a pressure greater than systolic pressure for 2 minutes. The resultant oxygen deficiency precipitates overt tetany as demonstrated by **carpal-pedal spasms.** This is called **Trousseau sign** (Fig. 4-14A). Another test is to tap the facial nerve, which evokes facial muscle spasms **(Chvostek sign).**

Treatment of hypoparathyroidism is difficult because of the lack of readily available effective human PTH. The disorder is frequently treated with a

BOX 4-2
SYMPTOMS OF HYPOPARATHYROIDISM

- Tetany, convulsions, paresthesias, muscle cramps
- Decreased myocardial contractility
- First-degree heart block
- Central nervous system problems, including irritability and psychosis
- Intestinal malabsorption
- Low serum calcium concentration; high serum phosphate concentration

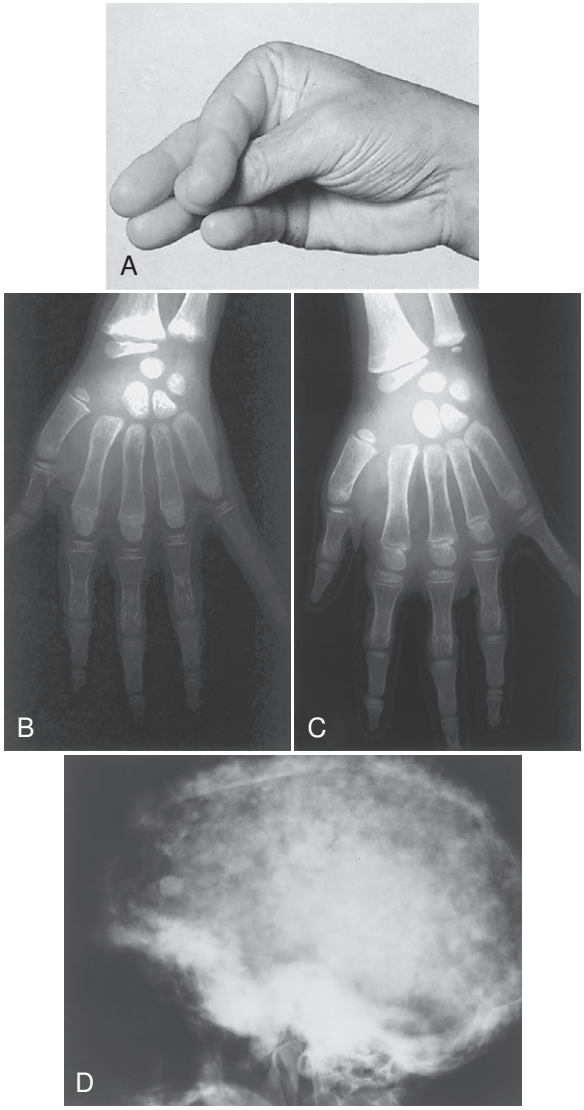


FIGURE 4-14 ■ **A,** Position of hand in hypocalcemic tetany. **B,** Radiograph of left hand of 9-year-old boy with rickets caused by malnutrition. He would eat only potato chips. All the bony structures are osteopenic. Note widening of space between provisional zone of calcification and epiphysis of left radius. **C,** After 2 months of force feedings, rickets has subsided. Note decreased width of space between provisional zone of calcification and epiphysis of radius and increased bone calcification. **D,** Radiograph of skull of patient with Paget disease. Thickness of skull is increased, and sclerotic changes are seen scattered throughout skull, consistent with healing phase of Paget disease. *(**A** from Hall R, Evered DC:* Color Atlas of Endocrinology, *2nd ed., London, 1990, Mosby-Wolfe. **B** to **D,** Courtesy of Dr. C. Joe.)*

high-calcium diet, vitamin D, and occasionally thiazide diuretics to decrease renal calcium clearance. Thiazide diuretics increase calcium reabsorption in the thick ascending limb of the loop of Henle. Acute hypocalcemia can be treated with intravascular calcium gluconate infusion.

Hypomagnesemia resulting from either severe malabsorption or chronic alcoholism can cause hypoparathyroidism. Hypomagnesemia impairs the secretion of PTH and decreases the biologic response to PTH.

Vitamin D Deficiency

Vitamin D deficiency produces hypocalcemia and hypomagnesemia and decreases GI absorption of calcium and phosphate. The drop in the serum calcium level stimulates PTH secretion, which stimulates renal phosphate clearance, thereby aggravating the serum phosphate loss. Because the level of the calcium-phosphate product in serum, and hence in body fluids, is low, bone mineralization is impaired, and demineralization is increased. This leads to osteomalacia in adults or rickets in children. The secondary elevation in PTH can produce osteoporosis. Rickets and osteomalacia are disorders in which bone mineralization is defective. Osteoid is formed, but it does not mineralize adequately. If the calcium-phosphate product level or the pH in bone fluid bathing the osteoid is low, demineralization rather than mineralization is favored. *Rickets* is caused by a vitamin D deficiency before skeletal maturation; it involves problems in not only the bone but also the cartilage of the growth plate (Fig. 4-14B and C). *Osteomalacia* is the term used when inadequate bone mineralization occurs after skeletal growth is complete and the epiphyses have closed.

Paget Disease

Paget disease results in bone deformities. It is characterized by an increase in bone resorption followed by an increase in bone formation. The new bone is generally abnormal and often irregular. Serum alkaline phosphatase and osteocalcin levels are increased, as are those of urinary hydroxyproline. Pain, bone deformation, and bone weakness can occur (Fig. 4-14D).

Bone Problems of Renal Failure (Renal Osteodystrophy)

Approximately 0.9 g, or more than 50% of dietary phosphate, is normally lost in the urine in a day. Consequently, the kidney serves as the major excretory route for phosphate. As renal function, and hence phosphate clearance, decreases, the serum phosphate concentration rises. The increase in serum phosphate concentration will lower serum calcium levels by exceeding the solubility product and hence increasing calcium-phosphate precipitation. A drop in the serum calcium level is an effective stimulus for PTH, and serum PTH levels also rise (Fig. 4-15). In addition, vitamin D activation by 1α-hydroxylase occurs in the renal proximal tubules. In kidney failure, vitamin D activation is impaired, which decreases GI absorption of calcium and phosphate. This results in a further drop in the serum calcium level and aggravates the preexisting problem with excess PTH secretion. The result is to stimulate bone resorption and demineralization. As bone demineralization occurs, it aggravates the hyperphosphatemia because the renal mechanisms of counteracting the hyperphosphatemia are now defective.

Figure 4-16 shows the effect of renal impairment on phosphate, calcium, vitamin D, and PTH.

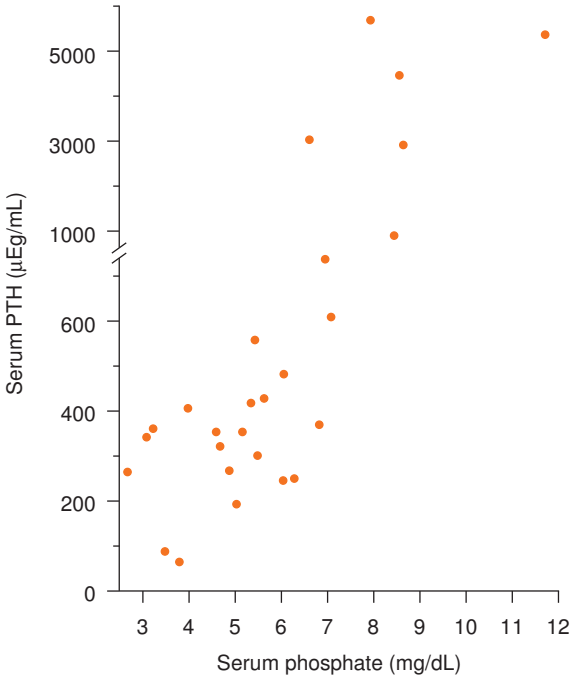


FIGURE 4-15 ■ Relationship between serum parathormone (PTH) level and serum phosphate level in patients with renal failure. *(Redrawn from Bordier PF, Marie PF, Arnaud CD: Evolution of renal osteodystrophy: Correlation of bone histomorphometry and serum mineral and immunoreactive parathyroid hormone values before and after treatment with calcium carbonate or 25-hydroxycholecalciferol.* Kidney Int *7 [Suppl 2]:102, 1975.).*

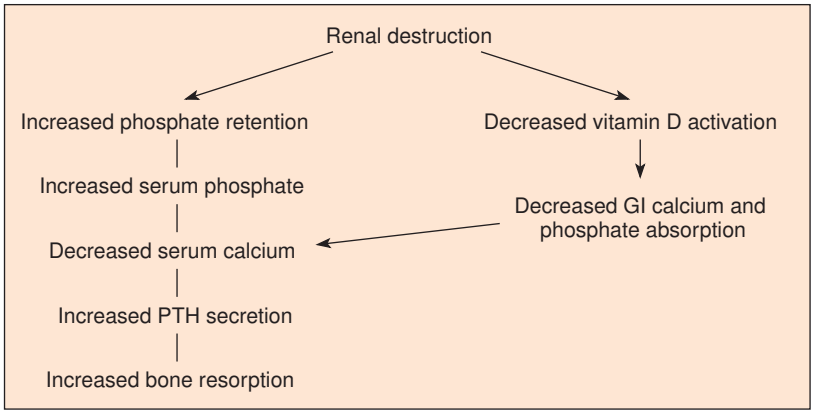


FIGURE 4-16 ■ The physiologic basis of bone loss in renal failure. GI, gastrointestinal; PTH, parathyroid hormone.

SUMMARY

1. Serum calcium levels are a function of the rate at which calcium enters from GI absorption and bone demineralization and leaves through bone mineralization, GI excretion, and renal excretion. Serum calcium levels are normally maintained within a narrow range.
2. Serum phosphate levels are determined by the rate at which phosphate enters from GI absorption, soft tissue efflux, and bone demineralization and leaves through GI excretion, soft tissue influx, bone mineralization, and renal excretion. Serum phosphate levels normally fluctuate over a relatively wide range.
3. The major physiologic hormones regulating serum calcium and phosphate levels are parathyroid hormone (PTH) and 1,25-dihydroxyvitamin D (calcitriol). Calcitonin is an important pharmacologic regulator of calcium and phosphate.
4. PTH is an 84-amino acid peptide, whose expression is regulated by serum Ca^{2+} through the Ca^{2+}-sensing receptor (CaSR) and 1,25-dihydroxyvitamin D. PTH is made by the principal cells of the parathyroid glands. PTH binds to the PTH/PTHrP receptor, which is primarily coupled to a Gs/cAMP/PKA pathway.
5. Vitamin D can be synthesized from 7-dehydrocholesterol in skin in the presence of ultraviolet light. It is hydroxylated to 25-hydroxycholecalciferol (calcifediol) in the liver and activated by renal 1α-hydroxylase to 1,25-dihydroxycholecalciferol (calcitriol). PTH stimulates the renal 1α-hydroxylase activity, whereas 1,25-dihydroxyvitamin D negatively feeds back on the enzyme. 1,25-Dihydroxyvitamin D binds to a nuclear vitamin D receptor (VDR), which regulates specific gene expression.
6. 1,25-Dihydroxyvitamin D strongly promotes intestinal Ca^{2+} absorption and weakly increases Pi absorption. PTH has little if any direct effect at the intestine.
7. Osteoblasts are bone-forming cells that are of mesenchymal origin. They synthesize bone matrix and produce an environment that promotes bone mineralization. Osteocytes are osteoblasts that have become entrapped in bone. Osteoclasts are large, multinucleate cells derived from hematopoietic stem cells. Mature, activated osteoclasts secrete enzymes and acid that resorb bone.
8. The flux of Ca^{2+} and Pi into and out of bone is determined by the relative activities of osteoblasts versus osteoclasts, which exist as basic multicellular units at about 2 million sites within bone. Bone resorption is initiated by osteoblasts, which recruit and activate monocyte-macrophage-lineage cells to become mature polykaryonic osteoclasts. This occurs through the expression of M-CSF and RANKL by osteoblasts, which bind to their receptors, C-FMS and RANK, respectively, on osteoclasts. Bone repair is initiated by the release of osteoprotegerin (OPG) from osteoblasts, which acts as a soluble decoy for RANKL. This causes osteoclasts to stop bone resorption and either move to a new resorption site or undergo apoptosis. Osteoblasts then secrete osteoid, which undergoes calcification. As osteoblasts secrete osteoid in a lamellar, outside-in, configuration, they reform osteons.
9. The PTH/PTHrP receptor is expressed on the osteoblast, not the osteoclast. PTH promotes osteoblast differentiation, proliferation, and survival, and intermittent administration of PTH promotes bone formation. PTH also increases M-CSF and RANKL expression by osteoblasts, and a chronic high level of PTH shifts the balance in favor of bone resorption.
10. 1,25-Dihydroxyvitamin D acts through the VDR on osteoblasts to increase osteoblast differentiation, promote secretion of normal osteoid components, and sensitize osteoblasts to PTH.
11. PTH increases the fractional reabsorption of calcium at the distal nephron. However, because the filtered load of calcium increases, renal excretion of calcium typically increases after PTH administration. PTH decreases renal Pi and bicarbonate reabsorption. 1,25-Dihydroxyvitamin D increases both Ca^{2+} and Pi reabsorption by the kidney.
12. Calcitonin acts on osteoclasts to inhibit their bone-resorptive function and on the kidney to inhibit Ca^{2+} reabsorption. Although active analogs

of calcitonin are useful pharmacologic agents for the treatment of imbalances in Ca^{2+} homeostasis (e.g., Paget disease), endogenous human calcitonin does not play an important role in Ca^{2+}/Pi homeostasis.

13. Other factors that regulate Ca^{2+} and/or Pi levels, either physiologically or pathophysiologically, include PTHrP, FGF23, gonadal steroids, and adrenal steroids.
14. Patients with hyperparathyroidism typically have hypercalcemia, hypophosphatemia, hyperchloremia, and acidosis. They are prone to kidney stones because of hypercalciuria and hyperphosphaturia.
15. Patients with hypoparathyroidism typically have hypocalcemia, hyperphosphatemia, hypochloremia, and alkalosis. They may show symptoms of increased neuromuscular excitability such as paresthesias, muscle cramps, and tetany.
16. Children with a vitamin D deficiency are prone to develop rickets, whereas adults with a vitamin D deficiency develop osteomalacia. The vitamin D deficiency results in decreased GI absorption of calcium, phosphate, and magnesium.

SELF-STUDY PROBLEMS

1. How would the loss of 1,25-dihydroxyvitamin D directly and indirectly alter PTH secretion?
2. What is the relationship between osteoblasts and bone resorption?
3. Why does unregulated overproduction of PTHrP (e.g., produced by a tumor) cause hypercalcemia?
4. What would be the effect of overproduction of osteoprotegerin on bone density?
5. How does vitamin D deficiency affect Pi levels? Why?
6. What is the physiologic basis for polyuria and nocturia in hyperparathyroidism?
7. Why are the urinary calcium level and fractional reabsorption of calcium typically low in hypoparathyroidism?

KEYWORDS AND CONCEPTS

- 1,25-Dihydroxyvitamin D
- Adrenal corticoids (cortisol)
- Autosomal-recessive hypophosphatemic rickets

For full list of keywords and concepts see Student Consult

SUGGESTED READINGS

Brannon PM, Picciano MF: Vitamin D in pregnancy and lactation in humans, *Annu Rev Nutr* 31:89–115, 2011.

Chopra S, Cherian D, Jacob JJ: The thyroid hormone, parathyroid hormone and vitamin D associated hypertension, *Indian J Endocrinol Metab* 15(Suppl 4):S354–S360, 2011.

Kohli SS, Kohli VS: Role of RANKL-RANK/osteoprotegerin molecular complex in bone remodeling and its immunopathologic implications, *Indian J Endocrinol Metab* 15:175–181, 2011.

Krishnan AV, Feldman D: Mechanisms of the anti-cancer and anti-inflammatory actions of vitamin D, *Annu Rev Pharmacol Toxicol* 51:311–336, 2011.

McGreevy C, Williams D: New insights about vitamin D and cardiovascular disease: A narrative review, *Ann Intern Med* 155:820–826, 2011.

Sikjaer T, Rejnmark L, Mosekilde L: PTH treatment in hypoparathyroidism, *Curr Drug Saf* 6:89–99, 2011.

KEYWORDS AND CONCEPTS

- Basic multicellular units
- Ca^{2+}-sensing receptor (CaSR)
- Calcitonin gene–related peptide (CGRP)
- Calbindin-D_{9K}
- Calbindin-Ds
- Calciotropic hormones
- Calcitriol
- Calcium channels
- Calcium response element
- Cancellous (trabecular) bone
- Carpal-pedal spasms
- Cholecalciferol
- Chvostek sign
- Cleidocranial dysplasia
- Compact cortical bone
- Estradiol-17β
- Familial benign hypocalciuric hypercalcemia (FBHH)
- Fibroblast growth factor-23 (FGF23)
- Gonadal/adrenal steroid hormones
- Hypercalcemia
- Hyperchloremic acidosis
- Hyperphosphatemia
- Hypocalcemia
- Inorganic phosphate (Pi)
- Monocyte colony-stimulating factor
- Neonatal severe hyperparathyroidism
- Nuclear vitamin D receptor (VDR)
- Osteitis fibrosa cystica
- Osteoblasts
- Osteoclasts
- Osteoid
- Osteoporosis
- Oxyphil cell
- Paget disease
- Parafollicular C cells
- Parathyroid glands
- Parathyroid hormone (PTH)
- Parathyroid hormone–related peptide (PTHrP)
- Plasma membrane calcium ATPase (PMCA)
- PreproPTH
- Principal (chief) cell
- ProPTH
- PTH/PTHrP receptor
- Sodium-calcium exchanger (NCX)
- Sodium-phosphate cotransporter (NPT2)
- Solubility product
- Trousseau sign
- TrpV5
- TrpV6
- Tumor-induced rickets/osteomalacia
- Vitamin D–responsive elements
- Vitamin D–binding protein (DBP)
- X-linked hypophosphatemia

5 HYPOTHALAMUS-PITUITARY COMPLEX

OBJECTIVES

1. Discuss the embryology and anatomy of the pituitary gland.
2. Review the function of the neurohypophysis (posterior pituitary), including the synthesis, regulation, and function of two neurohormones: antidiuretic hormone (ADH; also called vasopressin) and oxytocin.
3. Describe the neurovascular connection between the hypothalamus and the adenohypophysis (anterior pituitary).
4. Develop the concept of an endocrine axis.
5. Describe the cytology of the adenohypophysis, along with the structure and function of the six hormones produced by the adenohypophysis.
6. Discuss the significant direct effects of growth hormone and prolactin on nonendocrine organs.
7. Present some forms of pituitary pathophysiology.

The **pituitary gland** (also called the **hypophysis**) is a small (about 0.5 g in weight) yet complex endocrine structure at the base of the forebrain (Fig. 5-1A and B). It is composed of an epithelial component, called the **adenohypophysis** or **anterior pituitary**, and a neural structure, called the **neurohypophysis**. The caudal end of the neurohypophysis is called the pars nervosa, or posterior pituitary. The anterior pituitary contains five cell types that secrete six hormones. The neurohypophysis acts as a site of release of multiple neurohormones. All endocrine functions of the pituitary gland are regulated by the **hypothalamus** and by **negative and positive feedback loops**.

EMBRYOLOGY AND ANATOMY

Microscopic examination of the pituitary reveals two distinct types of tissues: epithelial and neural (Fig. 5-1C and D). This dual nature of the gland is best understood by reviewing its development. During development, a caudal extension of the primitive forebrain (i.e., the diencephalon) grows toward the roof of the primitive oral cavity (Fig. 5-2). This neural downgrowth, called the **infundibulum**, secretes factors that induce the epithelium of the roof of the oral cavity to extend cranially toward the base of the developing brain. This extension of the oral ectoderm is called **Rathke pouch**. As Rathke pouch moves upward, the following events occur:

1. Rathke pouch loses its contact with the oral cavity, and by doing so, becomes a ductless endocrine structure. Remnants of Rathke pouch may persist and can give rise to craniopharyngiomas.
2. Rathke pouch comes into direct contact with the infundibulum. The cells on the posterior side of the pouch lumen facing the infundibulum give rise to the **pars intermedia** in the fetus. These cells degenerate in the adult human pituitary. The cells on the anterior side of the pouch lumen facing away

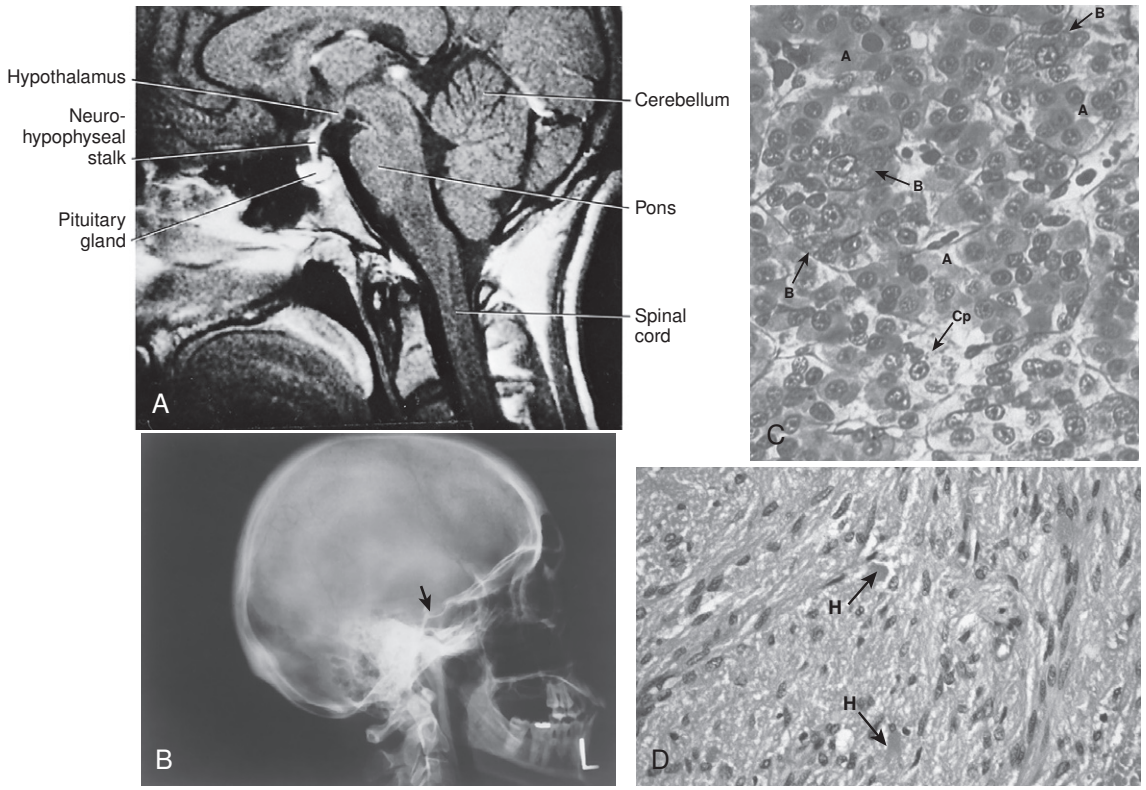


FIGURE 5-1 ■ A, Magnetic resonance image of the head shows the proximity of the hypothalamus and pituitary gland and their connection by a neurohypophyseal (pituitary) stalk. **B,** Pituitary gland is located in the sella turcica *(arrow)*. **C,** Histology of pars distalis. **D,** Histology of pars nervosa. A, acidophil; B, basophil; Cp, chromophobe; H, Herring bodies. *(**A,** Courtesy of Dr. Steven Weiner. From Berne RM, Levy MN, Koeppen BM, et al:* Physiology, *5th ed., St. Louis, 2004, Mosby, 2004. **B,** Courtesy of Dr. C. Joe. **C** and **D,** From Young B, Lowe JS, Stevens A, et al:* Wheater's Functional Histology, *5th ed., Edinburgh, 2006, Churchill Livingstone, 2006.)*

from the infundibulum expand considerably and give rise to the **pars distalis**. The pars distalis makes up almost all of the adenohypophysis in the adult and is also referred to as the **anterior pituitary**. A third division of Rathke pouch develops into the **pars tuberalis** and is composed of a thin layer of cells that wrap around the infundibular stalk at the superior end of the anterior pituitary. To summarize, the adenohypophysis (i.e., the **epithelial** portion of the pituitary, or anterior pituitary) develops from epithelial cells (the oral ectoderm) and is composed of the pars distalis, a thin layer called the pars tuberalis, and the pars intermedia, which is lost in adult humans.

3. The infundibular process expands at its lower end to give rise to a structure called the **pars nervosa**. The pars nervosa is also called the **posterior lobe of the pituitary** (or simply, the **posterior pituitary**). At the superior end of the infundibulum, a funnel-shaped swelling develops called the **median eminence**. The rest of the infundibular process, which extends from the medium eminence down to the pars nervosa, is called the infundibulum. To summarize, the neurohypophysis develops from a downgrowth of **neural tissue** at the base of the diencephalon (corresponding to the hypothalamus in the adult) and gives rise to the pars nervosa, the infundibulum, and the median eminence. The

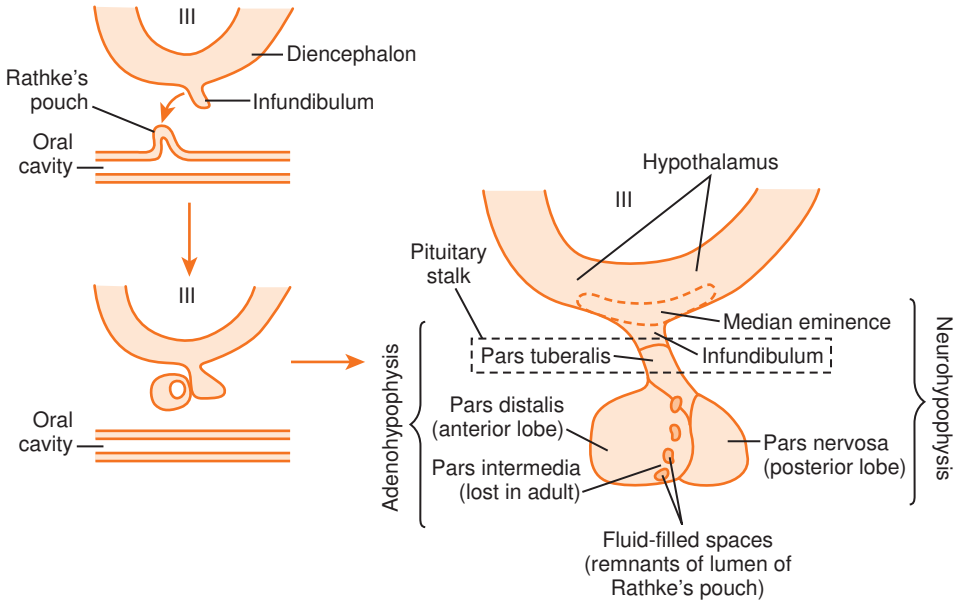


FIGURE 5-2 ■ Development of the pituitary gland from neural and epithelial sources.

infundibulum and the pars tuberalis make up the **pituitary stalk**.

CLINICAL BOX 5-1

With development, the pituitary gland becomes encased in the sphenoid bone in a structure called the **sella turcica** (see Fig. 5-1). The pituitary stalk emerges superiorly out of the sella turcica in the vicinity of the optic nerves and optic chiasm. Generally, cancers of the pituitary have only one way to expand, which is superiorly into the brain and against the **optic nerves**. Thus, any increase in the size of the pituitary is often associated with **dizziness** or **vision problems**. The sella turcica is sealed off from the brain by a membrane called the **diaphragma sellae**. Defective development of the diaphragma sellae can allow cerebrospinal fluid to enter the sellar cavity and encroach on developing pituitary tissue. This can give rise to **empty sella syndrome,** which represents a reduction of pituitary tissue (but not always pituitary function) within the sella turcica.

NEUROHYPOPHYSIS

The pars nervosa is a **neurovascular** structure that is the site of release of neurohormones adjacent to a rich bed of fenestrated capillaries. The peptide hormones that are released are **antidiuretic hormone (ADH)** (also called **vasopressin**) and **oxytocin**. The cell bodies of the neurons that project to the pars nervosa are located in the **supraoptic nuclei (SON)** and **paraventricular nuclei (PVN)** of the hypothalamus (in this context, a *nucleus* refers to a collection of neuronal cell bodies residing within the central nervous system [CNS]—a *ganglion* is a collection of neuronal cell bodies residing outside the CNS). The cell bodies of these neurons are described as **magnocellular** (i.e., large cell bodies) and are equipped with enough biosynthetic capacity to produce a short-lived peptide hormone that is released into and diluted by the peripheral circulation. The magnocellular neurons project axons down the infundibular stalk as the **hypothalamohypophyseal tracts**. These axons terminate in the pars nervosa (Fig. 5-3). In addition to axonal processes and termini from the SON and PVN, there are glial-like supportive cells called **pituicytes**. As is typical of endocrine organs, the posterior pituitary is extensively vascularized, and the capillaries are fenestrated, thereby facilitating diffusion of hormones into the vasculature.

Synthesis of Antidiuretic Hormone (Vasopressin) and Oxytocin

ADH and oxytocin are nonapeptides (9 amino acids) and are similar in structure, differing in only 2 amino acids (Fig. 5-4). They have limited overlapping

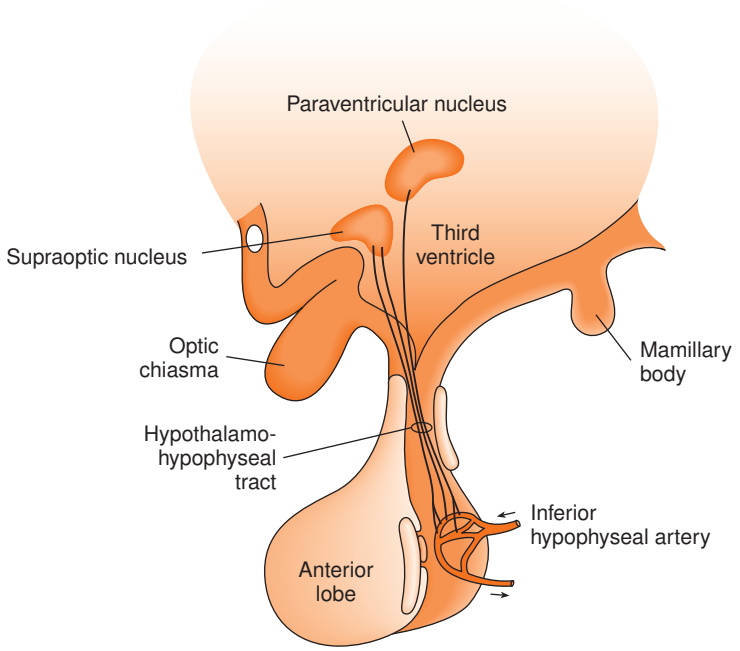


FIGURE 5-3 ■ Axonal projections from the paraventricular nuclei (PVN) and supraoptic nuclei (SON) to the pars nervosa.

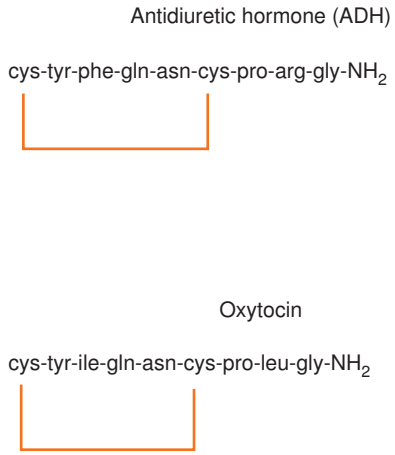


FIGURE 5-4 ■ Structure of antidiuretic hormone and oxytocin.

activity. ADH (vasopressin) and oxytocin are synthesized as preprohormones. Each prohormone harbors the structure of oxytocin or ADH, each of which is composed of 9 amino acids and a co-secreted peptide called either **neurophysin-II** (associated with ADH) or **neurophysin-I** (associated with oxytocin). These preprohormones are called **preprovasophysin** and **prepro-oxyphysin**. The N-signal peptide is cleaved as the peptide is transported into the endoplasmic reticulum. The prohormone is packaged in the endoplasmic reticulum and Golgi in a membrane-bound secretory granule in the cell bodies within the SON and PVN (Fig. 5-5). The secretory granules are conveyed intra-axonally through a "fast" (i.e., mm/hour) adenosine triphosphate (ATP)-dependent transport mechanism down the infundibular stalk to the axonal termini in the pars nervosa. During transit of the secretory granule, the prohormones are proteolytically cleaved, producing equimolar amounts of hormone and neurophysin. Secretory granules containing fully processed peptides are stored in the axonal termini. Axonal swellings due to the storage of secretory granules can be observed by light microscopy with certain stains and are termed **Herring bodies** (see Fig. 5-1D).

ADH and oxytocin are released at the pars nervosa in response to stimuli that are primarily detected at the cell bodies and their dendrites in the SON and PVN of the hypothalamus. The stimuli are primarily in the form of neurotransmitters released from hypothalamic interneurons. On sufficient stimulus, the neurons will depolarize and propagate an action potential down the axon.

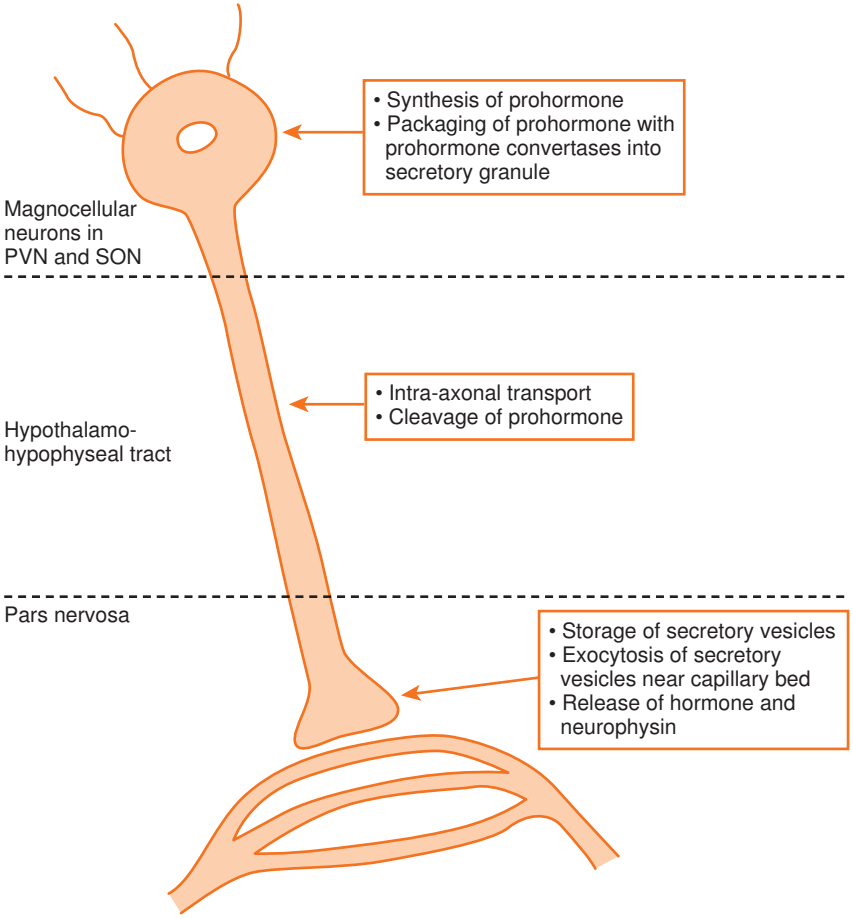


FIGURE 5-5 ■ Intra-axonal transport and processing of preprovasophysin and prepro-oxyphysin.

At the axonal termini, the action potential increases intracellular Ca^{2+} and results in a stimulus-secretion response, with the exocytosis of ADH or oxytocin, along with neurophysins, into the extracellular fluid (ECF) of the pars nervosa (see Fig. 5-5). Both hormones and neurophysins gain access to the peripheral circulation, and both can be measured in the blood.

CLINICAL BOX 5-2

There is no known biologic function for circulating neurophysins. However, several mutations in **familial diabetes insipidus** (in which ADH production is deficient) have been mapped to mutations within the neurophysin structure, suggesting that the sequence and structure of the neurophysin portion are important for the correct processing of the prohormone.

Because posterior pituitary hormones are synthesized in the hypothalamus rather than the pituitary, hypophysectomy (pituitary removal) does not necessarily permanently disrupt synthesis and secretion of these hormones. Immediately after hypophysectomy, secretion of the hormones decreases. However, over a period of weeks, the severed proximal end of the tract will show histologic modification, and pituicytes will form around the neuron terminals. Secretory vacuoles are visible, and secretion of hormone resumes from this proximal end. Secretion of hormone can actually potentially return to normal levels. In contrast, a lesion higher up on the pituitary stalk can lead to loss of the neuronal cell bodies in the PVN and SON.

Antidiuretic Hormone

Actions of ADH (Mosby Physiology Monograph Series cross reference: Chapter 9 in Renal Physiology, Third Ed., BM Koeppen and BA Stanton)

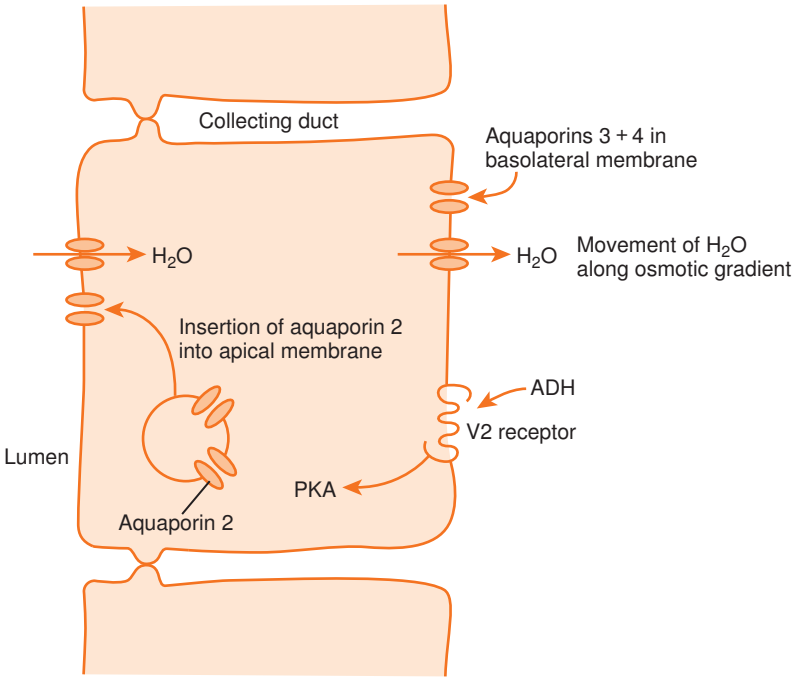


FIGURE 5-6 ■ Mechanism of antidiuretic hormone action on the kidney to promote water retention (i.e., antidiuresis).

The primary functions of ADH in humans are maintenance of normal osmolality of body fluids and maintenance of normal blood volume. The primary target cells of the ADH are the cells lining the distal renal tubule and the principal cells of the collecting ducts in the kidney. ADH binds to the **vasopressin-2 (V2) receptor** on the basal side of renal cells (Fig. 5-6). The V2 receptor is a G-protein-coupled receptor (GPCR) linked to the Gs-cAMP-PKA pathway. Signaling from the V2 receptor induces the insertion of vesicles containing the water channel protein, called **aquaporin-2**, into the apical membrane of the principal cells, thereby increasing water permeability of this membrane. ADH also increases the gene expression and new synthesis of aquaporin-2. As the basolateral side of the target cells constitutively expresses aquaporin-3 and -4, the ADH-induced increase in apical membrane aquaporin-2 enhances the transepithelial flow of water from the lumen toward the renal interstitium. Therefore, in the presence of ADH, urine flow decreases (**antidiuresis**), and urine osmolality approaches that of the medullary epithelium (about 1200 mOsm/kg). In the absence of ADH, urine flow increases (**diuresis**), and urine osmolality decreases.

ADH increases mesangial cell contraction, which lowers the filtration coefficient of the glomerular membrane and therefore decreases the glomerular filtration rate. This action will further decrease the volume of urine flow. ADH inhibits renin release, a response that could be beneficial in compensation for an increase in ECF osmolality.

As part of its role in the defense against the cardiovascular consequences of severe volume depletion, ADH levels increase to supraphysiologic levels (i.e., increase by greater than 100-fold) during **vasodilatory shock**. At these levels, ADH binds to the **V1 receptor** on vascular smooth muscle. The V1 receptor is coupled to a Gq-phospholipase C-intracellular Ca^{2+} signaling pathway, which increases vascular smooth muscle contraction. Thus, the vasopressive actions of ADH become important during early states of vasodilatory shock.

Regulation of ADH Secretion ADH is released in response to increased ECF osmolality or decreased blood volume and pressure. **Osmoreceptive neurons**, probably in the hypothalamus or circumventricular organs, innervate the magnocellular neurons of the PVN and SON. These osmoreceptive neurons respond to changes

in ECF osmolality by shrinkage or swelling. Thus, increased osmolarity indirectly stimulates the magnocellular cells and action potential frequency increases in the neuronal axons constituting the hypothalamohypophyseal tract, with a resultant increase in posterior pituitary ADH release. Because the actual stimulus is cellular dehydration, the response to the hyperosmolality depends on the nature of the solutes. Solutes such as sodium, sucrose, and mannitol that do not readily enter the osmoreceptor cells are effective stimulators, whereas urea, to which the cells are more permeable, has about one third the potency of sodium. These effects may be demonstrated with the following relationship:

↑ ECF osmolality →↑ ADH →↑ Renal water reabsorption
→↓ ECF osmolality

The regulatory system is sensitive to serum osmolality changes in the range of 280 to 295 mOsm/kg (Fig. 5-7). Within this range, a rise in as little as 1% in serum osmolality will stimulate a measurable increase in ADH secretion.

ADH release can also be stimulated by a drop in effective blood volume. The receptors for this stimulus are the **cardiovascular volume receptors**, including low-pressure receptors in the atria of the heart, great veins, and pulmonary vasculature and high-pressure receptors in the aortic arch and carotid sinus baroreceptors (Fig. 5-8). Although all of these volume receptors are

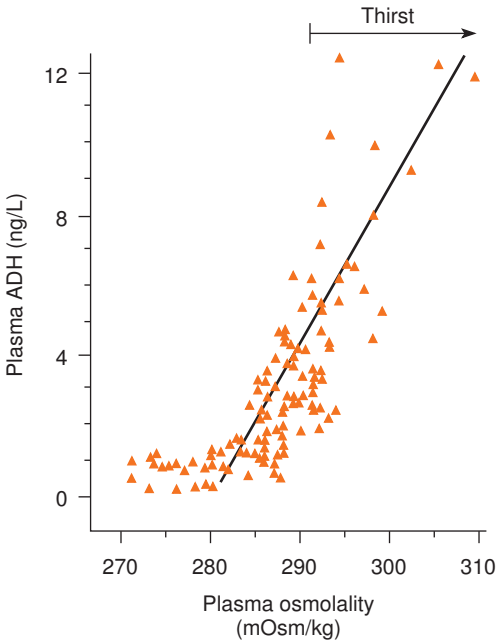


FIGURE 5-7 ■ Relation between plasma osmolality and plasma antidiuretic hormone. *(Redrawn from Wilson JD, Foster DW, Kronenberg HM, et al, editors:* Williams' Textbook of Endocrinology, *9th ed., Philadelphia, 1998, WB Saunders.)*

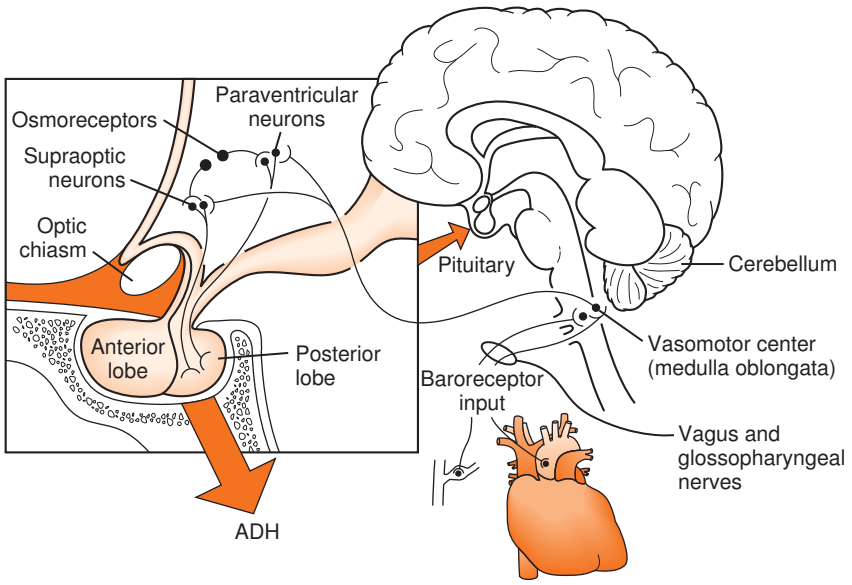


FIGURE 5-8 ■ Anatomy of the hypothalamus and pituitary gland (midsagittal section) depicting the pathways for antidiuretic hormone secretion. The *closed box* illustrates an expanded view of the hypo-thalamus and pituitary gland. *(From Koeppen BM, Stanton BA:* Renal Physiology, *3rd ed., St. Louis, 2001, Mosby.)*

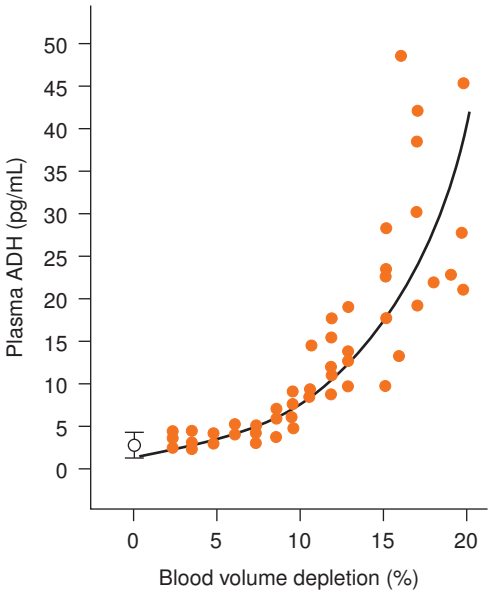


FIGURE 5-9 ■ Relation between blood volume and plasma antidiuretic hormone (ADH). *(Redrawn from Greenspan FS, Strewler GJ:* Basic and Clinical Endocrinology, *5th ed., Norwalk, CT, 1997, Appleton & Lange.)*

capable of regulating ADH secretion, the predominant regulator appears to be the atrial volume receptors. The sensitivity of the system to volume change is low at small volume changes. However, volume change does become a significant stimulus when circulating blood volume decreases 8% to 10% or more (Fig. 5-9). This becomes the only mechanism of ADH stimulation during hemorrhage. A decrease in effective blood volume increases the sensitivity of ADH secretion to an increase in ECF osmolality.

Relationship Between Osmotic and Volume Stimuli Vascular volume influences the sensitivity of the system to osmotic stimuli. At lower vascular volumes, the system becomes more sensitive to a rise in serum osmolality. In turn, as vascular volume increases, the sensitivity of ADH release to osmotic stimuli decreases.

Other Factors Altering ADH Secretion Several drugs, including barbiturates, nicotine, and opiates, increase ADH secretion. Alcohol is an effective suppressor of ADH secretion. For this reason, consumption of alcoholic beverages can lead to dehydration rather than volume expansion. Nausea increases ADH secretion, affording a protective effect against imminent volume loss due to vomiting. The hormones atrial natriuretic peptide (ANP) and cortisol (see Chapter 7) inhibit ADH secretion.

Regulation of Thirst The regulation of thirst and drinking behavior is an important component of body fluid balance regulation. Thirst is regulated by many of the same factors that regulate ADH secretion. Increased serum osmolality, decreased vascular volume, and ADH secretion are effective stimuli for thirst. The osmoreceptors regulating thirst involve medial hypothalamic regions that approximate the osmoreceptors regulating ADH secretion. Angiotensin II is also thought to play a major role in the regulation of thirst. There are many components to the regulation of drinking, which include, in humans, chemical factors, social factors, and pharyngeal and gastrointestinal factors.

Degradation ADH is predominantly destroyed by proteolysis in the kidney and liver. The circulating half-life of ADH is about 15 to 20 minutes.

CLINICAL BOX 5-3

A deficiency in ADH production results in **diabetes insipidus** (**DI**). People with DI are unable to concentrate urine normally and, therefore, excrete a large volume of urine. These individuals can have urinary flow rates as high as 25 L/day. Thirst increases as a result of the dehydration caused by the high urinary flow. Diabetes insipidus differs from **osmotic diuresis** in that in the former, the urinary osmolality (or specific gravity) is much lower than plasma, whereas in the latter, the urinary osmolality approaches that of plasma.

Neurogenic (Pituitary-Hypothalamic) Diabetes Insipidus

Neurogenic DI is due to mutations in the **preprovasophysin gene** or to destruction of either the hypothalamus (e.g., by hypothalamic tumors) or the posterior pituitary (e.g., by metastatic disease). Thus, excessive water is lost in the urine. People with neurogenic diabetes insipidus have a high urine volume and a low urinary osmolality (Table 5-1), accompanied by a high plasma osmolality with inappropriately low ADH levels. If fluids are withheld, these patients continue to produce an excessive urinary volume and a dilute urine. Note that the receptor for ADH (**vasopressin-2, or V2,**

receptor) is intact and will respond to exogenous ADH administration. Thus, ADH treatment will decrease urinary volume and increase urinary osmolality and will decrease plasma osmolality.

Nephrogenic Diabetes Insipidus

Individuals with **nephrogenic DI** have normal ADH production but lack a normal renal ADH response. The two primary defects in congenital nephrogenic DI are mutations in the **V2 receptor** and **aquaporin-2** (see Fig. 5-6). **Acquired nephrogenic DI** can occur from disruption of renal architecture with washout of the medullary gradient or by certain drugs (e.g., lithium) that impair the signaling pathway from the V2 receptor. Blood ADH levels are normal or elevated in patients with nephrogenic DI, and administration of exogenous ADH analogs does not decrease the urinary flow rate.

Psychogenic Diabetes Insipidus

Those with psychogenic DI are compulsive water drinkers. If water is withheld, the ADH secretion increases and urinary flow decreases, whereas osmolality increases. Individuals with this disorder respond to treatment with ADH.

Syndrome of Inappropriate Secretion of Antidiuretic Hormone

Many disorders can produce inappropriately high ADH concentrations relative to plasma osmolality. Some **neoplasms** produce ADH and release it into plasma. This is particularly common with pulmonary carcinomas, but it can occur in other types of tumors, including nonmalignant tumors. In addition, there are many other causes of the **syndrome of inappropriate secretion of antidiuretic hormone (SIADH)**. Pulmonary tuberculosis is often associated with SIADH, as are trauma, anesthesia, and pain. In SIADH, falling serum osmolality does not inhibit ADH secretion because control of ADH secretion is no longer linked to the normal regulatory mechanisms.

In an individual with SIADH who consumes a normal amount of water, water is retained because of the inappropriately high ADH levels. The resultant increase in blood volume and hence blood pressure increases renal glomerular filtration and therefore increases the loss of sodium in the urine. The **hypervolemia** stimulates release of **atrial natriuretic peptide (ANP)**, which promotes renal sodium loss. The person consequently becomes **hyponatremic** (low blood sodium) and has a low serum osmolality. The urine osmolality is inappropriately high (the free water clearance decreases). If water is restricted in an individual with this condition, serum sodium and osmolality will return to normal.

Oxytocin

The nonapeptide oxytocin is structurally similar to ADH, and there is some overlap in biologic activity. Although the major actions of oxytocin are on uterine motility and milk release, many other biologic actions have been proposed.

Oxytocin and Uterine Motility Oxytocin stimulates contraction of the uterine myometrium. The magnitude of the oxytocin action depends on the phase of the menstrual cycle. Estrogens increase the uterine response to oxytocin, and progestins decrease the response. Although uterine responsiveness to oxytocin increases around the time of parturition, oxytocin is not thought to be a factor initiating labor. Oxytocin secretion does not increase until after labor has begun. Once labor begins, the stretching of the

TABLE 5-1

Analysis of Various Types of Diabetes Insipidus

	NEUROGENIC	NEPHROGENIC	PSYCHOGENIC
Plasma osmolality	↑	↑≠	↓
Urine osmolality	↓	↓	↓
Plasma ADH	Low	Normal to high	Low
Urine osmolality after mild water deprivation	No change	No change	↑
Plasma ADH after water deprivation	No change	↑≠	↑
Urine osmolality after administration of ADH	↑≠	No change	↑

ADH, antidiuretic hormone.

vagina and cervix stimulates oxytocin release, which facilitates labor. This is referred to as a **neuroendocrine reflex**, which in this case has a positive feedback nature. Whereas negative feedback loops confer stability, positive feedback loops confer instability—that is, "something has to give." In the case of labor, increasing labor contractions stimulate the cervix and vagina, stimulating more oxytocin, increasing labor contractions, and so on. The pregnancy becomes unstable and is terminated by the delivery of the baby.

Sexual intercourse can stimulate oxytocin release in both men and women. Although the exact role of oxytocin in men is not entirely understood, the increased release of oxytocin during intercourse in women may aid in sperm transport in the female reproductive tract by stimulating uterine motility.

Oxytocin and Milk Letdown The role of in the mammary glands during nursing is discussed in Chapter 11.

Degradation Like ADH, oxytocin circulates unbound. It has a relatively short half-life of 3 to 5 minutes. Its degradation occurs primarily in the liver and kidney. However, it can also be degraded in other tissues, including the mammary glands and uterus.

Pathologic Conditions Involving Oxytocin No known pathologic problems are associated with excess levels of oxytocin. Although a deficiency of oxytocin does not cause major problems, it can prolong labor and produce lactational difficulties as a result of poor milk ejection in some women. Oxytocin release is inhibited by several forms of stress.

ADENOHYPOPHYSIS

Because the pars distalis makes up most of the adenohypophysis in the adult human, the terms **adenohypophysis**, **pars distalis,** and **anterior pituitary** are often used synonymously. The anterior pituitary is composed of five endocrine cell types that produce six hormones (Table 5-2). Because of the tinctorial characteristics of the cell types, the corticotropes, thyrotropes, and gonadotropes are referred to as **pituitary basophils**, whereas the somatotropes and lactotropes are referred to as **pituitary acidophils** (see Fig. 5-1C). All but one of these hormones are part of an **endocrine axis**.

Endocrine Axes

A major part of the endocrine system is organized into endocrine axes (Fig. 5-10), which contain three levels of hormonal output: The highest level of hormonal output is actually neurohormonal, and is made up of several hypothalamic nuclei, collectively referred to as the **hypophysiotropic region** of the hypothalamus, that regulate the adenohypophysis. These nuclei are distinguished from the magnocellular neurons of the PVN and SON that project to the pars nervosa in that they have small **parvicellular neuronal cell bodies** that project axons to the **median eminence**. Parvicellular neurons release neurohormones called **releasing hormones** at the median eminence (Fig. 5-11). The median eminence is like the pars nervosa in that it represents another neurovascular organ. Releasing hormones secreted from axonal endings at the median eminence enter a primary plexus of fenestrated capillaries. Hypothalamic-releasing hormones are then conveyed from the median eminence to a second capillary plexus located in the pars distalis by the **hypothalamohypophyseal portal vessels** (a "portal" vessel is defined as a vessel that begins and ends in capillaries without going through the heart). With one exception (see later) all releasing hormones are short-lived peptides (see Table 5-2) and reach significant levels only in the *private* portal system between the hypothalamus and the pituitary gland. At the secondary capillary plexus, the releasing hormones diffuse out of the vasculature and bind to their specific receptors on specific cell types within the anterior pituitary.

CLINICAL BOX 5-4

The **neurovascular link** (i.e., the pituitary stalk) between the hypothalamus and pituitary is somewhat fragile and can be disrupted by physical trauma, surgery, or hypothalamic disease. Damage to the stalk and subsequent functional isolation of the anterior pituitary result in the decline of all anterior pituitary tropic hormones except prolactin (see later).

The cells of the anterior pituitary make up the second, intermediate level of an endocrine axis. The anterior pituitary secretes protein hormones that are referred to as **tropic hormones—adrenocorticotropic**

TABLE 5-2

Endocrine Cell Types of the Adenohypophysis

CELL TYPE	CORTICOTROPE	THYROTROPE	GONADOTROPE	SOMATOTROPE	LACTOTROPE
Primary hypothalamic regulation	Corticotropin-releasing hormone (CRH) (41-aa peptide) stimulatory	Thyrotropin-releasing hormone (TRH) (tripeptide) stimulatory	Gonadotropin-releasing hormone (GnRH) (decapeptide) stimulatory	Growth hormone-releasing hormone (GHRH) (44-aa peptide) stimulatory and somatostatin (tetradecapeptide) inhibitory	Dopamine (catecholamine) inhibitory Prolactin (PRL) releasing factor (stimulatory)
Tropic hormone secreted	Adrenocorticotropic hormone (ACTH) (39-aa peptide)	Thyroid-stimulating hormone (TSH) (glycoprotein hormone)	Follicle-stimulating hormone and luteinizing hormone (FSH LH) and (glycoprotein hormone)	Growth hormone (GH) (ca. 22-kDa protein)	Prolactin (ca. 23-kDa protein)
Receptor	MC2R (Gs-linked GPCR)	TSH receptor (Gs-linked GPCR)	FSH and LH receptors (Gs-linked GPCRs)	GH receptor (JAK/STAT-linked cytokine receptor)	PRL receptor (JAK/STAT-linked cytokine receptor)
Target endocrine gland	Zona fasciculata and zona reticularis of the adrenal cortex	Thyroid epithelium	Ovary (theca and granulosa*) Testis (Leydig and Sertoli)	Liver (but also direct actions—especially in terms of metabolic effects)	No endocrine target organ—not part of an endocrine axis
Peripheral hormone involved in negative feedback	Cortisol	Triiodothyronine (T_3)	Estrogen,† progesterone, testosterone, inhibin‡	IGF-1	None

*Both follicular and luteinized thecal and granulosa cells.

†Estrogen can also have a positive feedback in women.

‡Inhibin selectively inhibits FSH release from the gonadotrope. IGF-1, insulin-like growth factor-1.

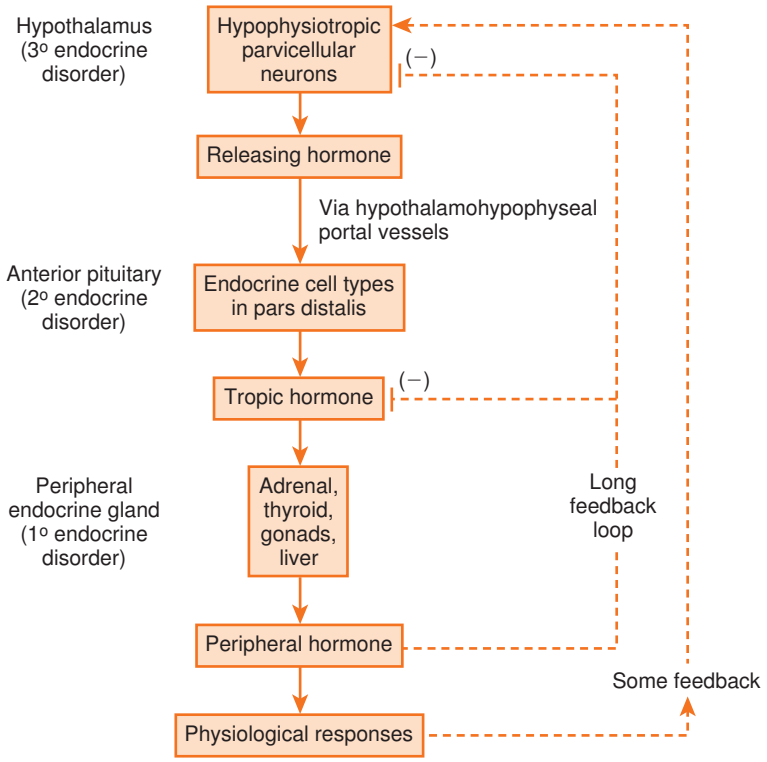


FIGURE 5-10 ■ Configuration of an endocrine axis.

hormone (ACTH), thyroid-stimulating hormone (TSH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), growth hormone (GH), and prolactin (PRL) (see Table 5-2). With a few exceptions, tropic hormones bind their receptors on peripheral endocrine glands. Because of this arrangement, pituitary tropic hormones generally do not *directly* regulate physiologic responses.

The third level of an endocrine axis involves the **peripheral endocrine organs**, which include the **thyroid gland**, the **adrenal cortex**, the **ovary**, the **testis**, and the **liver**. These peripheral endocrine glands are stimulated by pituitary tropic hormones to secrete **thyroid hormone**, **cortisol**, **estrogen**, **progesterone**, **testosterone**, and **insulin-like growth factor-1 (IGF-1)**. Thus, we refer to the following endocrine axes: hypothalamus-pituitary-adrenal axis, hypothalamus-pituitary-thyroid axis, hypothalamus-pituitary-ovary axis, hypothalamus-pituitary-testis axis, and hypothalamus-pituitary-liver axis. These axes, through the peripheral hormones they regulate, have a broad range of effects on growth, metabolism, homeostasis, and reproduction, as discussed in Chapters 6, 7, 9, and 10. The endocrine axes have the following important features:

1. The activity of a specific axis is normally maintained at a **set-point** (which in truth is a normal **range** of activity). The set-point is determined primarily by the integration of hypothalamic stimulation and peripheral hormone negative feedback. Importantly, the negative feedback is not exerted primarily by the physiologic responses regulated by a specific endocrine axis, but rather from the peripheral hormone acting on the pituitary and hypothalamus (see Fig. 5-10). Thus, if the level of a peripheral hormone drops, the secretion of hypothalamic-releasing hormones and pituitary tropic hormones will increase. As the level of peripheral hormone rises, the hypothalamus and pituitary will decrease secretion owing to negative feedback. Although some nonendocrine physiologic parameters (e.g., acute hypoglycemia) can regulate some endocrine axes, the axes function semiautonomously with respect to the physiologic changes they

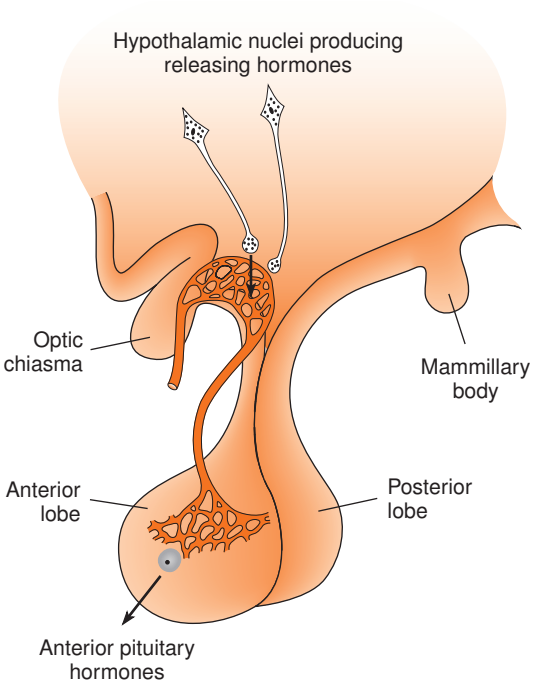


FIGURE 5-11 ■ Hypophyseotropic hormones (also called hypothalamic releasing hormones) are secreted into hypophyseal portal circulation and then transported to anterior pituitary. *(Modified from Aron DC, Findling JW, Tyrrell JB, et al: Hypothalamus and pituitary. In Greenspan FS, Strewler GJ, editors:* Basic and Clinical Endocrinology, *5th ed., Norwalk, CT, 1997, Appleton & Lange.)*

produce. This configuration means that a peripheral hormone (e.g., thyroid hormone) can evolve to regulate multiple organ systems, without those organ systems exerting competing negative feedback regulation on the hormone. Clinically, this partial autonomy means that multiple aspects of a patient's physiology are at the mercy of whatever derangements exist within a specific axis.

2. Hypothalamic hypophysiotropic neurons often secrete in a **pulsatile** manner and are entrained to daily and seasonal rhythms through CNS inputs. Additionally, hypothalamic nuclei receive various neuronal inputs from higher and lower levels of the brain. These can be short term (e.g., various stresses/infections) or long term (e.g., onset of reproductive function at puberty). Thus, the inclusion of the hypothalamus in an endocrine axis allows for the integration of a considerable amount of information in determining or changing the set-point of that axis.
3. The loss of a peripheral hormone (e.g., thyroid hormone) may be due to a defect at the level of the peripheral endocrine gland (e.g., thyroid), the pituitary gland, or the hypothalamus, which are referred to as **primary**, **secondary**, and **tertiary endocrine disorders**, respectively (see Fig. 5-10). A thorough understanding of the feedback relationships within an axis allows the physician to determine where the defect lies. Primary endocrine deficiencies tend to be the most severe because they often involve complete absence of the peripheral hormone. Disorders can also be due to excessive secretion at the primary, secondary, or tertiary level of an axis. This is usually due to a hormone-producing tumor (e.g., Cushing disease is due to an ACTH-producing pituitary tumor).

CLINICAL BOX 5-5

Clinically, the inclusion of the hypothalamus within an endocrine axis means that a broad range of complex, neurogenic states can alter pituitary function. **Psychosocial dwarfism** is a striking example of this, in which children who are abused or under intense emotional stress have lower growth rates as a result of decreased growth hormone secretion by the pituitary gland.

Endocrine Function of the Anterior Pituitary

The anterior pituitary comprises the following endocrine cell types: **corticotropes, thyrotropes, gonadotropes, somatotropes**, and **lactotropes**. Each cell type is discussed subsequently in the context of hormonal production and action, hypothalamic regulation, and feedback regulation.

Corticotropes

Corticotropes stimulate (i.e., are *tropic to*) the **adrenal cortex**, as part of the **hypothalamus-pituitary-adrenal (HPA) axis**. Corticotropes produce the

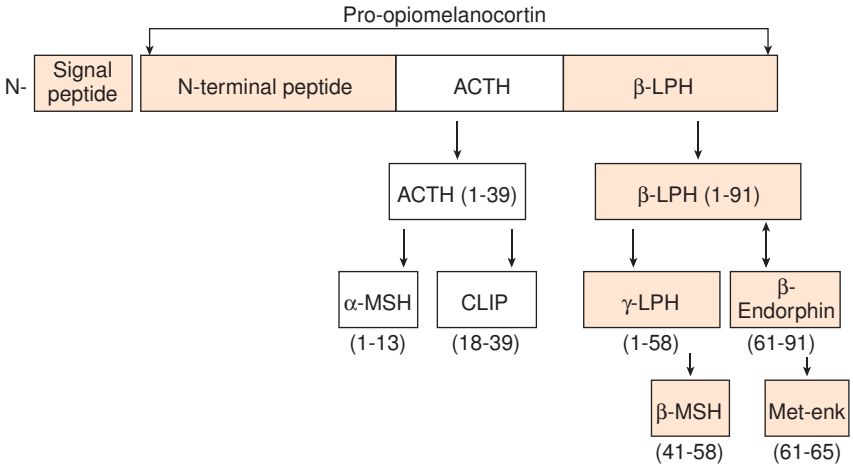


FIGURE 5-12 ■ Original gene transcript of pro-opiomelanocortin encodes the amino acid sequences of multiple bioactive compounds. Note that adrenocorticotropic hormone (ACTH) is the only bioactive peptide released by the human corticotrope. αMSH, α-melanocyte-stimulating hormone; βLPH, β-lipotrophic hormone; βMSH, β-melanocyte-stimulating hormone; CLIP, corticotropin-like intermediate peptide; γLPH, γ-lipotrophic hormone; Met-enk, metenkephalin.

hormone, **adrenocorticotropic hormone (ACTH; corticotropin)**, which stimulates two zones of the adrenal cortex (see Chapter 7).

ACTH is a 39-amino acid peptide that is synthesized as part of a larger prohormone, **pro-opiomelanocortin (POMC)**. Corticotropes are also referred to as **POMC cells** (note that POMC neurons were discussed in relation to appetite control in Chapter 3; these neurons release α—melanocyte-stimula-g hormone [α-MSH] as their neurotransmitter). POMC harbors the peptide sequence for ACTH, α- and β-MSH, endorphins (endogenous opioids), and enkephalins (Fig. 5-12). However, the human corticotrope expresses only the **prohormone convertases** capable of producing **ACTH** as the sole active hormone secreted from these cells in humans. The other fragments that are cleaved out of POMC and secreted by the corticotropes are the N-terminal fragment and β-lipotropic hormone (β-LPH). Neither of these latter two fragments appears to play a physiologic role in humans.

ACTH circulates as an unbound hormone and has a short half-life of about 10 minutes. ACTH binds to the **melanocortin-2 receptor (MC2R)** on cells in the **adrenal cortex** (Fig. 5-13). MC2R is a GPCR coupled to the Gs-cAMP-PKA signaling pathway. ACTH acutely increases cortisol and adrenal androgen production but also increases expression of steroidogenic enzyme genes and, in the long-term, promotes growth and survival of two zones of the adrenal cortex (see Chapter 7).

CLINICAL BOX 5-6

At supraphysiologic levels (e.g., Addison disease with loss of negative feedback by cortisol), ACTH causes darkening of light-colored skin. Normally, keratinocytes express the *POMC* gene, but secrete α-MSH instead of ACTH. Keratinocytes secrete α-MSH in response to ultraviolet light, and α-MSH acts as a paracrine factor on neighboring melanocytes to darken the skin. α-MSH binds to the MC1R on melanocytes. However, at high levels, ACTH can also cross-react with the MC1R receptor on skin melanocytes (see Fig. 5-13). Thus, darkening of skin is a clinical sign of excessive ACTH levels, especially in the presence of low cortisol).

ACTH is under stimulatory control by the hypothalamus. A subset of parvicellular hypothalamic neurons expresses the peptide, **procorticotropin-releasing hormone (pro-CRH)**. Pro-CRH is processed to an amidated 41-amino acid peptide, **CRH**. CRH binds to the CRH receptor, **CRH-R1**, on corticotropes. CRH-R1 is a GPCR linked to a Gs-cAMP-PKA signaling pathway. CRH acutely stimulates ACTH secretion and increases transcription of the *POMC* gene. The parvicellular neurons that express CRH also coexpress **ADH**. ADH binds to **V3 receptors** on corticotropes. The V3 receptor is GPCR linked to a Gq–phospholipase C signaling pathway. ADH potentiates the action of CRH on corticotropes.

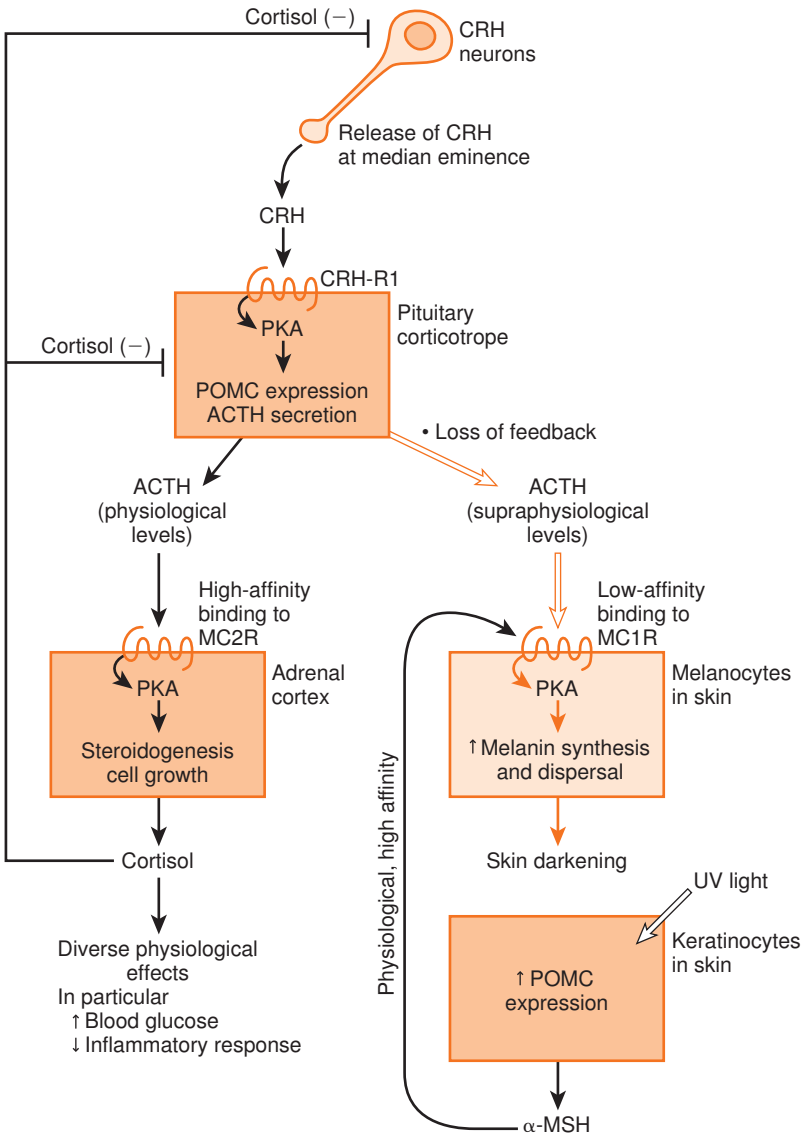


FIGURE 5-13 ■ Normal levels of adrenocorticotropic hormone (ACTH) act on the MC2R to increase cortisol. Supraphysiologic levels of ACTH (due to loss of cortisol) act on both the MC2R and the MC1R on melanocytes, causing skin darkening.

ACTH secretion shows a pronounced diurnal pattern, with a peak in early morning and a valley in late afternoon (Fig. 5-14). In addition, secretion of CRH, and hence secretion of ACTH, is pulsatile. There are multiple regulators of the HPA axis, and many of them are mediated through the CNS (Fig. 5-15). Many types of **stress**, both **neurogenic** (e.g., fear) and **systemic** (e.g., infection), stimulate ACTH secretion. The stress effects are mediated through CRH and vasopressin and the CNS. The response to many forms of severe stress can persist despite negative feedback from high cortisol levels. This means that the hypothalamus has the ability to reset the set-point of the HPA axis in response to stress. Severe, chronic depression can cause such a resetting of the HPA axis due to hypersecretion of CRH and is, in fact, a factor in the development of **tertiary hypercortisolism** (i.e., excess cortisol production due to hypothalamic dysfunction). Because cortisol has profound effects on the immune system (see Chapter 7), the HPA axis and the immune system are

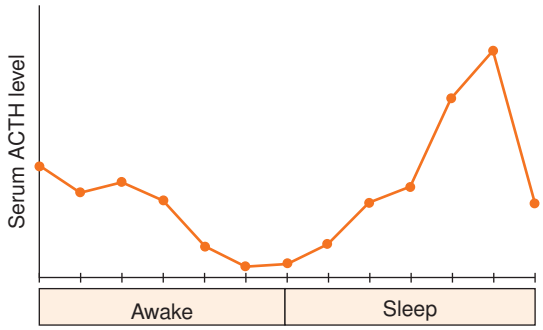


FIGURE 5-14 ■ Diurnal pattern for serum adrenocorticotropic hormone (ACTH).

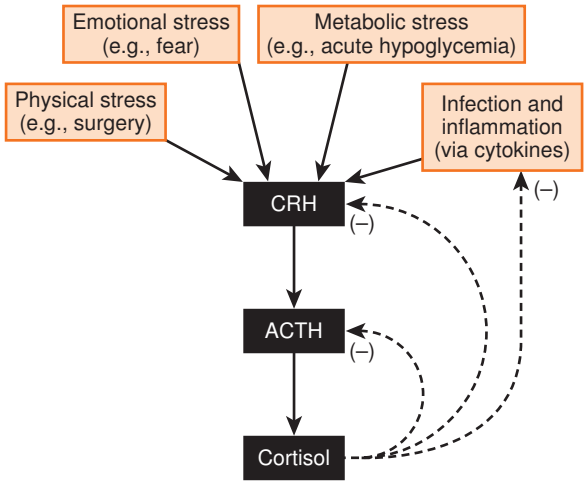


FIGURE 5-15 ■ Hypothalamic-pituitary-adrenal axis illustrating factors regulating secretion of corticotropin-releasing hormone (CRH). ACTH, adrenocorticotropic hormone.

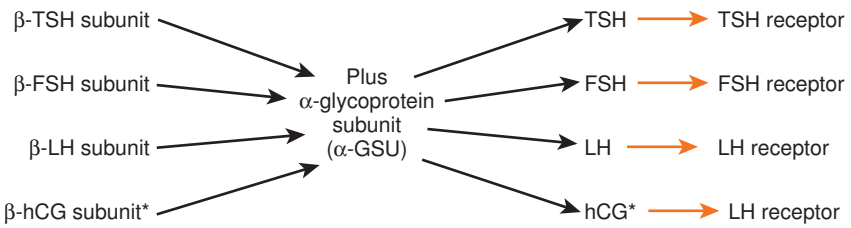
closely coupled, and **cytokines**—particularly interleukin-1 (IL-1), IL-2, and IL-6—stimulate the HPA axis.

Cortisol exerts a negative feedback on the pituitary, where it suppresses *POMC* gene expression and ACTH secretion, and on the hypothalamus, where it decreases pro-*CRH* gene expression and CRH release. As mentioned earlier, ACTH has a long-term effect on the growth and survival of adrenocortical cells. This means that long-term administration of exogenous corticosteroids will cause the adrenal cortex to atrophy because of the negative feedback of the exogenous hormone on ACTH secretion. In such a patient, termination of exogenous corticosteroid therapy must be gradual in order to allow the adrenal cortex to regain its normal functional capacity.

Thyrotropes

Thyrotropes regulate thyroid function by secreting the hormone **TSH** (also called **thyrotropin**) as part of the **hypothalamus-pituitary-thyroid axis**. TSH is one of three **pituitary glycoprotein hormones** along with FSH and LH (see later). TSH is a heterodimer composed of an α-subunit, called the **α-glycoprotein subunit** (**α-GSU**), and a **β-subunit** (**β-TSH**) (Fig. 5-16). The α-GSU is common to TSH, FSH, and LH, whereas the β-subunit is specific to the hormone (i.e., **β-TSH**, **β-FSH**, and **β-LH** are all unique).

Glycosylation (in particular, terminal sialylation) of the subunits increases their stability in the circulation. The half-lives of TSH, FSH, and LH (and an LH-like placental glycoprotein hormone, **human chorionic gonadotropin** [**hCG**]) are relatively long, ranging from tens of minutes to several hours.



*hCG is human chorionic gonadotropin. hCG is made by the placenta, and binds to the LH receptor.

FIGURE 5-16 ■ Pituitary glycoprotein hormones. Human chorionic gonadotropin (hCG) is made by the placenta and binds to the luteinizing hormone (LH) receptor. FSH, follicle-stimulating hormone; TSH, thyroid-stimulating hormone.

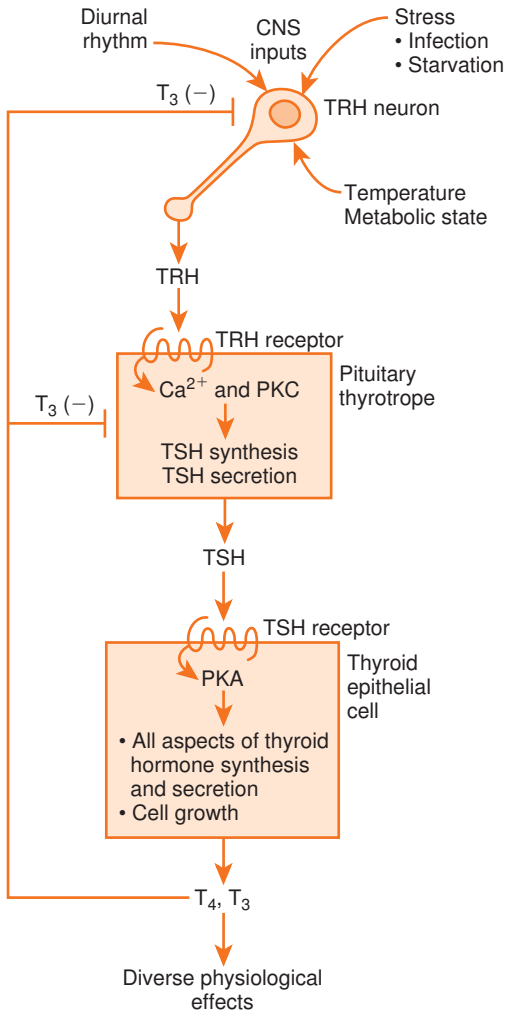


FIGURE 5-17 ■ Hypothalamus-pituitary-thyroid axis. PKA, protein kinase A; PKC, protein kinase C; TRH, thyrotropin-releasing hormone; TSH thyroid-stimulating hormone; T_4, tetraiodothyronine; T_3, triiodothyronine (active form of thyroid hormone).

Glycosylation also serves to increase the affinity and specificity of the hormones for their receptors.

TSH binds to the **TSH receptor** on thyroid epithelial cells (Fig. 5-17). The TSH receptor is a GPCR linked to a Gs-cAMP-PKA signal transduction pathway. As discussed in Chapter 6, the production of thyroid hormones is a complex, multistep process. TSH stimulates essentially every aspect of thyroid function. TSH also has a strong tropic effect, stimulating hypertrophy, hyperplasia, and survival of thyroid epithelial cells. Indeed, in geographic regions of low iodide availability (iodide is required for thyroid hormone synthesis), TSH levels become elevated because of reduced negative feedback. Elevated TSH levels can produce noticeable growth of the thyroid, causing a bulge in the neck, called a **goiter**.

The pituitary thyrotrope is stimulated by the **thyrotropin-releasing hormone (TRH)**. TRH is produced by a subset of parvicellular hypothalamic neurons. TRH is a tripeptide, with cyclization of a glutamine at its N-terminus (pyroGlu), and an amidated C-terminus (similar to the structure of gastrin termini; see Chapter 2). TRH is synthesized as a larger prohormone, which contains six copies of TRH within its sequence. TRH binds to the **TRH receptor** on the thyrotropes (see Fig. 5-19). The TRH receptor is a GPCR linked to a Gq–phospholipase C signaling pathway. TRH neurons are regulated by numerous CNS-mediated stimuli. TRH is released according to a diurnal rhythm (highest during overnight hours, lowest around dinnertime). TRH is regulated by various stresses, but unlike with CRH, stresses inhibit TRH secretion. These include physical and mental stress, starvation, and infection. The active form of thyroid hormone, triiodothyronine (T_3), negatively feeds back on both pituitary thyrotropes and TRH-producing neurons. T_3 represses both β-TSH expression and the sensitivity of thyrotropes to TRH. T_3 also inhibits TRH production and secretion.

Gonadotropes

The **gonadotrope** is a dual hormone producer in that the same cell secretes **FSH** and **LH**. FSH and LH are also referred to as **gonadotropins** and have the same nomenclature in men and women, while being named for their actions in women. The gonadotrope regulates the function of gonads in both sexes. As such, the gonadotrope plays an integral role in the **hypothalamus-pituitary-testis axis** and the **hypothalamus-pituitary-ovary axis** (Fig. 5-18).

As discussed earlier, FSH and LH are pituitary glycoprotein hormones composed of a common **α-GSU** heterodimerized with a unique **β-FSH** or

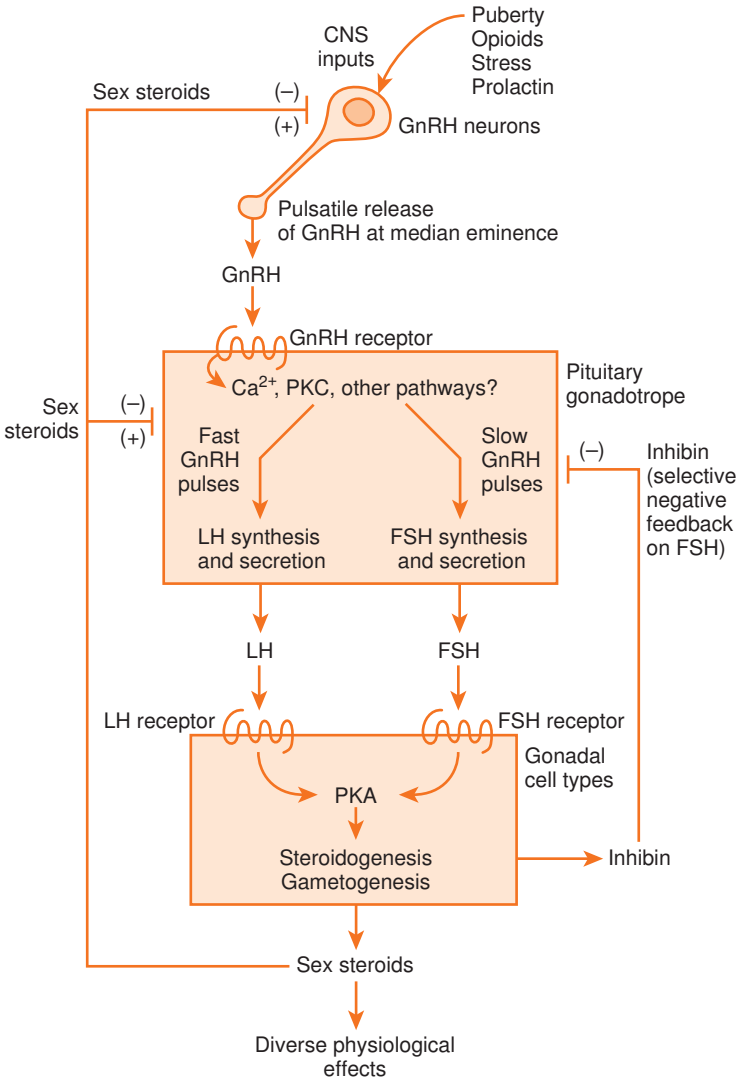


FIGURE 5-18 ■ Hypothalamus-pituitary-gonadal axis. FSH, follicle-stimulating hormone; GnRH, gonadotropin-releasing hormone; LH, luteinizing hormone.

β-LH subunit. Importantly, FSH and LH are segregated to a large degree into different secretory granules and are not co-secreted in equimolar amounts (in contrast to ADH and neurophysin, for example). This allows for the modulation of the ratio of FSH/LH secretion by the gonadotropes. FSH and LH bind to their respective receptors, which are both GPCRs primarily coupled to Gs-cAMP-PKA signaling pathways. The actions of FSH and LH on gonadal function are complex, especially in women, and are discussed in detail in Chapters 9 and 10. In general, gonadotropins promote testosterone production in men and estrogen and progesterone secretion in women. FSH also increases the secretion of a TGF-β-related protein hormone, called **inhibin**, in both sexes.

FSH and LH secretion are regulated by one hypothalamic releasing hormone, **gonadotropin-releasing hormone (GnRH)**. GnRH is a 10-amino acid peptide produced by a subset of parvicellular hypothalamic GnRH neurons. GnRH is produced as a larger prohormone, and as part of its processing to a decapeptide, is modified with a cyclized glutamine (pyroGlu) at its amino terminus and has an amidated carboxy terminus.

CLINICAL BOX 5-7

During embryonic development, the GnRH neurons migrate to the mediobasal hypothalamus from the nasal placode. Patients with **Kallmann syndrome** have **tertiary hypogonadotropic hypogonadism**, often associated with loss of sense of smell (**anosmia**). This is due to a mutation in the *KAL* gene, which results in the failure of the GnRH neuronal precursors to properly migrate to the hypothalamus from the region of the nasal placode and to establish a neurovascular link to the pars distalis.

GnRH binds to the **GnRH receptor**, which is a GPCR coupled primarily to a Gq–phospholipase C signaling pathway. GnRH is released in a pulsatile manner, and both the pulsatile secretion and the frequency of the pulses have important effects on the gonadotrope. Continuous infusion of GnRH downregulates the GnRH receptor, resulting in a decrease in FSH and LH secretion. In contrast, pulsatile secretion does not desensitize the gonadotrope to GnRH, and FSH and LH secretion are normal. At a frequency of 1 pulse per hour, GnRH preferentially increases LH secretion (Fig. 5-19). At a slower frequency of 1 pulse per 3 hours, GnRH preferentially increases FSH secretion. The mechanism by which the frequency of GnRH secretion determines the ratio of FSH to LH levels in the blood is poorly understood but may involve multiple signaling pathways linked to the GnRH receptor, leading to differential synthesis or glycosylation, or both, of FSH versus LH.

GnRH secretion is inhibited by numerous drugs (e.g., opioids, selective serotonin reuptake inhibitors) and by intense exercise and mental stress (depression). GnRH secretion is also regulated by the program of puberty. During infancy and childhood, GnRH is under inhibitory signaling from the CNS. At puberty, this inhibition is lost, and GnRH levels increase. The hormone leptin, which acts as a gauge of adipose mass and thus energy supplies, plays a permissive role in puberty.

CLINICAL BOX 5-8

Anorexia nervosa is an eating disorder in which patients (usually early adolescent girls) develop an extreme resistance to eating and a distorted body image in which they perceive themselves as fat even in the face of emaciation. A diagnostic criterion for anorexia nervosa in postmenarchal girls is **amenorrhea**, as defined by the absence of three consecutive menstrual cycles. The amenorrhea is due to loss of **GnRH** secretion in response to an **extreme energy deficit**.

Gonadotropins increase sex steroid synthesis. In men, testosterone and estrogen negatively feed back at the level of the pituitary and the hypothalamus.

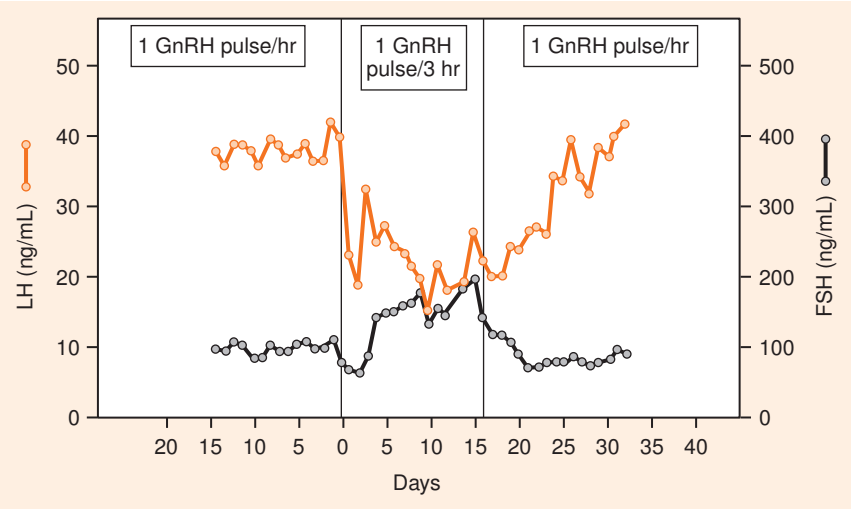


FIGURE 5-19 ■ Frequency encoded regulation of follicle-stimulating hormone (FSH) and luteinizing hormone (LH). A higher frequency of GnRH pulses preferentially stimulates LH secretion, whereas a slower frequency preferentially stimulates FSH secretion. *(From Larsen PR, Kronenberg HM, Melmed S, et al, editors:* Williams Textbook of Endocrinology, *10th ed., Philadelphia, 2003, Saunders, 2003; and Koeppen BM, Stanton BA, editors:* Berne & Levy Physiology, *6th ed., Philadelphia, 2010, Mosby, p 719.)*

Exogenous progesterone also inhibits gonadotropin function in men and could act as a possible ingredient in a male contraceptive pill. Additionally, inhibin negatively feeds back selectively on FSH secretion in men and women. In women, progesterone and testosterone negatively feed back on gonadotropic function at the level of hypothalamus and pituitary. At low doses, estrogen also exerts a negative feedback on FSH and LH secretion. However, high estrogen levels (e.g., 500 pg/mL) maintained for 3 days cause a surge of LH and, to a lesser extent, FSH secretion (see Chapter 10). This positive feedback is observed at the hypothalamus and pituitary. At the hypothalamus, GnRH pulse amplitude and frequency increase. At the pituitary, high estrogen levels greatly increase the sensitivity of the gonadotrope to GnRH by increasing GnRH receptor levels and by enhancing postreceptor signaling pathway components.

Somatotropes

The **somatotropes** produce **GH** (also called **somatotropin**). A major target of GH is the **liver**, where it stimulates the production of **IGF-1**. Thus, the somatotrope is part of the **hypothalamus-pituitary-liver axis** (Fig. 5-20). However, GH also has several direct actions at physiologic levels on nonendocrine organs (Box 5-1).

GH is a 191-amino acid protein that is similar to PRL and human placental lactogen (hPL), and there is some overlap in activity among these hormones. Multiple forms of GH are seen in serum, thereby

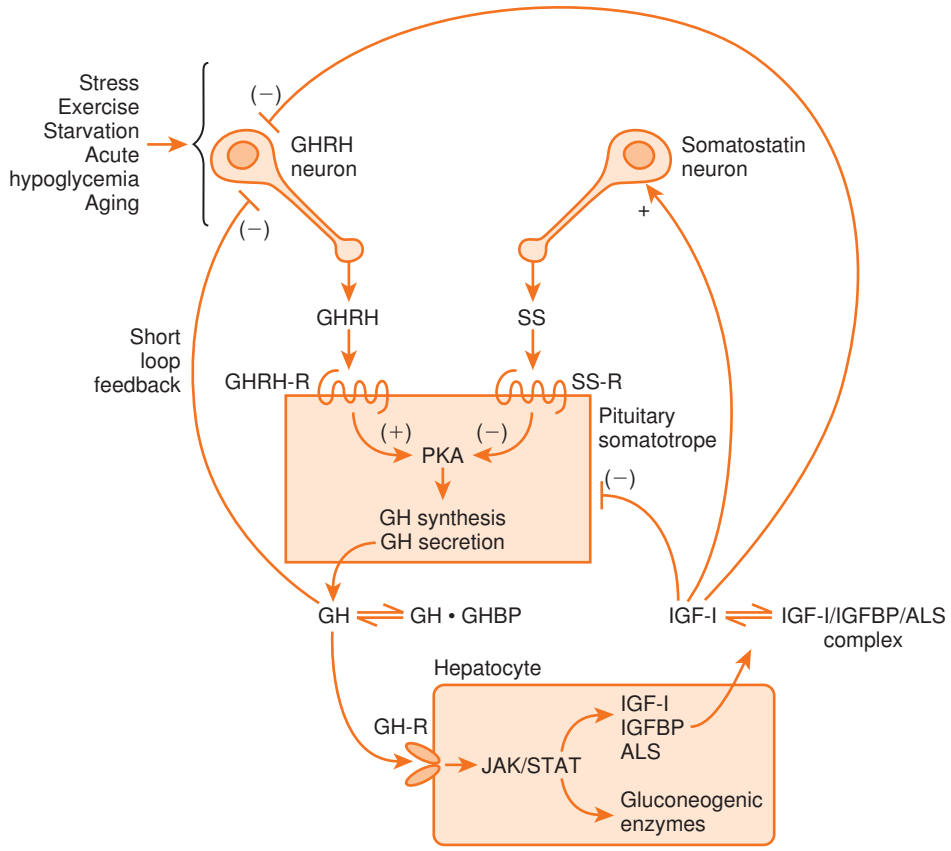


FIGURE 5-20 ■ Hypothalamus-pituitary-liver axis. ALS, acid labile subunit; GHBP, growth hormone–binding protein; GHRH, growth hormone–releasing hormone; IGF-1, insulin-like growth factor-1; IGFBP, insulin-like growth factor-binding protein; SS, somatostatin.

BOX 5-1

METABOLIC ACTIONS OF GROWTH HORMONE

CARBOHYDRATES

Increase blood glucose
Decrease peripheral insulin sensitivity
Increase hepatic output of glucose
Administration results in increased serum insulin levels

PROTEINS

Increase tissue amino acid uptake
Increase incorporation into proteins
Decrease urea production
Produce positive nitrogen balance

LIPIDS

Promotes lipolysis
Can be ketogenic after long-term administration, particularly if insulin is deficient

INSULIN-LIKE GROWTH FACTOR

Stimulates IGF production
Stimulates growth
Is mitogenic

constituting a "family of hormones," with the 191-amino acid (22-kDa) form representing about 75% of the circulating GH. The **GH receptor** is a member of the cytokine-GH-PRL-erythropoietin receptor family and, as such, is linked to the JAK-STAT signaling pathway. Human GH can also act as an agonist for the **PRL receptor**.

About 50% of the 22-kDa form of GH in serum is bound to the N-terminal portion (the extracellular domain) of the GH receptor, called **GH-binding protein (GHBP)**. **Laron dwarfism** is characterized by the absence of or decrease in normal GH receptors and GHBP and by normal GH secretion. GHBP reduces renal clearance and thus increases the biologic half-life of GH. The circulating half-life for GH is only about 20 minutes. The liver and kidney are major sites of hormone degradation.

GH secretion is under dual control by the hypothalamus (see Fig. 5-20). The hypothalamus predominantly stimulates GH secretion through the peptide, **growth hormone–releasing hormone (GHRH)**. GHRH is a member of the vasoactive intestinal peptide (VIP)-secretin-glucagon family and is processed into a 44-amino acid peptide with an amidated C-terminus from a larger prohormone. GHRH binds to the **GHRH receptor**, which is coupled to a Gs-cAMP-PKA signaling pathway. GHRH enhances GH secretion and *GH* gene expression. The hypothalamus inhibits pituitary GH synthesis and release through the peptide **somatostatin**. Somatostatin is a cyclic tetradecapeptide that is found in many locations in the body (see Chapter 2). Somatostatin in the anterior pituitary inhibits GH and TSH release. Somatostatin binds to the **somatostatin receptor**, which lowers cyclic adenosine monophosphate (cAMP) through a Gi-linked signaling pathway.

The primary negative feedback on the somatotrope is exerted by IGF-1. GH stimulates IGF-1 production by the liver, and IGF-I then inhibits GH synthesis and secretion at the pituitary and hypothalamus by a classic long feedback loop. In addition, GH exerts negative feedback on GHRH release through a short feedback loop. GH also increases somatostatin release.

GH secretion, like that of ACTH, shows prominent diurnal rhythms, with peak secretion occurring in the early morning just before awakening (see Fig. 5-20). Its secretion is stimulated during deep, slow-wave sleep (stages III and IV). GH secretion is lowest during the day. This rhythm is entrained to sleep-wake patterns rather than light-dark patterns, so a phase shift occurs in people who work night shifts. As is typical of anterior pituitary hormones, GH secretion is pulsatile. The levels of GH in serum vary widely (0 to 30 ng/mL, with most values usually falling between 0 and 3). Because of this marked variation, serum GH values are of minimal clinical value unless the sampling time is known. Frequently, rather than measuring GH, the clinician measures IGF-1 because its secretion is regulated by GH, and IGF-1 has a relatively long circulating half-life that buffers pulsatile and diurnal changes in secretion.

GH secretion is also regulated by several different physiologic states (see Fig. 5-20). GH is classified as one of the stress hormones and is increased by neurogenic and physical stress. As discussed later, GH promotes lipolysis, increases protein synthesis, and antagonizes the ability of insulin to reduce blood glucose. It is not surprising, therefore, that hypoglycemia is a stimulus for GH secretion, and GH is classified as a **hyperglycemic hormone**. Although its secretion is not regulated by minor variations in serum glucose levels, its release is stimulated by falling glucose levels or by

hypoglycemia. Falling blood glucose levels are such an effective stimulus that insulin-induced hypoglycemia can be used as a provocative test of a person's ability to secrete GH. A rise in certain serum amino acids also serves as an effective stimulus for GH secretion. Arginine is one of these amino acids, and the GH response to arginine infusion may be used to evaluate GH secretion. In contrast, an increase in blood glucose or free fatty acids inhibits GH secretion. Obesity also inhibits GH secretion, in part because of insulin resistance (relative hyperglycemia) and increased circulating free fatty acids. Conversely, exercise is an effective stimulator of GH secretion. GH is also increased during starvation. Other hormonal regulators of GH include estrogen, androgens, and thyroid hormone, which enhance GH secretion; they have direct effects on IGF-1 secretion and bone maturation as well.

GH secretion is also regulated by **ghrelin**, which is primarily produced by the stomach (see Chapter 3). Ghrelin is expressed in response to an empty stomach during fasting. GH is also secreted during fasting in response to decreased nutrient levels, especially hypoglycemia. Thus, ghrelin not only promotes eating but also reinforces the secretion of GH. GH, in turn promotes lipolysis in adipose tissue and opposes the actions of insulin on glucose uptake by muscle and adipose tissue (see later).

Direct Versus Indirect Actions of Growth Hormone

Direct Actions of GH on Metabolism GH acts directly on the liver, muscle, and adipose tissue to regulate energy metabolism (Fig. 5-21). It shifts metabolism to lipid use for energy, thereby conserving carbohydrates and proteins.

GH is a protein anabolic hormone that increases cellular amino acid uptake and incorporation into protein and represses proteolysis. Consequently, it produces nitrogen retention (a positive nitrogen balance) and a decreased urea production. The muscle wasting that occurs concomitant with aging has been proposed to be caused, at least in part, by the decrease in GH secretion that occurs with aging.

GH is a lipolytic hormone. It activates hormone-sensitive lipase and therefore mobilizes neutral fats from adipose tissue. As a result, serum fatty acid levels rise after GH administration. More fats are used for energy production. Fatty acid uptake and oxidation increase in skeletal muscle and liver. GH can be

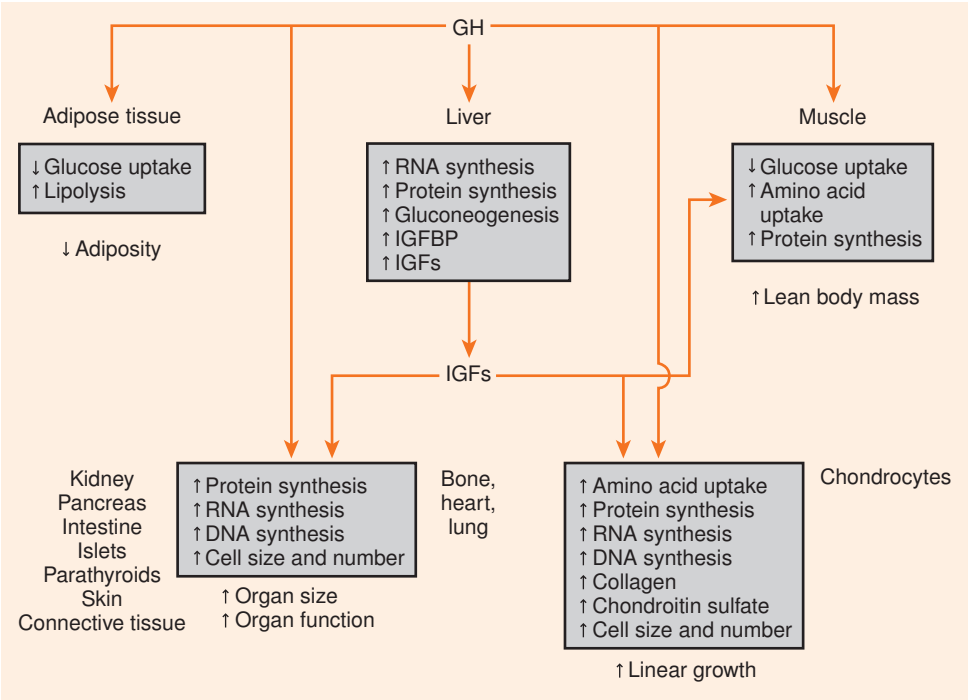


FIGURE 5-21 ■ Biologic actions of GH. *(From Koeppen BM, Stanton BA editors: Berne & Levy Physiology, 6th ed., Philadelphia, 2010, Mosby.)*

ketogenic as a result of the increase in fatty acid oxidation (the ketogenic effect of GH is not seen when insulin levels are normal). If insulin is given along with GH, the lipolytic effects of GH are abolished.

GH alters carbohydrate metabolism. Many of its actions may be secondary to the increase in fat mobilization and oxidation; an increase in serum free fatty acids inhibits glucose uptake and utilization in skeletal muscle and adipose tissue. After GH administration, blood glucose rises. The hyperglycemic effects of GH are mild and slower than those of glucagon and epinephrine. The increase in blood glucose results, in part, from decreased glucose uptake and use in skeletal muscle and adipose tissue. Liver glucose output increases, and this is probably not a result of glycogenolysis. In fact, glycogen levels can rise after GH administration. However, the increase in fatty acid oxidation and, hence, the rise in liver acetyl coenzyme A (acetyl CoA) stimulate gluconeogenesis, followed by increased glucose production from substrates such as lactate and glycerol.

GH antagonizes the action of insulin at the postreceptor level in skeletal muscle and adipose tissue (but not liver). Hypophysectomy (removal of the pituitary gland) can improve diabetic management because GH, like cortisol, decreases insulin sensitivity. Because GH produces insulin insensitivity, it is considered a **diabetogenic hormone**. When secreted in excess, GH can cause insulin resistance and ultimately type 2 diabetes. Normal levels of GH are required for normal pancreatic function and insulin secretion. In the absence of GH, insulin secretion declines.

Indirect Effects of GH on Growth GH promotes the growth of bones and visceral organs. GH administration increases skeletal and visceral growth; children without GH show growth stunting or dwarfism. If given in vivo, GH results in increased cartilage growth, long-bone length, and periosteal growth. Most of these are mediated by a group of hormones called IGFs (see Fig. 5-21).

Insulin-like Growth Factors **IGFs** are multifunctional hormones that regulate cellular proliferation, differentiation, and cellular metabolism. These protein hormones resemble insulin in structure and function. The two hormones in this family, **IGF-1** and **IGF-2**, are produced in many tissues and have autocrine, paracrine, and endocrine actions. IGF-1 is the major form produced in most adult tissues, and IGF-2 is the major form produced in the fetus. Both compounds are structurally similar to proinsulin, with IGF-1 having 42% structural homology with proinsulin. IGFs and insulin cross-react with each other's receptors, and IGFs in high concentration mimic the metabolic actions of insulin. Both IGF-1 and IGF-2 act through **type 1 IGF receptor**s. However, IGF-2 also binds to the **type 2 IGF–mannose-6-phosphate receptor**. This receptor does not resemble the insulin receptor and does not have intrinsic tyrosine kinase. Binding to these receptors probably facilitates internalization and degradation of the growth factor. IGFs stimulate glucose and amino acid uptake and protein and DNA synthesis. They were initially called somatomedins because they mediate GH (somatotropin) action on cartilage and bone growth. IGFs have many other actions, and GH is not the only regulator of IGF formation. Initially, IGFs were thought to be produced in the liver in response to a GH stimulus. It is now known that IGFs are produced in many tissues and that their many actions are autocrine or paracrine. The liver is probably the predominant source of circulation IGFs (see Fig. 5-20).

Essentially all circulating IGFs are transported in serum bound to **insulin-like growth factor–binding proteins (IGFBPs)**. IGFBPs bind to IGFs and then associate with another protein, called **acid labile subunit (ALS)**. GH stimulates the hepatic production of IGF-1, IGFBPs, and ALS (see Fig. 5-20). The **IGFBP–ALS–IGF-1 complex** mediates transport and bioavailability of the IGF-1. Although IGFBPs generally inhibit IGF action, they greatly increased the biologic half-life of IGFs (up to 12 hours). IGFBP proteases degrade IGFBP and probably play a role locally in generating free (i.e., active) IGFs. This is of interest in the context of IGF-responsive cancers (e.g., prostate cancer), which may overexpress one or more IGFBP proteases.

Although GH is an effective stimulator for IGF production, the correlation between GH and IGF-1 is greater than the correlation between GH and IGF-2. During puberty, when GH levels increase, IGF-1 levels increase in parallel.

IGFs have profound effects on bone and cartilage (see Fig. 5-21). They stimulate the growth of bones, cartilage, and soft tissue and regulate essentially all aspects of the metabolism of the cartilage-forming cells, called chondrocytes. IGFs are mitogenic. Although appositional growth of long bones continues after

closure of the epiphyses, growth in length ceases. IGFs stimulate osteoblast replication and collagen and bone matrix synthesis. Serum IGF levels correlate well with growth in children.

Interaction of Role of Growth Hormone, Insulin-like Growth Factor, and Insulin in Different Metabolic States

When ample supplies of nutrients are available, the high serum amino acid levels stimulate GH and insulin secretion, and the high serum glucose levels stimulate insulin secretion. The high serum GH, insulin, and nutrient supplies stimulate IGF production, and these conditions are appropriate for growth.

However, if the diet is high in calories but low in amino acids, the conditions change. Whereas the high carbohydrate availability results in high insulin availability, the low serum amino acid levels inhibit GH and IGF production. These conditions allow dietary carbohydrates and fats to be used, but conditions are unfavorable for tissue growth.

During fasting, when nutrient availability decreases, serum GH levels rise, and serum insulin levels fall (because of hypoglycemia). Importantly, GH cannot stimulate IGF production in the absence of insulin. This means that during starvation, with very low levels of insulin, IGF secretion is effectively inhibited, and the conditions are not favorable for growth. Under these circumstances, the decrease in IGF-1 reduces a negative feedback on GH. The consequent rise in GH secretion is beneficial because it promotes fat mobilization while minimizing tissue protein loss. In the absence of insulin, peripheral tissue glucose use decreases, thereby conserving glucose for essential tissues such as brain.

Pathologic Conditions Involving Growth Hormone

GH is necessary for growth before adulthood. Deficiencies can produce **dwarfism,** and excesses can produce **gigantism**. Normal growth requires not only normal levels of GH but also normal levels of thyroid hormones, sex steroids, and insulin.

Dwarfism If a GH deficiency occurs before puberty, growth is severely impaired (Fig. 5-22). Individuals with this condition are relatively well proportioned and have normal intelligence. If the anterior pituitary deficiency is limited to GH, they can have a normal life span. They are sometimes pudgy because they lose GH-induced lipolysis. If they have **panhypopituitary dwarfism** (all anterior pituitary hormones are deficient) so that gonadotropins are deficient, they may not mature sexually and remain infertile. People with dwarfism show few metabolic abnormalities other than a tendency toward hypoglycemia, insulinopenia, and increased insulin sensitivity. There are multiple potential sites of impairment. GH secretion may be reduced, GH-stimulated IGF production may decrease, or IGF action may be

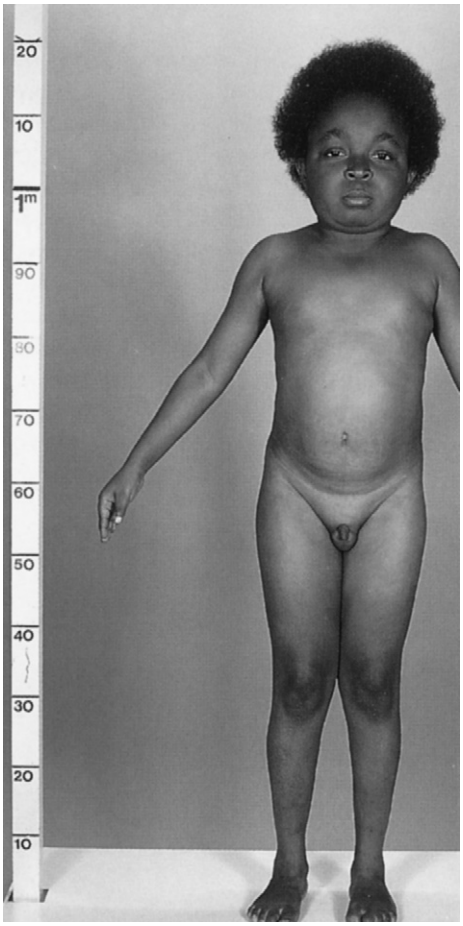


FIGURE 5-22 ■ A 17-year-old boy with growth hormone deficiency associated with hypopituitarism. The patient has short stature for his age (note scale bar) and underdeveloped genitalia. *(From Besser GM, Thorner MO:* Clinical Endocrinology, *London, 1994, Mosby-Wolfe.)*

deficient. **Laron dwarfs** are GH resistant because of a genetic defect in the expression of the GH receptor so that response to GH is impaired. Hence, although the serum GH levels are normal to high, they do not produce IGFs in response to GH. Treating patients afflicted by Laron dwarfism with GH will not correct the growth deficiency.

The **African pigmy** represents another example of abnormal growth. Individuals with this condition have normal serum GH levels, but they do not exhibit the normal rise in IGF that occurs at puberty. However, the IGF-2 levels are normal. Unlike the Laron dwarfs, they do not totally lack the IGF response to GH.

Growth Hormone Deficiency in Adults GH deficiency in adults is only currently becoming recognized as a pathologic syndrome. If the GH deficiency occurs after the epiphyses close, growth is not impaired. A GH deficiency is one of many possible causes of **hypoglycemia**. Recent studies have shown that extended deficiencies of GH lead to body composition changes. The percentage of the body weight that is fat increases, whereas the percentage that is protein decreases. In addition, muscle weakness and early exhaustion are symptoms of GH deficiency.

Because the muscle loss that occurs with aging may result from an age-related decline in GH production, GH is being used experimentally in elderly people to delay the physical decline associated with aging. The efficacy of this treatment in humans has not been established.

Growth Hormone Excess Before Puberty Excessive GH production before puberty results in **gigantism**. Individuals with this condition can reach heights greater than 8 feet. The GH excess results in an increase in body weight as well as height. Many complications are associated with gigantism. These individuals frequently have **glucose intolerance** and **hyperinsulinemia**. Overt clinical diabetes mellitus can develop, but ketoacidosis is rare. They have cardiovascular problems, including cardiac hypertrophy (all viscera increase in size); they are more susceptible to infections than normal; and they rarely live past their 20s. Hypersecretion of GH generally results from pituitary tumors; tumor growth eventually compresses other components of the anterior pituitary, decreasing secretion of other anterior pituitary hormones.

Figure 5-23 shows Robert Wadlow, the Alton Giant. At 1 year, he weighed 62 pounds. His adult size was 8 feet, 11 inches and 475 pounds. Note the long

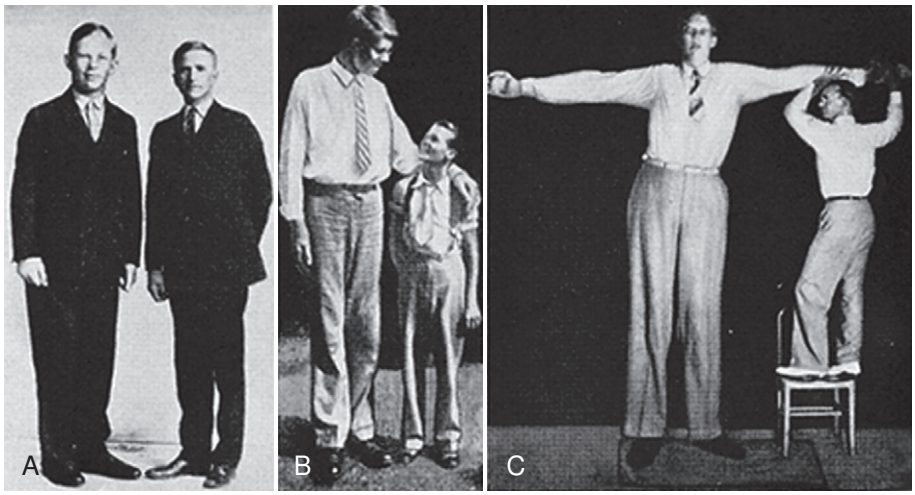


FIGURE 5-23 ■ A notable example of growth hormone excess was Robert Wadlow, later known as the "Alton Giant." Although he weighed only 9 pounds at birth, he grew rapidly, and by 6 months of age, he weighed 30 pounds. At 1 year of age, he weighed 62 pounds. Growth continued throughout his life. Shortly before his death at age 22 years from cellulitis of the feet, he was 8 feet, 11 inches tall and weighed 475 pounds. *(**A** and **B,** from Fadner F:* Biography of Robert Wadlow, *Boston, MA, 1944, Bruce Humphreys.* ***C,** Courtesy Dr. C. M. Charles and Dr. C. M. MacBryde.)*

extremities. The androgen deficiency secondary to the gonadotropin deficiency caused delayed puberty, resulting in late closure of the epiphyses and contributing to long bone growth.

Acromegaly Excessive GH secretion after the epiphyses close results in appositional bone growth, but not a further lengthening of long bones. Cartilage and membranous bones continue to grow, and gross deformities can result. In addition, soft tissue growth increases, and the abdomen protrudes as a result of visceral enlargement. Brain weight increases, with a resultant decrease in ventricular size. There is an increase in the growth of the nose, ears, and mandible, with the mandibular enlargement producing prognathism and widely spaced teeth (Fig. 5-24). The calvarium thickens, and the frontal sinuses enlarge, resulting in protrusion of the frontal ridge of the orbit of the eye. The characteristic enlargement of the hands and feet is the basis for the name **acromegaly** (*acro,* end or extremity; *megaly,* enlargement). The excessive bone and cartilage growth can produce carpal tunnel syndrome and joint problems. The voice deepens because of laryngeal growth. Acromegaly usually results from a **functional tumor of the somatotropes**. Because it is generally slow in onset, patients typically do not seek medical help for 13 to 14 years. Unfortunately, by that time, they typically have permanent physical deformities, including coarsening of facial features (see Fig. 5-24). People with gigantism eventually exhibit acromegaly if the condition is not corrected before puberty. A person with untreated acromegaly has a shortened life expectancy.

Extended treatment of adults with GH results in changes in body composition, with the percentage of body protein increasing and the percentage of body fat decreasing.

Lactotropes

The **lactotrope** produces the hormone, PRL, which is a 199-amino acid single-chain protein. The lactotrope differs from the other endocrine cell types of the adenohypophysis in two major ways:

- The lactotrope is not part of an endocrine axis. This means that PRL acts directly on nonendocrine cells to induce physiologic changes.
- The production and secretion of PRL is predominantly under inhibitory control by the hypothalamus through the neurotransmitter, **dopamine**. Thus, disruption of the pituitary stalk and the hypothalamohypophyseal portal vessels (e.g., due to surgery or physical trauma) results in an increase in PRL levels, but a decrease in ACTH, TSH, FSH, LH, and GH.

PRL circulates unbound to serum proteins and thus has a relatively short half-life of about 20 minutes. As is typical of protein hormones, there is heterogeneity of circulating forms of PRL, and the 199-amino acid form represents only 60% to

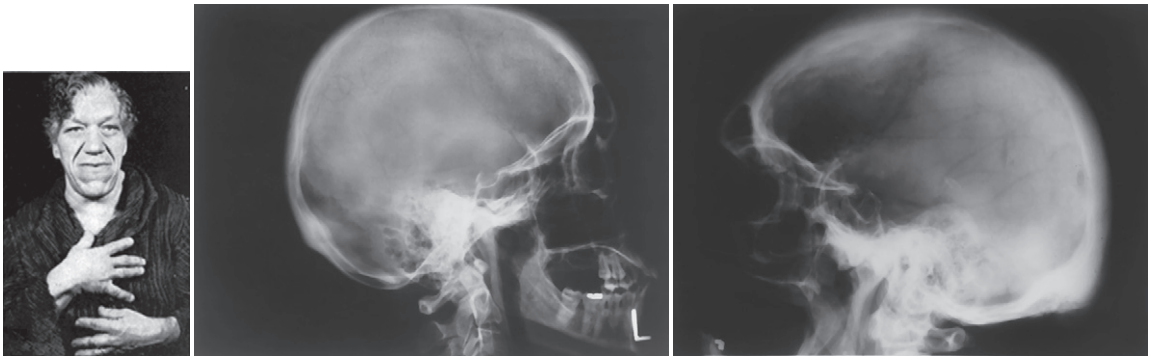


FIGURE 5-24 ■ **Left,** Severe acromegaly. **Middle,** Radiograph of a normal skull. **Right,** Radiograph of skull of a woman with acromegaly that demonstrates effects of acromegaly on morphologic features of skull. The sella turcica is enlarged as a result of growth of pituitary adenoma. The skull is thicker than normal, and protrusion of frontal ridge with enlargement of frontal sinuses is evident. *(**Left,** From Clinico-pathologic conference: acromegaly, diabetes, hypermetabolism, proteinuria and heart failure.* Am J Med *20:133, 1956; **Middle** and **Right,** Courtesy of Dr. C. Joe.)*

80% of the PRL measured by radioimmunoassays. Normal basal serum concentrations are similar in men and women.

PRL release is normally under tonic inhibition by the hypothalamus. This is exerted by dopaminergic tracts that secrete dopamine at the median eminence. Dopamine binds to the D2 receptor, which is linked to a Gi signaling pathway. There is also evidence for the existence of a prolactin-releasing factor (PRF). The exact nature of this compound is not known, although many factors, including TRH and hormones in the glucagon family (secretin, glucagon, VIP, and gastroinhibitory peptide [GIP]), can stimulate PRL release.

The regulation of PRL secretion during pregnancy and lactation is discussed in Chapter 11.

Drugs that interfere with dopamine synthesis or action increase PRL secretion. Many commonly prescribed antihypertensive drugs and tricyclic antidepressant drugs are dopamine inhibitors. **Bromocriptine** is a dopamine agonist that can be used to inhibit PRL secretion. Somatostatin, TSH, and GH also inhibit PRL secretion.

The **PRL receptor** belongs to the cytokine-GH-PRL-erythropoietin receptor superfamily. Therefore, PRL acts through a JAK-STAT signaling pathway (see Chapter 1). At least 85 different actions have been proposed for PRL; which actions are seen often depends on the dose of hormone used and the species studied. In humans, the predominant physiologic role of PRL is the regulation of essentially every aspect of postnatal breast development and function, as discussed in Chapter 11.

CLINICAL BOX 5-9

PRL-secreting tumors (**prolactinomas**) account for about 70% of all anterior pituitary tumors. Furthermore, many drugs interfere with dopamine production or action and hence increase PRL release. For these reasons, **hyperprolactinemia** is a common disorder in humans. Hyperprolactinemia in women is associated with **oligomenorrhea** or **amenorrhea and infertility**. GnRH release, the gonadotrope response to GnRH, and the ovarian response to LH all decrease. In the early stages of the pathologic condition, PRL suppresses follicular maturation, leading to an inadequate corpus luteum and a short luteal phase. As the hyperprolactinemia persists, the preovulatory estrogen peak is lost, thereby lengthening the cycle and leading to oligomenorrhea and anovulatory cycles. Hyperprolactinemia can produce **infertility in men**. Although breast enlargement in men can occur, true **gynecomastia** (inappropriate growth of breasts) and **galactorrhea** (inappropriate flow of milk) are rare.

The primary symptoms causing men and postmenopausal women with PRL-secreting tumors to seek medical attention may be those resulting from compression by the pituitary mass. These patients may experience **severe headaches** or **visual disturbances** that can include bitemporal hemianopia (defect in vision in the temporal half of the field of vision in both eyes). Both men and women may complain of decreased libido and signs of hypogonadism.

Dopamine agonists not only inhibit PRL production but also repress lactotrope growth and proliferation. Thus, dopamine agonists can be used as an antitumor drug in the case of dopamine-responsive prolactinomas.

Hypopituitarism

Panhypopituitarism There are many causes of hypopituitarism, which can involve either hypothalamic or pituitary problems. The deficiencies can be variable for the different anterior pituitary hormones. The symptoms of hypopituitarism are slow in onset and are reflected in deficiencies in the target organs of the anterior pituitary. **Hypogonadism, hypothyroidism, hypoadrenalism**, and **growth impairment** (in children) may be present. People with panhypopituitarism tend to have sallow complexions because of the **ACTH deficiency,** and they become particularly sensitive to the actions of insulin because of the decreased secretion of the insulin antagonists GH and cortisol. They are prone to develop **hypoglycemia**, particularly when stressed. **Hypogonadism** is manifested by amenorrhea in women, impotence in men, and loss of libido in both men and women. Some of the clinical manifestations of **hypothyroidism** are cold, dry skin, constipation, hoarseness, and bradycardia. The myxedema (nonpitting edema) associated with severe hypothyroidism is rare. **Adrenal insufficiency** caused by the ACTH deficiency can result in weakness, mild postural hypotension, hypoglycemia, and a loss of pubic and axillary

hair. The only symptom associated with the PRL deficiency is the incapacity for postpartum lactation. Finely wrinkled skin is characteristic of a deficiency of both gonadotropin and GH. The GH deficiency can also lead to fasting hypoglycemia in adults and children. In children, growth is impaired, and the relative increase in adipose tissue and decrease in muscle mass may produce a chubby appearance. The symptoms of the endocrine deficiencies are not as severe as they are in primary thyroid, adrenal, and gonadal deficiencies.

Growth

Normal growth is a complex process that requires normal endocrine function. There are definitive patterns for normal growth. The most rapid growth occurs during fetal development, and the exact fetal growth regulators have not yet been well established. Postnatally, the most rapid growth occurs in the neonate. The next period of rapid growth occurs at **puberty**. It is during late puberty that the rising estrogen levels act to stimulate closure of the epiphyses and hence cause the termination of long-bone growth.

The role of GH in growth regulation has been discussed. However, appropriate levels of **thyroid hormones, insulin, and cortisol** are also required for normal growth. The growth deficiencies associated with **hypothyroidism** are discussed in Chapter 6. Causes of retarded growth in children are listed in Box 5-2. In the absence of normal **insulin** levels, intermediary metabolism is impaired, and IGF production decreases. Both of these hormones are important for normal growth. **Hypercortisolism** is associated with growth impairment. **Gonadal steroids** are potent growth stimulators in puberty, but they also terminate long-bone growth by stimulating closure of the epiphyses. Growth is stunted if nutrition is not adequate. In either starvation or malnutrition, IGF-1 production is low, and growth is slowed.

BOX 5-2
CAUSES OF RETARDED GROWTH IN CHILDREN

GH deficiency
- IGF-1 deficiency
- Impaired IGF-1 action

Thyroid deficiency
Insulin deficiency
Cortisol excess
Malnutrition, undernutrition
Psychosocial growth retardation
Constitutional delay
Chronic disease
Genetic disorders characterized by short stature

GH, growth hormone; IGF-1, insulin-like growth factor-I.

Another cause of growth impairment is **psychosocial dwarfism**. Infants who are not stimulated and nurtured or children developing in a hostile environment can demonstrate poor growth. These children have an immature appearance and often have unusual eating and drinking habits. Pituitary function in these children is suppressed. However, when such children are removed from the poor environment, normal pituitary function and growth resume.

In many cases, deficient growth is merely a result of **constitutional delay**. This is not a pathologic condition but a genetic variation from the average. **Chronic illnesses** also impair growth.

SUMMARY

1. The pituitary gland (also called the hypophysis) is derived from a neural structure (the infundibulum) and an epithelial structure (Rathke pouch). The infundibulum develops into the neurohypophysis, which includes the median eminence, infundibular stalk, and pars nervosa (also called the posterior pituitary). Rathke pouch develops into the adenohypophysis (also called anterior pituitary), which includes the pars distalis, pars tuberalis, and pars intermedia (the pars intermedia is lost in the adult human). The pituitary gland sits in a pocket of the sphenoid bone, called the sella turcica, at the base of the forebrain.
2. Magnocellular hypothalamic neurons in the paraventricular and supraoptic nuclei project axons down the infundibular stalk and terminate in the pars nervosa. The pars nervosa is a neurovascular organ, wherein neurohormones are released and diffuse into the vasculature.
3. Two neurohormones, antidiuretic hormone (ADH; also called vasopressin) and oxytocin, are synthesized in the hypothalamus in the magnocellular neuronal cell bodies. ADH and oxytocin are transported intra-axonally down the hypothalamohypophyseal tracts to the pars nervosa. Stimuli perceived by the cell bodies and dendrites in the hypothalamus control the release of ADH and oxytocin at the pars nervosa.
4. The primary action of ADH is to promote water reuptake at the distal nephron and collecting duct. ADH also has vasopressive actions, which are important during vasodilatory shock.
5. Diabetes insipidus (DI) is a disease in which either there is deficient ADH (central DI) or deficient response to ADH at the kidney (nephrogenic DI). DI is associated with increased urine flow, dehydration, and increased thirst. The syndrome of inappropriate ADH secretion (SIADH) is characterized by high ADH levels, which increase volume and blood pressure, and a low serum osmolarity.
6. Oxytocin acts on the breast to cause milk letdown during nursing and on the uterus to cause muscular contractions during labor.
7. The adenohypophysis secretes several tropic hormones, which are part of the endocrine axes. An endocrine axis includes the hypothalamus, the pituitary, and a peripheral endocrine gland. The set-point of an axis is largely controlled by the negative feedback of the peripheral hormone on the pituitary and hypothalamus.
8. The adenohypophysis contains five endocrine cell types: corticotropes, thyrotropes, gonadotropes, somatotropes, and lactotropes. Corticotropes secrete ACTH, thyrotropes secrete TSH, gonadotropes secrete FSH and LH, somatotropes secrete GH, and lactotropes secrete PRL.
9. The predominant control exerted by the hypothalamus on the anterior pituitary is mediated by releasing hormones. These small peptides are carried through the hypophyseal portal system to the anterior pituitary, where they control synthesis and release of the pituitary hormones ACTH, TSH, FSH, LH, and GH. PRL is under predominantly inhibitory control by the hypothalamus through the catecholamine dopamine.
10. GH stimulates growth primarily through the regulation of the growth-promoting hormones IGF-1 and IGF-2. GH also has metabolic actions. It raises blood glucose by decreasing peripheral tissue utilization. It is protein anabolic and lipolytic.
11. The predominant action of PRL in humans is the initiation and maintenance of lactation.
12. Normal growth is a complex process that requires normal endocrine function. Consequently, growth deficiencies are associated with many endocrine disorders in children.

SELF-STUDY PROBLEMS

1. Explain the origins of the neurohypophysis and adenohypophysis.
2. How is a portion of the neurohypophysis critical to the normal function of the anterior pituitary?
3. Which is ADH secretion more sensitive to: changes in volume or changes in osmolality?
4. Describe the production of ADH in terms of synthesis, processing, and secretion. Where does each of these occur?
5. How does SIADH result in hyponatremia?
6. Name two proteins that are mutated in congenital nephrogenic diabetes insipidus.
7. Cushing disease is hypercortisolism due to hypothalamic-independent excessive ACTH production by corticotropes. Is this a primary, secondary, or tertiary endocrine disorder?
8. McCune-Albright syndrome is a genetically mosaic condition in which some cells express a Gs protein with an activating mutation. Explain why this can lead to excessive growth of somatotropes and high GH levels.
9. Why is GH referred to as a diabetogenic hormone?
10. How does stress (in any form) affect the secretion of the following: CRH, TRH, GnRH, and GHRH?
11. How is skin darkening related to primary hypocortisolism?
12. What is the effect of hypoglycemia on GH and IGF-1 secretion?

KEYWORDS AND CONCEPTS

- Acid labile subunit (ALS)
- Acidophils
- Acromegaly

For full list of keywords and concepts see Student Consult

SUGGESTED READINGS

Appel JG, Bergsneider M, Vinters H, et al: Acromegaly due to an ectopic pituitary adenoma in the clivus: Case report and review of literature, *Pituitary* Sep 30. Epub ahead of print.

Birzniece V, Nelson AE, Ho KK: Growth hormone and physical performance, *Trends Endocrinol Metab* 22:171–178, 2011.

Cariboni A, Maggi R, Parnavelas JG: From nose to fertility: The long migratory journey of gonadotropin-releasing hormone neurons, *Trends Neurosci* 30:638–644, 2007.

Clayton PE, Banerjee I, Murray PG, et al: Growth hormone, the insulin-like growth factor axis, insulin and cancer risk, *Nat Rev Endocrinol* 7:11–24, 2011.

Fideleff HL, Boquete HR, Suarez MG, et al: Prolactinoma in children and adolescents, *Horm Res* 72:197–205, 2009.

Pendleton C, Adams H, Salvatori R, et al: On the shoulders of giants: Harvey Cushing's experience with acromegaly and gigantism at the Johns Hopkins Hospital, 1896–1912, *Pituitary* 14:53–60, 2011.

Raff H: Cushing's syndrome: Diagnosis and surveillance using salivary cortisol, *Pituitary* 15:64–70, 2012.

KEYWORDS AND CONCEPTS

- Adenohypophysis
- Adrenal cortex
- Adrenocorticotropic hormone (ACTH)
- African pigmy
- α-Glycoprotein subunit (α-GSU)
- Anterior pituitary
- Antidiuresis
- Antidiuretic hormone
- Aquaporin-2
- Atrial natriuretic peptide (ANP)
- Basophils
- Bitemporal hemianopia
- Corticotrope
- Corticotropin-releasing hormone (CRH)
- Cortisol
- Craniopharyngioma
- Diabetes insipidus
- Diabetogenic hormone
- Diaphragma sellae
- Dopamine
- Dwarfism
- Empty sella syndrome
- Endocrine axes
- Follicle-stimulating hormone (FSH)
- GH-binding protein (GHBP)
- Ghrelin
- Gigantism
- Gonadotrope
- Gonadotropin-releasing hormone (GnRH)
- Growth hormone (GH)
- Growth hormone–releasing hormone (GHRH)
- Herring bodies
- Human chorionic gonadotropin (hCG)
- Human placental lactogen (hPL)
- Hyperglycemic hormone
- Hypophysectomy
- Hypophysiotropic region of the hypothalamus
- Hypophysis
- Hypothalamohypophyseal portal vessels
- Hypothalamohypophyseal tract
- Hypothalamus-pituitary-adrenal (HPA) axis
- Hypothalamus-pituitary-thyroid axis
- IGF-binding protein (IGFBP)
- IGFBP proteases
- IGF-2
- Infundibulum
- Insulin-like growth factor-1 (IGF-1)
- *KAL* gene
- Kallmann syndrome
- Lactational amenorrhea
- Lactotrope
- Laron dwarfism
- Lipolytic hormone
- Liver
- Luteinizing hormone (LH)
- Magnocellular neurons
- Median eminence
- Melanocortin-2 receptor (MC2R)
- Milk ejection (letdown)
- Neuroendocrine reflex
- Neurohypophysis
- Neurophysin-I
- Neurophysin-II
- Ovary
- Oxytocin
- Panhypopituitarism
- Panhypopituitary dwarfism
- Paraventricular nucleus (PVN)
- Pars distalis
- Pars intermedia
- Pars nervosa
- Pars tuberalis
- Parvicellular neurons
- Peripheral endocrine organs
- Pituicyte
- Pituitary apoplexy
- Pituitary gland
- Pituitary glycoprotein hormone
- Positive feedback
- Posterior lobe of the pituitary
- Prepro-oxyphysin
- Preprovasophysin
- Primary endocrine disorder
- Progesterone
- Prolactin (PRL)
- Prolactin-releasing factor (PRF)
- Pro-opiomelanocortin (POMC)
- Protein anabolic hormone
- Psychosocial dwarfism
- Psychosocial Rathke pouch
- Releasing hormones

- Secondary endocrine disorder
- Sella turcica
- Set-point
- Sheehan syndrome
- Somatomedin
- Somatostatin
- Somatotrope
- Stress hormones
- Supraoptic nucleus (SON)
- Syndrome of inappropriate secretion of antidiuretic hormone (SIADH)
- Tertiary endocrine disorder
- Tertiary hypercortisolism
- Tertiary hypogonadotropic hypogonadism
- Testis
- Testosterone
- Thyroid gland
- Thyroid hormone
- Thyroid-stimulating hormone (TSH)
- Thyrotrope
- Thyrotropin
- Thyrotropin-releasing hormone (TRH)
- Tropic hormones
- Vasopressin-2 (V2) receptor

6 THE THYROID GLAND

OBJECTIVES

1. Explain the mechanism of synthesis of the thyroid hormones.
2. Describe the regulation of thyroid function and the actions of thyroid hormones.
3. Compare and contrast the functions of thyroxine and triiodothyronine.
4. Draw the regulatory feedback loop for the regulation of thyroid function.
5. Understand the etiology, major symptoms, and pathophysiology of the symptoms for Graves disease, Hashimoto thyroiditis, sporadic congenital hypothyroidism, and cretinism.

The thyroid gland produces the prohormone, tetraiodothyronine (T_4), and the active hormone, triiodothyronine (T_3). The synthesis of T_4 and T_3 requires iodine, which can be a limiting factor in some parts of the world. Much of T_3 is also made by peripheral conversion of T_4 to T_3. T_3 acts primarily through a nuclear receptor that regulates gene expression. T_3 is critical for normal brain development and has broad effects on metabolism and cardiovascular function in the adult.

ANATOMY AND HISTOLOGY OF THE THYROID GLAND

The thyroid gland is composed of right and left lobes that sit anterolaterally to the trachea (Fig. 6-1). Normally, the lobes of the thyroid gland are connected by a midventral isthmus. The functional unit of the thyroid gland is the **thyroid follicle**, a spherical structure, about 200 to 300 μm in diameter, which is surrounded by a single layer of thyroid epithelial cells (Fig. 6-2). The epithelium sits on a basal lamina, which is the outermost structure of the follicle and is surrounded by a rich capillary supply. The apical side of the follicular epithelium faces the lumen of the follicle. The follicular lumen itself is filled with **colloid** composed of **thyroglobulin**, which is secreted and **iodinated** by the thyroid epithelial cells. The size of the epithelial cells and the amount of colloid are dynamic features that change with the activity of the gland. The thyroid gland contains another type of cell in addition to follicular cells. Scattered within the gland are **parafollicular cells**, called **C cells**. These cells are the source of the polypeptide hormone, **calcitonin**, whose function is unclear in humans.

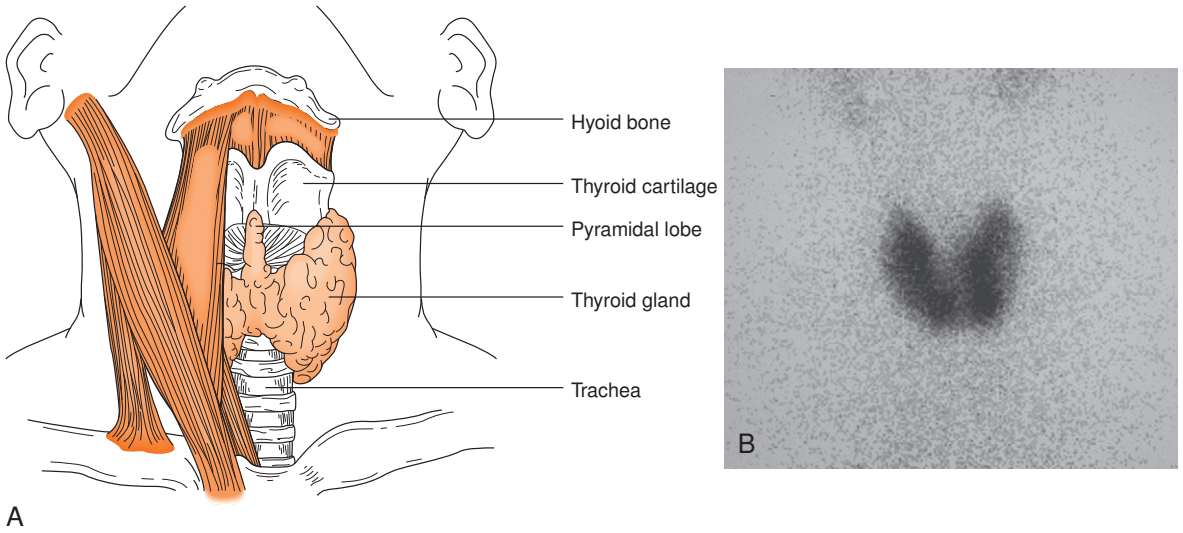


FIGURE 6-1 ■ **A,** Anatomy of the thyroid gland. **B,** Image of pertechnetate uptake by a normal thyroid gland. *(Modified from Drake RL, Vogl W, Mitchell AWM:* Gray's Anatomy for Students, *Philadelphia, 2005, Churchill Livingstone.)*

CLINICAL BOX 6-1

Although parafollicular C cells and calcitonin may be of minimal importance in normal humans, C cells can give rise to **medullary thyroid carcinoma**. Medullary cancer is the most aggressive form of thyroid cancer, and spread to lungs, liver, bone, or other organs drastically reduces survival. Although most medullary cancer is sporadic, about one fifth of cases are familial. The familial forms of medullary thyroid cancer are due to activating mutations of the ***RET* protooncogene**. RET is a tyrosine kinase receptor that interacts with co-receptors and is activated by glial-derived neurotrophic factor and related proteins. Familial medullary thyroid cancer can occur independently of other endocrine glands or as part of a **multiple endocrine neoplasia (MEN)**, which in this case also involves adrenal medulla chromaffin cells (pheochromocytoma), parathyroid glands, and/or neuromas. These cancers remain differentiated enough to secrete **calcitonin**, and assay of calcitonin in the blood is useful in assessing treatment and for follow-up examinations for recurrence. Medullary thyroid cancer is treated by total thyroidectomy and removal of regional lymph nodes. Effective chemotherapy regimens that target the tyrosine kinase receptor, c-ret, have also been developed.

PRODUCTION OF THYROID HORMONES

The secretory products of the thyroid gland are **iodothyronines** (Fig. 6-3), a class of hormones resulting from the coupling of two iodinated tyrosine molecules. About 90% of the thyroid output is **3,5,3′,5′-tetraiodothyronine** (**thyroxine**, or $\mathbf{T_4}$). T_4 is a prohormone. About 10% is **3,5,3′-triiodothyronine** ($\mathbf{T_3}$), which is the active form of thyroid hormone. Less than 1% of thyroid output is **3,3′,5′-triiodothyronine** (**reverse $\mathbf{T_3}$**, or **$\mathbf{rT_3}$**), which is inactive. Normally, these three hormones are secreted in the same proportions as they are stored in the gland.

Because the primary product of the thyroid gland is T_4, yet the active form of thyroid hormone is T_3, the thyroid axis relies heavily on **peripheral conversion** through the action of **thyronine-specific deiodinases** (see Fig. 6-3). Most conversion of T_4 to T_3 by **type 1 deiodinase** occurs in tissues with high blood flows and rapid exchanges with plasma, such as liver, kidneys, and skeletal muscle. This process supplies circulating T_3 for uptake by other tissues in which local T_3 generation is too low to provide sufficient thyroid

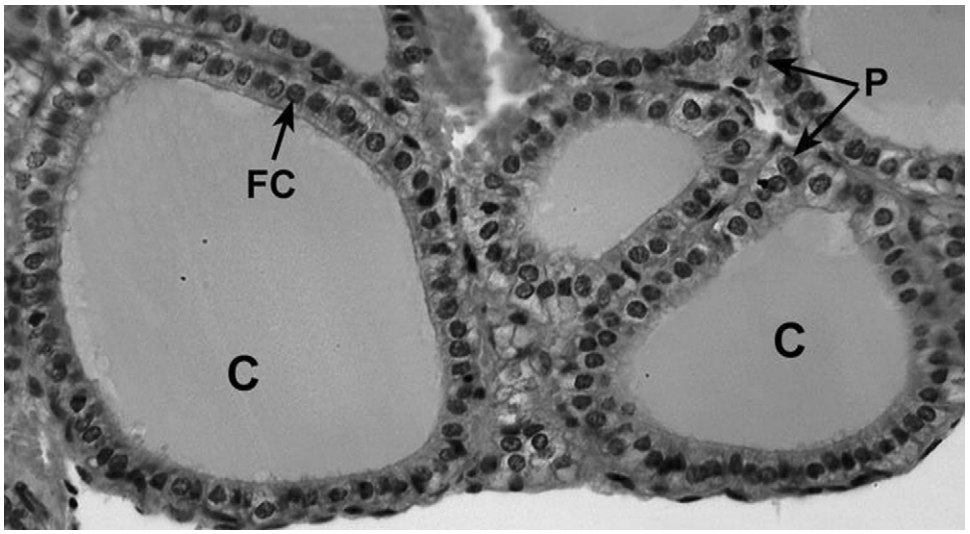


FIGURE 6-2 ■ Normal rat thyroid. Single layer of cuboidal epithelial cells (follicular cells; FC) surround colloid (C). Parafollicular cells (P) produce calcitonin (see Chapter 4).

hormone. Type 1 deiodinase is also expressed in the thyroid (again, where T_4 is abundant) and has a relatively low affinity (i.e., a Km of 1 μM) for T_4. The levels of type 1 deiodinase are paradoxically increased in hyperthyroidism, which contributes to elevated circulating T_3 levels in this disease.

The brain maintains constant intracellular levels of T_3 by a high-affinity deiodinase called **type 2 deiodinase**, which is expressed in glial cells in the central nervous system (CNS). Type 2 deiodinase has a Km of 1 nM and maintains intracellular concentrations of T_3 even when free T_4 falls to low levels. Type 2 deiodinase is also present in the thyrotropes of the pituitary. In the pituitary, type 2 deiodinase acts as a "thyroid axis sensor" that mediates the ability of circulating T_4 to feed back on TSH secretion (see later). The expression of type 2 deiodinase is increased during hypothyroidism, which helps maintain constant T_3 levels in the brain.

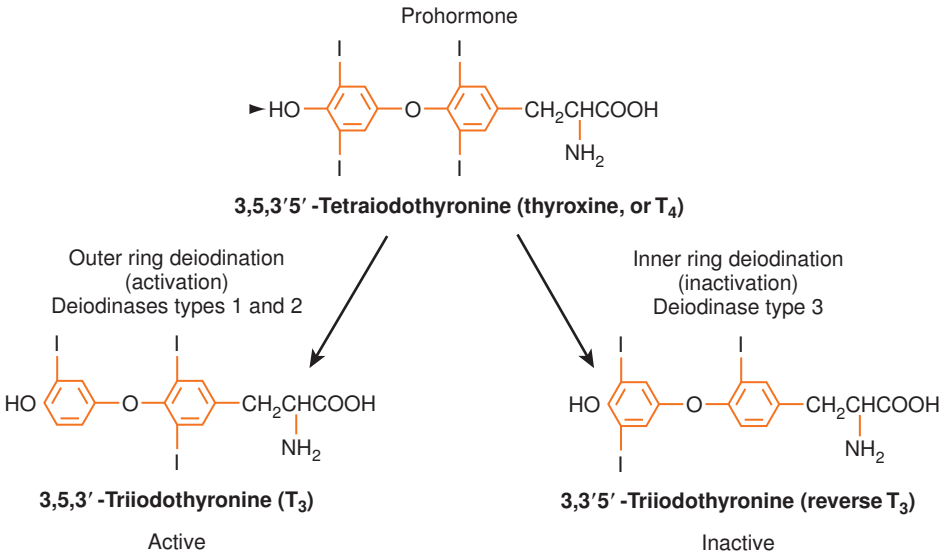


FIGURE 6-3 ■ Structure of iodothyronines, T_4, T_3, and reverse T_3.

There also exists an "inactivating" deiodinase, called **type 3 deiodinase**. Type 3 deiodinase is a high-affinity, inner-ring deiodinase that converts T_4 to the inactive rT_3. Type 3 deiodinase is increased during hyperthyroidism, which helps to blunt the overproduction of T_4. All forms of iodothyronines are further deiodinated, eventually to noniodinated thyronine.

Iodide Balance

Because of the unique role of **iodide (I^-; the water-soluble, ionized form of iodine, or I_2)** in thyroid physiology, a description of thyroid hormone synthesis requires some understanding of iodide turnover (Fig. 6-4). An average of 400 μg of iodide per person is ingested daily in the United States, compared with a minimum daily requirement of 150 μg for adults, 90 to 120 μg for children, and 200 μg for pregnant women. In the steady state, virtually the same amount, 400 μg, is excreted in the urine. Iodide is actively concentrated in the thyroid gland, salivary glands, gastric glands, lacrimal glands, mammary glands, and choroid plexus. About 70 to 80 μg of iodide is taken up daily by the thyroid gland from a circulating pool that contains about 250 to 750 μg of iodide. The total iodide content of the thyroid gland averages 7500 μg, virtually all of which is in the form of iodothyronines. In the steady state, 70 to 80 μg of iodide, or about 1% of the total, is released from the gland daily. Of this amount, 75% is secreted as thyroid hormone, and the remainder is secreted as free iodide. The large ratio (100:1) of iodide stored in the form of hormone to the amount turned over daily protects the individual from the effects of iodide deficiency for about 2 months. Iodide is also conserved by a marked reduction in the renal excretion of iodide as the concentration in serum falls.

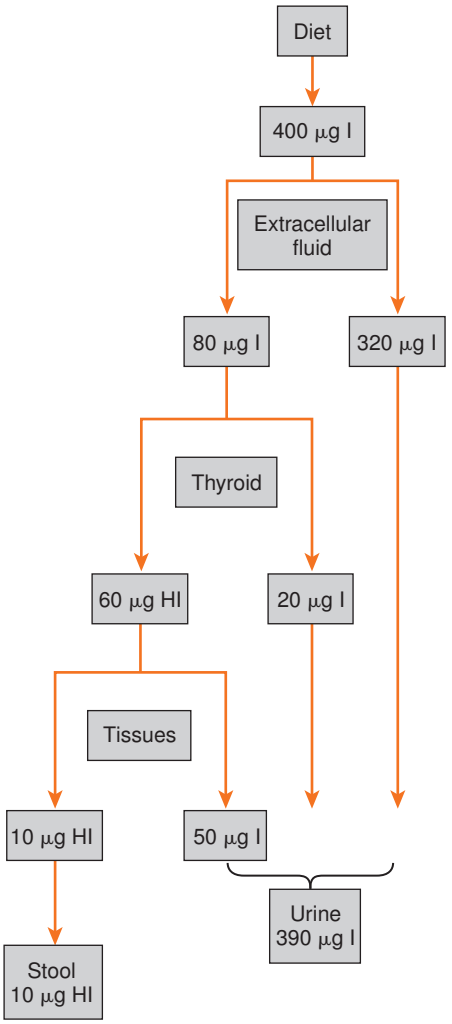


FIGURE 6-4 ■ Iodine distribution and turnover in humans.

Overview of Thyroid Hormone Synthesis

To understand thyroid hormone synthesis and secretion, one must appreciate the directionality of each process as it relates to the **polarized thyroid epithelial cell** (Fig. 6-5). Synthesis of thyroid hormone requires two precursors, **thyroglobulin** and **iodide**. Iodide is transported across cells from the basal (vascular) side to the apical (follicular luminal) side of the thyroid epithelium. Amino acids are assembled by translation into thyroglobulin, which is then secreted from the apical membrane into the follicular lumen. Thus, synthesis involves a basal-to-apical movement of precursors into the follicular lumen (see Fig. 6-5; *black arrows*). Actual synthesis of **iodothyronines** occurs enzymatically in the follicular lumen close to the apical membrane of the epithelial cells (see later). Secretion involves fluid phase endocytosis of **iodinated thyroglobulin** and apical-to-basal movement of the endocytic vesicles and their fusion with lysosomes.

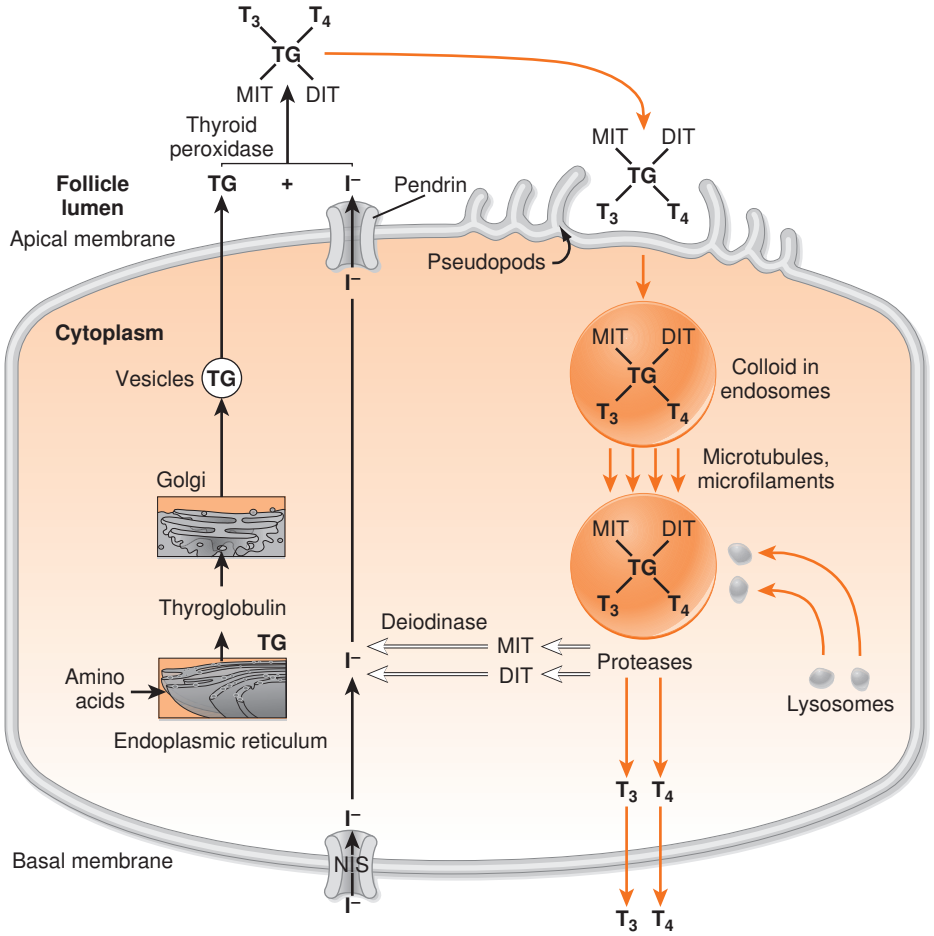


FIGURE 6-5 ■ Synthesis *(black arrows)* and secretion *(orange arrows)* of thyroid hormones by the thyroid epithelial cell. *Open arrows* denote pathways involved in the conservation of iodine and amino acids.

Thyroglobulin is then enzymatically degraded, which results in the release of **thyroid hormones** from the thyroglobulin peptide backbone. Finally, thyroid hormones move across the basolateral membrane, probably through a specific transporter, and ultimately into the blood. Thus, secretion involves an apical-to-basal movement (see Fig. 6-5; *orange arrows*). There are also scavenger pathways within the epithelial cell that reuse iodine and amino acids after enzymatic digestion of thyroglobulin (see Fig. 6-5; *open arrows*).

Synthesis of Iodothyronines Within a Thyroglobulin Backbone

Iodide is transported into the gland against chemical and electrical gradients by an **Na^+-I^- symporter (NIS)** located in the basolateral membrane of thyroid epithelial cells. Normally, a thyroid-to-plasma free iodide ratio of 30 is maintained. This so-called **iodide trap** is highly expressed in the thyroid gland, but NIS is also expressed at lower levels in the placenta, salivary glands, and actively lactating breast. One iodide ion is transported uphill against an iodide gradient, whereas two sodium ions move down the electrochemical gradient from the extracellular fluid into the thyroid cell. The energy source for this secondary active transporter is provided by a Na^+, K^+-ATPase in the plasma membrane. Expression of the *NIS* gene is inhibited by iodide and stimulated by thyroid-stimulating hormone (TSH; see below). Numerous inflammatory cytokines also suppress *NIS* gene expression. A reduction in dietary iodide intake depletes the circulating iodide pool and greatly enhances the

activity of the iodide trap. When dietary iodide intake is low, the rates of thyroid uptake of iodide can reach 80% to 90%.

The steps in thyroid hormone synthesis are shown in Figure 6-6. After entering the gland, iodide rapidly moves to the apical plasma membrane of the epithelial cells. From there, iodide is transported into the lumen of the follicles by a sodium-independent iodide-chloride transporter, named **pendrin**.

CLINICAL BOX 6-2

Pendred syndrome refers to condition due to an autosomal recessive mutation in the pendrin gene (referred to as *PDS* or *SLC26A4*). Because iodide is not efficiently transported into the follicular lumen, patients develop **hypothyroidism**. Some patients exhibit enlarged thyroid glands called **goiters**. This form of hypothyroidism can be treated with replacement thyroxine. Unfortunately, pendrin is also expressed in the inner ear and is required for normal structural development of the inner ear. Thus, patients with Pendred syndrome experience hearing loss in infancy or early childhood.

Once in the follicular lumen, iodide (I^-) is immediately oxidized and incorporated into tyrosine residues within the primary structure of **thyroglobulin**. Thyroglobulin is continually synthesized and exocytosed into the follicular lumen and is **iodinated** to form either **monoiodotyrosine (MIT)** or **diiodotyrosine (DIT)** (see Fig. 6-6). After iodination, two DIT molecules are **coupled** to form $\mathbf{T_4}$, or one MIT and one DIT molecule are coupled to form $\mathbf{T_3}$. The coupling also occurs between iodinated tyrosines that remain part of the primary structure of thyroglobulin. This entire sequence of reactions is catalyzed by **thyroid peroxidase** (**TPO**), which is an enzyme complex that spans the apical membrane. The immediate oxidant (electron acceptor) for the reaction is hydrogen peroxide (H_2O_2). The mechanism whereby H_2O_2 is generated in the thyroid gland involves **NADPH oxidase** that is also localized to the apical membrane.

When iodide availability is restricted, the formation of T_3 is favored. This response provides more active hormone per molecule of organified iodide. The proportion of T_3 is also increased when the gland is hyperstimulated by TSH or other activators.

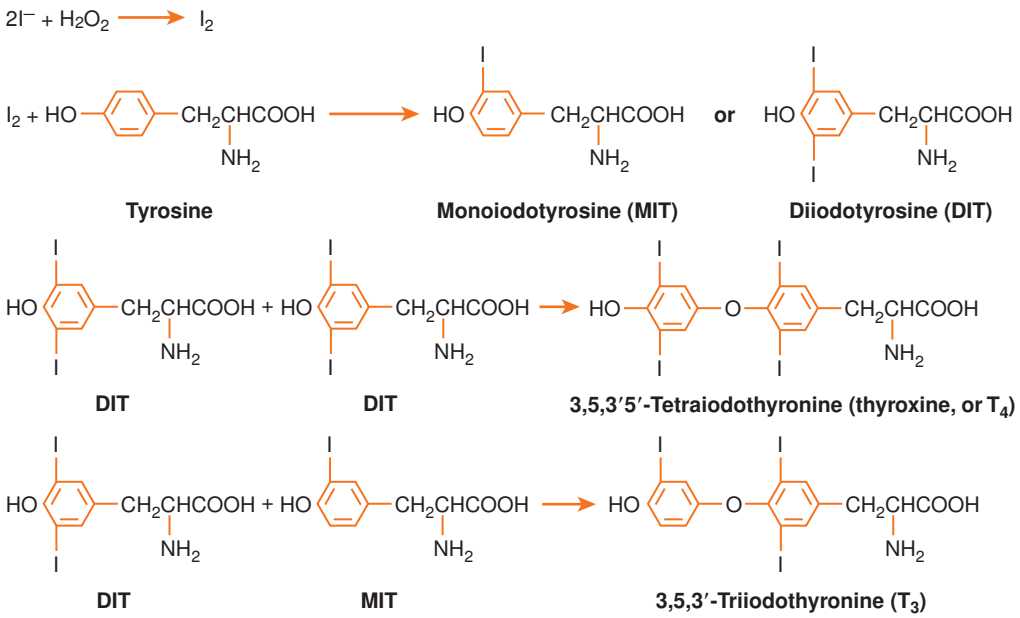


FIGURE 6-6 ■ Reactions involved in the generation of iodide, MIT, DIT, T_3, and T_4.

Secretion of Thyroid Hormones

After thyroglobulin has been iodinated, it is stored in the lumen of the follicle as colloid (see Fig. 6-2). Release of the T_4 and T_3 into the bloodstream requires endocytosis and lysosomal degradation of thyroglobulin (see Fig. 6-5; *orange arrows*). Enzymatically released T_4 and T_3 then leave the basal side of the cell and enter the blood.

The MIT and DIT molecules, which also are released during proteolysis of thyroglobulin, are rapidly deiodinated within the follicular cell by the enzyme, **intrathyroidal deiodinase** (see Fig. 6-5; *open arrows*). This deiodinase is specific for MIT and DIT and cannot use T_4 and T_3 as substrates. The iodide is then recycled into T_4 and T_3 synthesis. Amino acids from the digestion of thyroglobulin reenter the intrathyroidal amino acid pool and can be reused for protein synthesis. Only minor amounts of intact thyroglobulin leave the follicular cell under normal circumstances.

CLINICAL BOX 6-3

Because of its ability to **trap** and incorporate iodine into thyroglobulin (called **organification**), the activity of the thyroid can be assessed by **radioactive iodine uptake** (**RAIU**). For this, a tracer dose of ^{123}I is administered, and the RAIU is measured by placing a gamma detector on the neck after 4 to 6 hours and after 24 hours. In the United States, where the diet is relatively rich in iodine, the RAIU is about 15% after 6 hours and 25% after 24 hours (Fig. 6-7). Abnormally high RAIU (>60%) after 24 hours indicates hyperthyroidism. Abnormally low RAIU (<5%) after 24 hours indicates hypothyroidism. In individuals with extreme chronic stimulation of the thyroid (Graves disease–associated thyrotoxicosis), iodide is trapped, organified, and released as hormone very rapidly. In these cases of elevated turnover, the 6-hour RAIU will be very high, but the 24-hour RAIU will be lower (see Fig. 6-8). A number of anions, such as thiocyanate (CNS^-), perchlorate ($HClO_4^-$), and pertechnetate ($TcO^4\_$), are inhibitors of iodide transport through the NIS. If iodide cannot be rapidly incorporated into tyrosine (**organification defect**) after its uptake by the cell, administration of one of these anions will, by blocking further iodide uptake, cause a rapid release of the iodide from the gland (see Fig. 6-8). This release occurs as a result of the high thyroid-to-plasma concentration gradient of iodide.

The thyroid can be imaged using the iodine isotopes ^{123}I or ^{131}I, or the iodine mimic, pertechnetate (^{99m}Tc), followed by imaging with a rectilinear scanner or gamma camera. Imaging can display the size and shape of the thyroid (see Fig. 6-1B), as well as heterogeneities of active versus inactive tissue within the thyroid gland. Such heterogeneities are often due to the development of **thyroid nodules**, which are regions of enlarged follicles with evidence of regressive changes indicating cycles of stimulation and involution. Particular **hot nodules** (i.e., nodules that display a high RAIU) on imaging, are usually not cancerous but may lead to thyrotoxicosis (hyperthyroidism; see later). **Cold nodules** are 10 times more likely to be cancerous than hot nodules. Such nodules can be sampled for pathology analysis by **fine-needle aspiration biopsy**.

The thyroid can also be imaged by **ultrasonography**, which is superior in resolution to RAIU imaging. Ultrasonography is used to guide the physician during fine-needle aspiration biopsy of a nodule. Highest resolution of the thyroid is achieved with **magnetic resonance imaging**.

TRANSPORT AND METABOLISM OF THYROID HORMONES

Secreted T_4 and T_3 circulate in the bloodstream almost entirely bound to proteins. Normally, only about 0.04% of total plasma T_4 and 0.4% of total plasma T_3 exist in the free state (Table 6-1). Free T_3 is biologically active and mediates thyroid hormone effects on peripheral tissues as well as in negative feedback on the pituitary and hypothalamic (see later). The major binding protein is **thyroxine-binding globulin** (**TBG**). TBG is synthesized in the liver and binds one molecule of T_4 or T_3.

About 70% of circulating T_4 and T_3 is bound to TBG; 10% to 15% is bound to another specific thyroid-binding protein, called **transthyretin** (**TTR**). **Albumin** binds 15% to 20%, and 3% is bound to lipoproteins. Ordinarily, only alterations in TBG concentration significantly affect total plasma T_4 and T_3 levels. Two important biologic functions have been ascribed to TBG. First, it maintains a large circulating **reservoir** of T_4, which buffers any acute changes in thyroid gland function. Second, the binding of plasma T_4 and T_3 to proteins prevents the loss of these relatively small hormone molecules in the urine and thereby helps conserve iodide. TTR, in particular, provides thyroid hormones to the CNS.

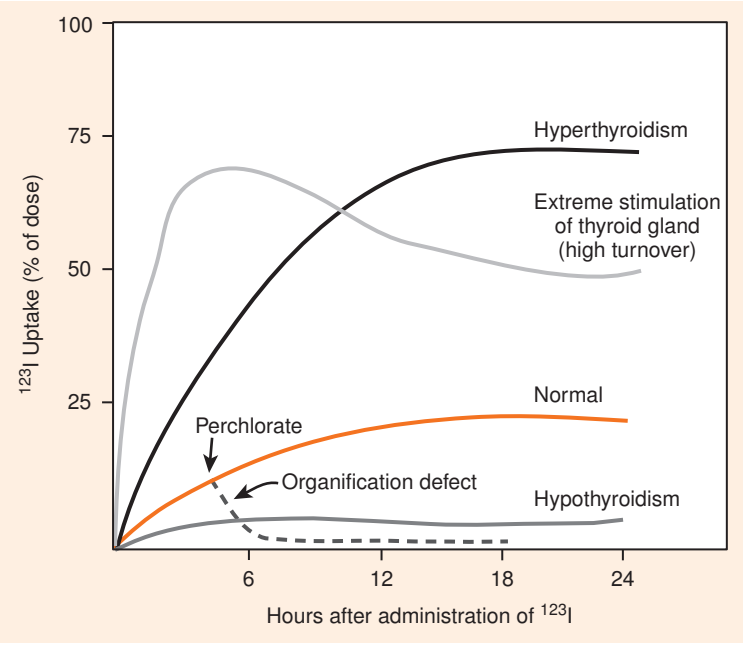


FIGURE 6-7 ■ Thyroid gland iodothyronine uptake curves for normal, hypothyroid, hyperthyroid, and organification defective states.

CLINICAL BOX 6-4

There are several transporters that mediate thyroid hormone transport across cell membranes **Thyroid hormone transporters** include sodium taurocholate co-transporting polypeptide (NCTP), organic anion transporting polypeptide (OATP), L-type amino acid transporter (LAT), and the monocarboxylate transporter (MCT). These transporters show specificity with respect to T_4 versus T_3 binding and cell-specific expression. MCT8 is required for neuronal uptake of thyroid hormones. Mutations in MCT8 are linked to severe psychomotor retardation (Allan-Herndon-Dudley syndrome) that cannot be treated with exogenous T_3 or T_4.

Regulation of Thyroid Function

The most important regulator of thyroid gland function and growth is the **hypothalamic-pituitary-thyroid axis** (see Chapter 5). TSH stimulates every aspect of thyroid function. TSH has immediate, intermediate, and long-term actions on the thyroid epithelium. Immediate actions of TSH involve induction of pseudopod extension, endocytosis of colloid, and formation of colloid droplets in the cytoplasm, which represent thyroglobulin within endocytic vesicles. Shortly thereafter, iodide uptake and TPO activity increase. Concurrently, TSH also stimulates glucose entry into the hexose monophosphate shunt pathway, which generates the NADPH that is needed for the

TABLE 6-1
Average Thyroid Hormone Turnover

	T_4	T_3	rT_3
Daily production (μg)	90	35	35
From thyroid (%)	100	25	5
From T_4 (%)	–	75	95
Extracellular pool (μg)	850	40	40
Plasma concentration			
Total (μg/dL)	8.0	0.12	0.04
Free (ng/dL)	2.0	0.28	0.20
Half-life (days)	7	1	0.8
Metabolic clearance (L/day)	1	26	77
Fractional turnover per day (%)	10	75	90

peroxidase reaction. TSH also stimulates the proteolysis of thyroglobulin and the release of T_4 and T_3 from the gland. Intermediate effects of TSH on the thyroid gland occur after a delay of hours to days and involve protein synthesis and the expression of numerous genes, including those encoding NIS, thyroglobulin, and TPO. Sustained TSH stimulation leads to the long-term effects of hypertrophy and hyperplasia of the follicular cells. Capillaries proliferate, and thyroid blood flow increases. These actions, which underlie the growth-promoting effects of TSH on the gland, are supported by the local production of growth factors. A noticeably enlarged thyroid gland is called a **goiter** (Fig. 6-8).

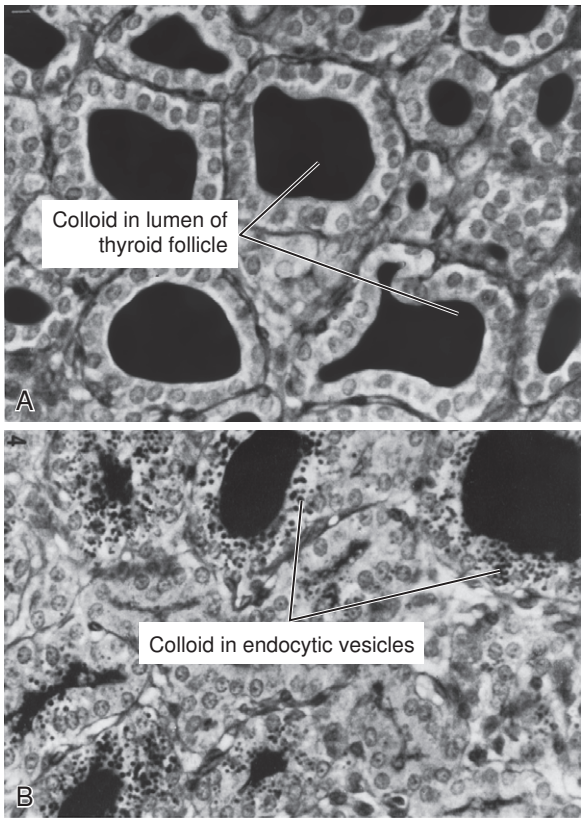


FIGURE 6-8 ■ The thyroid gland is located in the anterior neck, where it is easily visualized and palpated when it is enlarged (goiter).

CLINICAL BOX 6-5

Goiter can develop in response to multiple imbalances and disease within the hypothalamus-pituitary-thyroid axis, coexisting with hypothyroidism, euthyroidism (normal), and hyperthyroidism. These imbalances include the following:

Primary Hypothyroidism

- Lack of adequate iodine in the diet (nontoxic goiter, endemic goiter)
- Benign nodules or mutation of growth-related gene (nontoxic goiter)
- Sporadic hypothyroidism of unknown etiology (nontoxic goiter)
- Chronic thyroiditis (Hashimoto disease; autoimmune-induced deficiency in thyroid function)

Hyperthyroidism

- Excessive stimulation of the TSH receptor by an autoantibody (Graves disease)
- Excessive secretion of TSH from a TSH-producing tumor (i.e., secondary hyperthyroidism)
- Thyroid hormone–producing (toxic) adenoma (nodular) or toxic multinodular goiter
- An inactivating mutation in the TRβ-2 (see later)

The regulation of thyroid hormone secretion by TSH is under exquisite negative feedback control. Circulating thyroid hormones act on the pituitary gland to decrease TSH secretion, primarily by repressing TSH-β subunit gene expression. The pituitary gland expresses the high-affinity type 2 deiodinase. Thus, small changes in free T_4 in the blood result in significant changes in intracellular T_3 in the pituitary thyrotrope. Because the diurnal variation of TSH secretion is small, thyroid hormone secretion and plasma concentrations are relatively constant. Only small nocturnal increases in secretion of TSH and release of T_4 occur. Thyroid hormones also feed back on the hypothalamic TRH-secreting neurons. In these neurons, T_3 inhibits the expression of the prepro-*TRH* gene.

Another important regulator of thyroid gland function is iodide itself, which has a biphasic action. At relatively low levels of iodide intake, the rate of

thyroid hormone synthesis is directly related to iodide availability. However, if the intake of iodide exceeds 2 mg/day, the intraglandular concentration of iodide reaches a level that suppresses NADPH oxidase activity and the *NIS* and *TPO* genes, and thereby the mechanism of hormone biosynthesis. This autoregulatory phenomenon is known as the **Wolff-Chaikoff effect**. As the intrathyroidal iodide level subsequently falls, *NIS* and *TPO* genes are derepressed, and the production of thyroid hormone returns to normal. In unusual instances, the inhibition of hormone synthesis by iodide can be great enough to induce thyroid hormone deficiency. The temporary reduction in hormone synthesis by excess iodide can also be used therapeutically in hyperthyroidism.

Thyroid hormones increase oxygen use, energy expenditure, and heat production. Therefore, it is logical to expect that the availability of active thyroid hormone correlates with changes in the body's caloric and thermal status. In fact, ingestion of excess calories, particularly in the form of carbohydrate, increases the production and plasma concentration of T_3 as well as the individual's metabolic rate, whereas prolonged fasting leads to corresponding decreases. Because most T_3 arises from circulating T_4 (see Table 6-1), peripheral mechanisms are important in mediating these changes. However, starvation also gradually lowers T_4 levels in humans.

CLINICAL BOX 6-6

Graves disease represents the most common form of **hyperthyroidism**; it occurs most frequently between the ages of 20 and 50 years and is 10 times more common in women than in men. Graves disease is an autoimmune disorder in which autoantibodies are produced against the TSH receptor. The nature of specific autoantibodies depends on the epitope that they are directed against. The most critical type is called the **thyroid-stimulating immunoglobulin (TSI)**. The hyperthyroidism is often accompanied by a diffuse goiter due to hyperplasia and hypertrophy of the gland. The follicular epithelial cells become tall columnar cells, and the colloid shows a scalloped periphery indicative of rapid turnover.

The primary clinical state found in Graves disease is **thyrotoxicosis**—the state of excessive thyroid hormone in the blood and tissues. The patient with thyrotoxicosis presents one of the most striking pictures in clinical medicine. The large increase in metabolic rate is accompanied by the highly characteristic symptom of weight loss despite an increased intake of food. The increased heat production causes discomfort in warm environments, excessive sweating, and a greater intake of water. The increase in adrenergic activity is manifested by a rapid heart rate, hyperkinesis, tremor, nervousness, and a wide-eyed stare. Weakness is caused by a loss of muscle mass as well as by an impairment of muscle function. Other symptoms include a labile emotional state, breathlessness during exercise, and difficulty in swallowing or breathing due to compression of the esophagus or trachea by the enlarged thyroid gland (goiter). The most common cardiovascular sign is sinus tachycardia. There is an increased cardiac output associated with widened pulse pressure due to a positive inotropic effect coupled with a decrease in vascular resistance. Major clinical signs in Graves disease are **exophthalmos** (abnormal protrusion of the eyeball; Fig. 6-9) and **periorbital edema** due to recognition by the anti-TSH receptor antibodies of a similar epitope within the orbital cells (probably fibroblasts).

Graves disease is diagnosed by an elevated serum free and total T_4 or T_3 level (i.e., thyrotoxicosis) and the clinical signs of diffuse goiter and ophthalmopathy. In most cases, the thyroid uptake of iodine or pertechnetate is excessive and diffuse. Serum TSH levels are low, because the hypothalamus and the pituitary gland are inhibited by the high levels of T_4 and T_3. Assaying TSH levels, and for the presence of circulating TSI, will distinguish Graves disease (a primary endocrine disorder) from a rare adenoma of the pituitary thyrotrophs (a secondary endocrine disease). The latter etiology generates elevated TSH levels unaccompanied by TSI.

Treatment of Graves disease is usually removal of the thyroid tissue, followed by lifelong replacement therapy with T_4. Thyroid tissue can be ablated by either the radiation effects of ^{131}I or by surgery. Surgical removal of the gland rarely but potentially precipitates a massive release of hormone, causing a **thyroid storm**, which causes death in 30% of patients due primarily to cardiac failure and arrhythmia. An alternative to removal of thyroid tissue is administration of **antithyroid drugs** that inhibit TPO activity.

Mechanism of Thyroid Hormone Action

Free T_4 and T_3 enter cells by a carrier-mediated, energy-dependent process. The transport of T_4 is rate limiting for the intracellular production of T_3. Within the cell, most, if not all, of the T_4 is converted to T_3 (or rT3).

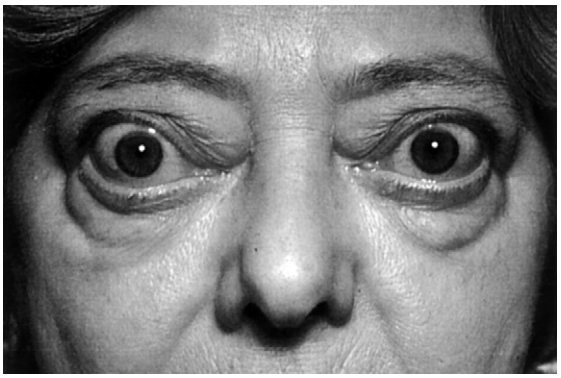


FIGURE 6-9 ■ Severe exophthalmos of Graves disease. Note lid retraction, periorbital edema, and proptosis. *(From Hall R, Evered DC:* Color atlas of endocrinology, *2nd ed., London, 1990, Mosby-Wolfe.)*

Many of the T_3 actions are mediated through its binding one of members of the **thyroid hormone receptor (TR) family.** The TR family belongs to the nuclear hormone receptor superfamily of transcription factors. TRs bind to a specific DNA sequence, termed a thyroid-response element (TRE), usually as a heterodimer with retinoid X receptor (RXR). As discussed in Chapter 1, gene activation by T_3 involves (1) the unliganded TR/RXR bound to a TRE and recruiting co-repressor protein that deacetylate DNA in the vicinity of the regulated gene; (2) binding of T_3 and the dissociation of co-repressor proteins; and (3) recruitment of coactivator proteins that, in part, acetylate DNA and activate the gene in question (see Fig. 1-24 in Chapter 1). However, T_3 also represses gene expression, indicating that other mechanisms exist, probably in a cell type–specific and gene-specific manner.

In humans, there are two TR genes, ***THRA*** and ***THRB***, located on chromosomes 17 and 3, respectively, that encode the classic nuclear thyroid hormone receptors. THRA encodes **TRα**, which is alternatively spliced to form two main isoforms. **TRα1** is a bona fide TR, whereas the other isoform does not bind T_3. THRB encodes **TRβ1** and **TRβ2**, both of which are high-affinity receptors for T_3. The tissue distribution of TRα1 and TRβ1 is widespread. TRα1 is especially expressed in cardiac and skeletal muscle, and TRα1 is the dominant TR that transduces thyroid hormone actions on the heart. By contrast, TRβ1 is expressed more in the brain, liver, and kidney. TRβ2 expression is restricted to the pituitary and critical areas of the hypothalamus, as well as the cochlea and retina. T_3-bound TRβ2 is responsible for inhibiting the expression of the prepro-*TRH* gene in the paraventricular neurons of the hypothalamus and of the β-subunit *TSH* gene in pituitary thyrotropes. Thus, negative feedback effects of thyroid hormone on both TRH and TSH secretion are largely mediated by TRβ2. T_3 also down regulates *TRβ2* gene expression in the pituitary gland.

CLINICAL BOX 6-7

An understanding of TR subtypes and tissue expression is of more than academic interest because inactivating mutant genes have been found increasingly to be causes of clinical syndromes manifested by **resistance to thyroid hormone (RTH) syndrome**. The most common mutations occur in the pituitary-hypothalamus-specific TRβ2 subtype. In these patients, there is incomplete negative thyroid hormone feedback at the hypothalamic-pituitary level. Thus, T_4 levels are elevated, but TSH is not suppressed. When the resistance is purely at the hypothalamic-pituitary level, the patient may exhibit signs of hyperthyroidism due to excess effects of high thyroid hormone levels on peripheral tissue, particularly on the heart through TRα1. These individuals have clinical signs such as goiter, short stature, decreased weight, tachycardia, hearing loss, monochromatic vision, and decreased IQ.

Physiologic Effects of Thyroid Hormone

Thyroid hormone acts on essentially all cells and tissues, and imbalances in thyroid function represent one of the most common endocrine diseases. Thyroid hormone has many direct actions, but it also acts in more subtle ways to optimize the actions of several other hormones and neurotransmitters.

Cardiovascular Effects

Perhaps the most clinically important actions of thyroid hormone are those on cardiovascular physiology. T_3 increases cardiac output, ensuring sufficient oxygen delivery to the tissues (Fig. 6-10). The resting heart rate and the stroke volume are increased. The speed and force of myocardial contractions are enhanced

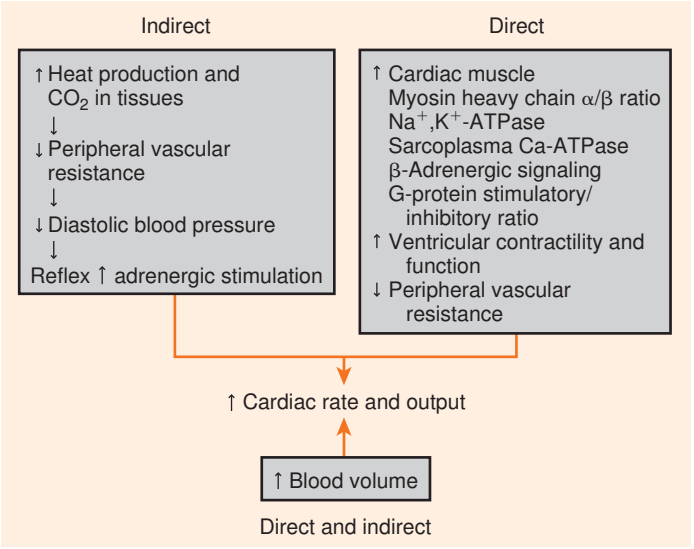


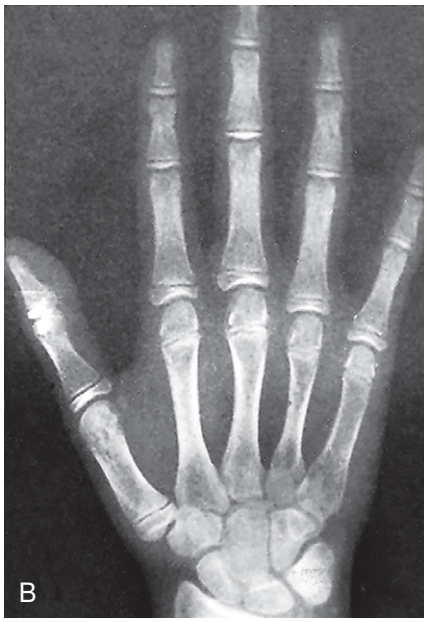
FIGURE 6-10 ■ Mechanisms by which thyroid hormone increases cardiac output. The indirect mechanisms are probably quantitatively more important.

(positive chronotropic and inotropic effects, respectively), and the diastolic relaxation time is shortened (positive lusitropic effect). Systolic blood pressure is modestly augmented, and diastolic blood pressure is decreased. The resultant widened pulse pressure reflects the combined effects of the increased stroke volume and the reduction in total peripheral vascular resistance that result from blood vessel dilation in skin, muscle, and heart. These effects in turn are partly secondary to the increase in tissue production of heat and metabolites that thyroid hormone induces (see later). In addition, however, thyroid hormone decreases systemic vascular resistance by dilating resistance arterioles in the peripheral circulation. Total blood volume is increased by activating the renin-angiotensin-aldosterone axis and thereby increasing renal tubular sodium reabsorption (see Chapter 7).

The cardiac inotropic effects of T_3 are indirect, through enhanced responsiveness to catecholamines (see Chapter 7), and direct (Fig. 6-11). Myocardial calcium uptake is increased, which enhances contractile force. Thyroid hormone inhibits expression of the **Na-Ca antiporter**, thereby increasing intramyocellular Ca^{2+} concentrations. T_3 increases the velocity and strength of myocardial contraction. T_3 promotes the expression of the faster and stronger α-isoform and represses the slower, weaker β-isoform of cardiac myosin heavy chain. T_3 also increases the **ryanodine Ca^{2+} channels** in the sarcoplasmic reticulum, promoting Ca^{2+} release from the sarcoplasmic reticulum during systole. The **calcium adenosine triphosphatase (ATPase) of the sarcolemmal reticulum (SERCA)** is increased by T_3, which facilitates sequestration of calcium during diastole and shortens the relaxation time.

CLINICAL BOX 6-8

Thyroid hormone levels in the normal range are necessary for optimal cardiac performance. **Hypothyroidism** in humans reduces stroke volume, left ventricular ejection fraction, cardiac output, and the efficiency of cardiac function. The latter defect is shown by the fact that the stroke work index [(stroke volume/left ventricular mass) × peak systolic blood pressure] is decreased even more than is myocardial oxidative metabolism. The rise in systemic vascular resistance may contribute to this cardiac debility. On the other hand, **hyperthyroidism** increases cardiac output and reduces peripheral resistance, generating a widened pulse pressure. T_3 increases UCP2 and UCP3 in cardiac muscle, which uncouples ATP production from oxygen use during the β-oxidation of free fatty acids. This can cause high-output cardiac failure. When aging individuals develop hyperthyroidism, the cardiac effects of thyroid hormone may include rapid atrial arrhythmias, flutter, and fibrillation.



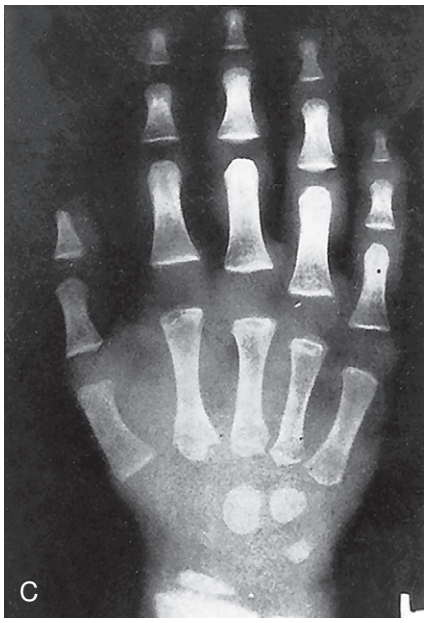


FIGURE 6-11 ■ A, A normal 6-year-old child *(left)* and a congenitally hypothyroid 17-year-old child *(right)* from the same village in an area of endemic cretinism. Note especially the short stature, obesity, malformed legs, and dull expression of the mentally retarded hypothyroid child. Other features are a prominent abdomen, a flat and broad nose, a hypoplastic mandible, dry and scaly skin, delayed puberty, and muscle weakness. Hand radiographs of a 13-year-old normal child (**B**) and a 13-year-old hypothyroid child (**C**). Note that the hypothyroid child has a marked delay in development of the small bones of the hands, in growth centers at either end of the fingers, and in the growth center of the distal end of the radius. *(**A,** From Delange FM: Endemic cretinism. In Braverman LE, Utiger RD, editors:* Werner and Ingbar's the Thyroid, *7th ed., Philadelphia, 1996, Lippincott-Raven. **B,** From Tanner JM, Whitehouse RH, Marshall WA, et al:* Assessment of skeletal maturity and prediction of adult height (TW2 method), *New York, 1975, Academic Press. **C,** From Andersen HJ: Nongoitrous hypothyroidism. In Gardner LI, editor:* Endocrine and Genetic Diseases of Childhood and Adolescence, *Philadelphia, 1975, Saunders.)*

Effects on Basal Metabolic Rate

Thyroid hormones increase the **basal rate of oxygen consumption** and **heat production** (**e.g., basal metabolic rate**). As mentioned earlier, thyroid hormone increases the expression of mitochondrial **uncoupling proteins** (**UCPs**). This action is demonstrated in all tissues except the brain, gonads, and spleen. Glucose and fatty acid uptake and oxidation are increased overall, as are lactate-glucose recycling and fatty acid–triglyceride recycling. Thyroid hormone does not augment diet-induced oxygen use, and it may not change the efficiency of energy use during exercise.

Thermogenesis must also increase concomitantly with oxygen use (see earlier). Thus, changes in body temperature parallel fluctuations in thyroid hormone availability. The potential increase in body temperature, however, is moderated by a compensatory increase in heat loss through appropriate thyroid hormone-mediated increases in **blood flow**, **sweating**, and **ventilation**. Hyperthyroidism is accompanied by **heat intolerance**, whereas hypothyroidism is accompanied by **cold intolerance**.

Increased oxygen use ultimately depends on an increased supply of substrates for oxidation. T_3 augments glucose absorption from the gastrointestinal tract and increases glucose turnover (glucose uptake, oxidation, and synthesis). In adipose tissue, thyroid hormone enhances lipolysis by increasing the number of β-adrenergic receptors (see later). Thyroid hormone also enhances clearance of chylomicrons. Thus, lipid turnover (free fatty acid release from adipose tissue and oxidation) is augmented in hyperthyroidism.

Protein turnover (release of muscle amino acids, protein degradation, and to a lesser extent, protein synthesis and urea formation) is also increased by thyroid hormones. T_3 potentiates the respective stimulatory effects of epinephrine, norepinephrine, glucagon, cortisol, and growth hormone on gluconeogenesis, lipolysis, ketogenesis, and proteolysis of the labile protein pool. The overall metabolic effect of thyroid hormone has been aptly described as accelerating the response to starvation.

T_3 regulates lipoprotein metabolism and cholesterol synthesis and clearance. Hypothyroidism is associated with an increase in TG-rich lipoproteins and low-density lipoprotein and a decrease in high-density lipoproteins.

The metabolic clearance of adrenal and gonadal steroid hormones, some B vitamins, and some administered drugs is also increased by thyroid hormone.

Respiratory Effects

T_3 stimulates oxygen use and also enhances oxygen supply. Appropriately, T_3 increases the **resting respiratory rate**, **minute ventilation**, and the **ventilatory responses** to hypercapnia and hypoxia. These actions maintain a normal arterial P_{O_2} when O_2 use is increased, and a normal P_{CO_2} when CO_2 production is increased. T_3 promotes erythropoietin production, hemoglobin synthesis, and absorption of folate and vitamin B_{12} from the gastrointestinal tract. Thus, hypothyroidism is accompanied by different **anemias**. Hypothyroidism in women is also associated with loss of iron due to excessive uterine bleeding (menorrhagia; see later), which further contributes to the anemic state.

Skeletal Muscle Effects

Normal function of skeletal muscles also requires optimal amounts of thyroid hormone. This requirement may also be related to the regulation of energy production and storage. In hyperthyroidism, glycolysis and glycogenolysis are increased, and glycogen and creatine phosphate are reduced. The inability of muscle to take up and phosphorylate creatine leads to increased urinary excretion of creatine. Muscle pain and weakness can occur in both hypothyroidism and hyperthyroidism.

Effects on the Autonomic Nervous System and Catecholamine Action

There is synergism between catecholamines and thyroid hormones. Thyroid hormones are synergistic with catecholamines in increasing metabolic rate, heat production, heart rate, motor activity, and CNS excitation. T_3 may enhance sympathetic nervous system activity by increasing the number of β-adrenergic receptors in heart muscle and by increasing the generation of intracellular second messengers, such as cyclic adenosine monophosphate (cAMP).

Effects on Growth and Maturation

Another major effect of thyroid hormone is to promote growth and maturation. A small but crucial amount of thyroid hormone crosses the placenta, and the fetal thyroid axis becomes functional at midgestation. Thyroid hormone is extremely important for normal neurologic development and for proper bone formation in the fetus. Insufficient fetal thyroid

hormone causes cretinism in the infant, characterized by irreversible mental retardation and short stature.

Effects on Bone, Hard Tissues, and Dermis

Thyroid hormone stimulates endochondral ossification, linear growth of bone, and maturation of the epiphyseal bone centers. T_3 enhances the maturation and activity of chondrocytes in the cartilage growth plate, in part by increasing local growth factor production and action. Although thyroid hormone is not required for linear growth until after birth, it is essential for normal maturation of growth centers in the bones of the developing fetus. T_3 also stimulates adult bone remodeling.

The progression of tooth development and eruption depends on thyroid hormone, as does the normal cycle of growth and maturation of the epidermis, its hair follicles, and nails. The normal degradative processes in these structural and integumentary tissues are also stimulated by thyroid hormone. Thus, either too much or too little thyroid hormone can lead to hair loss and abnormal nail formation.

Thyroid hormone alters the structure of subcutaneous tissue by inhibiting the synthesis, and increasing the degradation, of mucopolysaccharides (glycosaminoglycans) and fibronectin in the extracellular connective tissue. In hypothyroidism, the skin is thickened, cool, and dry, and the face becomes puffy because of the accumulation of subcutaneous glycosaminoglycans and other matrix molecules (**myxedema**).

Effects on the Nervous System

Thyroid hormone regulates the timing and pace of development of the CNS. Thyroid hormone deficiency in utero and in early infancy decreases growth of the cerebral and cerebellar cortex, proliferation of axons, and branching of dendrites, synaptogenesis, myelinization, and cell migration. Irreversible brain damage results when thyroid hormone deficiency is not recognized and treated promptly after birth. The structural defects described earlier are paralleled by biochemical abnormalities. Decreased thyroid hormone levels reduce cell size, RNA and protein content, tubulin- and microtubule-associated protein, protein and lipid content of myelin, local production of critical growth factors, and the rates of protein synthesis.

Thyroid hormone also enhances wakefulness, alertness, responsiveness to various stimuli, auditory sense, awareness of hunger, memory, and learning capacity. Normal emotional tone also depends on proper thyroid hormone availability. Furthermore, the speed and amplitude of peripheral nerve reflexes are increased by thyroid hormone, as is the motility of the gastrointestinal tract.

CLINICAL BOX 6-9

Hypothyroidism in the fetus or early childhood leads to **cretinism**. Affected individuals present with severe mental retardation, short stature with incomplete skeletal development (see Fig. 6-11), coarse facial features, and a protruding tongue. The most common cause of hypothyroidism in children is iodide deficiency. Iodide is not plentiful in the environment, and deficiency of iodide is a major cause of hypothyroidism in certain mountainous regions of South America, Africa, and Asia. This tragic form of **endemic cretinism** can be easily prevented by public health programs that add iodide to table salt or that provide yearly injections of a slowly absorbed iodide preparation. **Congenital defects** are a less common cause of neonatal and childhood hypothyroidism. In most cases, the thyroid gland simply does not develop (**thyroid gland dysgenesis**). Less frequent causes of childhood hypothyroidism are mutations in genes involved in thyroid hormone production (e.g., genes for NIS, TPO, thyroglobulin, and pendrin) and blocking antibodies to the TSH receptor (see later). The severity of neurologic and skeletal defects is closely linked to the time of diagnosis and replacement treatment with thyroid hormone (T_4), with early treatment resulting in a normal IQ with subtle neurologic deficits. Hypothyroid babies usually appear normal at birth because of maternal thyroid hormones. However, in geographic areas of endemic iodide deficiency, even the mother may be somewhat hypothyroid and unable to make up for the fetal defects. Alternatively, maternal hypothyroidism can cause mild mental retardation in euthyroid fetuses. **Neonatal screening** (T_4 or TSH levels) has played a major role in the prevention of severe cretinism. If hypothyroidism at birth remains untreated for only 2 to 4 weeks, the central nervous system will not mature normally in the first year of life. Developmental milestones, such as sitting, standing, and walking, will be late, and severe irreversible mental retardation can result.

Effects on Reproductive Organs and Endocrine Glands

In both women and men, thyroid hormone plays an important, permissive role in the regulation of reproductive function. The normal ovarian cycle of follicular development, maturation, and ovulation; the homologous testicular process of spermatogenesis; and the maintenance of the healthy pregnant state are all disrupted by significant deviations of thyroid hormone levels from the normal range. In part, these deleterious effects may be caused by alterations in the metabolism or availability of steroid hormones. For example, thyroid hormone stimulates hepatic synthesis and release of sex steroid–binding globulin.

Thyroid hormone also has significant effects on other parts of the endocrine system. Pituitary production of growth hormone is increased by thyroid hormone, whereas that of prolactin is decreased. Adrenocortical secretion of cortisol (see Chapter 7), as well as the metabolic clearance of this hormone, is stimulated, but plasma free cortisol levels remain normal. The ratio of estrogens to androgens (see Chapter 9) is increased in men (in whom breast enlargement may occur with hyperthyroidism). Decreases in both parathyroid hormone and in 1,25-$(OH)_2$-vitamin D production are compensatory consequences of the effects of thyroid hormone on bone resorption (see Chapter 4).

Kidney size, renal plasma flow, glomerular filtration rate, and transport rates for a number of substances are also increased by thyroid hormone.

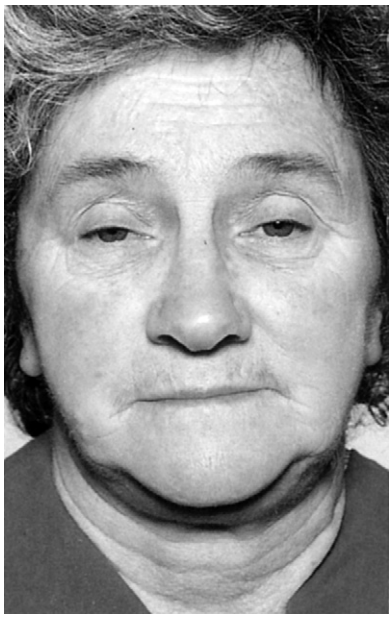


FIGURE 6-12 ■ Adult hypothyroidism. Note puffy face, puffy eyes, frowzy hair, and dull, apathetic appearance. *(From Hall R, Evered DC:* Color Atlas of Endocrinology, *2nd ed., London, 1990, Mosby-Wolfe.)*

CLINICAL BOX 6-10

Hypothyroidism in adults who do not have iodide deficiency most often results from idiopathic atrophy of the gland, which is thought to be preceded by a chronic autoimmune inflammatory reaction. In this form of **lymphocytic (Hashimoto) thyroiditis**, the antibodies that are produced may block hormone synthesis or thyroid gland growth, or they may have cytotoxic properties. Other causes of hypothyroidism include iatrogenic causes (e.g., radiochemical damage or surgical removal for treatment of hyperthyroidism), nodular goiters, and pituitary or hypothalamic disease.

The clinical picture of hypothyroidism in adults is in many respects the exact opposite of that seen in hyperthyroidism. The lower-than-normal metabolic rate leads to weight gain without an appreciable increase in caloric intake. The decreased thermogenesis lowers body temperature and causes intolerance to cold, decreased sweating, and dry skin. Adrenergic activity is decreased, and therefore bradycardia may occur. Movement, speech, and thought are all slowed, and lethargy, sleepiness, and a lowering of the upper eyelids (ptosis) occur. An accumulation of mucopolysaccharides—extracellular matrix—in the tissues also causes an accumulation of fluid. This nonpitting **myxedema** produces puffy features (Fig. 6-12); an enlarged tongue; hoarseness; joint stiffness; effusions in the pleural, pericardial, and peritoneal spaces; and pressure on peripheral and cranial nerves, entrapped by excess ground substance, with consequent thyroid dysfunction. Constipation, loss of hair, menstrual dysfunction, and anemia are other signs. In adults lacking thyroid hormone, positron emission tomography demonstrates a generalized reduction in cerebral blood flow and glucose metabolism. This abnormality may explain the psychomotor retardation and depressed affect of hypothyroid individuals.

Replacement therapy with T_4 is curative in adults. T_3 is not needed because it will be generated intracellularly from the administered T_4. Furthermore, giving T_3 raises plasma T_3 to unphysiologic levels.

SUMMARY

1. The thyroid gland is situated in the ventral neck, composed of right and left lobes anterolateral to the trachea and connected by an isthmus.
2. The thyroid gland is the source of tetraiodothyronine (thyroxine, T_4) and triiodothyronine (T_3).
3. The basic endocrine unit in the gland is a follicle that consists of a single spherical layer of epithelial cells surrounding a central lumen that contains colloid or stored hormone.
4. Iodide is taken up into thyroid cells by a sodium iodide symporter in the basolateral plasma membrane.
5. T_4 and T_3 are synthesized from tyrosine and iodide by the enzyme complex, thyroid peroxidase. Tyrosine is incorporated in peptide linkages within the protein thyroglobulin. After iodination, two iodotyrosine molecules are coupled to yield the iodothyronines.
6. Secretion of stored T_4 and T_3 requires retrieval of thyroglobulin from the follicle lumen by endocytosis. To support hormone synthesis, iodide is conserved by recycling the iodotyrosine molecules that escape coupling within thyroglobulin.
7. More than 99.5% of the T_4 and T_3 circulates bound to the following proteins: thyroid-binding globulin (TBG), transthyretin, and albumin. Only the free fractions of T_4 and T_3 are biologically active.
8. T_4 functions as a prohormone whose disposition is regulated by three types of deiodinases. Monodeiodination of the outer ring yields 75% of the daily production of T_3, which is the principal active hormone. Alternatively, monodeiodination of the inner ring yields reverse T_3, which is biologically inactive. Proportioning of T_4 between T_3 and reverse T_3 regulates the availability of active thyroid hormone.
9. Thyrotropin (TSH) acts on the thyroid gland through its plasma membrane receptor and cAMP to stimulate all steps in the production of T_4 and T_3. These steps include iodide uptake, iodination and coupling, and retrieval from thyroglobulin. TSH also stimulates glucose oxidation, protein synthesis, and growth of the epithelial cells.
10. TSH is increased by hypothalamic TRH. T_3 negatively feeds back on TSH and, to a lesser extent, TRH.
11. T_3 binds to thyroid hormone receptor (TR) subtypes that exist linked to thyroid regulatory elements (TREs) in target DNA molecules. As a result, induction or repression of gene expression increases or decreases a large number of enzymes, as well as structural and functional proteins.
12. Thyroid hormone increases and is a major regulator of the basal metabolic rate. Additional important actions of thyroid hormone are to increase heart rate, cardiac output, and ventilation and to decrease peripheral resistance. The corresponding increase in heat production leads to increased sweating. Substrate mobilization and disposal of metabolic products are enhanced. As part of normal cardiopulmonary function, T_3 is required for erythrocyte production and function.
13. T_3 is absolutely required for normal development and function of the CNS. In the absence of the hormone, brain development is retarded, and cretinism results. In the adult, T_3 optimizes normal brain function. Hypothyroidism and hyperthyroidism can cause erratic behavior and depression.
14. T_3 also regulates skeletal development and is crucial to normal growth. In hypothyroidism, growth is retarded and the bones fail to mature. In adults, T_3 increases the rates of bone resorption and degradation of skin and hair. T_3 is required for normal muscle function and normal integrity of the skin, nails, and hair.
15. T_3 regulates several organs within the endocrine system. T_3 is required for normal reproductive function, including fertility, normal menstrual cycling and blood loss, ovulation, spermatogenesis, and erectile function.

SELF-STUDY PROBLEMS

1. What is a likely cause of goiter associated with low T_4 and T_3 levels? What is a likely cause of goiter associated with elevated T_4 and T_3 levels?
2. Explain how a thyroid hormone receptor mutation can result in a deficiency in cardiac function without any change in TSH.
3. What is the relationship of the NIS to pendrin? How would inactivating mutations in each protein alter radioiodide uptake tests?
4. Why do serum T_4 levels approximately double in pregnancy? Are pregnant women hyperthyroid?
5. How does T_3 affect cardiac function?

KEYWORDS AND CONCEPTS

- Basal metabolic rate (BMR)
- Bruit
- Colloid

For full list of keywords and concepts see Student Consult

SUGGESTED READINGS

Kopp P, Pesce L, Solis SJ: Pendred syndrome and iodide transport in the thyroid, *Trends Endocrinol Metab* 19:260–268, 2008.

Liu YY, Brent GA: Thyroid hormone crosstalk with nuclear receptor signaling in metabolic regulation, *Trends Endocrinol Metab* 21:166–173, 2010.

Michalek K, Morshed SA, Latif R, et al: TSH receptor autoantibodies, *Autoimmun Rev* 9:113–116, 2009.

Patel J, Landers K, Li H, et al: Delivery of maternal thyroid hormones to the fetus, *Trends Endocrinol Metab* 22:164–170, 2011.

KEYWORDS AND CONCEPTS

- Coupling
- Diiodotyrosine (DIT)
- Endemic cretinism
- Euthyroid (or hyperthyroid)
- Exophthalmos
- Extrathyroidal pools
- Follicular cells
- Glycosaminoglycan (GAG)
- Goiter
- Goitrogens
- Graves disease
- Hashimoto thyroiditis
- Hypothyroid
- Iodide
- Iodide trap
- Iodothyronines
- Iodotyrosines
- Lid retraction
- Monoiodotyrosine (MIT)
- Myxedema
- Myxedema madness
- Organification
- Phagosome (endosome)
- Pretibial myxedema
- Radioactive iodide uptake (RAIU)
- Reverse T_3 (rT_3)
- Sporadic congenital hypothyroidism
- Subacute thyroiditis
- T/S [I^-]
- T_2, T_1, T_0
- T_3-amine
- T_4-amine
- Tetrac
- Thiourea (propylthiouracil [PTU])
- Thyroglobulin (TG)
- Thyroid deiodinase (D1, D2, D3)
- Thyroid peroxidase (TPO)
- Thyroid-responsive element (TSab)
- Thyrotropin, thyroid-stimulating hormone (TSH)
- Thyrotropin-releasing hormone (TRH)
- Thyroxine (T_4)
- Thyroxine-binding globulin (TBG)
- Transthyretin (TTR) (thyroxine-binding prealbumin)
- Triac
- Triiodothyronine (T_3)
- Wolff-Chaikoff effect

7

THE ADRENAL GLAND

OBJECTIVES

1. Discuss the anatomy of the adrenal gland, including the vascular supply and cortical zonation.
2. Discuss the synthesis and regulated release of catecholamines in the chromaffin cell.
3. Explain the action of catecholamines on different adrenergic receptors and the integrated effects of catecholamines during exercise.
4. Outline the differences between the steroidogenic pathways in each zone of the adrenal cortex.
5. Describe the physiologic actions of cortisol, aldosterone, DHEAS, and other adrenal androgens.
6. Describe the regulation of the zona fasciculata and zona reticularis by the pituitary.
7. Describe the regulation of the zona glomerulosa by the renin–angiotensin II system.
8. Describe the pathophysiology of adrenal hormone excess and underproduction.

In the adult, the adrenal glands emerge as fairly complex endocrine structures (Box 7-1) that produce two structurally distinct classes of hormones: **steroids** and **catecholamines**. The catecholamine hormone, **epinephrine**, acts as a rapid responder to stresses such as hypoglycemia and exercise to regulate multiple parameters of physiology, including energy metabolism and cardiac output. Stress is also a major secretogogue of the longer-acting steroid hormone, cortisol, which regulates glucose use, immune and inflammatory homeostasis, and numerous other processes. The adrenal glands also regulate salt and volume homeostasis through the steroid hormone, aldosterone. The adrenal gland secretes a large amount of the androgen precursor, dehydroepiandrosterone sulfate (DHEAS), which plays a major role in fetoplacental estrogen synthesis and as a substrate for peripheral androgen synthesis in women.

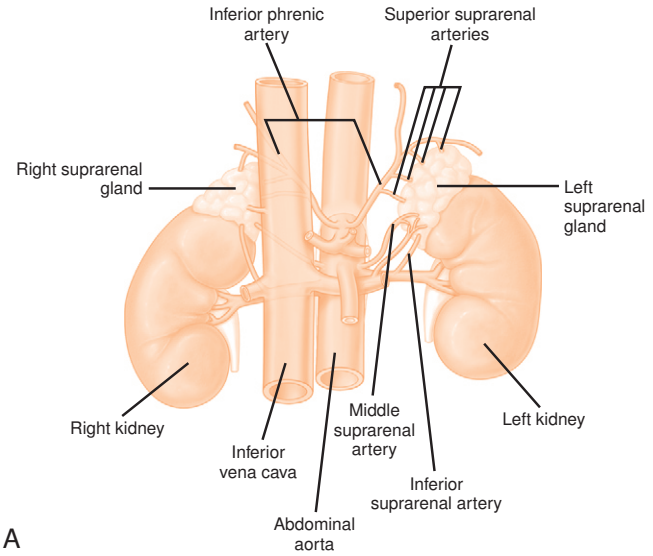
ANATOMY

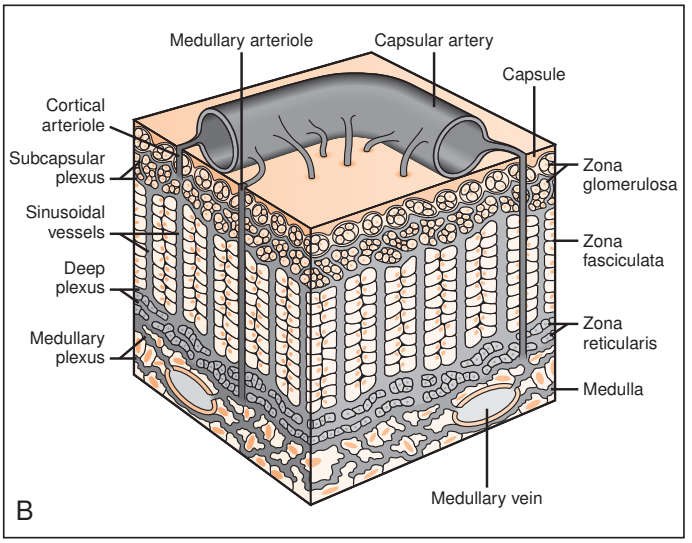
The adrenal glands are bilateral structures located immediately superior to the kidneys (*ad*, towards; *renal*, kidney) (Fig. 7-1A). In humans, they are also referred

BOX 7-1

OVERVIEW

The adrenal gland is a hybrid gland consisting of a cortex and a medulla. The hormones of the adrenal gland are important regulators of metabolism and serve an important role in adaptation to stress. The hormone aldosterone is critical to normal salt balance and hence water balance. Because of the anti-inflammatory and immunosuppressive actions of adrenal corticosteroids, synthetic analogs are widely used in the treatment of disorders ranging from skin rashes to arthritis.





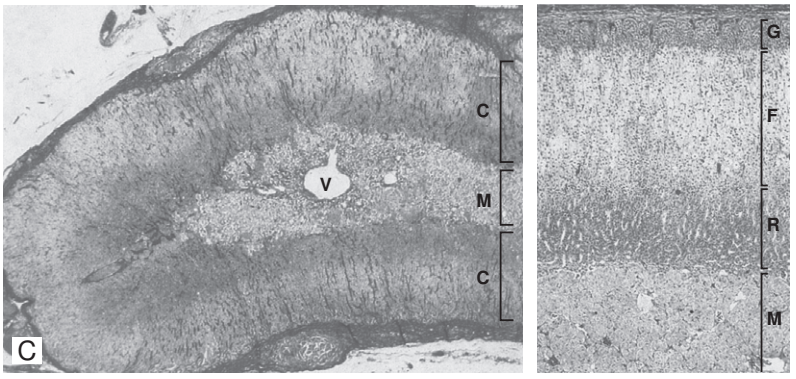


FIGURE 7-1 ■ A, Anatomy of human adrenal glands. Adrenals sit on superior poles of kidneys and thus are also referred to as suprarenal glands. Adrenal glands receive a rich arterial supply from the inferior, middle, and superior suprarenal arteries. In contrast, adrenals are drained by a single suprarenal vein. **B,** Blood flow through the adrenal gland. Capsular arteries give rise to sinusoidal vessels that carry blood centripetally through the cortex to the medulla. **C,** *Left,* Low magnification of adrenal histology. *Right,* histologic zonation of adrenal gland (C, cortex; G, zona glomerulosa; F, zona fasciculata; M, medulla; R, zona reticularis; V, central vein). *(**A,** From Drake RL, Vogl W, Mitchell AWM:* Gray's Anatomy for Students, *Philadelphia, 2005, Churchill Livingstone. **B,** From Stevens A, Lowe J:* Human Histology, *3rd ed., Philadelphia, 2005, Mosby. **C,** From Young B, Lowe JS, Stevens A, et al:* Wheater's Functional Histology, *Philadelphia, 2006, Churchill Livingstone.)*

to as the **suprarenal glands** because they sit on the superior pole of each kidney. The adrenal glands are similar to the pituitary, in that they are derived from both neuronal tissue and epithelial (or epithelial-like) tissue. The outer portion of the adrenal gland, called the **adrenal cortex**, develops from mesodermal cells in the vicinity of the superior pole of the developing kidney. These cells form cords of epithelioid endocrine cells. The cells of the cortex develop into steroidogenic cells (see Chapter 1) and produce **mineralocorticoids**, **glucocorticoids**, and **adrenal androgens** (Fig. 7-2; see Fig. 7-1C).

Soon after the cortex forms, neural crest–derived cells that are associated with the sympathetic ganglia—called **chromaffin cells** because they stain with chromium stains—migrate into the cortical cells and become encapsulated by them. Thus, the chromaffin cells establish the inner portion of the adrenal gland, which is called the **adrenal medulla** (see Fig. 7-1C). The chromaffin cells of the adrenal medulla have the potential of developing into postganglionic sympathetic neurons. They are innervated by cholinergic preganglionic sympathetic neurons and can synthesize the catecholamine neurotransmitter, **norepinephrine**, from tyrosine. However, the cells of the adrenal medulla are exposed to high local concentrations of cortisol from the cortex. Cortisol inhibits neuronal differentiation of the medullary cells so that they fail to form dendrites and axons. Additionally, cortisol induces the expression of an additional enzyme, **phenylethanolamine-*N*-methyl transferase (PNMT)**, in the catecholamine biosynthetic pathway. This enzyme adds a methyl group to norepinephrine, producing the catecholamine hormone, **epinephrine**, which is the primary hormonal product of the adrenal medulla (see Fig. 7-2).

The high local concentration of cortisol in the medulla is maintained by the vascular configuration within the adrenal gland. The outer connective tissue capsule of the adrenal gland is penetrated by a rich arterial supply coming from three main arterial branches (i.e., the inferior, middle, and superior suprarenal arteries; see Fig. 7-1A). These give rise to the following two types of blood vessels that carry blood from the cortex to the medulla (see Fig. 7-1B):

1. Relatively few medullary arterioles that provide high oxygen and nutrient blood directly to the medullary chromaffin cells
2. Relatively numerous cortical sinusoids, into which cortical cells secrete steroid hormones (including cortisol)

Both vessel types fuse to give rise to the medullary plexus of vessels that ultimately drain into a single suprarenal vein. Thus, secretions of the adrenal cortex

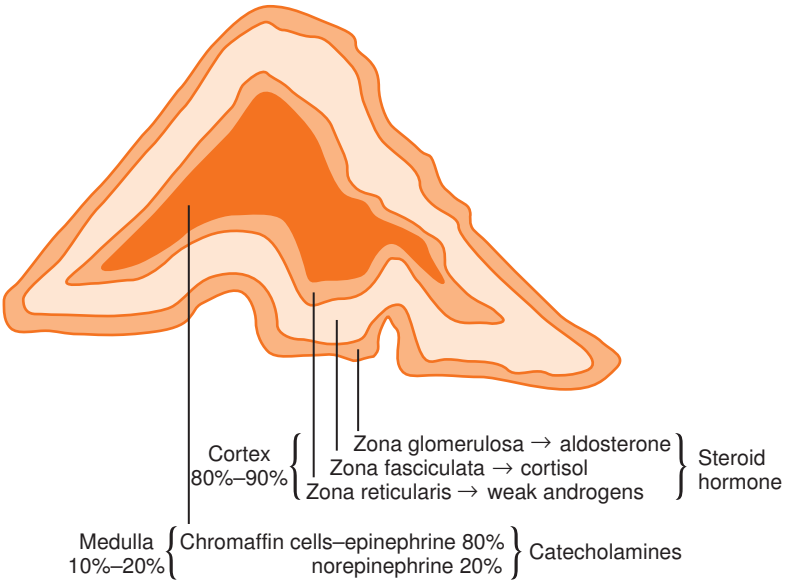


FIGURE 7-2 ■ Zonation and corresponding endocrine function of the adrenal gland.

percolate through the chromaffin cells, bathing them in high concentrations of cortisol before leaving the gland and entering the inferior vena cava.

ADRENAL MEDULLA

Together, the two adrenal medullae weigh about 1 g. As described, the adrenal medulla is similar to a sympathetic ganglion without postganglionic processes. Instead of being secreted near a target organ and acting as neurotransmitters, adrenomedullary catecholamines are secreted into the blood and act as hormones. About 80% of the cells of the adrenal medulla secrete **epinephrine,** and the remaining 20% secrete **norepinephrine**. Although circulating epinephrine is derived entirely from the adrenal medulla, only about 30% of the circulating norepinephrine comes from the medulla. The remaining 70% is released from postganglionic sympathetic nerve terminals and diffuses into the vascular system. Because the adrenal medulla is not the sole source of catecholamine production, this tissue is not essential for life.

Synthesis of Epinephrine

The enzymatic steps in the synthesis of epinephrine are shown in Figure 7-3. Synthesis begins with the sodium-linked transport of the amino acid, **tyrosine**, into the chromaffin cell cytoplasm (see Fig. 7-3) and the subsequent hydroxylation of tyrosine by the rate-limiting enzyme, **tyrosine hydroxylase**, to produce dihydroxyphenylalanine (DOPA). DOPA is converted to dopamine by the cytoplasmic enzyme, aromatic amino acid decarboxylase, and is then transported into the secretory vesicle (also called the **chromaffin granule**). Within the granule, dopamine is converted to **norepinephrine** by the enzyme, dopamine β-hydroxylase. This is an efficient reaction, so essentially all of chromaffin granule dopamine is converted to norepinephrine. In most adrenomedullary cells, essentially all of the norepinephrine diffuses out of the chromaffin granule by facilitated transport and is methylated by the cytoplasmic enzyme, **PNMT**, to form **epinephrine**. Epinephrine is then transported back into the granule by vesicular monoamine transporters (VMATs).

Multiple molecules of epinephrine, and to a lesser extent norepinephrine, are stored in the chromaffin granule complexed with adenosine triphosphate (ATP), Ca^{2+}, and proteins called **chromogranins**. These multimolecular complexes are thought to decrease the osmotic burden of storing individual molecules of epinephrine within chromaffin granules.

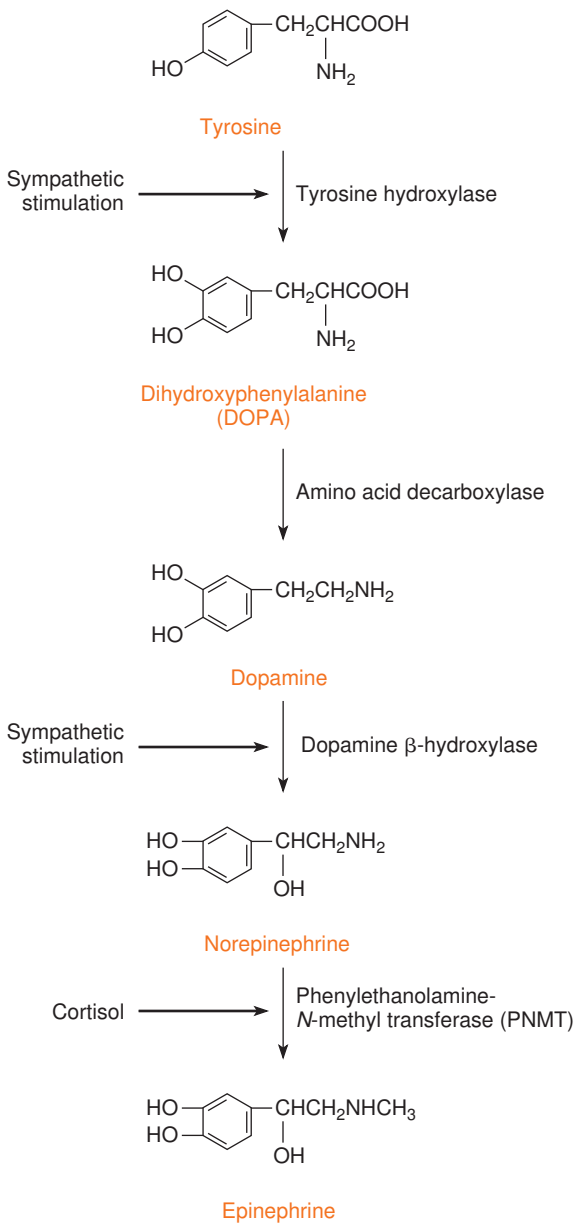


FIGURE 7-3 ■ Enzymatic steps and sites of regulation in the synthesis of catecholamines.

Chromogranins play a role in the biogenesis of secretory vesicles and the organization of components within the vesicles. Circulating chromogranins can be used as a marker of sympathetic paraganglion-derived tumors (paragangliomas). Chromaffin cells also synthesize several secretory peptides, including adrenomedullin and enkephalins, which can have local, subtle effects on sympathetic input and adrenomedullary response.

Secretion of epinephrine and norepinephrine from the adrenal medulla is regulated primarily by descending sympathetic signals in response to various forms of stress, including exercise, hypoglycemia, and surgery. The primary autonomic centers that initiate sympathetic responses reside in the hypothalamus and brainstem, and they receive inputs from the cerebral cortex, the limbic system, and other regions of the hypothalamus and brainstem.

The chemical signal for catecholamine secretion from the adrenal medulla is **acetylcholine (ACh)**, which is secreted from preganglionic sympathetic neurons and binds to **nicotinic receptors** on chromaffin cells. Nicotinic receptors are G-protein-coupled receptors (GPCRs) that are coupled to a Gs-cAMP-PKA pathway. ACh increases the activity of the rate-limiting enzyme, tyrosine hydroxylase, in chromaffin cells (see Fig. 7-3). ACh also increases the activity of dopamine β-hydroxylase and stimulates exocytosis of the chromaffin granules. Synthesis of epinephrine and norepinephrine is closely coupled to secretion so that the levels of intracellular catecholamines do not change significantly, even in the face of changing sympathetic activity. As discussed earlier, cortisol regulates epinephrine production by maintaining adequate expression of the *PNMT* gene in chromaffin cells (see Fig. 7-3).

Mechanism of Action of Catecholamines

Catecholamines act through membrane GPCRs (see Chapter 1). The individual types of adrenergic receptors were first classified based on their pharmacology, and this classification scheme has been supported by genetics and molecular cloning. Adrenergic receptors are generally classified as α- and β-adrenergic receptors, and these are further divided into α_1 and α_2 receptors and β_1, β_2, and β_3 receptors (Table 7-1). These receptors can be characterized according to the following:

1. The relative potency of endogenous and pharmacologic agonists and antagonists. The α receptors and β_3 receptors respond better to norepinephrine than epinephrine. The β_1 receptor responds equally to the two catecholamines, whereas epinephrine is more potent than norepinephrine for the β_2 receptor. A large number of synthetic selective and nonselective adrenergic agonists and antagonists now exist.
2. Downstream signaling pathways. Table 7-1 shows the primary pathways that are coupled to the different adrenergic receptors. This is an oversimplification because differences in signaling pathways for a given receptor have been linked to duration of agonist exposure and cell type.
3. Location and relative density of receptors. Importantly, different receptor types predominate in different tissues. For example, although both α and β receptors are expressed by islet β cells, the

TABLE 7-1

Catecholamine Receptors

RECEPTOR TYPE	PRIMARY MECHANISM OF ACTION	EXAMPLES OF TISSUE DISTRIBUTION	AGONIST POTENCY
α_1	↑IP_3, DAG	Vascular smooth muscle	Epinephrine ≈ norepinephrine
α_2	↓cAMP	Pancreatic β cells	Epinephrine ≈ norepinephrine
β_1	↑cAMP	Heart	Epinephrine = norepinephrine
β_2	↑cAMP	Liver	Epinephrine >> norepinephrine
β_3	↑cAMP	Adipose	Norepinephrine >> epinephrine

cAMP, cyclic adenosine monophosphate; DAG, diacylglycerol; IP_3, inositol triphosphate.

predominant response to a sympathetic discharge is mediated by α_2 receptors.

Physiologic Actions of Adrenomedullary Catecholamines

Because the adrenal medulla is directly innervated by the autonomic nervous system, adrenomedullary responses are very rapid. Because of the involvement of several centers in the central nervous system (CNS), most notably the cerebral cortex, adrenomedullary responses can precede onset of the actual stress (i.e., they can be anticipated). For example, a sprinter at the starting line can experience an adrenomedullary response in anticipation of the starter's gun and of the intense exertion of sprinting. In many cases, the adrenomedullary output, which is primarily epinephrine, is coordinated with sympathetic nervous activity as determined by the release of norepinephrine from postganglionic sympathetic neurons. However, some stimuli (e.g., hypoglycemia) evoke a stronger adrenomedullary response than a sympathetic nervous response, and vice versa.

Many organs and tissues are affected by a sympathoadrenal response. An informative example of the major physiologic roles of catecholamines is the sympathoadrenal response to exercise. Exercise is similar to the fight-or-flight response, but without the subjective element of fear. Exercise increases circulating levels of both norepinephrine and epinephrine. The overall goal of the sympathoadrenal system during exercise is to meet the increased energy demands of skeletal and cardiac muscle while maintaining sufficient oxygen and glucose supply to the brain. The response to exercise includes three of the following major physiologic actions of norepinephrine and epinephrine (Fig. 7-4):

1. Increased blood flow to the muscles is achieved by the integrated actions of norepinephrine and epinephrine on the heart, veins and lymphatics, and the nonmuscular (e.g., splanchnic) and muscular arteriolar beds. Norepinephrine and epinephrine act on β_1 receptors at the heart to increase the rate (chronotropy) and strength (inotropy) of contractions and facilitate ventricular relaxation during diastole (lusitropy). Catecholamines also induce vasoconstriction through α-adrenergic receptors of high-capacity vessels (veins and lymphatics), thereby increasing venous return to the heart. All these effects increase **cardiac output**. Catecholamines shunt blood away from the gastrointestinal (GI) tract through vasoconstriction of splanchnic arterioles (α receptors) and increase blood flow to skeletal muscle by inducing vasodilation of muscle arteriolar beds through β_2 receptors.
2. Epinephrine promotes **glycogenolysis** in muscle through β_2 receptors. Exercising muscle can also use free fatty acids (FFAs), and epinephrine and norepinephrine act through β_2 and β_3 receptors, respectively, to promote **lipolysis** in adipose tissue. The actions just described increase circulating levels of lactate and glycerol, which can be used by the liver as gluconeogenic substrates to increase glucose. Epinephrine does, in fact, increase blood glucose by increasing hepatic glycogenolysis and gluconeogenesis through β_2 receptors. The promotion of lipolysis in adipose tissue is also coordinated with an epinephrine-induced increase in hepatic **ketogenesis**. Finally, the effects of catecholamines on metabolism are reinforced by the fact that they stimulate glucagon secretion (β_2 receptors) and inhibit insulin secretion (α_2 receptors). Efficient production of ATP during normal exercise (i.e., a 1-hour workout) also requires efficient exchange of gases with an adequate supply of oxygen to exercising muscle. Epinephrine promotes this by relaxation of bronchiolar smooth muscle through β_2 receptors.
3. Catecholamines decrease energy demand by visceral smooth muscle. In general, a sympathoadrenal response decreases overall motility of the smooth muscle in the GI tract and urinary tract, thereby conserving energy where it is not needed.

Metabolism of Catecholamines

There are two primary enzymes involved in the degradation of catecholamines: monoamine oxidase (MAO) and catechol-*O*-methyltransferase (COMT). Although MAO is the predominant enzyme in neuronal mitochondria, both enzymes are found in many non-neuronal tissues, including liver and kidney. The neurotransmitter norepinephrine is degraded by MAO and COMT after uptake of the compound into the presynaptic terminal. This mechanism is also

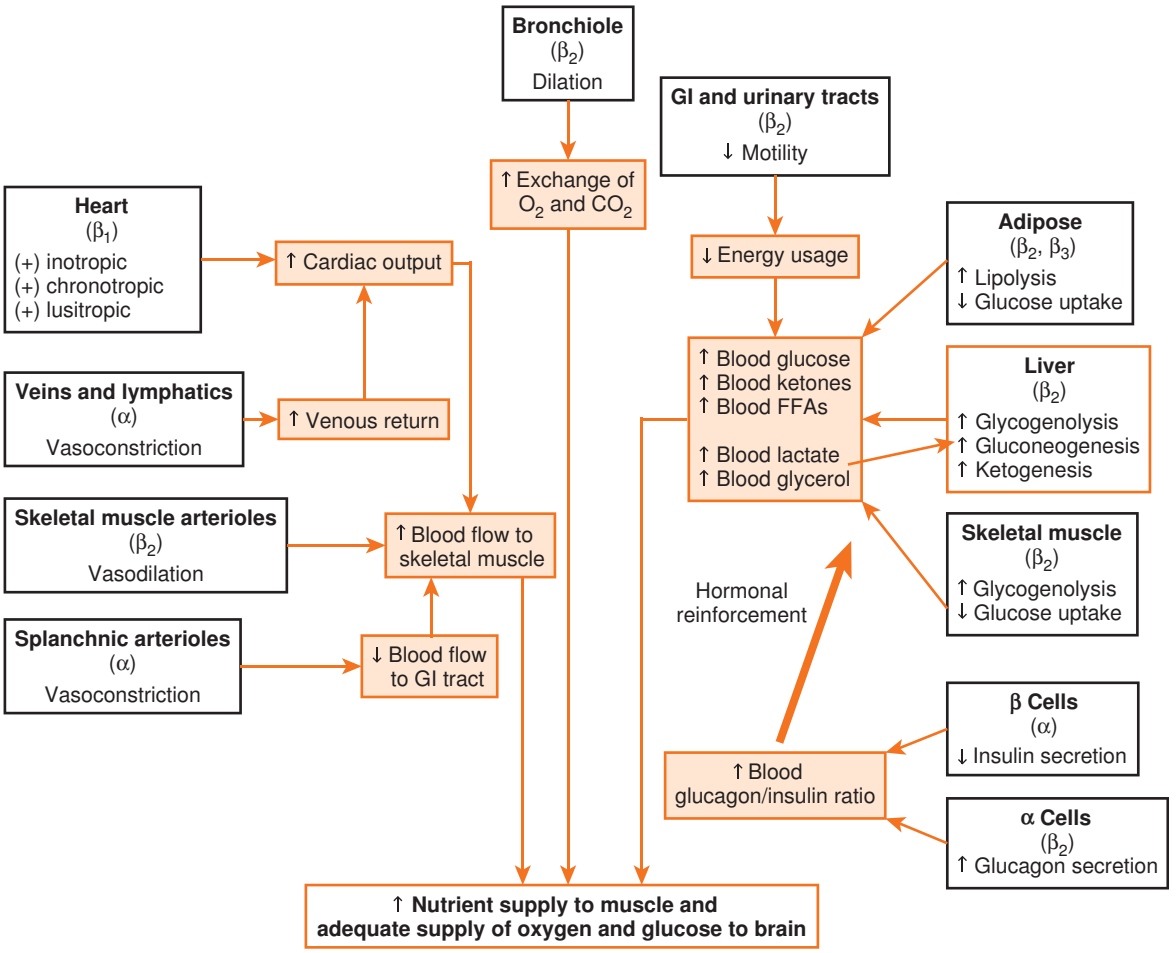


FIGURE 7-4 ■ Some of the individual actions of catecholamines that contribute to the integrated sympathoadrenal response to exercise.

involved in the catabolism of circulating adrenal catecholamines. However, the predominant fate of adrenal catecholamines is methylation by COMT in non-neuronal tissues such as the liver and kidney. The metabolism of catecholamines is shown in Figure 7-5. Urinary vanillylmandelic acid (VMA) and metanephrine are sometimes used clinically to assess the level of catecholamine production in a patient. Much of the urinary VMA and metanephrine is derived from neuronal, rather than adrenal, catecholamines.

CLINICAL BOX 7-1

A **pheochromocytoma** is a tumor of chromaffin cells (also called **pheochromocytes**) of the adrenal medulla that produces excessive quantities of epinephrine and norepinephrine. **Paragangliomas** are derived from nonadrenal sympathetic ganglia and secrete only norepinephrine. Although pheochromocytomas are not common tumors, they are the most common source of hyperadrenal medullary function and are often used as an example to demonstrate the functions of the adrenal medulla. For unknown

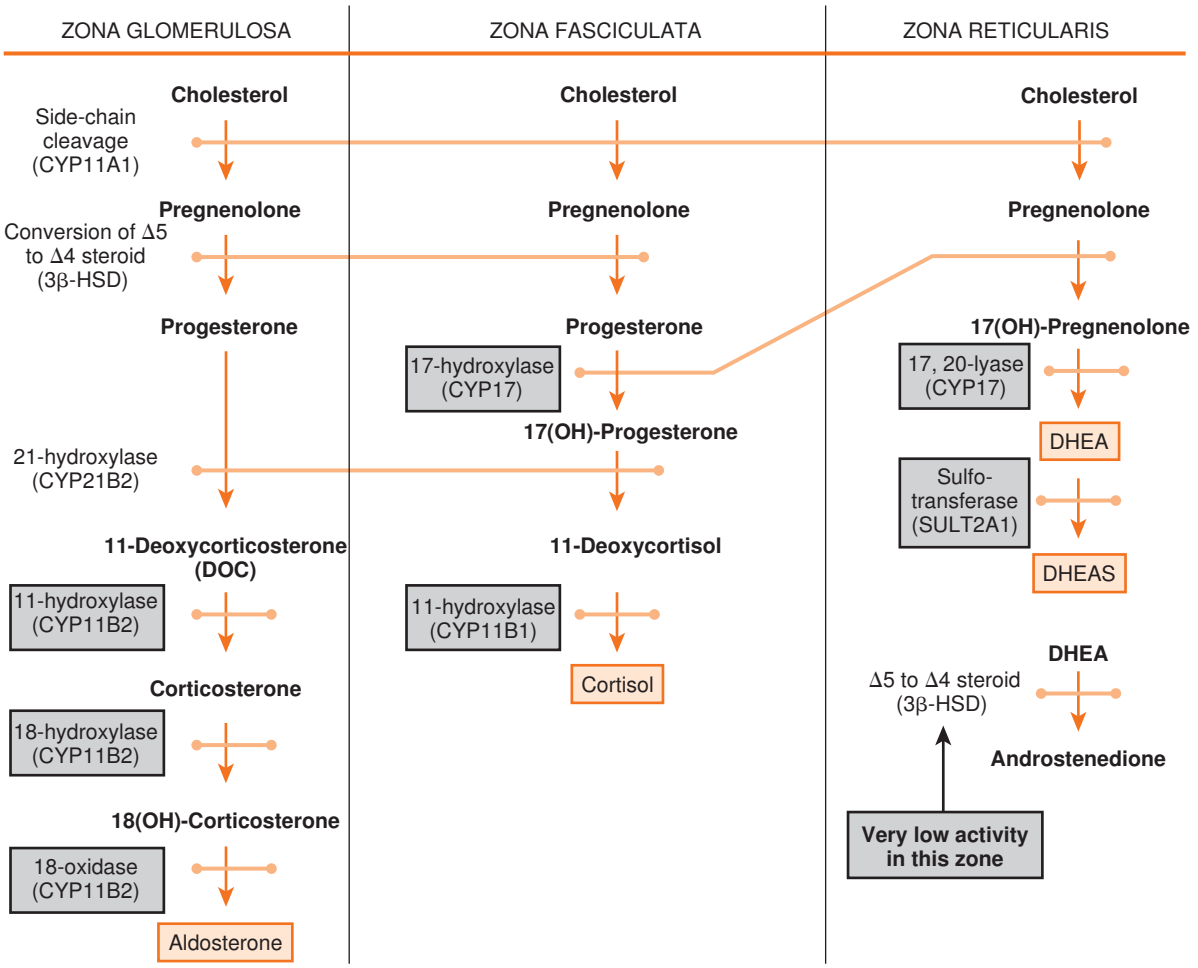


FIGURE 7-5 ■ Summary of the steroidogenic pathways for each of the three zones of the adrenal cortex. The major products of each zone are shown in *orange boxes*. Zone-specific enzymes are in *gray boxes*.

reasons, the symptoms of excessive catecholamine secretion (Box 7-2) are often sporadic rather than continuous. The symptoms include hypertension, headaches (from hypertension), sweating, anxiety, palpitations, and chest pain. In addition, patients with this disorder may experience orthostatic hypotension (despite the tendency for hypertension). This occurs because hypersecretion of catecholamines can decrease the postsynaptic response to norepinephrine as a result of down regulation of the receptors. Consequently, the baroreceptor response to the blood shifts that occur on standing is blunted.

ADRENAL CORTEX

The cortex of the adult human adrenal shows distinct zonation with respect to histologic appearance, steroidogenesis, and regulation. The adrenal cortex is made up of three zones: the outer zona glomerulosa, the middle zona fasciculata, and the inner zona reticularis (see Fig. 7-2). Each zone expresses a distinct complement of steroidogenic enzymes, resulting in the production of a different steroid hormone as the major endocrine product for each zone as summarized in Figure 7-5. Recall from Chapter 1 that steroid

hormones are derived from **cholesterol**, which is enzymatically modified in a cell type–specific manner. This means that the steroidogenic endocrine cells are characterized by the steroidogenic enzymes they express, as well as their final hormonal product. Associated with the production of a different steroid hormone, each zone has unique aspects concerning its regulation and the configuration of the feedback loop. An understanding of the steroidogenic pathways for each steroid hormone and steroidogenic cell type is required to understand the consequences of specific mutations in genes encoding steroidogenic enzymes and in states of dysregulation of specific steroidogenic pathways.

Zona Fasciculata

The Zona Fasciculata Makes Cortisol The largest and most actively steroidogenic zone is the middle **zona fasciculata** (see Figs. 7-1B and 7-2). The zona fasciculata produces the **glucocorticoid hormone**, **cortisol**. This zone is composed of straight cords of large cells. These cells have a foamy cytoplasm because they are filled with lipid droplets that represent stored **cholesterol esters**. Although the cells make some cholesterol de novo from acetate, they are very efficient at capturing cholesterol from the blood circulating in the form of **low-density lipoprotein** particles (delivery by high-density lipoprotein [HDL] is minimal in humans). Free cholesterol is then esterified by the enzyme **acyl CoA cholesterol transferase (ACAT)** and stored in lipid droplets (Fig. 7-6). The stored cholesterol is continually turned back into free cholesterol by **hormone sensitive lipase (HSL),** a process that is increased by adrenocorticotropic hormone (ACTH; see later).

Free cholesterol is modified by five reactions within a steroidogenic pathway to form cortisol. However, cholesterol is stored in the cytoplasm, and the first enzyme of the pathway, **CYP11A1**, is located on the inner mitochondrial membrane (see Fig. 7-6). Thus, the rate-limiting reaction in steroidogenesis is the transfer of cholesterol from the outer mitochondrial membrane to the inner mitochondrial membrane and its conversion to **pregnenolone (P5)**. Although several proteins appear to be involved, one protein, called **steroidogenic acute regulatory protein (StAR protein)**, is indispensable in the process of transporting cholesterol to the inner mitochondrial membrane (see Fig. 7-6). StAR is associated with the outer mitochondrial membrane and phosphorylation by ACTH-Gs-cAMP-PKA signaling increases its activity.

CLINICAL BOX 7-2

Endocytosed LDL particles are enzymatically digested by lysosomal enzymes. Free cholesterol, but not cholesterol esters, is transported out of the lysosome and enters the cellular cholesterol pool. Cholesterol esters are cleaved by **lysosomal acid lipase (LAL)** encoded by the ***LIPA*** gene. Mutations in the ***LIPA*** gene cause **cholesterol ester storage disease** and the more severe variant, **Wolman disease**. Wolman disease affects numerous organs and is ultimately fatal. With respect to the adrenal cortex, Wolman disease causes **adrenal insufficiency** due to the inability of cells to use LDL cholesterol for steroidogenesis. This underscores the importance of LDL cholesterol for steroidogenesis.

The **Niemann-Pick disease C transporters (NPC1 and NCP2)** are required for transport of free cholesterol out of the lysosome after receptor-mediated endocytosis of the LDL receptor. NPC disease is caused by a mutation in either the ***NPC1*** gene or, at a much lower frequency, the ***NPC2*** gene. NPC disease leads to progressive neurodegeneration and death within the first decade of life.

StAR protein is encoded by the ***StarD1*** gene. Inactivating mutations in *StarD1* cause cells of the **adrenal cortex** and **gonads** to become excessively laden with lipid ("lipoid") because cholesterol cannot be accessed by CYP11A1 within the mitochondria and used for hormone synthesis. Loss of cortisol increases ACTH, causing adrenal hypertrophy. Thus, mutations in *StarD1* lead to **lipoid congenital adrenal hyperplasia**. Elevated ACTH also increases cholesterol synthesis and transport of cholesterol into the cell cytoplasm through LDL receptor–mediated endocytosis, worsening the engorgement of the cell with lipid. Affected individuals make a small amount of **cortisol**, **aldosterone,** or **gonadal steroid hormones** as a result of StAR-independent transport. Aldosterone insufficiency represents the most serious deficit because it leads to salt wasting, reduced blood volume, and hypotension (especially orthostatic). Hypoaldosteronism also causes hyperkalemia and metabolic acidosis (see later). Hypocortisolism is especially a serious threat in the face of infection, trauma, surgery, or extended fasting (see later).

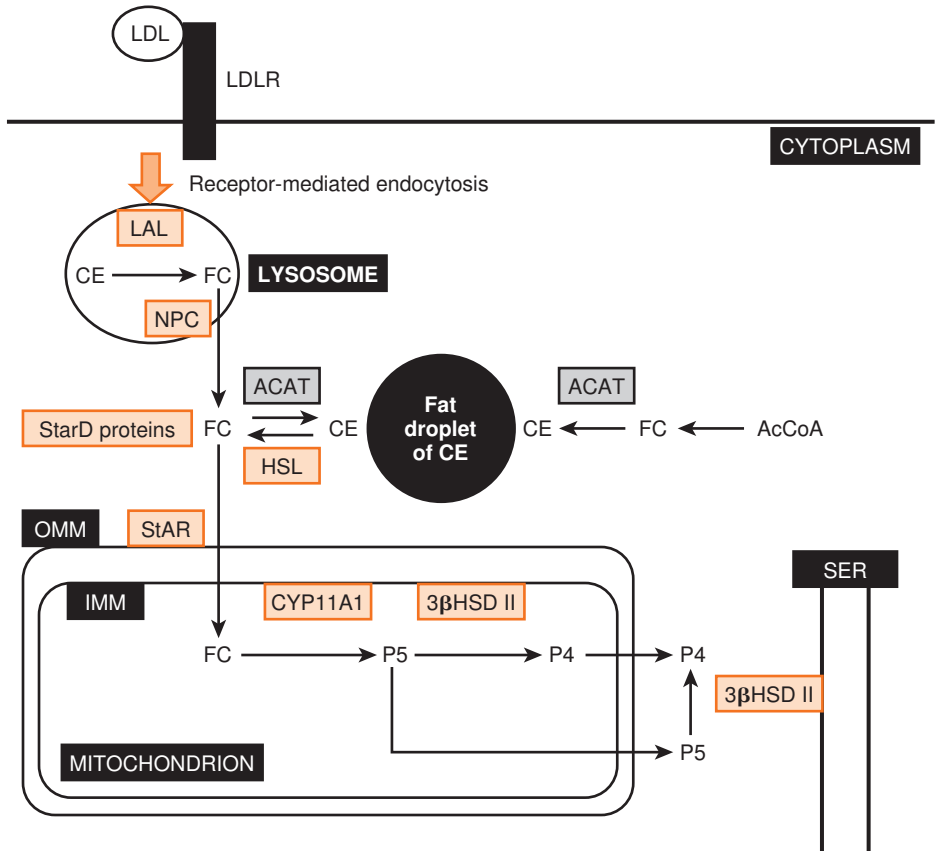


FIGURE 7-6 ■ Events involved in the first reaction (conversion of cholesterol to pregnenolone [P5]) in the steroidogenic pathway in zona fasciculata cells. Cholesterol is made de novo from acetyl CoA (AcCoA) to a limited extent (not shown), and a significant amount of cholesterol is imported from low-density lipoprotein particles (LDL) through receptor-mediated endocytosis of the LDL receptor (LDLR). Within endolysosomes, cholesterol esters (CE) released from LDL particles are converted to free cholesterol (FC). Free cholesterol is transported out of the lysosome by Niemann-Pick C1 (NPC1) and NPC2 proteins. Free cholesterol (FC) is converted to the storage form of cholesterol esters (CEs) by the enzyme, acyl CoA cholesterol acyltransferase (ACAT). CEs coalesce to form lipid droplets in the cytoplasm. FC is mobilized for steroidogenesis by hormone-sensitive lipase (HSL) and transported to the outer mitochondrial membrane by one or more cytoplasmic carrier proteins of the *StarD* gene family. FC must then be transported from the outer mitochondrial membrane (OMM) to the inside of the inner mitochondrial membrane (IMM) where CYP11A1 (also called P-450 side-chain cleavage enzyme) is localized. The critical protein that carries out this transport is steroidogenic acute regulatory (StAR) protein. The second reaction that converts P5 to progesterone (P4) can occur in the mitochondria or at the cytoplasmic surface of the smooth endoplasmic reticulum (SER) by 3β-hydroxysteroid dehydrogenase type II (3βHSDII).

The pathway by which cortisol is synthesized involves three enzymes that are not specific to the adrenal and two enzymes that are specifically adrenocortical in their expression. Four of these enzymes belong to the **cytochrome P-450 mono-oxidase** gene family and thus are referred to as **CYPs**. The fifth enzyme is **3β-hydroxysteroid dehydrogenase type 2 (3β-HSD2)**.

The steroidogenic pathway from cholesterol to cortisol is as follows (refer to Fig. 1-4 in Chapter 1

for structure of cholesterol and numbering of cholesterol carbons):

- **Reaction 1.** The side chain of cholesterol (carbons 22 to 27) is removed by **CYP11A1** (also called **P-450 side-chain cleavage**) to generate a C21 steroid intermediate, pregnenolone (Fig. 7-7). Generating a C21 intermediate is a key step because cortisol (as well as aldosterone and progesterone) is a 21-carbon steroid.
- **Reactions 2a/b and 3a/b.** The next two enzymes compete with each other for pregnenolone, so they will be presented as reactions 2a and 2b. The products of reactions 2a and 2b are then modified by the reciprocal enzymes in reactions 3a and 3b, to generate the final product, **17-hydroxyprogesterone** (see Fig. 7-7).
- **Reaction 2a.** Pregnenolone is a substrate for the enzyme, **3β-HSD2**, which converts the hydroxyl group on the 3 carbon to a ketone and moves the double bond from the 5-6 (Δ5) position to the 4-5 (Δ4) position. All active steroid hormones must be converted to Δ4 structures. This reaction converts **pregnenolone** (also called **P5**, because it is a Δ5 steroid) to **progesterone** (also called **P4**, because it is a Δ4 steroid).
- **Reaction 3a.** Progesterone is then hydroxylated to **17-hydroxyprogesterone** by **CYP17**. 17-Hydroxylation is an indispensable step for the formation of cortisol (and sex steroids). We will see that the presence or absence of CYP17 plays an important role in defining the nature of steroidogenic tissue.
- **Reaction 2b.** Pregnenolone can also be hydroxylated by CYP17 to **17-hydroypregnenolone** (this is called the Δ5 pathway).
- **Reaction 3b.** 17-Hydroxypregnenolone can then be converted to **17-hydroxyprogesterone** by **3β-HSD**. Note that **CYP17** has two separate activities: a **17-hydroxylase function**, and a **17,20-lyase function**. This latter function removes the 20 and 21 carbons, reducing the steroid to a 19-carbon precursor of active androgens. The zona fasciculata does not express cofactors that promote the 17,20-lyase activity of CYP17 and therefore does not produce

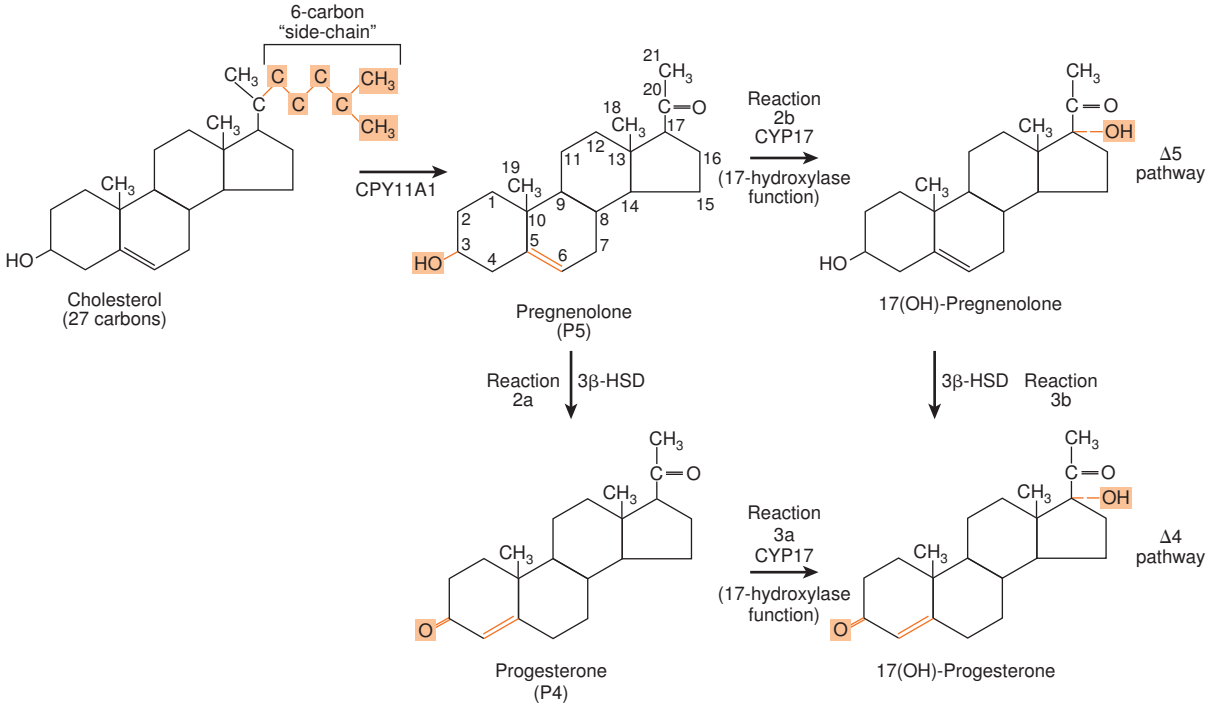


FIGURE 7-7 ■ Reaction 1, catalyzed by CYP11A1, in making cortisol. Reactions 2a/b and reactions 3a/b, involving CYP17 (17-hydroxylase function) and 3β-hydroxysteroid dehydrogenase (3β-HSD), in making cortisol.

significant amounts of androgen precursors. Instead, 17-hydroxyprogesterone is efficiently funneled into the cortisol-specific pathway, which involves two subsequent hydroxylations by adrenocortical-specific enzymes.

- **Reactions 4 and 5.** 17-Hydroxyprogesterone is hydroxylated on the 21 carbon by **CYP21**, producing **11-deoxycortisol**. 11-Deoxycortisol is then efficiently hydroxylated on the 11 carbon by **CYP11B1**, producing **cortisol** (Fig. 7-8). Note that **progesterone**

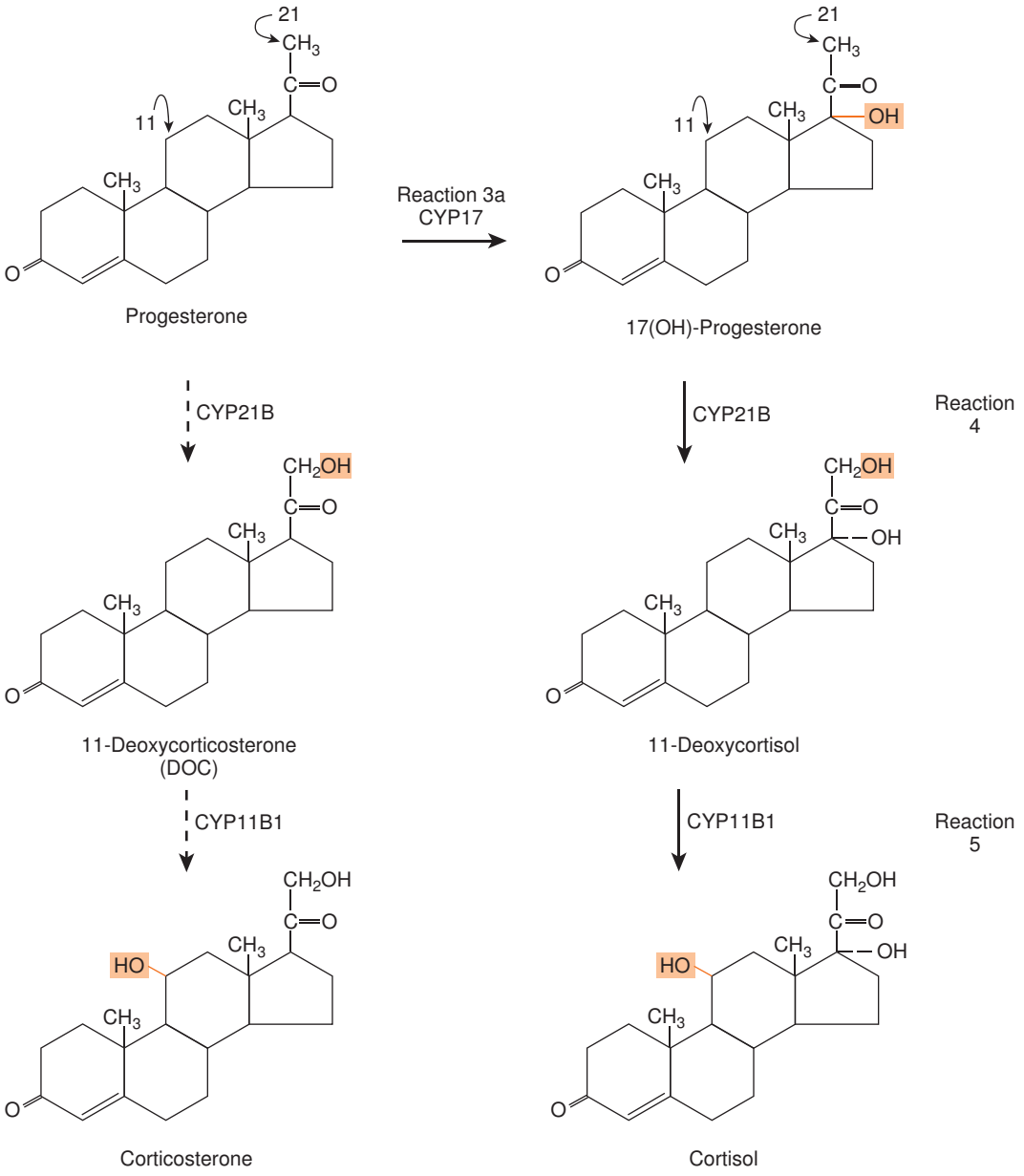


FIGURE 7-8 ■ Reactions 4 and 5, involving CYP21 and CYP11B1, that carry out the last two steps of the synthesis of cortisol. Also shown is the minor pathway leading to the synthesis of corticosterone in the zona fasciculata.

(the product of reaction 2a) can also enter this pathway of 21- and 11-hydroxylations, producing **deoxycorticosterone (DOC)** and **corticosterone**, respectively (see Fig. 7-5). However, CYP17 activity is robust in the human zona fasciculata, so DOC and corticosterone are normally minor products.

Transport and Metabolism of Cortisol Cortisol is transported in blood predominantly bound to proteins. These are primarily **corticosteroid-binding globulin (CBG)** (also called **transcortin**), which binds about 90%, and **albumin**, which binds 5% to 7%, of the circulating hormone. As stated for thyroid hormones in Chapter 6, it is the unbound *(free)* form of the hormone that exerts biologic effects within target cells. It is also the *free* form of cortisol that feeds back on the pituitary and hypothalamus. Thus, changes in CBG levels are usually counteracted by changes in the hypothalamus-pituitary-adrenal axis.

The **liver** is the predominant site of **steroid inactivation**, which occurs through several enzymatic steps. The liver also conjugates 95% of active and inactive steroids with glucuronide or sulfate so that they can be excreted more readily by the kidney (see Chapter 1). The circulating half-life of cortisol is about 70 minutes.

Cortisol is reversibly inactivated by conversion to **cortisone**. This is catalyzed by the enzyme, **11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2)**. Inactivation of cortisol protects the mineralocorticoid receptor (MR) in aldosterone-responsive cells (e.g., distal convoluted tubule cells of the kidney) because cortisol binds to the MR with high affinity. The inactivation of cortisol by 11β-HSD2 is reversible in that another enzyme, **11β-HSD1**, converts cortisone back to cortisol. This *activation* of cortisol occurs in tissues expressing the glucocorticoid receptor (GR), including liver, adipose, skin, and CNS.

Mechanism of Action of Cortisol Cortisol acts primarily through the GR, which regulates gene transcription (see Fig. 1-24 in Chapter 1). In the absence of hormone, the GR resides in the cytoplasm in a stable complex with several **molecular chaperones**, including heat-shock protein 90 and cyclophilins. Cortisol-GR binding promotes dissociation of the chaperone proteins, followed by the following:

1. Rapid translocation of the cortisol-GR complex into the nucleus.
2. Dimerization and binding to the **glucocorticoid-response elements (GREs)** near the basal promoters of cortisol-regulated genes.
3. Recruitment of coactivator or co-repressor proteins, followed by covalent modification of chromatin (e.g., histone acetylation for activation; histone deacetylation for inactivation).
4. A change (increase or decrease) in the assembly of the general transcription factors, leading to changes in the transcription rate of the targeted genes.
5. Phosphorylation, followed by nuclear export and/or degradation of the GR, thereby terminating the signal.

Physiologic Actions of Cortisol Cortisol has a broad range of actions on several organ systems (see Box 7-2). Several of the actions of cortisol were put forth as an integrated response to stress by Hans Selye in the 1930s, and cortisol is often characterized as a **stress hormone**. In general, cortisol maintains blood glucose, CNS function, and cardiovascular function during fasting, and it increases blood glucose during stress at the expense of muscle protein. Cortisol protects the body against the self-injurious effects of unbridled inflammatory and immune responses.

BOX 7-2
BIOLOGIC ACTIONS OF CORTISOL

Metabolic
Hyperglycemic
Glycogenic
Gluconeogenic
Lipolytic
Protein catabolic
Insulin antagonist in muscle and adipose tissue
Inhibits bone formation, stimulates bone resorption
Necessary for vascular response to catecholamines
Anti-inflammatory
Suppresses immune system
Inhibits antidiuretic hormone secretion and action
Stimulates gastric acid secretion
Necessary for integrity and function of gastrointestinal tract
Stimulates red blood cell production
Alters mood and behavior
Permissive for calorigenic, lipolytic effects of catecholamine

Cortisol also partitions energy to cope with stress by inhibiting reproductive function. As stated subsequently, cortisol has several other effects on bone, skin, connective tissue, the GI tract, and the developing fetus that are independent of its stress-related functions.

Metabolic Actions As the term **glucocorticoid** implies, cortisol is a steroid hormone from the adrenal cortex that regulates blood glucose. Cortisol increases blood glucose by stimulating **gluconeogenesis**. Cortisol enhances the gene expression of the hepatic gluconeogenic enzymes, **phosphoenolpyruvate carboxykinase (PEPCK)** and **glucose-6-phosphatase,** through direct actions and by increasing responsiveness to glucagon and catecholamines. Cortisol also decreases **GLUT4-mediated glucose uptake** in skeletal muscle and adipose tissue. During a fast (low insulin-to-glucagon ratio), cortisol promotes glucose sparing by potentiating the effects of catecholamines on **lipolysis**, thereby making FFAs available as an energy source and glycerol available for gluconeogenesis. Cortisol inhibits protein synthesis and significantly increases **proteolysis**, especially in skeletal muscle, thereby providing a rich source of carbon for hepatic gluconeogenesis. Excessive cortisol causes **muscle wasting**.

During the fed state, when the insulin-to-glucagon ratio is high, cortisol synergizes with insulin in promoting **hepatic glycogen synthesis.** This ensures that glycogen stores are replete at a time of stress or fasting. Cortisol also synergizes with insulin to promote **preadipocyte differentiation into adipocytes**, an effect that is dependent on PPARγ. Again, this ensures storage of excess calories during the fed state that can be mobilized at a time of stress or fasting. The effect on adipose tissue is region specific: cortisol specifically increases **abdominal** and **interscapular adipose tissue**.

Cardiovascular Actions Cortisol reinforces the enhancement of the delivery of blood glucose to the brain by its positive effects on the cardiovascular system. Cortisol is permissive on the actions of catecholamines and thereby increases **cardiac output** and **blood pressure**. Cortisol stimulates **erythropoietin synthesis** and, hence, increases red blood cell production. Anemia occurs when cortisol is deficient and polycythemia occurs when cortisol levels are excessive.

Anti-inflammatory and Immunosuppressive Actions Inflammation and immune responses are often part of a response to stress. However, inflammation and immune responses have the potential of doing significant harm, even to the extent of causing death, to the organism they are designed to protect if they are not held in homeostatic balance. As a stress hormone, cortisol plays an important role in maintaining immune homeostasis. Cortisol, along with epinephrine and norepinephrine, represses the production of **proinflammatory cytokines** and stimulate the production of **anti-inflammatory cytokines**.

The inflammatory response to injury consists of local dilation of capillaries and increased capillary permeability with a resultant local edema and accumulation of white blood cells. These steps are mediated by **prostaglandins, thromboxanes,** and **leukotrienes**. Cortisol inhibits **phospholipase A_2**, a key enzyme in prostaglandin, leukotriene, and thromboxane synthesis. Cortisol also stabilizes lysosomal membranes, thereby decreasing the release of the proteolytic enzymes that augment local swelling. In response to injury, **leukocytes** normally migrate to the site of injury and leave the vascular system. These changes are inhibited by cortisol, as is the phagocytic activity of the **neutrophils**, although bone marrow release of neutrophils is stimulated. Cortisol decreases the number of circulating **eosinophils**. The proliferation of connective tissue **fibroblasts** involved in inflammation is also inhibited. This latter response is important in the formation of barriers to the spread of certain infectious agents. Analogs of glucocorticoid are frequently used pharmacologically because of their anti-inflammatory properties. When cortisol levels are high, many of the body's defense mechanisms against infection are inhibited. For this reason, glucocorticoid therapy is contraindicated as the sole medication for the treatment of infections.

Cortisol inhibits the immune response and, for this reason, glucocorticoid analogs have been used as **immunosuppressants** in organ transplantations. High cortisol levels decrease the number of circulating **T lymphocytes** (particularly helper T lymphocytes) and decrease their ability to migrate to the site of antigenic stimulation. Glucocorticoids promote atrophy of the thymus and other lymphoid tissue. Although corticosteroids inhibit cell-mediated immunity, antibody production by B lymphocytes does not appear to be impaired.

Figure 7-9 contrasts the normal role of cortisol in response to a stress and the effects of chronically elevated

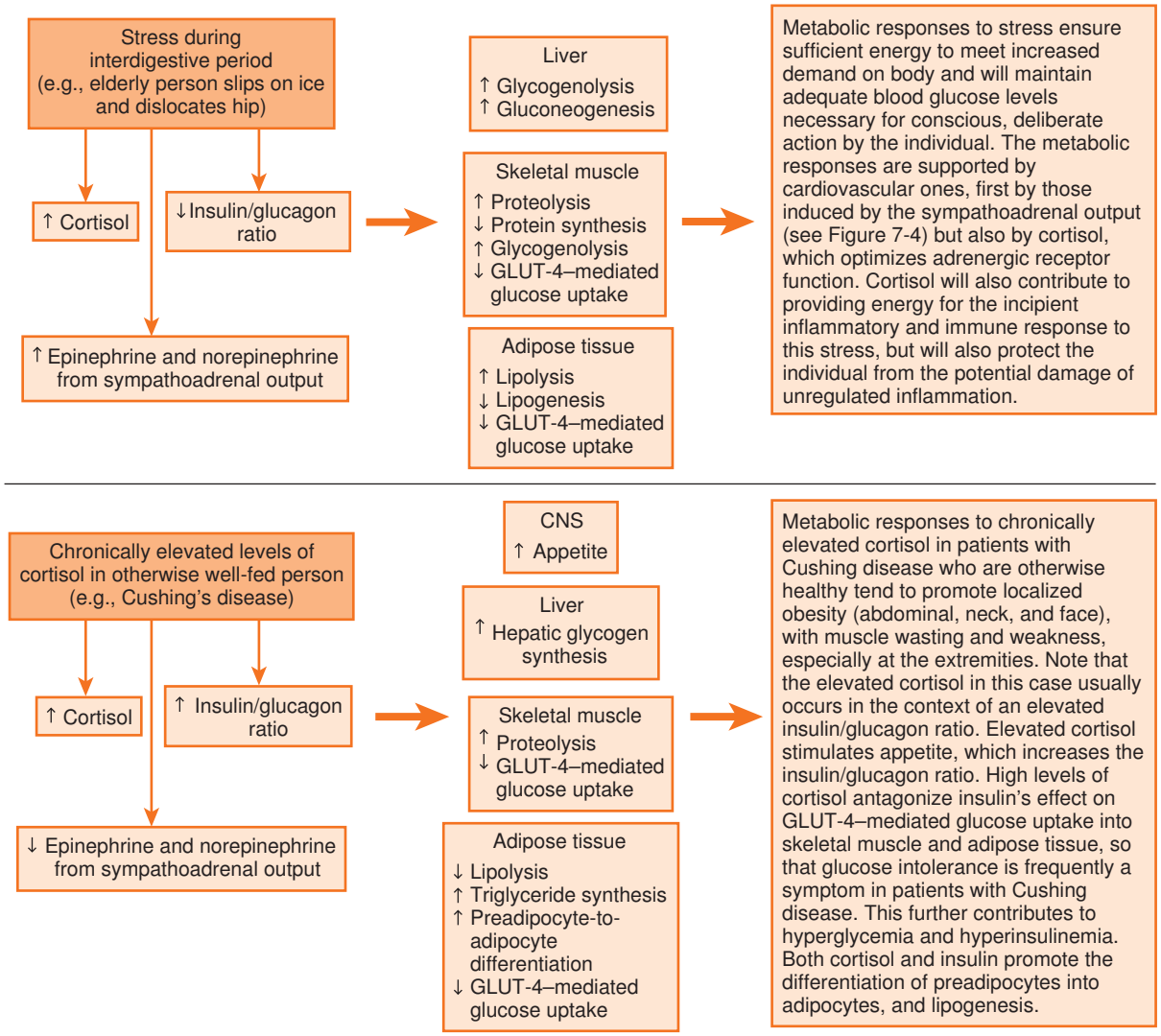


FIGURE 7-9 ■ Metabolic actions of cortisol (integrated with catecholamines and glucagon) in response to stress *(upper panel)* and contrasted to actions of chronically elevated cortisol (integrated with insulin) in an otherwise healthy individual *(lower panel)*.

cortisol due to a pathologic condition. There are important differences in the overall metabolic effects of cortisol between these two states, particularly with respect to lipid metabolism. During stress, cortisol synergizes with catecholamines and glucagon to promote a **lipolytic** and **gluconeogenic** metabolic response, while synergizing with catecholamines to promote an appropriate cardiovascular response. During chronically elevated levels of cortisol owing to a pathologic overproduction, cortisol synergizes with **insulin** in the context of elevated levels of glucose (because of an increased appetite) and **hyperinsulinemia** (because of elevated glucose and increased glucose intolerance) to promote truncal (abdominal, visceral) and interscapular adiposity.

Action on Reproductive Systems Reproduction exacts a considerable energy cost on the organism. In humans, reproductive behavior and function are dampened in response to stress. Cortisol decreases the function of the reproductive axis at the hypothalamic, pituitary, and gonadal levels.

Actions on Bone Glucocorticoids increase bone resorption. They have multiple actions that alter bone metabolism. Glucocorticoids decrease intestinal calcium absorption and decrease renal calcium reabsorption. Both mechanisms lower serum calcium concentrations. As the serum calcium level drops, the secretion of parathyroid hormone (PTH) increases, and PTH mobilizes calcium from bone both by stimulating resorption of bone. In addition to this action, glucocorticoids directly inhibit osteoblast bone-forming functions (see Chapter 4). Although glucocorticoids are useful for treating the inflammation associated with arthritis, excessive use will result in bone loss (osteoporosis).

Actions on Connective Tissue Cortisol inhibits **fibroblast** proliferation and collagen formation. In the presence of excessive amounts of cortisol, the skin thins and is more readily damaged. The connective tissue support of capillaries is impaired, and capillary injury (bruising) is increased.

Actions on Kidney Cortisol inhibits **antidiuretic hormone (ADH) secretion and action**, so it is an **ADH antagonist**. In the absence of cortisol, the action of ADH is potentiated, making it difficult to increase the free-water clearance in response to a water load and increasing the likelihood of water intoxication. As discussed earlier, cortisol binds to the mineralocorticoid receptor with high affinity, but this action is normally blocked by the inactivation of cortisol to cortisone by the enzyme 11β-HSD2. However, the mineralocorticoid activity (i.e., Na^+ and water retention, K^+ and H^+ excretion) of cortisol depends on the relative amount of cortisol (or synthetic glucocorticoids) and the activity of 11β-HSD2. Certain agents (such as compounds in black licorice) inhibit 11β-HSD2 and thereby increase the mineralocorticoid activity of cortisol. Cortisol increases the glomerular filtration rate by increasing cardiac output and acting directly on the kidney.

Actions on Muscle Cortisol actions on muscle are complex. When cortisol levels are excessive, muscle weakness and pain are common symptoms. The weakness has multiple origins. In part it is a result of the **excessive proteolysis** (muscle wasting) that cortisol produces. High cortisol levels can result in hypokalemia (through the mineralocorticoid actions), which can produce muscle weakness because it hyperpolarizes and stabilizes the muscle cell membrane, thereby making stimulation more difficult.

Gastrointestinal Actions Cortisol exerts a trophic effect on the GI mucosa. In the absence of cortisol, GI motility decreases, GI mucosa degenerates, and GI acid and enzyme production decrease. Because cortisol stimulates appetite, hypercortisolism is frequently associated with weight gain. The cortisol-mediated stimulation of gastric acid and pepsin secretion increases the risk for ulcer development.

Psychological Actions The normal range of daily cortisol levels maintains optimal psychological function in humans. Psychiatric disturbances are associated with either excessive or deficient levels of corticosteroids. Excessive corticosteroids can initially produce a feeling of well-being, but continued excessive exposure eventually leads to emotional lability and depression. Frank psychosis can occur with either excess or deficient hormone. Cortisol has been shown to increase the tendency for insomnia and decrease rapid eye movement (REM) sleep. People who are deficient in corticosteroids tend to be depressed, apathetic, and irritable.

Actions of Cortisol During Fetal Development Cortisol is required for normal development of the CNS, retina, skin, GI tract, and lungs. The best-studied system is the lungs, in which cortisol induces differentiation and maturation of **type 2 alveolar cells**. These cells produce **surfactant** during late gestation that reduces surface tension in the lungs and thus allows for the onset of breathing at birth.

Regulation of Cortisol Production

Cortisol production by the zona fasciculata is regulated by the hypothalamus-pituitary-adrenal axis involving **CRH**, **ACTH**, and **cortisol** (see Chapter 5). The hypothalamus and pituitary stimulate cortisol production and cortisol negatively feeds back on the hypothalamus and pituitary to maintain its set-point.

A subset of the hypophysiotropic parvicellular neurons secrete corticotropin-releasing hormone (CRH), which binds to the Gs-coupled CRH receptor on

pro-opiomelanocortin (POMC) cells (also called corticotropes) in the pars distalis (see Chapter 5). Both **neurogenic** (e.g., fear) and **systemic** (e.g., hypoglycemia, hemorrhage, cytokines) forms of stress stimulate CRH release (Box 7-3). CRH is also under **strong diurnal rhythmic** regulation emerging from the suprachiasmatic nucleus so that cortisol levels surge during early predawn and morning hours and then continually decline throughout the day and evening (refer to Fig. 5-15 in Chapter 5, which shows diurnal variation for ACTH). CRH acutely ACTH release and chronically increases *POMC* gene expression and corticotrope hypertrophy and proliferation. Some parvicellular neurons coexpress CRH and **vasopressin** (also called **ADH**). Vasopressin that reaches the anterior pituitary binds to the Gq-coupled vasopressin-3 receptor (V3 receptor) on corticotropes and potentiates the actions of CRH.

ACTH binds to the **melanocortin-2 receptor (MC2R)** located on cells in the zona fasciculata. The MC2R is coupled primarily to a Gs-cAMP-PKA signaling pathway. The effects of ACTH can be subdivided into three phases:

1. **Acute effects of ACTH** occur within minutes. Cholesterol is rapidly mobilized from lipid droplets by posttranslational activation of hormone-sensitive lipase and transported to the outer mitochondrial membrane. ACTH both rapidly increases StAR protein gene expression and activates StAR protein through PKA-dependent phosphorylation. Collectively, these acute actions of ACTH increase pregnenolone levels.
2. **Chronic effects of ACTH** occur over a period of several hours. These involve increasing the transcription of the genes encoding the steroidogenic enzymes and their coenzymes. ACTH also increases the expression of the LDL receptor.
3. **Trophic actions of ACTH** on the zona fasciculata and reticularis occur over a period of weeks and months. This last effect is exemplified by atrophy of the zona fasciculata in patients receiving therapeutic (i.e., supraphysiologic) levels of glucocorticoid analogs for at least 3 weeks. Under these conditions, the exogenous corticosteroids completely repress CRH and ACTH production, resulting in the **atrophy of the zona fasciculata** and decline in endogenous cortisol production (Fig. 7-10). At the end of therapy, such patients need to be gradually weaned from exogenous glucocorticoids to allow the hypothalamus-pituitary-adrenal axis to reestablish itself and the zona fasciculata to enlarge and produce adequate amounts of cortisol.

BOX 7-3
STIMULI FOR CORTICOTROPIN-RELEASING HORMONE SECRETION

- Diurnal input from suprachiasmatic nucleus
- Proinflammatory cytokines/infection
- Hypoglycemia
- Hemorrhage
- Neurogenic stress (e.g., fear)
- Physical stress (e.g., surgery)

Cortisol inhibits both ***POMC* gene expression** at the corticotropes and **pro-*CRH*** gene expression at the hypothalamus. However, intense stress can override the negative feedback effects of cortisol at the hypothalamus, thereby resetting the set-point at a higher level.

Zona Reticularis

The innermost zone, the **zona reticularis**, begins to appear after birth at about age 5 years. **Adrenal androgens**, especially **DHEAS**, the main product of the zona reticularis, become detectable in the circulation at about 6 years of age. This onset of adrenal androgen production is called **adrenarche** and contributes to appearance of axillary and pubic hair at about age 8 years. DHEAS levels continue to increase, peak during the mid-20s, and then progressively decline with age.

The Zona Reticularis Makes Adrenal Androgens The zona reticularis differs from the zona fasciculata in several important ways with respect to steroidogenic enzyme activity. First, **3β-HSD** is expressed at a much lower level in the zona reticularis than in the zona fasciculata, so the **Δ5 pathway** predominates in the zona reticularis.

Second, the zona reticularis expresses cofactors or conditions that enhance the **17,20-lyase function of CYP17**, thereby generating the 19-carbon androgen precursor molecule, **dehydroepiandrosterone (DHEA)**, from 17-hydroxypregnenolone. Additionally, the zona

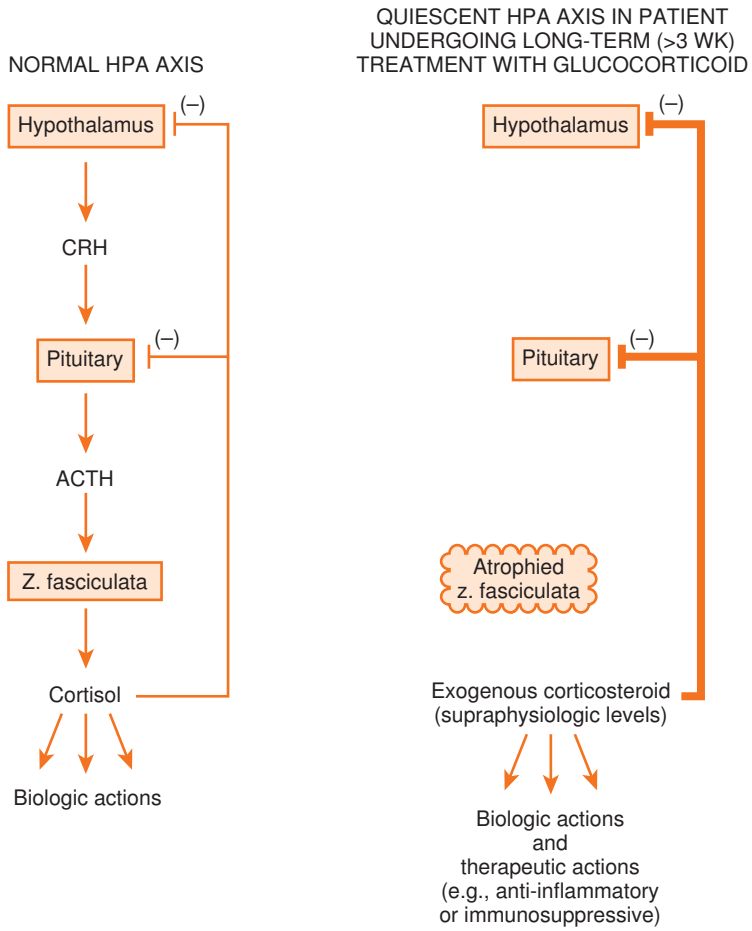


FIGURE 7-10 ■ Comparison of normal hypothalamus-pituitary-adrenal (HPA) axis to quiescent HPA in an individual receiving exogenous glucocorticoid therapy. The latter causes the zona fasciculata to atrophy after 3 weeks, requiring a careful withdrawal regimen that allows rebuilding of the adrenal tissue before total cessation of exogenous corticosteroid administration.

reticularis expresses **DHEA-sulfotransferase** (***SULT2A1* gene**), which converts DHEA into **DHEAS** (Fig. 7-11). A limited amount of the Δ4 androgen, **androstenedione**, is also made in the zona reticularis. Small amounts of potent androgens (e.g., testosterone) or 18-carbon estrogens are normally produced by the human adrenal cortex (see Fig. 7-5 for summary).

Metabolism and Fate: DHEAS and DHEA DHEAS can be converted back to DHEA by peripheral **sulfatases.** Importantly, several peripheral tissues (e.g., hair follicle, breast) express steroidogenic enzymes that can convert DHEA and androstenedione to the potent androgens, **testosterone** and **dihydrotestosterone** (see Chapter 9), or to the potent estrogen, **estradiol** (see Chapter 10). This peripheral conversion of DHEA, DHEAS, and androstenedione is the basis for masculinization of a female fetus or an adult woman by excessive adrenal androgen production.

DHEA binds to albumin and other transport globulins with low affinity and so is excreted efficiently by the kidney. The half-life of DHEA is 15 to 30 minutes. In contrast, DHEAS binds to albumin with very high affinity and has a half-life of 7 to 10 hours.

Physiologic Actions of Adrenal Androgens In men, the peripheral conversion of adrenal androgens to active androgens is much lower than testicular production of active androgens. However, in women, the adrenal gland contributes to about 50% of circulating active androgens, which are required for axillary and pubic hair growth as well as libido. Under conditions of adrenal androgen excess (adrenal tumor, Cushing syndrome, congenital adrenal hyperplasia),

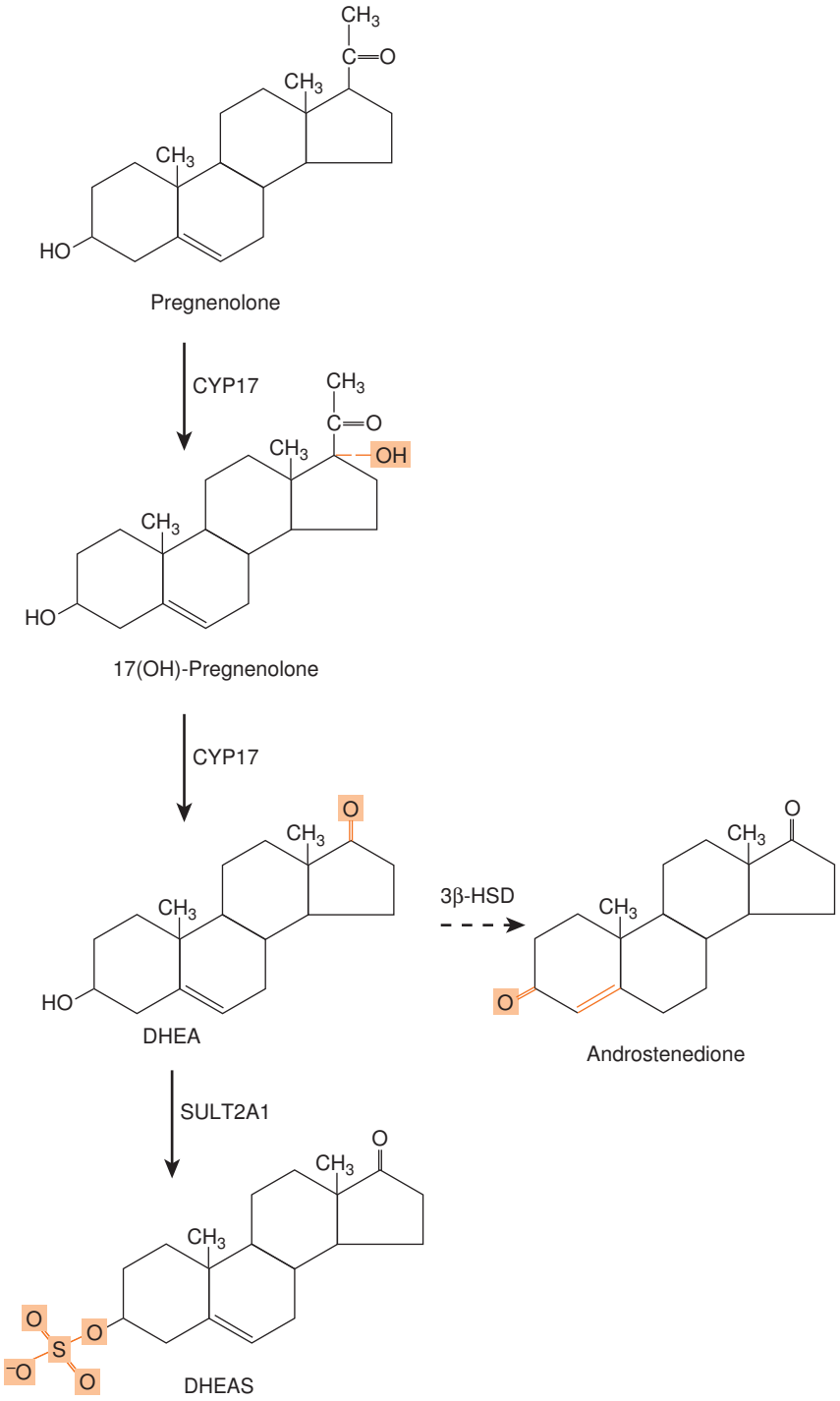


FIGURE 7-11 ■ Steroidogenic pathways in the zona reticularis. The first common reaction in the pathway, conversion of cholesterol to pregnenolone by CYP11A1, is not shown. The expression of 3β-hydroxysteroid dehydrogenase (3β-HSD) is relatively low in the zona reticularis, so androstenedione is a minor product compared with DHEA and DHEAS. The zona reticularis also makes a small amount of testosterone and estrogens (not shown).

masculinization of women can occur. This involves masculinization of external genitalia (e.g., enlarged clitoris) in utero and excessive facial and body hair (called **hirsutism**) and acne in adult women. Excessive adrenal androgens also appear to play a role in **ovarian dysovulation** (i.e., polycystic ovary syndrome). The ability of the breast to convert adrenal androgens into the active estrogen, estradiol, is a complicating factor in estrogen receptor–positive forms of breast cancer.

Apart from providing androgen precursors, it is not clear what other roles, if any, the zona reticularis plays in the adult human. DHEAS is the most abundant circulating hormone in young adults. DHEAS increases steadily until it peaks in the mid-20s and then steadily declines thereafter. Thus, there has been considerable interest in the possible role of DHEAS in the aging process. However, the function of this abundant steroid in young adults and the potential impact of its gradual disappearance on aging are still poorly understood. It should be noted that the age-related decline in DHEA and DHEAS has been associated with the popular use of these steroids as dietary supplements, even though there are few (if any) studies to support the efficacy or safety of this practice.

Regulation of Zona Reticularis Function **ACTH** promotes hormone production by the zona reticularis. Both DHEA and androstenedione display the same diurnal rhythm as cortisol (DHEAS does not because of its long circulating half-life). The zona reticularis shows the same atrophic changes as the zona fasciculata under conditions of little or no ACTH. However, other factors must regulate adrenal androgen function. Adrenarche occurs in the face of constant ACTH and cortisol levels, and the rise and decline of DHEAS is not associated with a similar pattern of ACTH or cortisol production. However, the other factors, whether extra-adrenal or intra-adrenal, remain unknown.

A crucial clinical aspect of the regulation of the zona reticularis is that, although ACTH stimulates production of adrenal androgens, neither adrenal androgens nor their more potent metabolites (e.g., testosterone, dihydrotestosterone, estradiol-17β) negatively feed back on ACTH or CRH (Fig. 7-12). This means that an enzymatic defect associated with the synthesis of cortisol (e.g., CYP21B deficiency) is associated with a dramatic increase in both ACTH (no negative feedback from cortisol) and adrenal androgens (because of the elevated ACTH). It is this "loophole" in the hypothalamus-pituitary-adrenal axis that gives rise to **congenital adrenal hyperplasia** (discussed further later).

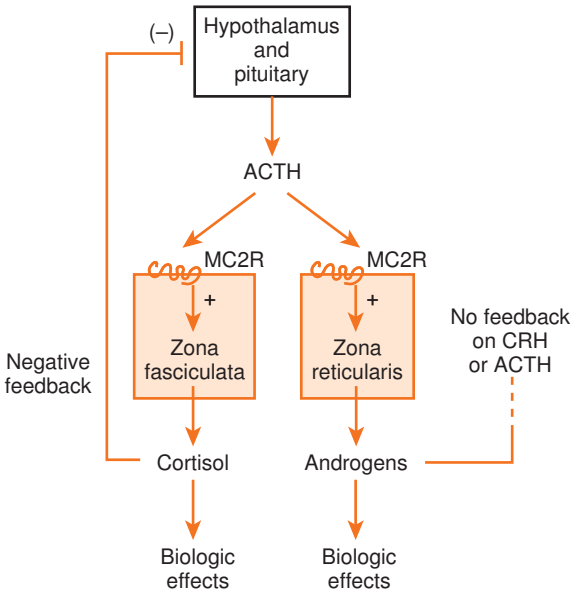


FIGURE 7-12 ■ The *loophole* in the hypothalamus-pituitary-adrenal axis. ACTH stimulates the production of both cortisol and adrenal androgens but only cortisol negatively feeds back on ACTH and CRH. Thus, if cortisol production is blocked (i.e., CYP11B1 deficiency), ACTH levels increase, along with adrenal androgens.

ZONA GLOMERULOSA

The thin, outermost zone is called the **zona glomerulosa**. This zone produces the **mineralocorticoid**, **aldosterone**, which regulates salt and volume homeostasis. The zona glomerulosa is only secondarily influenced by ACTH. Rather, it is regulated primarily by the **renin-angiotensin system**, extracellular K^+, and **atrial natriuretic peptide (ANP)**.

The Zona Glomerulosa Makes Aldosterone

An important feature in the steroidogenic capacity of the zona glomerulosa is that it **does not express CYP17**. Therefore, zona glomerulosa cells never make cortisol—nor do they make adrenal androgens in any form. Pregnenolone is converted to progesterone and

deoxycorticosterone (DOC) by 3β-HSD and CYP21, respectively (Fig. 7-13).

A completely unique feature of the zona glomerulosa among the steroidogenic glands is the expression of **CYP11B2**. The *CYP11B2* gene lies close to *CYP11B1* gene which encodes the enzyme that catalyzes the 11-hydroxylation of 11-deoxycortisol in the zona fasciculata to form cortisol (see Fig. 7-5) on the same chromosome in humans. However, CYP11B2 has a different promoter that is regulated by different signaling pathways. The enzyme itself, called **aldosterone synthase**, catalyzes the last **three reaction**s from **DOC** to **aldosterone** within the zona glomerulosa. These reactions are 11-hydroxylation of **DOC** to form **corticosterone**, **18-hydroxylation** to form **18-hydroxycorticosterone**, and 18-oxidation to form **aldosterone** (see Fig. 7-13).

Transport and Metabolism of Aldosterone Aldosterone binds to transport proteins (albumin, corticosteroid-binding protein) with low affinity and therefore has a short biologic half-life of about 20 minutes. Almost all of aldosterone is inactivated by the liver in one pass, conjugated to a glucuronide group, and excreted by the kidney.

Mechanism of Aldosterone Action Aldosterone acts much like cortisol (and other steroid hormones) in that its primary mechanism of action is through binding to a specific intracellular receptor, the **MR**. After dissociation of chaperone proteins, nuclear translocation, dimerization, and binding to **mineralocorticoid-response element**, the aldosterone-MR complex regulates the expression of specific genes.

Cortisol binds equally well to the MR and activates the same genes as does aldosterone. However, as discussed earlier, these cells also express **11β-HSD2**, which converts **cortisol** to the inactive steroid, **cortisone** (Fig. 7-14).

Cortisone can be converted back to cortisol by 11β-HSD1, which is expressed in several glucocorticoid-responsive tissues, including the liver and skin.

Physiologic Actions of Aldosterone*

Actions on Kidney The primary action of aldosterone is to increase the **reabsorption of Na^+**, followed by **H_2O**, by the distal nephron. About 95% of Na^+ reabsorption in the nephron occurs before the distal nephron, independently of aldosterone regulation. However, the amount of Na^+ reabsorbed by the distal nephron can be regulated by a few percentages to match changes in dietary Na^+ intake. Na^+ uptake at the distal nephron is accompanied by Cl^- and H_2O. As emphasized in the *Mosby Renal Physiology* monograph, "a 2% change in the fractional excretion of Na^+ would produce more than a 3 liter change in the volume of the extracellular fluid." Salt wasting and dehydration occur in patients with aldosterone insufficiency.

Aldosterone increases Na^+ reabsorption at the distal nephron (the latter portion of the distal convoluted tubule and the cortical collecting duct) primarily by increasing the expression of the α-subunit of the **epithelial Na^+ channel (ENaC)**. Aldosterone also increases the **stability of ENaC** in the apical (luminal) membrane (Fig. 7-15). This action of aldosterone is mediated by the aldosterone-inducible serine/threonine kinase, **SGK1**. *SGK1* gene expression is rapidly and profoundly increased by aldosterone. SGK1 prevents the ability of a protein, called Nedd 4-2, from targeting ENaC for degradation.

CLINICAL BOX 7-3

The importance of ENaC in the actions of aldosterone is made apparent by forms of **aldosterone resistance** (**type 1 pseudohypoaldosteronism**; **PHA1**). PHA1 is characterized by symptoms related to lack of aldosterone (salt wasting, dehydration, hyperkalemia, hypotension, with very high levels of renin, angiotensin, and aldosterone; see later). Some cases of PHA1 are due to inactivating mutations in one of the subunits of the ENaC. In the presence of these mutations, aldosterone cannot efficiently increase Na^+ reabsorption.

In contrast to PHA1, **Liddle syndrome** is characterized by hypertension, hypokalemia, and low renin and aldosterone levels. In these patients, ENaC subunits have mutations that prevent Nedd 4-2 from interacting with them and targeting them for degradation. Therefore, in Liddle syndrome, the ENaCs reside in the apical membrane much longer and transport more Na^+ independently of aldosterone.

Aldosterone also promotes Na^+ reabsorption by increasing the activity of the basolateral Na^+/K^+ ATPase in the distal nephron, although the hormone does not acutely increase gene expression of this transporter.

*For more information on this subject, see Koeppen BM, Stanton BA: *Renal Physiology,* 3rd ed., St. Louis, 2001, Mosby.

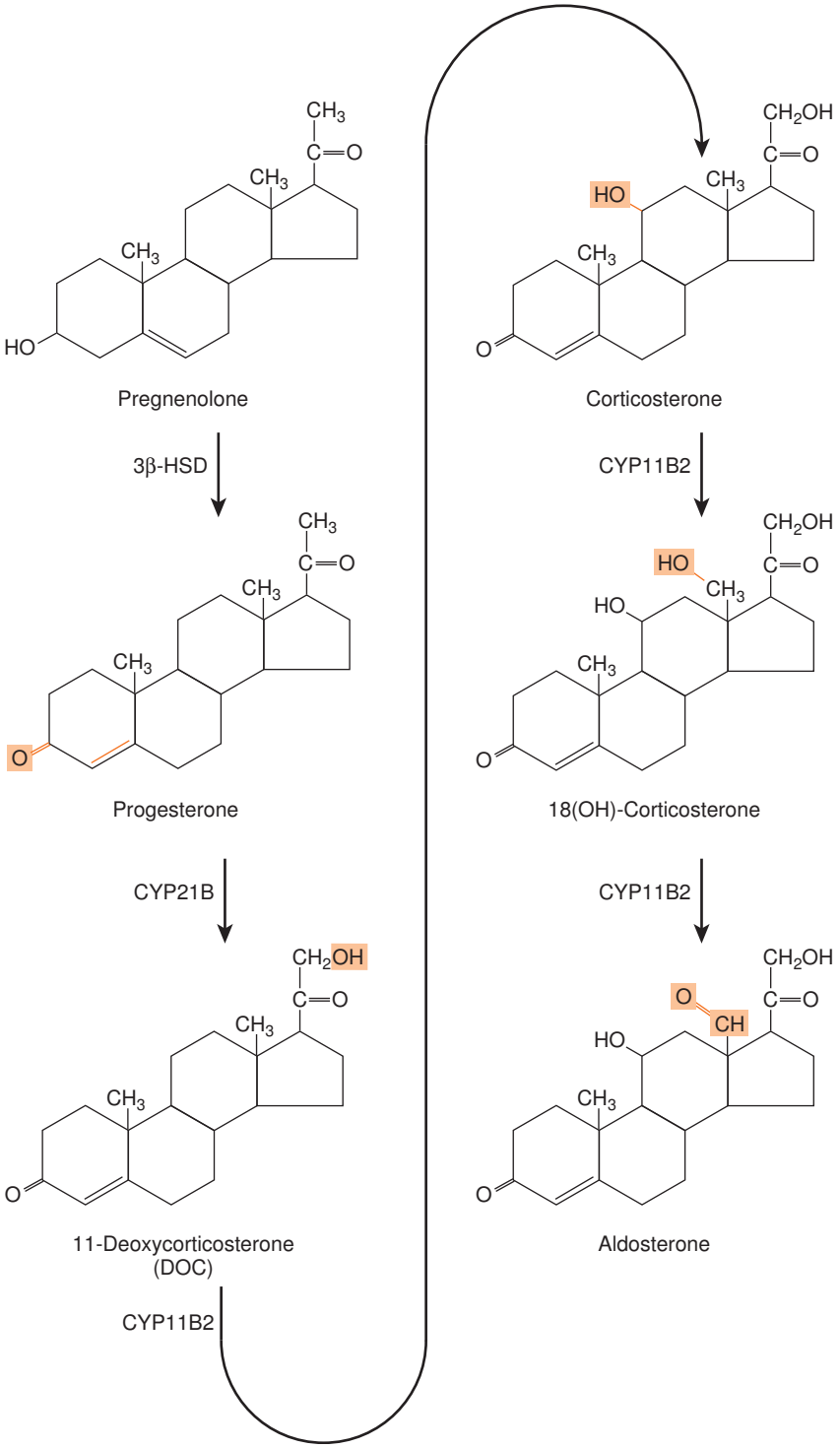


FIGURE 7-13 ■ Steroidogenic pathways in the zona glomerulosa. The first common reaction in the pathway, conversion of cholesterol to pregnenolone by CYP11A1, is not shown. Note that the last three reactions are catalyzed by CYP11B2.

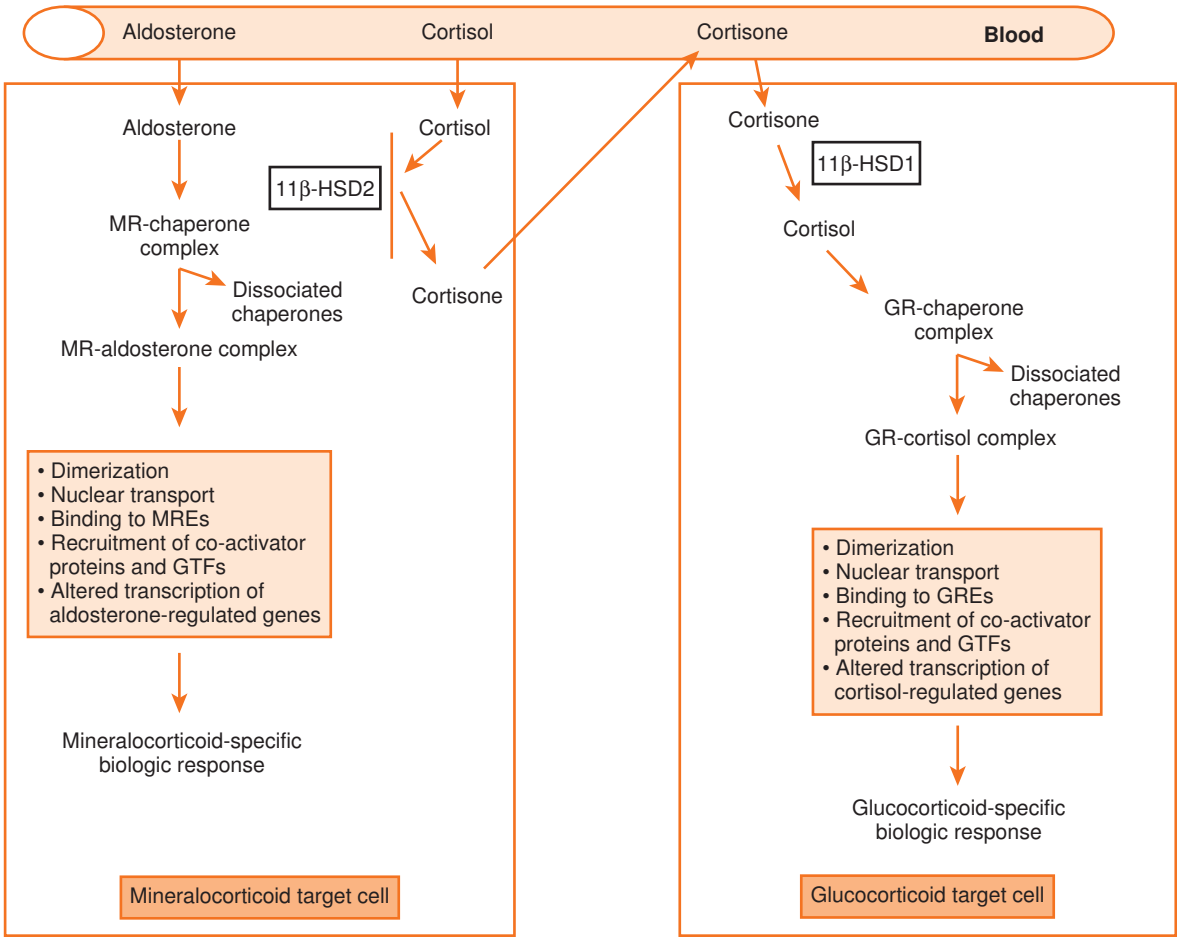


FIGURE 7-14 ■ The mineralocorticoid receptor (MR) is protected from activation by cortisol by the enzyme, 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2), which converts cortisol to inactive cortisone. Cortisone can be converted back to cortisol in glucocorticoid target cells by the enzyme, 11β-HSD1. Cortisol binds to the glucocorticoid receptor (GR) in cortisol target cell.

Aldosterone also stimulates **K^+** and **H^+ excretion.** Aldosterone increases gene expression of the **renal outer medullary K^+ (ROMK) channel** and the density of this channel in the apical membrane of the distal nephron (see Fig. 7-15). The excretion of K^+ is linked to the reabsorption of Na^+, in that the ENaC and the Na^+, K^+-ATPase establish the electrochemical conditions for apical secretion of K^+. In this sense, SGK1 indirectly promotes K^+ secretion. Additionally, SGK1 increases ROMK channel insertion into the apical membrane and increases its transporting activity. The importance of aldosterone on K^+ and H^+ homeostasis is emphasized by the findings that **hyperaldosteronism** leads to **hypokalemia** and **metabolic alkalosis.**

Continuous aldosterone administration results in aldosterone *escape* in 2 to 3 days. Initially, sodium retention and volume expansion are promoted, but the volume expansion does not continue indefinitely. As extracellular volume and therefore vascular volume increases, the glomerular filtration rate increases. This increases the rate of sodium delivery to the nephron and therefore the rate of renal sodium excretion, which limits the ability of aldosterone to continue expanding extracellular volume. The increase

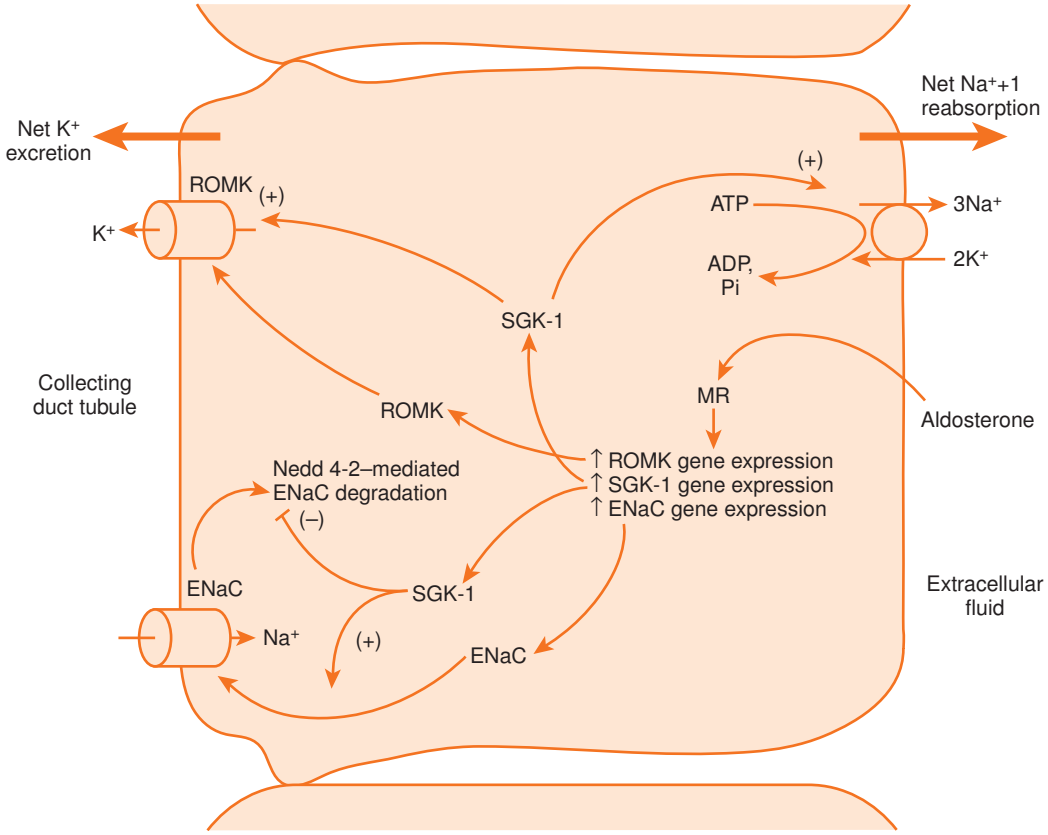


FIGURE 7-15 ■ Effects of aldosterone on collecting duct. Aldosterone increases gene expression of the kinase SGK1, the epithelial sodium channel ENaC, and the renal outer medullary potassium channel ROMK. SGK1 kinase activity reinforces aldosterone actions by increasing ENaC insertion into the membrane and inhibiting Nedd 4-2–dependent degradation of ENaC. SGK1 also increases the activity of ROMK and the basolateral sodium-potassium ATPase.

in vascular volume will stimulate the release of **ANP**, which promotes renal Na^+ excretion. However, escape from the effects of aldosterone on potassium and hydrogen ion excretion does not occur, and potassium depletion and metabolic alkalosis can persist.

Actions on Other Epithelia The colon is an important extrarenal site in terms of aldosterone regulation of salt and water homeostasis. As in the distal nephron, aldosterone increases sodium and water reabsorption and increases K^+ excretion in the colon. Aldosterone has similar effects on epithelia of **salivary glands**, **sweat glands**, and **gastric glands**.

Actions on Heart Muscle Clinical studies in humans have revealed a deleterious effect of aldosterone on cardiovascular function independent of its effects on renal sodium and water reabsorption. Aldosterone has a proinflammatory, profibrotic effect on the cardiovascular system and promotes left ventricular hypertrophy and remodeling. This effect of aldosterone is associated with increased morbidity and mortality in patients with essential hypertension.

Regulation of Aldosterone Secretion Given that Na^+ reabsorption and water uptake represent major actions of aldosterone, it would make sense that Na^+ levels and volume would feed back on aldosterone production (Box 7-4). This occurs through the **renin-angiotensin system (RAS)**. In the kidney, the vascular smooth muscle cells of the afferent arteriole adjacent to the glomerulus, called **juxtaglomerular (JG) cells**, are specialized to secrete a proteolytic enzyme called

BOX 7-4

REGULATION OF ALDOSTERONE PRODUCTION BY ZONA GLOMERULOSA CELLS

STIMULATORS

Angiotensin II
Extracellular K^+
Acute elevated adrenocorticotropic hormone (ACTH)

INHIBITORS

Atrial natriuretic peptide (ANP)
Chronic elevated ACTH

renin. JG cells release renin in response to a decrease in blood pressure in the afferent arteriole—as detected by **baroreceptors** in the wall of the afferent arteriole (Fig. 7-16). JG cells also release renin in response to decreased systemic blood pressure—as detected by baroreceptors. Decreased systemic blood pressure also leads to activation of sympathetic fibers that directly innervate JG cells through **β_1-adrenergic receptors**. In addition to stimulation by decreased blood pressure, decreased delivery of Na^+ to specialized cells of the ascending loop of Henle, collectively called the **macula densa**, causes these cells to signal to the JG cells to release renin.

Once secreted, renin acts on circulating **angiotensinogen** (renin substrate) to produce the decapeptide, **angiotensin I**. Angiotensin I is converted to **angiotensin II** (8 amino acids) by **angiotensin-converting enzyme (ACE)** in the lungs (see Fig. 7-16). Angiotensin II binds to the Gq-coupled **angiotensin I receptor** on zona glomerulosa cells and most vascular smooth muscle cells. **Angiotensin II** is a potent stimulus for aldosterone production. Angiotensin II increases **StAR** and **CYP11B2 (aldosterone synthase)** expression. As the name suggests, angiotensin II is also a potent vasoconstrictor and plays a direct role in compensation for vascular volume depletion.

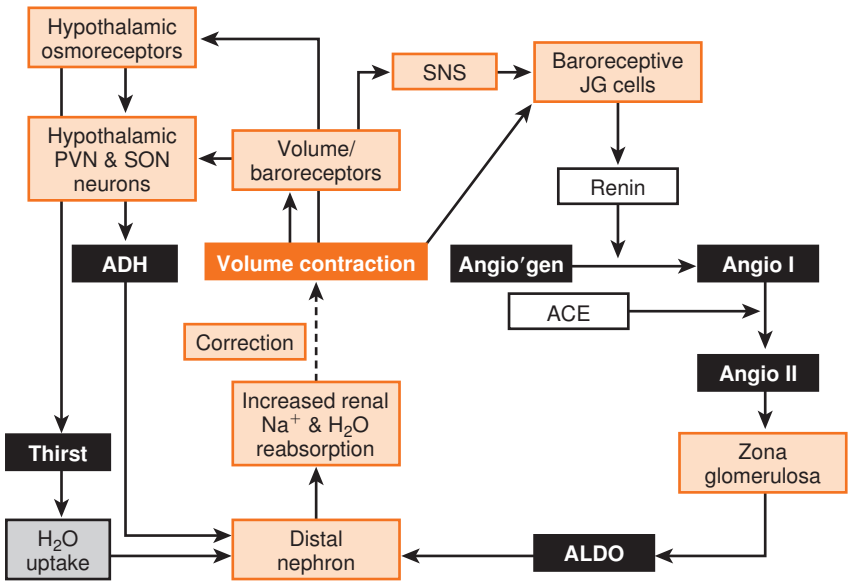


FIGURE 7-16 ■ Integrated response to volume contraction. Volume change and increased osmolarity will activate specific receptors that lead to the release of ADH and an increase in thirst. ADH promotes water reabsorption by the distal nephron (see Chapter 5). Volume contraction will also activate the juxtaglomerular cells directly through decreased blood pressure in the afferent arteriole of the glomerulus and through activation of the sympathetic nervous system (SNS). Renin converts angiotensinogen (Angio'gen) to angiotensin I (Angio I). Angiotensin I–converting enzyme (ACE) in the lung then generates angiotensin II (Angio II). Angiotensin II directly stimulates aldosterone (ALDO) production at the zona glomerulosa. Aldosterone acts on the distal nephron to increase Na^+ reabsorption, which results in an increase in water reabsorption due to osmotic drag.

Two other regulators of aldosterone production are **extracellular K^+** and **ANP**. Rising **serum K^+** levels depolarize the glomerulosa cell membrane, thereby stimulating voltage-sensitive calcium channels to open. The resultant calcium influx stimulates aldosterone production. In contrast to angiotensin II and extracellular K^+, **ANP** is a signal of too much aldosterone causing an expanded extracellular fluid volume and increased blood pressure. ANP is secreted by the heart and acts directly on zona glomerulosa cells to inhibit aldosterone production. Note that ANP also inhibits aldosterone indirectly by inhibiting renin release and plays an important role in the aldosterone escape response (see earlier).

PATHOLOGIC CONDITIONS INVOLVING THE ADRENAL CORTEX

Adrenocortical Insufficiency (Addison Disease)

Addison disease is a primary adrenal insufficiency in which the levels of both mineralocorticoids and glucocorticoids are usually extremely low. In North America and Europe, the most prevalent cause of Addison disease is autoimmune destruction of the adrenal cortex. Because of the cortisol deficiency, ACTH secretion increases. Elevated levels of ACTH can compete for the **MC1R** in melanocytes, causing an increase in **skin pigmentation**, particularly in skin creases, scars, and gums (Fig. 7-17; also see Chapter 5). The loss of the mineralocorticoids results in contraction of extracellular volume, producing **circulatory hypovolemia** and therefore a drop in blood pressure. Because the loss of cortisol decreases the **vasopressive response to catecholamines**, peripheral resistance drops, adding to the tendency toward hypotension. Hypotension predisposes people to **circulatory shock**. These people are also prone to have **hypoglycemia** when stressed or fasting. The hyperglycemic actions of other hormones, such as glucagon, epinephrine, and growth hormone, generally will prevent hypoglycemia at other times. Although volume depletion occurs because of the loss of mineralocorticoids, water intoxication can develop if a water load is given. The loss of cortisol impairs the ability to increase free-water clearance in response to a water load and hence rid the body of the excess water. Patients with Addison disease exhibit **hyperkalemic acidosis**. Because cortisol is important for muscle function, muscle weakness occurs in cortisol deficiency. The loss of cortisol results in anemia, decreased GI motility and secretion, and decreased iron and vitamin B_{12} absorption. The appetite decreases because of the cortisol deficiency, and this decreased appetite, coupled with the GI dysfunction, will predispose these persons to weight loss. These patients often show disturbances in mood and behavior and are more susceptible to depression (Box 7-5). Treatment involves replacement therapy with glucocorticoid and mineralocorticoid analogs.

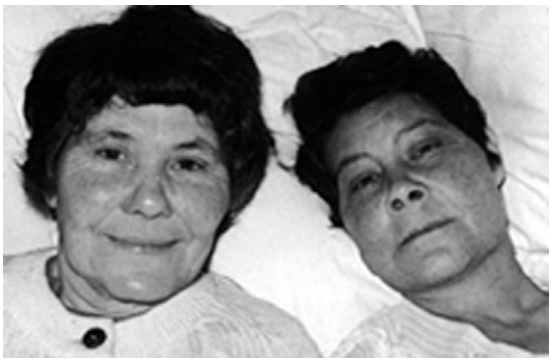


FIGURE 7-17 ■ Woman on the *right* has Addison disease. Note her increased pigmentation relative to her healthy twin sister on the *left*. *(From Hall R, Evered DC:* Color Atlas of Endocrinology, *2nd ed., London, 1990, Mosby-Wolfe.)*

Adrenocortical Excess

Cushing Syndrome **Hypercortisolism** is termed Cushing syndrome. Pharmacologic use of **exogenous corticosteroids** is now the most common cause of Cushing syndrome. The next most prevalent cause is **ACTH-secreting tumors** (pituitary or extra-pituitary). The form of Cushing syndrome caused by a functional pituitary adenoma is called **Cushing disease**. A fourth cause is **primary hypercortisolism** resulting from a functional adrenal tumor. If the disorder is primary or a result of corticosteroid treatment, ACTH secretion will be suppressed, and increased skin pigmentation will not occur. However, if the hypersecretion of the adrenal is a result of an ACTH-secreting nonpituitary tumor, ACTH levels sometimes become high enough to increase skin pigmentation even in the presence of hypercortisolism.

Increased cortisol secretion causes a tendency to gain weight, with a characteristic **abdominal** and

BOX 7-5

MANIFESTATIONS OF PRIMARY ADRENOCORTICAL INSUFFICIENCY

CORTISOL DEFICIENCY

Gastrointestinal disturbances
- Anorexia
- Nausea
- Vomiting
- Diarrhea
- Abdominal pain
- Weight loss

Mental disturbances
- Apathy
- Psychosis
- Confusion

Metabolic disturbances
- Hypoglycemia, especially under stress or fasting
- Impaired gluconeogenesis
- Increased insulin sensitivity

Cardiovascular/renal disorders
- Impaired free-water clearance
- Impaired pressor response to catecholamines
- Hypotension

Pituitary
- Increased adrenocorticotropic hormone secretion
- Hyperpigmentation

ALDOSTERONE DEFICIENCY
- Inability to conserve sodium
- Decreased extracellular fluid volume
- Decreased blood volume
- Weight loss
- Decreased cardiac output
- Increased renin production
- Hypotension
- Shock
- Impaired renal secretion of potassium and hydrogen
- Hyperkalemia
- Metabolic acidosis

TABLE 7-2

Clinical Manifestations of Hypercortisolism

SYMPTOM	METABOLIC RESULTS
Weight gain	Centripetal fat distribution, increased appetite
Protein wasting	Thin skin, abdominal striae
Capillary fragility (ecchymoses)	
Muscle wasting, muscle weakness	
Osteoporosis	
Poor wound healing	
Growth retardation	
Carbohydrate intolerance	Impaired glucose use, hyperglycemia
Insulin resistance	
Mineralocorticoid effects of cortisol	Hypertension, hypokalemia
Immunologic suppression	Increased susceptibility to infections
Other manifestations	Hirsutism, oligomenorrhea, polycythemia, personality changes

interscapular (buffalo hump) fat distribution (Table 7-2). The face appears round **(moon face)**, and the cheeks may be reddened **(plethora)**, in part because of the **polycythemia** and in part due to thinning of the skin (Fig. 7-18A). The limbs will be thin as a result of **skeletal muscle wasting** (from increased proteolysis), and **muscle weakness** will be evident (from muscle proteolysis and hypokalemia). Proximal muscle weakness is apparent, so the patient may have difficulty with stair climbing or rising from a sitting position. The abdominal fat accumulation, coupled with atrophy of the abdominal muscles and thinning of the skin, will produce a large, protruding abdomen. Purple abdominal **striae** are seen as a result of the damage to the skin by the prolonged proteolysis, increased intra-abdominal fat, and loss of abdominal muscle tone (see Fig. 7-18B).

Capillary fragility is seen as a result of damage to the connective tissue supporting the capillaries. Patients are likely to show signs of **osteoporosis** and **poor wound healing**. They have metabolic disturbances that include **glucose intolerance, hyperglycemia,** and **insulin resistance**. Prolonged hypercortisolism can lead to manifestations of **diabetes mellitus**. However, the lipolytic effect of cortisol by itself is so minor that if high insulin levels are present, lipogenesis, rather than lipolysis, predominates.

Insulin probably plays an important role in the increased adipose tissue mass typically seen with hypercortisolism. Cortisol interacts with insulin to promote the differentiation of preadipocytes into adipocytes. For reasons not fully understood, hypercortisolism is associated with a peculiar pattern of fat deposition, which is called centripetal fat distribution because the adipose tissue is concentrated in the trunk, whereas

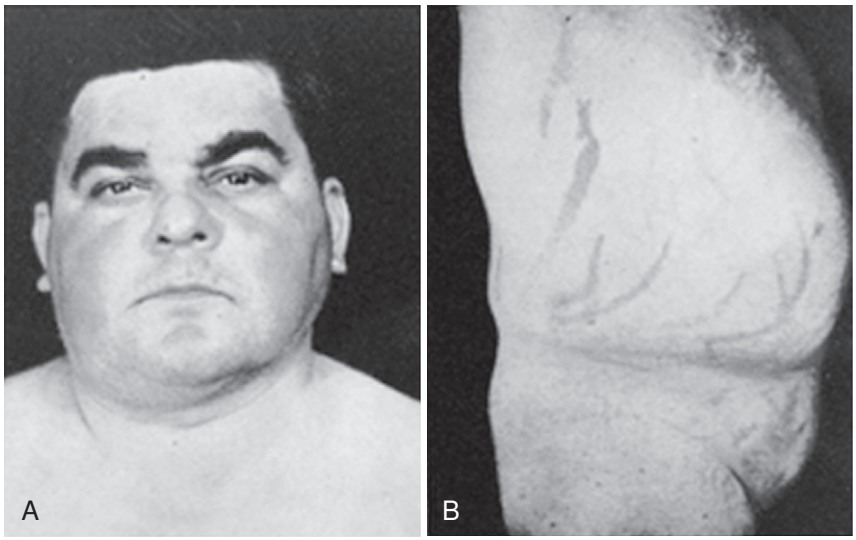


FIGURE 7-18 ■ **A,** Cushing syndrome with typical moon face and reddish cheeks. **B,** Truncal obesity and abdominal striae. *(From Wilson JD, Foster DW:* Williams' Textbook of Endocrinology, *8th ed., Philadelphia, 1992, Saunders.)*

wasting is seen in the arms and legs. Adipose tissue tends to accumulate in the abdomen. Visceral adipose tissue expresses a high level of 11β-HSD1, thereby efficiently converting cortisone to cortisol and increasing differentiation of preadipocytes to adipocytes. However, other mechanisms are likely to contribute. Also, hypercortisolism increases the size of interclavicular fat pads, producing the buffalo hump characteristic of this endocrine imbalance (see Table 7-2).

Because there are many hyperglycemic hormones, a cortisol deficiency is not likely to produce hypoglycemia unless fasting occurs or the person is stressed. However, cortisol is essential for proper mobilization of proteins for glucose production. Changes in the serum after cortisol administration include increased blood urea nitrogen; decreased serum alanine (because it is used in gluconeogenesis); the increased branched-chain amino acids leucine, isoleucine, and valine; and increased serum fatty acid levels. The change in branched-chain amino acid levels is indicative of decreased muscle protein synthesis and increased proteolysis, whereas the increase in fatty acids reflects adipose tissue lipolysis. Because of the suppression of the immune system caused by the glucocorticoids, patients are more susceptible to infection. Mineralocorticoid activities of the glucocorticoids and the possible elevation of aldosterone secretion produce salt retention and subsequent water retention, resulting in hypertension.

Because ACTH also regulates the zona reticularis, Cushing disease is associated with excessive **adrenal androgen secretion.** In women, this can produce **hirsutism**, **male pattern baldness**, and **clitoral enlargement** (**adrenogenital syndrome**).

Conn Syndrome **Primary hyperaldosteronism** is called **Conn syndrome**. It frequently occurs as a result of **aldosterone-secreting tumors**. Excessive mineralocorticoid secretion results in **potassium depletion, sodium retention, muscle weakness, hypertension, and hypokalemic alkalosis**. Although extracellular fluid volume increases, edema is not common because of hypervolemia-induced ANP release that results in natriuresis.

Congenital Adrenal Hyperplasia Any enzyme blockage that decreases cortisol synthesis will increase ACTH secretion and produce **adrenal hyperplasia**. The most common form of **congenital adrenal hyperplasia** occurs as a result of a deficiency of the enzyme **21-hydroxylase (CYP21)**. These individuals cannot produce normal quantities of cortisol, deoxycortisol, DOC, corticosterone, or aldosterone. Because of impaired cortisol production, ACTH will be elevated. High ACTH will drive adrenal androgen production by the zona reticularis. As shown in Figure 7-12, androgens do not feed back on ACTH or CRH and thus stay elevated. A female fetus will be **masculinized**. Conversely, conversion of adrenal androgens to estrogen can cause feminization (e.g., breast development, called **gynecomastia**) in males.

Because patients with Addison disease are unable to produce the mineralocorticoids, aldosterone, DOC,

and corticosterone, they have difficulty retaining salt and maintaining extracellular volume. Consequently, they are likely to be **hypotensive**. If the blockage is at the next step, 11β-hydroxylase (CYP11B1), DOC will be formed, and the levels of DOC will accumulate. Because DOC has significant mineralocorticoid activity and the levels become high, these individuals tend to retain salt and water at an excessive rate and become **hypertensive**. If there is a **deficiency of CYP17**, neither cortisol nor sex hormones are produced. The inability of the gonads to produce normal androgen levels during fetal development can result in a **female phenotype** for both males and females. A complete **deficiency of 3β-HSD2** is embryonic lethal. An incomplete deficiency results in the inability to produce adequate quantities of mineralocorticoids, glucocorticoids, androgens, and estrogens. The adrenal produces large quantities of the weak androgen DHEA, which can lead to masculinization of females because of the expression of 3β−HSD1 in some nonendocrine tissues.

SUMMARY

1. The adrenal gland is composed of a cortex, which is of mesodermal origin, and a medulla, which is of neuroectodermal origin. The cortex produces steroid hormones (cortisol, aldosterone, and inactive adrenal androgens), and the medulla produces catecholamines (epinephrine and, to a lesser extent, norepinephrine).
2. Regulated enzymes in medullary catecholamine synthesis are tyrosine hydroxylase and β-dopamine hydroxylase, which are induced by sympathetic stimulation, and phenylethanolamine *N*-methyltransferase, which is induced by cortisol.
3. Catecholamines increase serum glucose and fatty acid levels. They stimulate gluconeogenesis, glycogenolysis, and lipolysis. Catecholamines increase cardiac output and bronchiolar dilation but have selective effects on blood flow to different organs.
4. A pheochromocytoma is a tumor of chromaffin tissue from the adrenal medulla that produces excessive quantities of catecholamines. Symptoms of pheochromocytoma are often sporadic and include hypertension, headaches, sweating, anxiety, palpi-tations, chest pain, and orthostatic hypotension.
5. The adrenal cortex displays clear structural and functional zonation: the zona glomerulosa produces the mineralocorticoid, aldosterone; the zona fasciculata produces the glucocorticoid, cortisol; and the zona reticularis produces the androgen precursors, DHEAS, DHEA, and, to a much lesser extent, androstenedione.
6. Cortisol acts by binding to the glucocorticoid receptor. During stress, cortisol increases blood glucose by increasing gluconeogenic gene expression in the liver and breaking down muscle protein to supply gluconeogenic precursors. Cortisol also decreases glucose uptake by muscle and adipose tissue and has permissive actions on glucagon and catecholamines. Cortisol has multiple effects on other tissue. From a pharmacologic point of view, the immunosuppressive and anti-inflammatory effects are the most important.
7. Cortisol is regulated by the CRH-ACTH-cortisol axis. Cortisol negatively feeds back at the hypothalamus on CRH-producing neurons and on the pituitary corticotropes. CRH is regulated by several forms of stress, including proinflammatory cytokines, hypoglycemia, neurogenic stress, and hemorrhage, and by diurnal inputs.
8. Adrenal androgens—DHEA, DHEAS, and androstenedione—are androgen precursors. They can be converted to active androgens peripherally and provide about 50% of circulating androgens in women. In adult men, the role of adrenal androgens, if any, remain obscure. In women, adrenal androgens promote pubic and axillary hair growth and libido. Excessive adrenal androgens in women can lead to various degrees of virilization and ovarian dysfunction.
9. The zona glomerulosa of the adrenal cortex is the site of aldosterone production. Aldosterone is the strongest naturally occurring mineralocorticoid in humans. Aldosterone promotes Na^+ and water uptake by the distal nephron, while promoting renal K^+ and H^+ excretion. Aldosterone promotes Na^+ and water uptake in the colon and salivary glands. Aldosterone has a proinflammatory, profibrotic effect on the cardiovascular system and causes left ventricular hypertrophy and remodeling.
10. Major actions of angiotensin II on the adrenal cortex are increased growth and vascularity of the zona

glomerulosa, increased StAR and CYP11B2 enzyme activity, and increased aldosterone synthesis.

11. Major stimuli for aldosterone production are a rise in angiotensin II and a rise in serum potassium concentration. The major inhibitory signal is ANP.
12. Addison disease is adrenocortical insufficiency. Common symptoms include hypotension, hyperpigmentation, muscle weakness, anorexia, hypoglycemia, and hyperkalemic acidosis.
13. Cushing syndrome results from hypercortisolemia. If the basis of the disorder is increased pituitary adrenocorticotropin secretion, the disorder is called Cushing disease. Common symptoms of Cushing syndrome include centripetal fat distribution, muscle wasting, proximal muscle weakness, thin skin with abdominal striae, capillary fragility, insulin resistance, and polycythemia.
14. Congenital adrenal hyperplasia results from a congenital enzyme deficiency that blocks production of cortisol. The enzyme blockage results in elevated ACTH secretion, which stimulates adrenal cortical growth and secretion of precursors produced before the block. A 21-hydroxylase (CYP21B) deficiency is the most common form.

SELF-STUDY PROBLEMS

1. How does epinephrine influence metabolic pathways in the liver? Adipose tissue?
2. Explain how catecholamines can cause vasoconstriction in some blood vessels, while causing vasodilation in others.
3. Why does the adrenal cortex undergo atrophy in response to prolonged administration of synthetic glucocorticoids?
4. Why does masculinization of women (adrenogenital syndrome) often occur in patients with Cushing disease?
5. Explain the interaction of ENaC and SGK1 in the actions of aldosterone.
6. Explain the differences between the causes of orthostatic hypotension in patients with pheochromocytoma and those with Addison disease.

KEYWORDS AND CONCEPTS

- 3β-hydroxylase dehydrogenase (3β-HSD)
- 3β-hydroxysteroid dehydrogenase (3β-HSD)
- 11-deoxycortisol

For full list of keywords and concepts see Student Consult

SUGGESTED READINGS

Ellison DH, Brooks VL: Renal nerves, WNK4, glucocorticoids, and salt transport, *Cell Metab* 13:619–620, 2011.

Fourkiotis VG, Hanslik G, Hanusch F, et al: Aldosterone and the kidney, *Horm Metab Res* 44:194–201, 2012.

Heinlen JE, Buethe DD, Culkin DJ, et al: Multiple endocrine neoplasia 2a presenting with pheochromocytoma and pituitary macroadenoma, *ISRN Oncol* 2011:732452, 2011.

Lemke U, Krones-Herzig A, Berriel Diaz M, et al: The glucocorticoid receptor controls hepatic dyslipidemia through Hes1, *Cell Metab* 8:212–223, 2008.

Rauch A, Seitz S, Baschant U, et al: Glucocorticoids suppress bone formation by attenuating osteoblast differentiation via the monomeric glucocorticoid receptor, *Cell Metab* 11:517–531, 2010.

Shimizu N, Yoshikawa N, Ito N, et al: Crosstalk between glucocorticoid receptor and nutritional sensor mTOR in skeletal muscle, *Cell Metab* 13:170–182, 2011.

Simões E, Silva AC, Flynn JT: The renin-angiotensin-aldosterone system in 2011: Role in hypertension and chronic kidney disease, *Pediatr Nephrol* Sep 23. Epub ahead of print.

Takahashi H, Yoshika M, Komiyama Y, et al: The central mechanism underlying hypertension: A review of the roles of sodium ions, epithelial sodium channels, the renin-angiotensin-aldosterone system, oxidative stress and endogenous digitalis in the brain, *Hypertens Res* 34:1147–1160, 2011.

KEYWORDS AND CONCEPTS

- 11β-HSD1
- 11β-Hydroxylase (CYP11B1)
- 11β-Hydroxysteroid dehydrogenase type 2 (11β-HSD2)
- 17,20-Lyase function
- 17-Hydroxylase function
- 17-Hydroxyprogesterone
- 18-Hydroxycorticosterone
- 21-Hydroxylase (CYP21)
- α- and β-Adrenergic receptors
- Δ4 pathway
- Δ5 pathway
- Acetylcholine (ACh)
- Addison disease
- Adrenal androgens
- Adrenal cortex
- Adrenal glands
- Adrenal medulla
- Adrenarche
- Adrenocortical insufficiency
- Adrenocorticotropic hormone (ACTH)
- Adrenogenital syndrome
- Aldosterone
- Aldosterone escape
- Aldosterone synthase
- Aldosterone-inducible serine/threonine kinase, SGK-1
- Androstenedione
- Anemia
- Angiotensin I
- Angiotensin II
- Angiotensin-converting enzyme (ACE)
- Angiotensinogen (renin substrate)
- Anti-inflammatory and immunosuppressive actions
- Anti-inflammatory properties
- Aromatic amino acid decarboxylase
- Angiotensin II receptor
- Atrial natriuretic peptide (ANP)
- Bone resorption
- Buffalo hump
- Cardiac output
- Catechol-*O*-methyltransferase (COMT)
- Cholesterol ester hydroxylase
- Chromaffin cells
- Chromaffin granule
- Chromogranins
- Coactivator proteins
- Congenital adrenal hyperplasia
- Conn syndrome
- Corticosteroid-binding globulin (CBG; transcortin)
- Corticosterone
- Corticotropin-releasing hormone (CRH)
- Cortisol
- Cortisone
- CRH receptor
- Cushing disease
- Cushing syndrome
- CYP11A1
- CYP11B1
- CYP11B2
- CYP21
- Cytochrome P-450 mono-oxidase gene family (CYPs)
- Dehydroepiandrosterone sulfate (DHEAS)
- Deoxycorticosterone (DOC)
- Deoxycortisol
- DHEA-sulfotransferase (*SULT2A1* gene)
- Dihydroxyphenylalanine (DOPA)
- Dopamine
- Epinephrine
- Epithelial Na^+ channel (ENaC)
- Erythropoietin
- Extracellular K^+
- Fight-or-flight response
- Glucocorticoid
- Glucocorticoid receptor (GR)
- Glucocorticoid-response elements (GREs)
- Glucose intolerance
- GLUT4-mediated glucose uptake
- Glycolysis
- Hepatic gluconeogenic enzymes
- Hepatic glycogenolysis and gluconeogenesis
- Hepatic ketogenesis
- Hirsutism
- Hyperinsulinemia
- Hypokalemia and metabolic alkalosis
- Hypothalamus-pituitary-adrenal axis
- Immune homeostasis
- Immunosuppressants

- Increased appetite
- Inflammation and immune responses
- Juxtaglomerular cells
- LDL receptor
- Liddle syndrome
- Lipogenesis
- Lipolysis
- Macula densa
- Masculinization of women
- Melanocortin-2 receptor (MC2R)
- Metanephrine
- Mineralocorticoid
- Mineralocorticoid receptor (MR)
- Mineralocorticoid-response element
- Molecular chaperones
- Monoamine oxidase (MAO)
- Norepinephrine
- Phenylethanolamine-*N*-methyl transferase (PNMT)
- Pheochromocytoma
- Phospholipase A_2
- Preganglionic sympathetic neurons
- Pregnenolone (P5)
- Progesterone (P4)
- Proinflammatory, profibrotic effect (of aldosterone)
- Pro-opiomelanocortin (POMC) cells (corticotropes)
- Proteolysis
- Renal outer medullary K^+ (ROMK) channel
- Renin
- Renin-angiotensin system (RAS)
- Salt wasting and dehydration
- Scavenger receptor-BI (SR-BI; the HDL receptor)
- Side chain of cholesterol
- Steroidogenic acute regulatory protein (StAR protein)
- Sulfatases
- Suprarenal glands
- Truncal (abdominal, visceral) adiposity
- Type 1 pseudohypoaldosteronism; PHA1
- Tyrosine
- Tyrosine hydroxylase
- Vanillylmandelic acid (VMA)
- Vasopressin (ADH)
- Zona fasciculata
- Zona glomerulosa
- Zona reticularis

8 LIFE CYCLE OF THE MALE AND FEMALE REPRODUCTIVE SYSTEMS

OBJECTIVES

1. Present an overview of meiosis.
2. Describe the general anatomic components of the male and female reproductive systems.
3. Describe the development of the male and female reproductive systems in utero.
4. Describe the regulation of puberty.
5. Describe the changes in adolescent boys and girls that occur during puberty (Tanner stages).
6. Discuss the causes and physiologic changes that occur during the female menopause.
7. Discuss the decline of androgens in men (andropause).

Many of the functional deficiencies and clinical syndromes associated with reproduction are linked to the following in some way (as a cause or as a consequence):

- **Embryonic development** of the male and female systems
- Onset of reproductive maturity and activity at **puberty**
- Age-related changes that lead to a decline in reproductive function with aging (**menopause** and **andropause**)

Thus, before discussing the male and female reproductive systems in detail, it is useful to familiarize oneself with the general components of each system and their changes in development and function during life.

GENERAL COMPONENTS OF A REPRODUCTIVE SYSTEM

The two anatomic components of the reproductive system are the **gonads** and the **reproductive tracts**. The gonads (**testes** in men, **ovaries** in women) perform an endocrine function. The major hormonal product of the testes is **testosterone**. The major hormonal products of the ovaries are **estrogen** before ovulation and **progesterone plus estrogen** after ovulation. Like the thyroid and adrenal glands, adult gonadal endocrine function is regulated within a **hypothalamus-pituitary-gonadal axis**. The female reproductive axis is remarkable in that it involves both negative and positive feedback.

The **gonads** are distinct from other endocrine glands in that they also perform an exocrine (**gametogenic**) function. The testes produce **sperm** essentially in a continuous manner, whereas the ovaries produce **eggs** in a discontinuous manner at a rate of about one egg per month.

The **reproductive tracts** (also referred to as internal and external genitalia) serve to transport gametes (**sperm** and **eggs**) and, in women, allow for fertilization, implantation, gestation, and labor. Because clinically unassisted fertilization in humans involves internal insemination of the female tract by the male,

the male tract includes an intromittent organ, the **penis**, and the female tract includes the copulatory organ (and birth canal), the **vagina**. The external portions of the tracts, called the **external genitalia**, receive innervation that is associated with sexual excitement and orgasm, thereby reinforcing human sex drive. The **mammary glands** are an adjunct to the female reproductive system, providing nourishment and immune protection to the newborn and infant. Normal gametogenesis in the gonads, the development and physiology of the male and female reproductive tracts, and postpubertal development of the breasts are absolutely dependent on the endocrine function of the gonads.

OVERVIEW OF MEIOSIS

Sexual reproduction in humans requires the production of specialized **haploid cells** (called **gametes**) through the process of **meiosis** (Fig. 8-1). The male gametes are called **sperm** and are produced in the testes. The female gametes are called **eggs** (or ova) and are produced in the ovaries. **Sexual reproduction**

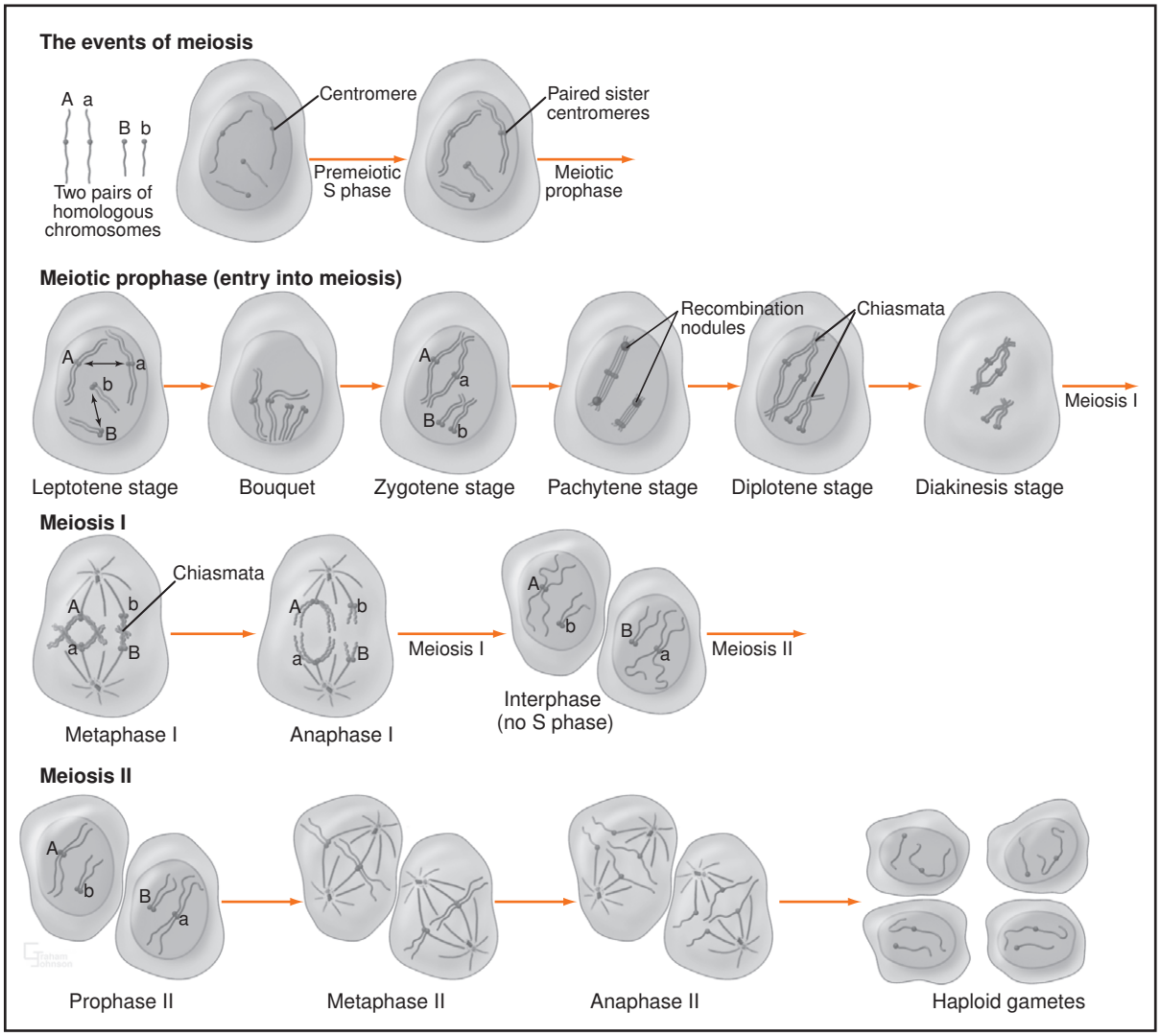


FIGURE 8-1 ■ The events of meiosis. *(Adapted from Pollard TD, Earnshaw WC:* Cell Biology, *2nd ed., Philadelphia, 2008, Saunders.)*

has the advantage of generating new genotypes within the progeny, thereby increasing the **genetic diversity** of a species. Genetic diversity in humans is advantageous primarily because it dilutes out the dosage and effects of deleterious mutations, although it also has adaptive value. The diversification of genetic material occurs during sexual reproduction at three levels:

1. In humans, the 23 chromosomes from the father are complemented by 23 homologous chromosomes from the mother after fertilization. This generates 46,XX or 46,XY individuals with DNA from two unrelated individuals (barring **consanguinity** or inbreeding).
2. Meiosis promotes the random recombination of chromosomes. During production of gametes, the 23 pairs of chromosomal homologs undergo **independent assortment** during meiosis so that the possible combinations of chromosomes in a haploid gamete equals 2^{23}, or 8,400,000 genetically distinct gametes.
3. Before entry into meiosis, chromosomes are replicated, generating **sister chromatids** that are closely adhered to each other along their length. During the first meiotic prophase, homologous chromosomes (now as sister chromatids) **pair**, forming a **bivalent**. Before or during pairing, double-stranded breaks are induced in a chromatid, followed by the complex process of **crossing-over** between homologous chromosomes. This process occurs at two or three sites in each chromosome and introduces **genetic recombination**, thereby further scrambling the DNA that will reside in a gamete.

As shown in Figure 8-1, meiosis consists of two phases. During the premeiotic prophase, chromosomes are replicated and consist of **sister chromatids** that are tightly adhered to each other at the centromeres and along their entire length. **Prophase I** involves the complex process of **crossing-over**, followed by **alignment** and **disjunction** (separation) of the homologous chromosomes. Because of crossing-over, points of chromosomal adhesion, called **chiasmata**, occur. Chiasmata resist separation of homologous chromosomes and thereby help to guide the alignment of chromosomes along the metaphase plate. Chiasmata are disassembled during **anaphase I,** allowing the disjunction (separation) of homologous chromosomes.

A **kinetochore** is a large protein complex associated with the **centromere** of a chromosome and connects to **microtubule spindles** that pull the chromatids to opposite poles. Importantly, in meiosis I, the kinetochores of the sister chromatids rotate and fuse to each other. This results in the movement of sister chromatids to the same pole at anaphase I. Consequently, homologous chromosomes are separated during meiosis I, but chromatids are not (see Fig. 8-1). After meiosis I, cells enter a brief interphase, but do not replicate DNA (i.e., no S phase). During this time, sister chromatids become separated, except at their centromeres. **Meiosis II** proceeds much more rapidly and involves the separation of chromatids. This involves the loss of cohesion between sister **centromeres** at **anaphase II** so that kinetochores can become attached to spindles on opposite poles. The final product is haploid gametes.

As discussed later, there are striking sex-specific differences in the details of meiotic induction and progression, as well as in gamete maturation, interaction with somatic nurse cells, and release.

CLINICAL BOX 8-1

Although meiosis and sexual reproduction confer concrete advantages to humans, correct **disjunction** or separation of chromosomes must occur at both phases of meiosis to maintain the integrity of the genotype (i.e., maintenance of **euploidy**). Failure of correct separation in either meiosis I or meiosis II is called **nondisjunction** and results in the addition of genetic material at one pole and the deletion of genetic material at the opposite pole. Nondisjunction causes **aneuploidy** (one or more chromosomes are not diploid) or **polyploidy** (all chromosomes are not diploid). It is likely that the resulting gametes, if they participate in fertilization and embryogenesis, will result in spontaneous abortion. The mistake of nondisjunction occurs with alarming frequency, in that 50% of conceptions are estimated to result in spontaneous abortion, of which more than 60% are caused by aneuploidy. Overall, spontaneous abortion acts as a safeguard against mistakes in chromosome number. In rare cases, aneuploid individuals survive. For example, gain of an extra chromosome 21 (**trisomy 21**) is compatible with life and results in **Down syndrome**. These individuals suffer mental retardation and a decreased life expectancy.

BASIC ANATOMY OF THE REPRODUCTIVE SYSTEMS

Overview of the Male Reproductive System

The **testes** are the male gonads. Each testis (right and left) resides outside of the abdominopelvic cavity within the scrotum (Fig. 8-2). The testis produces sperm within tubules (seminiferous tubules). The lumina of the tubules empty into the anastomosing network of tubules called the **rete testis**, which in turn empty into about 20 **efferent ductules**. Ultimately, efferent ductules converge into one lumen in the **epididymis**. The right and left **epididymides**, **vas deferens,** and **ejaculatory ducts** transport sperm from each testis to the midline **male urethra**.

The male tract also contains two large accessory sex glands, the **seminal vesicles** and the **prostate**. These glands produce most of the volume of the **semen**, which mixes with spermatozoa as they enter the urethra. Semen components largely provide a buffered, bacteriostatic, and nutrient-rich microenvironment for sperm after ejaculation into the vagina.

The male urethra becomes the distal portion of the male tract and consists of **prostatic** (intrapelvic), **membranous** (a short segment within the deep perineal space), and **penile** (extrapelvic in superficial perineal space) segments. The urethra receives lubricating and cleansing secretions from the paired bulbourethral glands and the paraurethral glands (glands of Littre). The penile segment courses through the length of the penis. The **penis** serves as an intromittent organ designed for internal insemination within the vagina. Sensory (pudendal) innervation of the penis leads to **orgasm** during coitus, thereby reinforcing libido.

The Female Reproductive System

The **ovaries** are the female gonads. Each ovary (right and left) resides within the pelvic cavity (Fig. 8-3). There are no tubules in the ovary. Instead, the gametes (primary oocytes) become invested with epithelial and stromal cells that make up an **ovarian follicle**. With growth, the follicle ultimately creates its own private fluid-filled lumen (called an **antrum**). At the time of gamete release, the gamete (now an egg arrested in meiotic metaphase II) and a thin covering of epithelial cells become free-floating within this lumen. Ovulation involves a complex set of events that essentially

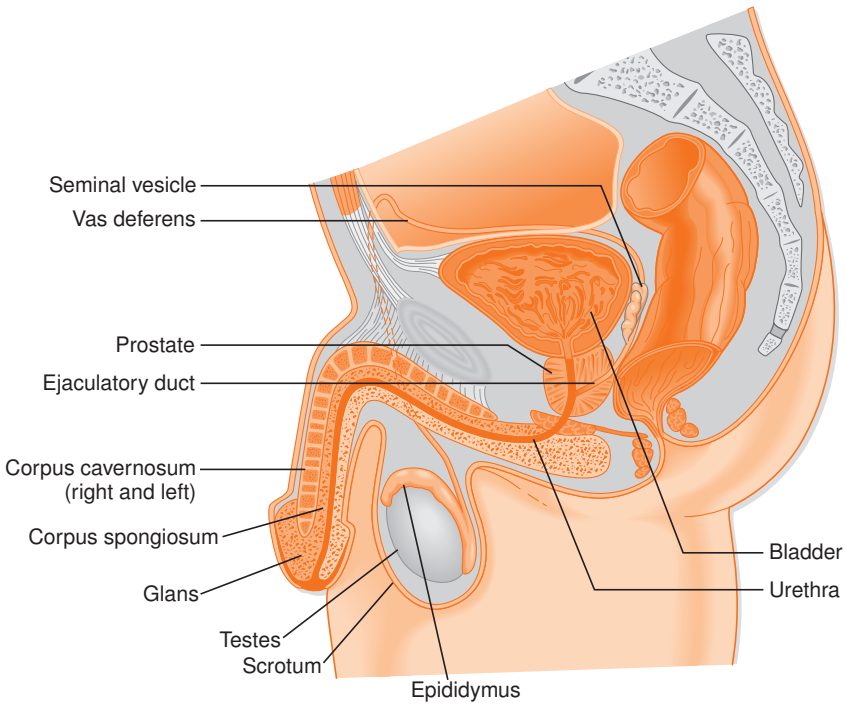


FIGURE 8-2 ■ The anatomy of the male reproductive tract. *(Adapted from Koeppen BM, Stanton BA:* Berne and Levy Physiology, *6th ed., updated edition, Philadelphia, 2010, Mosby.)*

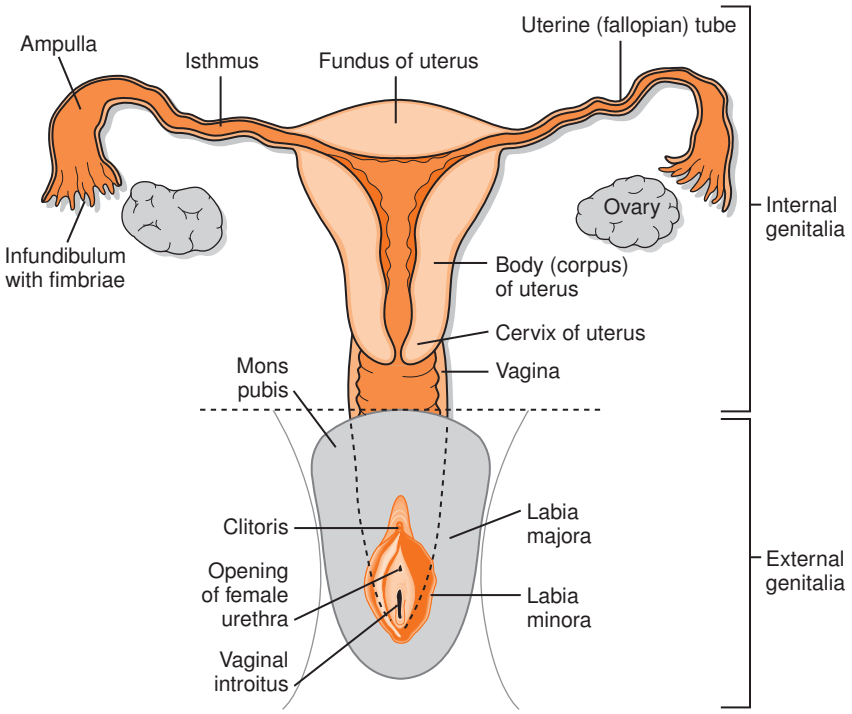


FIGURE 8-3 ■ The anatomy of the female reproductive tract. *(Adapted from Koeppen BM, Stanton BA:* Berne and Levy Physiology, *6th ed., updated edition, Philadelphia, 2010, Mosby.)*

erode the follicular and ovarian walls at the point where the follicle is pushing against the ovarian surface, followed by the release of the egg.

Unlike the male tract, the egg is released into the pelvic cavity and has to be captured by the proximal segment of the female tract called the **oviduct** (see Fig. 8-3). The oviduct has an opening at its proximal, free end (the infundibulum), through which the captured egg is transported. There is usually only one egg ovulated, either from the right or left side, depending on which side had the largest follicle at the beginning of the menstrual cycle. The oviduct transports the egg toward the midline **uterus** and allows for the movement of sperm from the uterus laterally toward the egg. Fertilization and early development (5 to 6 days) normally occur in the oviduct. The early embryo (blastocyst) eventually moves into the uterine lumen and implants into the uterine mucosa. The growing fetus is supported in part by the elastic and fibrous inferior end of the uterus called the **cervix**. At term, the newborn is expelled from the uterus through the cervix and vagina.

The **vagina** acts as both the copulatory organ and the birth canal. The female external genitalia surround the superficial opening of the vagina (called introitus). The **labia majora** are homologous to an unclosed scrotum. **Vestibular bulbs** (deep to the **labia minora**) and the **clitoris** represent structures homologous to the erectile tissue of the penis. However, unlike the penis, erection of these structures is not required for fertility. Sensory (pudendal) innervation of these structures and the vaginal wall may lead to **orgasm** during coitus, thereby providing reinforcement to libido.

SEXUAL DEVELOPMENT IN UTERO

The **genetic sex** of a fetus depends on the nature of the sex chromosomes contributed by the egg and the sperm. Normally, there are 46 chromosomes, consisting of 22 pairs of autosomes and one pair of sex chromosomes. The sex chromosomes are called X and Y chromosomes; **46,XX** is the normal karyotype for the female, and **46,XY** is the normal karyotype for the male. **Genetic sex determines gonadal sex**. The gonads then either produce **hormones** (if male) or **no hormones** (if female). The hormonal environment

determines the sex of the reproductive tract and external genitalia.

Male Development

During the first 6 weeks of development, mesodermal cells within the genital ridge develop into a **bipotential gonadal primordium** (Table 8-1). During this period, **primordial germ cells** migrate from the yolk sac endoderm into the gonadal tissue. By 6 weeks of gestation, the gonads contain germ cells, supporting cells, and stromal cells that will become androgen-producing steroidogenic cells in both sexes.

TABLE 8-1

Time Frame for Fetal Development of Male Reproductive System

GESTATIONAL AGE (WK)	DEVELOPMENTAL FEATURE
6-8	Differentiation of testes
8	Retention of wolffian ducts
	Regression of müllerian ducts
9-12	Development of male-type external genitalia

The short arm of the Y chromosome contains the ***SRY* gene (*s*ex-determining *r*egion on the *Y* chromosome)** that encodes the transcription factor, **SRY**. SRY, along with other transcription factors, plays a major role in the induction of differentiation of the bipotential gonad into a **testis** between weeks 6 and 7 of gestation (Fig. 8-4). With testicular development, the supportive epithelial cells differentiate into **Sertoli cells** and form the **seminiferous tubules.** Sertoli cells perform two critical functions in early embryogenesis: (1) they surround male germ cells (called **spermatogonia**) and produce an enzyme (CYP26B1; also produced by Leydig cells) that degrades locally produced **retinoic acid**, thereby preventing meiotic progression of spermatogonia; and (2) they express **antimüllerian hormone** (**AMH**, also referred to as **müllerian-inhibitory substance**), which causes the degeneration of the müllerian ducts (see later).

Hormones mediate **phenotypic gender** expression. The fetus originally develops with bipotential internal and external genitalia (Figs. 8-5 and 8-6). Internally, there are two **wolffian** (also called **mesonephric**)

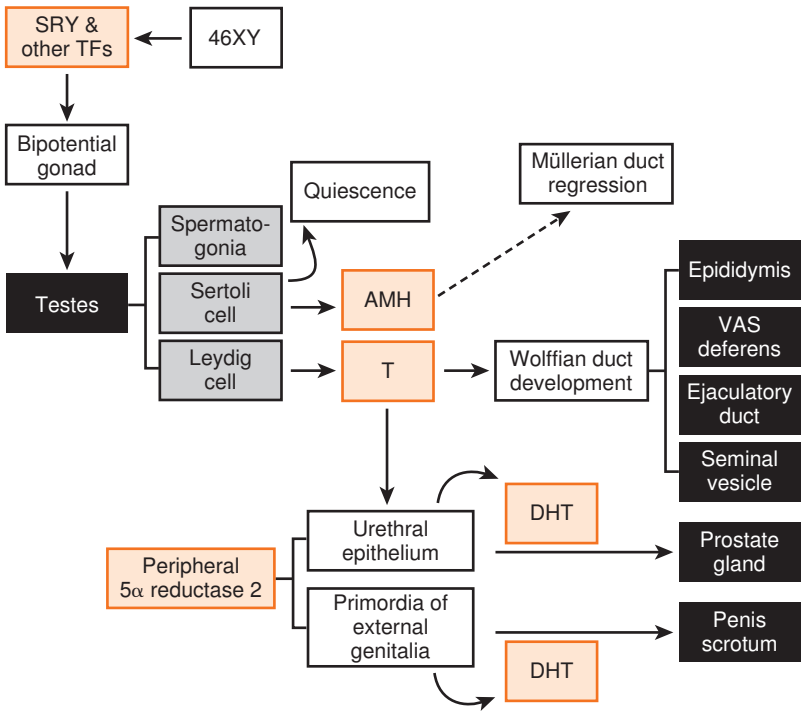


FIGURE 8-4 ■ Embryonic development of the male reproductive system. AMH, antimüllerian hormone; DHT, dihydrotestosterone; T, testosterone; TFs, transcription factors.

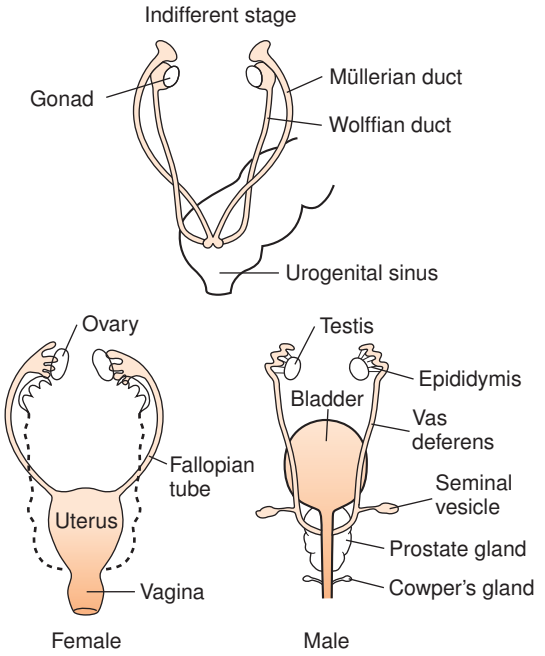


FIGURE 8-5 ■ Differentiation of internal genitalia and primordial ducts. *(Redrawn from George FW, Wilson JD: Embryology of the genital tract. In Walsh PC, Retik AB, Stamey TA, Vaughan ED, editors:* Campbell's Urology, *6th ed., Philadelphia, 1992, Saunders.)*

ducts, which have the potential for differentiating into the nonurethral segment of the male tract (epididymis, vas deferens, ejaculatory duct, and seminal vesicles), and two **müllerian** (also called **paramesonephric**) **ducts**, which have the potential for differentiating into most of the female reproductive tract (the oviducts, uterus, cervix, and proximal third of the vagina). Whether male or female reproductive tracts develop depends on the presence or absence of two hormones produced by the fetal testis-**testosterone** and **AMH** (Box 8-1).

The persistence and differentiation of the **wolffian ducts** into the proximal nonurethral male tract requires testosterone (see Fig. 8-4). By week 8, testosterone is produced by stromal cells that have differentiated into **Leydig cells**. Androgen production is first promoted by the placental, luteinizing hormone (LH)-like hormone, called **human chorionic gonadotropin (hCG)**, and later by fetal pituitary **LH**. At this time, testosterone acts more like a paracrine factor than a true hormone, in that testosterone from each testis promotes the differentiation of the ipsilateral tract (Fig. 8-7).

The degeneration of the müllerian ducts requires production of **AMH** by the Sertoli cells of the testis (see Fig. 8-4). AMH is a glycoprotein homodimer belonging to the transforming growth factor-β (TGF-β) gene family. Accordingly, AMH binds to a co-receptor with serine-threonine kinase activity. AMH binds specifically to the **AMH receptor type II (AMHR-II)**, which binds to and activates a type-I receptor. As described in Chapter 1, this activates a Smad-dependent signaling pathway.

CLINICAL BOX 8-2

Persistent müllerian duct syndrome (PMDS) occurs in 46,XY males. The persistence of müllerian derivatives (oviducts, uterus, cervix, vaginal tissue) frequently disrupts the descent of one or both testes, resulting in unilateral or bilateral **cryptorchidism**. Correction of cryptorchidism is performed by surgery. Mutations in both the *AMH* gene and AMH receptor II (AMHR-II) have been identified in families with PMDS. Most cases involve homozygous mutations that occur with a high frequency in certain Mediterranean and North African populations that have a high incidence of consanguinity.

The differentiation of the **prostate gland** and **male external genitalia** occurs between week 9 and week 11 (Box 8-2; see Figs. 8-5 and 8-6). This requires the expression of an enzyme, **5α-reductase-2**, within the primordia of these structures (urogenital sinus, genital tubercle urethral folds, and labioscrotal swellings). The 5α-reductase-2 catalyzes the peripheral conversion of testosterone to **dihydrotestosterone (DHT)**. Testosterone and DHT bind to the same androgen receptor, but DHT is required in these tissues. The cell-specific actions of testosterone and DHT are not completely understood but may depend on cell-specific expression of co-regulatory proteins, the chromatin context of cell-specific androgen-regulated genes, or cell-specific differences in the stability of the hormone-receptor complex. In any case, conversion of testosterone to DHT is absolutely required for the normal development of the prostate and external genitalia.

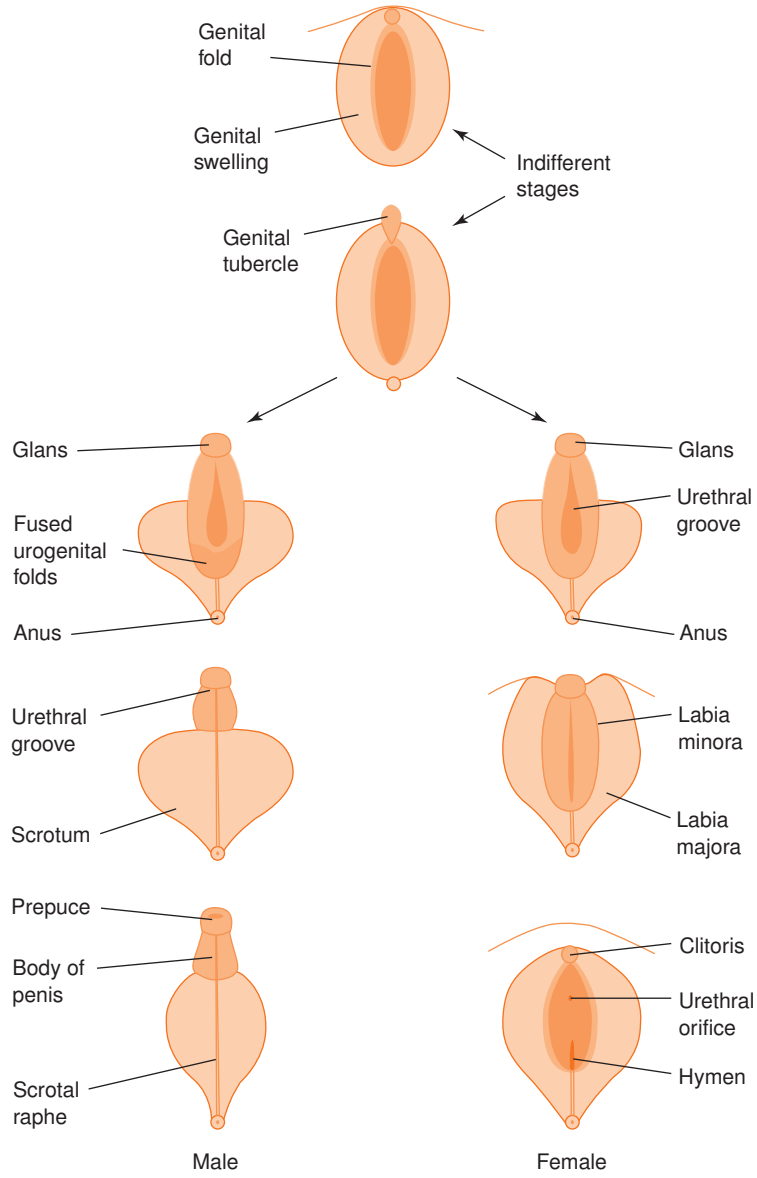


FIGURE 8-6 ■ Regulation of development of male and female external genitalia. In the presence of dihydrotestosterone (DHT) between 9 and 12 weeks of gestation, male external genitalia develop from the genital tubercle, genital fold, genital swelling, and urogenital sinus. In the absence of DHT, female external genitalia develop.

BOX 8-1

REGULATION OF DEVELOPMENT OF INTERNAL GENITALIA

- The wolffian ducts, when stimulated with testosterone, become the epididymis, vas deferens, seminal vesicles, and ejaculatory ducts.
- The müllerian ducts, in the absence of Sertoli cell müllerian-inhibiting substance, become the fallopian tubes, uterus, cervix, and upper one third of the vagina.

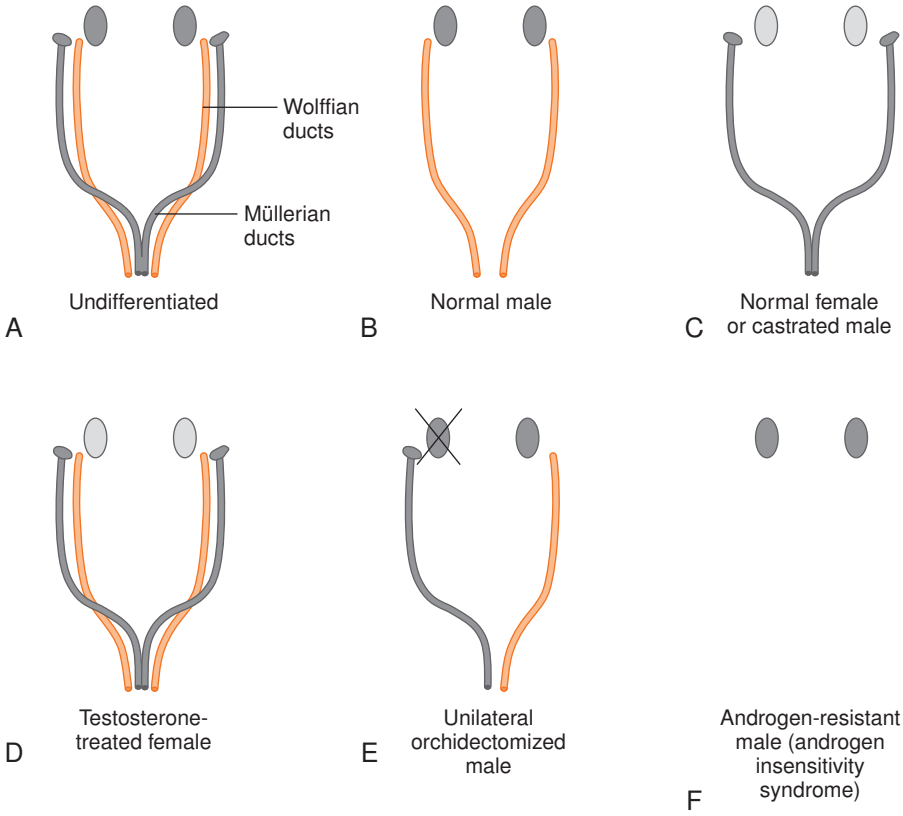


FIGURE 8-7 ■ Regulation of development of internal genitalia. **A,** Both wolffian and müllerian ducts are originally present in both male and female fetuses (undifferentiated). **B,** If functional testes are present, wolffian ducts develop, and müllerian ducts regress. **C,** If no testes are present, müllerian ducts develop, and wolffian ducts are lost. **D,** If a female fetus is exposed to testosterone, both ductile systems can remain. **E,** If a testis is removed unilaterally (orchidectomized), the müllerian duct will develop, and the wolffian duct will regress on one side. **F,** A male with functional testes but androgen insensitivity will show regression of both ductile systems.

CLINICAL BOX 8-3

A **5α-reductase-2 deficiency** results in decreased DHT formation. Affected persons typically have a well-developed internal male tract (except for the prostate gland), but incompletely masculinized external genitalia (i.e., **ambiguous genitalia**) with microphallus and hypospadias. The testes are either inguinal or labial. Severely affected patients can be mistaken for females at birth. The testosterone production is normal, and at puberty, when testosterone production greatly increases, some masculinization of the external genitalia may occur. Also, **5α-reductase-1** activity in the skin appears at puberty, which may generate enough DHT to induce the pubertal changes. These include increased muscle mass, deepening of the voice, and descent of the testis into the labioscrotal folds. Patients who are diagnosed in infancy are assigned a male sex and treated with androgen therapy to induce growth of the penis and surgical repair of hypospadias. Patients who are diagnosed after puberty may develop a male or female sexual identity and may require some form of corrective surgery. It is not uncommon for patients to switch from a female sexual identity to a male one after puberty.

BOX 8-2
REGULATION OF DEVELOPMENT OF EXTERNAL GENITALIA

- *In the presence of dihydrotestosterone*, the genital tubercle, genital fold, genital swelling, and urogenital sinus become the penis, scrotum, and prostate.
- *In the absence of dihydrotestosterone*, the genital tubercle, genital fold, genital swelling, and urogenital sinus become the labia majora, labia minora, clitoris, and lower two thirds of the vagina.

Female Development

In a normal 46,XX embryo, the absence of SRY allows for the expression of other transcription factors that induce the differentiation of the gonad into an ovary (Fig. 8-8). However, full ovarian differentiation does not occur until the end of the first trimester of

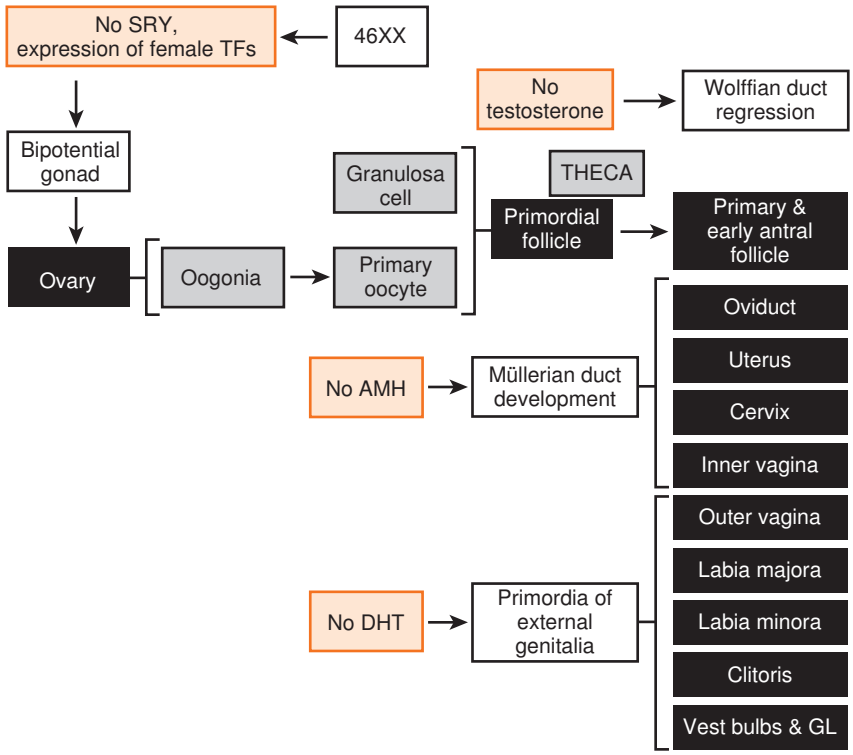


FIGURE 8-8 ■ Embryonic development of the female reproductive system. AMH, antimüllerian hormone; DHT, dihydrotestosterone; TFs, transcription factors; Vest bulbs & GL, vestibular bulbs and gland.

BOX 8-3

TIME FRAME FOR FETAL DEVELOPMENT OF THE FEMALE REPRODUCTIVE SYSTEM

7-12 WEEKS
- Meiosis of oogonia and arrest at first meiotic prophase
- Ovarian organogenesis
- Formation of primordial follicles

11-12 WEEKS
- Oviducts, uterus, cervix, external genitalia, and vagina

20-25 WEEKS
- Primary follicles in ovary

pregnancy (Box 8-3). The germ cells differentiate into **oogonia** and divide mitotically. Starting at about 11 weeks of gestation, all oogonia enter meiosis and become primary oocytes arrested in first meiotic prophase. Oocytes become surrounded by a single layer of supportive cells called **granulosa cells** and their basal lamina. These structures are called **primordial follicles** and appear in the second trimester. Although some follicular development (see Chapter 10) occurs during pregnancy, the ovary remains hormonally quiescent during gestation.

The absence of Sertoli cell–derived AMH allows for the persistence and development of the müllerian duct into oviducts, uterus, cervix, and the inner one third of the vagina (see Figs. 8-5 and 8-8; see Box 8-1). In the absence of a testis and testosterone, the wolffian duct degenerates. If high testosterone levels are present in a female fetus because of a **congenital adrenal hyperplasia** (see Chapter 7), or because of a maternal endocrine disorder, both sets of ducts can be retained (see Fig. 8-7).

In the absence of androgen exposure, the external genitalia develop into the labia majora, labia minora, clitoris, vestibular bulbs and glands, and outer two thirds of the vagina (see Figs. 8-6 and 8-8; see Box 8-2).

CLINICAL BOX 8-4

Kallmann syndrome type 1 is a **tertiary** (i.e., hypothalamic) endocrine disorder and a form of isolated **hypogonatropic hypogonadism**. This genetic disorder is often associated with anosmia (absence of sense of smell) or hyposmia (poor sense of smell). It is due to loss of a functional protein, called *anosmin,* that is encoded by the X-linked *KAL1* gene. The syndrome is caused by the inability of all or some of the **GnRH neurons** to properly migrate to the mediobasal hypothalamus from the nasal placode (see Chapter 5). Anosmin is similar to adhesion molecules, and so may play a guiding role in GnRH migration. Males are more frequently affected than females. Men affected with this disorder have undescended testes **(cryptorchism)**. Although there is normal embryonic differentiation of the wolffian duct–derived structures, penis development is deficient, and microphallus often develops. These effects probably result from the fact that early fetal development of the internal genitalia is controlled by testicular androgens that are initially regulated by **placental hCG** (see Chapter 11), rather than fetal LH. Adult growth and function of wolffian duct–derived structures and prostate gland are impaired. The inability of the fetus to secrete normal quantities of LH has an impact on testicular function later in development, when androgens regulate growth of the external genitalia. The severity of the impairment of LH secretion is variable, as is the severity of the reproductive problems associated with the disorder. Puberty is delayed.

PUBERTY

Regulation of Timing of Puberty

The reproductive axis is driven by **gonadotropin-releasing hormone (GnRH) neurons** in the mediobasal hypothalamus that secrete GnRH in a pulsatile pattern. GnRH, in turn, stimulates production of pituitary LH and follicle-stimulating hormone (FSH) (see Chapter 5). The GnRH neurons display activity during gestation, which decreases at parturition. GnRH neurons display a significant increase in activity in the first 2 years of infancy ("mini-puberty of infancy"), followed by about a decade of very low activity of the reproductive axis (Fig. 8-9). The resurgence in activity of the reproductive system during adolescence is called **puberty** and induces dramatic phenotypic and behavioral changes. Unfortunately, physical changes precede maturation of the personality and emotional stability.

The timing of puberty is variable among countries and among ethnic populations within countries. In the United States, the average age of puberty in girls has declined over the past half-century, probably owing to enhanced health and nutrition, and more recently possibly because of increased rates of childhood obesity (although this has not been firmly established). Black girls typically reach pubertal milestones about 6 months earlier than white girls. The clinical significance of the timing of puberty relates to

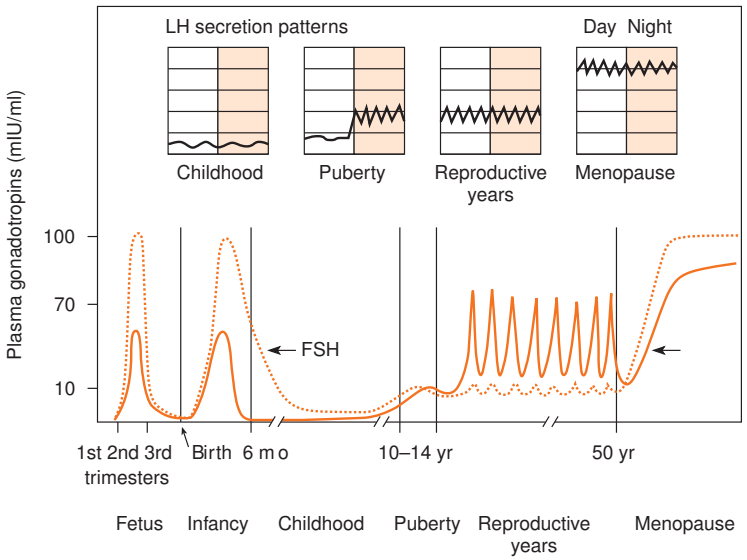


FIGURE 8-9 ■ Relative serum luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels across the life span in women. *(Redrawn from Braunwald E, et al:* Harrison's Principles of Internal Medicine, *4th ed., New York, 1987, McGraw-Hill.)*

underlying disorders or disease that induces the onset of puberty at an abnormally young age (called **precocious puberty**) or at an abnormally older age **(delayed puberty)** or not at all. The average age of breast development (first sign of puberty) in North American girls is about 10 years old. The average age of testicular enlargement (first sign of puberty) in North American boys is about 11.5 years old. The threshold for earliest onset of normal versus precocious puberty is 8 years in girls and 9 years in boys. These ages may be too high as the age of puberty declines.

After birth, the reproductive axes of both sexes show significant activity for 1 to 2 years (see Fig. 8-9). This is followed by the development of mechanisms that imposes a significant (but not absolute) suppression of GnRH neuronal activity. The exact nature of the inhibitory signals that result in the "nadir of childhood" reproductive activity remains poorly understood. The fact that some tumors that cause swelling or tissue destruction in the vicinity of the posterior hypothalamus can induce **gonadotropin-dependent precocious puberty** has led some to propose the existence of an inhibitory center within the central nervous system (CNS). There is also some evidence for an exquisite sensitivity of the hypothalamus and pituitary to negative feedback by steroid hormones (i.e., a low *set-point*, or gonadostat). This is indicated by the findings that primary agonadal children typically have higher gonadotropin levels than normal children (although gonadotropin levels are low in both cases because of CNS inhibition). At puberty, these inhibitory mechanisms are presumably terminated.

In addition to the release from CNS inhibition, there is also strong evidence for the activation of stimulatory centers in the CNS that induce pulsatile GnRH production at puberty. Recently, much attention has been given to the role of the neurotransmitter **kisspeptin (Kiss1)** as an inducer of GnRH pulsatile release at puberty. Kisspeptin is encoded by the ***KISS1* gene.** Kiss1 is a 54-amino acid peptide and is expressed by neurons in the hypothalamus. Kiss1 acts through a G-protein-coupled receptor called **Kiss1R** (previously called GPR54), which is expressed by GnRH neurons. Kiss1 levels increase in the hypothalamus at puberty, and intracerebral infusion of Kiss1 in female monkeys induced precocious puberty. Null mutations in Kiss1R have been described in individuals with compromised pubertal changes, whereas gain-of-function mutations in human Kiss1 and Kiss1R cause gonadotropin-dependent precocious puberty.

During the **peripubertal period**, pulsatile sleep-associated surges in GnRH begin to occur (see Fig. 8-9). Gonadotrope sensitivity to GnRH increases, resulting in an increase in LH secretion. At puberty, the frequency and amplitude of the GnRH pulses increase. This change increases LH and FSH secretion throughout the day and ultimately gonadal steroid hormone production. The maturation of the positive feedback by estrogen on GnRH and gonadotropin secretion in women occurs late in puberty (see Fig. 8-9; spikes during reproductive years). The first menstrual period **(menarche)** occurs 2.6 years after the onset of puberty.

Physiologic Changes Associated with Puberty

The developmental milestones associated with puberty in British boys and girls were objectively described by Marshall and Tanner and are referred to as **Tanner stages**. The progression of secondary sex characteristics was characterized as five stages, ending in adult as stage 5. For boys, these changes include testicular size, penis size (Table 8-2), and pubic hair.

TABLE 8-2

Tanner Pubertal Stages in Male

STAGE	GENITALS	PUBIC HAIR
1	Preadolescent	Preadolescent; no pubic hair
2	Scrotum and testes enlarge; change in scrotal skin texture	Sparse, long, downy pubic hair, chiefly at base of penis
3	Growth of penis in length and further growth of testes and scrotum	Hair darker and coarser
4	Growth of penis in length and breadth, darkening of scrotal skin	Adult-type hair, but area covered is less than that in adult
5	Adult-sized genitalia	Adult hair texture and quantity; hair is distributed in diamond-shaped escutcheon with hair extending up linea alba

Data from Marshall WA, Tanner JM: Variations in the pattern of pubertal changes in boys. *Arch Dis Child* 45:13, 1970.

For girls, these changes include breast development (Table 8-3) and pubic hair. Changes are driven by increasing sex steroids. Also, adrenal androgens increase (**adrenarche**) before gonadal gametogenesis and hormonogenesis (**gonadarche**), at about 6 to 7 years of age in girls and 7 to 8 years of age in boys. Adrenal androgens are converted to testosterone, DHT, and estradiol and therefore contribute to growth of pubic and axillary hair, particularly in girls.

Males

The first sign of puberty (Tanner stage 1) in males is an increase in **testicular volume** (Fig. 8-10). Before puberty, there are a few partially differentiated Leydig cells. During puberty, Leydig cells differentiate from mesenchymal peritubular cells and divide to form the typical clusters seen in the adult testis. Within the seminiferous tubules, meiosis and spermatogenesis are initiated at puberty, and as testosterone and FSH levels increase, the rate of spermatogenesis reaches adult levels. Sertoli cells divide and become more active at puberty, forming the occluding junctions and performing other hormonally dependent functions, including secretion of fluid. **Spermarche** represents the age at first ejaculation as nocturnal emissions and sperm in the urine.

Puberty is associated with numerous primary and secondary sexual changes, including growth and onset of function of the prostate and seminal vesicles; increased growth of the penis; increased muscle mass; thickening of vocal cords; appearance of pubic (**pubarche**), facial, and body hair; and development of libido (see Table 8-2). Many of these changes are dependent on the conversion of testosterone to DHT.

TABLE 8-3
Tanner Pubertal Stages in Female

STAGE	BREAST	PUBIC HAIR
1	Prepubertal	Prepubertal; no pubic hair
2	Breast bud and papilla elevated; small mound present	Slight growth of fine, downy hair
3	Enlargement of breast mound; palpable glandular tissue	Hair darker, coiled, denser
4	Areola and nipple elevated	Adult-type hair, but area covered is less than in adult
5	Adult breast	Adult-type hair with triangular-shaped distribution

Data from Marshall WA, Tanner JM: Variations in pattern of pubertal changes in girls. *Arch Dis Child* 44:291, 1969.

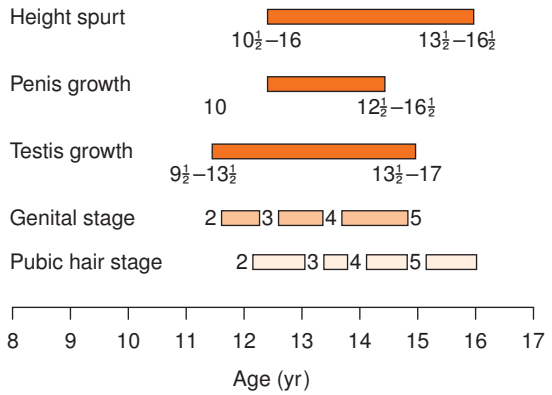


FIGURE 8-10 ■ Normal sequence of changes of male puberty. Numbers 2 to 5 refer to Tanner stages. *(Data from Marshall WA, Tanner JM: Variations in the pattern of pubertal changes in boys.* Arch Dis Child *45:13, 1970.)*

Females

The timing of puberty in females is influenced by the level of body fat. Lean girls tend to enter puberty later. Female athletes with low body fat levels often have amenorrhea. This may be due in part to the fact that adipose tissue expresses significant levels of CYP19 (aromatase), which aromatizes androgens to estrogens. Growing evidence, however, suggests that leptin, which also is produced by adipose tissue, plays a permissive role in hypothalamic maturation at puberty. Several years before menarche (onset of menstrual cycles), adrenarche occurs. This is manifested by the development of pubic hair and axillary hair.

A landmark of puberty in women is breast growth with some limited development (Fig. 8-11). At puberty, the increase in estrogen induces an enlargement and darkening of the areola, which is the pigmented, hairless circle surrounding the nipple, and some limited ductal growth beneath the areola and nipple. The onset of these mammary gland changes is referred to as **thelarche**. Once ovulatory cycles begin, progesterone stimulates further growth and maturation of the

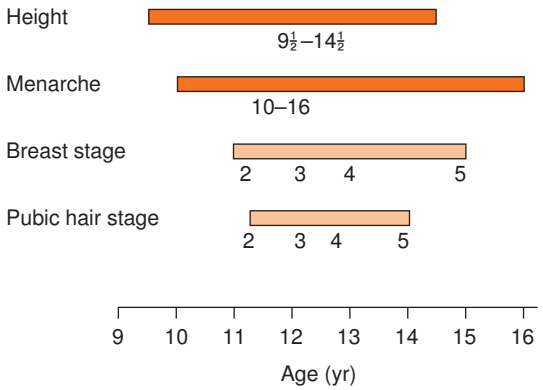


FIGURE 8-11 ■ Sequence of events during female puberty indicating ranges of ages at which each event normally occurs. Numbers 2 to 5 refer to Tanner stages. *(Data from Marshall WA, Tanner JM: Variations in the pattern of pubertal changes in boys.* Arch Dis Child *45:13, 1970.)*

mammary glands. Progesterone also increases stromal edema and vacuolation of the epithelia, thereby inducing a sensation of fullness or tenderness during the late luteal phase (see Chapter 10). Table 8-3 shows the Tanner stages of pubertal development for women, and Figure 8-11 shows the ages at which these changes occur.

The pubertal growth spurt refers to a significant increase in growth velocity. The growth spurt occurs early in puberty in girls, but toward the end of puberty in boys. The growth spurt is under complex hormonal regulation, involving triiodothyronine (T_3), growth hormone and its target, insulin-like growth factor-1 (IGF-1), and sex steroids. Estradiol promotes normal bone density in both sexes. Also, estradiol promotes the cessation of long bone growth by inducing the closure of the epiphyseal plates in both sexes. Men who either have a null mutation in the estrogen receptor (ERα) or are deficient in CYP19-aromatase continue long-bone growth well beyond their second decade.

MENOPAUSE AND ANDROPAUSE

Menopause

As discussed earlier, all ovarian oogonia commit to meiosis, so no stem cell population exists (as it does in the testis). Menopause generally is thought to result from primary ovarian deficiency due to depletion of a sufficient number of functional follicles. Menopause typically occurs between 46 and 55 years of age. It extends over several years. Initially, the cycles become irregular and are periodically anovulatory. This is referred to as the **menopausal transition**. The cycles tend to shorten, primarily in the follicular phase. Eventually, the woman ceases to cycle altogether. The final menstrual period is determined retrospectively after 1 year of amenorrhea, and the stage of life from this final period to death is termed **postmenopause**.

The observation that some morphologically normal oocytes can be present in the postmenopausal ovary, however, suggests that oocyte depletion is not the sole cause of menopause. These remaining follicles are hypothesized to be less sensitive to gonadotropins. It has been proposed recently that age-related changes in the CNS, including critical patterns of GnRH secretion, precede follicular depletion and may play an important role in menopause. Because follicles do not develop in response to LH and FSH secretion, estrogen and progesterone levels drop. Loss of the negative feedback inhibition of estrogen on GnRH, LH, and FSH results in a marked rise in serum LH and FSH. FSH levels rise more than LH levels. This may result from ovarian inhibin loss.

The serum estradiol levels drop to about one sixth the mean levels in younger cycling women, and progesterone levels drop to about one third those in the follicular phase in younger women. Production of these hormones does not cease entirely, but the primary source of estradiol and active androgens (testosterone, dihydrotestosterone) in the postmenopausal woman becomes the peripheral conversion of adrenal androgens. Because estrone is the primary estrogen produced in adipose tissue, it becomes the predominant circulating form of estrogen in postmenopausal women. The decrease in circulating levels of sex steroids and inhibin results in an increase in gonadotropins, especially FSH. FSH levels increase even before the menopausal transition.

Most of the signs and symptoms associated with menopause result from estrogen deficiency. Some of the consequences of estrogen loss at menopause are the following:

1. The vaginal epithelium atrophies, becomes dry, and with loss of vaginal flora, increases in pH. These

changes often result in increased incidence of vaginal and urinary tract infections, as well as pain during intercourse.

2. Bone loss is accelerated, potentially leading to osteoporosis. The effect of menopause on osteoporosis is staggering: greater than 1 million fractures per year occur in the United States due to menopausal osteoporosis. A significant fraction of these fractures are hip fractures, which greatly increase morbidity and mortality and increase the loss of independence.
3. Hot flushes result from periodic increases in core temperature, which produce peripheral vasodilation, sweating, and a feeling of malaise. Hot flushes are also correlated with sleep disturbances, which can significantly affect a woman's daily productivity and quality of life. Hot flushes are correlated with LH pulses, and current thinking proposes that a CNS mechanism leads to both events. Hot flushes typically subside within 1 to 5 years of the onset of menopausal symptoms.
4. The incidence of cardiovascular disease increases markedly after menopause. This is due, in part, to the reversal of the beneficial effect of estrogen on high-density lipoprotein levels. However, lifestyle plays an important role, and menopause can affect lifestyle—if a woman is losing sleep because of hot flushes, she is less likely to exercise during the day. There are other psychosocial effects of menopause, including fatigue and depression.

Most of the menopausal sequelae respond well to estrogen replacement therapy (ERT), or estrogen and progesterone replacement therapy (hormone replacement therapy, or HRT). Results from the Women's Health Initiative and other studies, however, have provided conflicting findings on the safety of estrogens and progestins (which were not in the form of bioidentical estradiol-17β and progesterone) for HRT in postmenopausal women, emphasizing the need for physicians to fully consider each woman's individual medical and family history before deciding on the course of therapy for the alleviation of postmenopausal symptoms. In general, use of the lowest effective dose and for the shortest effective duration is standard. New analysis from the Women's Health Initiative indicates that ERT given before the age of 60 years (i.e., short-term ERT) was not associated with an increased risk for thromboembolic events, stroke, or breast cancer.

Andropause

There is no distinct andropause in men. As men age, however, gonadal sensitivity to LH decreases and androgen production drops. As this occurs, serum LH and FSH levels rise. Although sperm production typically begins to decline after age 50 years, many men can maintain reproductive function and spermatogenesis throughout life.

SUMMARY

1. The male and female reproductive systems are composed of a gonad (testis and ovary) and a reproductive tract.
2. The gonads perform an endocrine function, which is regulated within a hypothalamic-pituitary-gonad axis.
3. The gonads perform an exocrine function by the production of gametes. This function is absolutely dependent on gonadal hormones.
4. Meiosis is central to sexual reproduction. Meiosis generates haploid gametes through two divisions, meiosis I and meiosis II, with no DNA synthesis in between. The major advantage of sexual reproduction is the recombination of genetic material. This occurs by independent segregation of chromosomes, combining the haploid genome of eggs with the haploid genome of sperm, and through crossing-over.
5. The process of crossing-over involves synapsis of homologous chromosomes (each with two sister chromatids) to form a bivalent, followed by a complex process that exchanges DNA sequence information between homologous strands. Chiasmata form at these sites, which help organize chromosomes at the metaphase plate.

6. Chromosomes must undergo accurate disjunction (separation) in both divisions. Defects in disjunction lead to nondisjunction, resulting in aneuploidy or polyploidy.
7. The male reproductive system includes the testis, in which the seminiferous tubules generate spermatozoa. These are guided out of the testis by the rete testis and efferent ductules. Sperm then enter the epididymis, vas deferens, ejaculatory duct, and male urethra. Sperm receive seminal fluid (semen) from the seminal vesicles and the prostate. The tract ends in the penis, which is an intromittent organ designed for internal semination.
8. The female reproductive system includes the ovaries, oviducts, uterus, cervix, vagina, and external genitalia. The female tract does **not** join with the female urethra.
9. The gamete within the ovary is a primary oocyte arrested in prophase of meiosis I. Just before ovulation, the oocyte completes meiosis I and becomes arrested at metaphase of meiosis II (now called an egg).
10. Release of an egg involves a complex process that involves the focal breakdown of the follicular and ovarian walls.
11. Early embryos have a bipotential gonad, into which primordial germ cells have migrated from the yolk sac. Genetic sex (46,XY or 46,XX) determines gonadal sex. Gonadal sex will create a hormonal environment that determines the development of the reproductive tract and external genitalia.
12. SRY is a major factor involved in male development (formation of a testis) at 6 to 7 weeks of gestation.
13. Formation of a testis involves the organization of seminiferous tubules lined by spermatogonia and Sertoli cells. Leydig cells develop in the peritubular compartment.
14. Meiosis is blocked in the male by the production of CYP26B1, which breaks down and inactivates retinoic acid.
15. Sertoli cells secrete AMH, thereby inducing the regression of the female müllerian tract.
16. Leydig cells, under stimulation by hCG and, later, fetal LH, produce testosterone. Testosterone promotes the differentiation of the wolffian tract into the epididymis, vas deferens, ejaculatory duct, and seminal vesicle.
17. Testosterone also induces descent of the testis into the scrotum.
18. Testosterone is peripherally converted to DHT by 5α-reductase-2. DHT induces the differentiation of the urogenital sinus into the proximal male urethra and the prostate gland. DHT induces the primordia of the external genitalia to form the penis and scrotum.
19. A deficiency of 5α-reductase-2 may lead to female or ambiguous external genitalia in a 46,XY fetus. The female phenotype changes to a male phenotype at puberty, requiring reassessment of sexual identity, followed by appropriate surgery and hormonal replacement.
20. Lack of SRY in a 46,XX female embryo allows female-specific transcription factors to drive ovarian differentiation. This is completed much later than testicular differentiation.
21. Oogonia develop from primordial germ cells and divide mitotically to generate about 1 million oogonia per ovary. All oogonia enter meiosis and become oocytes arrested at the prophase of meiosis I. Oocytes become surrounded by epithelial cells and their basal lamina, generating a structure called the primordial follicle.
22. Absence of AMH allows the female müllerian duct to develop into the oviducts, uterus, cervix, and the inner one third of the vagina. Absence of testosterone causes the male wolffian duct to regress. Absence of DHT allows the external genitalia to form female structures (labia majora, labia minora, clitoris, vestibular bulbs and glands) and the outer two thirds of the vagina.
23. The human reproductive axis involves hypothalamic pulsatile GnRH neurons and pituitary gonadotropes that secrete LH and FSH. After significant activity in early infancy, the reproductive axis diminishes to very low activity for about a decade. This may be due to both active inhibition from the CNS and exquisite sensitivity to negative feedback.
24. Puberty involves a significant activation of the reproductive axes and the reproductive system. This

probably involves cessation of CNS inhibition, along with stimulation by other CNS centers.

25. Kisspeptin (Kiss1) is produced in the hypothalamus and binds to its receptor, Kiss1R, on GnRH neurons. Null mutation in Kiss1R cause deficient GnRH-dependent pubertal changes. Gain-of-function mutations in both Kiss1 and Kiss1R have been linked to precocious puberty.
26. The progression of puberty is semi-quantified by the five Tanner stages. In boys, these involve assessment of testicular size (volume), penile growth, and pubic hair growth. In girls, Tanner staging involves assessment of breast development and pubic hair growth.
27. The pubertal growth spurt begins in early puberty in girls and in late puberty in boys. This involves an increase in the growth velocity, which is dependent on increased growth hormone, IGF-1, T_3, and sex steroids.
28. Menopause involves the great diminution of ovarian steroid and inhibin output, probably owing to insufficient number of gonadotropin-responsive follicles. LH and FSH levels increase.
29. Menopause may be accompanied by thinning of the vagina, vasomotor instability (hot flushes), sleep disturbances, personality changes, osteoporosis, and an increased risk for cardiovascular disease.
30. Men do not experience an abrupt cessation in the reproductive axis. Some men are fertile at advanced ages. Some men experience a decrease in androgen production (an incomplete andropause), along with symptoms such as low libido and erectile dysfunction.

SELF-STUDY PROBLEMS

1. How does sexual reproduction increase genetic diversity?
2. Explain how a 46,XX embryo would develop in the presence of an SRY-autosome translocation (i.e., gain of SRY).
3. Explain the gonadal, internal tract, and external genitalia in a 46,XY individual with a null mutation in the AMH receptor.
4. Compare the adult male and adult female reproductive systems in terms of onset and progression of meiosis.
5. Explain why wolffian duct derivatives develop in embryos with Kallmann syndrome type 1.
6. How do gain-of-function mutations in Kiss1R affect the timing of puberty?
7. Why do women become infertile at menopause, whereas men may remain fertile into their ninth decade?
8. List the consequences of menopause.

KEYWORDS AND CONCEPTS

- 46,XX
- 46,XY
- 5α-reductase-1

For full list of keywords and concepts see Student Consult

SUGGESTED READINGS

Griswold SL, Behringer RR: Fetal Leydig cell origin and development, *Sex Dev* 3:1–15, 2009.

Teles MG, Silveira LF, Tusset C, et al: New genetic factors implicated in human GnRH-dependent precocious puberty: The role of kisspeptin system, *Mol Cell Endocrinol* 346:84–90, 2011.

KEYWORDS AND CONCEPTS

- 5α-reductase-2
- 5α-reductase-2 deficiency
- Adrenarche
- Alignment
- Ambiguous genitalia
- AMH receptor type II (AMHR-II)
- Anaphase I
- Anaphase II
- Andropause
- Aneuploidy
- Antimüllerian hormone (AMH)
- Antrum
- Bipotential gonadal primordium
- Bivalent
- Centromere
- Cervix
- Chiasmata
- Chromatids
- Clitoris
- Congenital adrenal hyperplasia
- Consanguinity
- Crossing-over
- Cryptorchidism
- Cryptorchism
- Delayed puberty
- Dihydrotestosterone (DHT)
- Disjunction
- Down syndrome
- Efferent ductules
- Eggs
- Ejaculatory ducts
- Embryonic development
- Epididymides
- Epididymis
- Estrogen
- Euploidy
- Gametes
- Gametogenic
- Genetic diversity
- Genetic recombination
- Genetic sex
- GnRH neurons
- Gonadal sex
- Gonadarche
- Gonadotropin-dependent precocious puberty
- Gonads
- Granulosa cells
- Haploid cells
- Human chorionic gonadotropin (hCG)
- Hypogonatropic hypogonadism
- Hypothalamus-pituitary-gonadal axis
- Independent assortment
- Kallmann syndrome type 1
- Kinetochore
- *KISS1* gene
- Kiss1R
- Kisspeptin (Kiss1)
- Labia majora
- Labia minora
- Leydig cells
- Luteinizing hormone (LH)
- Male external genitalia
- Male urethra
- Mammary glands
- Meiosis I
- Meiosis II
- Membranous
- Menopausal transition
- Menopause
- Mesonephric ducts
- Microtubule spindles
- Müllerian ducts
- Müllerian-inhibitory substance
- Nondisjunction
- Oogonia
- Orgasm
- Ovarian follicle
- Ovaries
- Oviduct
- Pair
- Paramesonephric ducts
- Penile
- Penis
- Peripubertal period
- Persistent müllerian duct syndrome (PMDS)
- Phenotypic gender
- Placental hCG
- Polyploidy
- Postmenopause
- Precocious puberty

- Primordial follicles
- Primordial germ cells
- Progesterone plus estrogen
- Prophase I
- Prostate
- Prostate gland
- Prostatic
- Pubarche
- Puberty
- Reproductive tracts
- Rete testis
- Retinoic acid
- Semen
- Seminal vesicles
- Seminiferous tubules
- Sertoli cells
- Sexual reproduction
- Sister chromatids
- Sperm
- Spermarche
- Spermatogonia
- *SRY* gene
- Tanner stages
- Tertiary
- Testes
- Testicular volume
- Testis
- Testosterone
- Thelarche
- Trisomy 21
- Uterus
- Vagina
- Vas deferens
- Vestibular bulbs
- Wolffian ducts

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9 THE MALE REPRODUCTIVE SYSTEM

OBJECTIVES

1. Describe the organization of the male gonad, the testis, and the process of spermatogenesis, and discuss how this process is supported by Sertoli cells.
2. Describe the steroidogenic pathway of Leydig cells that produces testosterone, the peripheral conversion of testosterone to estradiol-17β or dihydrotestosterone, and the actions of these steroids in men.
3. Discuss the regulation of testicular function by the hypothalamic-pituitary-testicular axis.
4. Describe the role of the proximal male reproductive tract, especially the epididymis, in the further development of sperm.
5. Discuss the more distal segments of the male reproductive tract, including the accessory sex glands, in the context of emission and ejaculation.
6. Describe the neurovascular events in the penis that are involved in erection.
7. Discuss the following pathologic conditions of the male reproductive system: Klinefelter syndrome and androgen insensitivity that is coupled to testicular feminization.

In men, the reproductive system has evolved for continuous, lifelong gametogenesis, coupled to occasional internal insemination with a high density of sperm (greater than 60×10^6/mL in 3 to 5 mL of semen). This means that in adult men, the basic roles of gonadal hormones are as follows:

- Support of gametogenesis (spermatogenesis)
- Maintenance of the male reproductive tract and production of semen
- Maintenance of secondary sex characteristics and libido. There is no overall cyclicity of this activity in men.

HISTOPHYSIOLOGY OF THE TESTIS

A major difference between the testes and the ovaries is that the testes reside in the **scrotum** outside of the abdominopelvic cavity and are connected to the intrapelvic male tract by the spermatic cord (see Fig. 8-2 in Chapter 8). Various mechanisms, including the cremasteric reflex, a venous countercurrent exchanger in the spermatic cord (pampiniform plexus), folding of scrotal skin, and sweat glands within the scrotal skin, cooperate to maintain a testicular temperature at about 35° C, which is crucial for sperm development. Failure of the testes to descend through the

inguinal canal into the scrotum during development results in depressed spermatogenesis.

The human testis is covered by a connective tissue capsule and is divided into about 300 **lobules** by fibrous septa. Within each lobule are two to four loops of **seminiferous tubules**. Each loop empties into an anastomosing network of tubules called the **rete testis**. The rete testis is continuous with small ducts, the **efferent ductules** that lead the sperm out of the testis into the head of the **epididymis** on the superior pole of the testis (Fig. 9-1). Once in the epididymis, the sperm pass from the head, to the body, to the tail of the epididymis and then to the **vas (ductus) deferens**. Spermatozoa are stored in the tail of the epididymis and the vas deferens for several months as viable sperm.

The presence of the seminiferous tubules in the lobules of the testis creates two compartments within each lobule: an **intratubular compartment**, which is composed of the **seminiferous epithelium** of the seminiferous tubule; and a **peritubular compartment**, which is composed of neurovascular elements, connective tissue cells, immune cells, and the **interstitial cells of Leydig**, whose main function is to produce **testosterone**.

The Intratubular Compartment

The seminiferous tubule is lined by a complex **seminiferous epithelium** (Fig. 9-2) composed of two cell types:

- Sperm cells in various stages of spermatogenesis
- The Sertoli cell, which is a nurse cell in intimate contact with all sperm cells and which regulates many aspects of spermatogenesis

Developing Sperm Cells The entire developmental process by which spermatogonia give rise to spermatozoa is called **spermatogenesis**. Spermatogenesis begins at puberty and involves the processes of mitosis and meiosis (see Fig. 9-2). **Stem spermatogonia** (also called **prespermatogonia**) reside at the basal level of the seminiferous epithelium. Stem spermatogonia divide mitotically to generate daughter spermatogonia (**spermatocytogenesis**). These mitotic divisions are initially asymmetrical, in that one daughter cell remains a *stem* spermatogonium (thereby undergoing self-renewal throughout life), whereas the second daughter cell will divide several times to amplify its population. After several mitotic divisions, the daughter spermatogonia complete S phase (DNA replication) and commit to meiotic division. Of note, these amplifying divisions are accompanied by **incomplete cytokinesis**, so all spermatogonia daughter cells and subsequent sperm cells (at different stages) remain interconnected by a cytoplasmic bridge. This configuration contributes to the synchrony of development of a clonal population of sperm cells.

Spermatogonia migrate apically away from the basal lamina as they enter the first meiotic prophase (see Fig. 9-1). At this time, they are called **primary spermatocytes**. During the first meiotic prophase, the hallmark processes of sexual reproduction involving **synapsis**, **crossing-over**, formation of **chiasmata**, and the first **disjunction** occur (see Chapter 8). Completion of the first meiotic division gives rise to **secondary spermatocytes**, which quickly (within 20 minutes) complete the second meiotic division.

The initial products of meiosis are haploid **spermatids**, which reside apically within the seminiferous epithelium, close to the lumen of the seminiferous tubule (see Fig. 9-2). Spermatids are small, round cells with a round nucleus. Spermatids undergo a remarkable metamorphosis called **spermiogenesis** that results in a spermatozoon (Fig. 9-3). The spermatozoon contains the following parts:

1. A **head**. The head consists of two major components:
   a. A condensed and streamlined **nucleus**. The chromatin of the nucleus is highly heterochromatic (i.e., condensed), and the nucleosomal histones are replaced by **protamines**. The DNA of a spermatozoan is transcriptionally silent.
   b. An **acrosomal vesicle**. The acrosomal vesicle contains hydrolytic enzymes transported to it from the Golgi. These enzymes will play an important role in fertilization and the prevention of polyspermy (see Chapter 11). The acrosomal vesicle attaches to the forward pole of the nucleus and descends along the side of the nucleus so that it partially covers the nucleus.
2. The **neck**. This contains two **centrioles** (proximal and distal). The proximal centriole attaches to the nucleus, and the distal centriole will generate a "9+2" configuration of microtubules that is called the **axoneme**.
3. The **tail** (also called the **flagellum**). The tail has a continuous axonemal core but is composed of

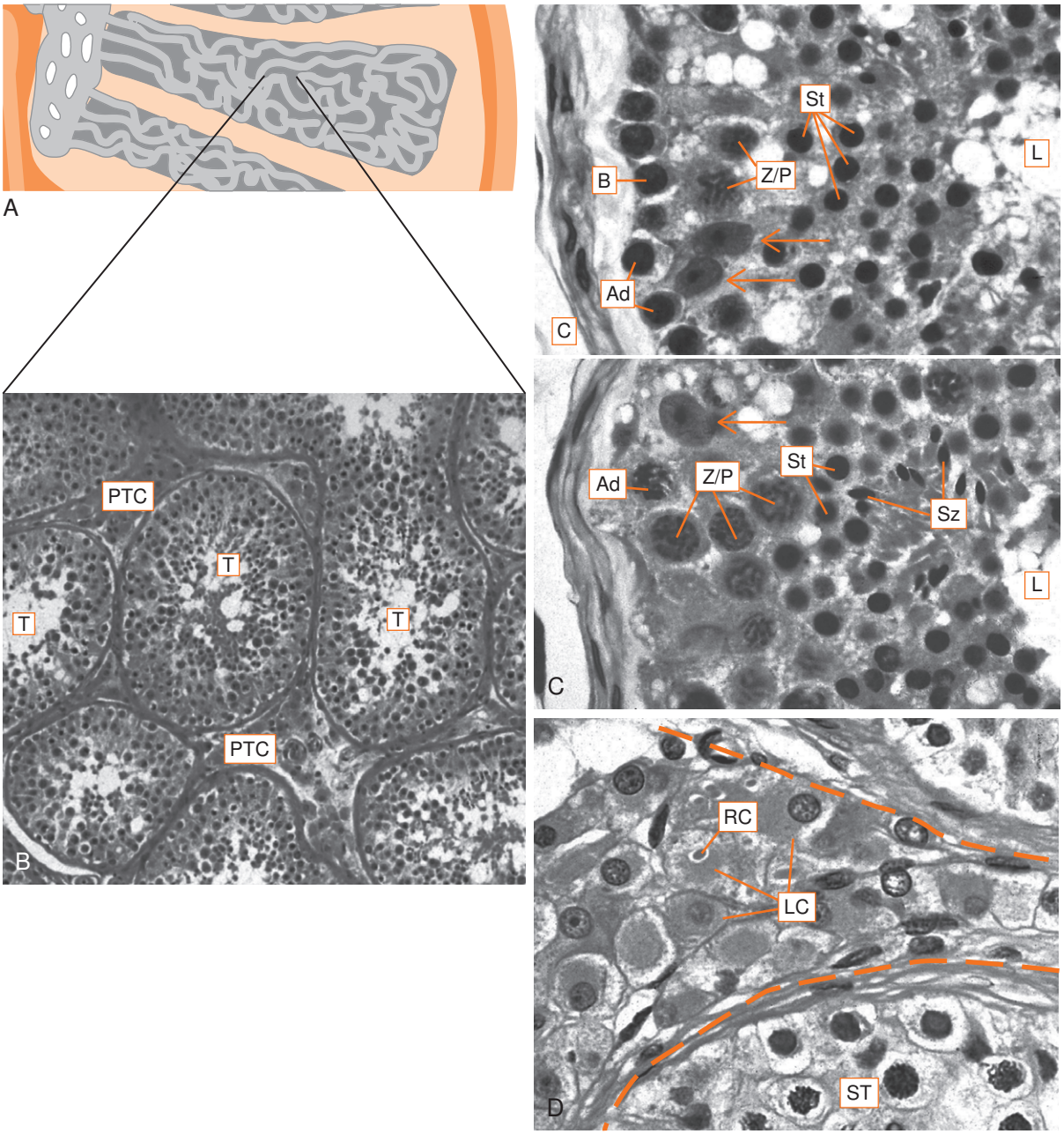


FIGURE 9-1 ■ **A,** Low-magnification drawing of testicular lobules containing coils of seminiferous tubules. **B,** Higher magnification of histologic organization of a section from the testicular lobule (as drawn in **A**), showing several seminiferous tubules (T), which collectively make up the intratubular compartment, and the peritubular compartment (PTC). **C,** Higher magnification of histologic organization of two seminiferous tubules, showing Sertoli cells *(arrows)*, spermatogonia (Ad and B), primary spermatocytes (Z/P), spermatids (St), and spermatozoa (Sz). L, lumen of tubule. Note that the association of sperm cells differs in two adjacent tubules as a result of the difference in their stage of the spermatogenic cycle. **D,** Higher magnification of histologic organization of the peritubular compartment (between *dashed lines*) showing a cluster of Leydig cells (LC). *(**A,** From Porterfield SP:* Endocrine Physiology, *2nd ed., St. Louis, 2001, Mosby. **B** to **D,** From Stevens A, Lowe J:* Human Histology, *3rd ed., Philadelphia, 2005, Mosby.)*

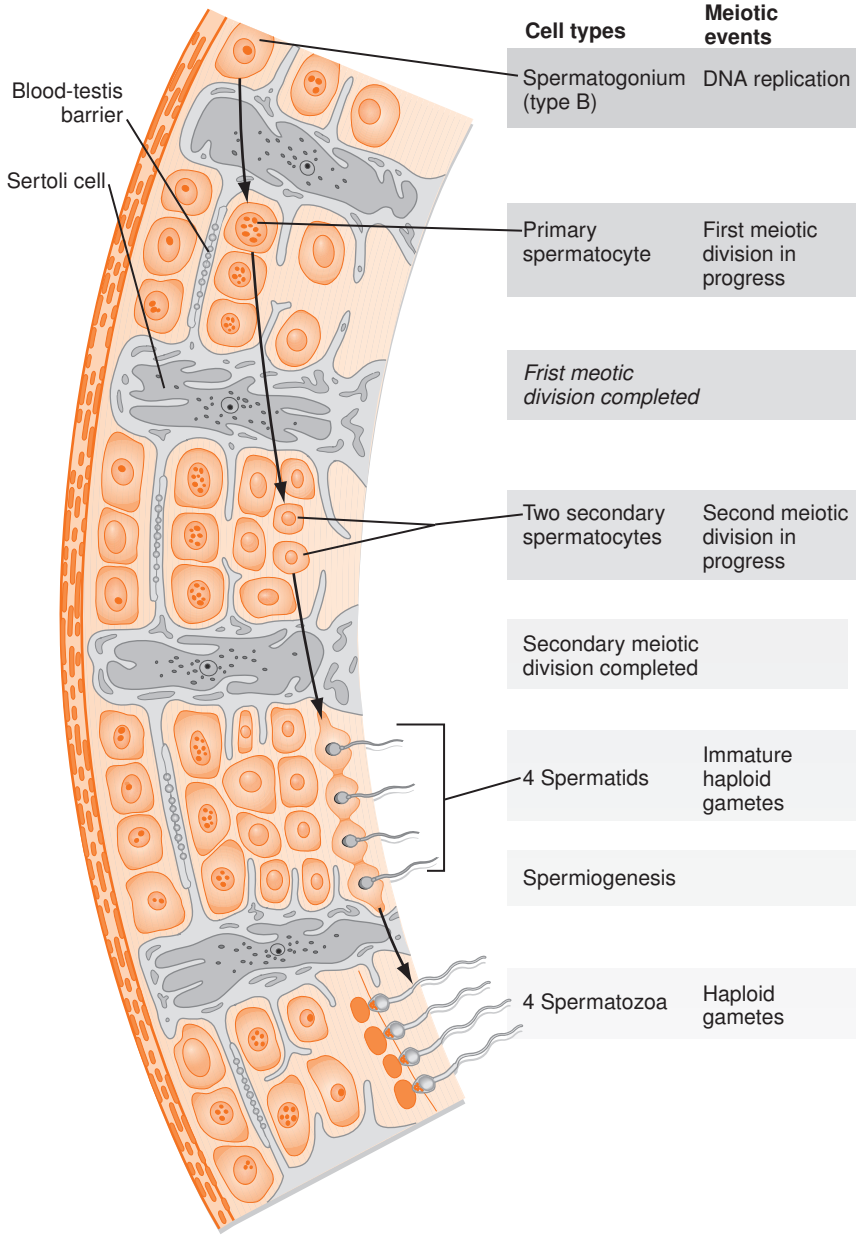


FIGURE 9-2 ■ Seminiferous epithelium. Spermatogonia undergo mitosis (spermatocytogenesis) to produce both a reservoir of spermatogonia and maturing spermatogonia that differentiate into primary spermatocytes. These spermatocytes remain joined by cytoplasmic bridges (not shown). Primary spermatocytes undergo the complex process of the first meiotic division to become secondary spermatocytes, and then a rapid second meiotic division (reduction-division) to become haploid spermatids. Spermatids mature into spermatozoa by the process of spermiogenesis. Sertoli cells extend from the basal to the apical sides of the epithelium and create the blood-testis barrier between spermatogonia and primary spermatocytes. *(From Koeppen B, Stanton B:* Berne and Levy Physiology, *updated 6th ed., Philadelphia, 2010, Mosby.)*

structurally distinct regions called the middle piece, principal piece, and end piece. The middle piece is the thickest and contains a collar of mitochondria that will deliver adenosine triphosphate (ATP) for flagellar beating and motility. The outer circumference of the middle piece contains dense fibers. The principal piece and the end piece lack the mitochondrial sheath, and the end piece lacks the outer dense fibers.

The process of **spermatogenesis** takes about 72 days. A cohort of adjacent spermatogonia enters the process every 16 days, so the process is staggered along the length of a seminiferous tubule. Consequently, spermatogonia do not enter the process of

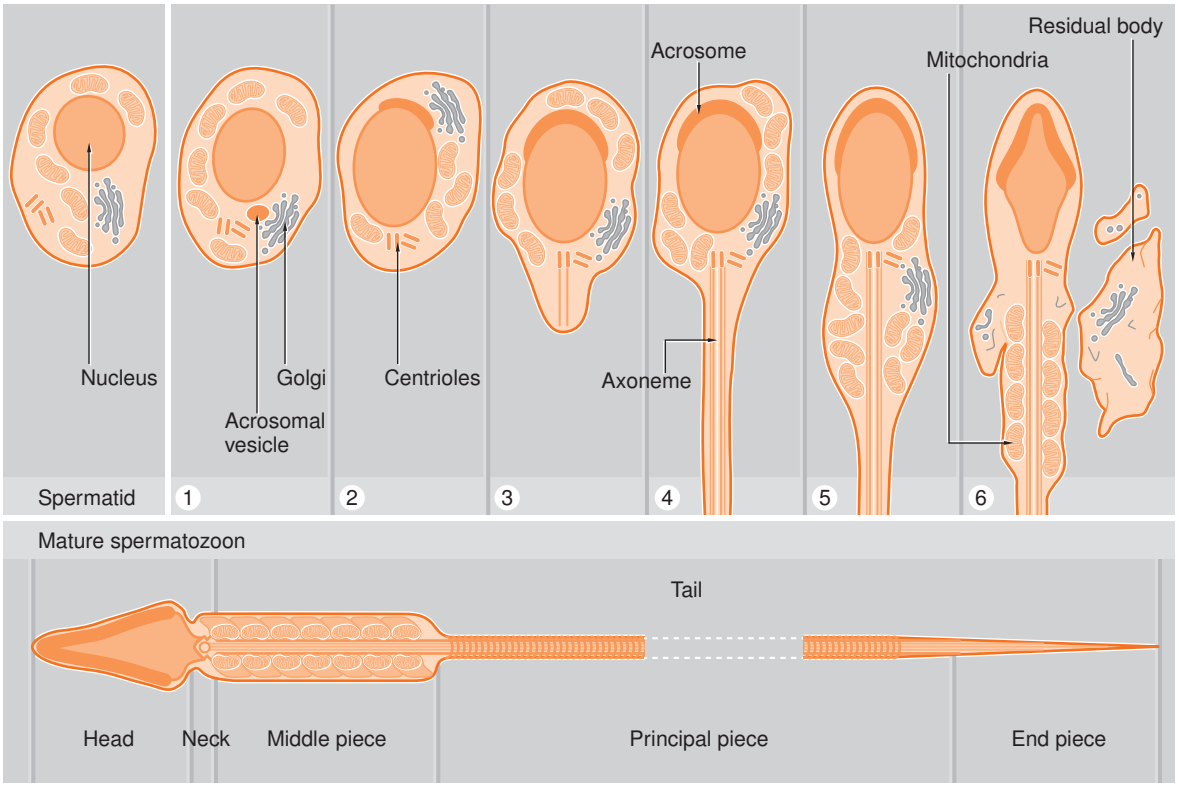


FIGURE 9-3 ■ Spermiogenesis and structure of a spermatozoon. The residual body is phagocytized by Sertoli cells. *(From Young B, Lowe J, Stevens A, et al:* Wheater's Functional Histology, *5th ed., Edinburgh, 2007, Churchill Livingstone.)*

spermatogenesis at the same time along the entire length of the tubule, or in synchrony with every other tubule (there are about 500 seminiferous tubules per testis). Because the seminiferous tubules within one testis total about 400 meters in length, spermatozoa are continually being generated at many sites within the testis at any given time. Histologic examination of the seminiferous tubules reveals that there are specific associations, called **spermatogenic stages**, of sperm cells at any one point in time. In humans, there are six different stages that progress and repeat as a cycle at one point within the seminiferous tubule. This is referred to as the **spermatogenic cycle**. The stages are staggered spatially along the length of the seminiferous tubule, before repeating themselves. This spatial configuration of cycles is called a **spermatogenic wave**.

The final release of sperm, called **spermiation**, is an active process involving dissolution of adhesion between Sertoli cells and spermatozoa. It is important to note that testicular spermatozoa after spermiation are not fully mature. Testicular spermatozoa are barely motile, and leave the seminiferous tubule passively within fluid produced by the Sertoli cells.

The Sertoli Cell The **Sertoli cell** represents the true epithelial cell of the seminiferous epithelium and extends from the basal lamina to the lumen (Box 9-1; see Fig. 9-3). Sertoli cells surround sperm cells, providing structural support within the epithelium, and form adherens-type junctions and gap junctions with all stages of sperm cells (see Fig. 9-3). Through the formation and breakdown of these junctions, Sertoli cells guide sperm cells toward the lumen as they advance to later stages in spermatogenesis. Accordingly, major secretory products of Sertoli cells include proteases and protease inhibitors. Spermiation requires the final breakdown of Sertoli cell–sperm cell junctions.

Another important structural feature of Sertoli cells is the formation of tight junctions between adjacent Sertoli cells (see Fig. 9-3). These occluding junctions divide the seminiferous epithelium into a **basal**

BOX 9-1

FUNCTIONS OF SERTOLI CELLS

SUPPORTIVE ("NURSING")

- Maintaining, breaking, and re-forming multiple junctions with developing sperm
- Maintaining blood-testis barrier
- Phagocytosis
- Transfer of nutrients and other substances from blood to developing sperm cells
- Expression of paracrine factors and receptors for sperm-derived paracrine factors

EXOCRINE

- Production of fluid to move immobile sperm out of testis toward epididymis
- Production of androgen-binding protein
- Determination of release of spermatozoa (spermiation) from seminiferous tubule

ENDOCRINE

- Expression of androgen receptor and follicle-stimulating hormone receptor
- Production of müllerian-inhibiting substance, also called antimüllerian hormone
- Aromatization of testosterone to estradiol-17β (this has local effect, not strictly endocrine)

compartment, containing the spermatogonia and early-stage primary spermatocytes, and an **adluminal compartment**, containing later-stage primary spermatocytes and all subsequent stages of sperm cells. As early primary spermatocytes move apically from the basal compartment to the adluminal one, the tight junctions need to be disassembled and reassembled. These tight junctions form the physical basis for the **blood-testis barrier**, which creates a specialized, immunologically safe microenvironment for developing sperm. By blocking paracellular diffusion, the tight junctions restrict movement of substances between the blood and the developing germ cells through a trans–Sertoli cell transport pathway and in this manner allow the Sertoli cell to control nutrient availability to germ cells. Accordingly, Sertoli cells also have the responsibility for providing nutrients to this environment, such as transferrin, iron, and lactate. For example, spermatogonia and released spermatozoa use fructose and glucose for energy. However, sperm undergoing meiosis cannot efficiently use glucose as an energy source. Sertoli cells acquire glucose by the GLUT1 transporter, metabolize it to lactate, and transfer it to developing sperm, which express a sperm-specific lactate transporter. This process is dependent on hormonal stimulation (follicle-stimulating hormone [FSH] and testosterone; see later) but also appears to be optimized by local sperm cell–generated paracrine factors.

Thus, healthy Sertoli cell function is essential for sperm cell viability and development. In this respect, it should be noted that spermatogenesis is absolutely dependent on testosterone produced by peritubular Leydig cells (see later), yet it is the Sertoli cells that express the **androgen receptor**, not the developing sperm cells. Similarly, the pituitary hormone FSH is also required for maximal sperm production, and again, it is the Sertoli cell that expresses the **FSH receptor**, not the developing sperm. Thus, these hormones support spermatogenesis indirectly through stimulation of Sertoli cell function.

Sertoli cells have multiple additional functions. Sertoli cells express the enzyme **CYP19** (also called **aromatase**), which converts Leydig cell–derived testosterone to the potent estrogen, **estradiol-17β** (see later). This local production of estrogen may enhance spermatogenesis in humans. Sertoli cells also produce **androgen-binding protein** (**ABP**). ABP is encoded by the same gene as for sex hormone–binding globulin (SHBG; see later) but has different carbohydrate groups and is specifically expressed intratesticularly. ABP maintains a high androgen level within the adluminal compartment, the lumina of the seminiferous tubules, and the proximal part of the male reproductive tract. Sertoli cells also produce a large amount of fluid. This fluid provides an appropriate bathing medium for the sperm and assists in moving the immotile spermatozoa from the seminiferous tubule into the epididymis. Sertoli cells perform an important **phagocytic function**. This allows Sertoli cells to engulf **residual bodies**, which represent cytoplasm that is shed by spermatozoa during spermiogenesis, as well as dead sperm cells.

Finally, the Sertoli cell has an important endocrine role. During development, Sertoli cells produce **antimüllerian hormone** (**AMH**), also called **müllerian-inhibiting substance** (**MIS**), which induces regression

of the embryonic müllerian duct that is programmed to give rise to the female reproductive tract (see Chapter 8). The Sertoli cells also produce the hormone **inhibin**. Inhibin is a heterodimer protein hormone related to the transforming growth factor-β (TGF-β) family. FSH stimulates inhibin production, which then exerts negative feedback on gonadotropes to inhibit FSH production. Thus, inhibin keeps FSH levels within a specific range (see later).

The Peritubular Compartment

The peritubular compartment (see Fig. 9-1) contains the primary endocrine cell of the testis, the **Leydig cell**. This compartment also contains common cell types of loose connective tissue and an extremely rich peritubular capillary network that must provide nutrients to the seminiferous tubules (by way of Sertoli cells) while conveying testosterone away from the testes to the peripheral circulation.

The Leydig Cell **Leydig cells** are steroidogenic stromal cells. These cells synthesize cholesterol de novo, as well as acquiring it through low-density lipoprotein (LDL) receptors and, to a lesser extent, high-density lipoprotein (HDL) receptors (the HDL receptor is also called **scavenger receptor-BI [SR-BI]**), and store cholesterol as cholesterol esters, as described for adrenocortical cells (see Chapter 7). Free cholesterol is generated by a cholesterol **hormone-sensitive lipase (HSL)** and transferred to the outer mitochondrial membrane, and then to the inner mitochondrial membrane in a **steroidogenic acute regulatory protein (StAR)**-dependent manner (refer to Fig. 7-7 in Chapter 7). As in all steroidogenic cells, cholesterol is converted to pregnenolone by **CYP11A1**. Pregnenolone is then processed to progesterone, 17α-hydroxyprogesterone, and androstenedione by **3β-hydroxysteroid dehydrogenase type 2 (3β-HSD2)** and **CYP17** (Fig. 9-4). Recall from Chapter 7 that CYP17 is a bifunctional enzyme, with a 17-hydroxylase activity and a 17, 20-lyase activity. CYP17 displays a robust level of both activities in the Leydig cell. In this respect, the Leydig cell is similar to the zona reticularis cell, except that it expresses a higher level of 3β-HSD, so that the Δ4 pathway is ultimately favored. Another major difference is that the Leydig cell expresses a Leydig cell–specific isoform of 17β-hydroxysteroid dehydrogenase (**17β-HSD3**), which converts androstenedione to **testosterone** (see Fig. 9-4). Mutation of this specific gene in men results in a form of **disorders of sexual development** (**DSD**; see later).

TRANSPORT, ACTIONS, AND METABOLISM OF ANDROGENS

Intratesticular Androgen

The testosterone produced by Leydig cells has several metabolic fates and multiple actions (Box 9-2; Table 9-1). Because of the proximity of Leydig cells to the seminiferous tubules, significant amounts of testosterone diffuse into the seminiferous tubules and become concentrated within the adluminal compartment by ABP (see Fig. 9-4). Testosterone within the seminiferous tubules is maintained at a significantly higher level than the circulating testosterone level. This concentration of intratubular testosterone is absolutely required for normal spermatogenesis. As noted, Sertoli cells express the enzyme CYP19 (aromatase), which converts a small amount of testosterone into the highly potent estrogen estradiol-17β. Human sperm cells express at least one isoform of the estrogen receptor (ER), and there is some evidence from CYP19-aromatase-deficient men that this locally produced estrogen optimizes spermatogenesis in humans.

Peripheral Conversion to Estrogen

In several tissues (especially adipose tissue), testosterone is converted to **estrogen** (Fig. 9-5) by the enzyme **CYP19 (also called aromatase)**. This peripheral conversion is the primary source of estrogen production in men.

CLINICAL BOX 9-1

Studies in men with **CYP19-aromatase deficiency** have shown that inability to produce estrogen results in tall stature, owing to lack of epiphyseal closure in long bones, and osteoporosis. Thus, peripheral estrogen plays an important role in bone maturation and biology in men. These studies also implicated estrogen in promoting insulin sensitivity, improving lipoprotein profiles (i.e., increasing HDL, decreasing triglycerides and LDL), and exerting negative feedback on pituitary gonadotropins.

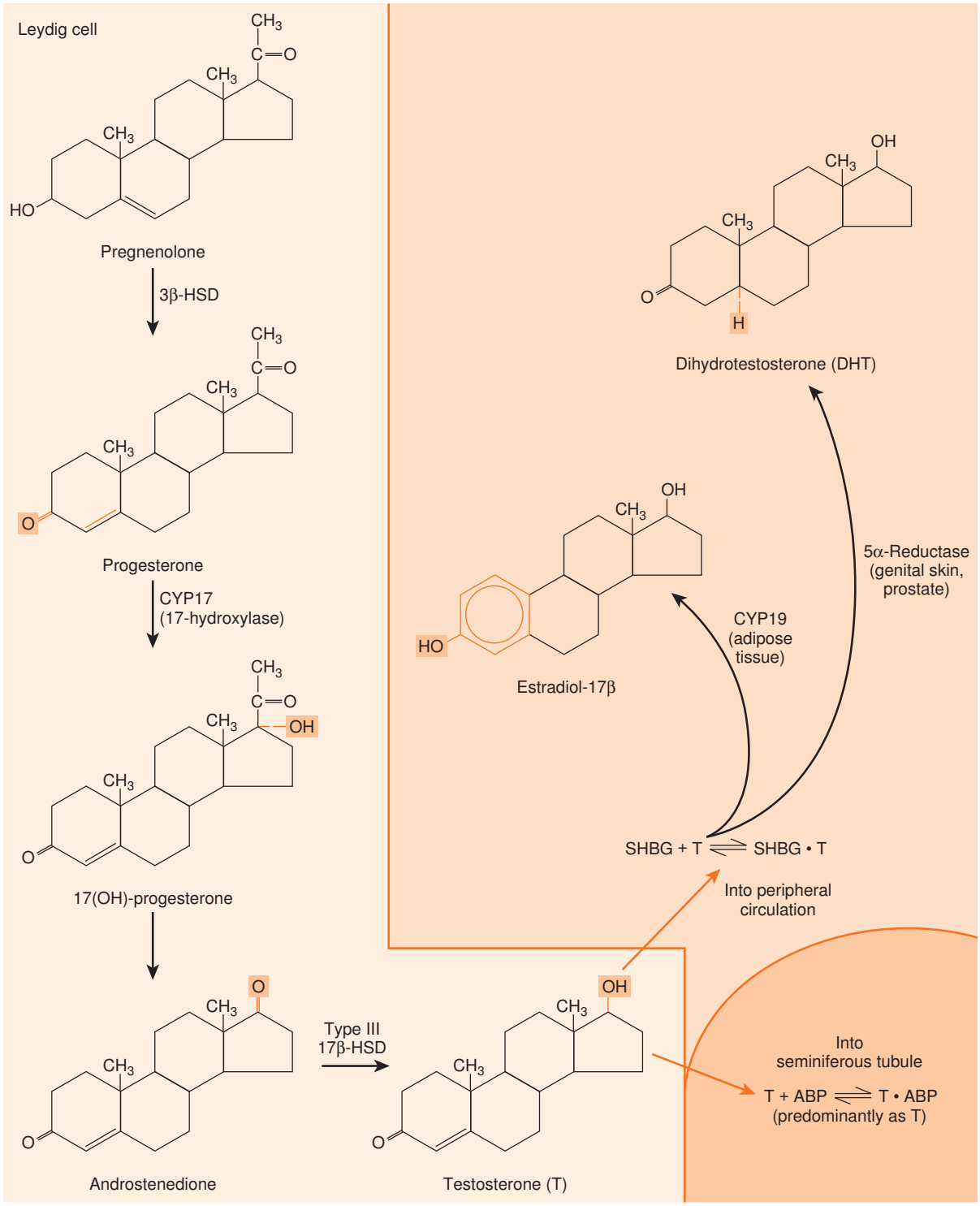


FIGURE 9-4 ■ Steroidogenic pathway in Leydig cells leading to testosterone production (conversion of cholesterol to pregnenolone is not shown). Testosterone (T) diffuses both into the neighboring seminiferous tubules and into the peritubular capillary network to be carried into the peripheral circulation. In the lumina of seminiferous tubules, T is concentrated by binding to androgen-binding protein (ABP). T is carried in the peripheral circulation by sex hormone–binding globulin (SHBG) and albumin. The Leydig cell makes limited amounts of DHT and estradiol-17β, but considerably more of these two steroids is made by peripheral conversion.

BOX 9-2

ACTIONS OF ANDROGENS

- Regulation of differentiation of male internal and external genitalia in fetus
- Stimulation of growth, development, and function of male internal and external genitalia
- Stimulation of sexual hair development
- Stimulation of sebaceous gland secretion
- Stimulation of erythropoietin synthesis
- Control of protein anabolic effects
- Stimulation of bone growth
- Closure of epiphyses as estrogen
- Initiation and maintenance of spermatogenesis
- Stimulation of androgen-binding protein synthesis (synergizes with follicle-stimulating hormone)
- Maintenance of secretions of sex glands
- Regulation of behavioral effects, including libido

TABLE 9-1

Approximate Hormone Production Rates in Adult Man

Testosterone	5 mg/day
Estradiol	10-15 μg/day
Dihydrotestosterone	50-100 μg/day

Peripheral Conversion to Dihydrotestosterone

Testosterone can also be converted into a potent, nonaromatizable androgen, **5α-dihydrotestosterone (DHT)**, by the enzyme **5α-reductase** (see Fig. 9-5). There are two isoforms of 5α-reductase: type 1 and type 2. Major sites of **5α-reductase-2** expression are the male urogenital tract, genital skin, hair follicles, and liver. 5α-Reductase-2 generates DHT, which is required for masculinization of the external genitalia and development of the prostate gland in utero, and in many of the changes associated with **puberty** (see Chapter 8), including growth and activity of the prostate gland, growth of the penis, darkening and folding of the scrotum, growth of pubic and axillary hair, facial and body hair, and increased muscle mass (see Fig. 9-5). Onset of 5α-reductase-1 expression occurs at puberty. This isozyme is expressed primarily in the skin and contributes to sebaceous gland activity and acne associated with puberty.

CLINICAL BOX 9-2

Because DHT has strong growth-promoting (i.e., trophic) effects on its target organs, the development of selective 5α-reductase-2 inhibitors has benefited the treatment of prostatic hypertrophy and prostatic cancer.

Peripheral Testosterone Actions

Individuals with 5α-reductase-2 deficiency are born with ambiguous or feminized external genitalia, thereby demonstrating the need for conversion of testosterone to DHT for an effect on some androgen-responsive tissues. However, testosterone can act as itself in several cell types (see Fig. 9-5). As mentioned previously, testosterone regulates Sertoli cell function. Testosterone induces the development of the male reproductive tract from the mesonephric duct (see Chapter 8) in the absence of 5α-reductase. Testosterone has several metabolic effects, including increasing very-low-density lipoprotein (VLDL) and LDL while decreasing HDL, promoting the deposition of abdominal adipose tissue, increasing red blood cell production, promoting bone growth and health, and having a protein anabolic effect on muscle. Testosterone is sufficient to maintain erectile function and libido.

Mechanism of Androgen Action

Testosterone and DHT act through the same **androgen receptor (AR)**. As described for other steroid hormone receptors (see Chapter 1), the AR resides in the cytoplasm bound to chaperone proteins in the absence of ligand. Testosterone-AR binding or DHT-AR binding causes dissociation of chaperone proteins, followed by nuclear translocation of the androgen-AR complex, dimerization, binding to an androgen-response element (ARE), and recruitment of co-regulatory proteins to the vicinity of a specific gene's promoter.

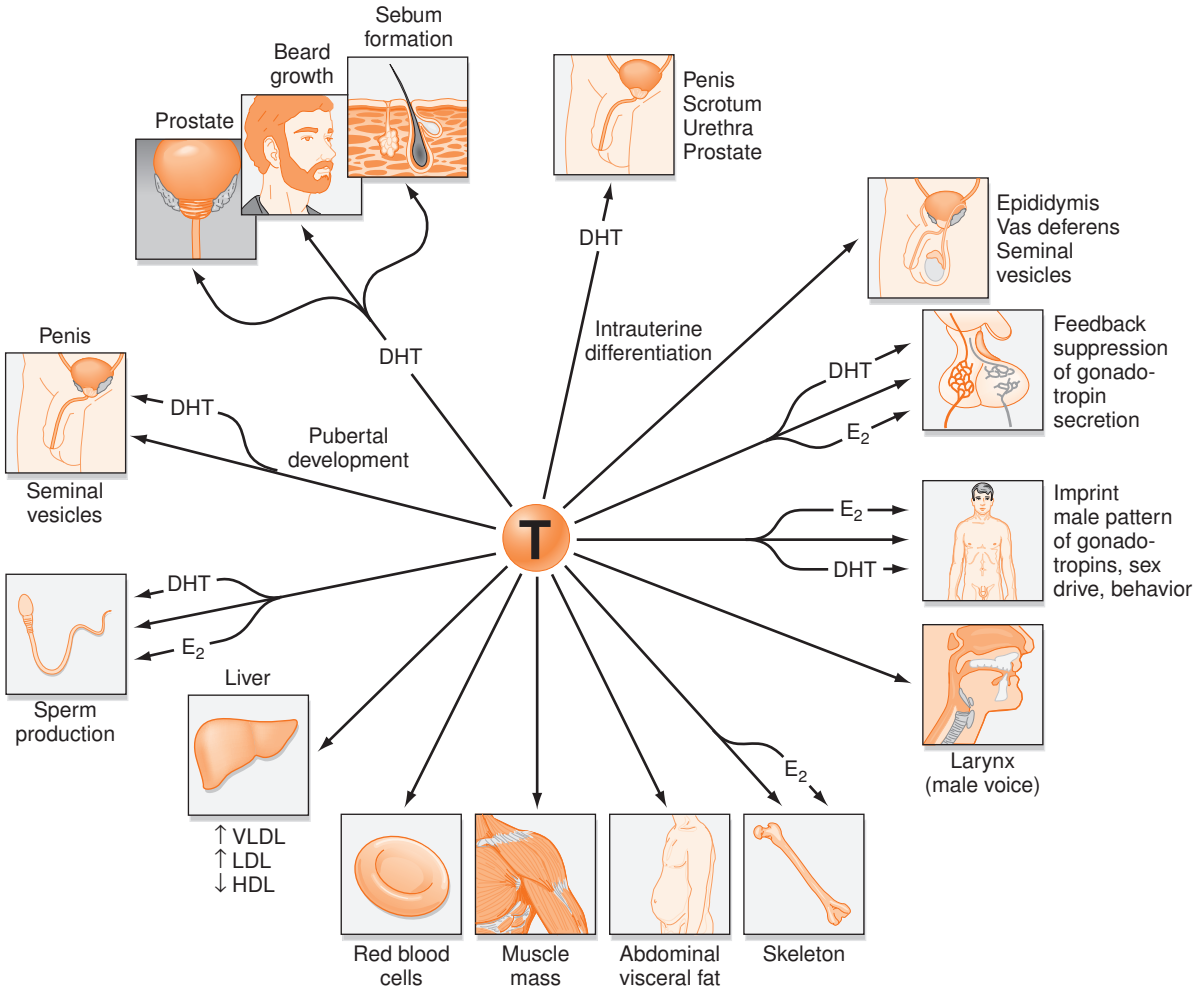


FIGURE 9-5 ■ Actions of testosterone, dihydrotestosterone (DHT), and estradiol in men. *(From Koeppen B, Stanton B:* Berne and Levy Physiology, *updated 6th ed., Philadelphia, 2010, Mosby.)*

It remains unclear how testosterone and DHT differ in their ability to activate the AR in the context of different cell types.

Transport and Metabolism of Androgens

As testosterone enters the peripheral circulation, it quickly reaches equilibrium with serum proteins. About 60% of circulating testosterone is bound to **SHBG**, 38% is bound to albumin, and about 2% remains as *free* hormone (see Fig. 9-5). Testosterone and its metabolites are excreted primarily in the urine. About 50% of excreted androgens are found as **urinary 17-ketosteroids**, with most of the remainder being conjugated androgens or diol or triol derivatives. Only about 30% of the 17-ketosteroids in urine are from the testis; the rest are produced from adrenal androgens. Androgens are **conjugated** with glucuronate or sulfate in the liver, and these conjugated steroids are excreted in the urine.

Androgen analogs are administered orally, sublingually, by intramuscular injection, by transdermal patch, and by subdermal slow-release pellets.

HYPOTHALAMUS-PITUITARY-TESTIS AXIS

The testis is regulated by an endocrine axis involving parvicellular hypothalamic **gonadotropin-releasing hormone** (**GnRH**)-secreting neurons and pituitary gonadotropes that produce both **luteinizing hormone** (**LH**) and **FSH** (Fig. 9-6). Recall from Chapter 5 that LH and FSH are pituitary glycoprotein hormones. They are heterodimers, composed of a common α-subunit—the α-glycoprotein subunit (αGSU)—and a specific β-subunit (either LH-β or FSH-β).

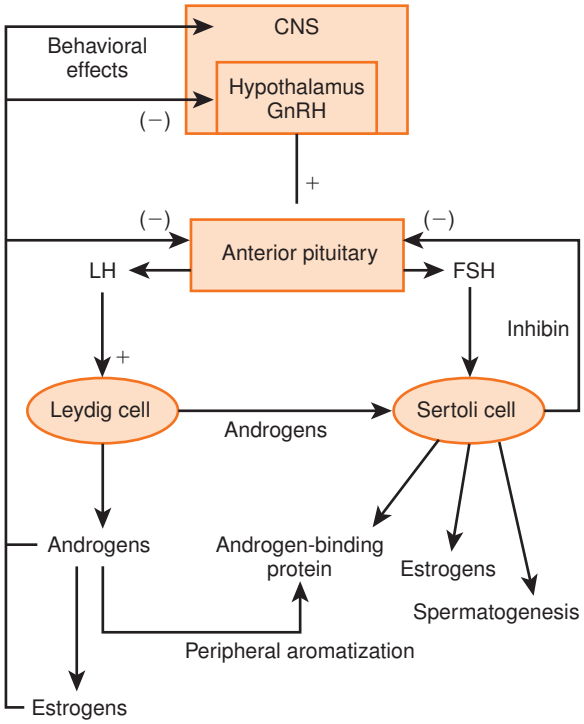


FIGURE 9-6 ■ Summary of regulation of testicular function. Gonadotropin-releasing hormone (GnRH) stimulates secretion of the anterior pituitary hormones luteinizing hormone (LH) and follicle-stimulating hormone (FSH). These hormones act on Leydig and Sertoli cells to stimulate production of hormones, androgen-binding protein, and sperm. Androgens (testosterone [T] and dihydrotestosterone [DHT]), estradiol, and inhibin control the production of LH and FSH through negative feedback. CNS, central nervous system.

Regulation of Leydig Cell Function

The Leydig cell expresses the **LH receptor**. LH acts on Leydig cells much like adrenocorticotropic hormone (ACTH) does on zona fasciculata cells (see Chapter 7). The LH receptor is coupled to a Gs-cyclic adenosine monophosphate cAMP-PKA signaling pathway (see Chapter 1). **Rapid effects** include hydrolysis of cholesterol esters and new expression of StAR. **Less acute effects** include an increase in steroidogenic enzyme gene expression and in the expression of the LDL receptor and SR-BI. Over the **long-term**, LH promotes Leydig cell growth and proliferation.

Testosterone has a negative feedback effect on LH production by the pituitary gonadotrope as testosterone, DHT, and estradiol-17β (see Fig. 9-7). All three steroid hormones inhibit the expression of LH-β, the GnRH receptor, and to a lesser extent, FSH-β. These steroids also inhibit the release of GnRH by the hypothalamic neurons.

Regulation of Sertoli Cell Function

Although testosterone, DHT, and estrogen exert negative feedback on both LH and FSH, they selectively inhibit LH more effectively than FSH. From a historical standpoint, this finding raised the possibility that a Sertoli cell–derived factor might feed back on FSH production. The Sertoli cell is stimulated by both testosterone and FSH. The FSH receptor also is coupled primarily to a Gs-cAMP-PKA pathway. In addition to stimulating the synthesis of proteins involved in the *nurse cell* aspect of Sertoli cell function (e.g., ABP), FSH stimulates the synthesis of the dimeric protein **inhibin**. Inhibin has a common α-subunit, coupled with either a β_A-subunit, called inhibin A, or a β_B-subunit, called inhibin B. Only inhibin B is expressed in men. Inhibin B expression is stimulated by FSH, and inhibin B exerts a negative feedback on the gonadotrope to selectively inhibit FSH production.

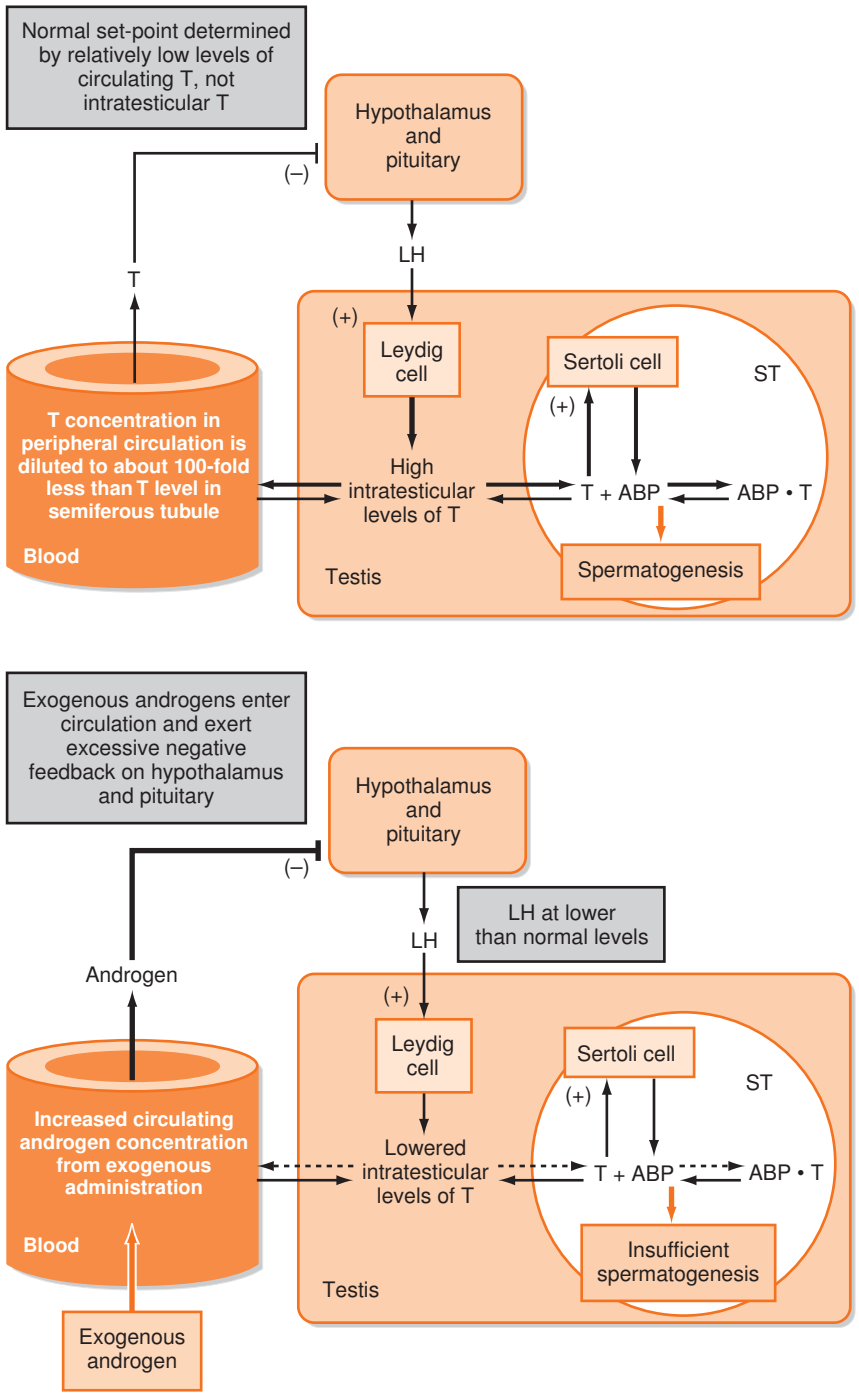


FIGURE 9-7 ■ The difference in intratesticular testosterone versus circulating testosterone concentrations and its importance in the hypothalamus-pituitary-testis axis. *Upper panel*, Feedback loop in a normal adult man. *Lower panel*, Administration of testosterone (or an androgenic analog) increases circulating testosterone (androgen) levels, which in turn increase negative feedback on release of luteinizing hormone (LH). Decreased LH levels diminish Leydig cell activity and intratesticular production of androgen. Lowered intratesticular testosterone levels result in reduced sperm production and can cause infertility. Note that the inhibin feedback loop has been omitted from this diagram. *(From Koeppen B, Stanton B:* Berne and Levy Physiology, *updated 6th ed., Philadelphia, 2010, Mosby.)*

CLINICAL BOX 9-3

There exists an important *loophole* in the male reproductive axis, which is based on the fact the **intratesticular levels of testosterone** need to be greater than 100-fold higher than circulating levels of the hormone to maintain normal rates of spermatogenesis, but it is the **circulating levels of testosterone** that provide the negative feedback to the pituitary and hypothalamus. This means that exogenous administration of testosterone can raise circulating levels sufficient to inhibit LH but not sufficient to concentrate in the testis at the required concentration for normal spermatogenesis. The decreased LH levels, however, will diminish intratesticular production of testosterone by Leydig cells, which will result in reduced levels of spermatogenesis (see Fig. 9-8). This *loophole* is currently being investigated as a possible strategy for developing a male oral contraceptive. It also is the basis for sterility in some cases of steroid abuse in men.

MALE REPRODUCTIVE TRACT

Once spermatozoa emerge from the **efferent ductules**, they leave the gonad and enter the extratesticular portion of the male reproductive tract (Fig. 9-8; see also Fig. 8-2 in Chapter 8). The segments of the tract are as follows: the **epididymis** (head, body, and tail), the **vas deferens**, the **ejaculatory duct**, the **prostatic urethra**, the **membranous urethra**, and the **penile urethra**. Unlike in the female tract:

- There is a continuous lumen from the seminiferous tubule to the end of the male tract (i.e., the tip of the penile urethra).
- The male tract connects to the distal urinary tract (i.e., male urethra).

In addition to conveying sperm, the primary functions of the male reproductive tract are as follows:

1. Sperm spend about a month in the **epididymis**, where they undergo further maturation. The epithelium of the epididymis is actively secretory and adds numerous proteins and glycolipids to the seminal fluid. Spermatozoa that enter the head of the epididymis are weakly motile but are **strongly unidirectionally motile** by the time they exit the tail. Spermatozoa also may undergo the process of **decapacitation**, which involves stabilization of their cell membranes to prevent spermatozoa from undergoing the acrosomal reaction before contact with an egg (see Chapter 11). Sperm become capacitated by the female reproductive tract within the oviduct (see Chapter 10). The function of the epididymis is dependent on **luminal testosterone-ABP complexes** that come from the seminiferous tubules and on testosterone from the blood. Of note, the epididymal epithelium is extremely tight, so a **blood-epididymis barrier** exists.
2. Sperm are stored in the tail of the epididymis and vas deferens. Sperm can be stored for several months without loss of viability. The primary function of the vas deferens, besides providing a storage site, is to propel sperm during sexual intercourse into the male urethra. The vas deferens has a very thick muscularis that is richly innervated by sympathetic nerves. Normally in response to repeated tactile stimulation of the penis during coitus, the muscularis of the vas deferens receives bursts of sympathetic stimulation, causing peristaltic contractions. The emptying of the contents of the vas deferens into the prostatic urethra is called **emission**. Emission immediately precedes **ejaculation**, which is the propulsion of semen out of the male urethra.
3. During emission, contraction of the vas deferens coincides with contraction of the muscular coats of the two accessory sex glands: the **seminal vesicles** (right and left) and the **prostate gland** (which surrounds the prostatic urethra). At this point, sperm become mixed with all the components of semen. The seminal vesicles secrete about 60% of the volume. These glands are the primary source of **fructose**, a critical nutrient for sperm. Seminal vesicles also secrete semenogelins, which induce coagulation of semen immediately after ejaculation. The alkaline secretions of the prostate, which make up about 30% of the volume, are high in citrate, zinc, spermine, and acid phosphatase. **Prostate-specific antigen (PSA)** is a serine protease that liquefies coagulated semen after a few minutes. PSA can be detected in the blood under conditions of prostatic infection, benign prostatic hypertrophy, and prostatic carcinoma and is currently used as

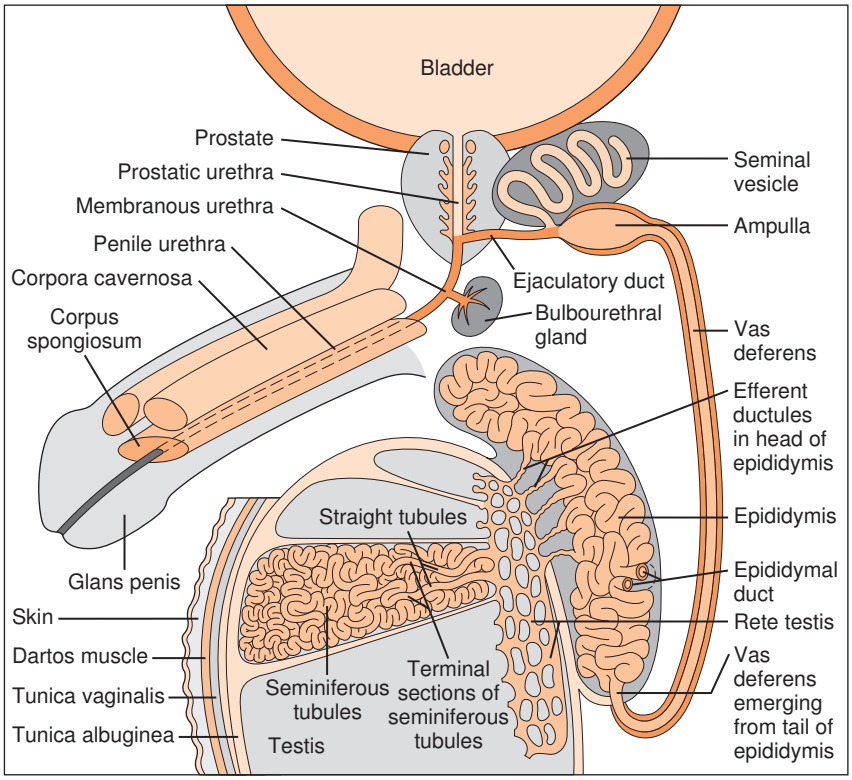
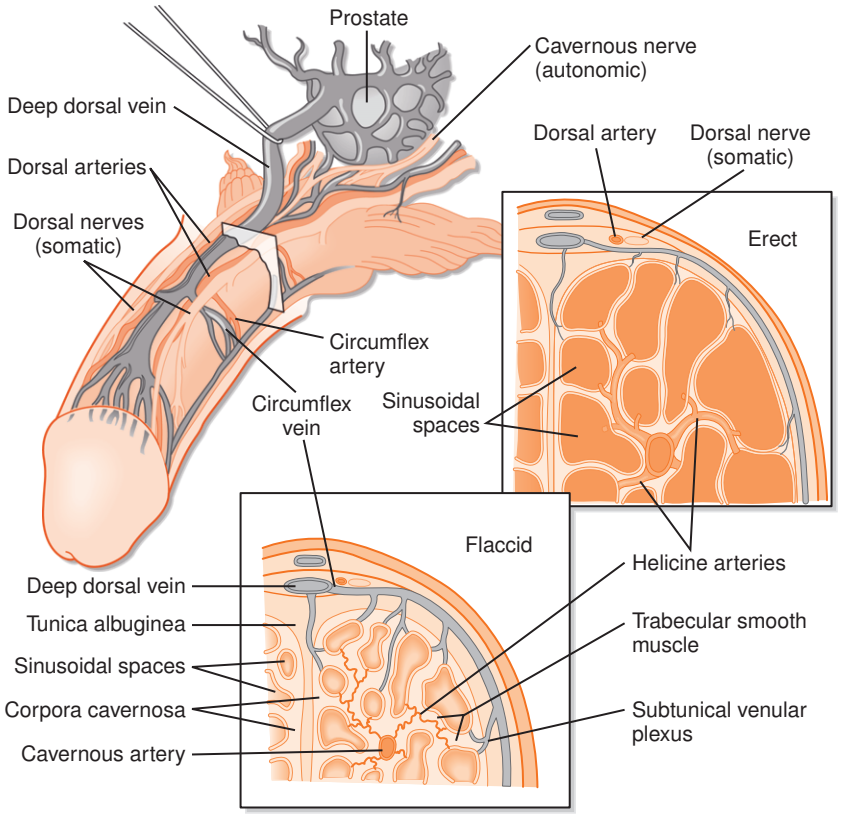


FIGURE 9-8 ■ Diagram of male reproductive tract, including the intratesticular portion of the tract. *(Redrawn from Stevens A, Lowe J:* Human Histology, *3rd ed., Philadelphia, 2005, Mosby.)*

one indicator of prostatic health. The predominant buffers in semen are phosphate and bicarbonate. The **bulbourethral glands** (also called Cowper glands) empty into the penile urethra in response to sexual excitement before emission and ejaculation. **Paraurethral glands** (glands of Littre) similarly secrete along the length of the male urethra. The mucous bulbourethral and paraurethral secretions lubricate, cleanse, and buffer the urethra. Average sperm counts are reported to be from 60 to 100 million/mL semen. Men with sperm counts below 20 million/mL, less than 50% motile sperm, or less than 60% normally formed sperm usually are infertile.

4. As noted, emission and ejaculation occur during coitus in response to a reflex arc that involves sensory stimulation from the penis (through the **pudendal nerve**) followed by **sympathetic motor stimulation** to the smooth muscle of the male tract and **somatic motor stimulation** (through the **pudendal nerve**) to the musculature associated with the base of the penis. However, for sexual intercourse to occur in the first place, the male partner has to achieve and maintain an **erection of the penis**. The penis has evolved as an intromittent organ designed to separate the walls of the vagina, pass through the potential space of the vaginal lumen, and deposit semen at the deep end of the vaginal lumen near the cervix. This process of internal insemination can be performed only if the penis is stiffened from the process of erection.

Erection is a neurovascular event (Fig. 9-9). The penis is composed of three erectile bodies: two **corpora cavernosa** and one **corpus spongiosum**. The penile urethra runs through the corpus spongiosum (and is also called the **spongy urethra**). These three bodies are composed of **erectile tissue**—an anastomosing network of potential **cavernous vascular spaces** lined with continuous endothelia within a loose connective tissue support. During the flaccid state, blood flow to the cavernous spaces is minimal (see Fig. 9-9A), because of vasoconstriction of vasculature that shunts blood flow away from the cavernous spaces. In



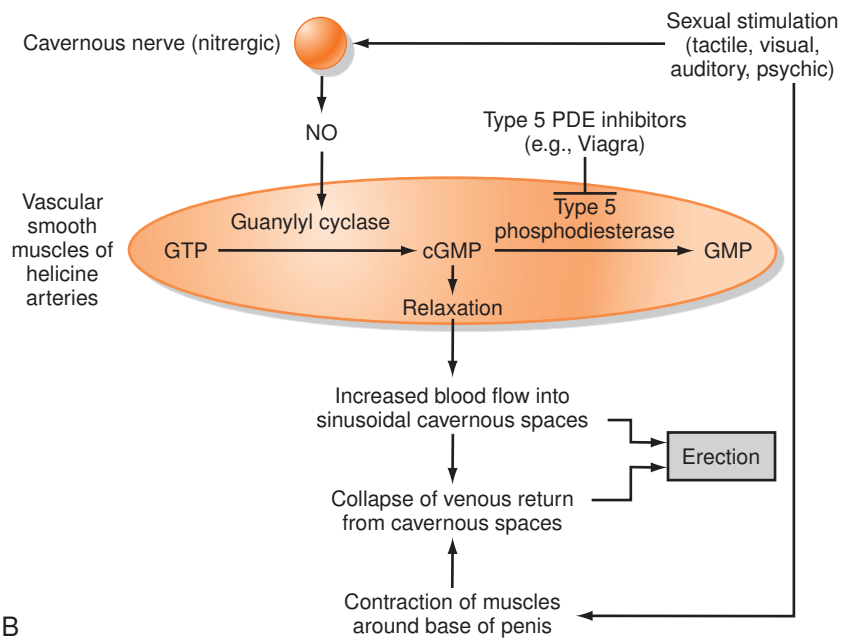


FIGURE 9-9 ■ A, Arrangement of the vasculature and cavernous tissue within the penis. During the flaccid state, blood flow into the cavernous spaces is limited by contraction of the helicine arteries. **B,** Outline of neurovascular events leading to penile erection. *(From Bhasun S, et al. In Larsen P, Kronenberg H, Melmed S, et al, editors:* Williams Textbook of Endocrinology, *10th ed., Philadelphia, 2003, Saunders.)*

response to sexual arousal, parasympathetic nerves innervating the vascular smooth muscle of the helicine arteries that supply blood to the cavernous spaces release **nitric oxide (NO)**. NO activates guanylyl cyclase, increasing cyclic guanosine monophosphate (cGMP), which decreases intracellular Ca^{2+} and causes relaxation of the vascular smooth muscle (see Fig. 9-9B). The vasodilation allows blood to flow into the spaces, causing engorgement and erection (see Fig. 9-9. The veins in the penis course to the circumference of the penis before emptying into the deep dorsal vein. During erection, the engorged tissue presses the veins against a noncompliant outer fascia, thereby reducing venous drainage. Finally, somatic stimulation increases contraction of muscles at the base of the penis, further promoting erection.

CLINICAL BOX 9-4

Inability to achieve or maintain an erection is termed **erectile dysfunction** (**ED**) and is one cause of **infertility**. Multiple factors can lead to ED, such as insufficient androgen production; neurovascular damage (e.g., from diabetes mellitus, spinal cord injury); structural damage to the penis, perineum, or pelvis; psychogenic factors (e.g., depression, performance anxiety); prescribed medications; and recreational drugs, including alcohol and tobacco. A major development in the treatment of some forms of ED is availability of selective cGMP phosphodiesterase inhibitors, which assist in the maintenance of an erection.

DISORDERS INVOLVING THE MALE REPRODUCTIVE SYSTEM

Klinefelter Syndrome (XXY Seminiferous Tubule Dysgenesis)

Men with an extra X chromosome have the genetic disorder called **Klinefelter syndrome** (also called **seminiferous tubular dysgenesis**). Although there are multiple permutations of the disorder, the most common form results in a 47,XXY karyotype. Affected persons are phenotypically male because of the presence of the Y chromosome, and they appear normal at birth. At puberty, increased levels of gonadotropins fail to induce normal testicular growth and spermatogenesis. Instead, the testis becomes fibrotic and hyalinized and remains small and firm. The seminiferous tubules are largely destroyed, resulting in infertility. However, some patches of tubules may exist, allowing for extraction of sperm to be used in intracytoplasmic sperm injection (ICSI) into an egg as part of an assisted reproductive procedure. Androgen production is usually low (but this is highly variable among patients), whereas the levels of gonadotropins are elevated, thereby indicating primary hypogonadism. A small penis and lack of body hair are two signs of reduced androgen production (Fig. 9-10). An elevated estradiol-to-testosterone ratio can lead to moderate feminization, including the potential for limited

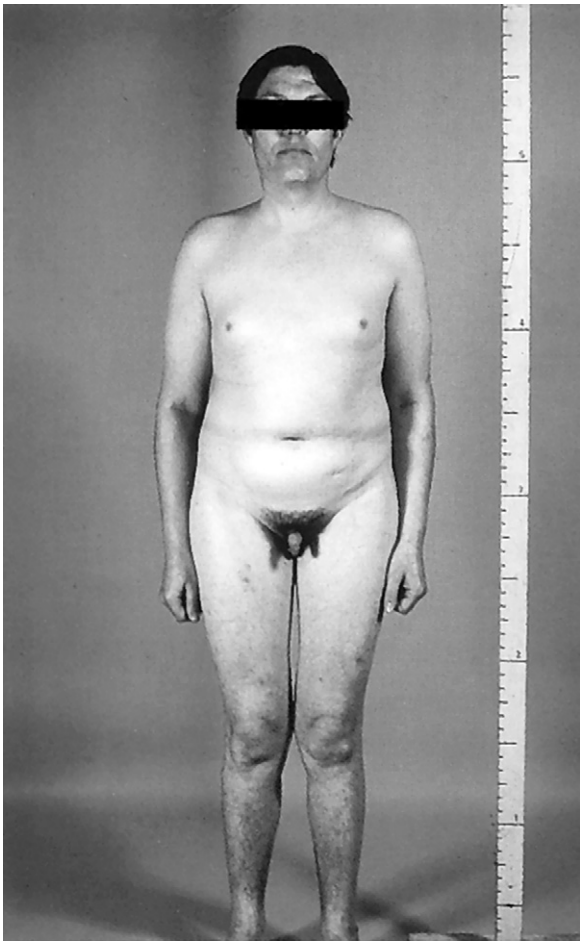


FIGURE 9-10 ■ Klinefelter syndrome in a young man. Limited gynecomastia is present, and body shape is somewhat feminine. *(From Besser GM, Thorner MO:* Clinical Endocrinology, *2nd ed., London, 1994, Mosby-Wolfe.)*

gynecomastia (inappropriate development of breasts). Klinefelter syndrome is associated with a compromised intellectual development, behavioral problems, alterations in bone growth and density, and several other comorbidities. Androgen replacement to induce virilization is the most common treatment.

Androgen Insensitivity Syndrome Androgen insensitivity syndrome (AIS) results from a hereditary defect of the X chromosome gene controlling AR expression. Because the defect can range from partial to complete inability of the AR to respond to androgens, the degree of feminization of AIS is variable. Because the karyotype is 46,XY, the gonad develops into the testis, which produces testosterone and MIS in utero. The mesonephric (wolffian) duct does not develop into male structures, however, because androgen action is deficient, and MIS causes the müllerian duct to regress. Consequently, there are no functional internal genitalia.

The external genitalia typically develop as female, and the phenotype is female (Fig. 9-11). In severe AIS, the affected person has labia, a clitoris, and a short, blind vagina. Pubic and axillary hair is absent or sparse because the development of sexual hair is androgen dependent. Menstruation does not occur (see Chapter 10), and serum androgen levels are high or normal. When androgen production rises at puberty, estradiol production increases, both from the testes and from peripheral aromatization of androgens. LH levels remain elevated throughout adulthood because testosterone and DHT cannot exert negative feedback on the pituitary and hypothalamus because of a defective AR. The increase leads to dividing, hypertrophic Leydig cells that produce enhanced amounts of androstenedione, testosterone, and estradiol-17β. The androgens are peripherally converted to estrogens, which feminize the individual in a manner unopposed by androgenic actions. The phenotype that is derived from hyperstimulated Leydig cells secreting steroids that are converted into estrogens and lead to feminization is called **testicular feminization**. The overall condition generally is referred to as **male pseudohermaphroditism**. The designator "male" is appropriate because the genotype of the affected person is XY. The term pseudohermaphroditism refers to the presence of an incomplete mix of external genitalia, which are feminine in this case, and internal genitalia, which is a male gonad in this case.

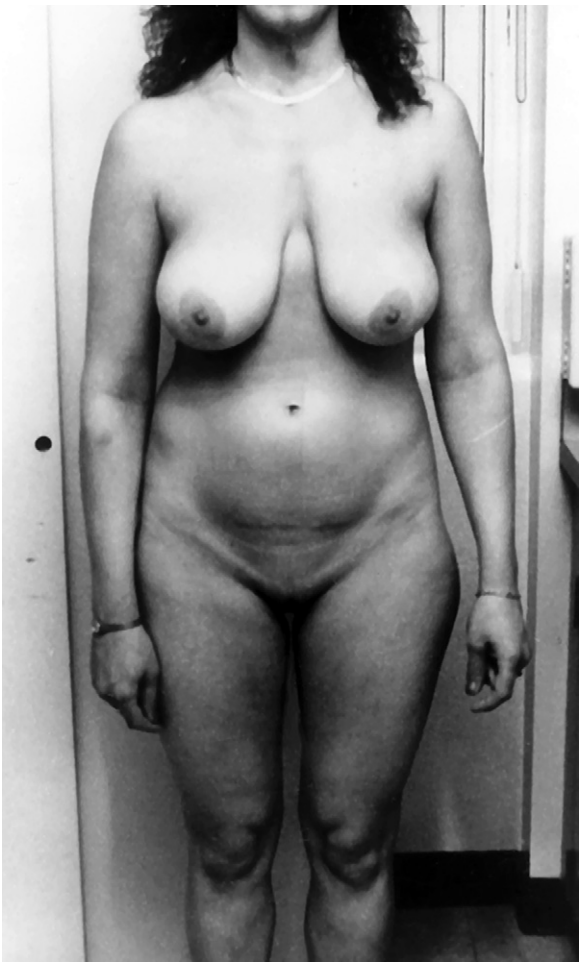


FIGURE 9-11 ■ A 46, *XY* patient with complete androgen insensitivity and female phenotype. Full breast development and female body form (e.g., widened pelvis) constitute evidence of testicular feminization. *(From Quigley CA, De Bellis A, Marschke KB, et al: Androgen receptor defects: Historical, clinical, and molecular perspectives.* Endocr Rev *16:271, 1995.)*

This terminology is being replaced in favor of the less embarrassing (to the patient) and pejorative term **disorder of sexual development (DSD)/androgen insensitivity syndrome**.

Note that the testes typically remain in the abdomen because androgen action is required for testicular descent. Because of gonadotropic hyperstimulation, the gonads represent a probable site for cancerous growth and are surgically removed as a precaution.

SUMMARY

1. Seminiferous tubules (the intratubular compartment) contain Sertoli cells and developing sperm. Sertoli cells have a *supportive* function, providing the proper microenvironment for sperm development. Tight junctions between adjacent Sertoli cells create the blood-testis barrier. Sertoli cells also have an important exocrine function, producing fluid and androgen-binding protein. In addition, Sertoli cells have an endocrine function, producing antimüllerian hormone and inhibin. Sertoli cells express the androgen receptor and the FSH receptor.
2. Spermatogenesis involves mitosis and meiosis. The final product is haploid spermatozoa. Normal spermatogenesis is dependent on FSH and high intratesticular levels of testosterone. However, sperm cells do not express the androgen receptor or the FSH receptor and are completely dependent on Sertoli cells for their development.
3. Leydig cells reside in the peritubular compartment. Leydig cells express the LH receptor and produce testosterone, as well as small amounts of DHT and estradiol-17β.
4. Testosterone can be converted peripherally to DHT (e.g., in the prostate gland) or estradiol-17β (e.g., in adipose tissue). Testosterone and DHT regulate secondary sex characteristics and are required for the normal development, growth, and function of the male reproductive tract. Estrogen is required for normal bone mineralization and epiphyseal plate closure in men, and for modulation of lipoprotein profile (lowered VLDL and LDL, increased HDL).
5. The endocrine function is regulated within a hypothalamus-pituitary-testis axis. GnRH is produced by hypothalamic neurons and stimulates LH and FSH production by pituitary gonadotropes. Circulating levels of testosterone, and to some extent DHT and estradiol-17β, exert a negative feedback at both the pituitary and the hypothalamus.
6. The male reproductive tract includes the epididymis, the vas deferens, the ejaculatory duct, and the male urethra. The male tract also includes accessory sex glands, the seminal vesicles, and the prostate gland. The secretions of these glands produce most of the volume of semen. Semen serves to provide bulk to sperm, maintain an alkaline environment for sperm, provide nutrients to sperm, prevent sperm capacitation, and inhibit sperm motility in the male reproductive tract. Emission and ejaculation are achieved through primarily sympathetic stimulation of the muscularis of the male tract and somatic stimulation of pelvic muscles.
7. The male tract also includes a copulatory organ, the penis. Erection of the penis is required for internal insemination of the female tract. Erection of the penis is a neurovascular process, involving parasympathetic stimulation of erectile tissue arterioles leading to vasodilation and engorgement of the cavernous spaces. Multiple factors can lead to erectile dysfunction.
8. Klinefelter syndrome (gonadal dysgenesis) results when men have an extra X chromosome. Fibrotic changes in the testis destroy most of the seminiferous tubules.
9. Androgen insensitivity syndrome results from a hereditary defect in the gene controlling androgen receptor expression. As a result of diminished feedback, LH levels are elevated, as are testosterone levels. More testis-derived testosterone is converted to estrogens, resulting in a female phenotype (enhanced breast development, female pelvis). This process is called testicular feminization. The overall condition (high estrogen levels in the absence of androgen effects) gives rise to male pseudohermaphroditism.

SELF-STUDY PROBLEMS

1. What is the relationship of Sertoli cells to the basal and adluminal compartments of the seminiferous tubules?
2. Name two endocrine products of Sertoli cells and their function.
3. Describe the structure of a spermatozoon. What is the process from spermatid to spermatozoon called?
4. Explain how the congenital loss of 17β-hydroxysteroid dehydrogenase (type 3) would affect the following: spermatogenesis, external genitalia, breast development.
5. How does abuse of androgens cause low sperm count?
6. Name one event that occurs in developing sperm cells during the following: spermatocytogenesis, spermiogenesis, passage through the epididymis, emission.
7. What is the role of the seminal vesicles and prostate. What is PSA?
8. How does cGMP control erection?

KEYWORDS AND CONCEPTS

- 3β-hydroxysteroid dehydrogenase type 2 (3β-HSD2)
- 5α-Dihydrotestosterone (DHT)
- 5α-Reductase

For full list of keywords and concepts see Student Consult

SUGGESTED READINGS

Hu GX, Lian QQ, Ge RS, et al: Phthalate-induced testicular dysgenesis syndrome: Leydig cell influence, *Trends Endocrinol Metab* 20:139–145, 2009.

Lee YS, Cheng AW, Ahmed SF, et al: Genital anomalies in Klinefelter's syndrome, *Horm Res* 68:150–155, 2007.

Miner MM: Erectile dysfunction: A harbinger or consequence. Does its detection lead to a window of curability? *J Androl* 32:125–134, 2011.

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KEYWORDS AND CONCEPTS

- 5α-Reductase-2
- 17β-HSD3
- Acrosomal vesicle
- Adluminal compartment
- Androgen insensitivity syndrome
- Androgen receptor (AR)
- Androgen-binding protein (ABP)
- Antimüllerian hormone (AMH)
- Aromatase
- Axoneme
- Basal compartment
- Blood-epididymis barrier
- Blood-testis barrier
- Bulbourethral glands
- Cavernous vascular spaces
- Centrioles
- Chiasmata
- Circulating levels of testosterone
- Conjugated
- Corpora cavernosa
- Corpus spongiosum
- Crossing-over
- CYP11A1
- CYP17
- CYP19
- CYP19-aromatase deficiency
- Decapacitation
- Disjunction
- Disorder of sexual development (DSD)
- Efferent ductules
- Ejaculation
- Ejaculatory duct
- Emission
- Epididymis
- Erectile dysfunction (ED)
- Erectile tissue
- Erection
- Estradiol-17β
- Estrogen
- Flagellum
- Follicle-stimulating hormone (FSH)
- Fructose
- FSH receptor
- Gonadotropin-releasing hormone (GnRH)
- Gynecomastia
- Head
- Hormone-sensitive lipase (HSL)
- Incomplete cytokinesis
- Infertility
- Inhibin
- Interstitial cells of Leydig
- Intratesticular levels of testosterone
- Intratubular compartment
- Klinefelter syndrome
- Less acute effects
- Leydig cell
- LH receptor
- Lobules
- Long-term
- Luminal testosterone-ABP complexes
- Luteinizing hormone (LH)
- Male pseudohermaphroditism
- Membranous urethra
- Müllerian-inhibiting substance (MIS)
- Neck
- Nitric oxide (NO)
- Nucleus
- Paraurethral glands
- Penile urethra
- Peritubular compartment
- Phagocytic function
- Prespermatogonia
- Primary spermatocytes
- Prostate gland
- Prostate-specific antigen (PSA)
- Prostatic urethra
- Protamines
- Puberty
- Pudendal nerve
- Rapid effects
- Residual bodies
- Rete testis
- Scavenger receptor-BI (SR-BI)
- Scrotum
- Secondary spermatocytes
- Seminal vesicles
- Seminiferous epithelium
- Seminiferous tubular dysgenesis
- Seminiferous tubules
- Sertoli cell

- Sex hormone–binding globulin (SHBG)
- Somatic motor stimulation
- Sperm cells
- Spermatids
- Spermatocytogenesis
- Spermatogenesis
- Spermatogenic cycle
- Spermatogenic stages
- Spermatogenic wave
- Spermiation
- Spermiogenesis
- Spongy urethra
- Stem spermatogonia
- Steroidogenic acute regulatory protein (StAR)
- Strongly unidirectionally motile
- Sympathetic motor stimulation
- Synapsis
- Tail
- Testicular feminization
- Testosterone
- Urinary 17-ketosteroids
- Vas (ductus) deferens

10 THE FEMALE REPRODUCTIVE SYSTEM

OBJECTIVES

1. Describe the anatomy and histology of the ovary and the development of the ovarian follicle.
2. Describe the steroidogenic pathways in the ovarian follicle and the functions of the ovarian steroids, estradiol-17β and progesterone.
3. Discuss the hypothalamus-pituitary-ovarian axis in the context of the monthly menstrual cycle.
4. Discuss the changes in the physiology of the female reproductive tract throughout the menstrual cycle.
5. Describe the anatomy and function of the female external genitalia during the female sexual response.
6. Discuss pathophysiologic conditions of the female reproductive system, including Turner syndrome and polycystic ovarian syndrome.

The physiology of pregnancy and the development and functions of the placenta and mammary glands are discussed in Chapter 11.

The female reproductive system is composed of the gonads, called **ovaries**, and the female reproductive tract. The mammary glands (breasts) are also part of the female reproductive system. Like the male gonads, the ovaries perform an **endocrine** function and a **gametogenic** function. The endocrine function is regulated within a hypothalamic-pituitary-ovarian axis, and ovarian hormones are absolutely necessary for the health and normal function of the female tract. The female reproductive system differs from the male system in several important general aspects (Box 10-1).

ANATOMY AND HISTOLOGY OF THE OVARY

The **ovary** is located within a fold of peritoneum called the broad ligament, usually close to the lateral wall of the pelvic cavity (Fig. 10-1). The ovary extends into the peritoneal cavity, and ovulated eggs briefly reside within the peritoneal cavity before they are captured by the oviducts. Nerves and blood vessels enter and exit the ovary at both its lateral and medial poles.

The ovary can be roughly divided into an outer cortex and an inner medulla (Fig. 10-2). The neurovascular elements run into the medulla of the ovary. The cortex of the ovary is composed of a densely cellular stroma. Within this stroma reside the **ovarian follicles**, which contain a primary oocyte surrounded by follicle cells (see later). The cortex is covered by a connective tissue capsule, called the tunica albuginea, and a layer of simple epithelium, called **ovarian surface epithelial cells**. There are no ducts emerging from the ovary to convey its gametes to the reproductive tract. Thus, the process of ovulation involves an inflammatory event that erodes the wall of the ovary and follicle. After ovulation, the ovarian surface epithelial cells rapidly divide to repair the wall. It is this highly mitogenic population of cells, the ovarian surface epithelial cells, which gives rise to more than 80% of cases of ovarian cancer.

BOX 10-1

MAJOR DIFFERENCES BETWEEN MALE AND FEMALE REPRODUCTIVE SYSTEMS

MALE REPRODUCTIVE SYSTEM	FEMALE REPRODUCTIVE SYSTEM
Gonads (testes) reside outside of abdominal cavity, in scrotum	Gonads (ovaries) reside within abdominal cavity
Gonad is continuous with reproductive tract	Gonad is not continuous with reproductive tract
Release of gametes (sperm) from gonads is continuous	Release of gamete (egg) from gonads occurs once per month
Gametic reserve is replenished throughout life	Gametic reserve is finite and exhausted by menopause
Testosterone exerts negative feedback on secretion of pituitary LH and FSH	Estrogen exerts both negative and positive feedback on secretion of pituitary LH and FSH
Male tract serves only male gamete transport and maturation and delivery	Female tract serves male and female gamete transport and maturation, fertilization, placentation, and gestation
Activity of male tract does not show rhythm	Activity of female tract is based on the monthly menstrual cycle, or on the length of a pregnancy (normally about 9 months)
Testosterone is always the primary gonadal steroid	Estrogen is the primary gonadal steroid in the first half of the monthly cycle, and progesterone in the second half
The male reproductive system does not prepare for newborn	The female reproductive system prepares for newborn with breast development and milk production and is involved in breastfeeding of the newborn

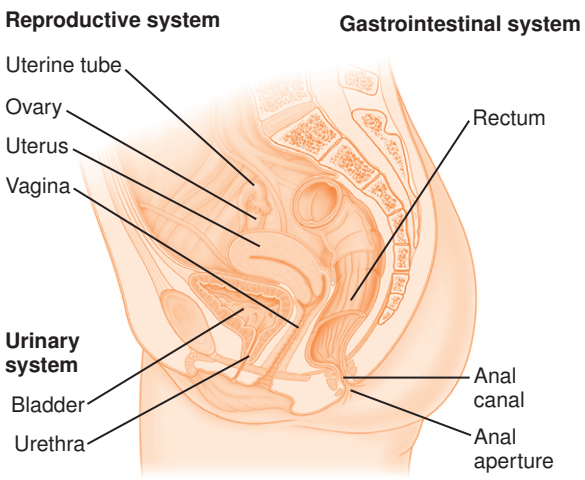

FIGURE 10-1 ■ Anatomy of the female pelvis and midsagittal section. *(From Drake RL, Vogl W, Mitchell AWM:* Gray's Anatomy for Students, *Philadelphia, 2005, Churchill Livingstone.)*

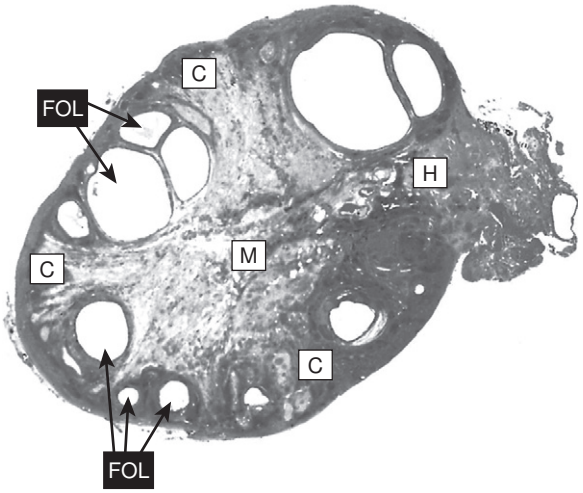

FIGURE 10-2 ■ Histologic features of the ovary. Micrograph of the ovary shows the hilum (H), medulla (M), and cortex (C). Follicular (FOL) formation and maturation occur in the cortex and are responsible for the cystic spaces seen here. *(Modified from Stevens A, Lowe J:* Human Histology, *3rd ed., Philadelphia, 2005, Mosby.)*

GROWTH, DEVELOPMENT, AND FUNCTION OF THE OVARIAN FOLLICLE

The **ovarian follicle** is the functional unit of the ovary, performing both gametogenic and endocrine functions. A histologic section of the ovary from a premenopausal cycling woman contains follicular structures at many different points of their development. The life history of a follicle can be divided into the following stages:

1. Resting primordial follicle
2. Growing preantral (primary and secondary) follicle
3. Growing antral (tertiary) follicle
4. Dominant (preovulatory, graafian) follicle
5. Dominant follicle within the periovulatory period
6. Corpus luteum (of menstruation or of pregnancy)
7. Atretic follicles

In this section, follicular biology is discussed in terms of the following:

- Growth and structure of the follicle
- State of the gamete
- Endocrine function of the follicle cells

Resting Primordial Follicle

Growth and Structure Resting primordial follicles represent the earliest and simplest follicular structure in the ovary. Primordial follicles appear during midgestation through the interaction of gametes and somatic cells. The approximately 7 million oogonia enter the process of meiosis, thereby becoming primary oocytes (see Chapter 8). The primary oocytes arrest in prophase of meiosis I.

During this time, the primary oocytes become surrounded by a simple epithelium of somatic follicle cells, thereby creating primordial follicles (Fig. 10-3). The follicle cells (also called **pregranulosa cells**) establish **gap junctions** with each other and the oocyte. The follicle cells themselves represent a true avascular epithelium, surrounded by a basal lamina. As in Sertoli cell–sperm interactions, the granulosa cells remain intimately attached to the oocyte throughout its development. Granulosa cells provide nutrients such as amino acids, nucleic acids, and pyruvate to support oocyte maturation.

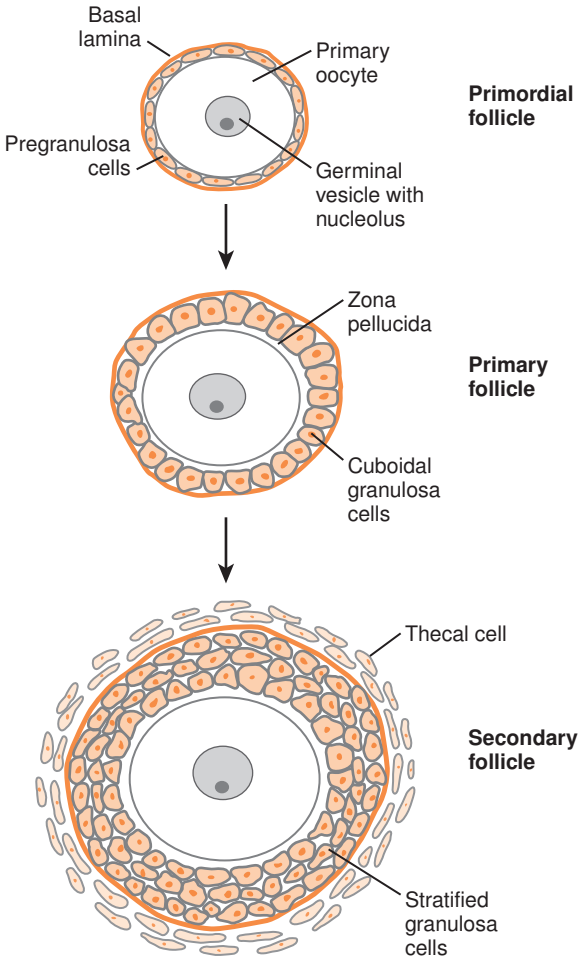

FIGURE 10-3 ■ Early follicular development from a primordial follicle to a secondary, preantral follicle.

Primordial follicles represent the **ovarian reserve** of follicles (Fig. 10-4). This is reduced from a starting number of about 7 million to less than 300,000 follicles at reproductive maturity. Of these, a woman will ovulate about 450 between menarche (first menstrual cycle) and menopause (cessation of menstrual cycles). At menopause, less than 1000 primordial follicles are left in the ovary. Primordial follicles are lost primarily from death due to follicular **atresia**. A small subset of primordial follicles, however, will enter follicular growth in waves. Because the ovarian follicular reserve represents a fixed, finite number, the rate at which

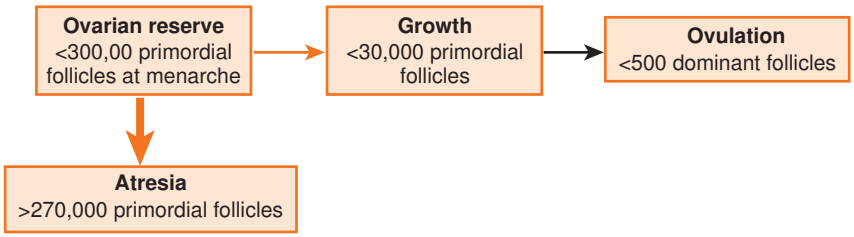

FIGURE 10-4 ■ Fate of the ovarian reserve in women.

resting primordial follicles die or begin to develop will determine the reproductive life span of a woman.

CLINICAL BOX 10-1

Determination of the age at which a woman will reach menopause has a strong genetic component but also is influenced by environmental factors. For example, cigarette smoking significantly depletes the ovarian reserve. An overly rapid rate of atresia or development also depletes the reserve, giving rise to **premature ovarian failure,** defined as entering **menopause** before the age of 40 years. Premature ovarian failure can also be caused by severe infections or tumors of the pelvis, by chemotherapy and radiation, and by endocrine factors that disrupt the hypothalamus-pituitary-ovarian axis.

The rate at which resting primordial follicles enter the growth process appears to be independent of pituitary gonadotropins. There is evidence in mice that follicle cells stimulate oocyte growth through paracrine factors. Reciprocal regulation of granulosa cell growth by the oocyte also probably occurs. Additional evidence indicates that factors from growing follicles provide restraint on the development of too many primordial follicles. One such factor appears to be **antimüllerian hormone (AMH)**. AMH-knockout mice deplete their ovarian reserve more rapidly than do wild-type mice, as a result of a high rate of follicular development. In summary, whether a resting follicle enters the early growth phase is dependent primarily on intraovarian paracrine factors that are produced by both the follicle cells and oocytes.

The Gamete As mentioned previously, the gamete in primordial follicles is derived from oogonia that have entered the first meiotic division and are now called **primary oocytes**. These primary oocytes progress through most of prophase of the first meiotic division (termed **prophase I**) over a 2-week period and then arrest in the diplotene stage. This stage is characterized by decondensation of chromatin, which supports transcription needed for oocyte maturation. Meiotic arrest at this stage, which may last up to 50 years, appears to be due to "maturational incompetence," or the lack of necessary cell cycle proteins to support the completion of meiosis. The nucleus of the oocyte, called the germinal vesicle, remains intact at this stage.

Endocrine Function Although primordial follicles release paracrine factors, they do not produce ovarian steroid hormones.

Growing Preantral Follicles

Growth and Structure The first stage of follicular growth involves the **preantral follicle**, which refers to development that occurs before the formation of a fluid-filled antral cavity. One of the first visible signs of follicle growth is the appearance of **cuboidal granulosa cells**. At this point, the follicle is referred to as a **primary follicle** (see Fig. 10-3). As granulosa cells proliferate, they form a multilayered (i.e., stratified) epithelium around the oocyte. At this stage, the follicle is referred to as a **secondary follicle** (see Fig. 10-3).

Once a secondary follicle acquires three to six layers of granulosa cells, it secretes paracrine factors that induce nearby stromal cells to differentiate into epithelioid **thecal cells**. Thecal cells form a flattened layer of cells around the follicle. Once a thecal layer forms, the follicle is referred to as a mature preantral follicle (see Fig. 10-3). In humans, it takes several months for a primary follicle to reach the mature preantral stage.

Follicular development is associated with an inward movement of the follicle from the outer cortex to the inner cortex, closer to the vasculature of the ovarian medulla. Follicles release angiogenic factors that

induce the development of one or two arterioles, which generate a vascular wreath around the follicle.

The Gamete During the preantral stage, the oocyte begins to grow and produce cellular and secreted proteins. The oocyte initiates secretion of extracellular matrix glycoproteins, called **ZP1**, **ZP2**, and **ZP3**, that form the **zona pellucida** (see Fig. 10-3). The zona pellucida ultimately increases to a thickness of 13 μm in humans and provides a species-specific binding site for sperm during fertilization (see Chapter 11). Of importance, granulosa cells and the oocyte project cellular extensions through the zona pellucida and maintain gap junctional contacts. The oocyte also continues to secrete paracrine factors that regulate follicle cell growth and differentiation.

Endocrine Function The granulosa cells express the FSH receptor during this period but are dependent primarily on factors from the oocyte to grow. They do not produce ovarian hormones at this early stage of follicular development.

The newly acquired thecal cells are analogous to testicular Leydig cells (see Chapter 9), in that they reside outside of the epithelial *nurse cells*, express the LH receptor, and produce androgens. The main difference between Leydig cells and thecal cells is that thecal cells do not express high levels of a 17β-hydroxysteroid dehydrogenase (17β-HSD). Thus, the major product of the thecal cells is androstenedione, as opposed to testosterone. Androstenedione production at this stage is absent or minimal.

Growing Antral Follicles

Growth and Structure Mature preantral follicles develop into early **antral follicles** (Fig. 10-5) over a period of about 25 days, growing from a diameter of approximately 0.1 to 0.2 mm. Once the granulosa epithelium increases to six or seven layers, fluid-filled spaces appear between cells and coalesce into the antrum. Over a period of about 45 days, this wave of small antral follicles will continue to grow to **large, recruitable antral follicles** that are 2 to 5 mm in diameter. This period of growth is characterized by about a 100-fold increase in granulosa cells (from about 10,000 to 1,000,000 cells). It also is characterized by swelling of the antral cavity, which increasingly

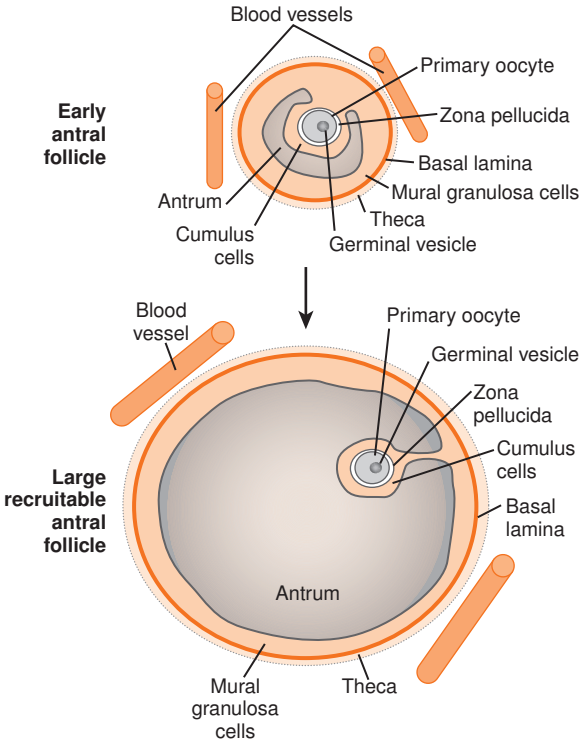

FIGURE 10-5 ■ Late follicular development from an early antral follicle to a large antral follicle.

divides the granulosa cells into two discrete populations (Fig. 10-6; see Fig. 10-5):

1. The **mural granulosa cells** (also called **stratum granulosum**) are those that form the outer wall of the follicle. The basal layer is adhered to the basal lamina and in close proximity to the outer-lying thecal layers. Mural granulosa cells become highly steroidogenic and remain in the ovary after ovulation to differentiate into the corpus luteum.
2. The **cumulus cells** are the inner cells that surround the oocyte (they are also referred to as the **cumulus oophorus** or **corona radiata**). The innermost layer (relative to the oocyte) of cumulus cells maintains gap and adhesion junctions with the oocyte. Cumulus cells are released from the ovary with the oocyte (collectively referred to as the **cumulus-oocyte complex**) during the process of **ovulation**. Cumulus cells are crucial for the ability of the fimbriated end of the oviduct to "capture" and

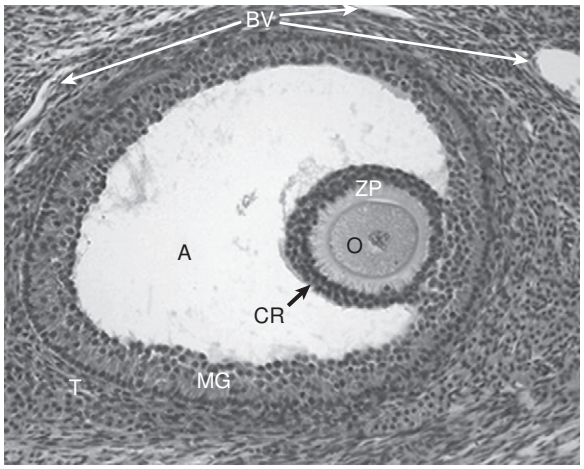

FIGURE 10-6 ■ Histologic features of ovarian graafian follicle. Ovum (O) is surrounded by zona pellucida (ZP). As a result of shrinkage artifacts, the zona pellucida appears larger than normal. Cumulus cells (C) are indicated, as is the large antrum (A). BV, blood vessels in outer thecal stroma; MG, mural glomerulosa cell; T, thecal cells.

move the oocyte by a ciliary transport mechanism along the length of the oviduct to the site of fertilization.

Early antral follicles are dependent on **pituitary FSH** for normal growth. Large antral follicles become **highly** dependent on pituitary FSH for their growth and sustained viability. As discussed later (under Dominant Follicle), 2- to 5-mm follicles are recruited to enter a rapid growth phase by a transient increase in follicle-stimulating hormone (FSH) that occurs toward the end of a previous menstrual cycle.

The Gamete The oocyte grows rapidly in the early stages of antral follicles; growth then slows in larger follicles. During the antral stage, the oocyte synthesizes sufficient amounts of cell cycle components, such as **cyclin-dependent kinase-1 (CDK1)** and **cyclin B**, and the oocyte becomes competent to complete meiosis I at ovulation. Thus, in preantral follicles, the oocyte fails to complete meiosis I because of a dearth of specific meiosis-associated proteins (i.e., they are incompetent to complete meiosis I). Larger antral follicles, however, gain **meiotic competence** but still maintain **meiotic arrest** until the midcycle luteinizing hormone (LH) surge. Meiotic arrest is achieved by the maintenance of elevated **cyclic adenosine monophosphate (cAMP)** levels in the mature oocyte. The oocyte expresses a constitutively active (i.e., active without a ligand) G-protein-coupled receptor, called **GPR3**, that maintains high cAMP levels (Figs. 10-7 and 10-8). Through a cAMP-PKA phosphorylation cascade, the **cyclin B–cyclin-dependent kinase, CDK1**, complex (also called **maturation-promoting factor,** or **MPF**) is kept inactive. As discussed in Chapter 1, intracellular cAMP levels are determined by the activity of adenylyl cyclase, which generates cAMP, and by the activity of **phosphodiesterases (PDEs)**, which metabolize cAMP to AMP. **Cyclic guanosine monophosphate (cGMP)** contributes to the maintenance elevated cAMP levels in competent oocytes by inhibiting the oocyte-specific PDE, PDE3A. Cyclic GMP is made in the cumulus cells and mural granulosa cells and is transferred to the oocyte through **gap junctions** (see Fig. 10-8).

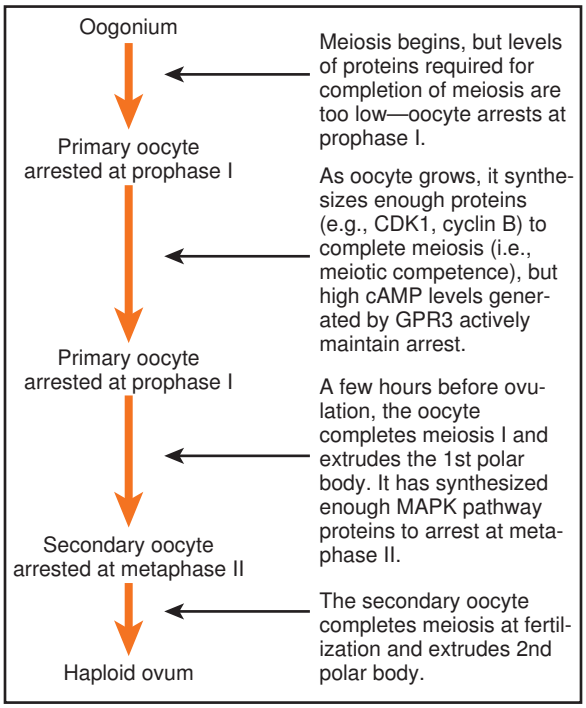

FIGURE 10-7 ■ Phases of oocyte development.

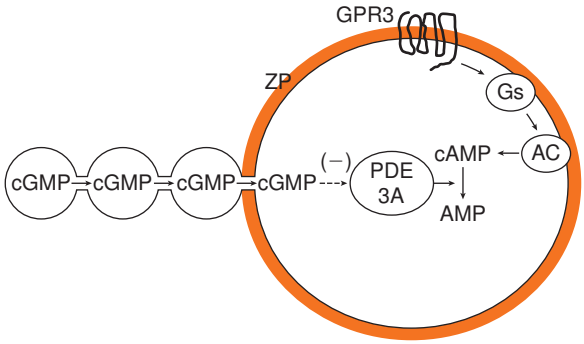

FIGURE 10-8 ■ Mechanisms involved in meiotic arrest of a meiotically competent primary oocyte. The smaller cells represent cumulus, stalk, and mural granulosa cells—all connected by gap junctions and all contributing to elevated cyclic guanosine monophosphate in the oocyte. AC, adenylyl cyclase; PDE 3A, phosphodiesterase 3A; ZP, zona pellucida.

Endocrine Function **Thecal cells** of large antral follicles produce significant amounts of **androstenedione** and, to a much lesser extent, **testosterone** (Fig. 10-9). This is due to high expression of CYP17 with both 17-hydroxylase and 17,20-lyase activities (Fig. 10-10A). Androgens are converted to **estradiol-17β** by the **mural granulosa cells** (see Fig. 10-9). At this stage, FSH stimulates proliferation of granulosa cells and induces the expression of **CYP19-aromatase** (Fig. 10-10B) required for estrogen synthesis. Additionally, the mural granulosa cells of the large antral follicles produce increasing amounts of **inhibin B** during the early follicular phase. Low levels of estrogen and inhibin exert a negative feedback effect on FSH secretion, thereby contributing to the selection of the follicle with the most FSH-responsive cells.

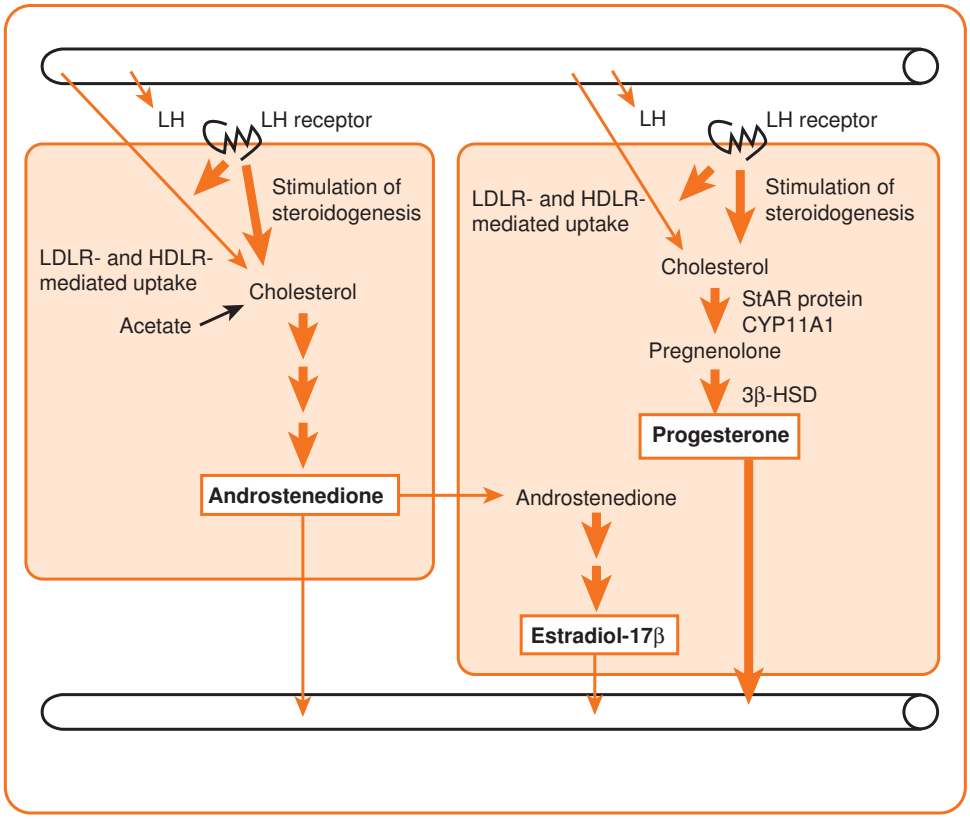

FIGURE 10-9 ■ Two-cell model of ovarian steroidogenesis. 3β-HSD, 3β-hydroxysteroid dehydrogenase; 17β-HSD, 17β-hydroxysteroid dehydrogenase; FSH, follicle-stimulating hormone; HDLR, high-density lipoprotein receptor; LDLR, low-density lipoprotein receptor; LH, luteinizing hormone; StAR protein, steroidogenic acute regulatory protein.

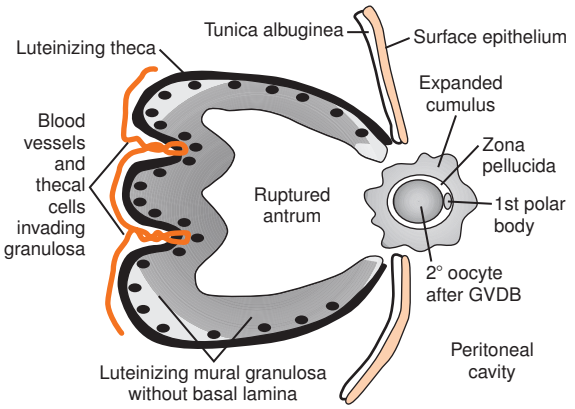

FIGURE 10-10 ■ Ovulation.

Dominant Follicle

Growth and Structure At the end of a previous menstrual cycle, a crop of large (2- to 5-mm) antral follicles (see Fig. 10-4) is recruited to begin rapid, gonadotropin-dependent development. The total number of recruited follicles in both ovaries can be as high as 20 in a younger woman (<33 years of age), but rapidly declines at older ages. The number of recruited follicles is reduced to the **ovulatory quota** (which is one in humans) by the process of **selection**. As FSH levels decline, the rapidly growing follicles progressively undergo atresia, until one follicle is left. Generally, the largest follicle with the most FSH receptors of the recruited crop becomes the **dominant follicle**. Selection occurs during the early follicular phase. By midcycle, the dominant follicle becomes a large preovulatory follicle that is 20 mm in diameter and contains about 50 million granulosa cells by the midcycle gonadotropin surge.

The Gamete The oocyte is competent to complete meiosis I but remains arrested in the dominant follicle through the mechanisms described earlier. Growth of the oocyte continues, but at a slower rate—the human oocyte reaches a diameter of about 140 μm by ovulation. The stalk by which cumulus cells are attached to the mural granulosa cells becomes increasingly attenuated.

Endocrine Function The newly selected follicle emerges for the first time during its development as a significant steroidogenic *gland*. Ovarian steroidogenesis requires both theca and granulosa cells (see Fig. 10-9). As discussed earlier, **thecal cells** express **LH receptors** and produce **androgens**. Basal levels of LH stimulate the expression of steroidogenic enzymes, as well as the LDL receptor in the theca. Thecal cells show robust expression of **CYP11A1** (also called **P-450 cholesterol side-chain cleavage**), **3β-hydroxysteroid dehydrogenase (3β-HSD)**, and **CYP17**, with both **17-hydroxylase activity** and **17,20-lyase activity**. Androgens (primarily **androstenedione** but also some **testosterone**) released from the theca can diffuse into the mural granulosa cells or can enter the vasculature surrounding the follicle.

The mural granulosa cells of the selected follicle have a high number of **FSH receptors** and are very sensitive to FSH signaling. FSH strongly up-regulates **CYP19 (aromatase)** gene expression and activity (see Fig. 10-9). CYP19 converts androstenedione to the weak estrogen, **estrone**, and converts testosterone to the potent estrogen, estradiol-17β. Granulosa cells express activating isoforms of **17β-HSD**, which ultimately drives steroidogenesis toward the production of **estradiol-17β** (see later). FSH also induces the expression of **inhibin B** during the follicular phase.

Of importance, FSH also induces the expression of **LH receptors** in the mural granulosa cells during the second half of the follicular phase (see Fig. 10-9). Thus, mural granulosa cells become responsive to both gonadotropins, allowing these cells to maintain high levels of CYP19 in the face of declining FSH levels. Acquisition of LH receptors also ensures that mural granulosa cells will respond to the LH surge (see later).

The Dominant Follicle During the Periovulatory Period

The **periovulatory period** can be defined as the time from the onset of the **LH surge** to the expulsion of the cumulus-oocyte complex out of the ovary (i.e., **ovulation**). This process lasts 32 to 36 hours in women. Starting at the same time, and superimposed on the process of ovulation, is a change in the steroidogenic function of the theca and mural granulosa cells. This process is called **luteinization** and culminates in the formation of a **corpus luteum** that is capable of producing large amounts of **progesterone**, along with estrogen, within a few days after ovulation.

Growth and Structure The LH surge induces dramatic structural changes in the dominant follicle that involve its rupture, ovulation of the cumulus-oocyte complex,

and the biogenesis of a new structure called the corpus luteum from the remaining thecal cells and mural granulosa cells. Major structural changes occur during this transition (see Fig. 10-10):

1. Before ovulation, the large preovulatory follicle presses against the ovarian surface, generating a poorly vascularized bulge of the ovarian wall called the **stigma**. The LH surge induces the release of inflammatory cytokines and hydrolytic enzymes from the theca and granulosa cells. These secreted components lead to the breakdown the follicle wall, tunica albuginea, and surface epithelium in the vicinity of the stigma. At the end of this process, the antral cavity becomes continuous with the peritoneal cavity.
2. The stalk-like attachment of the cumulus cells to the mural granulosa cells detaches, and the **cumulus-oocyte complex** becomes free-floating within the antral cavity. As an indirect response to the LH surge (i.e., in response to LH-dependent paracrine factors), the oocyte releases the transforming growth factor-β (TGF-β)-related factor, GDF9. **GDF9** (see earlier) stimulates the cumulus cells to secrete hyaluronic acid and other extracellular matrix components. These secreted components enlarge the entire cumulus-oocyte complex, a process called **cumulus expansion**. This enlarged cumulus-oocyte complex is more easily captured and transported by the oviduct. The expanded cumulus also makes the cumulus-oocyte complex easier for spermatozoa to find. The cumulus-oocyte complex is released through the ruptured stigma in a slow, gentle process, indicating that the follicular fluid in the antrum is not under increased pressure. The specific forces that lead to expulsion of the cumulus-oocyte complex are unknown.
3. The **basal lamina** of the mural granulosa cells is enzymatically degraded, and blood vessels and outer-lying theca can push into the granulosa cells. Granulosa cells secrete **angiogenic factors**, such as **vascular endothelial growth factor (VEGF)**, **angiopoietin-2**, and **basic fibroblast growth factor (bFGF)**, which significantly increase the blood supply to the new corpus luteum.

The Gamete Before ovulation, the primary oocyte is competent to complete meiosis but is arrested in prophase I as a result of high cAMP levels (see Fig. 10-8). The LH surge induces the oocyte to progress to **metaphase of meiosis II**. The oocyte subsequently arrests at metaphase II until fertilization. LH receptors are only present on the mural granulosa cells. The LH surge induces a series of events that eventually lead to a decrease in cGMP and closure of gap junctions (except for those between the oocyte and immediately adjacent cumulus cells) in mural granulosa and cumulus cells. As a result, cGMP levels fall in the oocyte, allowing **PDE3A** to degrade cAMP.

The decrease in cAMP and protein kinase A (PKA) activity ultimately leads to activation of **MPF**, composed of **CDK1** and **cyclin B**. Cyclin B synthesis is elevated during the periovulatory period, thereby increasing CDK1 activity. CDK1 activity is further enhanced by dephosphorylation, which is an indirect result of decreased PKA activity. The fully active MPF drives nuclear events that complete meiosis I with the extrusion of the **first polar body**. The **secondary oocyte** (called an **egg**) then arrests in **metaphase of meiosis II**. This is achieved by an increase in an activity called **cytostatic factor (CSF)**. It is now known that CSF is composed of the kinase, **c-Mos**, its target **mitogen-activated kinase kinase (MAPKK)**, also called **MEK1** (see Chapter 1), and **MAPK**. Thus, elevation of the MAPK signaling pathway is required for arrest at metaphase II, and **fertilization** leads to the rapid degradation of MAPK. It should be emphasized that our understanding of normal oocyte maturation has had a major impact on the ability to treat infertile couples through the process of in vitro fertilization (IVF). Normal oocyte biology dictates the type of hormonal treatment, the timing of egg retrieval, and the meiotic stage of eggs used for fertilization.

Endocrine Function Both thecal and mural granulosa cells express LH receptors at the time of the LH surge. The LH surge induces terminal differentiation (called **luteinization**) of the granulosa cells—a process that will continue for several days after ovulation. During the periovulatory period, the LH surge induces the following shifts in the steroidogenic activity of the mural granulosa cells (Fig. 10-11):

1. It transiently inhibits CYP19 expression and, consequently, estrogen production. The rapid decline in estrogen helps to turn off the positive feedback on LH secretion.

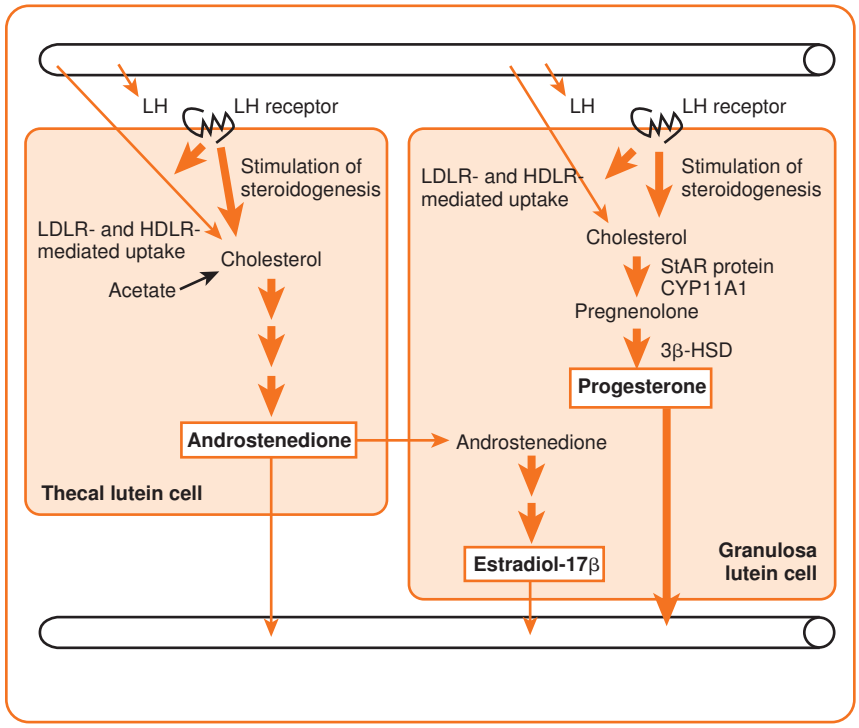

FIGURE 10-11 ■ Two-cell model of ovarian steroidogenesis during the luteal phase. 3β-HSD, 3β-hydroxysteroid dehydrogenase; HDLR, high-density lipoprotein receptor; LDLR, low-density lipoprotein receptor; LH, luteinizing hormone; StAR protein, steroidogenic acute regulatory protein. Note the absence of a basal lamina.

2. By inducing the breakdown of the basal lamina, the LH surge causes the direct vascularization of the granulosa cells. This makes **LDL** and **HDL cholesterol** accessible to these cells for steroidogenesis. The LH surge also increases the expression of the **LDL receptor** and **HDL receptor (SR-BI)** in granulosa cells.
3. The LH surge increases the expression of **StAR protein**, **CYP11A1 (side-chain cleavage enzyme)**, and **3β-HSD**. Because CYP17 activity, especially the 17,20-lyase function, is largely absent in granulosa cells, these cells begin to secrete progesterone, and progesterone levels will gradually increase over the next week.

The Corpus Luteum

Growth and Structure After ovulation, the remnant of the antral cavity fills with blood from damaged blood vessels in the vicinity of the stigma. This gives rise to a **corpus hemorrhagicum** with clotted blood within the former antral lumen (Fig. 10-12). Within a few days, red blood cells and debris are removed by macrophages, and fibroblasts fill in the antral cavity with a hyaline-like extracellular matrix. In the mature **corpus luteum**, the granulosa cells, now called **granulosa lutein cells**, enlarge and become filled with lipid (cholesterol esters). The enlarged granulosa lutein cells collapse into and partially fill in the old antral cavity. Proliferation of these cells is very limited. The theca, along with blood vessels, mast cells, macrophages, leukocytes, and other resident connective tissue cells, infiltrates the granulosa layer at multiple sites.

The human corpus luteum is programmed to live for 14 ± 2 days (**corpus luteum of menstruation**), unless *rescued* by the LH-like hormone, human chorionic gonadotropin (hCG), that originates from an implanting embryo (hCG is the protein that is detected in pregnancy tests; see Chapter 11). If rescued, the **corpus luteum of pregnancy** will remain viable for as long as the pregnancy (usually about 9 months). The mechanism by which the corpus luteum of menstruation regresses in 14 days is not fully understood, although the timing is very consistent. In response to

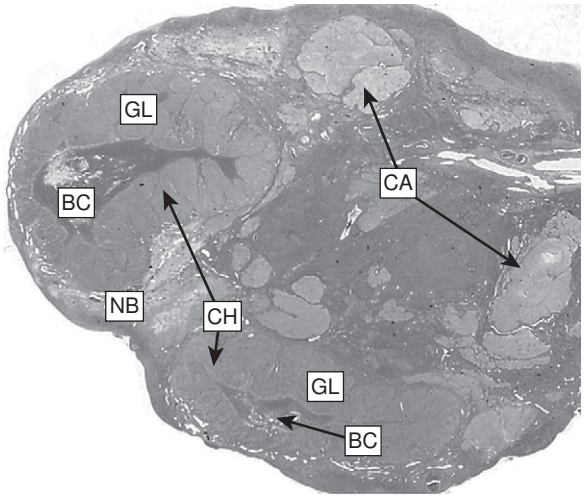

FIGURE 10-12 ■ Histologic features of the corpus hemorrhagicum. Micrograph of an ovary containing two corpora hemorrhagicum (CH). These arise from the co-ovulation of two dominant follicles, creating the potential for nonidentical twins. Each corpus luteum shows a central blood clot (BC) surrounded by a thick layer of lipid-rich granulosa lutein cells (GL). Degenerating corpora lutea are called corpora albicans (CA), which look like scar tissue and are eventually degraded. *(Modified from Stevens A, Lowe J:* Human Histology, *3rd ed., Philadelphia, 2005, Mosby.)*

paracrine factors and, perhaps, declining progesterone production, the corpus luteum becomes progressively unresponsive to pituitary LH and needs the extra amount of hCG to remain viable. The corpus luteum ultimately is turned into a scar-like body called the **corpus albicans** (see Fig. 10-12), which sinks into the medulla of the ovary and is slowly absorbed.

The Gamete The LH surge induces two parallel events, ovulation and luteinization. If ovulation occurs normally, the corpus luteum is devoid of a gamete.

Endocrine Function Progesterone production by the corpus luteum (see Fig. 10-11) increases steadily from the onset of the LH surge and peaks during the midluteal phase. The main purpose of this timing is to transform the uterine lining into an adhesive and supportive structure for implantation and early pregnancy. As discussed in Chapter 11, the midluteal phase is synchronized with early embryogenesis, so the uterus is optimally primed when a blastocyst tumbles into the uterus around day 22 of the menstrual cycle. Estrogen production transiently decreases in response to the LH surge but then rebounds and also peaks at midluteal phase.

Luteal hormonal output is absolutely dependent on basal LH levels (see Fig. 10-11). In fact, progesterone output is closely correlated with the pulsatile pattern of LH release in women. Both FSH and LH are reduced to basal levels during the luteal phase by the negative feedback from progesterone and estrogen. Also, granulosa lutein cells secrete **inhibin A**, which further represses FSH secretion. The elevated estrogen levels at midluteal phase may be responsible for the decrease in the sensitivity of the corpus luteum to LH, so progesterone and estrogen levels decline during the second half of the luteal phase unless an increase in circulating LH-like activity (i.e., in the form of hCG) compensates for the decreased sensitivity to LH.

CLINICAL BOX 10-2

The corpus luteum must generate large amounts of progesterone for an adequate number of days in order to support **implantation** and early **pregnancy**. Thus, the duration of the life of the corpus luteum (14 days) is very regular, and a shortened luteal phase typically leads to **infertility**. The quality of the corpus luteum is largely dependent on the size and health of the dominant follicle from which the corpus luteum developed. Dominant follicle development, in turn, is dependent on normal hypothalamic and pituitary stimulation during the follicular phase. Numerous factors that perturb hypothalamic and pituitary output during the follicular phase, including **heavy exercise**, **starvation**, **high prolactin levels**, and **abnormal thyroid function**, can lead to **luteal phase deficiency (LPD)** and **infertility**.

The corpus luteum of other mammalian species also produces an insulin-like hormone called **relaxin**. The human corpus luteum produces very low levels of relaxin, however, and the physiologic role of circulating relaxin in humans has not been established.

Follicular Atresia

Follicular atresia refers to the demise of an ovarian follicle and represents by far the predominant process in the ovary. During atresia, the granulosa cells and

oocytes undergo apoptosis. The thecal cells typically persist and repopulate the cellular stroma of the ovary. The thecal cells retain LH receptors and the ability to produce androgens and collectively are referred to as the **interstitial gland of the ovary**. Follicles can undergo atresia at any time during development.

Follicular Development and the Monthly Menstrual Cycle

The first half of the monthly menstrual cycle is referred to as the **follicular phase of the ovary** and is characterized by the recruitment and growth of 15 to 20 large, antral follicles (2 to 5 mm in diameter), selection of one of these follicles as the dominant follicle, and growth of the dominant follicle until ovulation. The dominant follicle must contain a fully developed, meiotically competent oocyte and somatic follicle cells that secrete high levels of estrogen. It takes several months for a primordial follicle to reach the size of a large antral follicle that can be recruited (Fig. 10-13). Therefore, it should be noted that much of follicular development occurs independently of the monthly menstrual cycle. The second half of the monthly menstrual cycle is referred to as the **luteal phase of the ovary** and is dominated by the hormonal secretions of the corpus luteum. Nevertheless, small follicles continue to develop within the ovarian stroma during the luteal phase.

THE HUMAN MENSTRUAL CYCLE

As stated earlier, late follicular development and luteal function are absolutely dependent on normal hypothalamic and pituitary function. As in the male, hypothalamic neurons secrete **gonadotropin-releasing hormone (GnRH)** in a **pulsatile** manner. GnRH, in turn, stimulates **LH** and **FSH** production by pituitary gonadotropes. A high frequency of GnRH pulses (one pulse every 60 to 90 minutes) selectively promotes LH production, whereas a slow frequency (one pulse every 120 minutes) selectively promotes FSH production. A major difference between the male and the female reproductive axes is the **midcycle gonadotropin surge** in females, which is dependent on a high level of estrogen over a specific duration coming from the dominant follicle.

A highly dynamic *conversation* occurs among the ovary, pituitary, and hypothalamus, which orchestrates the events of the menstrual cycle (Fig. 10-14). This section outlines the main points of events involving the ovary and pituitary gonadotrope that regulate the menstrual cycle, with an overview of hypothalamic involvement. In the next section, the effects of the hormonal changes on the female reproductive tract, especially the uterus, are discussed.

The following outline of events, numbered as depicted in Figure 10-14, begins with the ovary at

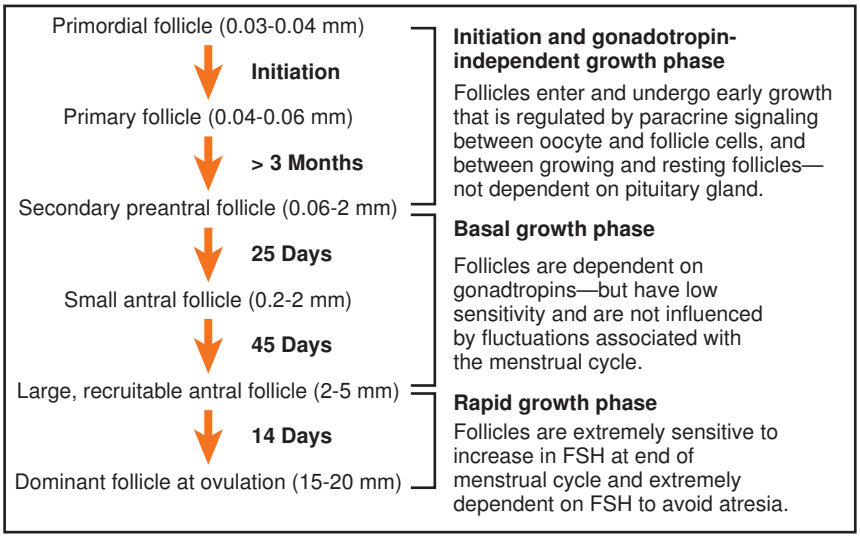

FIGURE 10-13 ■ Timing and phases of follicular growth.

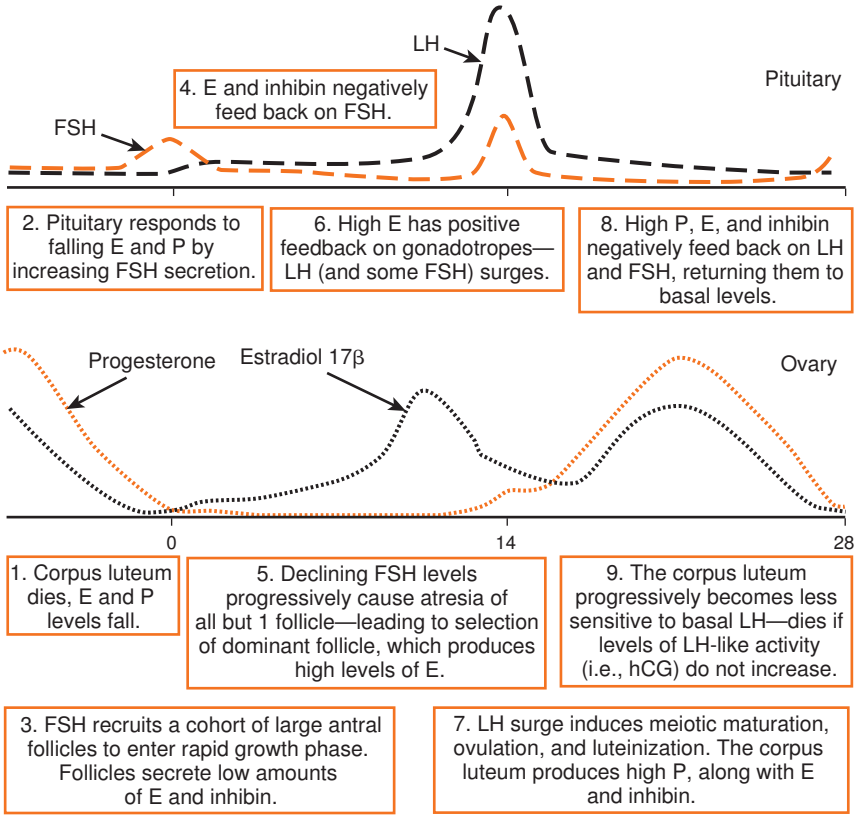

FIGURE 10-14 ■ The human menstrual cycle—a *conversation* between the ovary and the pituitary, with the hypothalamus as a facilitator. See text for comments on the involvement of the hypothalamus. E, estrogen; FSH, follicle-stimulating hormone; hCG, human chorionic gonadotropin; LH, luteinizing hormone; P, progesterone.

the end of the luteal phase of a previous, nonfertile cycle:

Ovary—event 1: In the absence of fertilization and implantation, the corpus luteum regresses and dies (a phenomenon called **luteolysis**). This leads to a drastic decline in the levels of progesterone, estrogen, and inhibin A by day 24 of the menstrual cycle.

Pituitary gonadotrope—event 2: The gonadotrope perceives the end of luteal function as a release from negative feedback. This permits a rise in **FSH** that occurs about 2 days before the onset of menstruation. The basis for the selective increase in FSH is incompletely understood but may result from the **slow frequency of GnRH pulses** during the luteal phase, which is due to high progesterone levels.

Ovary—event 3: The rise in FSH levels recruits a crop of large (2- to 5-mm) antral follicles to begin rapid, highly gonadotropin-dependent growth. These follicles produce low levels of **estrogen** and **inhibin B**.

Pituitary gonadotrope—event 4: The gonadotrope responds to the slowly rising levels of estrogen and inhibin B by decreasing FSH secretion. Loss of high levels of progesterone and estrogen causes an increase in the frequency of GnRH pulses, thereby selectively increasing **LH** synthesis and secretion by the gonadotrope. Thus, the **LH/FSH ratio** slowly increases throughout the follicular phase.

Ovary—event 5: The ovary's response to declining FSH levels is **follicular atresia** of all the recruited follicles, except for one **dominant follicle**. Thus, the process of selection is driven by an extreme dependency of follicles on FSH in the face of declining FSH secretion. Usually, only the largest follicle with the most FSH receptors and best blood supply (i.e., most angiogenic) survives. This follicle produces increasing amounts of estradiol-17β and inhibin B during the second half of the follicular phase. FSH also induces the expression of **LH receptors**

in the mural granulosa cells of the dominant follicle.

Pituitary gonadotrope—event 6: Once the dominant follicle causes the circulating estrogen levels to exceed 200 pg/mL for about 50 hours in women, estrogen exerts a **positive feedback** on the gonadotrope, producing the midcycle LH surge. This is enhanced by the small amount of progesterone starting to be made at midcycle. The exact mechanism of the positive feedback is unknown, but it occurs largely at the level of the pituitary. **GnRH receptors** and the **sensitivity to GnRH signaling** increase dramatically in the gonadotropes. The hypothalamus contributes to the gonadotropin surge by increasing the frequency of GnRH pulses. There is some evidence that other neurons in the hypothalamus (e.g., kisspeptin neurons; see Chapter 8) respond to high levels of estrogen by increasing the frequency and amount of GnRH released.

Ovary—event 7: The LH surge drives three general events in the ovary:

1. The primary oocyte completes meiosis I and arrests at metaphase of meiosis II. This is associated with **germinal vesicle breakdown** (**GVBD0;** the germinal vesicle refers to the nucleus of the oocyte), which is the dissolution of the nuclear membrane and interphase nuclear structure. GVBD occurs about 30 hours after the onset of the LH surge.
2. The wall of the follicle and of the ovary at the stigma is broken down, and the free-floating cumulus-oocyte complex is extruded from the ovary (i.e., ovulation). This occurs about 32 to 36 hours after the onset of the LH surge.
3. The mural granulosa cells and theca cells are restructured to form the corpus luteum. This involves direct vascularization of the granulosa cells and their differentiation into progesterone- and estrogen-producing cells. Note that estrogen production transiently drops for about 2 days after the onset of LH production, which may terminate the positive feedback. The granulosa cells also secrete inhibin A. The process of luteinization continues for several days after the onset of the LH surge. The small amount of progesterone secreted during the periovulatory period contributes to the magnitude of the LH surge.

Pituitary gonadotrope—event 8: Rising levels of progesterone, estrogen, and inhibin A by the mature corpus luteum negatively feed back on the pituitary gonadotrope. Even though estrogen levels exceed the 200 pg/mL threshold for positive feedback, the **high progesterone levels** block any positive feedback. Consequently, both FSH and LH levels decline to basal levels.

Ovary—event 9: **Basal levels of LH** (but not FSH) are absolutely required for normal corpus luteum function. The corpus luteum becomes progressively insensitive to LH signaling, however, and dies unless LH-like activity (i.e., hCG from an implanted embryo) increases. In a nonfertile cycle, the corpus luteum of menstruation will regress in 14 days, and progesterone and estrogen levels will start to decline by about 10 days.

Pituitary gonadotrope—event 10: Removal of negative feedback causes an increase in FSH at the end of the cycle, and the entire process begins again.

From this sequence of events, it is evident that the **ovary** is the primary clock for the menstrual cycle. The timing of the two main pituitary-based events—the transient rise in FSH that recruits large antral follicles and the LH surge that induces ovulation—is determined by two respective ovarian events:

- Highly regular life span of a corpus luteum and its demise after 14 days
- Growth of the dominant follicle to a point at which it can maintain a sustained high production of estrogen that induces a switch to positive feedback at the pituitary

The hypothalamic release of GnRH changes over the cycle. The frequency of GnRH pulses increases during the second half of the follicular phase and decreases during the luteal phase.

FEMALE REPRODUCTIVE TRACT

The female reproductive tract does not connect directly to the ovaries (Fig. 10-15). The internal portion of the tract consists of right and left oviducts and the following midline structures: uterus, cervix, and vagina. The external opening of the vagina is surrounded by the external genitalia.

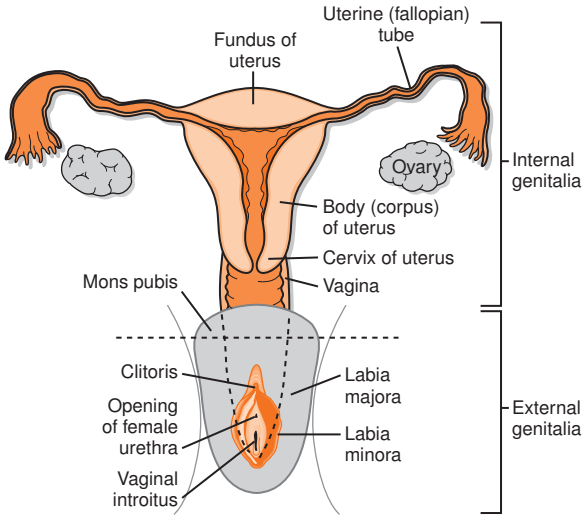

FIGURE 10-15 ■ The internal and external genitalia of the female reproductive tract. *(Modified from Drake RL, Vogl W, Mitchell AWM:* Gray's Anatomy for Students, *Philadelphia, 2005, Churchill Livingstone.)*

The Oviduct

Structure and Function The **oviducts** (also called the **uterine tubes** or **fallopian tubes**) are muscular tubes that are opened at both ends. The end of the oviduct close to the surface of each ovary has finger-like projections, called **fimbriae**. The opposite end pierces the wall of the uterus and opens into the uterine lumen. The oviducts can de divided into four sections (Fig. 10-16). Going from ovary to uterus, these sections are named as follows:

1. **Infundibulum**, which includes the fimbriae
2. **Ampulla**, which has a relatively wide lumen and extensive folding of the mucosa
3. **Isthmus**, which has a relatively narrow lumen and less mucosal folding
4. **Intramural or uterine segment**, which extends through the uterine wall at the superior corners (horns) of the uterus

The wall of the oviduct is composed of a mucosa called the **endosalpinx**, a two-layered muscularis called the **myosalpinx**, and outer-lying connective tissue, the **perisalpinx, that contains numerous blood vessels** (Fig. 10-17). The endosalpinx is lined by a simple epithelium made up of two cell types: **ciliated cells**

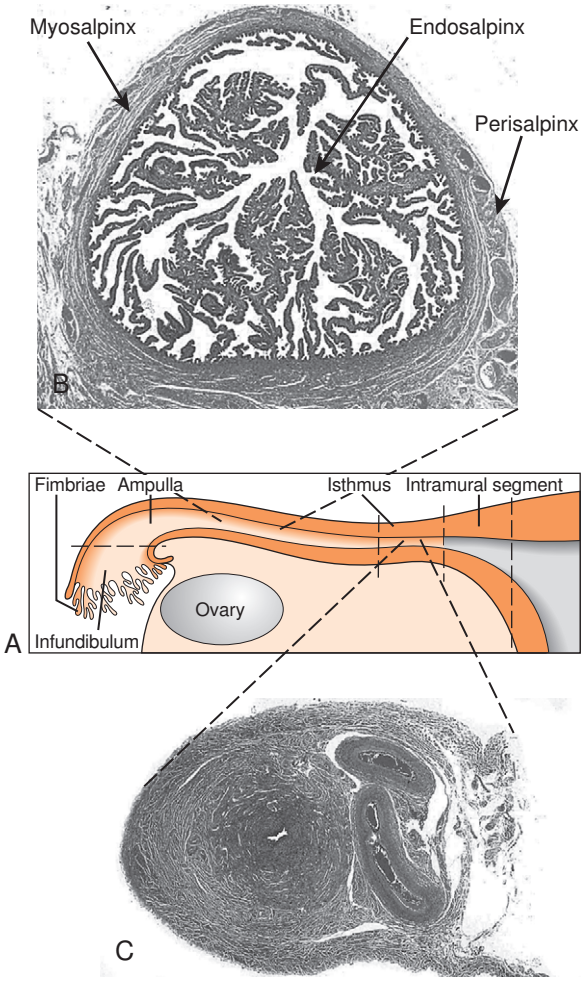

FIGURE 10-16 ■ **A,** Structures of the oviduct. **B,** Cross section of the oviduct at the ampulla, with a large lumen filled with mucosal (endosalpinx) folds. **C,** Cross section of the oviduct at the isthmus, showing a much smaller lumen but a thicker muscularis (myosalpinx). *(Modified from Stevens A, Lowe J:* Human Histology, *3rd ed., Philadelphia, 2005, Mosby.)*

and **secretory cells**. The cilia are most numerous at the ovarian end (infundibulum and ampulla) and beat toward the uterus. The cilia on the fimbriae are the sole mechanism for transport of the ovulated cumulus-oocyte complex into the oviduct. Once the complex passes through the ostium of the oviduct and enters the ampulla, it is moved by both cilia and peristaltic contractions of the myosalpinx.

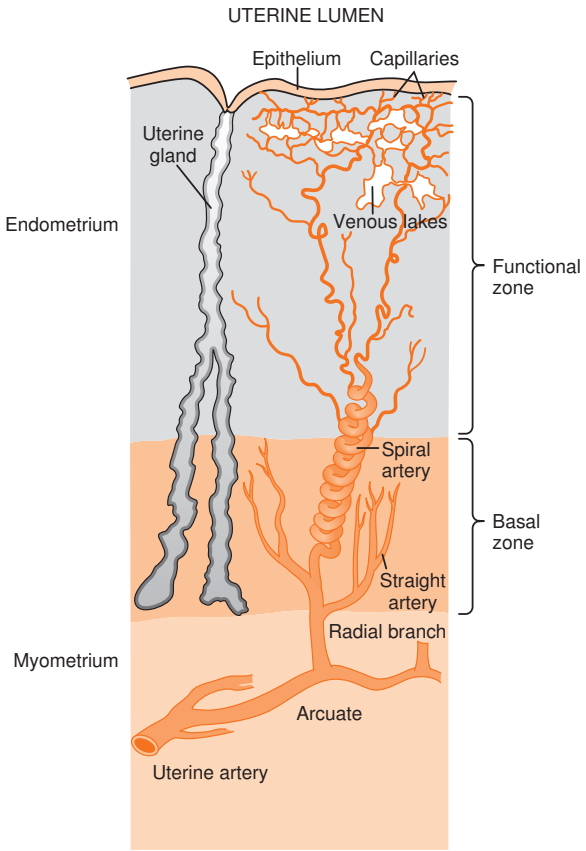

FIGURE 10-17 ■ Structure of the uterine endometrium. *(Modified from Strauss J III, Coutifaris C: The endometrium and myometrium: Regulation and dysfunction. In Yen SSC, Jaffe RB, Barbieri RL, editors:* Reproductive Endocrinology, *4th ed., Philadelphia, 1999, Saunders, pp 191-217.)*

As shown in Figure 10-17, the ovarian end of the oviduct (infundibulum and ampulla) has a wide lumen partially filled with a highly folded myosalpinx. This allows the cumulus-oocyte complex to be transported while in intimate contact with ciliated mucosal cells. The uterine end of the oviduct (isthmus and intramural segment) has a narrow lumen and a relatively thicker muscularis. This allows for slow transport of an early embryo to the uterus primarily by peristaltic waves of the muscularis.

The main functions of the oviducts are the following:

1. **Capture of the cumulus-oocyte complex** at ovulation and **transport** of the cumulus-oocyte complex to a midway point (the ampullary-isthmus junction), where fertilization takes place. Oviductal secretions coat and infuse the cumulus-oocyte complex and may be required for viability and fertilizability.
2. Providing a site for **sperm storage**. Women who ovulate up to 5 days after sexual intercourse can become pregnant. Sperm remain viable by adhering to the epithelial cells lining the isthmus. The secretions of the oviduct also induce **capacitation** and **hyperactivity of sperm** (see Chapter 11).
3. Providing nutritional support to the preimplantation embryo by the oviductal secretions. Also, the timing of the movement of the embryo into the uterus is critical because the human uterus has an implantation window of about 3 days. The oviduct needs to harbor the early embryo until it reaches the blastocyst stage (5 days after fertilization); then it allows the embryo to move into the uterine cavity (see Chapter 11).

The secretory cells produce a protein-rich mucus that is conveyed along the oviduct to the uterus by the cilia. This ciliary-mucus escalator maintains a healthy epithelium, moves the cumulus-oocyte complex toward the uterus, and may provide directional cues for swimming sperm. The movement of the cumulus-oocyte complex slows at the ampullary-isthmus junction, where fertilization normally takes place. This appears to be due in part to a thick mucus that is produced by the human isthmus and to an increased tone of the muscularis of the isthmus. The composition of oviductal secretions is complex and includes growth factors, enzymes, and oviduct-specific glycoproteins. Note that IVF has shown that the secretions of the oviduct are not absolutely necessary for fertility by in vitro techniques. However, normal oviductal function is absolutely required for both fertilization and implantation from in vivo insemination, and to minimize the risk for ectopic implantation (i.e., implantation outside of the uterus). In fact, the most common site of ectopic implantation is the oviduct.

CLINICAL BOX 10-3

Primary ciliary dyskinesia (also called **immotile cilia syndrome** or **Kartagener syndrome**) is a highly heterogeneous inherited disease caused by the absence or defect of one of the many components of the ciliary-flagellar axoneme. The mutation can cause no beating (ciliary immotility), abnormal beating

(ciliary dyskinesia), or loss of cilia (ciliary aplasia). The disease is primarily characterized by infections of the upper respiratory tract, nasal tract, and middle ear. The importance of ciliary transport in the oviduct is indicated by the finding that about 50% of women with primary ciliary dyskinesia are infertile or subfertile.

Hormonal Regulation During the Menstrual Cycle In general, estrogen secreted during the follicular phase increases endosalpinx epithelial cell size and height. Estrogen increases blood flow to the lamina propria of the oviducts, increases the production of oviduct-specific glycoproteins (whose functions are poorly understood), and increases ciliogenesis throughout the oviduct. Estrogen promotes the secretion of a thick mucus in the isthmus and increases tone of the muscularis of the isthmus, thereby keeping the cumulus-oocyte complex at the ampullary-isthmus junction for fertilization. High progesterone, along with estrogen, during the early to midluteal phase decreases epithelial cell size and function. Progesterone promotes deciliation. Progesterone also decreases the secretion of thick mucus and relaxes the tone in the isthmus.

The Uterus

Structure and Function The uterus is a single organ that sits in the midline of the pelvic cavity between the bladder and the rectum. The mucosa of the uterus is called the **endometrium**, the three-layered, thick muscularis is called the **myometrium**, and the outer connective tissue and serosa are called the **perimetrium**. The parts of the uterus are the **fundus**, which is that portion that rises superiorly from the entrance of the oviducts; the **body**, which makes up most of the uterus; the **isthmus**, a short narrowed part of the body at its inferior end; and the **cervix**, which extends into the vagina (see Figs. 10-1 and 10-15). Because the cervical mucosa is distinct from the rest of the uterus and does not undergo the process of menstruation, it is discussed separately.

The established functions of the uterus are related to supporting a **pregnancy** (see Chapter 11). The main functions of the uterus are as follows:

1. Provide a suitable site for attachment and implantation of the blastocyst, including a thick, nutrient-rich stroma
2. Limit the invasiveness of the implanting embryo so that it stays in the endometrium and does not reach the myometrium
3. Provide a maternal side of the mature placental architecture. This includes the basal plate, to which the fetal side attaches, and large, intervillous spaces that become filled with maternal blood after the first trimester
4. Grow and expand with the growing fetus so that the fetus develops within an aqueous, largely nonadhesive environment
5. Provide strong muscular contractions to expel the fetus and placenta at term

An understanding of the function of the uterus and hormonally induced uterine changes during nonfertile menstrual cycles requires a basic knowledge of the fine structure of the endometrium and of the relationship of the uterine blood supply to the endometrium (Fig. 10-17). The luminal surface of the endometrium is covered by a simple cuboidal-columnar epithelium. The epithelium is continuous with mucosal glands (called **uterine glands**) that extend deep into the endometrium. The mucosa is vascularized by **spiral arteries**, which are branches of the uterine artery that runs through the myometrium. The terminal arterioles of the spiral arteries project to a position just beneath the surface epithelium. These arterioles give rise to a subepithelial plexus of capillaries and venules, which have ballooned, thin-walled segments called venous lakes or **lacunae**. The lamina propria (i.e., the connective tissue and stroma of the mucosa supporting the epithelium) itself is densely cellular. The stromal cells of the lamina propria play important roles both during pregnancy and menstruation.

About two thirds of the luminal side of the endometrium is lost during menstruation and is called the **functional zone** (also called the **stratum functionalis**) (see Fig. 10-17). The basal one third of endometrium that remains after menstruation is called the **basal zone** (also called the **stratum basale**). The basal zone is fed by straight arteries that are separate from the spiral arteries and contains all of the cell types of the endometrium (i.e., epithelial cells from the remaining tips of glands, stromal cells, and endothelial cells).

Hormonal Regulation of the Uterine Endometrium During the Menstrual Cycle Phases the of uterine cycle are controlled by ovarian estrogen and progesterone.

Thus, phases of the endometrial cycle correspond to phases of the ovarian cycle.

The Proliferative Phase At the end of menses (days 3 to 5), the functional layer of the uterine endometrium has been shed, and the basal layer is undergoing reepithelialization (Fig. 10-18). In the ovary, the follicular phase is under way. By day 5 of the ovarian cycle, FSH has recruited a cohort of 2- to 5-mm large antral follicles that begin producing low but increasing levels of estradiol. Once the dominant follicle is selected at midfollicular phase, estradiol production increases dramatically (see Fig. 10-14). The estrogen produced by the follicular phase of the ovary drives the **proliferative phase** of the uterine endometrium. Estrogen induces all cell types in the basal layer to proliferate. In fact, the definition of an estrogenic compound has historically been one that is **uterotropic**. It is not clear whether estrogen stimulates the growth and differentiation of pluripotential stem cells or stimulates the growth of cells that are already defined as endothelial, epithelial, and stromal. Estrogen increases cell proliferation directly through **estrogen receptor-α (ERα)** and indirectly through the production of growth factors, such as insulin-like growth factor-1 (IGF-1). Estrogen also induces the expression of **progesterone receptors**, thereby **priming** the uterine endometrium so that it can respond to progesterone during the luteal phase of the ovary.

During the proliferative phase, the functional layer of the endometrium is rebuilt, and the endometrium increases from about 0.5 to 5 mm in thickness. Mitotic figures are found throughout the tissue. The uterine glands display a straight or coiled shape with narrow lumina (see Fig. 10-18).

The Secretory Phase By ovulation, the thickness of stratum functionalis has been reestablished under the proliferative actions of estradiol (see Fig. 10-18). After ovulation, the corpus luteum produces high levels of progesterone, along with estradiol. The **luteal phase** of the ovary switches the proliferative phase of the uterine endometrium to the **secretory phase**. In general, progesterone inhibits further endometrial growth and induces the differentiation of epithelial and stromal cells. Progesterone induces the uterine glands to secrete a nutrient-rich product, which will support implanting blastocyst viability. As the secretory phase proceeds, the mucosal uterine glands become corkscrewed and sacculated. Progesterone also induces changes in the adhesivity of the surface epithelium, thereby generating the **window of receptivity** for implantation (see Chapter 11). Progesterone also promotes the differentiation of the stromal cells into **predecidual cells,** which must be prepared to form the **decidua** of pregnancy, or to orchestrate menstruation in the absence of pregnancy.

Of importance, progesterone opposes the proliferative actions of estradiol. Progesterone down regulates the estrogen receptor. Progesterone also induces **inactivating isoforms of 17β-HSD**, thereby converting the active **estradiol** into the inactive **estrone**. Progesterone also up regulates the expression of a steroid **sulfotransferase** that sulfates and inactivates estrogen.

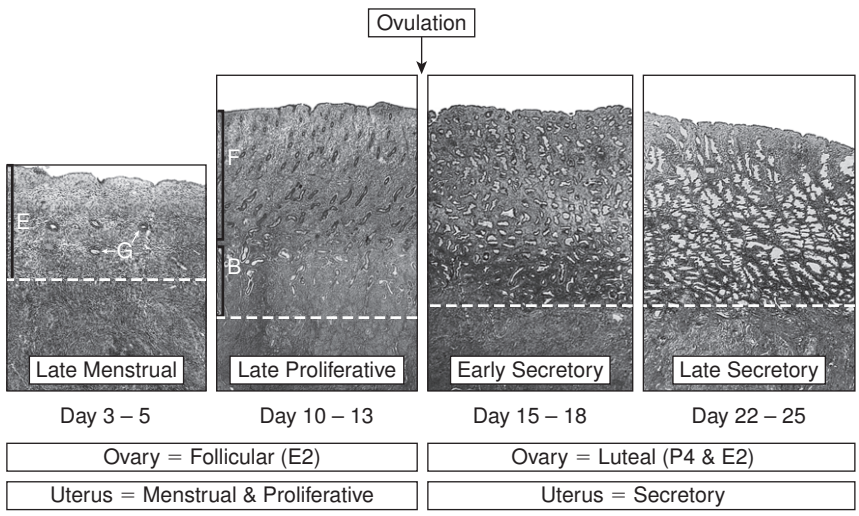

FIGURE 10-18 ■ Changes in endometrial glands at different phases of the uterine cycle, and the corresponding phases of the ovarian cycle. *(Modified partial images from Young B, Lowe J, Stevens A, et al:* Wheater's Functional Histology, *5th ed., Edin-burgh, 2006, Churchill Livingstone.)*

This opposition of the mitogenic actions of estradiol by progesterone is extremely important in protecting the uterine endometrium from estrogen-induced uterine cancer. By contrast, the administration of unopposed estrogen significantly increases the risk for uterine cancer in women.

The Menstrual Phase In a nonfertile cycle, death of the corpus luteum leads to a sudden withdrawal of progesterone and estrogen, which leads to changes in the uterine endometrium that result in the loss of the lamina functionalis. **Menstruation** normally lasts 3 to 5 days (called a **period**), and the volume of blood loss ranges from 25 to 35 mL. Menstruation coincides with the early follicular phase of the ovary.

The breakdown of the stratum functionalis is due to the up regulation of hydrolytic enzymes, called **matrix metalloproteases**, which destroy the extracellular matrix and basal lamina of the endometrium. These enzymes are produced by the three resident cell types of the endometrium: the **epithelial cell**, the **stromal cell**, and the **endothelial cell**. Matrix metalloproteases also are produced by **leukocytes**, which infiltrate into the endometrium just before menstruation. The other major component that leads to menstruation is the production of **prostaglandins**. The inducible enzyme required for prostaglandin synthesis, **cyclooxygenase-2 (COX-2)**, is increased in endothelial cells on progesterone withdrawal. This increases production of inflammatory prostaglandins, especially $\mathbf{PGF_{2\alpha}}$, which, in turn, promotes contraction of the smooth muscle cells of the myometrium and the vascular smooth muscle cells of the **spiral arteries**. Intermittent spiral artery contraction and dilation cause **hypoxic necrosis**, followed by reperfusion injury of weakened tissue. The degree of tissue loss and the onset of tissue repair appear to be dependent on increasing estrogen levels during the early follicular phase.

CLINICAL BOX 10-4

Disorders of menstruation are relatively common and include **menorrhagia** (loss of > 80 mL of blood) and **dysmenorrhea** (painful periods). The existence of few, irregular periods, called **oligomenorrhea**, and the absence of periods, called **amenorrhea**, often are due to dysfunction or cessation of the hypothalamus-pituitary-ovarian axis, as opposed to local pelvic pathophysiology.

Hormonal Regulation of the Myometrium The smooth muscle cells of the **myometrium** also are responsive to changes in steroid hormones. Peristaltic contractions of the myometrium favor movement of luminal contents from the cervix to the fundus at ovulation, and these contractions may play a role in rapid bulk transport of ejaculated sperm from the cervix to the oviducts. During menstruation, contractions propagate from the fundus to the cervix, thereby promoting expulsion of sloughed functional zone. The size and number of smooth muscle cells are determined by estrogen and progesterone. Healthy, cycling women maintain a robust myometrium, whereas the myometrium progressively thins in postmenopausal women.

The Cervix

Structure and Function The **cervix** represents the inferior extension of the uterus that projects into the vagina (see Figs. 10-1 and 10-15). It has a mucosa that lines the **endocervical canal**, which has a highly elastic lamina propria, and a muscularis that is continuous with the myometrium. The cervix acts as a gateway to the upper female tract; at midcycle, the endocervical canal facilitates sperm viability and entry. During the luteal phase, changes in the endocervical canal serve to impede the passage of sperm and microbes, thereby minimizing the chance of superimplantation of a second embryo, as well as inhibiting ascending infections into the placenta, fetal membranes, and fetus. The cervix physically supports the weight of the growing fetus. At term, cervical softening and dilation allow passage of the newborn and placenta from the uterus into the vagina.

Hormonal Regulation of Cervical Mucus During the Menstrual Cycle The endocervical canal is lined by a simple columnar epithelial gland that secretes cervical mucus in a hormonally responsive manner. Estrogen stimulates production of a copious quantity of thin, watery, slightly alkaline mucus that is an ideal environment for sperm. The macromolecules within this mucus are thought to facilitate sperm movement. Progesterone stimulates production of a scant, viscous, slightly acidic mucus that is hostile to sperm and does not "fern." This thick mucus forms a barrier within the endocervical canal during the secretory phase of the endometrium and during pregnancy, when the placenta produces high amounts of progesterone (see Chapter 11).

The Vagina

Structure and Function The vagina represents one of the **copulatory structures** in women and acts as the **birth canal** (see Fig. 10-16). Its mucosa is lined by a nonkeratinized, stratified squamous epithelium. The mucosa has a thick lamina propria enriched with elastic fibers and is well vascularized. There are no glands in the vagina, so lubrication during intercourse comes from the following:

- **Cervical mucus** (especially during midcycle)
- A **transudate** (i.e., ultrafiltrate) from the blood vessels of the lamina propria
- From the **vestibular glands** (see later)

The mucosa is surrounded by a relatively thin (i.e., relative to the uterus and cervix) two-layered muscularis and an outer connective tissue. The vaginal wall is innervated by branches of the pudendal nerve, which contribute to sexual pleasure and orgasm during intercourse.

Hormonal Regulation During the Menstrual Cycle The superficial cells of the vaginal epithelium are continually desquamating and the nature of these cells is influenced by the hormonal environment. Estrogen stimulates proliferation of the vaginal epithelium and increases their glycogen content. Estradiol also induces minimal keratinization of the apical layers. Progesterone increases the desquamation of the epithelial cells. The glycogen is metabolized to lactic acid by commensal lactobacilli, thereby maintaining an acidic environment. This relative acidity inhibits infections by noncommensal bacteria and fungi.

The External Genitalia

Structure and Function The female external genitals are surrounded by the **labia majora** (homologs of the scrotum) laterally and the **mons pubis** anteriorly (see Fig. 10-16). The **vulva** collectively refers to an area that includes the labia majora and the mons pubis, plus the labia minora, the clitoris, the vestibule of the vagina, the vestibular bulbs (glands), and the external urethral orifice (Fig. 10-19). The vulva also is referred to as the **pudendum** by clinicians. The structures of the vulva serve the functions of **sexual arousal** and **orgasm**, directing the flow of urine and providing a partial cover of the opening of the vagina, thereby inhibiting the entry of pathogens.

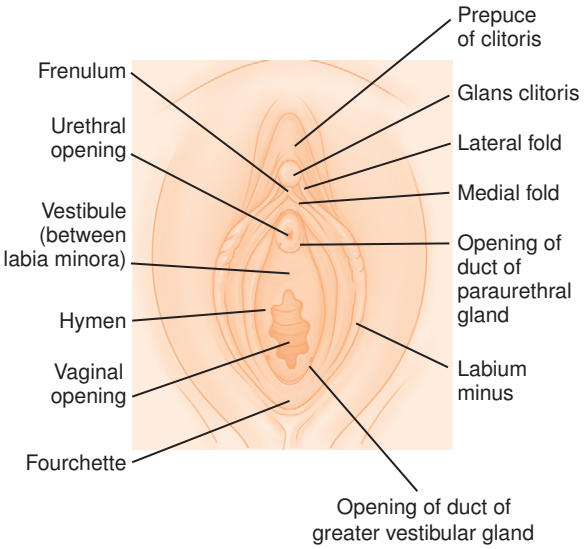

FIGURE 10-19 ■ External genitalia in women. *(From Drake RL, Vogl W, Mitchell AWM:* Gray's Anatomy for Students, *Philadelphia, 2005, Churchill Livingstone.)*

The **clitoris** is the homolog of the penis. The clitoris is composed of erectile tissue that undergoes the process of erection in essentially the same manner as the penis. Unlike the penis, clitoral tissue is completely separate from the urethra. Thus, the only function of the clitoris is involved with sexual arousal and climax at orgasm.

Hormonal Regulation During the Menstrual Cycle The structures of the vulva do not show marked changes during the menstrual cycle. The health and function of these structures, however, are dependent on hormonal support. The external genitalia and vagina appear to be responsive to androgens (testosterone and dihydrotestosterone), as well as estrogen. Androgens also act on the central nervous system (CNS) to increase libido in women.

BIOLOGY OF ESTRADIOL AND PROGESTERONE

Mechanisms of Estrogen and Progesterone Hormone Action

Estrogen and progesterone are steroid hormones; accordingly, their cognate receptors belong to the nuclear hormone receptor superfamily (see Chapter 1).

Biologic Effects of Estrogen and Progesterone

Although the levels of estradiol and progesterone fluctuate during the menstrual cycle, estrogen and progesterone levels are always higher in women than in men. Estradiol and progesterone have multiple effects that can be categorized according to whether they are directly related to the reproductive system. As discussed previously, both steroid hormones have profound effects on the ovary, oviduct, uterus, cervix, vagina, and external genitalia, and on the hypothalamus and pituitary. Estrogen and progesterone also have important effects on the following nonreproductive tissues:

Bone: **Estrogen** is required for closure of the epiphyseal plates of long bones in both sexes. **Estradiol** has a bone anabolic effect and a calciotropic effect at several sites. E2 stimulates intestinal calcium absorption and renal tubular calcium reabsorption. E2 also is one of the most potent regulators of osteoblast and osteoclast function. Estrogen promotes survival of osteoblasts and apoptosis of osteoclasts, thereby favoring bone formation over resorption. Loss of estradiol at menopause is frequently associated with osteoporosis.

Liver: The overall effect of **estradiol** on the **liver** is to improve circulating **lipoprotein** profiles. Estrogen increases expression of the **LDL receptor**, thereby increasing clearance of cholesterol-rich LDL particles by the liver. Estrogen also increases circulating levels of **high-density lipoprotein (HDL)** levels. Estrogen regulates hepatic production of several transport proteins, including cortisol-binding protein, thyroid hormone–binding protein, and sex hormone–binding protein.

Cardiovascular organs: Premenopausal women have significantly lower cardiovascular disease than men or postmenopausal women. **Estrogen** promotes **vasodilation** through increased production of nitric oxide, which relaxes vascular smooth muscle and inhibits platelet activation. Estrogen promotes angiogenesis in target tissues.

Integument: Overall, estrogen and progesterone maintain a healthy, smooth skin with normal **epidermal** and **dermal thickness**. Estrogen stimulates proliferation and inhibits apoptosis of **keratinocytes**. In the dermis, estrogen and progesterone increases collagen synthesis and inhibits (along with progesterone) the breakdown of collagen by suppressing matrix metalloproteases. Estrogen increases **glycosaminoglycan production** and deposition in the dermis. Estrogen also promotes **wound healing**.

CNS: In general, **estrogen** is **neuroprotective**—that is, it inhibits neuronal cell death in response to hypoxia or other insults. The positive effects on angiogenesis may account for some of the beneficial and stimulant-like actions of estrogen on the CNS. Currently, the proposed benefits of estrogen for the onset and severity of Parkinson disease and Alzheimer disease are controversial. **Progesterone** acts on the hypothalamus to increase the **set-point for thermoregulation**, thereby elevating body temperature by approximately 0.5° F. This is the basis for using body temperature measurements to determine whether ovulation has occurred. Progesterone generally acts as a depressant on the CNS. Loss of progesterone on demise of the corpus luteum of menstruation is the basis for **premenstrual syndrome (PMS)** and the severe variant **premenstrual dysphoria disorder (PMDD)** experienced by some women.

Adipose tissue: **Estrogen** decreases adipose tissue by decreasing **lipoprotein lipase activity** and increasing **hormone-sensitive lipase** (i.e., it has a lipolytic effect). Loss of estrogen results in an accumulation of adipose tissue, especially in the abdomen.

The actions of estrogen and progesterone on the maternal physiology and breast development and function are discussed in Chapter 11.

Transport and Metabolism of Ovarian Steroids

Steroid hormones are sparingly soluble in blood and are carried primarily associated with plasma proteins. About 60% of the estrogen is transported bound to **sex hormone–binding globulin (SHBG)**, 20% is bound to albumin, and 20% is in the free form.

Progesterone binds primarily to **cortisol-binding globulin (CBG)** (i.e., transcortin) and albumin. Because it has a relatively low binding affinity for these proteins, its circulating half-life ($t_{1/2}$) is about 5 minutes.

Although the ovary is the primary site of estrogen production, it is important to understand that peripheral aromatization of androgens to estrogens can

generate locally high levels of estradiol in specific tissues. For example, the fact that CYP19 (aromatase) is expressed in the adipose tissue of the breast is the basis for the use of **aromatase inhibitors** in the treatment of estrogen-dependent breast cancer.

Estrogens and progestins are degraded in the liver to inactive metabolites, conjugated with sulfate or glucuronide, and excreted in the urine. Major metabolites of estradiol include estrone, estriol, and catecholestrogens (2-hydroxyestrone and 2-methoxyestrone). The major metabolite of progesterone is pregnanediol, which is conjugated with glucuronide and excreted in the urine.

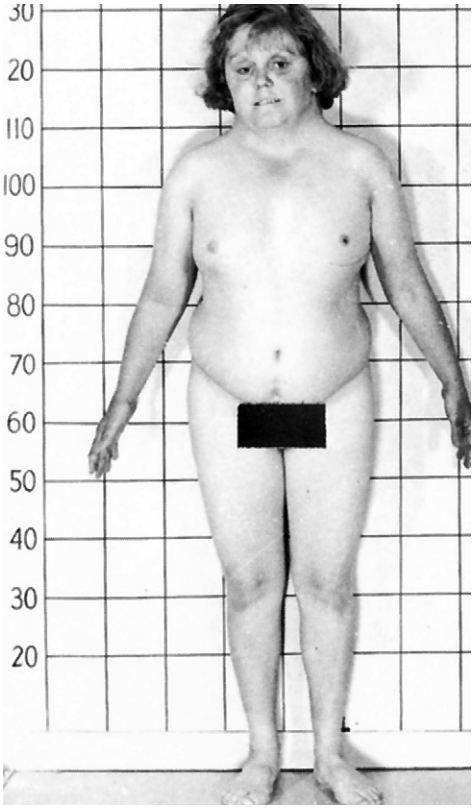

FIGURE 10-20 ■ Female with Turner syndrome. Note the characteristically broad, webbed neck. Stature is reduced, and sexual secondary characteristics are poorly developed. *(From Goodman RM, Gorlin RJ:* Atlas of the Face in Genetic Disorders, *2nd ed., St. Louis, 1977, Mosby.)*

OVARIAN PATHOPHYSIOLOGY

Turner Syndrome

Turner syndrome, or **gonadal dysgenesis**, is the most common cause of congenital hypogonadism. In about 50% of cases, it results from the complete absence of the second X chromosome, so the karyotype of the affected person is **45,XO**. The germ cells do not develop, and each gonad consists of a connective tissue-filled streak. The major clinical characteristics include short stature, a characteristic webbed neck, low-set ears, a shield-shaped chest, short fourth metacarpals, and sexual infantilism resulting from gonadal dysgenesis (Fig. 10-20). Internal and external genitalia typically are female.

Polycystic Ovarian Syndrome

Chronically anovulatory women with high circulating androgen, estrogen, and LH levels often have the disorder called **polycystic ovarian syndrome (PCOS)**. This syndrome may be caused by any of a broad array of underlying problems, and PCOS accounts for 75% of **anovulatory infertility**. Currently, the diagnosis of PCOS requires two of the following three conditions: **amenorrhea**, evidence of **excessive androgen secretion** (i.e., acne, hirsutism), and **polycystic ovaries**, as usually detected by sonogram (Fig. 10-21). The

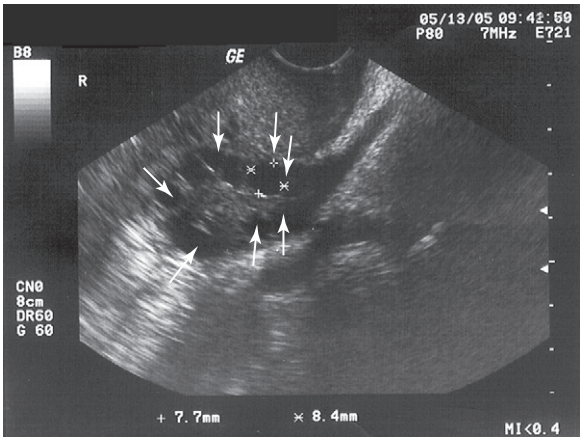

FIGURE 10-21 ■ Sonogram of polycystic ovary. Cysts *(arrows)* are due to large antral follicles in the cortex that failed to ovulate. *(Courtesy of Dr. Andrea DiLuigi, Department of Obstetrics and Gynecology, University of Connecticut Health Center, Farmington, Conn.)*

ovarian cysts represent large antral follicles that have failed to ovulate and luteinize. The continuous gonadotropin secretion leads to ovarian enlargement, and the ovaries typically show a thickened capsule and numerous follicles, many of which are undergoing atresia. FSH levels are low, which inhibits granulosa cell function, and the high intrafollicular androgen level inhibits follicular maturation. A significant proportion of the circulating estrogen, present in high levels, is estrone formed from peripheral aromatization of androstenedione. These high androgen levels can produce hirsutism and acne. Hirsutism is the abnormal formation of coarse sexual hair in regions atypical for a woman, such as the face, back, and chest.

The exact cause of PCOS is not well understood, but the primary defect appears to be inappropriate signals between the hypothalamic-pituitary axis and the ovary. A significant subset of patients with PCOS are overweight or obese and have insulin resistance and hyperinsulinemia. Insulin promotes ovarian androgen production, and hyperinsulinism may account for increased androgen production. Reduction of insulin levels (such as by weight loss, exercise, or metformin administration) ameliorates the hyperandrogenism and PCOS in these patients. Alternatively, an inadequate response of the follicle to FSH may be due to impaired IGF-1 or insulin signaling.

SUMMARY

1. The female reproductive system includes the ovary, oviducts, uterus, cervix, vagina, and external genitalia, along with the pituitary gonadotropes and the hypothalamic GnRH neurons. The mammary glands (breasts) also can be considered a part of the female reproductive system.
2. The ovarian phases of the human menstrual cycle are the follicular phase, the periovulatory period, and the luteal phase.
3. The ovarian follicle contains a primary oocyte arrested in meiotic prophase and variable layers of granulosa and thecal cells. Preantral and early antral follicular growth is gonadotropin independent. Intermediate antral follicular growth is dependent on a basal level of FSH, but not affected by fluctuations in FSH associated with the menstrual cycle. Large antral follicular development is exquisitely dependent on fluctuations of FSH. Follicles can degenerate at any phase during the process of atresia.
4. The dominant follicle is selected based on its size, number of FSH receptors, aromatase activity, and blood supply. The dominant follicle is the endocrine structure of the follicular phase. The thecal cells express the LH receptor, and LH stimulates the production of androgens (primarily androstenedione). The granulosa cells express the FSH receptor, and FSH promotes the aromatization of androgens to estrogens (primarily estradiol). FSH also induces the expression of the LH receptor in granulosa cells of the dominant follicle.
5. The dominant follicle signals that it is ready to ovulate by its estrogen production. High sustained levels of estrogen induce the midcycle LH surge through a positive feedback mechanism. This is due, in part, to a marked increase in the sensitivity of the pituitary gonadotropes to GnRH pulses.
6. The periovulatory period involves the meiotic maturation of the primary oocyte to a secondary oocyte (egg) arrested at metaphase II. Ovulation involves the rupture of the follicular wall at the stigma, release of the cumulus-oocyte complex, and differentiation of the remaining follicular cells into a corpus luteum.
7. The luteal phase is characterized by high progesterone production. The corpus luteum is programmed to die in 14 days, unless rescued by hCG.
8. Progesterone opposes the estradiol-dependent proliferation of the endometrial functional zone.
9. The oviduct functions to capture the cumulus-oocyte complex, transport and nurture both male and female gametes, promote fertilization and early embryonic development, and determine the timing of the movement of the blastocyst into the uterine cavity.

10. The mucosa of the uterus is called the endometrium. The function of the endometrium is to allow implantation and placentation. Estrogen produced during the mid- to late follicular phase of the ovary drives the proliferative phase of the uterus, during which the endometrium grows in thickness. The progesterone produced by the luteal phase of the ovary drives the secretory phase of the uterus. Loss of progesterone after the death of the corpus luteum causes the endometrium to break down. This represents the menstrual phase of the uterus.
11. The cyclic changes in ovarian steroids also alter cervical mucus and vaginal epithelium. The external genitalia are responsive to estrogen and androgens.
12. Estrogen and progesterone regulate numerous processes directly associated with reproduction. However, these steroids also regulate nonreproductive aspects of physiology, including bone growth and health, cardiovascular function, hepatic function, and others. Estrogen and progesterone act primarily through interaction with classic estrogen and progesterone receptors, which belong to the family of nuclear hormone receptors. Estrogen and progesterone also have rapid, membrane-initiated actions.
13. Turner syndrome (gonadal dysgenesis) is the most common cause of congenital hypogonadism. It typically results from the absence of the second X chromosome, so the karyotype of the affected person is 45,XO.
14. Polycystic ovarian syndrome produces chronic anovulation. Circulating androgen, estrogen, and LH levels typically are high.

SELF-STUDY PROBLEMS

1. During treatment for in vitro fertilization, the patient receives a daily injection of FSH for 8 to 10 days, followed by one injection of hCG. Cumulus-oocyte complexes are retrieved 35 hours after the hCG injection from ovarian follicles just before they ovulate. Because oocytes are retrieved before ovulation, and patients are treated with progesterone after retrieval, what is the purpose of the hCG injection?
2. When and in what cells is the LH receptor expressed during the menstrual cycle?
3. Describe the major ovarian processes that occur during the periovulatory period.
4. What is meant by the "two-cell model" of ovarian steroidogenesis?
5. Name three effects of estrogen on reproductive tissue and three effects on nonreproductive tissue.
6. What would be the outcome of a truncated luteal phase with early death of the corpus luteum on the uterine endometrium?
7. What is the ovarian reserve?
8. What is the response of the pituitary gonadotropes to the death of a corpus luteum of menstruation?
9. What is the response of the ovary to declining FSH levels during the early follicular phase?

KEYWORDS AND CONCEPTS

- 3β-Hydroxysteroid dehydrogenase (3β-HSD)
- 17β-Hydroxysteroid dehydrogenase (17β-HSD)
- 17-Hydroxylase activity

For full list of keywords and concepts see Student Consult

SUGGESTED READINGS

Arevalo MA, Santos-Galindo M, Lagunas N, et al: Selective estrogen receptor modulators as brain therapeutic agents, *J Mol Endocrinol* 46:R1–R9, 2011.

Conti M, Hsieh M, Musa Zamah A, et al: Novel signaling mechanisms in the ovary during oocyte maturation and ovulation, *Mol Cell Endocrinol* 356:65–73, 2012.

Mehlmann LM: Stops and starts in mammalian oocytes: Recent advances in understanding the regulation of meiotic arrest and oocyte maturation, *Reproduction* 130:791–799, 2005.

Pasquali R, Stener-Victorin E, Yildiz BO, et al: PCOS Forum: Research in polycystic ovary syndrome today and tomorrow, *Clin Endocrinol* 74:424–433, 2011.

KEYWORDS AND CONCEPTS

- 17,20-Lyase activity
- 45,XO
- Abnormal thyroid function
- Amenorrhea
- Ampulla
- Androgens
- Androstenedione
- Angiogenic factors
- Angiopoietin-2
- Anovulatory infertility
- Antimüllerian hormone (AMH)
- Antral follicles
- Aromatase inhibitors
- Atresia
- Basal lamina
- Basal levels of LH
- Basal zone (stratum basale)
- Basic fibroblast growth factor (bFGF)
- Birth canal
- Body
- Bone morphogenetic proten-15 (BMP15)
- Capacitation
- Capture of the cumulus-oocyte complex
- Cervical mucus
- Cervix
- Ciliated cells
- c-kit
- Clitoris
- c-Mos
- Copulatory structures
- Corona radiata
- Corpus albicans
- Corpus hemorrhagicum
- Corpus luteum
- Corpus luteum of menstruation
- Corpus luteum of pregnancy
- Cortisol-binding globulin (CBG)
- Cuboidal granulose cells
- Cumulus cells
- Cumulus expansion
- Cumulus oophorus
- Cumulus-oocyte complex
- Cyclic adenosine monophosphate (cAMP)
- Cyclic guanosine monophosphate (cGMP)
- Cyclin B
- Cyclin-dependent kinase-1 (CDK1)
- Cyclooxygenase-2 (COX-2)
- CYP11A1 (P-450 cholesterol side-chain cleavage)
- CYP11A1 (side-chain cleavage enzyme)
- CYP17
- CYP19-Aromatase
- Cytostatic factor (CSF)
- Decidua
- Dermal thickness
- Dominant follicle
- Dysmenorrhea
- Ectocervix
- Endocervical canal
- Endocervix
- Endocrine
- Endometrium
- Endothelial cell
- Epidermal
- Epithelial cell
- Estradiol
- Estradiol-17β
- Estrogen
- Estrogen receptor-α (ERα)
- Estrogen receptor antagonist
- Estrone
- Excessive androgen secretion
- Ferning
- Fertilization
- Fimbriae
- First polar body
- Follicle-stimulating hormone (FSH)
- Follicular atresia
- Follicular phase of the ovary
- FSH receptors
- Functional zone (stratum functionalis)
- Fundus
- Gametogenic
- Gap junctions
- GDF9
- Germinal vesicle breakdown (GVBD)
- Glycosaminoglycan production
- GnRH receptors
- Gonadotropin-releasing hormone (GnRH)
- GPR3

- GPR30
- Granulosa lutein cells
- Growth differentiation factor-9 (GDF9)
- HDL cholesterol
- HDL receptor (SR-BI)
- Heavy exercise
- High-density lipoprotein (HDL)
- High progesterone levels
- High prolactin levels
- Hormone-sensitive lipase
- Hyperactivity of sperm
- Hypoxic necrosis
- Implantation
- Inactivating isoforms of 17β-HSD
- Infertility
- Infundibulum
- Inhibin A
- Inhibin B
- Interstitial gland of the ovary
- Intramural or uterine segment
- Isthmus
- Keratinocytes
- Kit ligand
- Labia
- Labia majora
- Lacunae
- Large, recruitable antral follicles
- LDL cholesterol
- LDL receptor
- Leiomyomas
- Leukocytes
- LH receptors
- LH surge
- LH/FSH ratio
- Lipoprotein
- Lipoprotein lipase activity
- Liver
- Low-density lipoprotein (LDL)
- Luteal phase
- Luteal phase deficiency (LPD)
- Luteal phase of the ovary
- Luteinization
- Luteinizing hormone (LH)
- Luteolysis
- Matrix metalloproteases
- Maturation-promoting factor (MPF)
- Meiotic arrest
- Meiotic competence
- Menopause
- Menorrhagia
- Menstruation
- Metaphase II
- Midcycle gonadotropin surge
- Mitogen-activated kinase kinase (MAPKK, MEK1, MAPK)
- Mons pubis
- Mural granulose cells
- Mural granulose cells (stratum granulosum)
- Myometrium
- Myosalpinx
- Neuroprotective
- Nitric oxide
- Oligomenorrhea
- Orgasm
- Ovarian follicles
- Ovarian reserve
- Ovarian surface epithelial cells
- Ovaries
- Ovary gonadotrope—event 7
- Ovary—event 1
- Ovary—event 3
- Ovary—event 5
- Ovary—event 9
- Oviducts (uterine or fallopian tubes)
- Ovulation
- Ovulatory quota
- PDE3A
- Perimetrium
- Period
- Periovulatory period
- Perisalpinx
- $PGF_{2\alpha}$
- Phosphodiesterase (PDE)
- Pituitary FSH
- Pituitary gonadotrope—event 1
- Pituitary gonadotrope—event 2
- Pituitary gonadotrope—event 4
- Pituitary gonadotrope—event 6
- Polycystic ovarian syndrome (PCOS)
- Polycystic ovaries
- Positive feedback
- Preantral

- Predecidual cells
- Pregnancy
- Pregranulosa cells
- Premature ovarian failure
- Premenstrual dysphoria disorder (PMDD)
- Premenstrual syndrome (PMS)
- Primary ciliary dyskinesia (immotile cilia syndrome, Kartagener syndrome)
- Primary follicle
- Primary oocytes
- Priming
- Progesterone
- Progesterone receptors
- Proliferative phase
- Prophase I
- Prostaglandins
- Pudendum
- Pulsatile
- Raloxifene
- Relaxin
- Secondary follicle
- Secondary oocyte (egg)
- Secretory cells
- Secretory phase
- Selection
- Selective estrogen receptor modulator (SERM)
- Sensitivity to GnRH signaling
- Set-point for thermoregulation
- Sex hormone–binding globulin (SHBG)
- Sexual arousal
- Slow frequency of GnRH pulses
- Sperm storage
- Spinnbarkeit
- Spiral arteries
- StAR protein
- Starvation
- Stromal cell
- Sulfotransferase
- Tamoxifen
- Testosterone
- Thecal cells
- Transport
- Transudate
- Turner syndrome (gonadal dysgenesis)
- Uterine fibroids
- Uterine glands
- Uterotropic
- Vascular endothelial growth factor (VEGF)
- Vasodilation
- Ventilatory response
- Vestibular glands
- Vulva
- Window of receptivity
- Wound healing
- Zona pellucida (ZP)
- ZP1
- ZP2
- ZP3

11 FERTILIZATION, PREGNANCY, AND LACTATION

OBJECTIVES

1. Describe the synchronization among fertilization, early embryonic events, and the human menstrual cycle.
2. Describe the events involved in fertilization.
3. Explain how implantation and placentation occur.
4. Discuss the endocrine and transport functions of the placenta.
5. Describe the development of the fetal endocrine system.
6. Discuss maternal endocrine changes during pregnancy.
7. Discuss the current models for the initiation and progression of labor (parturition) in humans.
8. Describe the development and regulation of the mammary glands.
9. Discuss the endocrine basis for contraception, the "morning after" pill, and the abortion pill.

Human reproduction involves internal insemination, internal fertilization, and internal gestation, all within the female tract. Internal gestation also involves the development of a transient organ, the placenta. The placenta is remarkable in that it is composed of tissues from two organisms:

- An extraembryonic membrane (called the chorion) of the fetus
- Endometrial tissue (called decidua) of the mother

From an endocrine point of view, pregnancy represents a state in which three separate endocrine systems—**maternal**, **fetal**, and **placental**—interact to promote adequate growth and nutrition of the fetus, the timing of parturition, and preparation of the maternal mammary glands to support extrauterine life of the fetus.

FERTILIZATION, EARLY EMBRYOGENESIS, IMPLANTATION, AND PLACENTATION

Synchronization with Maternal Ovarian and Reproductive Tract Function

Fertilization, **early embryogenesis**, **implantation**, and **early gestation** all are synchronized with the human menstrual cycle (Fig. 11-1). Just before ovulation, the ovary is in the **late follicular stage** and produces high levels of **estrogen**. Estrogen promotes growth of the uterine endometrium and induces expression of the progesterone receptor. Estrogen ultimately induces the **luteinizing hormone** (**LH**) **surge**, which in turn induces **meiotic maturation** of the oocyte and **ovulation** of the cumulus-oocyte complex.

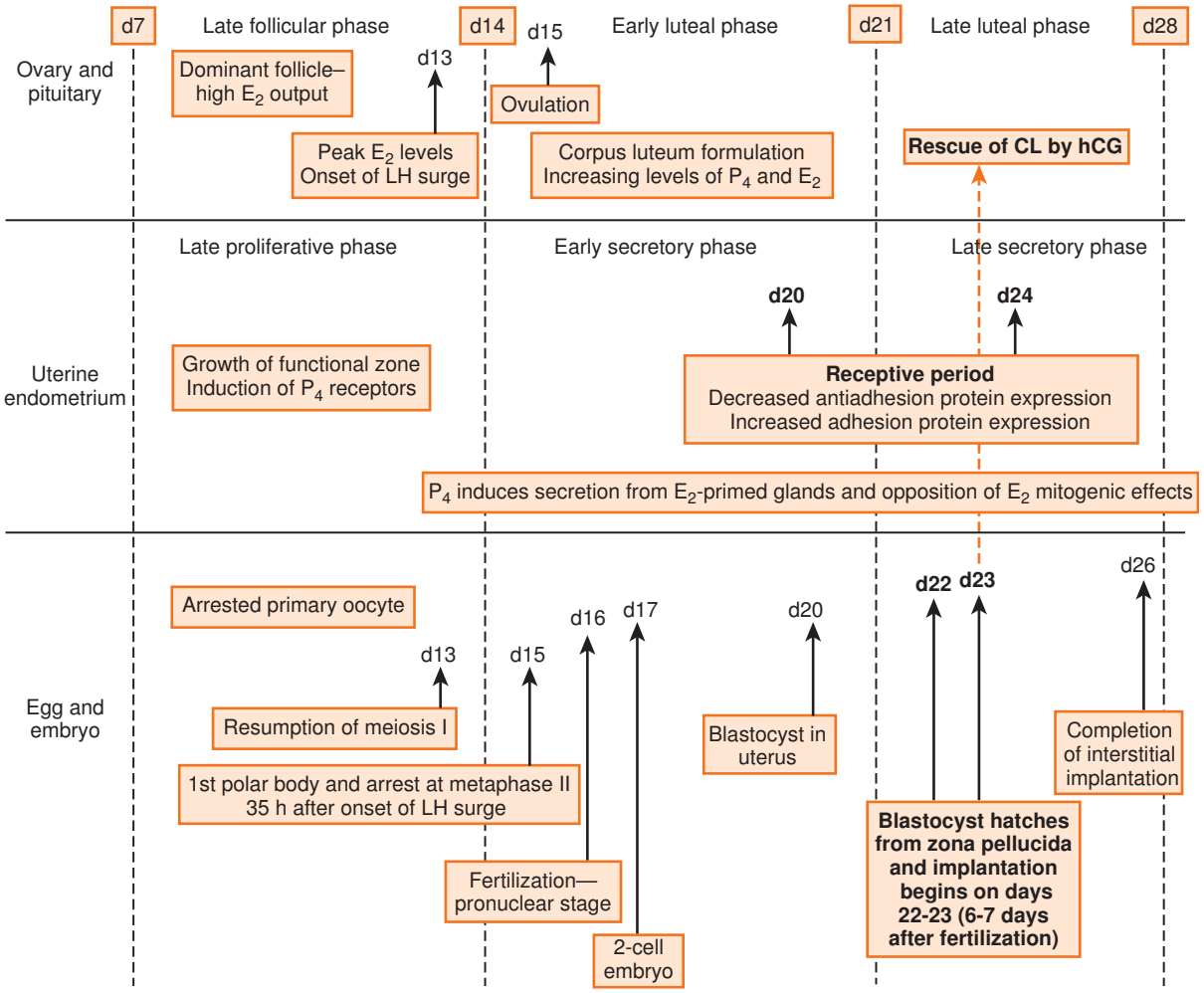

FIGURE 11-1 ■ Synchronization of the human menstrual cycle with fertilization and early embryogenesis. CL, corpus luteum; E, estrogen; E2, estrone; hCG, human chorionic gonadotropin; LH, luteinizing hormone; P4, progesterone.

The events between fertilization and implantation take about 6 days to complete, and implantation occurs at about day 21 of the menstrual cycle. At this time, the ovary is in the **midluteal phase**, secreting large amounts of **progesterone**. Progesterone stimulates secretion from uterine glands, which provide nutrients to the embryo. This is referred to as **histotrophic nutrition** and is an important mode of maternal-to-fetal transfer of nutrients for about the first trimester of pregnancy, after which it is replaced by hemotrophic nutrition. Progesterone inhibits myometrial contraction and prevents the release of paracrine factors that lead to menstruation.

Progesterone induces the **window of receptivity** in the uterine endometrium, which exists from about day 20 to 24 of the menstrual cycle. This receptive phase is associated with increased adhesivity of the endometrial epithelium and involves the formation of cellular extensions, called **pinopodes**, on the apical surface of endometrial epithelia, along with increased expression of **adhesive proteins** (e.g., integrins, cadherins) and decreased expression of antiadhesive proteins (e.g., mucins) in the apical cell membrane.

Thus, during the time it takes a fertilized egg to implant in the uterus, the uterine endometrium is at

its full thickness, actively secretory, and capable of tightly adhering to the implanting embryo. It also should be noted that the uterine endometrium is well vascularized at the time of implantation. **Spiral arteries** extend to the basal lamina of the surface epithelium (see Fig. 10-19 in Chapter 10) and give rise to rich capillary beds and postcapillary venous lakes (also called **lacunae**). Apart from its nutrient supply to all cells of the endometrium, the extensive blood supply immediately adjacent to the surface epithelium plays a critical role in capturing embryonic **human chorionic gonadotropin (hCG)** and transporting hCG to the ovary, where it *rescues* the corpus luteum. The rich endometrial blood supply also is important for efficient delivery of progesterone to the endometrium.

Fertilization

Fertilization accomplishes both the recombination of genetic material to form a new, genetically distinct organism and the initiation of events that begin embryonic development. There are several steps that must occur for successful fertilization to be achieved. The **sperm** must find its way to the **egg**, and the sperm and the egg must contact, recognize one another, and fuse. After sperm-egg fusion, an intracellular signaling cascade occurs within the egg that has two major consequences. First, it allows the egg to regulate sperm entry such that only one sperm can fuse with the egg. This prevents **polyspermy**, which is lethal. Second, it "wakes up" the metabolically quiescent egg so that it can resume meiosis and begin embryonic development. This process is called **egg activation**.

Spermatozoa present in the male ejaculate enter the vagina near the cervix and must reach the **ampulla of the oviduct** where fertilization occurs.

Sperm transport is largely dependent on the female reproductive tract and, while the sperm are still in the uterus, is independent of swimming. Large numbers of sperm in the ejaculate generally are required for successful fertilization of the egg by one sperm—of the 300 million sperm typically ejaculated, only about 200 reach the oviduct. Clinically, males with fewer than 20 million sperm per milliliter of ejaculate are considered to be **infertile**.

The female reproductive tract is an important regulator of sperm transport. Toward the end of the follicular phase of the menstrual cycle, before ovulation, estrogen levels are high. Estrogen causes the cervix to produce a **watery mucus**, often called "egg white cervical mucus" because of its consistency. This mucus forms channels to aid the passage of sperm through the cervix, and only motile sperm can pass through this barrier. Estrogen also causes contractions of the **myometrium** to help propel sperm upward toward the oviduct (i.e., cervical-to-fundal contractions).

Sperm must undergo a process called **capacitation** in the female reproductive tract before they are able to fertilize the egg. Sperm capacitation is an incompletely understood, transient event that occurs largely in the **oviduct** and modifies the spermatozoan in several ways so that it becomes capable of fertilizing the egg. These changes include the following:

1. An altered membrane fluidity due to the removal of cholesterol from the sperm membrane
2. The removal of proteins and carbohydrates from the membrane that may otherwise block sites that bind to the egg
3. A change in membrane potential that may permit Ca^{2+} to enter the sperm and thereby facilitate the **acrosome reaction** (see later)
4. Phosphorylation of numerous proteins

Incapacitated sperm bind actively to the epithelial cells of the **oviductal isthmus** and become unbound when they are capacitated. This binding slows the capacitation process, extends the sperm life span, prevents too many sperm from reaching the egg, and increases the probability that sperm will be in the oviduct when the egg is ovulated. Sperm can therefore survive in the female reproductive tract for several days. **Hyperactivation** is another phenomenon associated with sperm capacitation. Hyperactivation involves a change in flagellar motion from a wavelike to a whiplike motion. This type of flagellar movement is necessary for the sperm to detach from the oviductal epithelium, is well suited to swimming through the thick oviductal fluid, and helps propel the sperm through the outer layers of the egg to reach the egg's plasma membrane.

Capacitated sperm reach the egg, surrounded by its expanded **cumulus cells**, in the ampulla of the oviduct. Fertilization involves the penetration of the

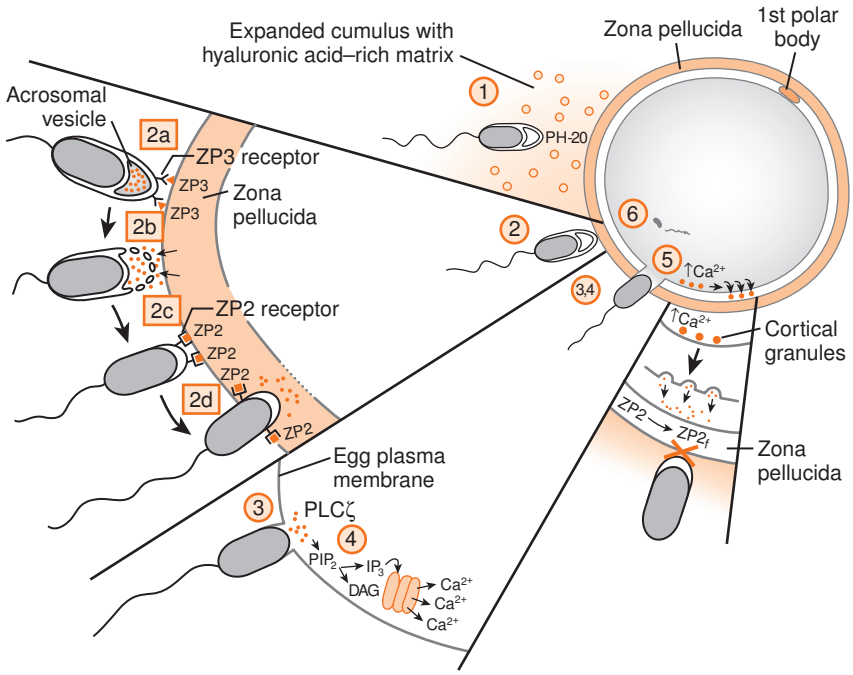

FIGURE 11-2 ■ Events of fertilization: **(1)** penetration of expanded cumulus; **(2)** acrosome reaction; **(3)** fusion of sperm membrane with egg membrane and **(4)** egg activation; **(5)** exocytosis of cortical granules and block to polyspermy. DAG, diacylglycerol; IP_3, inositol 1,4,5-triphosphate; PIP_3, phosphatidylinositol 4,5-biphosphate; PLCξ, phospholipase Cξ; ZP2, ZP3, zona pellucida glycoproteins 2 and 3; $ZP2_f$. *(Courtesy of Dr. Lisa Mehlmann, Department of Cell Biology, University of Connecticut Health Center, Farmington, Conn.)*

egg by the entire sperm. To do this, the sperm must breach three barriers (Fig. 11-2):

- Expanded cumulus
- Zona pellucida
- Plasma membrane of the egg (called the oolemma)

The cumulus cell matrix is composed predominantly of **hyaluronic acid**, and the sperm are able to digest through this layer with a membrane-bound hyaluronidase called **PH-20**. The next obstacle the sperm encounters is the zona pellucida, an extracellular coat made up of three glycoproteins called **ZP1**, **ZP2**, and **ZP3**. The sperm contains one or more **ZP3 receptors**. Sperm binding to ZP3 triggers the **acrosome reaction**, in which the inner sperm plasma membrane fuses with the outer acrosomal membrane to release the contents of the **acrosomal vesicle** (see Fig. 11-2). The enzymes released from the acrosomal vesicle then digest the zona pellucida. After the acrosome reaction, the sperm loses ZP3 receptors, and it undergoes a secondary binding instead to **ZP2**. The sperm is thus held in place as the enzymes from the acrosome digest a hole in the zona pellucida and the still-swimming sperm can go through to reach the egg plasma membrane. The molecular mechanisms involved in the interactions of the sperm and egg plasma membranes are not completely understood. **Sperm-egg fusion** is likely to involve **tetraspanin proteins** in the egg that bind to a sperm protein called **Izumo** (see Fig. 11-2).

The entire sperm enters the egg during fusion. The flagellum and mitochondria disintegrate, so most of the mitochondrial DNA in cells is maternally derived. Once the sperm is inside the egg, protamines associated with the tightly condensed sperm DNA are uncoiled by the highly reducing egg cytoplasm, causing **decondensation** of the sperm DNA. A membrane called the **pronucleus** forms around the sperm DNA as the newly activated egg completes the **second meiotic division**.

The egg is a metabolically quiescent cell that is "woken up" as a result of sperm-egg fusion, in a process called **egg activation**. All of the events associated with egg activation depend on **intracellular release of Ca^{2+} in the egg**, which occurs soon after sperm-egg fusion. Ca^{2+} release is stimulated by the production of **inositol 1,4,5-triphosphate (IP_3)** in response to the sperm phospholipase C enzyme, **PLCζ**. IP_3 binds to its receptor on the endoplasmic reticulum and opens Ca^{2+} channels (see Chapter 1).

One of the earliest Ca^{2+}-dependent events that occurs at fertilization of mammalian eggs is the

prevention of polyspermy. Enzyme-filled vesicles, called **cortical granules**, reside in the outermost, or cortical, region of the unfertilized egg. These vesicles translocate to the plasma membrane and release hydrolytic enzymes through exocytosis. These enzymes modify ZP2, generating ZPf, which blocks binding of any acrosome-reacted sperm. Thus, only one sperm usually enters the egg. Occasionally, more than one sperm does enter the egg. This results in a **triploid cell**, which is unable to develop further.

Ca^{2+} release also stimulates the egg to reenter the cell cycle, **complete meiosis**, and recruit **maternal messenger RNA (mRNA)** after fertilization. The unfertilized egg is held in meiotic arrest at **metaphase of meiosis II** by the cell cycle regulatory protein complex, **maturation-promoting factor (MPF)**, as well as **cytostatic factor (CSF)**, which contains components of the **mitogen-activating protein kinase (MAPK) pathway**. Ca^{2+}-calmodulin-dependent pathways inactivate both MPF and CSF so that the metaphase II chromosomes decondense, the anaphase-promoting complex becomes active, and the egg can form a **pronucleus**. The unfertilized egg is transcriptionally inactive, and Ca^{2+} release at fertilization also is needed for the recruitment of **stored maternal mRNAs** for translation into **maternally derived proteins** needed for early embryonic development.

The activated egg completes the second meiotic division as the sperm DNA decondenses and a pronucleus forms around it (Fig. 11-3). Once the egg has completed meiosis, a pronucleus forms around the female chromosomes as well. A **centrosome**, contributed by the sperm, becomes a **microtubule-organizing center** from which microtubules extend until they contact the female pronucleus. The male and female DNAs replicate as the two pronuclei are pulled together. Once the pronuclei contact each other, the nuclear membranes break down, the chromosomes align on a common metaphase plate, and the **first embryonic cleavage** occurs.

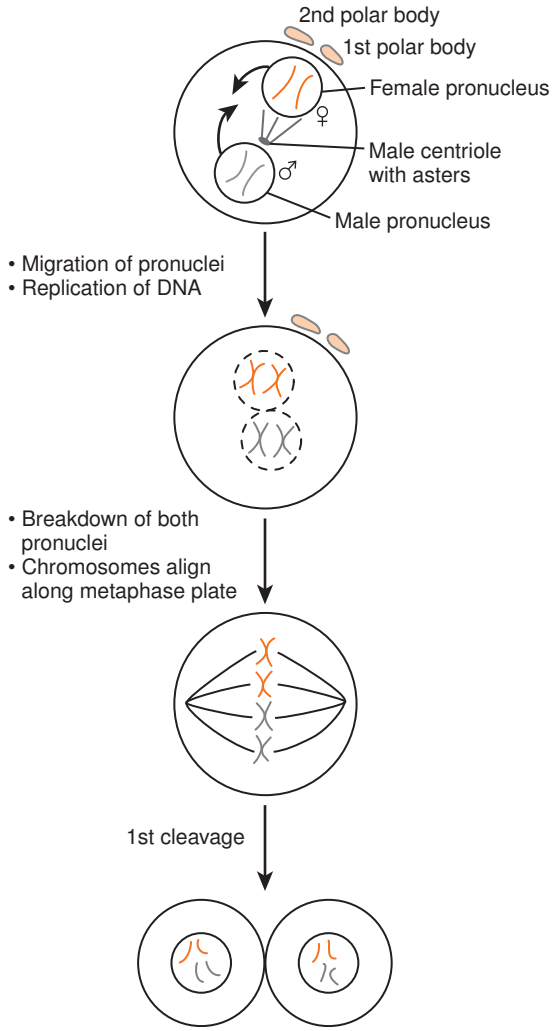

FIGURE 11-3 ■ Pronuclear formation and first embryonic cleavage.

Early Embryogenesis and Implantation

Fertilization typically occurs on day 15 or 16 of the menstrual cycle, and implantation occurs about 6 days later. Thus, the first week of embryogenesis occurs within the lumina of the oviduct and uterus (Fig. 11-4). For most of this time, the embryo remains encapsulated by the **zona pellucida**. The first two cleavages take about 2 days, and the embryo reaches a 16-cell **morula** by 3 days. The outer cells of the morula become tightly adhesive with each other and begin transporting fluid into the embryonic mass. During days 4 and 5, the transport of fluid generates a cavity, called the blastocyst cavity (blastocoele), and the embryo is now called a **blastocyst** (Fig. 11-5). The blastocyst is composed of two subpopulations of cells:

- The eccentric **inner cell mass**
- An outer, epithelial-like layer of **trophoblasts**. The region of trophoblast layer immediately adjacent

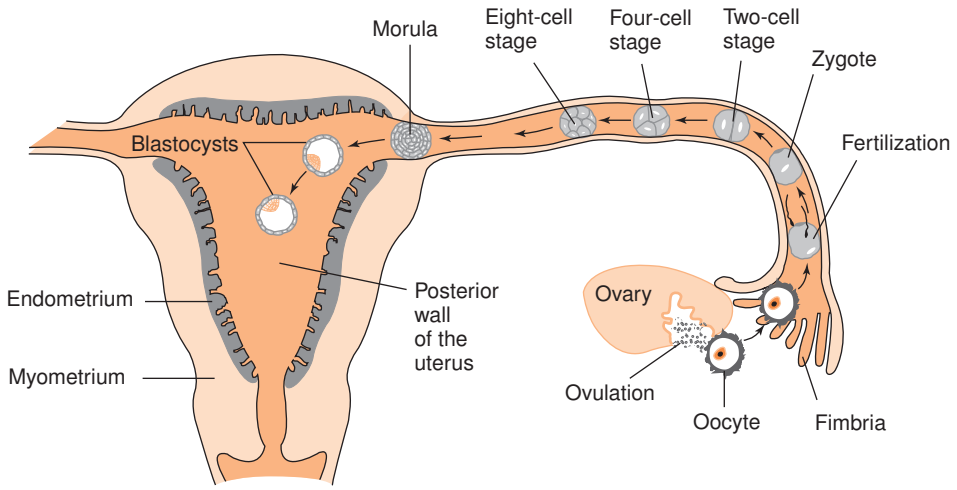

FIGURE 11-4 ■ Fertilization and human development during the first week.

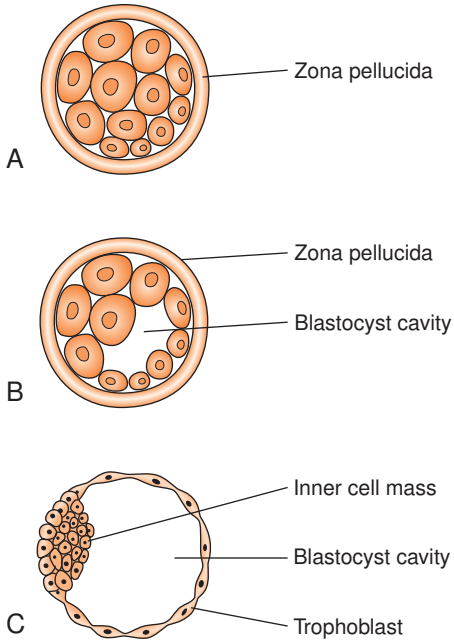

FIGURE 11-5 ■ Early cleavage stages in embryos. **A,** Morula. **B,** Early blastocyst with zona pellucida intact. **C,** Late blastocyst shows inner cell mass and blastocyst cavity.

to the inner cell mass is referred to as the **embryonic pole**, and it is this region that attaches to the uterine endometrium at implantation.

The embryo resides within the oviduct during the first 3 days and then enters the uterus. By 5 to 6 days of development, the trophoblasts of the blastocyst secrete proteases that digest the outer-lying zona pellucida. At this point, corresponding to about day 22 of the menstrual cycle, the **hatched blastocyst** (Fig. 11-6) is able to adhere to and implant into the receptive uterine endometrium.

At the time of attachment and **implantation**, the trophoblasts differentiate into two cell types: an inner layer of **cytotrophoblasts** and an outer layer of multinuclear and multicellular **syncytiotrophoblasts** (Fig. 11-7). The cytotrophoblasts initially provide a feeder layer of continuously dividing cells. Syncytiotrophoblasts perform three general types of functions: **adhesive**, **invasive**, and **endocrine**. Syncytiotrophoblasts express adhesive surface proteins (i.e., cadherins and integrins) that bind to uterine surface epithelia, and as the embryo implants, these bind to components of the uterine extracellular matrix. In humans, the embryo completely burrows into the superficial layer of the endometrium (see Fig. 11-7). This mode of implantation, called **interstitial implantation**, is the most invasive among placental mammals. Interstitial implantation involves adhesion-supported invasion and migration of syncytiotrophoblasts into the endometrium, along with the breakdown of extracellular matrix by the secretion of matrix metalloproteases and other hydrolytic enzymes.

The endocrine function begins with the onset of implantation, when syncytiotrophoblasts begin secreting the LH-like protein **hCG** (see later), which

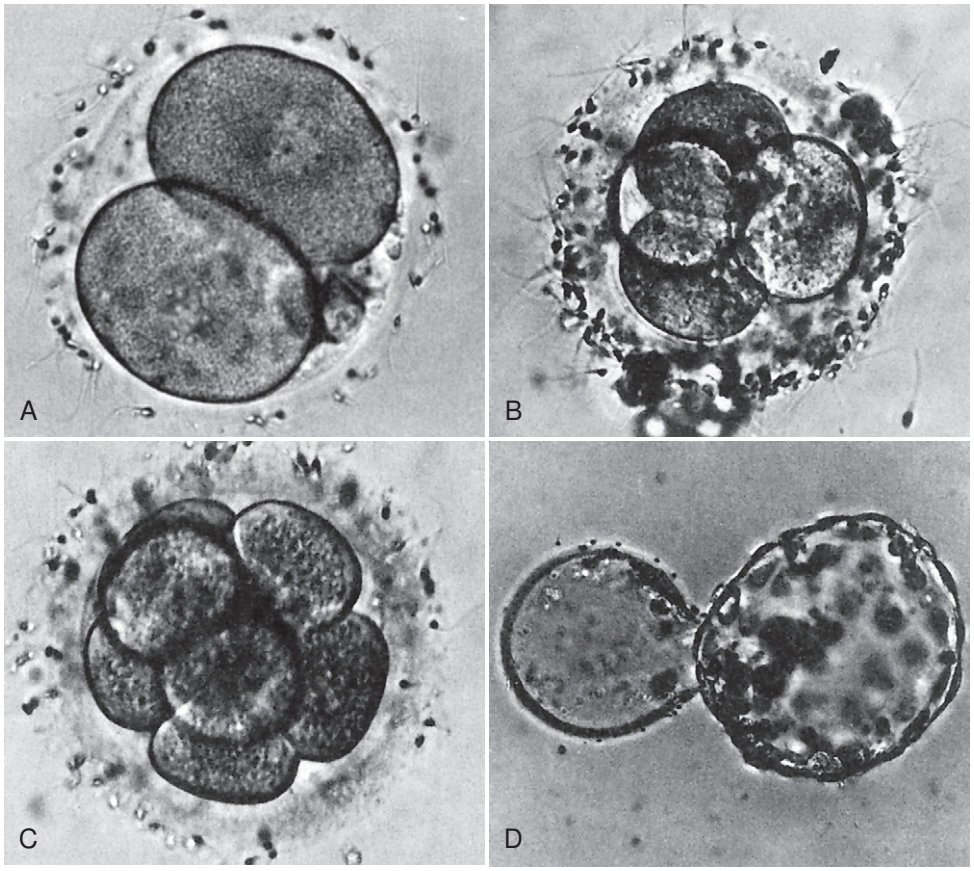

FIGURE 11-6 ■ Cleavage stages of human eggs fertilized in vitro. **A,** Two cells 39 hours after fertilization. Polar body is at right of boundary between the two cells. **B,** Four cells 42 hours after fertilization. **C,** Eight cells 49 hours after fertilization. **D,** Hatching blastocyst 123 hours after fertilization. In **A** to **C,** numerous spermatozoa can be seen clinging to zona pellucida. *(From Veeck LL:* Atlas of the Human Oocyte and Early Conceptus, *vol. 2, Baltimore, 1991, Williams & Wilkins.)*

maintains the viability of the corpus luteum (**corpus luteum of pregnancy**) and, thus, maintains **progesterone** secretion. Syncytiotrophoblasts become highly steroidogenic by 10 weeks and make progesterone at sufficient levels to maintain pregnancy independently of the corpus luteum. Syncytiotrophoblasts produce several other hormones, as well as enzymes that modify hormones (see later).

As implantation and placentation progress, syncytiotrophoblasts take on the important functions of **phagocytosis** (during histotrophic nutrition) and **bidirectional placental transfer** of gases, nutrients, and wastes. Exchange across the syncytiotrophoblasts involves diffusion (e.g., gases), facilitated transport (e.g., GLUT1-mediated transfer of glucose), active transport (e.g., amino acids by specific transporters), and pinocytosis-transcytosis (e.g., of iron-transferrin complexes).

There also is a maternal response to implantation, called **decidualization**, which involves the transformation of the **endometrial stroma** into enlarged and glycogen-filled **decidual cells** (the endometrium is now referred to as the **decidua**). The decidua forms an epithelial-like sheet with adhesive junctions that inhibits migration of the implanting embryo. The decidua also secretes factors, such as tissue inhibitors of metalloproteases (TIMPs), which moderate the activity of syncytiotrophoblastic-derived hydrolytic enzymes in the endometrial matrix. Consequently, decidualization allows for regulated invasion during implantation.

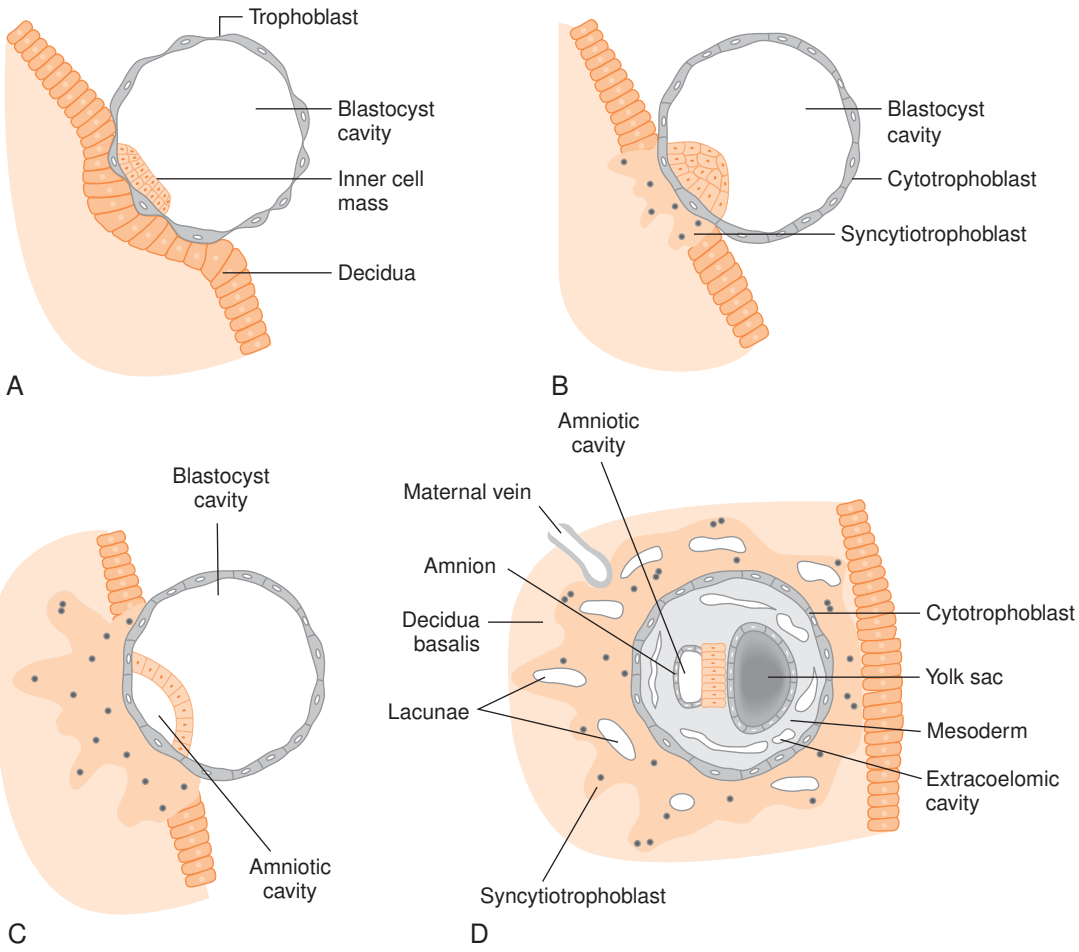

FIGURE 11-7 ■ Steps in implantation. **A,** Early implantation of blastocyst. **B,** Formation of cytotrophoblast and syncytiotrophoblast. **C,** Formation of amniotic cavity. **D,** Formation of lacunae with maternal venous penetration.

CLINICAL BOX 11-1

Normally, the implanting embryo and placenta do not extend to and involve the **myometrium**. Placental **accreta** is the destruction of the endometrium and adherence of the placenta to the myometrium, a condition associated with potentially life-threatening **postpartum hemorrhage**. It is important to note that the decidual response occurs only in the uterus. Thus, the highly invasive nature of the human embryo poses considerable risk to the mother in the case of ectopic implantations. The most common site of **ectopic implantation** is the oviduct (giving rise to a **tubal pregnancy**), but implantations also rarely occur in the ovary and cervix and within the abdominal cavity.

Structure of the Mature Placenta

The progression of placental development is complicated, and the reader is referred to embryology texts for a more complete discussion than the one presented here. It is useful to consider placental development with a focus first on the entire gravid uterus and then on the fine structure of the mature placenta.

Initially, the growing syncytiotrophoblasts extend evenly from the embryo into the outer-lying decidua. At about 9 days, spaces appear within the syncytiotrophoblast layer, called **lacunae**. These spaces become filled with the secretions of endometrial glands, maternal blood from degraded vessels, and the remnants of enzymatically digested matrix, referred to as the

embryotroph, which provides for histotrophic nutrition (Fig. 11-8).

By the end of the second week of development, the columns of syncytiotrophoblasts with a core of cytotrophoblasts are distinguishable as **primary villi** (Fig. 11-9). By this time, a new extraembryonic layer, called **extraembryonic mesoderm**, becomes associated with the cytotrophoblast and syncytiotrophoblast layers. The three layers are now referred to as the **chorionic membrane**. After primary villi gain a mesodermal core, they are referred to as **secondary villi**. The extraembryonic mesoderm provides a connection, called the **connecting stalk**, between the **chorion** and the **embryo**. It is within this mesoderm that the fetal **(umbilical) circulation** develops. Once villi contain umbilical blood vessels, they are referred to as **tertiary villi** (see Fig. 11-9). **Chorionic villi** represent the functional unit of the placenta and, through extensive branching, greatly increase the surface area for maternal-fetal exchange.

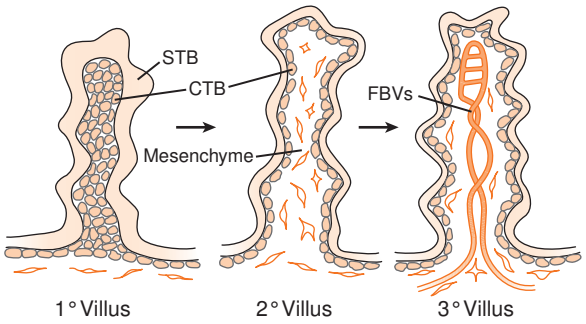

FIGURE 11-9 ■ Development of primary (1°), secondary (2°), and tertiary (3°) villi. CTB, cytotrophoblast; FBVs, fetal blood vessels; STB, syncytiotrophoblast.

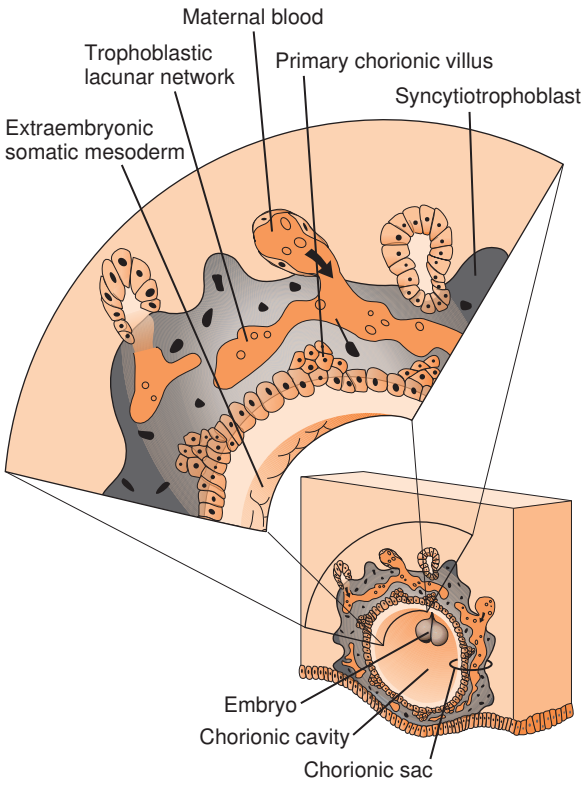

FIGURE 11-8 ■ Histotrophic nutrition *(arrows)* as the endometrial glands and spiral arteries are invaded and eroded by advancing syncytiotrophoblasts (at 14 days). *(Modified from Moore KL, Persaud TVN:* The Developing Human: Clinically Oriented Embryology, *Philadelphia, 2003, Saunders.)*

Although villi develop from the entire spherical chorionic membrane, they quickly degenerate around most of the chorion, forming a smooth chorion, or the **chorion laeve** (Fig. 11-10). In the region of the original embryonic pole, however, the chorion develops into a highly branching villous chorion, called the **chorion frondosum** (see Fig. 11-10). The chorion frondosum represents the fetal side of the mature placenta.

The uterine decidua immediately apposed to the chorion frondosum is called the **decidua basalis** and forms the maternal side of the mature placenta (see Fig. 11-10). The decidua that is subjacent to the chorion laeve is called the **decidua capsularis**. With time, the decidua capsularis fuses with the **decidua parietalis**, which is the part of the uterine endometrium that is not directly associated with the chorionic membrane (see Fig. 11-10). This means that the original uterine lumen is obliterated. The decidua capsularis ultimately degenerates.

Another extraembryonic membrane, called the **amnion**, grows and surrounds the developing fetus. The amnion becomes a fluid-filled sac, allowing for a nonadhesive environment in which the fetus can develop. By the beginning of the third trimester, the amnion fuses with the chorion, forming the **amniochorionic membrane**, which in turn fuses with the decidua parietalis (see Fig. 11-10). With the disappearance of

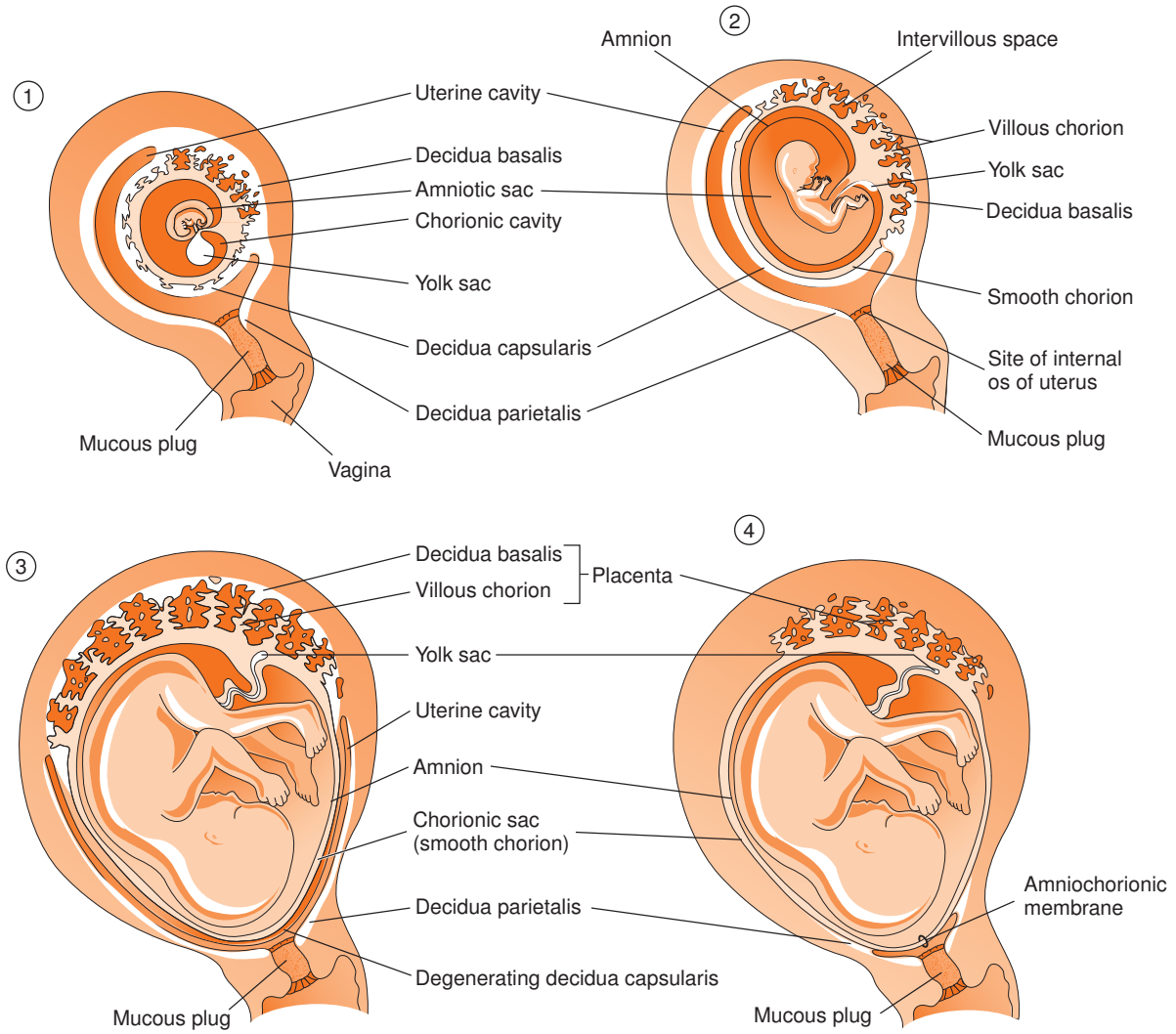

FIGURE 11-10 ■ Development and fusion of fetal membranes and decidua. *(From Moore KL, Persaud TVN:* The Developing Human: Clinically Oriented Embryology, *Philadelphia, 2003, Saunders.)*

the decidua capsularis, only the fetal amniochorionic membrane stretches across the internal opening of the cervical canal, and it is the amniochorionic membrane that ruptures during childbirth.

The mature placenta (Fig. 11-11) is composed of three major structures:

1. The **chorionic villi**, which are lined externally by the syncytiotrophoblast layer and contain the termini of umbilical blood vessels within their core. As chorionic villi branch, they become increasingly smaller, thinner, and more involved in maternal-fetal exchange. The smallest villi, called **terminal villi**, are the predominant sites of maternal-fetal exchange (Fig. 11-12). Terminal villi have an outer layer of syncytiotrophoblasts, which becomes extremely thin in certain regions. Subjacent to the thinnest regions of syncytiotrophoblasts, the

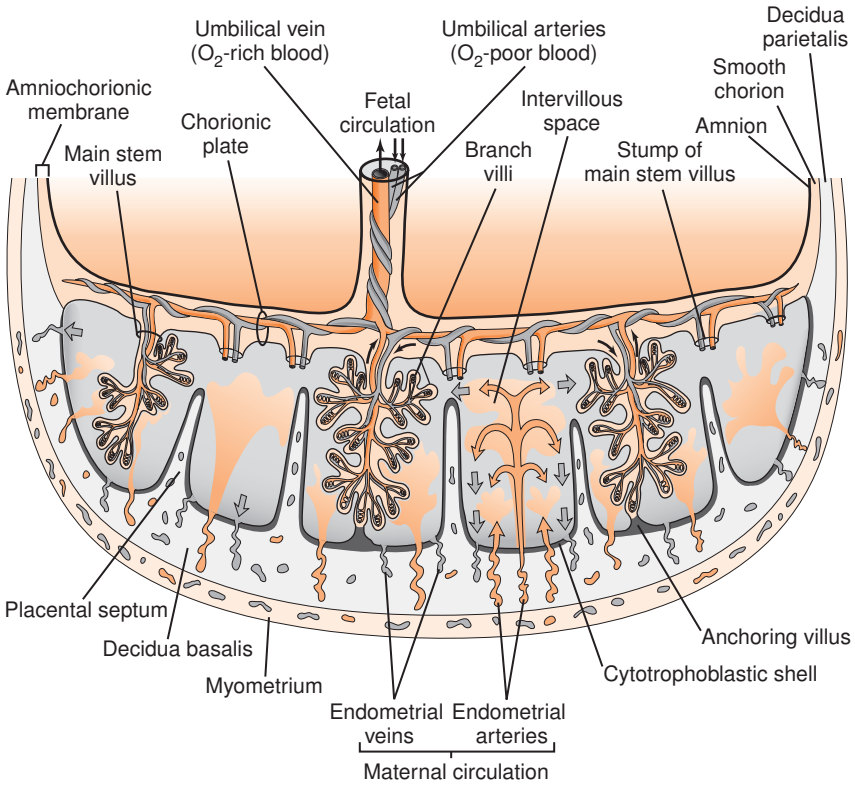

FIGURE 11-11 ■ Structure of the mature hemochorial placenta. Villi have been removed in some segments to show flow of maternal blood. *(From Moore KL, Persaud TVN:* The Developing Human: Clinically Oriented Embryology, *Philadelphia, 2003, Saunders.)*

cytotrophoblasts disappear, and an umbilical capillary presses against the syncytiotrophoblast layer. Thus, nutrients from the maternal blood that bathes the terminal villi (see intervillous space in next entry) have to cross only a single, flat layer of syncytiotrophoblast, the fused basal lamina of the syncytiotrophoblast and capillary endothelium, and a flattened umbilical endothelial cell. This barrier between maternal blood and the umbilical circulation is called the **placental membrane** (see Fig. 11-12) and is also called a **vasculosyncytial membrane**. It represents the thinnest barrier to maternal-fetal exchange among placental (i.e., eutherian) mammals.

2. The **intervillous space**, into which maternal blood flows from the open ends of **spiral arteries** (see Fig. 11-11). This blood bathes the chorionic villi and returns to maternal circulation through **endometrial veins**. Because the maternal side of the placenta is represented by maternal blood within the intervillous space, and the fetal side is represented by the chorionic vasculosyncytial membrane, human placentation is referred to as **hemochorial placentation**.
3. The **decidua basalis**. Some villi, called **anchoring villi**, extend through the intervillous space and anchor onto the decidua (see Fig. 11-11). Columns of cytotrophoblasts migrate out of the end of the anchoring villi and spread across the decidua basalis. These **extravillous cytotrophoblasts** form an adhesive layer, called the **cytotrophoblastic shell**, that anchors the **chorion frondosum** to the **decidua basalis** (see Fig. 11-11). Spiral arteries extend through the decidua basalis and open into the intervillous space through breaks in the cytotrophoblastic shell. During the first trimester, extravillous cytotrophoblasts migrate into the spiral arteries and plug them up. Thus, embryonic and early fetal development is supported primarily by histotrophic nutrition within a **hypoxic**

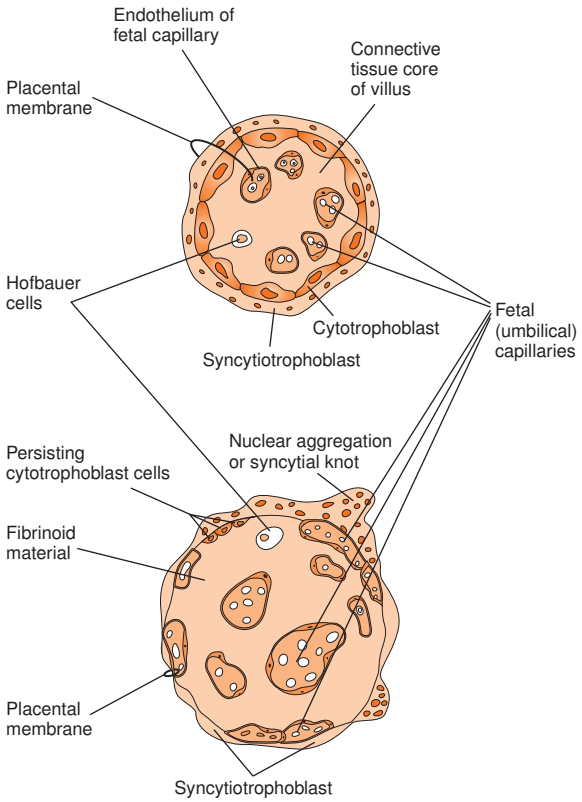

FIGURE 11-12 ■ Cross section of early *(upper)* and mature *(lower)* terminal villi. In the mature terminal villus, the cytotrophoblast layer becomes discontinuous, the fetal vessels assume an eccentric position subjacent to the syncytiotrophoblast layer, and the syncytiotrophoblast becomes very thinned out except for nuclear aggregations (also called syncytial knots). *(From Moore KL, Persaud TVN: The Developing Human: Clinically Oriented Embryology, Philadelphia, 2003, Saunders.)*

environment. During this time, the cytotrophoblasts that have invaded the spiral arteries replace the **tunica media** (i.e., the vascular smooth muscle layer) and **tunica intima** (i.e., the endothelia and their lamina propria), thereby converting the arteries into **low-resistance, high-capacitance vessels**. At the beginning of the second trimester, coincident with entry of the fetus into a rapid growth phase, the converted spiral arteries become unplugged, and **hemotrophic nutrition** predominates until parturition.

CLINICAL BOX 11-2

Preeclampsia, which is a form of hypertension and proteinuria of pregnancy, is often accompanied by a shallow invasion of the placenta and an inability of the cytotrophoblasts to convert the spiral arteries. This condition leads to **intrauterine growth restriction** (**IUGR**) of the fetus, increasing the risk for perinatal mortality.

Endocrine Function of the Placenta

The syncytiotrophoblasts of the placenta produce several steroid and protein hormones. The general functions of these hormones in pregnancy include the following:

- Maintaining the pregnant state of the uterus
- Stimulating lobuloalveolar growth and function of maternal breasts
- Adapting aspects of maternal metabolism and physiology to support a growing fetus
- Regulating aspects of fetal development
- Regulating the timing and progression of parturition

Human Chorionic Gonadotropin

The first hormone produced by the syncytiotrophoblasts is **hCG**. This hormone is structurally related to the pituitary glycoprotein hormones (see Chapter 5). As such, hCG is composed of a common **α-glycoprotein subunit** (**αGSU**) and a hormone-specific β-subunit, **β-hCG**. Antibodies used to detect hCG (as in laboratory assays and over-the-counter pregnancy tests) are designed to specifically detect the β-subunit. Human chorionic gonadotropin is most similar to LH and binds with high affinity to the **LH receptor**. The β-subunit of hCG is longer than that of LH and contains more sites for glycosylation, which greatly increases the half-life of hCG to up to 30 hours. The stability of hCG allows it to rapidly accumulate in the maternal circulation, so hCG is detectable within maternal serum within 24 hours of implantation. Serum hCG levels double every 2 days for the first 6 weeks until they peak at about 10 weeks. Serum hCG then declines to a constant level at about 50% of the peak value (Fig. 11-13).

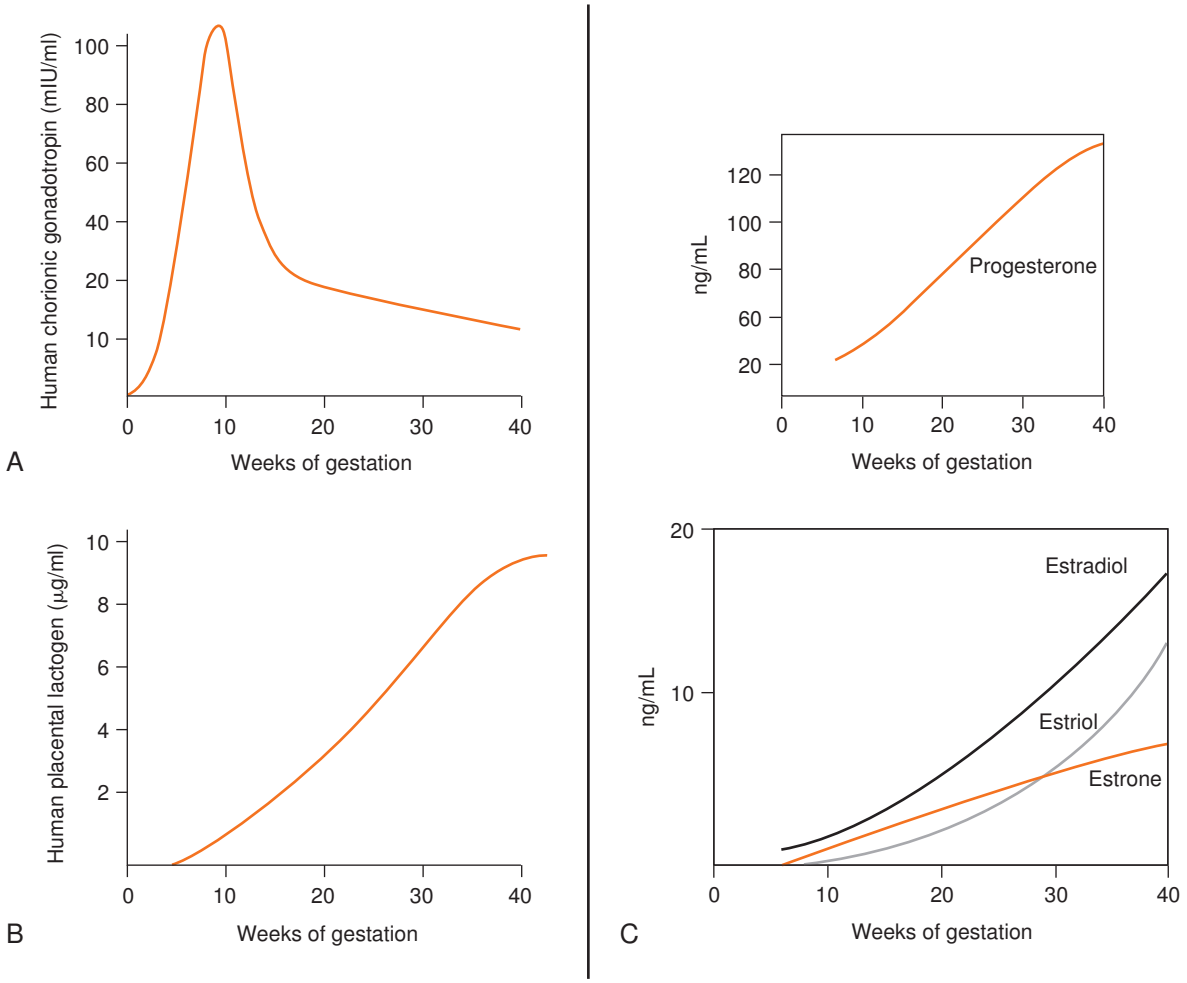

FIGURE 11-13 ■ **A,** Maternal serum human chorionic gonadotropin levels during pregnancy. **B,** Human placental lactogen levels during pregnancy. **C,** Progesterone and estrogen. *(**C,** From Koeppen B, Stanton B:* Berne and Levy Physiology, *updated 6th ed., Philadelphia, 2010, Mosby.)*

The primary action of hCG is to stimulate LH receptors on the **corpus luteum**. This prevents luteolysis and maintains a high level of luteal-derived progesterone production during the first 10 weeks, after which the syncytiotrophoblasts take over progesterone production. The rapid increase in hCG is responsible for the nausea of **morning sickness** associated with early pregnancy. Human chorionic gonadotropin binds weakly to the thyrotropin (thyroid-stimulating hormone [TSH]) receptor, so early pregnancy can be associated with a **transient gestational hyperthyroidism**. A small amount (i.e., 1% to 10%) of hCG enters into the fetal circulation. The hCG stimulates **fetal Leydig cells** to produce **testosterone** before the fetal gonadotropic axis is fully mature. hCG may also stimulate the fetal adrenal cortex during the first trimester.

CLINICAL BOX 11-3

Human chorionic gonadotropin is produced in high levels by cancers derived from **trophoblastic cells**, such as **molar disease** and **choriocarcinoma**. Thus, hCG levels can be used as a measure of the efficacy of chemotherapy.

Progesterone

The syncytiotrophoblasts express high levels of **CYP11A1 (side-chain cleavage enzyme)** and a **placenta-specific 3β-hydroxysteroid dehydrogenase type 1 (3β-HSD1)** but do not express CYP17 (Fig. 11-14). Syncytiotrophoblasts also express the receptors (e.g., low-density lipoprotein [LDL] receptor) that import cholesterol from the maternal blood. Consequently, the placenta produces a high amount of **progesterone**, which is absolutely required to maintain a quiescent myometrium and a pregnant uterus. Progesterone production by the placenta is largely unregulated—the placenta produces as much progesterone as the supply of cholesterol and the levels of CYP11A1 and 3β-HSD will allow. Of note, placental steroidogenesis differs from that in the adrenal cortex, ovaries, and testis, in that cholesterol is transported into the placental mitochondria by a mechanism that is independent of the labile **steroidogenic acute regulatory (StAR) protein**. Thus, this first step in steroidogenesis is not a regulated, rate-limiting step in the placenta as it is in other steroidogenic glands. This means that a fetus with an inactivating mutation in StAR protein will develop lipoid congenital adrenal hyperplasia (see Chapter 7) and hypogonadism but will have normal progesterone levels produced by their placenta. It also should be noted that progesterone production by the placenta does not require fetal tissue. Consequently, progesterone levels are largely independent of fetal health status and cannot be used as a measure of fetal well-being. Maternal progesterone levels continue to increase throughout pregnancy (see Fig. 11-13).

Progesterone is released primarily into the maternal circulation and is required for implantation and the maintenance of pregnancy. Progesterone also has several effects on maternal physiology and induces breast growth and differentiation (discussed later). The switch from corpus luteum–derived progesterone to placenta-derived progesterone (referred to as the **luteal-placental shift**) is complete at about the eighth week of pregnancy. Progesterone and pregnenolone are used by the definitive zone of the fetal cortex (see later) to make cortisol late in pregnancy.

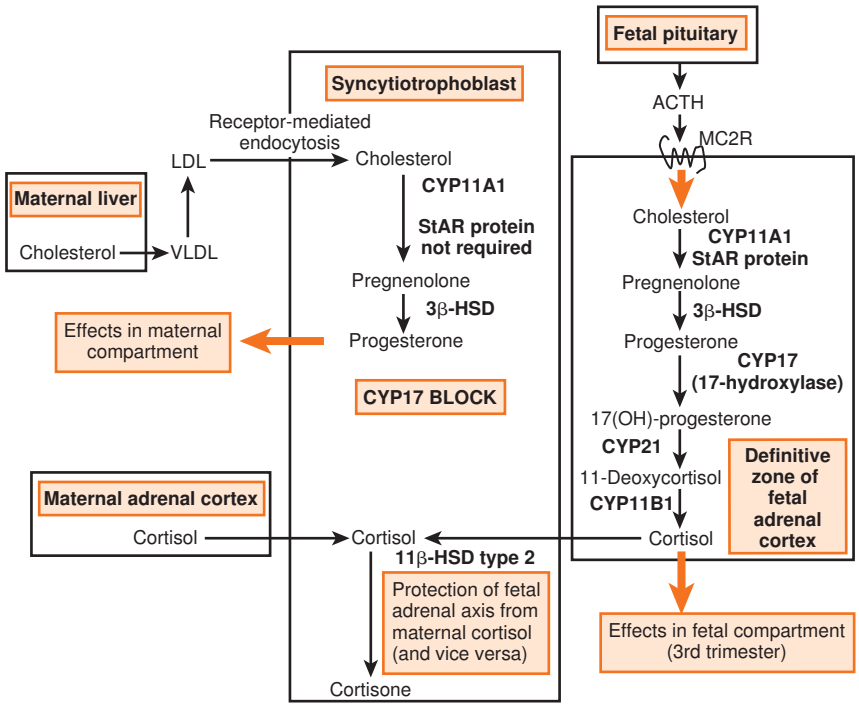

FIGURE 11-14 ■ Progesterone biosynthesis by the syncytiotrophoblast. ACTH, adrenocorticotropic hormone; 3β-HSD, 3β-hydroxysteroid dehydrogenase; 11β-HSD, 11β-hydroxysteroid dehydrogenase; LDL, low-density lipoprotein; MC2R, melanocortin-2 receptor (ACTH receptor); StAR protein, steroidogenic acute regulatory protein; VLDL, very-low-density lipoprotein.

Estrogen

Estrogens are produced by the syncytiotrophoblasts. Syncytiotrophoblasts lack CYP17 and are dependent on another cell type to provide 19-carbon androgens for aromatization. The ancillary, androgen-producing cell resides in the **fetal adrenal cortex**.

The cells of the fetal adrenal cortex emerge at week 5, and by 8 weeks there exists a distinct cortex and medulla. Initially, the cortex is composed of one zone, the **fetal zone** (Fig. 11-15). The fetal zone becomes surrounded by cells that make up the **definitive cortex**, which will differentiate into the adult zona glomerulosa and zona fasciculata. The fetal zone constitutes as much as 80% of the bulk of the large fetal adrenal gland. The fetal zone recedes after birth (see Fig. 11-15). The zona reticularis develops after 1 to 3 years, but does not secrete hormones until adrenarche at 6 to 8 years of age.

The fetal zone does not express **3β-HSD** and releases the sulfated form of the inactive androgen, **dehydroepiandrosterone sulfate (DHEAS)**, throughout most of gestation (Fig. 11-16). The production of DHEAS from the fetal adrenal is absolutely dependent on **fetal adrenocorticotropic hormone (ACTH)** from the fetal pituitary by the end of the first trimester.

The DHEAS released from the fetal zone has two fates (see Fig. 11-16). First, DHEAS can go directly to the **syncytiotrophoblast**, where it is desulfated by a placental **steroid sulfatase** and used as a 19-carbon substrate for the synthesis of **estradiol-17β** and **estrone**. The second fate of DHEAS is 16-hydroxylation in the **fetal liver** by the enzyme CYP3A7. 16-Hydroxyl-DHEAS is then converted by the syncytiotrophoblasts to the major estrogen of pregnancy, called **estriol** (see Fig. 11-16).

Because estrogen production is dependent on a healthy fetus, estriol levels can be used to assess fetal well-being. The **fetoplacental unit** represents the collective term for the tissues that make estrogen. Estrogens increase uteroplacental blood flow, enhance LDL receptor expression in syncytiotrophoblasts, and induce several components (e.g., prostaglandins, oxytocin receptors) involved in parturition. Estrogens increase the growth and development of the **mammary glands** directly, and also indirectly through the stimulation of **maternal pituitary prolactin** production (see later). Estrogens are not necessary for a normal pregnancy but are required for labor and parturition.

Human Placental Lactogen

Human placental lactogen (hPL), also called human chorionic somatomammotropin (hCS), is a 191-amino acid protein hormone produced in the syncytiotrophoblast that is structurally similar to growth hormone (GH) and prolactin (PRL). It can be detected within the syncytiotrophoblast by 10 days after conception and in maternal serum by 3 weeks of gestation

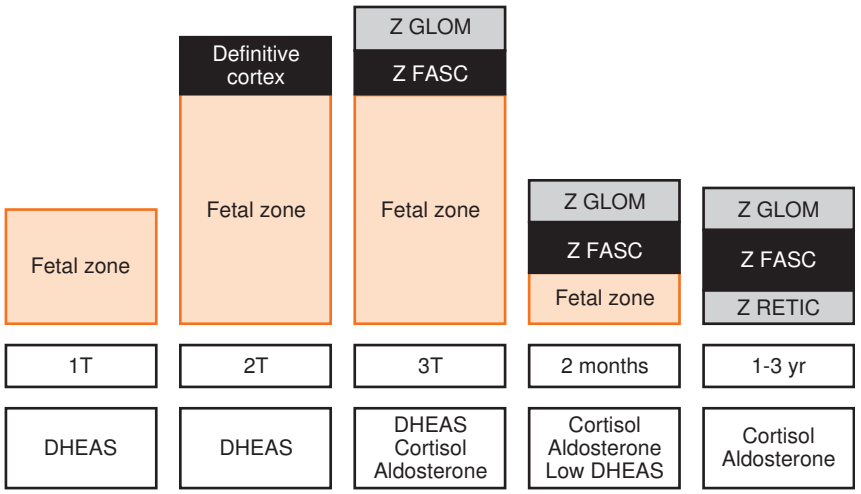

FIGURE 11-15 ■ The zones of the fetal adrenal cortex.

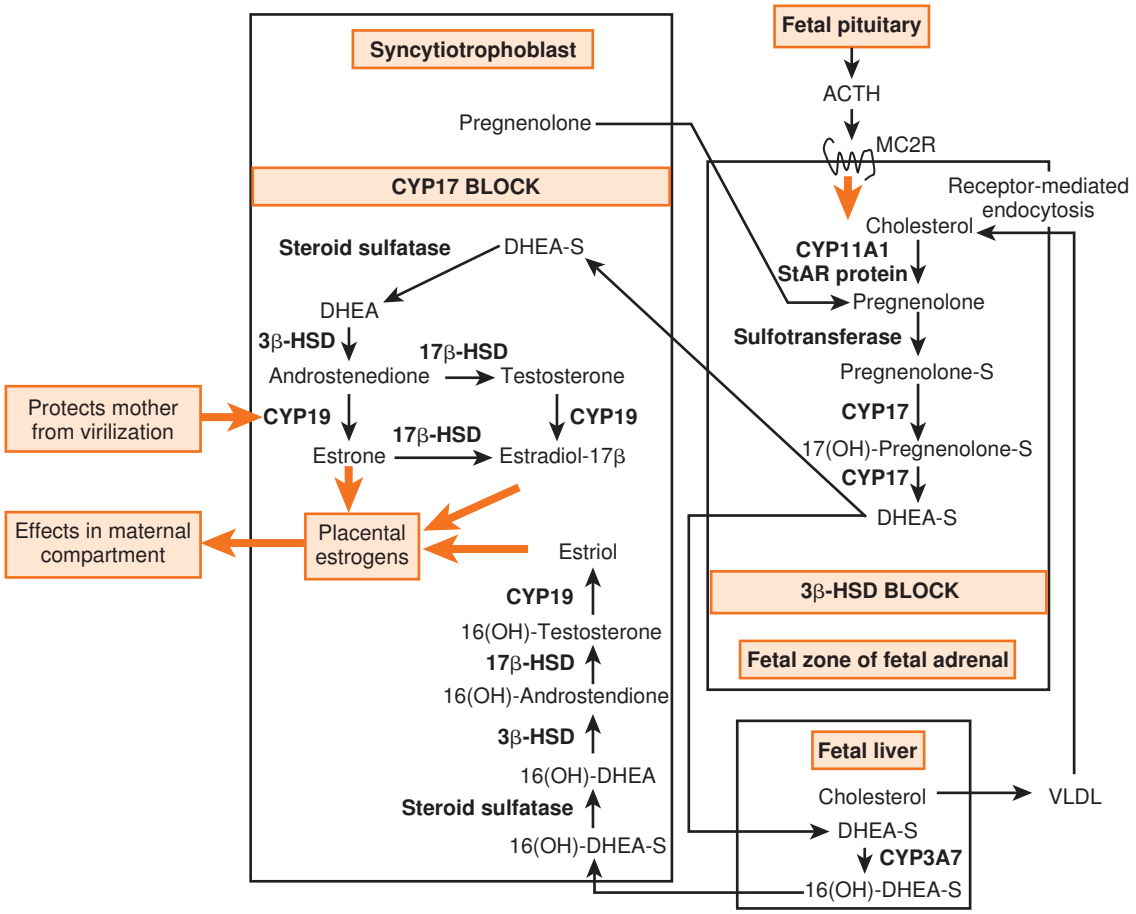

FIGURE 11-16 ■ Estrogen biosynthesis by the fetoplacental unit. ACTH, adrenocorticotropic hormone; DHEA, dehydroepiandrostenedione; DHEAS, dehydroepiandrostenedione sulfate; 3β-HSD, 3β-hydroxysteroid dehydrogenase; 17β-HSD, 17β-hydroxysteroid dehydrogenase; MC2R, melanocortin-2 receptor (ACTH receptor); 16(OH)-, 16-hydroxy-; StAR protein, steroidogenic acute regulatory protein; VLDL, very-low-density lipoprotein.

(see Fig. 11-13). Maternal serum levels rise progressively throughout the remainder of the pregnancy. As much as 1 g/day of hPL can be secreted late in gestation!

Like GH, hPL is protein-anabolic and lipolytic. Its antagonistic action to insulin contributes to the **diabetogenicity of pregnancy**. Glucose is a major energy substrate for the fetus, and hPL increases glucose availability by inhibiting maternal glucose uptake (Fig. 11-17). The lipolytic actions help the mother to shift to the use of free fatty acids for energy. Despite its very high levels in the maternal blood, hPL is probably not essential for normal pregnancy.

Other Placental Hormones

The placenta is a source of many other hormones. The placenta produces **parathyroid hormone–related protein (PTHrP)**, which increases placental calcium transport (see later). The role of **placental corticotropin-releasing hormone (CRH)** in the induction of **labor** is discussed later.

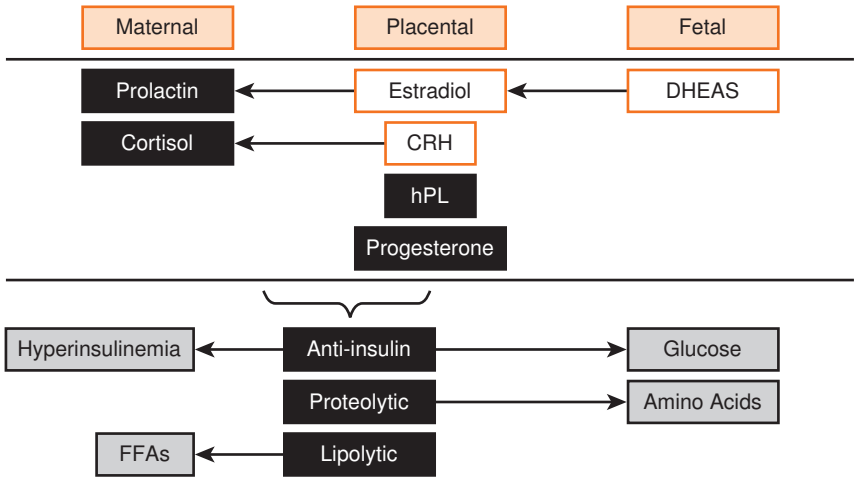

FIGURE 11-17 ■ Role of human placental lactogen (hPL), prolactin (PRL), and cortisol in altering maternal metabolism to provide amino acids and glucose to fetus during the second half of pregnancy.

PLACENTAL TRANSPORT

As discussed earlier, the thickness of placental membrane is minimized and the surface area maximized. Depending on the substance involved, transport can occur by simple diffusion, facilitated diffusion, active transport, or endocytosis.

Gases, **water**, and **many electrolytes** cross the placenta by **simple diffusion**. Because the placental membrane is considerably thicker than the diffusional surface of the lungs, placental gas transport efficiency is only about 1/50th that of the lung on a per-unit weight basis. A sizable gradient exists for oxygen between maternal and fetal blood. Fetal compensation for the low Po_2 is aided by a high fetal blood flow rate and the high oxygen affinity of **fetal hemoglobin**.

Carbon dioxide, on the other hand, is more soluble in body tissues, and the diffusion capacity is greater.

Amino acids are transported by selective **active transporters**. **Glucose** is transported by **facilitated diffusion**, primarily by **GLUT1**. Neutral fats do not cross the placenta, and LDLs are transported into the placenta by **LDL receptor–mediated endocytosis.**

The fat-soluble **steroid hormones** cross relatively readily, but protein hormone transport is minimal.

THE FETAL ENDOCRINE SYSTEM

The timing and some key aspects of the fetal endocrine system are listed in Table 11-1. The details of the development of the reproductive systems are presented in Chapter 8.

MATERNAL ENDOCRINE CHANGES DURING PREGNANCY

Pituitary Gland

PRL levels rise during pregnancy because estrogen stimulates PRL synthesis and secretion (see later). The **pituitary gland** increases significantly (i.e., more than twofold) in size during pregnancy. This pituitary enlargement is due to lactotroph hypertrophy and hyperplasia. Pituitary enlargement during pregnancy can cause dizziness and visual problems if the pituitary presses against the optic chiasm, and makes the pituitary susceptible to vascular insult and necrosis at parturition (Sheehan syndrome).

Pituitary production of **LH and follicle-stimulating hormone (FSH)** decreases during pregnancy because of negative feedback inhibition by the high levels of placentally produced **estrogens plus progesterone**.

TABLE 11-1

Development of the Fetal Endocrine System

ENDOCRINE GLAND	TIMING OF DEVELOPMENT	COMMENTS
Hypothalamus and pituitary gland	All **pituitary hormones** produced by 12 wk All **hypothalamic-releasing hormones** being produced by 12 wk	**Hypothalamohypophyseal portal system** is functional at 18 wk
Thyroid gland	**T_4** produced by 10-12 wk	Early neural development is dependent on some **maternal T_3**; even mild maternal hypothyroidism can cause neurologic deficits in fetus (e.g., lower IQ) Fetal blood **T_3** increases significantly after 30 wk due to **type 1 deiodinase** expression Fetus is protected from maternal hyperthyroidism by **placental type 3 (inner ring) deiodinase**
Parathyroid glands	Parathyroids form by 8-10 wk, secretion of **PTH** is inhibited by relative hypercalcemic state	**Decidual 1,25-dihydroxyvitamin D_3** promotes calcium absorption by maternal intestine and **placental PTHrP** promotes placental transfer of calcium into fetal compartment
Pancreatic islets	Secrete **insulin** and **glucagon** by 15 wk	**Fetal glucose** mostly determined by placental transport rather than fetal pancreatic hormones In latter half of pregnancy, maternal diabetes with poor glycemic control can induce hyperplasia of fetal islet cells and fetal hyperinsulinemia
Adrenal glands	Fetal cortex produces **DHEAS** by 7 wk, **cortisol** during second half or pregnancy and **aldosterone** near term Neural crest cells migrate into center of adrenal and differentiate into **chromaffin cells** before adrenal capsule forms by end of first trimester	As discussed above, fetal adrenal cortex is composed of large **fetal zone** and small **definitive cortex;** refer to Fig. 11-15 One major role of fetal cortisol is to induce production of **surfactant** in lung; untreated pre-term deliveries are associated with **respiratory distress syndrome** Complete or partial loss of **21-hydroxylase** activity encoded by the **CYP21A2** gene is the most common cause of **congenital adrenal hyperplasia (CAH)**. Loss of fetal **cortisol** during second half of pregnancy induces high fetal **ACTH** levels, hypertrophy of definitive cortex, and an increase in **adrenal androgen** production. In a female fetus, CAH causes **virilization** of external genitalia. Severe deficiency of 21-hydroxylase causes dangerous salt wasting and **hyponatremia** in neonate because of **aldosterone** deficiency.

Thyroid Gland

Thyroid size increases during pregnancy, and serum **total T_4** and **total T_3 levels** can double. The primary basis for the increase in total thyroid hormone levels is an estrogen-induced increase in liver **thyroxine-binding globulin (TBG)** production, which leads to an increase in hormone binding. Serum **free T_4 and T_3 levels** do not increase markedly during gestation. The effects of hCG on the maternal thyroid gland were discussed earlier.

Adrenal Gland

Estrogens not only stimulate liver TBG production but also nonspecifically stimulate liver production of many other serum proteins, such as **cortisol-binding globulin (CBG)** (also called **transcortin**). Consequently, **total serum cortisol** levels rise. Although maternal serum ACTH levels increase slightly during pregnancy, they typically remain within the normal nonpregnant range. Late in pregnancy, however, **serum free cortisol levels rise steadily to a peak at parturition that is about twice nonpregnancy levels.** The fetus is protected from maternal cortisol levels (and vice versa) by the presence of **placental 11β-dehydrogenase type 2**, which converts cortisol to the inactive cortisone (see Fig. 11-14).

Estrogen stimulates **liver angiotensinogen** production and renal **renin** production. Consequently, synthesis of **angiotensin II** and **aldosterone** increases. Estrogens potentiate the adrenal action of angiotensin II but antagonize the vasopressive actions. Aldosterone supports the volume expansion in the mother by increasing NaCl retention (by as much as 1000 mEq). However, because of elevated antidiuretic

hormone (ADH) and decreased threshold for thirst, the osmolality of the blood falls slightly.

MATERNAL PHYSIOLOGIC CHANGES DURING PREGNANCY

Physiologic changes occur in the pregnant woman both as a consequence of the size of the developing fetus and as a result of the endocrine and cardiovascular changes associated with the pregnancy (Box 11-1).

Cardiovascular Changes

Pregnancy is associated with an increase in cardiac output coupled to a reduction in peripheral resistance and an expansion of total body and plasma volume.

The **cardiac output** during pregnancy increases to about 40% more than preconception levels, with most of this increase occurring by 8 weeks of gestation. Cardiac output is due to both positive chronotropic and inotropic effects of pregnancy. These may be due to increased sympathetic tone and an increased sensitive to catecholamines.

Peripheral resistance declines in response to several factors. As discussed previously, the spiral arteries are converted to low-resistance, highly compliant vessels, allowing the uteroplacental blood flow to increase by an order of magnitude from the end of the first trimester to term (about 60 to 600 mL per minute). Overall, vascular resistance decreases throughout the maternal circulatory system, and diastolic pressure tends to drop. Blood pressure typically does not rise until late in pregnancy.

Total body volume increases by the retention of up to 8 L of water, which is partitioned among the maternal and growing fetal circulatory systems, uteroplacental circulation, and an enlarging amniotic sac. **Blood volume** increases by up to 50% during pregnancy to accommodate an increasing uteroplacental circulation. Bloating or mild edema is common in pregnancy.

BOX 11-1
PHYSIOLOGIC CHANGES IN PREGNANCY

CARDIOVASCULAR CHANGES
- Vascular volume
- Peripheral resistance
- Stroke volume
- Heart rate
- Contractility
- Cardiac output

RESPIRATORY CHANGES
- Minute volume
- Tidal volume
- P_{CO_2}
- Functional residual capacity
- Inspiratory reserve volume

RENAL CHANGES
- Antidiuretic hormone, renin, angiotensin II, aldosterone secretion
- Respiratory alkalosis

CLINICAL BOX 11-4

Because the growing uterus exerts pressure on the veins of the legs where these veins enter the abdomen, venous pressure in the lower extremities rises on standing, and edema and venous damage can occur. Lying in the supine position can result in compression of the inferior vena cava by the uterus, followed by maternal hypotension.

Respiratory Changes

As pregnancy proceeds, the **functional residual capacity** (volume of air in the lungs at the end of a quiet expiration) and the **residual volume** (volume remaining at the end of a maximal expiration) decrease, and respiratory rate remains unchanged. **Minute volume increases** and **tidal volume increases**, so P_{CO_2} decreases.

There are three major causes of the respiratory changes associated with pregnancy. The bulk of the growing fetus and uterus increases intra-abdominal pressure and forces the diaphragm upward. The high metabolic rate of the growing fetus increases maternal oxygen consumption and carbon dioxide production. In addition, **progesterone** acts on the central nervous system to lower the set-point for regulation of respiration by carbon dioxide, thereby increasing ventilation.

Renal Changes

The glomerular filtration rate increases about 60% over nonpregnant levels, causing the filtered load to increase, which can lead to **glucosuria** and **aminoaciduria** in pregnancy.

Maternal serum levels of the mineralocorticoid deoxycorticosterone (DOC) increase during pregnancy. This increase in circulating DOC levels results not from increased adrenal secretion but from renal conversion of placental progesterone to DOC. Increased levels of DOC and aldosterone (see earlier) stimulate renal salt and water retention.

Gastrointestinal Changes

Heartburn, or reflux of acidic gastric secretions into the esophagus, occurs for multiple reasons. The increased intra-abdominal pressure increases intragastric pressure, which increases the likelihood of reflux into the esophagus. Progesterone also decreases **lower esophageal sphincter tone**, thereby increasing reflux tendency.

Diabetogenicity of Pregnancy

Pregnancy represents an **insulin-resistant, hyperinsulinemic state** (see Fig. 11-17). During the last half of pregnancy, maternal energy metabolism shifts from an anabolic state in which nutrients are stored to a catabolic state, sometimes described as **accelerated starvation**, in which maternal energy metabolism shifts toward fat utilization with glucose sparing. The peripheral responsiveness to **insulin** decreases and pancreatic insulin secretion increases. **β-Cell hyperplasia** occurs in pregnancy. Although this usually does not lead to any clinical condition, pregnancy aggravates existing diabetes mellitus, and diabetes mellitus can develop for the first time in pregnancy. If the diabetes resolves spontaneously with delivery, the condition is referred to as **gestational diabetes**. Hormones contributing to the diabetogenicity of pregnancy are hPL, prolactin, cortisol, and progesterone (see Fig. 11-17).

PARTURITION

Human pregnancy lasts an average of 40 weeks from the beginning of the last menstrual period (gestational age). This corresponds to an average fetal age of 38 weeks. **Parturition** is the process whereby uterine contractions (**labor**) lead to childbirth. Labor consists of three stages:

1. Strong **uterine contractions** that force the fetus against the cervix, with **dilation and thinning of the cervix** (several hours)
2. **Delivery of the fetus** (< 1 hour)
3. **Delivery of the placenta**, along with contractions of the myometrium, which serve to halt bleeding (< 10 minutes).

Parturition control in humans is complex, and the exact mechanisms underlying parturition control are not well understood. In many species, such as sheep, the timing of parturition is controlled by fetus-derived signals, and fetal regulation is at least a factor in humans.

Placental Corticotropin-Releasing Hormone and the Fetal Adrenal Axis

The placenta produces **CRH**, which is identical to the 41-amino acid peptide produced by the hypothalamus. Placental CRH production and maternal serum CRH levels increase rapidly during late pregnancy and labor. Moreover, circulating CRH is either in the form of free CRH, which is bioactive, or complexed to a CRH-binding protein. Maternal levels of **CRH-binding protein** plummet during late pregnancy and labor, and **free CRH** levels increase. Placental CRH also accumulates in the fetal circulation and stimulates **fetal ACTH secretion**. ACTH stimulates both **fetal adrenal cortisol** production and **feto-placental estrogen** production. In contrast to the inhibitory effect of cortisol on hypothalamic CRH production, cortisol stimulates placental CRH production. This establishes a self-amplifying **positive feedback** loop. CRH itself promotes **myometrial contractions** through sensitizing the uterus to **prostaglandins** and **oxytocin**. **Estrogens** also directly and indirectly stimulate **myometrial contractility**. This model correlates with the onset of parturition with **cortisol-induced maturation of fetal systems**, including the lungs and gastrointestinal system.

Estrogen

Estrogen is required for labor. It increases receptors and sensitivity of the myometrium to oxytocin and increases prostaglandin production.

CLINICAL BOX 11-5

In male fetuses with **X-linked steroid sulfatase deficiency**, the fetoplacental unit cannot make estrogens. This results in maternal estrogen levels that are an order of magnitude less than those in normal pregnancies. These babies typically are delivered by cesarean section because the absence of estrogen results in a quiescent myometrium and a pregnancy that goes several weeks beyond the due date. Nevertheless, the pregnancy proceeds normally, and the newborn is normal except for the phenotype associated with sulfatase deficiency (**ichthyosis**, or **scaly skin**).

Oxytocin

Oxytocin stimulates powerful uterine contractions (Fig. 11-18). It is released in response to stretch of the cervix, and it stimulates uterine contractions, thereby facilitating delivery. Oxytocin is also used to induce parturition, and uterine sensitivity to oxytocin increases before parturition.

Prostaglandins

Prostaglandins and other cytokines increase uterine motility, and levels of these compounds increase during parturition, thereby facilitating delivery. The **prostaglandins** $\mathbf{PGF_{2\alpha}}$ and $\mathbf{PGE_2}$ increase uterine motility. Large doses of these compounds have been used to induce labor.

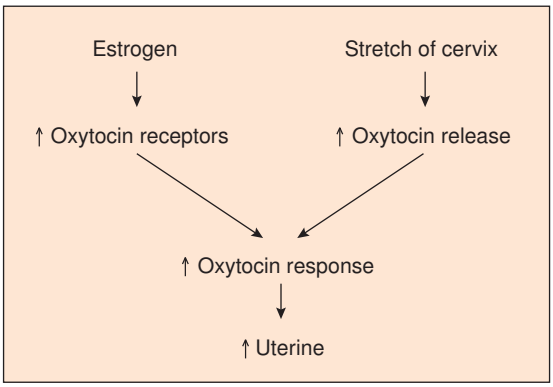

FIGURE 11-18 ■ Role of oxytocin in parturition.

Uterine Size

Uterine size is thought to be a factor regulating parturition because stretch of smooth muscle, including the uterus, increases muscle contraction. In addition, uterine stretch stimulates uterine prostaglandin production. Multiple births generally occur prematurely.

MAMMOGENESIS AND LACTATION

Structure of the Mammary Gland

The **mammary gland** is composed of about 20 lobes, each with an excretory lactiferous duct that opens at the nipple (Fig. 11-19). Lobes, in turn, are composed of several lobules, which contain secretory structures called **alveoli**, and the terminal portions of the **ducts**. The epithelia of the alveoli and ducts is composed of apical luminal ductal or alveolar cells and a

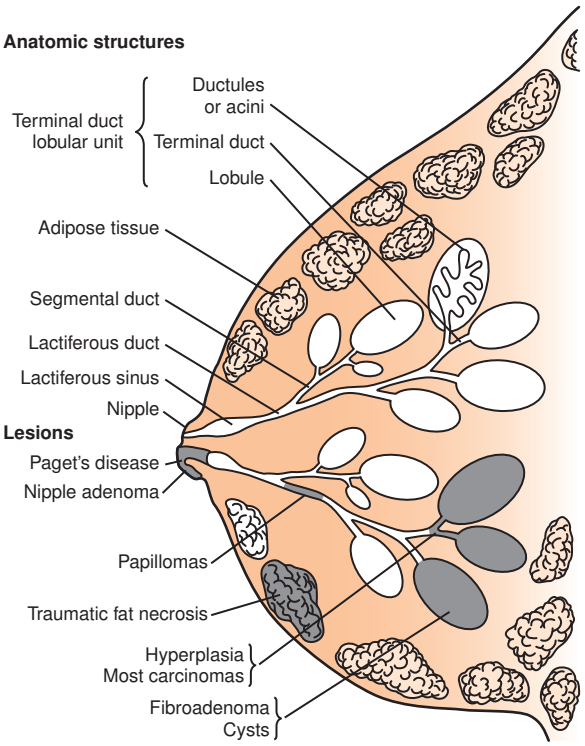

FIGURE 11-19 ■ Anatomy of the breast and major lesions at each site within the breast. *(From Cotran RS, Kumar V, Robbins SL:* Pathologic Basis of Disease, *5th ed., Philadelphia, 1994, Saunders.)*

myoepithelial cell layer on the basal side of the epithelium. Myoepithelial cells are stellate, smooth muscle–like cells, and contraction of these cells in response to oxytocin expels milk from the lumina of the alveoli and ducts. Myoepithelial cells produce the basal lamina of the epithelial layer and oppose the invasion of breast cancer cells from the lumen into the outer-lying stroma.

Lobes and lobules are supported within a connective tissue matrix. The density of this matrix can affect the resolution of **mammograms**. The other major stromal component of the breast is **adipose tissue**. The lactiferous ducts empty at the **nipple**, which is a highly innervated, hairless protrusion of the breast designed for suckling by an infant. The nipple is surrounded by a pigmented, hairless areola, which is lubricated by sebaceous glands. Protrusion of the nipple, called erection, is mediated by sympathetic stimulation of smooth muscle fibers in response to suckling and other mechanical stimulation, erotic stimulation, and cold.

Hormonal Regulation of Mammary Gland Development

The mammary glands develop in utero as **rudimentary mammary buds**. At puberty, **estrogen** increases **ductal growth** and **branching**. With the onset of luteal phases of the ovary, **progesterone and estrogen** induce further ductal growth and the formation of **rudimentary alveoli**. During nonpregnant cycles, the breasts develop somewhat and then regress. Estrogen also increases the deposition of adipose tissue. Adipose tissue expresses **CYP19 (aromatase)**, and accumulation of this tissue in the breast increases the **local production of estrogens** from circulating androgens.

The greatest degree of breast development occurs during pregnancy, during which **extensive ductal growth and branching and lobuloalveolar development** occur. High levels of placental-derived **estrogen** and **progesterone** stimulate the extensive development of the breast. Estrogen acts on the breast directly and also indirectly through increasing **maternal pituitary PRL production by lactotrophs**. Although the luminal alveolar epithelial cells begin to express genes encoding milk proteins and enzymes involved in milk production, progesterone inhibits the onset of milk production and secretion (called lactogenesis).

Immediately after parturition, the human breast excretes **colostrum**, which is enriched with antimicrobial and anti-inflammatory proteins. In the absence of placental progesterone, normal breast milk production occurs within a few days. The lobuloalveolar structures produce milk, which is subsequently modified by the ductal epithelium. **Lactogenesis** and the maintenance of milk production (**galactopoiesis**) require stimulation by pituitary **prolactin**, in the presence of normal levels of other hormones, including **insulin**, cortisol, and **thyroid hormone**. Whereas placental estrogen stimulates prolactin secretion during pregnancy, the stimulus for prolactin secretion during the nursing period is **suckling by the infant** (Fig. 11-20). The levels of prolactin are directly correlated with the frequency and duration of sucking at the nipple. The link between suckling at the nipple and

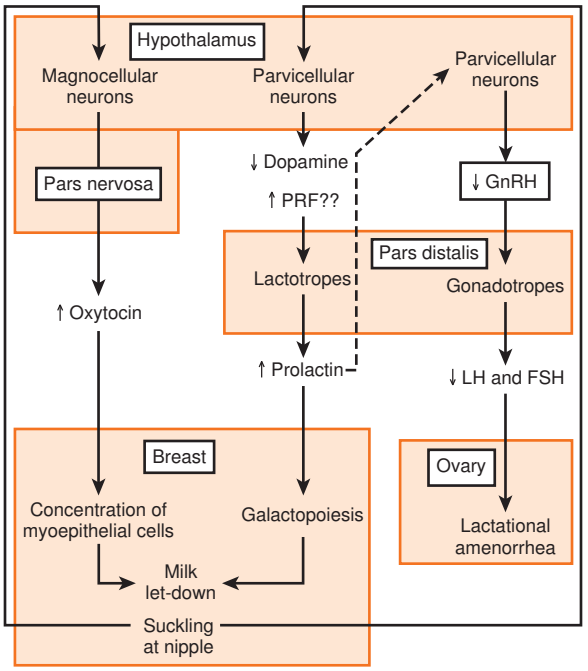

FIGURE 11-20 ■ The neuroendocrine reflex linking suckling at the nipple to oxytocin and prolactin release. FSH, follicle-stimulating hormone; GnRH, gonadotropin-releasing hormone; LH, luteinizing hormone; PRL, prolactin.

prolactin secretion involves a **neuroendocrine reflex**, in which **dopamine** (the **prolactin-release inhibitory factor**; see Chapter 5) secretion at the median eminence is inhibited. It also is possible that suckling increases the secretion of unidentified prolactin-releasing hormones.

Prolactin also inhibits **GnRH release**; consequently, nursing can be associated with **lactational amenorrhea** (see Fig. 11-20). This effect of prolactin has been called *nature's contraceptive* and may play a role in spacing out pregnancies. Only very regular nursing over a 24-hour period, however, is sufficient to allow for a prolactin-induced anovulatory state in the mother. Thus, lactational amenorrhea is not an effective or reliable form of birth control for most women.

CLINICAL BOX 11-6

The inhibition of GnRH by high levels of prolactin is important clinically. The prolactinoma is the most common form of hormone-secreting pituitary tumor, and hyperprolactinemia is a significant cause of infertility in both sexes. Hyperprolactinemia can also be associated with galactorrhea, or the inappropriate flow of breast milk, in men and women.

Suckling at the nipple also stimulates the release of **oxytocin** from the **posterior pituitary** (see Chapter 5) through a neuroendocrine reflex (see Fig. 11-20). **Oxytocin receptors** on the **myoepithelial cells** cause contractions that ultimately induce **milk letdown**, or the expulsion of milk from alveolar and ductal lumina. Oxytocin release and milk letdown can be induced by psychogenic stimuli, such as the sound of a baby crying on a television program or thinking about the baby. Such psychogenic stimuli do not affect prolactin release.

CLINICAL BOX 11-7

The breast epithelium represents a hormonally responsive, highly mitogenic population of cells. **Invasive breast cancer (IBC)** arises from breast epithelium (primarily from the epithelial cells lining small ducts). IBC represents a very common cancer in women in the United States and is a major cause of death among women older than 45 years. The most commonly diagnosed form of IBC is called **luminal A**, in which most cells express the α isoform of the estrogen receptor **ERα**. These tumors are highly dependent on estrogen and regress or fail to recur in response to antiestrogen treatment. This treatment involves the use of a **selective estrogen receptor modulator (SERM)** such as **tamoxifen** or **aromatase (CYP19) inhibitor**. Treatment of an early (not metastasized) form of luminal A IBC usually involves surgical resection of the cancer (lumpectomy, mastectomy), followed by several weeks of radiation therapy, followed by antiestrogen therapy for several years. The overall survival rate for early, lymph node–negative, ERα-positive luminal A IBC is well over 90% in North America.

CONTRACEPTION

Behavioral and Mechanical Approaches

There are multiple methods of contraception. These methods include the **age-old rhythm method**, which relies on abstinence from sexual intercourse during fertile periods around the time of ovulation. (The fertile period is considered to be the period extending from 3 to 4 days before the time of ovulation until 3 to 4 days afterward.) A second method is withdrawal before ejaculation, **coitus interruptus**. Both of these methods have higher failure rates (20% to 30%) than the barrier methods (2% to 12%), intrauterine devices (IUDs) (<2%), and oral contraceptives (<1%).

Barriers such as **condoms** or **diaphragms** are more effective as contraceptives when used with **spermicidal jellies**.

Among the various methods of contraception, **IUDs** are the most effective, except for oral contraceptives. These devices are thought to prevent implantation by producing a local inflammatory response in the endometrium. Some forms of IUDs contain copper, zinc, or progestins, which inhibit sperm transport or viability in the female reproductive tract.

Female (tubule ligation) and male (vasectomy) sterilization are also effective options, especially for couples who have children and do not wish for further procreation in the face of continued sexual activity.

Oral Contraceptives

Oral contraceptives have been marketed in the United States since the early 1960s. The doses of steroids used today are many-fold lower than those used in the initial preparations. Properly used, oral contraceptives have a low failure rate.

Many forms of oral contraceptives are marketed today. The trend over the years has been to decrease the dosage of steroids used because the side effects are dose dependent. All oral steroidal contraceptives contain a progestin analog, and some also contain an estrogen analog. The numerous formulations, applications (e.g., pills, patches), and configurations of the timing and duration available are beyond the scope of this chapter.

Oral contraceptives work through multiple mechanisms. Most block the **LH surge** that triggers ovulation. However, some pills, such as the progestin-only minipill, block fertility by changing the nature of **cervical mucus**, by altering **endometrial development**, and by regulating **fallopian tube motility**. Because these contraceptives suppress FSH, they impair early follicular development.

Side effects include bloating, breast tenderness, and unscheduled bleeding (breakthrough bleeding). Estrogen-progesterone combination pills appear to increase the risk for **venous thrombosis**. The effect of combination pills on breast cancer is minimal, and they decrease the incidence of ovarian and uterine cancer. However, cervical cancer is linked to the use of oral contraceptives.

Hormonal Treatment for Emergency Contraception and Abortion

Emergency contraception involves hormonal treatment designed to inhibit or delay ovulation, inhibit corpus luteum function, and disrupt the function of the oviducts and uterus. Candidates for emergency contraception include women who have been sexually assaulted or who experienced a failure of a barrier method (e.g., ruptured condom). The currently preferred medication is **levonorgestrel (Plan B)**, which is a synthetic progestin-only pill. The efficacy of the pill is inversely correlated with the time it is taken after intercourse. The exact mechanism of action is not known. Treatment has no effect if implantation has occurred.

Medical (hormonal) termination of pregnancy (abortion) can be achieved until up to 49 days of gestation by administration of **mifepristone (RU-486)**. Mifepristone is a **progesterone receptor antagonist** (i.e., an **antiprogestin**), which induces collapse of the pregnant endometrium. Mifepristone is followed 48 hours later by ingestion or vaginal insertion of a synthetic prostaglandin E (e.g., misoprostol), which induces myometrial contractions.

IN VITRO FERTILIZATION

The development of **in vitro fertilization (IVF)** and other assisted pregnancy procedures was made possible through our increased understanding of the basic science of reproduction. A general description of IVF is available online at Student Consult.

SUMMARY

1. The events of fertilization, early embryogenesis, and implantation are synchronized with the hormonal changes of the human menstrual cycle, ultimately ensuring a receptive uterus at the time of implantation.
2. Spermatozoa bind to the epithelium of the oviductal isthmus, which secretes factors that capacitate the sperm. Hyperactivation allows the sperm to detach and swim to the cumulus-oocyte complex in the ampulla. Fertilization involves the penetration by a spermatozoon of the expanded cumulus as mediated by a membrane hyaluronidase, penetration of the zona pellucida as mediated by the acrosome reaction, and fusion with the oocyte membrane as mediated by specific membrane fusion proteins. The egg is activated, completes the second meiotic division, and releases cortical granule enzymes that prevent polyspermy. The female and male pronuclei are drawn together, line up on a metaphase plate, and undergo the first cleavage.
3. The embryo undergoes cleavage and formation of a morula within the oviduct. The embryo enters the uterus on day 3, forms a blastocyst, degrades

the zona pellucida (hatches), and implants on day 6 or 7.

4. The trophoblast layer differentiates into the cytotrophoblastic layer and an outer syncytiotrophoblastic layer. The syncytiotrophoblasts secrete invasive enzymes, express adhesion molecules, produce protein and steroid hormones, and ultimately become the primary cell involved in maternal-fetal exchange. With the addition of an extraembryonic mesoderm, the trophoblast layers become the chorion. The fetal (umbilical) blood vessels develop within the mesenchyme of the extraembryonic mesoderm. The chorion ultimately gives rise to protrusions, the chorionic villi, which constitute the functional unit of the placenta.
5. The uterine endometrium decidualizes in response to implantation. The decidua impose restraint on the invading embryo.
6. The intervillous spaces become filled with maternal blood from eroded spiral arteries. This gives rise to a hemochorial placenta. Spiral arteries are plugged up by extravillous cytotrophoblasts for the first trimester so that the embryo/early fetus develops in a relatively hypoxic environment and receives histotrophic nutrition. The cytotrophoblasts convert the spiral arteries to low-resistance, high-capacitance vessels that supply blood to the placenta during the second and third trimesters.
7. The first hormone produced by the placenta is hCG. This hormone is structurally similar to LH but has a longer half-life. Its function is to rescue the corpus luteum, which is needed to produce progesterone for the first 10 weeks. Human chorionic gonadotropin also stimulates male fetal gonadal production of testosterone and the early fetal adrenal.
8. Progesterone production is taken over by the placenta. Syncytiotrophoblasts use maternal cholesterol to make progesterone. Because no fetal tissues are involved in progesterone synthesis, progesterone levels are not a measure of fetal health. Progesterone is required for pregnancy. Progesterone maintains a quiescent uterus and affects several aspects of maternal physiology. Progesterone is used by the fetal adrenal cortex for cortisol synthesis.
9. The fetal adrenal cortex is different from the adult. The large inner zone is called the fetal zone. The fetal zone produces DHEAS, which is then converted by the syncytiotrophoblasts to estradiol and estrone. DHEAS also is converted to 16-hydroxy-DHEAS by the fetal liver, and this steroid is further converted to estriol by the syncytiotrophoblasts. This multiorgan pathway for estrogen synthesis is referred to as the fetoplacental unit. Maternal serum estriol levels can be used as an indicator of fetal health. Estrogen is not required for pregnancy. The primary function of estrogen is to prepare the uterus for parturition. Both estrogen and progesterone play important roles in mammary gland development during pregnancy.
10. The outer definitive zone begins to make cortisol at midgestation and to make aldosterone close to term. Cortisol production increases toward late pregnancy, playing a role in fetal lung surfactant synthesis, fetal gastrointestinal tract maturation, and other aspects of late fetal development.
11. Human placental lactogen (hPL) is structurally and functionally similar to both GH and PRL. It is an insulin antagonist and is lipolytic.
12. Glucose crosses the placenta by GLUT1 carrier-mediated facilitated diffusion. Amino acid transport is by carrier-mediated secondary active transport, and LDL transport is by receptor-mediated endocytosis. Gas transport is by simple diffusion.
13. Cardiovascular changes in pregnancy include increased vascular volume; decreased peripheral resistance; and increased heart rate, cardiac contractility, and cardiac output.
14. Respiratory changes in pregnancy include increased minute volume and increased tidal volume.
15. During pregnancy, ADH, renin, angiotensin II, and aldosterone secretion all increase. These changes produce sodium and water retention.
16. The exact mechanism underlying initiation of parturition in humans has not been defined. Possible stimuli include increases in placental CRH production, fetal ACTH and cortisol production, uterine size, oxytocin receptor concentration, and uterine prostaglandin production. Parturition requires estrogen, which stimulates prostaglandin synthesis and oxytocin receptor expression.

17. Mammary glands are lobuloalveolar structures. Estrogen and progesterone promote ductal and alveolar growth, whereas progesterone and prolactin stimulate alveolar development. The extensive mammary development in pregnancy is driven by PRL, estradiol, and progesterone. Milk production in pregnancy is blocked by progesterone.
18. After parturition, sucking at the nipple is required for prolactin and oxytocin secretion. Prolactin maintains milk production (galactopoiesis), and oxytocin causes the myoepithelial cells to contract. Prolactin inhibits GnRH secretion—the basis for lactational amenorrhea and for both male and female infertility due to a prolactinoma with associated hyperprolactinemia.
19. Breast cancer is often a hormonally responsive cancer in the earlier stages, so antiestrogens and aromatase inhibitors are effective as adjuvant therapy.
20. Elucidation of the basic science of reproductive endocrinology has led to the development of oral contraceptives, emergency contraceptives, medical abortion pills, and in vitro fertilization procedures.

SELF-STUDY PROBLEMS

1. What is meant by *egg activation*?
2. Describe the initial events of implantation.
3. How does placental progesterone synthesis differ from placental estriol synthesis?
4. What is the role of estrogen in pregnancy and parturition?
5. What is the basis for hyperthyroidism during the first trimester of some pregnancies? Why is it transient?
6. How does progesterone affect maternal respiration?
7. What is the relationship between infertility and PRL in a physiologic situation? In a pathologic condition?

KEYWORDS AND CONCEPTS

- 1,25-Dihydroxyvitamin D
- 3β-Hydroxysteroid dehydrogenase (3β-HSD)
- α-Chain of hCG

For full list of keywords and concepts see Student Consult

SUGGESTED READINGS

Axlund SD, Sartorius CA: Progesterone regulation of stem and progenitor cells in normal and malignant breast, *Mol Cell Endocrinol* 257:71–79, 2012.

Lee HJ, Ormandy CJ: Interplay between progesterone and prolactin in mammary development and implications for breast cancer, *Mol Cell Endocrinol* 357:101–107, 2012.

Taylor HS, Manson JE: Update in hormone therapy use in menopause, *J Clin Endocrinol Metab* 96:255–264, 2011.

Wetendorf M, Demayo FJ: The progesterone receptor regulates implantation, decidualization, and glandular development via a complex paracrine signaling network, *Mol Cell Endocrinol* 357:108–118, 2012.

KEYWORDS AND CONCEPTS

- α-Glycoprotein subunit (αGSU)
- Accelerated starvation
- Accrete
- Acrosomal vesicle
- Acrosome reaction
- ADH secretion
- Adhesive
- Adhesive proteins
- Adipose tissue
- Aldosterone
- Alveoli
- Amino acids
- Aminoaciduria
- Amniochorionic membrane
- Amnion
- Ampulla of the oviduct
- Angiotensin II
- Anterior pituitary trophic hormones
- Antidiuretic hormone (ADH)
- Aromatase (CYP19) inhibitors
- β-Chain
- β-hCG
- β-Cell hyperplasia
- Bidirectional placental transfer
- Blastocyst
- Blood volume
- Branching
- Capacitation
- Cardiac output
- Carrier-mediated facilitated diffusion
- Carrier-mediated secondary active transport
- Centrosome
- Cervical mucus
- Choriocarcinoma
- Chorion
- Chorion frondosum
- Chorion laeve
- Chorionic membrane
- Chorionic villi
- Coitus interruptus
- Colostrum
- Complete meiosis
- Condom
- Congenitally hypothyroid fetus
- Connecting stalk
- Corpus luteum
- Corpus luteum of pregnancy
- Cortical granules
- Corticosteroids
- Corticotropin-releasing hormone (CRH)
- Cortisol-binding globulin (CBG) (transcortin)
- Cortisol-induced maturation of fetal systems
- CRH-binding protein
- Cumulus cells
- CYP11A1 (side-chain cleavage enzyme)
- CYP19 (aromatase)
- Cytostatic factor (CSF)
- Cytotrophoblastic shell
- Cytotrophoblasts
- Decidua
- Decidua basalis
- Decidua capsularis
- Decidua parietalis
- Decidual cells
- Decidualization
- Decondensation
- Definitive cortex
- Dehydroepiandrosterone sulfate (DHEAS)
- Delivery of the fetus
- Delivery of the placenta
- DHEAS
- Diabetogenicity of pregnancy
- Diaphragm
- Dilation and thinning of the cervix
- Dopamine (prolactin-release inhibitory factor)
- Ductal growth
- Duct
- Early embryogenesis
- Early gestation
- Ectopic implantation
- Egg
- Egg activation
- Electrolytes
- Embryo
- Embryonic pole
- Embryotroph
- Emergency contraception
- Endocrine
- Endometrial development
- Endometrial stroma

- Endometrial veins
- ERα
- Estradiol-17β
- Estriol
- Estrogen
- Estrogen plus progesterone
- Estrone
- Expanded cumulus
- Expansion of total body and plasma volume
- Extensive ductal growth
- Extraembryonic mesoderm
- Extravillous cytotrophoblasts
- Fallopian tube motility
- Fertilization
- Fetal ACTH secretion
- Fetal adrenal cortex
- Fetal adrenal cortisol
- Fetal adrenocorticotropic hormone (ACTH)
- Fetal anterior pituitary
- Fetal cortisol
- Fetal hemoglobin
- Fetal Leydig cells
- Fetal liver
- Fetal lungs
- Fetal neurologic development
- Fetal PRL
- Fetal thyroid gland
- Fetal zone
- Fetoplacental estrogen
- Fetoplacental unit
- First embryonic cleavage
- Follicle-stimulating hormone (FSH)
- Free CRH
- Free T_3 levels
- Free T_4 levels
- Functional residual capacity
- Galactopoiesis
- Gases
- Gastric emptying rate
- Gestational diabetes
- GH levels
- Glucose
- Glucosuria
- GnRH agonist or antagonist
- GnRH release
- Growth hormone variant (GH-V)
- Hatched blastocyst
- hCG
- Hemochorial placentation
- Hemotrophic nutrition
- High dose of FSH
- Histotrophic nutrition
- Human chorionic gonadotropin (hCG)
- Human placental lactogen (hPL)
- Hyaluronic acid
- Hyperactivation
- Hypophyseal portal system
- Hypothalamic-releasing or -inhibiting hormones
- Hypothalamus
- Hypoxic environment
- Ichthyosis (scaly skin)
- Implantation
- In vitro fertilization (IVF)
- Increase in cardiac output
- Infertility
- Inner cell mass
- Inositol 1,4,5-triphosphate (IP_3)
- Insulin
- Insulin glucagon
- Insulin-resistant state
- Interstitial implantation
- Infertile
- Intervillous space
- Intracellular release of Ca^{2+} in the egg
- Intracytoplasmic sperm injection (ICSI)
- Intrauterine device (IUD)
- Intrauterine growth restriction (IUGR)
- Invasive
- Invasive breast cancer (IBC)
- Labor
- Lactational amenorrhea
- Lactogenesis
- Lacunae
- Late follicular stage
- LDL receptor–mediated endocytosis
- Levonorgestrel (Plan B)
- Liver angiotensinogen
- Lobuloalveolar development
- Lower esophageal sphincter tone
- Low-resistance, high-capacitance vessels
- Luteal-placental shift
- Luteinizing hormone (LH)

- Luteinizing hormone (LH) surge
- Mammary glands
- Mammograms
- Maternal hyperthyroidism
- Maternal hypothyroidism
- Maternal mRNA
- Maternal pituitary prolactin
- Maturation-promoting factor (MPF)
- Medical (hormonal) termination of pregnancy (abortion)
- Meiotic maturation
- Metaphase of meiosis II
- Microtubule-organizing center
- Midluteal phase
- Mifepristone (RU-486)
- Milk letdown
- Minute volume increases
- Mitogen-activating protein kinase (MAPK) pathway
- Molar disease
- Morning sickness
- Morula
- Multiple births
- Myoepithelial cells
- Myometrial contractility
- Myometrial contractions
- Myometrium
- Neuroendocrine reflex
- Nipple
- Oral contraceptives
- Ovarian hyperstimulation syndrome (OHSS)
- Oviduct
- Oviductal isthmus
- Ovulation
- Oxytocin
- Oxytocin receptors
- Parathyroid
- Parathyroid hormone–related protein (PTHrP)
- Parturition
- Peripheral resistance
- PH-20
- Phagocytosis
- Pinopodes
- Pituitary gland
- Pituitary lactotrophs
- Placental 11β-dehydrogenase type 2
- Placental corticotrophin-releasing hormone (CRH)
- Placental membrane
- Placental PTHrP
- Placental type 3 (inner ring) deiodinase
- Placenta-specific 3β-hydroxysteroid dehydrogenase type 1 (3β-HSD1)
- Plasma membrane of the egg (oolemma)
- Polyspermy
- Positive feedback
- Posterior pituitary
- Postpartum hemorrhage
- Preeclampsia
- Primary villi
- Progesterone
- Progesterone receptor antagonist (antiprogestin)
- Prolactin (PRL)
- Pronucleus
- Prostaglandins
- Prostaglandins $PGF_{2\alpha}$ and PGE_2
- Reduction in peripheral resistance
- Residual volume
- Respiratory distress syndrome (RDS)
- Reverse T_3 (rT_3)
- Rhythm method
- Rudimentary alveoli
- Rudimentary mammary buds
- Second meiotic division
- Secondary villi
- Selective estrogen receptor modulator (SERMs)
- Serum-free cortisol levels
- Side effects
- Simple diffusion
- Sperm
- Sperm-egg fusion
- Spermicidal jellies
- Spiral arteries
- Steroid hormones
- Steroidogenic acute regulatory (StAR) protein
- Stored maternal mRNA
- Suckling
- Surfactant
- Syncytiotrophoblasts
- Tamoxifen
- Terminal villi
- Tertiary villi
- Thirst

- Thyroid hormones
- Thyroid size
- Thyroxine (T_4)
- Thyroxine-binding globulin (TBG)
- Tidal volume increases
- Total body volume
- Total serum cortisol
- Total T_3 levels
- Total T_4 levels
- Transient gestational hyperthyroidism
- Triiodothyronine (T_3)
- Triploid cell
- Trophoblastic cells
- Trophoblasts
- TSH receptors
- Tubal pregnancy
- Tubule ligation (female sterilization)
- Tunica intima
- Tunica media
- Type 3 deiodinase
- Type 1 monodeiodinase
- Umbilical (fetal circulation)
- Uterine contractions
- Uterine size
- Vaginal tenting
- Vaginal transudate
- Vasculosyncytial membrane
- Vasectomy (male sterilization)
- Venous thrombosis
- Water
- Watery mucus
- Window of receptivity
- X-linked steroid sulfatase deficiency
- Zona pellucida
- ZP1
- ZP2
- ZP3
- ZP3 receptors

APPENDIX A
Answers to Self-Study Problems

CHAPTER 1

1. Protein hormones are stored within secretory vesicles and are secreted in response to a stimulus. Steroid hormones freely diffuse out of cells. Their synthesis, as opposed to secretion, is regulated by stimuli.
2. Hormone-binding proteins in the serum generally increase the circulating half-life of a hormone. It is the *free* fraction (i.e., unbound) that is considered to be active.
3. Increasing the GTPase activity of Gs would result in a more rapid inactivation of Gs, thereby decreasing adenylate cyclase activity and cAMP levels. Thus, the cell would demonstrate resistance to hormones that act through Gs-coupled GPCRs.
4. The IRS protein is recruited to and phosphorylated by the insulin receptor. The phosphotyrosines within the IRS protein recruit and activate the Ras-Raf-MAPK pathway, which transduces insulin receptor binding into a growth response. Other phosphotyrosines on IRS protein activate the PI3 kinase–PKB pathway, which is linked primarily to metabolic actions of insulin (including GLUT4 translocation to the membrane).
5. Cytokines and TGF-β-related hormones (e.g., inhibin) signal by phosphorylating a transcription factor (cytokines use STATs; TGF-β-related hormones use SMADs). The transcription factors then translocate to the nucleus (as dimers) and activate the expression of specific genes.
6. Hormone binding to a GPCR induces a conformational change in the receptor, which makes the receptor a substrate for serine/threonine GPCR kinases (GRKs). β-Arrestins then bind to the phosphorylated residue and link the receptor to clathrin-coated pits and clathrin-mediated endocytosis. Receptors within endosomes may recycle back to the membrane, or may be destroyed by lysosomal enzymes if the endosome fuses with a lysosome.
7. Guanosine nucleotide exchange factors (GEFs) allow a small G protein to exchange a bound GDP (inactive state) for GTP (active state). GPCRs are essentially ligand-activated GEFs for the trimeric G-protein complexes. Without this activity, GPCRs could not activate the α subunits of the trimeric G protein complex.
8. Phospholipase C (PLC) cleaves the membrane phospholipid, phosphatidylinositol, into diacylglycerol (DAG) and inositol-3-phosphate (IP3). DAG activates certain isoforms of protein kinase C, whereas IP_3 binds to its receptor in the endoplasmic reticulum to induce release of Ca^{2+} into the cytoplasm.

CHAPTER 2

1. Cephalic, gastric, and intestinal. The greatest release of gastrin occurs during the gastric phase. This occurs because (1) the presence of amino acids in the stomach directly stimulates gastrin release from G cells; (2) the presence of food promotes gastrin release through pressor receptors in the stomach wall; (3) the presence of food buffers acidity, thereby decreasing inhibitory somatostatin release; and (4) there is minimal inhibitory signaling from the small intestine and colon.
2. (a) Stimulation. (b) Stimulation. (c) Inhibition. (d) Stimulation. (e) Stimulation.
3. S cells secrete secretin, I cells secrete CCK. CCK inhibits gastric motility. Secretin may act as an enterogastrone to inhibit gastrin secretion but has a minimal effect on gastric emptying.

4. (a) Some stimulation. (b) Strong stimulation. (c) No effect. (d) Increase. (e) Decrease.
5. Both glucagon-like peptide-1 (GLP-1) and glucagon are encoded within the same gene that encodes the preproglucagon prohormone. Glucagon is secreted by α cells of the endocrine pancreas and binds to the glucagon receptor. GLP-1 is released by intestinal L cells and acts through the GLP-1 receptor.
6. An incretin is a hormone (peptide) that is released in response to food (especially glucose) in the intestine and enhances the ability of glucose to promote insulin release from the pancreatic β cells. Two incretins are GIP and GLP-1.
7. Hypertrophy and hyperplasia of the gastric mucosa and rugae (submucosal folds). Enterochromaffin-like (ECL) cells show the greatest degree of proliferation.
8. Erythromycin binds to and activates the motilin receptor.

CHAPTER 3

1. In the fed state, glycolysis in the liver leads to de novo fatty acid and triglyceride synthesis. In the adipose tissue, glycolysis generates glycerol-3-phosphate, which is used to reesterify fatty acids (from the digestion of chylomicrons by LPL) into triglycerides. Glycolysis is also used to generate ATP.
2. Glycogen is a storage form of glucose. In the liver, glycogenolysis contributes directly to blood glucose because the liver can dephosphorylate glucose-6-phosphate to glucose. In the muscle, glycogen is used during exercise to provide ATP by glycolysis. Skeletal muscle cannot dephosphorylate glucose-6-phosphate and thus cannot directly contribute to blood glucose levels.
3. Ketone bodies. The liver produces ketone bodies from free fatty acids.
4. Accumulation of mitochondrial citrate (in times of plentiful ATP) can be transferred to the cytoplasm, where it can generate cytoplasmic acetyl CoA used for lipogenesis.
5. Lipoprotein lipase (insulin-dependent activation) and hormone-sensitive lipase (insulin-dependent inhibition).
6. LDL particles lose apoprotein E and bind to LDL receptors through apoprotein B100. LDL receptors remove these high cholesterol particles from the blood by receptor-mediated endocytosis. The liver plays the major role in LDL receptor–mediated removal of LDL particles, although steroidogenic cells and proliferating cells (i.e., cells that need cholesterol) also take up LDL particles by the LDL receptor. Loss of LDL receptor results in a high LDL concentration in the blood and, therefore, high cholesterol content in the blood.
7. Malonyl CoA inhibits the carnitine-palmitoyl transferase-I transporter. This prevents the futile cycle of synthesizing fatty acids only to have them transported into the mitochondria for β-oxidation.
8. Decreased glucokinase activity would inhibit glycolysis and therefore ATP production in β cells. Lower ATP would result in less insulin secreted. Heterozygous null mutations of glucokinase cause one from of MODY.
9. a. Glucokinse—insulin increases gene expression through SREBP-1C;
   b. Fructose-1,6-bisphosphatase—insulin represses gene expression through inactivation of FOXO1 and inhibits enzyme activity by increasing the level of fructose-2,6-bisphosphate, which is an allosteric inhibitor of fructose-1, 6-bisphosphatase.
   c. Pyruvate kinase—insulin both increases *PK* gene expression through SREBBP-1C and increases PK activity through protein phosphatase-mediated dephosphorylation.
   d. Acetyl CoA carboxylase—insulin increases *ACC1* and *ACC2* gene expression through SREBP-1C, and increases activity by phosphatase-mediated dephosphorylation.
   e. PEPCK—insulin inhibits *PEPCK* gene expression, at least in part by inactivation of FOXO1.
10. In the presence of a low I/G ratio, more fatty acids are released from adipose tissue. These fatty acids are transported to the liver where they are metabolized. β-Oxidation increases. The low I/G ratio inhibits glycolysis and lipogenesis. Thus, malonyl CoA levels remain low. Malonyl CoA is an inhibitor of carnitine-palmitoyl transferase-I: as malonyl CoA levels drop, carnitine-palmitoyl transferase-I activity increases. Mitochondria

contain the enzymes for β-oxidation and ketogenesis. The elevated acetyl CoA production from β-oxidation, along with the decreased TCA cycle activity caused by NAD depletion, results in increased ketone body production. T1DM also results in an increased hepatic glucose production; enhanced gluconeogenic flux involves an enhanced efflux of oxaloacetate (as malate) from the mitochondria, further making acetyl CoA available for ketogenesis (as opposed to citrate production).

11. Obesity is associated with the accumulation of ectopic TGs in skeletal muscle and liver. By-products of TG synthesis and turnover (especially diacylglycerol and ceramide) activate signaling pathways (serine/threonine kinases) that phosphorylate and desensitize the insulin receptor and insulin-receptor substrate.

CHAPTER 4

1. 1,25-Dihydroxyvitamin D directly represses *PTH* gene expression. 1,25-Dihydroxyvitamin D also increases gene expression of the Ca^{2+}-sensing receptor, which represses PTH in response to elevated serum calcium. Therefore, loss of 1,25-dihydroxyvitamin D would lead to an increase of PTH secretion. PTH would also increase in response to lowered Ca^{2+}, primarily owing to less absorption by the GI tract.
2. Osteoclasts perform the bone resorption phase of bone remodeling. Osteoblasts promote the differentiation of monocyte-macrophage lineage cells into osteoclast precursors (through secretion of M-CSF) and the maturation of osteoclast precursors into actively resorbing osteoclasts (through membrane expression and secretion of RANKL). Note that PTH/PTHrP receptors are expressed by osteoblasts, not osteoclasts.
3. PTH-related peptide (PTHrP) binds to the same receptor as PTH (the PTH/PTHrP receptor). PTHrP normally acts as a paracrine factor. However, high levels of PTHrP can be produced by neoplasms, thereby causing hypercalcemia (as in hyperparathyroidism).
4. Osteoprotegerin acts as a decoy inhibitor of RANKL, thereby inhibiting osteoclast-mediated bone resorption. Overexpression of osteoprotegerin would cause overly dense bone (osteopetrosis).
5. Vitamin D (i.e., 1,25-dihydroxyvitamin D) elevates Pi levels, promoting the proper calcification of osteoid. Pi absorption by the GI tract occurs largely unregulated. However, Pi reabsorption by the proximal tubule in the kidney is enhanced by vitamin D. This is because the sodium-phosphate exchanger expressed in the kidney is NPT2a, which is under strong hormonal regulation.
6. Hyperparathyroidism increases renal excretion of calcium and phosphate, and the presence of the additional osmotically active electrolytes decreases renal water reabsorption.
7. The urinary calcium level is low because the low serum calcium levels result in a decreased filtered load for calcium even though fractional reabsorption of calcium is low in the absence of PTH.

CHAPTER 5

1. The neurohypophysis (posterior pituitary [pars nervosa], infundibular stalk, and median eminence) is derived from the infundibular downgrowth of the diencephalon. The adenohypophysis (anterior pituitary [pars distalis plus pars tuberalis]) is derived from Rathke pouch, a cranial outgrowth of the oral ectoderm.
2. The median eminence is where releasing hormones are released and enter the hypothalamohypophyseal portal vessels, which run down the infundibular stalk.
3. Osmolality.
4. ADH is synthesized in the hypothalamus, specifically in the cell bodies or magnocellular neurons of the SON and PVN. ADH is synthesized as preprovasophysin, which is proteolytically processed during intra-axonal transport down the stalk. ADH is released from the axonal termini at the pars nervosa.
5. ADH secretion is no longer regulated according to normal servomechanisms. The unregulated, inappropriately high ADH levels lead to excess volume and decrease osmolality. The increased volume stimulates ANP, promoting sodium loss. The decreased osmolality further contributes to hyponatremia.
6. Aquaporin-2 and the vasopressin-2 receptor.
7. Secondary.

8. The GHRH receptor is coupled to a Gs-cAMP-PKA assay and increases *GH* gene expression and somatotrope proliferation.
9. GH is a weak counter-regulatory hormone and opposes insulin-dependent glucose uptake.
10. Cortisol and GH are both "stress hormones" that maintain blood glucose during stress. As expected, stress increases CRH and GHRH. TRH is inhibited by cortisol and thus is decreased by stress—this would decrease metabolic demands during stress. Similarly, the reproductive system imposes significant metabolic demands, and its activity is decreased during stress. Thus, GnRH is decreased by stress.
11. ACTH binds to the MC1R on melanocytes with low affinity. However, primary hypocortisolism leads to high ACTH levels, sufficient to activate the MC1R.
12. Acute hypoglycemia increases GHRH and GH secretion. GH then increases IGF-1 production, especially at the liver.

CHAPTER 6

1. Iodine deficiency with impaired thyroid hormone synthesis is the most common cause of goiter in parts of the world that do not have access to iodized salt, seafood, kelp, dairy products, or other sources of iodine. Graves disease causes unregulated thyroid hormone production (hyperthyroidism) and thyroid hyperplasia and hypertrophy that develops into an enlarged thyroid gland (i.e., goiter).
2. The thyroid hormone receptor (TR) is in fact a gene family encoding TRα1, TRβ1, and TRβ2. TRα1 is expressed primarily in cardiac and skeletal muscle. An inactivating mutation in TRα1 would result in decreased cardiac output and cardiac hypofunction. However, the TRβ2 isoform is expressed in the pituitary thyrotropes and hypothalamic TRH neurons. If this isoform is not mutated, the feedback remains intact.
3. NIS transports iodide across the basal membrane into the thyroid epithelial cell, whereas pendrin transports iodide across the apical membrane, out of the thyroid epithelial cell, and into the follicular lumen. An inactivating mutation in NIS would result in a very low curve for radioiodide uptake. An inactivating mutation in pendrin would result in a radioiodide uptake that is initially normal, but drops owing to leak of radioiodide out of the thyroid gland. This is essentially the same radioiodide uptake curve observed in an organification defect.
4. The increased estrogen production resulting from the pregnancy increases liver TBG production. As TBG levels increase, serum hormone binding increases. To maintain normal serum free T_4 levels, TSH increases T_4 production until a new equilibrium is established in which free hormone levels are close to normal and total levels (bound plus free) are high. Normal pregnant women have significant thyroidal changes during pregnancy, but they are not considered to be hyperthyroid.
5. T_3 increases cardiac output, resting heart rate, and stroke volume. The speed and force of myocardial contractions are enhanced (positive chronotropic and inotropic effects, respectively), and the diastolic relaxation time is shortened (positive lusitropic effect). T_3 induces a widened pulse pressure due to the combined effects of the increased stroke volume and the reduction in total peripheral vascular resistance that results from blood vessel dilation in skin, muscle, and heart. These effects in turn are partly secondary to the increase in tissue production of heat and metabolites that T_3 induces. T_3 also decreases systemic vascular resistance by dilating resistance arterioles in the peripheral circulation.

CHAPTER 7

1. Epinephrine acts as a counter-regulatory hormone at the liver—it stimulates glycogenolysis, gluconeogenesis, and ketogenesis. At the adipocyte, epinephrine has a strong lipolytic action, through the activation of hormone-sensitive lipase (HSL).
2. Catecholamines act through binding to adrenergic receptors. The β_2-adrenergic receptor is coupled to the Gs-cAMP-PKA pathway, which promotes vascular smooth muscle relaxation (through phosphorylation of myosin light-chain kinase) and, thus, vasodilation. Other vessels have a high density of α_1-adrenergic receptors, which are coupled to a Gq-PLC-IP_3-Ca^{2+} signaling pathway that promotes vasoconstriction.

3. Synthetic glucocorticoids inhibit CRH and ACTH production. Low ACTH levels lead to reduced production of endogenous glucocorticoids and adrenal androgens, but also to atrophy of the zona fasciculata and zona reticularis.
4. Excessive ACTH will drive adrenal androgen synthesis in the zona reticularis. The high levels of weak androgens lead to higher levels of testosterone and DHT being produced peripherally in such cells as hair follicle cells, causing hirsutism.
5. Aldosterone increases the synthesis of ENaC (α-subunit). Aldosterone also increases *SGK1* gene expression. SGK1 prevents the ability of a protein, called Nedd 4–2, from targeting ENaC for degradation. Thus, aldosterone promotes Na^+ reabsorption by increasing the synthesis and stability of ENaC in the apical membrane of the distal tubule.
6. A pheochromocytoma produces chronic high levels of catecholamines, which down regulate all adrenergic receptors. In Addison disease, very low levels of aldosterone deplete the intravascular volume, reducing blood pressure. Low cortisol will decrease angiotensinogen production by the liver and decreases adrenergic receptor expression (especially α_1) and signaling in blood vessels.

CHAPTER 8

1. (1) Pairing of chromosomes from genetically unrelated individuals. (2) Independent assortment of chromosomes during production of haploid gametes. (3) Crossing-over during prophase of meiosis I.
2. SRY will drive the testicular differentiation of the bipotential gonad. The testis will secrete testosterone and AMH, thereby causing the regression of the müllerian ducts and development of the wolffian ducts. Differentiation of the urogenital sinus and external genitalia will also be male. Thus, the individual will develop as if they were 46,XY. Psychosexual differentiation is most likely to be male.
3. This individual will have normal male development of the testis, internal tract, and external genitalia. However, the inability to respond to AMH will cause "persistent müllerian duct syndrome," in which the müllerian-derived structures will fail to regress. These structures can physically disrupt testicular descent of one or both testes. Treatment involves surgical removal of müllerian derivatives and correction of an undescended testis.
4. In the testis, meiosis is inhibited in early development by breakdown of retinoic acid. After puberty, meiosis is a continuous process from self-renewing stem cells (spermatogonia) that progress through all steps within about 70 days. In the ovary, all oogonia commit to meiosis, thereby generating a finite number of primary oocytes. Primary oocytes arrest at prophase of meiosis I. Meiosis is continued in response to the LH surge just before ovulation. The secondary oocyte (egg) arrests at metaphase of meiosis II. Meiosis is only completed on fertilization.
5. Kallmann syndrome type 1 is a form of tertiary hypogonadism resulting in low GnRH, LH, FSH, and testosterone (in males). Early production of testosterone is needed for initial development of wolffian structures. Placental hCG, as opposed to fetal pituitary LH, stimulates early testicular production of testosterone.
6. Gain of function mutations in the Kiss1R induce gonadotropin-dependent precocious puberty.
7. During the development of the ovary, retinoic acid induces all oogonia to commit to meiosis, and these primary oocytes become surrounded by prefollicle cells. As a result of follicular atresia, and to a much less extent ovulation, follicles are reduced in number during gestation and throughout infancy, childhood, adolescence, and adulthood. There is no self-renewal. At menopause, there are too few functional follicles to enter the menstrual cycle. In men, the spermatogonia remain meiotically quiescent owing to the presence of CYP26B1, which degrades retinoic acid. By the time spermatogonia enter meiosis at puberty, their microenvironment regulates their divisions, and they self-renew as well as differentiate into primary spermatogonia by the process of asymmetrical division.
8. Menopause is associated with the loss of estradiol and progesterone production by the ovary. This leads to vasomotor instability (hot flushes), decreased bone density (which may progress to osteoporosis), and genital atrophy and vaginal dryness. Postmenopausal women also lose the

beneficial effects of estrogen on lipoprotein profile (i.e., high HDL, low LDL) and develop a greater risk for cardiovascular disease.

CHAPTER 9

1. The Sertoli cells form occluding junctions just apical to the spermatogonia—it is these junctions between adjacent Sertoli cells that create the basal and adluminal compartments of the seminiferous epithelium.
2. Sertoli cells produce AMH, which cause the regression of the müllerian ducts, and inhibin, which feeds back negatively on FSH production by the pituitary gland.
3. Spermatozoa consist of a head with a condensed and streamlined nucleus and an acrosomal vesicle, and a neck with two centrioles (proximal and distal). The proximal centriole attaches to the nucleus and the distal centriole will generate a "9 + 2" configuration of microtubules that is called the axoneme. The tail (also called the flagellum), with a middle piece containing a collar of mitochondria, is the principal piece and the end piece. Spermiogenesis.
4. 17β-HSD3 is a testis-specific enzyme that catalyzes the conversion of androstenedione to testosterone. Without testosterone production, spermatogenesis would not occur, and the external genitalia would differentiate into female structures. Loss of testicular testosterone production would result in high levels of LH and high levels of circulating androstenedione. Androstenedione will be peripherally converted to estrone and estradiol, as well as to testosterone and DHT. Usually, conversion to estradiol (directly or through estrone) is greater than conversion to testosterone and DHT, resulting in significant breast development.
5. Normal spermatogenesis is absolutely dependent on LH-driven intratesticular production of testosterone, which leads to extremely high levels of testosterone within the seminiferous tubules. Exogenous androgens will increase blood testosterone levels enough to inhibit LH, which will actually result in decreased intratesticular levels of testosterone.
6. (1) Mitosis of spermatogonia. (2) Loss of most cytoplasm. (3) Incapacitation. (4) Mixing of sperm with secretions from seminal vesicles and prostate.
7. The seminal vesicles and the prostate gland produce most of the volume of semen. This contains buffer (citrate), antimicrobial agent (high Zn^{+}), fructose, etc. Also, semen contains semenogelins that in lower vertebrates create a fibrin-like plug in the vagina. PSA is a prostate-specific serine-protease that eventually breaks down the plug. In humans, these proteins represent evolutionary remnants. However, PSA can enter the blood when the prostate is infected or damaged by a growing prostatic tumor. PSA levels, especially rapid changes in these levels, are used to assess prostate gland health.
8. cGMP increases Ca^{2+} efflux from the vascular smooth muscle cells of the helicine arteries that empty into the cavernous spaces of the penis or clitoris. Decreased intracellular Ca^{2+} promotes vasodilation, allowing blood to enter the cavernous spaces.

CHAPTER 10

1. To induce meiotic maturation of the primary oocyte to an egg (secondary oocyte at metaphase II). Only this cell can be fertilized.
2. The LH receptor is always expressed on thecal cells. It is not expressed in granulosa cells until in a large preovulatory follicle. The LH receptor is expressed on luteinized theca and granulosa cells. LH expression is induced by FSH during the late follicular phase of the ovary.
3. The LH surge induces the following during the periovulatory period: (1) meiotic maturation of the oocyte; (2) cumulus expansion and breakdown of contact between cumulus and mural granulosa cells; (3) secretion of hydrolytic enzymes that erode the follicular and ovarian wall, and the basal lamina of the mural granulosa, allowing for direct vascularization; and (4) luteinization of the follicle cells, leading to the onset of progesterone secretion.
4. The theca cells convert cholesterol to androstenedione. However, theca express little 17β-HSD and essentially no CYP19 aromatase and, thus, cannot

produce estradiol. The androstenedione must enter the granulosa cells, which convert it to estradiol.

5. For example, estrogen has a negative and positive feedback on pituitary gonadotropes, stimulates growth of the uterine endometrium, stimulates ductal growth in the breasts, stimulates ciliogenesis in the oviduct, and stimulates secretion of a thin, watery mucus by the cervix. Nonreproductive actions of estrogen include bone mineralization, growth and epiphysial plate closure, increased HDL and decreased VLDL and LDL production, increased vasodilation in general, maintenance of healthy skin, and increased lipolysis.
6. The endometrium would not fully develop secretory activity, would not fully express surface proteins involved in implantation, and would undergo early menses.
7. The ovarian reserve indicates the number of primordial follicles in the ovary at any given time.
8. There is a selective rebound in FSH secretion.
9. Selection of recruited follicles.

CHAPTER 11

1. The process by which the sperm induces Ca^{2+} waves in the egg. This induces the cortical reaction, completion of meiosis, and synthesis of proteins involved in early embryogenesis.
2. The trophoblast differentiates of into cytotrophoblasts and syncytiotrophoblasts. Syncytiotrophoblasts express adhesion molecules and hydrolytic enzymes that allow for invasion, and hCG .
3. Placental progesterone is completed solely by the syncytiotrophoblast and is independent of fetal viability. Estriol requires the fetal hypothalamus, pituitary, adrenal, and liver, as well as the syncytiotrophoblast of the placenta. Thus, fetal and placental health impacts estriol synthesis.
4. Estrogen is not needed for a normal pregnancy, except for the development of the breasts for nursing and for a sufficiently responsive myometrium for labor to occur. This latter action involves upregulation of oxytocin receptors and increased prostaglandin synthesis.
5. hCG increases rapidly during the first trimester and cross-reacts with the TSH receptor on the maternal thyroid. hCG production declines to a lower steady level after the first trimester, thereby terminating the hyperthyroidism.
6. Respiratory changes in response to progesterone include increased minute volume and increased tidal volume.
7. Prolactin inhibits GnRH and thus promotes infertility. During very regular nursing, high prolactin levels cause lactational amenorrhea, which inhibits pregnancy while a newborn is being nursed. The most common type of pituitary tumor is the prolactinoma. This is associated with pathologically elevated prolactin, amenorrhea, and infertility.

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APPENDIX B
Comprehensive Multiple-Choice Examination

1. Which receptor resides in the cytoplasm in the absence of hormone?
   a. Insulin receptor
   b. Thyroid hormone receptor
   c. Prolactin receptor
   d. Glucocorticoid receptor
2. One general mode of an intracellular signaling pathway involves:
   a. Exocytosis of matrix molecules
   b. Protein synthesis of a hormone receptor
   c. Covalent phosphorylation of proteins or lipids
   d. Replication of DNA
3. G-protein-coupled receptors (GPCRs) function as:
   a. Ligand-activated tyrosine kinases
   b. Ligand-activated GEFs
   c. Ligand-activated phosphatases
   d. Ligand-activated phospholipases
4. GPCRs can be down regulated by:
   a. Ligand-induced endocytosis
   b. Transphosphorylation on tyrosine residues
   c. Dimerization within the cell membrane
   d. Activation of the Gs subunit
5. The transcription factors called STATs are activated by which class of receptor?
   a. Receptor tyrosine kinase
   b. Steroid hormone receptor
   c. GPCR
   d. Cytokine receptor
6. Which of the following is true concerning coactivator proteins?
   a. They relax chromatin coiling
   b. They reside in the cytoplasm
   c. They repress gene transcription
   d. They directly bind to steroid hormones
7. During the gastric phase, gastrin secretion is stimulated primarily by:
   a. Histamine
   b. Long-chain fatty acids
   c. Somatostatin
   d. Peptides
8. Erythromycin can be used to treat delayed gastric emptying through acting as an agonist for:
   a. Somatostatin
   b. Motilin
   c. CCK
   d. GLP-1
9. Zollinger-Ellison syndrome (gastrin-secreting tumor) causes the overgrowth of which cell type?
   a. S cell
   b. M cell
   c. Pancreatic acinar cell
   d. ECL cell
10. The term *incretin* is used to describe a hormone that:
    a. Sensitizes G cells to stomach distention
    b. Sensitizes intestinal K cells to long-chain fatty acids
    c. Sensitizes pancreatic β cells to glucose
    d. Sensitizes intestinal L cells to glucose
11. A 25-year-old patient was found to have abnormal overnight fasting blood values during a yearly physical exam. Genetic testing revealed the presence of a partial loss-of-function mutation in *GLUT2* gene. The finding from the initial blood work that ultimately pointed to GLUT2 was:
    a. Elevated liver enzymes
    b. Low cortisol
    c. Elevated LDL
    d. Elevated glucose

12. During acute hypoglycemia, insulin secretion is inhibited by low glucose and:
    a. Cholinergic signaling through the muscarinic receptor
    b. Catecholamine signalling through the α_2-adrenergic receptor
    c. Glucagon signaling through the glucagon receptor
    d. GLP-1 signaling through the GLP-1 receptor
13. The insulin receptor regulates metabolism primarily through a signaling pathway involving:
    a. Phosphoinositide-3,4,5-triphosphate
    b. Cyclic AMP
    c. Ca^{2+}
    d. Ras
14. A primary and direct stimulus for glucagon secretion during the FASTING state is:
    a. Low blood sugar
    b. Cholinergic innervation
    c. Low insulin
    d. Low blood free fatty acids
15. Insulin suppresses glucose-6-phosphatase through:
    a. Activation of SREBP-1
    b. Activation of protein phosphatase-1
    c. Inhibition of FOXO1
    d. Activation of cAMP-PKA
16. During the fed state, insulin activates which enzyme/transporter?
    a. Adipose tissue lipoprotein lipase
    b. Hepatic pyruvate carboxylase
    c. Muscle glycogen phosphorylase
    d. Hepatic GLUT2
17. Quantitatively, insulin maintains glucose tolerance through:
    a. Suppression of hepatic glycogenolysis
    b. Increased GLUT4-mediated glucose uptake in muscle
    c. Suppression of adipocyte hormone-sensitive lipase
    d. Increased hepatic glycolysis
18. A homozygous inactivating mutation in the CaSR results in:
    a. Hypercalciuria
    b. Low levels of 1,25-dihydroxyvitamin D_3
    c. Elevated PTH levels
    d. Hypophosphatemia
19. Intermittent treatment with PTH improves bone density through:
    a. Stimulation of RANKL by osteoblasts
    b. Induction of cell death of osteoclasts
    c. Inhibiting secretion of SOST by osteocytes
    d. Decreasing osteoprotegerin production by osteoblasts
20. The sodium-phosphate cotransporter (NPT) isoform 2a is expressed in the proximal renal tubules. NPT2a is regulated in the following manner:
    a. Increased by urine Pi
    b. Increased by calciuria
    c. Decreased by PTH
    d. Decreased by 1,25-dihydroxyvitamin D
21. The primary action of 1,25-dihydroxyvitamin D to increase serum Ca^{2+} is to:
    a. Stimulate of osteoblast RANKL production
    b. Increase intestinal Ca^{2+} absorption
    c. Increase renal phosphate excretion
    d. Stimulate PTH production
22. The expression of renal 1α-hydroxylase is directly stimulated by:
    a. FGF23
    b. CaSR
    c. PTH
    d. High serum Ca^{2+}
23. Damage to the pituitary stalk may result in an increase in:
    a. FSH
    b. GH
    c. ACTH
    d. PRL
24. The pituitary cell that produces two hormones is the:
    a. Thyrotrope
    b. Gonadotrope
    c. Corticotrope
    d. Somatotrope
25. Provasophysin in synthesized in magnocellular cells within the:
    a. Anterior pituitary
    b. Posterior pituitary
    c. Hypothalamus
    d. Infundibular stalk
26. During a fast, GH secretion is stimulated by:
    a. Insulin
    b. Glucagon

c. Ghrelin
d. Somatostatin

27. Tertiary hypercortisolism may result from:
a. Interleukin stimulation of CRH
b. A functional adrenocortical tumor
c. A functional pituitary corticotrope tumor
d. Genetic amplification of the *POMC* gene

28. A gain-of-function mutation in the type 3 deiodinase may cause:
a. Elevated blood T_3 levels
b. Suppressed T_4 synthesis
c. Elevated iodine uptake curve
d. Suppressed TSH levels

29. With respect to cardiovascular function, hypothyroidism causes:
a. Increased inotropy and increased peripheral resistance
b. Increased inotropy and decreased peripheral resistance
c. Decreased inotropy and increased peripheral resistance
d. Decreased inotropy and decreased peripheral resistance

30. Iodide is transported into the colloid by:
a. Sodium/iodide symporter (NIS)
b. Thyroid-specific iodotyrosine deiodinase
c. Thyroid peroxidase
d. Pendrin

31. How would acute chemical inhibition of thyroid peroxidase affect the iodine uptake curve?
a. Decreased initial uptake, then plateau over 24 hr
b. Normal initial uptake, with loss of iodine within 24 hr
c. Increased initial uptake, with loss of iodine within 24 hr
d. Increased initial uptake, then plateau over 24 hr

32. Maternal blood thyroid hormone levels in late pregnancy can be characterized as:
a. Elevated total T_4, normal free T_4
b. Elevated total T_4, elevated T_4
c. Normal total T_4, normal T_4
d. Normal total T_4, elevated T_4

33. In an individual with iodine deficiency–induced goiter and signs of cold intolerance and a decreased heart rate, one would expect to find the following circulating hormone levels:
a. High TSH and high T_3
b. High TSH and low T_3
c. Low TSH and high T_3
d. Low TSH and low T_3

34. In an individual with Graves disease–induced goiter and signs of heat intolerance and an elevated heart rate, one would expect to find the following circulating hormone levels:
a. High TSH and high T_3
b. High TSH and low T_3
c. Low TSH and high T_3
d. Low TSH and low T_3

35. A thyroid hormone receptor not bound to T_3 is located:
a. In the cytoplasm
b. Within the plasma membrane
c. In the nucleus
d. Within the endoplasmic reticulum membrane

36. During exercise, epinephrine and norepinephrine act to:
a. Increase muscle glycogenolysis
b. Increase hepatic glycolysis
c. Decrease adipocyte lipolysis
d. Decrease hepatic ketogenesis

37. A clinical sign or symptom of adrenocortical insufficiency (Addison disease) is:
a. Puffy, flushed face (“moon face”)
b. Skin darkening
c. Increased skin bruisability
d. Hyperglycemia

38. A congenital null mutation of CYP11B1 would result in:
a. Sodium wasting
b. Hyperglycemia
c. Masculinization of a female fetus
d. Atrophy of adrenal cortex

39. One treatment for hypertension is the use of aldosterone receptor antagonists (e.g., spironolactone). A side effect of this treatment can be:
a. Cardiac hypertrophy
b. Hyperkalemia
c. Metabolic alkalosis
d. Edema

40. Glucocorticoid analogs are used at high levels to suppress inflammation. A side effect of this treatment can be:
a. Adrenocortical hypertrophy

b. Hypoglycemia
c. Skin darkening
d. Osteoporosis

41. Cortisol is prevented from interacting with the mineralocorticoid receptor (MR) in the distal nephron through the action of:
a. Cortisol-binding protein
b. 11β-hydroxysteroid dehydrogenase type 2
c. 17β-hydroxysteroid dehydrogenase type 1
d. Serum and glucocorticoid-regulated kinase (SGK)

42. Aldosterone resistance (type 1 pseudohypoaldosteronism) can be due to an inactivating mutation in:
a. Nedd 4–2
b. Epithelial sodium channel (ENaC)
c. 11β-hydroxysteroid dehydrogenase type 2
d. Aquaporin-2

43. The basis for the generation of millions of genetically distinct gametes in a gonad is called:
a. Independent assortment
b. Genetic recombination
c. Disjunction
d. Euploidy

44. Phenotypic gender is directly regulated by:
a. XX or XY sex chromosomes
b. Reproductive tract differentiation
c. Sex steroids
d. Gonadal differentiation

45. The primary source of endogenous estradiol in a postmenopausal woman is the peripheral conversion of androgens made by:
a. The remaining few follicles
b. The ovarian stroma
c. The adrenal cortex
d. The adipose tissue

46. Congenital deficiency of 5α-reductase type 2 may lead to the following in a male:
a. Ovarian differentiation of the gonad
b. Poorly developed seminal vesicle
c. Persistence of müllerian duct derivatives
d. Poorly developed prostate gland

47. A mutation that renders the FSH receptor constitutively active would result in:
a. Elevated blood testosterone levels
b. Low blood LH levels
c. Elevated blood inhibin levels
d. Low androgen-binding protein (APB) in the seminiferous tubule

48. Administration of an exogenous androgen can result in:
a. Elevated sperm production
b. Elevated blood LH levels
c. Elevated hematocrit (polycythemia)
d. Elevated proteolysis in muscle

49. The Sertoli cell performs the following functions, *except:*
a. Regulates sperm development up to full motility
b. Expresses androgen-binding protein
c. Maintains blood-testis barrier
d. Expresses receptors for both FSH and testosterone

50. Congenital deficiency of 17β-hydroxysteroid dehydrogenase (HSD) type 3 in a 46,XY individual would result in the following:
a. Lack of pubic hair in the adult
b. Breast development at puberty
c. Precocious penile and testicular growth at puberty
d. Development of oviducts and uterus

51. DHT binds to the:
a. Estrogen receptor
b. DHT receptor
c. Androgen receptor
d. Prostate-specific antigen (PSA)

52. Up regulation of penile cyclic GMP phosphodiesterase would result in the following within the vascular smooth muscle in the helicine arteries:
a. Increased tone
b. Increased nitric oxide levels
c. Decreased intracellular Ca^{2+}
d. Increased cyclic GMP

53. Emission refers to sperm moving into the:
a. Vas deferens
b. Spongy urethra
c. Epididymis
d. Prostatic urethra

54. Testosterone has the following effect on the liver:
a. Up regulates LDL receptor
b. Increases VLDL production
c. Increases ApoA1 expression
d. Activates AMP kinase (AMPK)

55. In the late follicular phase, the LH receptor is expressed on:
    a. Theca cells only
    b. Granulosa cells only
    c. Theca and granulosa cells
    d. Theca, granulosa cells, and the oocyte
56. During the secretory phase of the uterus, progesterone induces:
    a. Inactivation of estradiol to estrone
    b. Proliferation of predecidual cells
    c. Myometrial contractions
    d. Release of matrix metalloproteases from stroma
57. The *ovarian reserve* is a collective term for:
    a. The number of preovulatory follicles
    b. The number of primordial follicles
    c. The ovarian stroma
    d. The number of oogonia
58. During her annual exam, a patient tells her gynecologist that she has noticed increased facial hair and acne. Ultrasound imaging of the ovaries reveals the presence of multiple "cysts," and the diagnosis of polycystic ovary syndrome (PCOS) is made. The patient's BMI is 32, indicating obesity. In this individual, the probable root cause of her PCOS is:
    a. Elevated estradiol production
    b. Hyperinsulinemia
    c. Peripheral conversion of estrone to androgens
    d. Elevated circulating FSH
59. In the two-cell model of ovarian steroidogenesis, thecal cells primarily produce:
    a. Estradiol
    b. Progesterone
    c. Testosterone
    d. Androstenedione
60. A key factor in the selection of a dominant follicle is high expression of which receptor?
    a. FSH receptor
    b. Androgen receptor
    c. LH receptor
    d. Progesterone receptor
61. During the periovulatory period, the following process occurs:
    a. Meiotic completion and formation of the second polar body
    b. Up regulation of CYP19-aromatase in granulosa cells
    c. Cumulus expansion
    d. Selection of a dominant follicle
62. The steroidogenic pathway of the mature corpus luteum is characterized by very low expression of:
    a. CYP19-aromatase
    b. CYP17 (17-hydroxylase)
    c. 3β-Hydroxysteroid dehydrogenase (3β-HSD)
    d. 17β-Hydroxysteroid dehydrogenase (17β-HSD; activating isoform)
63. The inability of the cumulus-oocyte complex to enter the female reproductive tract may be caused by an infection or inflammation of:
    a. The isthmus of the oviduct
    b. The ampulla of the oviduct
    c. The intramural portion of the oviduct
    d. The infundibulum of the oviduct
64. The priming of the endometrium by estrogen refers to:
    a. Development of vascular lacunae
    b. Induction of progesterone receptors
    c. Rapid proliferation of all cell types
    d. Expression of pinopods by the surface epithelial cells
65. The primary signal for the LH surge is:
    a. Increased frequency of GnRH pulses by hypothalamic GnRH neurons
    b. Decreased inhibin B production from both ovaries
    c. Persistently high blood levels of estradiol from the dominant follicle
    d. Rapid down regulation of the GnRH receptors on the gonadotropes
66. Sperm undergo the process of capacitation as they:
    a. Are stored in the tail of the epididymis
    b. Become adhered to the oviduct
    c. As they pass through the cervical mucus
    d. In the vagina just after ejaculation
67. An infertile couple undergo in vitro fertilization. Although the husband ejaculates several hundred million sperm, with greater than 50% normal phenotype, they fail to fertilize hatched oocytes from the wife's ovaries. This observation suggests a failure of:
    a. Surface hyaluronidase (PH-20) expression
    b. Binding to ZP3
    c. Sperm-egg fusion
    d. Sperm hyperactivation

68. The "window of receptivity" refers to:
    a. The desire on the part of the woman to have sex
    b. The adherent endometrium at midluteal phase
    c. The duration of a viable cumulus-oocyte complex in the oviduct
    d. The time of increased thickness of cervical mucus
69. The primary function of the endometrial lacunae and associated vessels is to:
    a. Capture hCG from the implanting embryo
    b. Provide nutrition and oxygen to the implanting embryo
    c. Deliver ovarian hormones to the implanting embryo
    d. Induce the differentiation of syncytiotrophoblasts around the implanting embryo
70. In humans, the placental barrier in the mature placenta includes the following cell types:
    a. Syncytiotrophoblast, fetal endothelial cell
    b. Maternal endothelial cell, syncytiotrophoblast, fetal endothelial cell
    c. Syncytiotrophoblast, cytotrophoblast, fetal endothelial cell
    d. Cytotrophoblast, fetal endothelial cell
71. In a fetus carrying a null mutation in the gene encoding StAR protein, the following would be observed during the third trimester:
    a. Maternal progesterone levels would be very low
    b. Maternal estriol levels would be very low
    c. Amniotic ACTH levels would be very low
    d. Maternal cortisol levels would be very low
72. In a fetus carrying a null mutation in the liver *CYP3A7* gene encoding 16-hydroxylase, maternal blood would show an absence of:
    a. Progesterone
    b. Estradiol
    c. Estriol
    d. Estrone
73. Transient gestational hyperthyroidism is due to:
    a. Deficiency in the placental type 1 deiodinase
    b. Induction of hypothalamic TRH by elevated levels of progesterone
    c. Cross-reaction of hCG with the TSH receptor
    d. Estradiol-induced hypertrophy and hyperplasia of thyroid epithelial cells
74. Reversible vision problems may be experienced by some women during late pregnancy. This is due to an enlarged pituitary gland that presses on the optic nerves. Pituitary enlargement is caused by:
    a. Estrogen-induction of lactotrope size and number
    b. Progesterone-induced edema within the sella turcica
    c. GnRH-induced growth of gonadotropes
    d. hCG-induced growth of thyrotropes
75. One factor that assists the mother in increasing her volume (for the umbilical circulation, growing fetus, and enlarging amniotic sac) is:
    a. Progesterone inhibition of antinatriuretic factor (ANF)
    b. hCG inhibition of ADH
    c. Estrogen induction of liver angiotensinogen
    d. Increased threshold for thirst
76. Lactational amenorrhea is similar to clinical amenorrhea and infertility due to:
    a. Elevated ovarian androgens
    b. Hyperprolactinemia
    c. An oxytocin-producing tumor
    d. Cessation of GnRH neuronal pulsatility
77. Tamoxifen is used to treat invasive breast cancer (after surgery and radiation). The mechanism of tamoxifen's action is:
    a. Inhibition of CYP19-aromatase
    b. Competitive inhibition of the progesterone receptor
    c. Increased inactivation of circulating estradiol
    d. Competitive inhibition of the estrogen receptor
78. Maternal cortisol levels increase during pregnancy and contribute to:
    a. Increased prolactin production by the pituitary gland
    b. Increased maternal tidal volume
    c. Increased maternal insulin levels
    d. Increased production of cortisol-binding protein by the liver

79. Fetal cortisol rises significantly just before term. This is due to positive feedback between cortisol and placental:
    a. CRH
    b. Progesterone
    c. Estrogen
    d. hPL

80. Extravillous cytotrophoblasts perform the following function during the first trimester:
    a. Induction of decidual cells to form the basal plate
    b. Phagocytosis of dead cells
    c. Conversion of spiral arteries
    d. Formation of a layer of the amniodecidual membrane

ANSWERS TO COMPREHENSIVE MULTIPLE-CHOICE EXAM

1. d	17. b	33. b	49. a	65. c
2. c	18. c	34. c	50. b	66. b
3. b	19. c	35. c	51. c	67. c
4. a	20. c	36. a	52. a	68. b
5. d	21. b	37. b	53. d	69. a
6. a	22. c	38. c	54. b	70. a
7. d	23. d	39. b	55. c	71. b
8. b	24. b	40. d	56. a	72. c
9. d	25. c	41. b	57. b	73. c
10. c	26. c	42. b	58. b	74. a
11. d	27. a	43. a	59. d	75. c
12. b	28. c	44. c	60. a	76. b
13. a	29. c	45. c	61. c	77. d
14. c	30. d	46. d	62. b	78. c
15. c	31. b	47. c	63. d	79. a
16. a	32. a	48. c	64. b	80. c

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APPENDIX C
Hormone Ranges

HORMONE	NORMAL RANGE, CONVENTIONAL UNITS
Adrenal steroids, plasma	
Aldosterone, supine, saline suppression	< 8.5 ng/dL
Aldosterone, upright, normal diet	5-20 ng/dL
Cortisol	
8 AM	5-25 µg/dL
4 PM	3-12 µg/dL
Overnight dexamethasone suppression	< 5 µg/dL
Dehydroepiandrosterone (DHEA)	2-9 µg/dL
DHEAS	50-250 µg/dL
Adrenal steroids, urine	
Aldosterone	5-19 µg/day
Cortisol, free	20-100 µg/day
17-Hydroxycorticosteroids	2-10 mg/day
17-Ketosteroids	
Men	7-25 mg/day
Women	4-16 mg/day
Angiotensin II, plasma	10-60 pg/mL
Antidiuretic hormone (ADH; vasopressin)	
Random fluid intake	1-3 pg/mL
Dehydration 18-24 hr	4-14 pg/mL
Calciferols (Vitamin D_3), plasma	
1,25-dihydroxyvitamin D_3	15-60 pg/mL
25-hydroxyvitamin D_3	8-40 ng/mL
Calcitonin, plasma	
Normal	< 19 pg/mL
Medullary thyroid cancer	> 100 pg/mL
Calcium, ionized serum	4-5.6 mg/dL
Catecholamines, urine	
Free catecholamines	< 100 µg/day
Epinephrine	< 50 µg/day
Metanephrine	< 1.3 ng/day
Norepinephrine	15-89 µg/day
Vanillylmandelic acid (VMA)	< 8 mg/day

Continued

HORMONE	NORMAL RANGE, CONVENTIONAL UNITS
Cholesterol, total plasma	
Desirable	<200 mg/dL
HDL cholesterol	
Desirable	>69 mg/dL
LDL cholesterol	
Desirable	<130 mg/dL
Corticotropin (ACTH), plasma, 8 AM	9-52 pg/mL
Electrolytes	
Chloride, serum	98-106 mEq/L
Sodium, serum	136-145 mEq/L
Free fatty acids, plasma	10.6-18 mg/dL
Gastrin, plasma	<120 pg/mL
Glucagon, plasma	50-100 pg/mL
Glucose, plasma	
Overnight fast, normal	75-100 mg/dL
Glucose tolerance test	
2-Hr plasma glucose, normal	<140 mg/dL
2-Hr plasma glucose, diabetes mellitus	>200 mg/dL
Gonadal steroids	
Dihydrotestosterone	
Women	0.05-0.3 ng/mL
Men	0.25-0.75 ng/mL
Estradiol	
Women, basal	20-60 pg/mL
Women, ovulatory surge	>200 pg/mL
Men	<50 pg/mL
Progesterone	
Women, luteal phase	2-20 ng/mL
Women, follicular phase	<2 ng/mL
Men	<2 ng/mL
Testosterone	
Women	<1 ng/mL
Men	3-10 ng/mL
Gonadotropins, plasma	
Follicle-stimulating hormone (FSH)	
Women, basal	1.4-9.6 mIU/mL
Women, ovulatory surge	2.3-21 mIU/mL
Women, postmenopausal	34-96 mIU/mL
Men	0.9-15 mIU/mL
Luteinizing hormone (LH)	
Women, basal	0.8-26 mIU/mL
Women, ovulatory surge	25-57 mIU/mL
Women, postmenopausal	40-104 mIU/mL
Men	1.3-13 mIU/mL

HORMONE	NORMAL RANGE, CONVENTIONAL UNITS
Growth hormone, plasma	
After 100 g glucose orally	< 2 mg/mL
After insulin-induced hypoglycemia	> 9 ng/mL
Human chorionic gonadotropin (hCG)	
Men and nonpregnant women	< 3 mIU/mL
Insulin, plasma, fasting	5-20 µU/ml
Insulin-like growth factor (IGF-1)	0.35-2.2 U/mL
Ketone bodies	
Acetoacetate	< 1 mg/dL
β-Hydroxybutyrate	< 3 mg/dL
Lactate, plasma	5-20 mg/dL
Magnesium, serum	1.8-3.0 mg/dL
Osmolality, plasma	285-295 mOsmol/L
Oxytocin, plasma	
Random	1.25-5 ng/mL
Women, ovulatory surge	5-10 ng/mL
Parathyroid hormone, serum (intact PTH)	10-65 pg/mL
Phosphorus, inorganic, serum	3.0-4.5 mg/dL
Prolactin, serum	
Nonpregnant women and men	2-15 ng/mL
Pyruvate, plasma	0.3-0.9 mg/dL
Renin activity, plasma, normal sodium intake	
Standing	9.3 ± 4.3 ng/mL/hr
Supine	3.2 ± 1 ng/mL/hr
Thyroid function tests	
Free thyroxine estimate	0.7-2.0 ng/dL
Radioactive iodine uptake, 24 hr	5%-30%
Resin T_3 uptake, serum	25%-35%
Reverse T_3 (rT_3), serum	10-40 ng/dL
Thyrotropin (TSH), serum	0.5-5 µU/mL
Thyroxine (T_4), serum	5-12 µg/dL
Triiodothyronine (T_3), serum	70-190 ng/dL
Triglycerides, plasma	< 160 mg/dL

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APPENDIX D
Abbreviations and Symbols

αGSU	alpha glycoprotein subunit
β-hCG	hormone-specific β-subunit of hCG
β-TSH	β-Thyroid-stimulating hormone
3β-HSD	3β-Hydroxysteroid dehydrogenase
11β-HSD2	11β-Hydroxysteroid dehydrogenase
17β-HSD	17β-Hydroxysteroid dehydrogenase
AA	amino acid
ABP	androgen-binding protein
ACE	angiotensin-converting enzyme
Ach	acetylcholine
ACTH	adrenocorticotropic hormone (corticotropin)
ADH	antidiuretic hormone
AIS	androgen insensitivity syndrome
ALS	acid-labile subunit
AMH	antimüllerian hormone
ANP	atrial natriuretic peptide
APO	apoprotein
AR	androgen receptor
ARE	androgen-response element
ATP	adenosine triphosphate
BAT	brown adipose tissue
bFGF	basic fibroblast growth factor
BMP-15	bone morphogenetic protein-15
BMR	basal metabolic rate
Ca^{2+}	calcium phosphate
CaMKII	Ca^{2+}-calmodulin-dependent protein kinase-II
cAMP	cyclic adenosine monophosphate
CaSR	Ca^{2+}-sensing receptor
CBG	corticosteroid-binding globulin (also transcortin)
CCK	cholecystokinin
CDK1	cyclin-dependent kinase-1
CETP	cholesterol ester transfer protein
cGMP	cyclic guanosine monophosphate
CGRP	calcitonin gene–related peptide
CNS	central nervous system
COMT	catechol-*O*-methyltransferase
COX-2	cyclooxygenase-2
CPT-1/CPT-2	carnitine-palmitoyl transferase
CREB protein	cAMP-response element–binding protein
CRH	corticotropin-releasing hormone
CSF	cytostatic factor
CYP11β	11β-Hydroxylase
CYP21β	21β-Hydroxylase
CYP	cytochrome P-450 mono-oxidase gene
DAG	diacylglycerol
DBP	vitamin D–binding protein
DHEAS	dehydroepiandrosterone sulfate
DHT	dihydrotestosterone
DI	diabetes insipidus
DIT	diiodotyrosine
DM	diabetes mellitus
DNA	deoxyribonucleic acid
DOC	deoxycorticosterone
DOPA	dihydroxyphenylalanine
ECL	enterochromaffin-like (cell)
ED	erectile dysfunction
EGF	epidermal growth factor
EnaC	epithelial Na^+ channel
ENS	enteric nervous system
ER	estrogen receptor
ERE	estrogen-response element
FAS	fatty acid synthase (complex)
FBHH	familial benign hypocalciuric hypercalcemia
FDA	Food and Drug Administration
FFA	free fatty acid

FGF-23	fibroblast growth factor-23
FSH	follicle-stimulating hormone
Gα	Gα-subunit
Gβ/γ	Gβ-subunit dimer
G-6-P	glucose-6-phosphate
GAG	glycosaminoglycan
GDF-9	growth differentiation factor-9
GEF	guanine nucleotide exchange factor
GFR	glomerular filtration rate
GH	growth hormone
GHRH	growth hormone–releasing hormone
GHS	growth hormone secretogogue
GH-V	growth hormone variant
GI	gastrointestinal
GIP	gastroinhibitory peptide
GLUT	glucose transporter
GnRH	gonadotropin-releasing hormone
GPCR	G-protein-coupled receptor
GPCR3	G-protein-coupled receptor-3
GR	glucocorticoid receptor
GRE	glucocorticoid-response element
GRK/RTK	GPCR kinases
GRP	gastrin-releasing peptide
GTP	guanosine nucleotide triphosphate
GVBD	germinal vesicle breakdown
HAD	histone diacetylase
HAT	histone acetyltransferase
HbA_{1c}	hemoglobin A_{1c}
hCG	human chorionic gonadotropin
HCO_3^-	bicarbonate ion
hCS	human chorionic somatomammotropin
HDL	high-density lipoprotein
HPA	hypothalamus-pituitary-adrenal
hPL	human placental lactogen
HRE	hormone-response element
HSD	hydroxysteroid dehydrogenase
HSL	hormone-sensitive lipase
ICSI	intracytoplasmic sperm injection
IDL	intermediate-density lipoprotein (particles)
IGF	insulin-like growth factor
IGF-1	insulin-like growth factor-1
IGFBP	insulin-like growth factor–binding proteins
IP_3	inositol 1,4,5-triphosphate
IR	insulin receptor
IRS	insulin receptor substrate
IUD	intrauterine device
IVF	in vitro fertilization
LDL	low-density lipoprotein
LH	luteinizing hormone
LPD	luteal phase deficiency
LPL	lipoprotein lipase
MAO	monoamine oxidase
MAP	mitogen-activated protein kinase (also ERK)
MAPK	mitogen-activated kinase
MC2R	melanocortin-2 receptor
MCsF	monocyte colony-stimulating factor
MIT	monoiodotyrosine
MMC	migrating myoelectric complex
MODY	mature onset of diabetes of the young
MPF	maturation-promoting factor
MR	mineralocorticoid receptor
MRE	mineralocorticoid-response element
MIS	müllerian-inhibiting substance
MPF	maturation-promoting factor
mRNA	messenger RNA
NCX	sodium-calcium exchanger
NO	nitric oxide
OHSS	ovarian hyperstimulation syndrome
OPG	osteoprotegerin
OxPhos	oxidative phosphorylation
P_{CO_2}	partial pressure of carbon dioxide
PCOS	polycystic ovary syndrome
PEPCK\t	PEP carboxykinase (phosphoenol-pyruvate carboxykinase)
PFK1	phosphofructokinase-1
PGE_2	prostaglandin E_2
$PGF_{2\alpha}$	prostaglandin $F_{2\alpha}$
PHEX	phosphate-regulating gene with homologies to endopeptidases on the X chromosome
Pi	phosphate
PI3K	phosphatidylinositol-3-kinase
PIP_3	phosphatidylinositol 3,4,5-triphosphate
PKA	protein kinase A

PKB	protein kinase B
PKG	protein kinase G
PLCζ	phospholipase Cζ
PMCA	plasma membrane calcium ATPase
PMS	premenstrual syndrome
PNMT	phenylethanolamine-*N*-methyl transferase
Po_2	partial pressure of oxygen
POMC	pro-opiomelanocortin
PPARγ	peroxisome proliferator-activated receptor-γ
PR	progesterone receptor
PRE	progesterone-response element
PRF	prolactin-releasing factor
PRL	prolactin
PSA	prostate-specific antigen
PTH	parathyroid hormone
PTHrP	parathyroid hormone–related peptide
PTU	propylthiouracil
PVH	paraventricular hypothalamus
PVN	paraventricular nuclei
RAIU	radioactive iodide uptake
RANKL	receptor activator of NF-KB ligand
RAS	renin-angiotensin system
RDS	respiratory distress syndrome
RGS	regulators of G-protein signaling
RIA	radioimmunoassay
RNA	ribonucleic acid
ROMK channel	renal outer medullary K^+ channel
ROS	reactive oxygen species
rT_3	reverse T_3
RTK	receptor tyrosine kinase
SERM	selective estrogen receptor modulator
SHBG	sex hormone–binding globulin
SIADH	syndrome of inappropriate secretion of antidiuretic hormone
SOCS	suppressor of cytokine signaling
SON	supraoptic nuclei
SRBEP-1C	sterol regulatory-binding element protein-1C
SRY	sex-determining region Y
StAR protein	steroidogenic acute regulatory protein
STAT	signal transducers and activator of transcription
SUR	ATP-binding subunit
$t_{1/2}$	half-life
T1DM	type 1 diabetes mellitus
T2DM	type 2 diabetes mellitus
T_3	triiodothyronine
T_4	thyroxine
TBG	thyroxine-binding globulin
TCA	tricarboxylic acid (cycle)
TGF	transforming growth factor
TGF-β	transforming growth factor-β
TG	thyroglobulin
TGs	triglycerides
TNF-α	tumor necrosis factor-α
TPO	thyroid peroxidase
TR	thyroid hormone receptor
TRE	thyroid hormone–response element
TRH	thyrotropin-releasing hormone (also thyroid-releasing hormone)
T/S	thyroid/serum (ratio)
T/S[I]	thyroid/serum (measured with radioactive iodide)
TSA	thyroid-stimulating antibody
TSAb	thyroid-stimulating antibody (abnormal)
TSH	thyroid-stimulating hormone (also called thyrotropin)
TTR	thyroxine-binding prealbumin
TZD	thiazolidinedione
VDR	vitamin D receptor
VEGF	vascular endothelial growth factor
VLDL	very-low-density lipoprotein
VMA	vanillylmandelic acid
WAT	white adipose tissue

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HORMONE	NORMAL RANGE, CONVENTIONAL UNITS
Adrenal steroids, plasma	
Aldosterone, supine, saline suppression	<8.5 ng/dL
Aldosterone, upright, normal diet	5-20 ng/dL
Cortisol	
8 AM	5-25 µg/dL
4 PM	3-12 µg/dL
Overnight dexamethasone suppression	<5 µg/dL
Dehydroepiandrosterone (DHEA)	2-9 µg/dL
DHEAS	50-250 µg/dL
Adrenal steroids, urine	
Aldosterone	5-19 µg/day
Cortisol, free	20-100 µg/day
17-Hydroxycorticosteroids	2-10 mg/day
17-Ketosteroids	
Men	7-25 mg/day
Women	4-16 mg/day
Angiotensin II, plasma	10-60 pg/mL
Antidiuretic hormone (ADH; vasopressin)	
Random fluid intake	1-3 pg/mL
Dehydration 18-24 hr	4-14 pg/mL
Calciferols (Vitamin D_3), plasma	
1,25-dihydroxyvitamin D_3	15-60 pg/mL
25-hydroxyvitamin D_3	8-40 ng/mL
Calcitonin, plasma	
Normal	<19 pg/mL
Medullary thyroid cancer	>100 pg/mL
Calcium, ionized serum	4-5.6 mg/dL
Catecholamines, urine	
Free catecholamines	<100 µg/day
Epinephrine	<50 µg/day
Metanephrine	<1.3 ng/day
Norepinephrine	15-89 µg/day
Vanillylmandelic acid (VMA)	<8 mg/day
Cholesterol, total plasma	
Desirable	<200 mg/dL
HDL cholesterol	
Desirable	>69 mg/dL
LDL cholesterol	
Desirable	<130 mg/dL
Corticotropin (ACTH), plasma, 8 AM	9-52 pg/mL
Electrolytes	
Chloride, serum	98-106 mEq/L
Sodium, serum	136-145 mEq/L

Continued

HORMONE	NORMAL RANGE, CONVENTIONAL UNITS
Free fatty acids, plasma	10.6-18 mg/dL
Gastrin, plasma	<120 pg/mL
Glucagon, plasma	50-100 pg/mL
Glucose, plasma	
Overnight fast, normal	75-100 mg/dL
Glucose tolerance test	
2-Hr plasma glucose, normal	<140 mg/dL
2-Hr plasma glucose, diabetes mellitus	>200 mg/dL
Gonadal steroids	
Dihydrotestosterone	
Women	0.05-0.3 ng/mL
Men	0.25-0.75 ng/mL
Estradiol	
Women, basal	20-60 pg/mL
Women, ovulatory surge	>200 pg/mL
Men	<50 pg/mL
Progesterone	
Women, luteal phase	2-20 ng/mL
Women, follicular phase	<2 ng/mL
Men	<2 ng/mL
Testosterone	
Women	<1 ng/mL
Men	3-10 ng/mL
Gonadotropins, plasma	
Follicle-stimulating hormone (FSH)	
Women, basal	1.4-9.6 mIU/mL
Women, ovulatory surge	2.3-21 mIU/mL
Women, postmenopausal	34-96 mIU/mL
Men	0.9-15 mIU/mL
Luteinizing hormone (LH)	
Women, basal	0.8-26 mIU/mL
Women, ovulatory surge	25-57 mIU/mL
Women, postmenopausal	40-104 mIU/mL
Men	1.3-13 mIU/mL
Growth hormone, plasma	
After 100 g glucose orally	<2 mg/mL
After insulin-induced hypoglycemia	>9 ng/mL
Human chorionic gonadotropin (hCG)	
Men and nonpregnant women	<3 mIU/mL
Insulin, plasma, fasting	5-20 μU/ml
Insulin-like growth factor (IGF-1)	0.35-2.2 U/mL
Ketone bodies	
Acetoacetate	<1 mg/dL
β-Hydroxybutyrate	<3 mg/dL

HORMONE	NORMAL RANGE, CONVENTIONAL UNITS
Lactate, plasma	5-20 mg/dL
Magnesium, serum	1.8-3.0 mg/dL
Osmolality, plasma	285-295 mOsmol/L
Oxytocin, plasma	
Random	1.25-5 ng/mL
Women, ovulatory surge	5-10 ng/mL
Parathyroid hormone, serum (intact PTH)	10-65 pg/mL
Phosphorus, inorganic, serum	3.0-4.5 mg/dL
Prolactin, serum	
Nonpregnant women and men	2-15 ng/mL
Pyruvate, plasma	0.3-0.9 mg/dL
Renin activity, plasma, normal sodium intake	
Standing	9.3 $\pm$ 4.3 ng/mL/hr
Supine	3.2 $\pm$ 1 ng/mL/hr
Thyroid function tests	
Free thyroxine estimate	0.7-2.0 ng/dL
Radioactive iodine uptake, 24 hr	5%-30%
Resin T_3 uptake, serum	25%-35%
Reverse T_3 (rT_3), serum	10-40 ng/dL
Thyrotropin (TSH), serum	0.5-5 μU/mL
Thyroxine (T_4), serum	5-12 μg/dL
Triiodothyronine (T_3), serum	70-190 ng/dL
Triglycerides, plasma	$<$160 mg/dL