# **34** Cholesterol Absorption, Synthesis, Metabolism, and Fate

Cholesterol is one of the most highly recognized molecules in human biology, in part because of a direct relationship between its concentrations in blood and tissues and the development of **atherosclerotic vascular disease**. Cholesterol, which is transported in the blood in **lipoproteins** because of its absolute insolubility in water, serves as a **stabilizing component of cell membranes** and as a precursor of the **bile salts** and **steroid hormones**. Precursors of cholesterol are converted to **ubiquinone**, **dolichol**, and, in the skin, to **cholecalciferol**, **the active form of vitamin D**. As a **major component of blood lipoproteins**, cholesterol can appear in its free, unesterified form in the outer shell of these macromolecules and as cholesterol esters in the lipoprotein core.

Cholesterol is obtained from the diet or synthesized by a pathway that occurs in most cells of the body, but to a greater extent in cells of the liver and intestine. The precursor for cholesterol synthesis is **acetyl CoA**, which can be produced from glucose, fatty acids, or amino acids. Two molecules of acetyl CoA form **acetoacetyl CoA**, which condenses with another molecule of acetyl CoA to form **hydroxymethylglutaryl CoA** (HMG-CoA). Reduction of HMG-CoA produces **mevalonate**. This reaction, catalyzed by HMG-CoA reductase, is the major ratelimiting step of cholesterol synthesis. Mevalonate produces isoprene units that condense, eventually forming **squalene**. Cyclization of squalene produces the steroid ring system, and a number of subsequent reactions generate cholesterol. The adrenal cortex and the gonads also synthesize cholesterol in significant amounts and use it as a precursor for steroid hormone synthesis.

Cholesterol is packaged in **chylomicrons** in the intestine and in **very-low-den**sity lipoprotein (VLDL) in the liver. It is transported in the blood in these lipoprotein particles, which also transport triacylglycerols. As the triacylglycerols of the blood lipoproteins are digested by lipoprotein lipase, chylomicrons are converted to **chylomicron remnants**, and VLDL is converted to **intermediate-density** lipoprotein (IDL) and subsequently to low-density lipoprotein (LDL). These products return to the liver, where they bind to receptors in cell membranes and are taken up by endocytosis and digested by lysosomal enzymes. LDL is also endocytosed by nonhepatic (peripheral) tissues. Cholesterol and other products of lysosomal digestion are released into the cellular pools. The liver uses this recycled cholesterol, and the cholesterol that is synthesized from acetyl CoA, to produce VLDL and to synthesize bile salts.

Intracellular cholesterol obtained from blood lipoproteins decreases the synthesis of cholesterol within cells, stimulates the storage of cholesterol as cholesterol esters, and decreases the synthesis of **LDL receptors**. LDL receptors are found on the surface of the cells and bind various classes of lipoproteins prior to endocytosis.

Although high-density lipoprotein (HDL) contains triacylglycerols and cholesterol, its function is very different from that of the chylomicrons and VLDL, which transport triacylglycerols. HDL exchanges proteins and lipids with the other lipoproteins in the blood. HDL transfers **apolipoprotein E (apoE) and apoC<sub>II</sub>** to chylomicrons and VLDL. After digestion of the VLDL triacylglycerols, apoE and apoC<sub>II</sub> are transferred back to HDL. In addition, HDL obtains cholesterol from other lipoproteins and from cell membranes and converts it to **cholesterol esters** by the **lecithin:cholesterol acyltransferase (LCAT)** reaction. Then HDL either directly transports cholesterol and cholesterol esters to the liver or transfers cholesterol esters to other lipoproteins via the **cholesterol ester transfer protein** (**CETP**). Ultimately, lipoprotein particles carry the cholesterol and cholesterol esters to the liver, where endocytosis and lysosomal digestion occur. Thus, "**reverse cholesterol transport**" (i.e., the return of cholesterol to the liver) is a major function of HDL.

Elevated levels of cholesterol in the blood are associated with the formation of **atherosclerotic plaques** that can occlude blood vessels, causing heart attacks and strokes. Although high levels of LDL cholesterol are especially atherogenic, high levels of HDL cholesterol are protective because HDL particles are involved in the process of removing cholesterol from tissues, such as the lining cells of vessels, and returning it to the liver.

Bile salts, which are produced in the liver from cholesterol obtained from the blood lipoproteins or synthesized from acetyl CoA, are secreted into the bile. They are stored in the gallbladder and released into the intestine during a meal. The bile salts emulsify dietary triacylglycerols, thus aiding in digestion. The digestive products are absorbed by intestinal epithelial cells from bile salt micelles, tiny microdroplets that contain bile salts at their water interface. After the contents of the micelles are absorbed, most of the bile salts travel to the ileum, where they are resorbed and recycled by the liver. Less than 5% of the bile salts that enter the lumen of the small intestine are eventually excreted in the feces.

Although the fecal excretion of bile salts is relatively low, it is a major means by which the body disposes of the steroid nucleus of cholesterol. Because the ring structure of cholesterol cannot be degraded in the body, it is excreted mainly in the bile as free cholesterol and bile salts.

The steroid hormones, derived from cholesterol, include the adrenal cortical hormones (e.g., cortisol, aldosterone, and the adrenal sex steroids dehydroepiandrosterone [DHEA] and androstenedione) and the gonadal hormones (e.g., the ovarian and testicular sex steroids, such as testosterone and estrogen).



## THE WAITING ROOM

At his current office visit, **Ivan Applebod's** case was reviewed by his physician. Mr. Applebod has several of the major risk factors for coronary heart disease (CHD). These include a sedentary lifestyle, marked obesity, hypertension, hyperlipidemia, and early non–insulin-dependent diabetes mellitus (NIDDM). Unfortunately, he has not followed his doctor's advice with regard to a diabetic diet designed to affect a significant loss of weight, nor has he followed an aerobic exercise program. As a consequence, his weight has gone from 270 to 281 lb. After a 14-hour fast, his serum glucose is now 214 mg/dL (normal, <110), and his serum total cholesterol level is 314 mg/dL (desired level is 200 or less). His serum triacylglycerol level is 295 mg/dL (desired level is 150 or less), and his serum HDL cholesterol is 24 mg/dL (desired level is 240 for a male). His calculated serum LDL cholesterol level is 231 mg/dL (desired level for a person with two or more risk factors for CHD is 130 mg/dL or less, unless one of the risk factors is diabetes mellitus, in which case, the LDL cholesterol level should be < 100 mg/dL).



lished heart disease is <100).

**Ann Jeina** was carefully followed by her physician after she survived her heart attack. Before discharge from the hospital, after a 14-hour fast, her serum triacylglycerol level was 158 mg/dL (slightly above the upper range of normal), and her HDL cholesterol level was low at 32 mg/dL (normal for women is  $\geq$ 50). Her serum total cholesterol level was elevated at 420 mg/dL (reference range,  $\leq 200$  for a female with known CHD). From these values, her LDL cholesterol level was calculated to be 356 mg/dL (desirable level for a person with estab-

Both of Ms. Jeina's younger brothers had "very high" serum cholesterol levels, and both had suffered heart attacks in their mid-forties. With this information, a tentative diagnosis of familial hypercholesterolemia, type IIA was made, and the patient was started on a step I diet as recommended by the National Cholesterol Education Program (NCEP) Adult Treatment Panel III. This panel recommends that decisions with regard to when dietary and drug therapy are initiated based on the serum LDL cholesterol level, as depicted in Table 34.1.

Because a Step I diet (Table 34.2) usually lowers serum total and LDL cholesterol levels by no more than 15%, it is likely that Ms. Jeina's diet will eventually have to be further restricted in cholesterol and fat and that one or more lipid-lowering drugs will have to be added to her treatment plan.

Vera Leizd is a 34-year-old woman in whom pubertal changes began at age 12, leading to the development of normal secondary sexual characteristics and the onset of menses at age 13. Her menstrual periods occurred on a monthly basis over the next 7 years, but the flow was scant. At age 20, she noted a gradual increase in the intermenstrual interval from her normal of 28 days to 32 to 38 days. The volume of her menstrual flow also gradually diminished. After 7 months, her menstrual periods ceased. She complained of increasing oiliness of her skin, the appearance of acnelike lesions on her face and upper back, and the appearance of short dark terminal hairs on the mustache and sideburn areas of her face. The amount of extremity hair also increased, and she noticed a disturbing loss of hair from her scalp.

#### INTESTINAL ABSORPTION OF CHOLESTEROL I.

Cholesterol absorption by intestinal cells is a key regulatory point in human sterol metabolism because it ultimately determines what percentage of the 1,000 mg of biliary cholesterol produced by the liver each day and what percentage of the

Risk Category	LDL Goal (mg/dL)	LDL level at which to initiate therapeutic lifestyle changes (mg/dL)	LDL level at which to consider drug therapy (mg/dL)
CHD or CHD risk equivalents (10-year risk >20%)	<100	≥100	≥130 (100 -129: drug optional)
2+ Risk factors (10-year risk ≤20%)	<130	≥130	10-Year risk 10%-20%: ≥130 10-year risk <10%: ≥160

Table 34.1. ATP III: LDL-C Goals and Cut Points for Therapy in Different Risk Categories

LDL-C = low-density lipoprotein cholesterol; CHD = coronary heart disease.

<160

0-1 risk factor

Source: Executive summary of the third report of the National Cholesterol Education Programs (NCEP) Expert panel on detection, evaluation, and treatment of high blood cholesterol in Adults (Adult Treatment Panel III). Final Report, Circulation 2002;106:3145-3457.

>160

>190

drug optional)

(160-189: LDL-lowering

Until recently, the concentration of LDL cholesterol could only be directly determined by sophisticated laboratory techniques not available for routine clinical use. As a consequence, the LDL cholesterol concentration in the blood was derived indirectly by using the Friedewald formula: the sum of the HDL cholesterol level and the triacylglycerol (TG) level divided by 5 (which gives an estimate of the VLDL cholesterol level) subtracted from the total cholesterol level.

#### LDL cholesterol =

Total cholesterol - [HDL cholesterol + (TG/5)]

This equation yields inaccurate LDL cholesterol levels 15 to 20% of the time and fails completely when serum triacylglycerol levels exceed 400 mg/dL.

A recently developed test called "LDL direct" isolates LDL cholesterol by using a special immunoseparation reagent. Not only is this direct assay for LDL cholesterol more accurate than the indirect Friedewald calculation, it also is not affected by mildly to moderately elevated serum triacylglycerol levels and can be used for a patient who has not fasted. It does not require the expense of determining serum total cholesterol, HDL cholesterol, and triacylglycerol levels.

Nutrient	Step I Diet	Step II Diet <sup>a</sup>	
Cholesterol <sup>b</sup>	<300 mg/day	<200 mg/day	
Total fat	≤30% <sup>b</sup>	30%	
Saturated fat	8–10%	<7%	
Polyunsaturated fat	≤10%	≤10%	
Monounsaturated fat	≤15%	≤15%	
Carbohydrates	≥55%	≥55%	
Protein	~15%	~15%	
Calories	To achieve and maintain desirable body weight		

Table 34.2: Dietary Therapy of Elevated Blood Cholesterol

Based on: NCEP. Second Report of the Adult Treatment Panel, JAMA, 1993;269(23):3015–3023. <sup>a</sup>The Step II diet is applied if 3 months on the Step I diet has failed to reduce blood cholesterol to the desired level (see Table 34.1).

<sup>b</sup>Except for the values given in mg/day, all the values are percentage of total calories eaten daily.

300 mg of dietary cholesterol entering the gut per day is eventually absorbed into the blood. In normal subjects, approximately 55% of this intestinal pool enters the blood through the enterocyte each day. The details of cholesterol absorption from dietary sources was outlined in Chapter 32.

Although the absorption of cholesterol from the intestinal lumen is a diffusioncontrolled process, there is also a mechanism to remove unwanted or excessive cholesterol and plant sterols from the enterocyte. The transport of sterols out of the enterocyte, and into the lumen, is related to the products of genes that code for the adenosine triphosphate (ATP)-binding cassette (ABC) protein family, ABC1, ABCG5, and ABCG8. These proteins couple ATP hydrolysis to the transport of unwanted or excessive cholesterol and plant sterols (phytosterols) from the enterocyte back into the gut lumen. Cholesterol cannot be metabolized to  $CO_2$  and water and is, therefore, principally eliminated from the body in the feces as unreabsorbed sterols and bile acids. ABC protein expression increases the amount of sterols present in the gut lumen, with the potential to increase elimination of the sterols into the feces. Patients with a condition known as phytosterolemia (a rare autosomal recessive disease, also known as sitosterolemia) have a defect in the function of either ABCG5 or ABCG8 in the enterocytes, thereby leading to the accumulation of cholesterol and phytosterols within these cells. These eventually reach the bloodstream, markedly elevating the level of cholesterol and phytosterol in the blood. This accounts for the increased cardiovascular morbidity in individuals with this disorder. From these experiments of nature, it is clear that agents that either amplify the expression of the ABC proteins within enterocytes, or block cholesterol absorption from the lumen, have therapeutic potential in the treatment of patients with hypercholesterolemia. Ezetimibe, now available for clinical use, is a compound that is structurally different from the sterols. Its primary action in lowering serum cholesterol levels is to block cholesterol absorption through a specific but as yet poorly characterized cholesterol absorption mechanism in the brush border of enterocytes. It also may induce ABC protein expression, but this action is relatively unimportant in reducing net cholesterol absorption. The reduction of cholesterol absorption from the intestinal lumen has been shown to reduce blood levels of LDL cholesterol.



**Fig. 34.1**. The basic ring structure of sterols; the perhydrocyclopentanophenanthrene nucleus. Each ring is labeled either A, B, C, or D.

## II. CHOLESTEROL SYNTHESIS

Cholesterol is an alicyclic compound whose basic structure includes the perhydrocyclopentanophenanthrene nucleus containing four fused rings (Figure 34.1). In its "free" form, the cholesterol molecule contains 27 carbon atoms, a simple hydroxyl group at C3, a double bond between C5 and C6, an eight-membered hydrocarbon chain attached to carbon 17 in the D ring, a methyl group (carbon 19) attached to carbon 10, and a second methyl group (carbon 18) attached to carbon 13 (Figure 34.2).



Fig. 34.2. The structure of cholesterol.

Approximately one third of plasma cholesterol exists in the free (or unesterified) form. The remaining two thirds exists as cholesterol esters in which a long-chain fatty acid (usually linoleic acid) is attached by ester linkage to the hydroxyl group at C-3 of the A ring. The proportions of free and esterified cholesterol in the blood can be measured using methods such as high-performance liquid chromatography (HPLC).

The structure of cholesterol suggests that its synthesis involves multimolecular interactions; yet all of the 27 carbons are derived from one precursor, acetyl CoA. Acetyl CoA can be obtained from several sources, including the beta oxidation of fatty acids, the oxidation of ketogenic amino acids, such as leucine and lysine, and the pyruvate dehydrogenase reaction. Carbons 1, 2, 5, 7, 9, 13, 15, 18, 19, 20, 22, 24, 26, and 27 of cholesterol are derived from the methyl group of acetyl CoA and the remaining 12 carbons of cholesterol from the carboxylate atom of acetyl CoA.

The synthesis of cholesterol requires significant reducing power, which is supplied in the form of NADPH. The latter is provided by glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase of the hexose monophosphate shunt pathway (see Chapter 29). Cholesterol synthesis occurs in the cytosol, requiring hydrolysis of high-energy thioester bonds of acetyl CoA and phosphoanhydride bonds of ATP. Its synthesis occurs in four stages.

### A. Stage 1: Synthesis of Mevalonate from Acetyl CoA

The first stage of cholesterol synthesis leads to the production of the intermediate mevalonate (Fig. 34.3). *The synthesis of mevalonate is the committed, rate-limiting step in cholesterol formation*. In this cytoplasmic pathway, two molecules of acetyl CoA condense, forming acetoacetyl CoA, which then condenses with a third molecule of acetyl CoA to yield the 6-carbon compound  $\beta$ -hydroxy- $\beta$ -methylglutaryl-CoA (HMG-CoA). The HMG-CoA synthase in this reaction is present in the cytosol and is distinct from the mitochondrial HMG-CoA synthase that catalyses HMG-CoA synthesis involved in ketone body production. *The committed step and major point of regulation of cholesterol synthesis in stage 1 involves reduction of HMG-CoA* reductase, an enzyme embedded in the membrane of the endoplasmic reticulum. HMG-CoA reductase contains eight membrane-spanning domains, and the amino terminal domain, which faces the cytoplasm, contains the enzymatic activity. The regulation of the activity of HMG-CoA reductase is controlled in multiple ways.

#### 1. TRANSCRIPTIONAL CONTROL

The rate of synthesis of HMG-CoA reductase messenger RNA (mRNA) is controlled by one of the family of sterol regulatory element binding proteins (SREBPs)(Fig. 34.4A). These transcription factors belong to the helix-loop-helix-



**Fig. 34.3**. The conversion of three molecules of acetyl-CoA to mevalonic acid.

Ann Jeina's serum total and LDL cholesterol levels improved only modestly after 3 months on a Step I diet. Three additional months on a more severe low-fat diet (Step II diet) brought little further improvement. The next therapeutic step would be to initiate lipid-lowering drug therapy (see Table 34.5).



**Fig. 34.4**. Regulation of HMG-CoA reductase activity. See text for details. A. Transcriptional control. B. Regulation by proteolysis. C. Regulation by phosphorylation.

leucine zipper (bHlH-Zip) family of transcription factors that directly activate the expression of more than 30 genes dedicated to the synthesis and uptake of cholesterol, fatty acids, triacylglycerols, and phospholipids as well as the production of the NADPH cofactors required to synthesize these molecules.

SREBP1-a specifically enhances transcription of genes required for HMG-CoA reductase expression by binding to the sterol regulatory element (SRE) upstream of the reductase gene. When bound, the rate of transcription is increased. SREBPs, after synthesis, are integral ER proteins, and the active component of the protein is released by two proteases, SCAP (SREBP cleavage-activating protein) and S2P (site 2 protease). Once released, the active amino terminal component travels to the nucleus to bind to SREs. The soluble SREBPs are rapidly turned over and need to be continuously produced to effectively stimulate reductase mRNA transcription. When cytoplasmic sterol levels rise, the sterols bind to SCAP and inactivate it, thereby leading to a decrease in transcription of the reductase gene, and less reductase protein being produced.

#### 2. PROTEOLYTIC DEGRADATION OF HMG-CoA REDUCTASE

Rising levels of cholesterol and bile salts in cells that synthesize these molecules also may cause a change in the oligomerization state of the membrane domain of HMG-CoA reductase, rendering the enzyme more susceptible to proteolysis (see Fig. 34.4B). This, in turn, decreases its activity. The membrane domains of HMG-CoA reductase contain sterol-sensing regions, which are similar to those in SCAP.

#### 3. REGULATION BY COVALENT MODIFICATION

In addition to the inductive and repressive influences cited above, the activity of the reductase is also regulated by phosphorylation and dephosphorylation (see Fig. 34.4C). Elevated glucagon levels increase phosphorylation of the enzyme, thereby inactivating it, whereas hyperinsulinemia increases the activity of the reductase by activating phosphatases, which dephosphorylate the reductase. Increased levels of intracellular sterols also may increase phosphorylation of HMG-CoA reductase, thereby reducing its activity as well (feedback suppression). Thyroid hormone also increases enzyme activity, whereas glucocorticoids decrease its activity. The enzyme that phosphorylates HMG-CoA reductase is the adenosine monophosphate (AMP)-activated protein kinase, which itself is regulated by phosphorylation by the AMP-activated protein kinase kinase. Thus, cholesterol synthesis decreases when ATP levels are low and increases when ATP levels are high. This will become very clear once the further reactions of the biosynthetic pathway of cholesterol are discussed.

## B. Stage 2: Conversion of Mevalonate to Two Activated Isoprenes

In the second stage of cholesterol synthesis, three phosphate groups are transferred from three molecules of ATP to mevalonate (Fig. 34.5). The purpose of these phosphate transfers is to activate both carbon 5 and the hydroxyl group on carbon 3 for further reactions in which these groups will leave the molecule. The phosphate group attached to the C-3 hydroxyl group of mevalonate in the 3phospho-5-pyrophosphomevalonate intermediate is removed along with the carboxyl group on C-1. This produces a double bond in the 5-carbon product,  $\Delta^3$ isopentenyl pyrophosphate, the first of two activated isoprenes necessary for the synthesis of cholesterol. The second activated isoprene is formed when  $\Delta^3$ isopentenyl pyrophosphate is isomerized to dimethylallyl pyrophosphate (see Fig. 34.5).

## C. Stage 3: Condensation of Six Activated 5-Carbon Isoprenes to Form the 30-Carbon Squalene

The next stage in the biosynthesis of cholesterol involves the head-to-tail condensation of isopentenylpyrophosphate and dimethylallyl pyrophosphate. In this reaction, one pyrophosphate group is displaced, and a 10-carbon chain, known as geranyl pyrophosphate, is generated (Fig. 34.6). (The "head" refers to the end to which pyrophosphate is joined.) Geranyl pyrophosphate then undergoes another head-totail condensation with isopentenyl pyrophosphate, resulting in the formation of the 15-carbon intermediate, farnesyl pyrophosphate. After this, two molecules of farnesyl pyrophosphate undergo a head-to-head fusion, and both pyrophosphate groups are removed to form squalene, a compound first isolated from the liver of sharks (genus Squalus). Squalene contains 30 carbons (24 in the main chain and 6 in the methyl group branches; see Fig. 34.6).

Farnesyl and geranyl groups can form covalent bonds with proteins, particularly the G proteins and certain protooncogene products involved in signal transduction. These hydrophobic groups anchor the proteins in the cell membrane.





Fig. 34.5. The formation of activated isoprene units ( $\Delta^3$ -isopentenyl pyrophosphate and dimethylallyl pyrophosphate) from mevalonic acid. Note the large ATP requirement for these steps.

## D. Stage 4: Conversion of Squalene to the Four-Ring **Steroid Nucleus**

The enzyme squalene monooxygenase adds a single oxygen atom from  $O_2$  to the end of the squalene molecule, forming an epoxide. NADPH then reduces the other oxygen atom of O2 to H2O. The unsaturated carbons of the squalene 2, 3- epoxide are aligned in a way that allows conversion of the linear squalene epoxide into a cyclic structure. The cyclization leads to the formation of lanosterol, a sterol with the four-ring structure characteristic of the steroid nucleus. A series of complex



**Fig. 34.6**. The formation of squalene from six isoprene units. The activation of the isoprene units drives their condensation to form geranyl pyrophosphate, farnesyl pyrophosphate, and squalene.

reactions, containing many steps and elucidated in the late 1950s, leads to the formation of cholesterol (Fig. 34.7).

#### **III. SEVERAL FATES OF CHOLESTEROL**

Almost all mammalian cells are capable of producing cholesterol. Most of the biosynthesis of cholesterol, however, occurs within liver cells, although the gut, the adrenal cortex, and the gonads (as well as the placenta in pregnant women) also produce significant quantities of the sterol. Although a fraction of hepatic cholesterol is used for the synthesis of hepatic membranes, the bulk of synthesized cholesterol is secreted from the hepatocyte as one of three moieties: cholesterol esters, biliary cholesterol, or bile acids. Cholesterol ester production in the liver is catalyzed by acyl-CoA-cholesterol acyl transferase (ACAT). ACAT catalyzes the transfer of a fatty acid from coenzyme A to the hydroxyl group on carbon 3 of cholesterol (Fig. 34.8). Cholesterol esters are more hydrophobic than is free cholesterol. The liver packages some of the esterified cholesterol into the hollow core of lipoproteins, primarily VLDL. VLDL is secreted from the hepatocyte into the blood and transports the cholesterol esters (and triacylglycerols, phospholipids, apoproteins, etc.) to the tissues that require greater amounts of cholesterol than they can synthesize de novo. These tissues then use the cholesterol for the synthesis of membranes,



**Fig. 34.7**. The conversion of squalene to cholesterol. Squalene is shown in a different conformation than that of Fig. 34.6 to better indicate how the cyclization reaction occurs.



**Fig. 34.8.** The ACAT reaction, producing cholesterol esters. ACAT = acyl-CoA:cholesterol acyl transferase.

for the formation of steroid hormones, and for the biosynthesis of vitamin D. The residual cholesterol esters not used in these ways are stored in the liver for later use. The hepatic cholesterol pool also serves as a source of cholesterol for the synthesis of the relatively hydrophilic bile acids and their salts. These derivatives of cholesterol are highly effective detergents because they contain both polar and nonpolar regions. They are introduced in the biliary ducts of the liver. They are stored and concentrated in the gallbladder and later discharged into the gut in response to the ingestion of food. They aid in the digestion of intraluminal lipids by forming micelles with them, which increases the surface area of lipids exposed to the digestive action of intraluminal lipases. Free cholesterol also enters the gut lumen via the biliary tract (approximately 1,000 mg daily, which mixes with 300 mg dietary cholesterol to form an intestinal pool, roughly 55% of which is resorbed by the enterocytes and enters the bloodstream daily). On a low-cholesterol diet, the liver will synthesize approximately 800 mg cholesterol per day to replace bile salts and cholesterol lost from the enterohepatic circulation into the feces. Conversely, a greater intake of dietary cholesterol suppresses the rate of hepatic cholesterol synthesis (feedback repression).

## **IV. SYNTHESIS OF BILE SALTS**

## A. Conversion of Cholesterol to Cholic Acid and Chenocholic Acid

Bile salts are synthesized in the liver from cholesterol by reactions that hydroxylate the steroid nucleus and cleave the side chain. In the first reaction, an  $\alpha$ hydroxyl group is added to carbon 7 (on the  $\alpha$  side of the B ring). The activity of the 7  $\alpha$ -hydroxylase that catalyzes this rate-limiting step is decreased by bile salts (Fig. 34.9).

In subsequent steps, the double bond in the B ring is reduced, and an additional hydroxylation may occur. Two different sets of compounds are produced. One set has  $\alpha$ -hydroxyl groups at positions 3, 7, and 12, and produces the cholic acid series



**Fig. 34.9**. The reaction catalyzed by  $7\alpha$ -hydroxylase. An  $\alpha$ -hydroxyl group is formed at position 7 of cholesterol. This reaction, which is inhibited by bile salts, is the rate-limiting step in bile salt synthesis.



**Fig. 34.10**. Synthesis of bile salts. Two sets of bile salts are generated; one with  $\alpha$ -hydroxyl groups at positions 3 and 7 (the chenocholate series), and the other with  $\alpha$ -hydroxyls at positions 3, 7 and 12 (the cholate series).

of bile salts. The other set has  $\alpha$ -hydroxyl groups only at positions 3 and 7 and produces the chenocholic acid series (Fig. 34.10). Three carbons are removed from the side chain by an oxidation reaction. The remaining 5-carbon fragment attached to the ring structure contains a carboxyl group (see Fig. 34.10).

The pK of the bile acids is approximately 6. Therefore, in the contents of the intestinal lumen, which normally have a pH of 6, approximately 50% of the molecules are present in the protonated form, and 50% are ionized, which forms bile salts. (The terms *bile acids* and *bile salts* are often used interchangeably, but *bile salts* actually refer to the ionized form of the molecule.)

## **B.** Conjugation of Bile Salts

The carboxyl group at the end of the side chain of the bile salts is activated by a reaction that requires ATP and coenzyme A (CoA). The CoA derivatives can react with either glycine or taurine (which is derived from cysteine), forming amides that are known as the conjugated bile salts. In glycocholic acid and glycochenocholic acid, the bile acids are conjugated with glycine. These compounds have a pK of approximately 4, so compared to their unconjugated forms, a higher percentage of the molecules is present in the ionized form at the pH of the intestine. The taurine conjugates, taurocholic and taurochenocholic acid, have a pK of approximately 2. Therefore, compared with the glycoconjugates, an even greater percentage of the molecules of these conjugates are ionized in the lumen of the gut (Fig. 34.11).

#### V. FATE OF THE BILE SALTS

The bile salts are produced in the liver and secreted into the bile (Fig. 34.12), They are stored in the gallbladder and released into the intestine during a meal, where they serve as detergents that aid in the digestion of dietary lipids (see Chapter 32).

Intestinal bacteria deconjugate and dehydroxylate the bile salts, removing the glycine and taurine residues and the hydroxyl group at position 7. The bile salts that lack a hydroxyl group at position 7 are called secondary bile salts. The deconjugated and dehydroxylated bile salts are less soluble and, therefore, less readily resorbed from the intestinal lumen than the bile salts that have not been subjected to bacterial



Fig. 34.11. Conjugation of bile salts. Conjugation lowers the pK of the bile salts, making them better detergents; i.e., they are more ionized in the contents of the intestinal lumen (pH  $\approx$  6) than are the unconjugated bile salts (pK  $\approx$  6). The reactions are the same for the chenocholic acid series of bile salts.



Fig. 34.12. Overview of bile salt metabolism.

action (Fig. 34.13). Lithocholic acid, a secondary bile salt that has a hydroxyl group only at position 3, is the least soluble bile salt. Its major fate is excretion.

Greater than 95% of the bile salts are resorbed in the ileum and return to the liver via the enterohepatic circulation (via the portal vein; see Fig. 34.12). The secondary bile salts may be reconjugated in the liver, but they are not rehydroxylated. The bile salts are recycled by the liver, which secretes them into the bile. This enterohepatic recirculation of bile salts is extremely efficient. Less than 5% of the bile salts entering the gut are excreted in the feces each day. Because the steroid nucleus cannot be degraded in the body, the excretion of bile salts serves as a major route for removal of the steroid nucleus and, thus, of cholesterol from the body.

## VI. TRANSPORT OF CHOLESTEROL BY THE BLOOD LIPOPROTEINS

Because they are hydrophobic and essentially insoluble in the water of the blood, cholesterol and cholesterol esters, like triacylglycerols and phospholipids, must be transported through the bloodstream packaged as lipoproteins. These macromolecules are water-soluble. Each lipoprotein particle is composed of a core of hydrophobic lipids such as cholesterol esters and triacylglycerols surrounded by a shell of polar lipids (the phospholipids), which allows a hydration shell to form around the lipoprotein (see Fig. 32.9). This occurs when the positive charge of the nitrogen atom of the phospholipid (phosphatidylcholine, phosphatidylethanolamine, or phosphatidylserine) forms an ionic bond with the negatively charged hydroxyl ion of the environment. In addition, the shell contains a variety of apoproteins that also increase the water solubility of the lipoprotein. Free cholesterol molecules are dispersed throughout the lipoprotein shell to stabilize it in a way that allows it to maintain its spherical shape. The major carriers of lipids are chylomicrons (see Chapter 32), VLDL, and HDL. Metabolism of VLDL will lead to IDL and LDL. Metabolism of chylomicrons leads to chylomicron remnant formation.

Through this carrier mechanism, lipids leave their tissue of origin, enter the bloodstream, and are transported to the tissues, where their components will be either used in synthetic or oxidative process or stored for later use. The apoproteins ("apo" describes the protein within the shell of the particle in its lipid-free form) not only add to the hydrophilicity and structural stability of the particle but have other functions as well: (1) they activate certain enzymes required for normal lipoprotein metabolism and (2) they act as ligands on the surface of the lipoprotein that target specific receptors on peripheral tissues that require lipoprotein delivery for their innate cellular function.

Ten principal apoproteins have been characterized. Their tissue source, molecular mass, distribution within lipoproteins, and metabolic functions are shown in Table 34.3.

The lipoproteins themselves are distributed among eight major classes. Some of their characteristics are shown in Table 34.4. Each class of lipoprotein has a specific function determined by its apolipoprotein content, its tissue of origin, and the proportion of the macromolecule made up of triacylglycerols, cholesterol esters, free cholesterol, and phospholipids (see Tables 34.3 and 34.4).

## A. The Chylomicrons

Chylomicrons are the largest of the lipoproteins and the least dense because of their rich triacylglycerol content. They are synthesized from dietary lipids (the "exogenous" lipoprotein pathway) within the epithelial cells of the small intestine and then secreted into the lymphatic vessels draining the gut (see Fig. 32.13). They enter the bloodstream via the left subclavian vein. The major apoproteins of chylomicrons are apoB-48, apoC<sub>II</sub>, and apoE (see Table 34.3). The apoC<sub>II</sub> activates lipoprotein lipase



**Fig. 34.13**. Structures of the primary and secondary bile salts. Primary bile salts form conjugates with taurine or glycine in the liver. After secretion into the intestine, they may be deconjugated and dehydroxylated by the bacterial flora, forming secondary bile salts. Note that dehydroxylation occurs at position 7, forming the deoxy family of bile salts.

Apoprotein	Primary Tissue Source	Molecular Mass (Daltons)	Lipoprotein Distribution	Metabolic Function
ApoA-1 ApoA-II	Intestine, liver Liver	28,016 17,414	HDL (chylomicrons) HDL (chylomicrons)	Activates LCAT; structural component of HDL Unknown
ApoA-IV	Intestine	46,465	HDL (chylomicrons)	Unknown (may facilitate transport of other apoproteins between HDL and chylomicrons)
ApoB-48	Intestine	264,000	Chylomicrons	Assembly and secretion of chylomicrons from small bowel
ApoB-100	Liver	540,000	VLDL, IDL, LDL	VLDL assembly and secretion structured protein of VLDL, IDL, and LDL ligand for LDL receptor
ApoC-1	Liver	6,630	Chylomicrons, VLDL, IDL, HDL	Unknown; may inhibit hepatic uptake of chylomicron and VLDL remnants
ApoC-II	Liver	8,900	Chylomicrons, VLDL, IDL, HDL	Cofactor activator of lipoprotein lipase (LPL)
ApoC-III	Liver	8,800	Chylomicrons, VLDL, IDL, HDL	Inhibitor of LPL; may inhibit hepatic uptake of chylomicrons and VLDL remnants
АроЕ	Liver	34,145	Chylomicron remnants, VLDL, IDL, HDL	Ligand for binding of several lipoproteins to the LDL receptor, to the LDL receptor-related protein (LRP) and possibly to a separate apo-E receptor.
Apo(a)	Liver		Lipoprotein "little" a (Lp(a))	Unknown

(LPL), an enzyme that projects into the lumen of capillaries in adipose tissue, cardiac muscle, skeletal muscle, and the acinar cells of mammary tissue. This activation allows LPL to hydrolyze the chylomicrons, leading to the release of free fatty acids derived from core triacylglycerides of the lipoprotein into these target cells. The muscle cells then oxidize the fatty acids as fuel while the adipocytes and mammary cells store them as triacylglycerols (fat) or, in the case of the lactating breast, use them for milk formation. The partially hydrolyzed chylomicrons remaining in the bloodstream (the **chylomicron remnants**), now partly depleted of their core triacylglycerols, retain their apoE and apoB48 proteins. Receptors in the plasma membranes of the liver cells bind to apoE on the surface of these remnants, allowing them to be taken up by the liver through a process of receptor-mediated endocytosis (see below).

### **B.** Very-Low-Density Lipoproteins (VLDL)

If dietary intake of fatty acids exceeds the immediate fuel requirements of the liver, the excess fatty acids are converted to triacylglycerols, which, along with free and esterified cholesterol, phospholipids, and a variety of apoproteins (see Table 34.3),

#### Table 34.4. CHARACTERISTICS OF THE MAJOR LIPOPROTEINS

	Density	Particle					
	Range	Diameter	Electrophoretic		Lipid (%)*		
Lipoprotein	(g/mL)	(MM) range	Mobility	TG	Chol	PL	Function
Chylomicrons Chylomicron remnants	0.930 0.930–1.006	75–1200 30–80	Origin Slow pre $\beta$	80–95	2–7	3–9	Deliver dietary lipids Return dietary lipids to the liver
VLDL IDL	0.930–1.006 1.006–1.019	30–80 25–35	Pre $\beta$ Slow pre $\beta$	55–80 20–50	5–15 20–40	10–20 15–25	Deliver endogenous lipids Return endogenous lipids to the liver; precursor of LDL
LDL	1.019-1.063	18–25	β	5–15	40–50	20–25	Deliver cholesterol to cells
HDL <sub>2</sub> HDL <sub>3</sub> Lip(a)	1.063–1.125 1.125–1.210 1.050–1.120	9–12 5–9 25	α α Pre β	5–10	15–25	20–30	Reverse cholesterol transport Reverse cholesterol transport

\*The remaining percent composition is composed of apoproteins.

Abbreviations: TG, Triacylglycerols; Chol, the sum of free and esterified cholesterol; PL, phospholipid; VLDL = very-low-density lipoproteins; IDL, intermediate-density lipoproteins; LDL, low-density lipoproteins; HDL, high-density lipoproteins.

including apoB-100, apoC<sub>II</sub>, and apoE, are packaged to form VLDL. These particles are then secreted from the liver (the "endogenous" pathway of lipoprotein metabolism) into the bloodstream (Fig. 34.14). The density, particle size, and lipid content of VLDL particles are given in Table 34.3. These particles are then transported from the hepatic veins to capillaries in skeletal and cardiac muscle and adipose tissue, as well as lactating mammary tissues, where lipoprotein lipase is activated by apoC<sub>II</sub> in the VLDL particles. The activated enzyme facilitates the hydrolysis of the triacylglycerol in VLDL, causing the release of fatty acids and glycerol from a portion of core triacylglycerols. These fatty acids are oxidized as fuel by muscle cells, used in the resynthesis of triacylglycerols in fat cells, and used for milk production in the lactating breast. The residual particles remaining in the bloodstream are called **VLDL remnants**. Approximately 50% of these remnants are taken up from the blood by liver cells through the binding of VLDL apoE to the hepatocyte plasma membrane apoE receptor followed by endocytic internalization of the VLDL remnant.

## C. Intermediate-Density Lipoprotein (IDL) and Low-Density Lipoproteins (LDL)

Approximately half of the VLDL remnants are not taken up by the liver but, instead, have additional core triacylglycerols removed to form IDL, a specialized class of VLDL remnants. With the removal of additional triacylglycerols from IDL through the action of hepatic triglyceride lipase within hepatic sinusoids, LDL is generated from IDL. As seen in Table 34.4, the LDL particles are rich in cholesterol and cholesterol esters. Approximately 60% of the LDL is transported back to the liver, where its apoB-100 binds to specific apoB-100 receptors in the liver cell plasma membranes, allowing particles to be endocytosed into the hepatocyte. The remaining 40% of LDL particles are carried to extrahepatic tissues such as adrenocortical



**Fig. 34.14.** Fate of VLDL. VLDL triacylglycerol (TG) is degraded by LPL, forming IDL. IDL can either be endocytosed by the liver through a receptor-mediated process or further digested, mainly by hepatic triacylglycerol lipase (HTGL), to form LDL. LDL may be endocytosed by receptor-mediated processes in the liver or in peripheral cells. LDL also may be oxidized and taken up by "scavenger" receptors on macrophages. The scavenger pathway plays a role in atherosclerosis. FA = fatty acids; Pi = inorganic phosphate.

and gonadal cells that also contain apoB-100 receptors, allowing them to internalize the LDL particles and use their cholesterol for the synthesis of steroid hormones. Some of the cholesterol of the internalized LDL is used for membrane synthesis and vitamin D synthesis as well. If an excess of LDL particles is present in the blood, this specific receptor-mediated uptake of LDL by hepatic and nonhepatic tissue becomes saturated. The "excess" LDL particles are now more readily available for nonspecific uptake of LDL by macrophages (scavenger cells) present near the endothelial cells of arteries. This exposure of vascular endothelial cells to high levels of LDL is believed to induce an inflammatory response by these cells, a process suggested to initiate the complex cascade of atherosclerosis discussed below.

#### D. High-Density Lipoprotein (HDL)

The fourth class of lipoproteins is HDL, which plays several roles in whole body lipid metabolism.

#### 1. SYNTHESIS OF HDL

HDL particles can be created by a number of mechanisms. The first is synthesis of nascent HDL by the liver and intestine as a relatively small molecule whose shell, like that of other lipoproteins, contains phospholipids, free cholesterol, and a variety of apoproteins, predominant among which are apoA1, apoAII, apoC<sub>I</sub>, and  $apoC_{II}$  (see Table 34.3). Very low levels of triacylglycerols or cholesterol esters are found in the hollow core of this early, or nascent, version of HDL.

A second method for HDL generation is the budding of apoproteins from chylomicrons and VLDL particles as they are digested by lipoprotein lipase. The apoproteins (particularly AI) and shells can then accumulate more lipid, as described below.

A third method for HDL generation is free apoprotein AI, which may be shed from other circulating lipoproteins. AI will acquire cholesterol and phospholipids from other lipoproteins and cell membranes, to form a nascent-like HDL particle within the circulation.

#### 2. MATURATION OF NASCENT HDL

In the process of maturation, the nascent HDL particles accumulate phospholipids and cholesterol from cells lining the blood vessels. As the central hollow core of nascent HDL progressively fills with cholesterol esters, HDL takes on a more globular shape to eventually form the mature HDL particle. The transfer of lipids to nascent HDL does not require enzymatic activity.

#### 3. REVERSE CHOLESTEROL TRANSPORT

A major benefit of HDL particles derives from their ability to remove cholesterol from cholesterol-laden cells and to return the cholesterol to the liver, a process known as reverse cholesterol transport. This is particularly beneficial in vascular tissue; by reducing cellular cholesterol levels in the subintimal space, the likelihood that foam cells (lipid-laden macrophages that engulf oxidized LDL-cholesterol and represent an early stage in the development of atherosclerotic plaque) will form within the blood vessel wall is reduced.

Reverse cholesterol transport requires a directional movement of cholesterol from the cell to the lipoprotein particle. Cells contain the protein ABC1 (ATP-binding cassette protein 1) which uses ATP hydrolysis to move cholesterol from the inner leaflet of the membrane to the outer leaflet. Once the cholesterol has reached the outer membrane leaflet, the HDL particle can accept it, but if the cholesterol is not modified within the HDL particle, the cholesterol can leave the particle by the same route that it entered. To trap the cholesterol within the HDL core, the HDL particle acquires the enzyme LCAT from the circulation (LCAT is synthesized and secreted by the liver). LCAT catalyzes the transfer of a fatty acid from the 2-position of lecithin (phosphatidylcholine) in the phospholipid shell of the particle to the 3-hydroxyl group of cholesterol, forming a cholesterol ester (Fig. 34.15). The cholesterol ester migrates to the core of the HDL particle and is no longer free to return to the cell.

Elevated levels of lipoprotein-associated cholesterol in the blood, particularly that associated with LDL but also that in the more triacylglycerol-rich lipoproteins, are associated with the formation of cholesterol-rich atheromatous plaque in the vessel wall, eventually leading to diffuse atherosclerotic vascular disease resulting in acute cardiovascular events, such as a myocardial infarction, a stroke, or symptomatic peripheral vascular insufficiency. High levels of HDL in the blood, therefore, are believed to be vasculoprotective, because these high levels increase the rate of reverse cholesterol transport "away" from the blood vessels and "toward" the liver ("out of harm's way").

#### 4. FATE OF HDL CHOLESTEROL

Mature HDL particles can bind to specific receptors on hepatocytes (such as the apoE receptor), but the primary means of clearance of HDL from the blood is through its uptake by the scavenger receptor SR-B1. This receptor is present on many cell types. It does not carry out endocytosis per se, but once the HDL particle



Fig. 34.15. The reaction catalyzed by LCAT. R1 = saturated fatty acid. R2 = unsaturated fatty acid.

Two genetically determined disorders, familial HDL deficiency and Tangier disease, result from mutations in the ATP-binding cassette 1 (ABC 1) protein. Cholesterol-depleted HDL cannot transport free cholesterol from cells that lack the ability to express this protein. As a consequence, HDL is rapidly degraded. These disorders have established a role for ABC 1 protein in the regulation of HDL levels in the blood.

Because Ann Jeina continued to experience intermittent chest pain, in spite of good control of her hypertension and a 20-lb weight loss, her physician decided that a 2-drug regimen to lower her blood LDL cholesterol level must be added to the dietary measures already in place. Consequently, treatment with cholestyramine, a resin that binds some of the bile salts in the intestinal lumen, and the HMG-CoA reductase inhibitor pravastatin was initiated.



**Fig. 34.16**. Functions and fate of HDL. Nascent HDL is synthesized in liver and intestinal cells. It exchanges proteins with chylomicrons and VLDL. HDL picks up cholesterol (C) from cell membranes. This cholesterol is converted to cholesterol ester (CE) by the LCAT reaction. HDL transfers CE to VLDL in exchange for triacylglycerol (TG). The cholesterol ester transfer protein (CETP) mediates this exchange. PL = phospholipids.



**Fig. 34.17**. Function of cholesterol ester transfer protein (CETP). CETP transfers cholesterol esters (CE) from HDL to VLDL in exchange for triacylglycerol (TG).

is bound to the receptor, its cholesterol and cholesterol esters are transferred into the cells. When depleted of cholesterol and its esters, the HDL particle dissociates from the SR-B1 receptor and re-enters the circulation. SR-B1 receptors can be upregulated in certain cell types that require cholesterol for biosynthetic purposes, such as the cells that produce the steroid hormones. The SR-B1 receptors are not downregulated when cholesterol levels are high.

#### 5. HDL INTERACTIONS WITH OTHER PARTICLES

In addition to its ability to pick up cholesterol from cell membranes, HDL also exchanges apoproteins and lipids with other lipoproteins in the blood. For example, HDL transfers apolipoprotein E (apoE) and apolipoprotein CII (apo $C_{II}$ ) to chylomicrons and to VLDL. The apoC<sub>II</sub> stimulates the degradation of the triacylglycerols of chylomicrons and VLDL by activating LPL (Fig. 34.16). After digestion of the chylomicrons and the VLDL triacylglycerols, apoE and apoCII are transferred back to HDL. When HDL obtains free cholesterol from cell membranes, the free cholesterol is esterified at the third carbon of the A ring via the LCAT reaction (see Fig. 34.14). From this point, HDL either transports the free cholesterol and cholesterol esters directly to the liver, as described above, or by CETP to circulating triacylglycerol-rich lipoproteins such as VLDL and VLDL remnants (see Fig. 34.16). In exchange, triacylglycerols from the latter lipoproteins are transferred to HDL (Fig. 34.17). The greater the concentration of triacylglycerol-rich lipoproteins in the blood, the greater the rate of these exchanges. Thus, the CETP exchange pathway may explain the observation that whenever triacylglycerol-rich lipoproteins are present in the blood in high concentrations, the amount of cholesterol reaching the liver via cholesterol-enriched VLDL and VLDL remnants increases, and a proportional reduction in the total amount of cholesterol and cholesterol esters that are directly transferred to the liver via HDL occurs. Mature

HDL particles are designated as HDL<sub>3</sub>; after the CETP reaction and loss of cholesterol and gain of triacylglycerol, the particles become larger and are designated as HDL<sub>2</sub> particles (see Table 34.4).

## VII. LIPOPROTEINS ENTER CELLS BY RECEPTOR-MEDIATED ENDOCYTOSIS

As stated earlier, each lipoprotein particle contains specific apoproteins on its surface that act as ligands for specific plasma membrane receptors on target tissues such as the liver, the adrenal cortex, the gonads, and other cells that require one or more of the components of the lipoproteins. With the exception of the scavenger receptor SR-B1, the interaction of ligand and receptor initiates the process of endocytosis depicted for LDL in Figure 34.18. The receptors for LDL, for example, are found in specific areas of the plasma membrane of the target cell for circulating lipoproteins. These are known as coated pits, and they contain a unique protein called clathrin. The plasma membrane in the vicinity of the receptor-LDL complex invaginates and fuses to form an endocytic vesicle. These vesicles then fuse with lysosomes, acidic subcellular vesicles that contain a number of degradative enzymes. The cholesterol esters of LDL are hydrolyzed to form free cholesterol, which is rapidly reesterified through the action of ACAT. This rapid reesterification is necessary to avoid the damaging effect of high levels of free cholesterol on cellular membranes. The newly esterified cholesterol contains primarily oleate or palmitoleate (monounsaturated fatty acids), unlike those of the cholesterol esters in LDL, which are rich in linoleate, a polyunsaturated fatty acid.



Fig. 34.18. Cholesterol uptake by receptor-mediated endocytosis.

As is true for the synthesis and activity of HMG CoA reductase, the synthesis of the LDL receptor itself is subject to feedback inhibition by increasing levels of cholesterol within the cell. One probable mechanism for this feedback regulation involves one or more of the SREBP described earlier. These proteins or the cofactors that are required for the full expression of genes that code for the LDL receptor are also capable of sensing the concentration of sterols within the cell. When sterol levels are high, the process that leads to the binding of the SREBP to the sterol regulatory element of these genes is suppressed (see Fig. 34.4). The rate of synthesis from mRNA for the LDL receptor is diminished under these circumstances. This, in turn, appropriately reduces the amount of cholesterol that can enter these cholesterol-rich cells by receptor-mediated endocytosis (downregulation of receptor synthesis). When the intracellular levels of cholesterol decrease, these processes are reversed, and cells act to increase their cholesterol levels. Both synthesis of cholesterol from acetyl CoA and synthesis of LDL receptors are stimulated. An increased number of receptors (upregulation of receptor synthesis) results in an increased uptake of LDL cholesterol from the blood, with a subsequent reduction of LDL-cholesterol levels. At the same time, the cellular cholesterol pool is replenished.

## VIII. LIPOPROTEIN RECEPTORS

The best-characterized lipoprotein receptor, the LDL receptor, specifically recognizes apoB-100 and apo E. Therefore, this receptor binds VLDL, IDL, and chylomicron remnants in addition to LDL. The binding reaction is characterized by its saturability and occurs with high affinity and a narrow range of specificity. Other receptors, such as the LDL receptor-related proteins (LRP) and the macrophage scavenger receptor (notably types SR-A1 and SR-A2, which are located primarily near the endothelial surface of vascular endothelial cells), have broad specificity and bind many other ligands in addition to the blood lipoproteins.

## A. The LDL Receptor

The LDL receptor has a mosaic structure encoded by a gene that was assembled by a process known as exon shuffling. It is composed of six different regions (Fig. 34.19). The first region, at the amino terminus, contains the LDL-binding region, a cysteine-rich sequence of 40 residues. Acidic side chains in this region bind ionic calcium. When these side chains are protonated, calcium is released from its binding sites. This release leads to conformational changes that allow the LDL to disconnect from its receptor docking site. Disulfide bonds, formed from the cysteine residues, have a stabilizing influence on the structural integrity of this portion of the receptor.

The second region of the receptor contains domains that are homologous with epidermal growth factor (EGF) as well as a complex consisting of six repeats that resemble the blades of the transducin beta subunit forming a propeller-like moiety.

The third region of the LDL receptor contains a chain of N-linked oligosaccharides, whereas the fourth region contains a domain that is rich in serine and threonine and contains O-linked sugars. This region may have a role in physically extending the receptor away from the membrane so that the LDL-binding region is accessible to the LDL molecule.

The fifth region contains 22 hydrophobic residues constituting the membranespanning unit of the receptor, whereas the sixth region extends into the cytosol, where it regulates the interaction between the C-terminal domain of the LDL receptor and the clathrin-containing coated pit where the process of receptor-mediated endocytosis is initiated.

The number of LDL receptors, the binding of LDL to its receptors, and the postreceptor binding process can be diminished for a variety of reasons, all of which



Fig. 34.19. Structure of the LDL receptor. The protein has six major regions.

may lead to an accumulation of LDL cholesterol in the blood and premature atherosclerosis. These abnormalities can result from mutations in one (heterozygousseen in approximately 1 in 500 people) or both (homozygous-seen in about 1 in 1 million people) alleles for the LDL receptor (familial hypercholesterolemia). Heterozygotes produce approximately half of the normal complement of LDL receptors, whereas the homozygotes produce almost no LDL receptor protein (receptor negative familial hypercholesterolemia). The latter have serum total cholesterol levels in the range of 500 to 800 mg/dL. In a subset of patients with familial hypercholesterolemia, the LDL receptor is normally synthesized and transported to the cell surface, but an amino acid substitution or deletion leads to changes in the protein's structure such that LDL-binding is impaired. As a result, cholesterol does not enter the target cell from the bloodstream and, therefore, cannot feedback negatively on cholesterol biosynthesis in the cell. As a result, the serum cholesterol level rises. A third form of familial hypercholesterolemia involves a genetic defect in the transport or migration mechanism that normally delivers the LDL receptor from its point of synthesis within the cell to its proper location in that cell's plasma membrane. Genetic mutations can lead to yet another form of familial hypercholesterolemia in which a structural change occurs in the carboxy terminus of the LDL receptor.



**Ann Jeina's** blood lipid levels (in mg/dL) were:

Triacylglycerol	158
Total cholesterol	420
HDL cholesterol	32
LDL cholesterol	356

She was diagnosed as having familial hypercholesterolemia (FH) type IIA, which is caused by genetic defects in the gene that encodes the LDL receptor (see Biochemical Comments). As a result of the receptor defect, LDL cannot readily be taken up by cells, and its concentration in the blood is elevated.

LDL particles contain a high percentage, by weight, of cholesterol and cholesterol esters, more than other blood lipoproteins. However, LDL triacylglycerol levels are low because LDL is produced by digestion of the triacylglycerols of VLDL and IDL. Therefore, individuals with a type IIA hyperlipoproteinemia have very high blood cholesterol levels, but their levels of triacylglycerols may be in or near the normal range (see Table 34.4).



Ivan Applebod's blood lipid levels were:

Triacylglycerol	295
Total cholesterol	314
HDL cholesterol	24
LDL cholesterol	231

The elevated serum levels of LDL cholesterol found in patients such as Ivan Applebod who have type 2 diabetes mellitus is multifactorial. One of the mechanisms responsible for this increase involves the presence of chronically elevated levels of glucose in the blood of poorly controlled diabetics. This prolonged hyperglycemia increases the rate of nonenzymatic attachment of glucose to various proteins in the body, a process referred to as glycation or glycosylation of proteins.

Glycation may adversely affect the structure or the function of the protein involved. For example, glycation of the LDL receptor and of proteins in the LDL particle may interfere with the normal "fit" of LDL particles with their specific receptors. As a result, less circulating LDL is internalized into cells by receptormediated endocytosis, and the serum LDL cholesterol level rises.

Although such patients are able to place structurally normal LDL receptors in the plasma membrane of the cell, they are unable to internalize the LDL-LDL receptor complex because they cannot properly translocate the complex into the clathrincontaining coated pits. The spectrum of mutations of the LDL receptor gene is shown in Figure 34.20.

## B. LDL Receptor-Related Protein (LRP)

LRP is structurally related to the LDL receptor but recognizes a broader spectrum of ligands. In addition to lipoproteins, it binds the blood proteins  $\alpha_2$ -macroglobulin (a protein that inhibits blood proteases) and tissue plasminogen activator (TPA) and its inhibitors. The LRP receptor recognizes the apoE of lipoproteins and binds remnants produced by the digestion of the triacylglycerols of chylomicrons and VLDL by LPL. Thus, one of its functions is believed to be the clearance of these remnants from the blood. The LRP receptor is abundant in the cell membranes of the liver, brain, and placenta. In contrast to the LDL receptor, synthesis of the LRP receptor is not significantly affected by an increase in the intracellular concentration of cholesterol. However, insulin causes the number of these receptors on the cell surface to increase, consistent with the need for removal of chylomicron remnants that otherwise would accumulate after eating a meal.

#### C. Macrophage Scavenger Receptor

Some cells, particularly the phagocytic macrophages, have nonspecific receptors known as "scavenger" receptors that bind various types of molecules, including oxidatively modified LDL particles. There are a number of different types of scavenger receptors. SR-B1 is used primarily for HDL binding, whereas the scavenger receptors expressed on macrophages are SR-A1 and SR-A2. Modification of LDL frequently involves oxidative damage, particularly of polyunsaturated fatty acyl groups (see Chapter 24). In contrast to the LDL receptors, the scavenger receptors



Fig. 34.20. Location of 353 point mutations and small deletions/insertions (<25 bp) in the LDL receptor gene in individuals with familial hypercholesterolemia (FH). Exons are shown as vertical boxes and introns as the lines connecting them. The figure was obtained from Goldstein JL Hobbs HH, Brown MS. Familial hypercholesterolemia. In: Scriver CR, Beaudet AL, Sly WS, Valle D, et al., eds. The Metabolic and Molecular Bases of Inherited Disease. 8th Ed., vol III. New York: McGraw-Hill, 2001:2863-2913.

are not subject to downregulation. The continued presence of scavenger receptors in the cell membrane allows the cells to take up oxidatively modified LDL long after intracellular cholesterol levels are elevated. When the macrophages become engorged with lipid, they are called foam cells. An accumulation of these foam cells in the subendothelial space of blood vessels form the earliest gross evidence of a developing atherosclerotic plaque known as a fatty streak.

The processes that cause oxidation of LDL involve superoxide radicals, nitric oxide, hydrogen peroxide, and other oxidants (see Chapter 24). Antioxidants, such as vitamin E, ascorbic acid (vitamin C), and carotenoids, may be involved in protecting LDL from oxidation.

## IX. ANATOMIC AND BIOCHEMICAL ASPECTS OF ATHEROSCLEROSIS

The normal artery is composed of three distinct layers (Fig. 34.21). That which is closest to the lumen of the vessel, the intima, is lined by a monolayer of endothelial cells that are bathed by the circulating blood. Just beneath these specialized cells lies the subintimal extracellular matrix, in which some vascular smooth muscle cells are embedded (the subintimal space). The middle layer, known as the tunica media, is separated from the intima by the internal elastic lamina. The tunica media contains lamellae of smooth muscle cells surrounded by an elastin- and collagen-rich matrix. The external elastic lamina forms the border between the tunica media and the outermost layer, the adventitia. This layer contains nerve fibers and mast cells. It is the origin of the vasa vasorum, which supply blood to the outer two thirds of the tunica media.

The initial step in the development of an atherosclerotic lesion within the wall of an artery is the formation of a fatty streak. The latter represents an accumulation of lipid-ladened macrophages or foam cells in the subintimal space. These fatty streaks are visible as a yellow-white linear streak that bulges slightly into the lumen of the vessel. These streaks are initiated when one or more known "vascular risk factors for atherosclerosis," all of which have the potential to injure the vascular endothelial cells, reach a critical threshold at the site of future lesions. Examples of such risk factors include elevated intra-arterial pressure (arterial hypertension), elevated circulating levels of various lipids such as LDL, chylomicron remnants, and VLDL remnants, or low levels of circulating HDL, cigarette smoking, chronic elevations in blood glucose levels, high circulating levels of the vasoconstricting octapeptide angiotensin II, and others. The resulting insult to



Fig. 34.21. The different layers of the arterial wall.



In addition to dietary therapy, aimed at reducing her blood cholesterol levels, Ann Jeina was treated with

pravastatin, an HMG-CoA reductase inhibitor. The HMG-CoA reductase inhibitors decrease the rate of synthesis of cholesterol in cells. As cellular cholesterol levels decrease, the synthesis of LDL receptors increases. As the number of receptors rises on the cell surface, the uptake of LDL is increased. Consequently, the blood level of LDL cholesterol decreases.



HDL is considered to be the "good cholesterol," because it accepts free

cholesterol from peripheral tissues, such as cells in the walls of blood vessels. This cholesterol is converted to cholesterol ester, part of which is transferred to VLDL by CETP, and returned to the liver by IDL and LDL. The remainder of the cholesterol is transferred directly as part of the HDL molecule to the liver. The liver reutilizes the cholesterol in the synthesis of VLDL, converts it to bile salts, or excretes it directly into the bile. HDL therefore tends to lower blood cholesterol levels. Lower blood cholesterol levels correlate with a lower rates of death of atherosclerosis.



In patients such as Ann Jeina and Ivan Applebod, who have elevated levels of VLDL or LDL, HDL levels are often low. These patients are predisposed to atherosclerosis and suffer from a high inci-

dence of heart attacks and strokes.

Exercise and estrogen administration both increase HDL levels. This is one of the reasons exercise is often recommended to aid in the prevention or treatment of heart disease, and estrogen replacement therapy (ERT) is often prescribed for postmenopausal women. Before menopause, the incidence of heart attacks is relatively low in women, but it rises after menopause and increases to the level found in men by the age of 65 or 70 years. Moderate consumption of ethanol (alcohol) has also been correlated with increased HDL levels. Recent studies suggest that the beneficial amount of ethanol may be quite low, about two small glasses of wine a day, and that beneficial effects ascribed to ethanol may result from other components of wine and alcoholic beverages. In spite of the evidence that postmenopausal estrogen replacement therapy decreases circulating levels of LDL and increases HDL levels, recent data suggest that ERT may actually increase the rate of atherosclerotic vascular disease in these women. As a result, the accepted indications for ERT are now limited to intolerable "hot flashes" or vaginal dryness.

endothelial cells may trigger these cells to secrete adhesion molecules that bind to circulating monocytes and markedly slow their rate of movement past the endothelium. When sufficiently slowed, these monocytic cells accumulate and have access to the physical spaces that exist between endothelial cells. This accumulation of monocytic cells resembles the classical inflammatory response to injury. These changes have led to the suggestion that atherosclerosis is, in fact, an inflammatory disorder and, therefore, is one that might be prevented or attenuated through the use of anti-inflammatory agents such as acetylsalicylic acid (e.g., aspirin) and HMG CoA reductase inhibitors (statins), which have been shown to suppress the inflammatory cascade as well as to inhibit the action of HMG CoA reductase.

The monocytic cells are transformed into macrophages that migrate through the spaces between endothelial cells. They enter the subintimal space under the influence of chemoattractant cytokines (e.g., chemokine macrophage chemoattractant protein I) secreted by vascular cells in response to exposure to oxidatively modified fatty acids within the lipoproteins.

The macrophages can replicate and exhibit augmented expression of receptors that recognize oxidatively modified lipoproteins. Unlike the classic LDL receptors on liver and many nonhepatic cells, these macrophage-bound receptors are highcapacity, low-specificity receptors (scavenger receptors). They bind to and internalize oxidatively modified fatty acids within LDLs to become subintimal foam cells as described previously. As these foam cells accumulate, they deform the overlying endothelium, causing microscopic separations between endothelial cells, exposing these foam cells and underlying extracellular matrix to the blood. These exposed areas serve as sites for platelet adhesion and aggregation. Activated platelets secrete cytokines that perpetuate this process and increase the potential for thrombus (clot) formation locally. As the evolving plaque matures, a fibrous cap forms over its expanding "roof," which now bulges into the vascular lumen, thereby partially occluding it. Vascular smooth muscle cells now migrate from the tunica media to the subintimal space and secrete additional plaque matrix material. The smooth muscle cells also secrete metalloproteinases that thin the fibrous cap near its "elbow" at the periphery of the plaque. This thinning progresses until the fibrous cap ruptures, allowing the plaque contents to physically contact the procoagulant elements present within the circulation. This leads to acute thrombus formation. If this thrombus completely occludes the remaining lumen of the vessel, an infarction of tissues distal to the occlusion (i.e., an acute myocardial infarction) may occur (Fig. 34.22). Most plaques that rupture also contain focal areas of calcification, which appears to result from the induction of the same cluster of genes as those that promote the formation of bone. The inducers for this process include oxidized sterols as well as transforming growth factor beta (TGF- $\beta$ ) derived from certain vascular cells.

Finally, high intraluminal shear forces develop in these thinning or eroded areas of the plaque's fibrous cap, inducing macrophages to secrete additional metalloproteinases that further degrade the arterial-fibrous cap matrix. This contributes further to plaque rupture and thrombus formation (see Fig. 34.22). The consequence is a macrovascular ischemic event such as an acute myocardial infarction (AMI) or an acute cerebrovascular accident (CVA).

Lipoprotein(a) is essentially an LDL particle that is covalently bound to apoprotein(a). It is called "lipoprotein little a" to avoid confusion with the apoprotein A found in HDL. The structure of apoprotein(a) is very similar to that of plasminogen, a precursor of the protease plasmin that degrades fibrin, a major component of blood clots. Lipoprotein(a), however, cannot be converted to active plasmin. There are reports that high concentrations of lipoprotein(a) correlate with an increased risk of coronary artery disease, even in patients in whom the lipid profile is otherwise normal.



**Fig. 34.22.** Evolution of an atherosclerotic plaque. Plaque capsule eroded near the "elbow" of plaque creating an early plaque fissure (A), which may heal as plaque increases in size (B) or may grow as thrombus expands, having an intraluminal portion and an intraintimal portion (C). If the fissure is not properly sealed, the thrombus may grow and completely occlude the vessel lumen (D), causing an acute infarction of tissues downstream of the vessel occlusion.

#### X. STEROID HORMONES

Cholesterol is the precursor of all five classes of steroid hormones: glucocorticoids, mineralcorticoids, androgens, estrogens, and progestins. These hormones are synthesized in the adrenal cortex, ovaries, testes, and ovarian corpus luteum. Steroid hormones are transported through the blood from their sites of synthesis to their target organs, where, because of their hydrophobicity, they cross the cell membrane and bind to specific receptors in either the cytoplasm or nucleus. The bound receptors then bind to DNA to regulate gene transcription (see Chapter 16, section III.C.2, and Fig. 16.13). Because of their hydrophobicity, steroid hormones must be complexed with a serum protein. Serum albumin can act as a nonspecific carrier for the steroid hormones, but there are specific carriers as well. The cholesterol used for steroid hormone synthesis is either synthesized in the tissues from acetyl CoA, extracted from intracellular cholesterol ester pools, or taken up by the cell in the form of cholesterol-containing lipoproteins (either internalized by the LDL-receptor, or absorbed by the SR-B1 receptor). In general, glucocorticoids and progestins contain 21 carbons, androgens contain 19 carbons, and estrogens contain 18 carbons. The specific complement of enzymes present in the cells of an organ determines which hormones the organ can synthesize.

The oxidative reactions that lead to the synthesis and secretion of glucocorticoids such as cortisol are stimulated by adrenal corticotrophic hormone (ACTH). The role of cortisol as a stress-released hormone is discussed in Chapter 43.

Mineralocorticoids such as aldosterone are also synthesized in the adrenal cortex and are secreted in response to angiotensin II or III, rising potassium levels in the blood, or hyponatremia (low levels of sodium ions in the blood). Aldosterone stimulates sodium reuptake in the kidney, sweat glands, salivary glands, and other



#### Vera Leizd consulted her gynecologist, who confirmed that her problems were probably the result of an

excess production of androgens (virilization) and ordered blood and urine studies to determine whether Vera's adrenal cortices or her ovaries were causing her virilizing syndrome.

Cytochrome P450<sub>C11</sub>, another enzyme located in the mitochondrial membrane, catalyzes *β*-hydroxylation at C11. Hydroxylations at C17 and C21 are catalyzed by two enzymes located in the membranes of the endoplasmic reticulum (P450<sub>C17</sub> for 17α-hydroxylation and P450<sub>C21</sub> for 21-hydroxylation).

tissues, with a resultant increase in extracellular fluid volume and eventually in blood pressure. The angiotensins are produced in response to a reduction in extracellular fluid volume, which may occur as a result of such things as excessive sweating, persistent vomiting without sufficient rehydration, or bleeding without adequate replacement of blood.

Androgens such as testosterone are synthesized in the Leydig cells of the testes and to a lesser extent in the ovary and are secreted in response to luteinizing hormone (LH). In males, testosterone is commonly converted to dihydrotestosterone, a higher-affinity form of the hormone, within specific target tissues. This active form of the hormone stimulates the production of sperm proteins in Sertoli cells and the development of secondary sex characteristics.

Estrogens such as  $17-\beta$ -estradiol are synthesized in the ovarian follicle and the corpus luteum, from which their secretion is stimulated by follicle-stimulating hormone (FSH). In the female, 17  $\beta$ -estradiol feeds back negatively on the synthesis and secretion of the pituitary gonadotropins, such as FSH. Estrogen and progesterone prepare the uterine endometrium for implantation of the fertilized ovum, and among other actions promotes differentiation of the mammary gland.

Progestogens such as progesterone are synthesized in the corpus luteum, and their secretion is stimulated by LH. As mentioned, in concert with estradiol, progesterone prepares the uterine endometrium for implantation of the fertilized ovum and acts as a differentiation factor in mammary gland development.

The biosynthesis of glucocorticoids and mineralocorticoids (in the adrenal cortex), and that of sex steroids (in the adrenal cortex and gonads), requires four cytochrome P450 enzymes (see Chapter 24). These monooxygenases are involved in the transfer of electrons from NADPH through electron transfer protein intermediates to molecular oxygen, which then oxidizes a variety of the ring carbons of cholesterol.

Cholesterol is converted to progesterone in the first two steps of synthesis of all steroid hormones. Cytochrome P450<sub>SCC</sub> side-chain cleavage enzyme (previously referred to as cholesterol desmolase) is located in the mitochondrial inner membrane and removes six carbons from the side chain of cholesterol, forming pregnenolone, which has 21 carbons (Fig. 34.23). The next step, the conversion of pregnenolone to progesterone, is catalyzed by 3  $\beta$ -hydroxysteroid dehydrogenase, an enzyme that is not a member of the cytochrome P450 family. Other steroid hormones are produced from progesterone by reactions that involve members of the P450 family. As the synthesis of the steroid hormones is discussed, notice how certain enzymes are used in more than one pathway. Defects in such enzymes will lead to multiple abnormalities in steroid synthesis, which, in turn, results in a variety of abnormal phenotypes.

## A. Synthesis of Cortisol

The adrenocortical biosynthetic pathway that leads to cortisol synthesis occurs in the middle layer of the adrenal cortex known as the zona fasciculata. Free cholesterol is transported by an intracellular carrier protein to the inner mitochondrial membrane of cells (Fig. 34.24), where the side chain is cleaved to form pregnenolone. Pregnenolone returns to the cytosol, where it forms progesterone.

In the membranes of the endoplasmic reticulum, the enzyme  $P450_{C17}$  catalyzes the hydroxylation of C17 of progesterone or pregnenolone and can also catalyze the cleavage of the 2-carbon side chain of these compounds at C17 (a C17-C20 lyase activity). These two separate functions of the same enzyme allow further steroid synthesis to proceed along two separate pathways: the 17-hydroxylated steroids that retain their side chains are precursors of cortisol (C21), whereas those from which the side chain was cleaved (C19 steroids) are precursors of androgens (male sex hormones) and estrogens (female sex hormones).



**Fig. 34.23.** Synthesis of the steroid hormones. The rings of the precursor, cholesterol, are lettered. Dihydrotestosterone is produced from testosterone by reduction of the carbon–carbon double bond in ring A. Structural changes between the precursor and final hormone are noted in blue. DHEA = dehydroepiandrosterone. The dashed lines indicate alternative pathways to the major pathways indicated. The starred enzymes are those that may be defective in the condition congenital adrenal hyperplasia.



**Fig. 34.24.** Cellular route for cortisol synthesis. Cholesterol is synthesized from acetyl CoA or derived from low-density lipoprotein (LDL), which is endocytosed and digested by lyso-somal enzymes. Cholesterol is stored in cells of the adrenal cortex as cholesterol esters. ACTH signals the cell to convert cholesterol to cortisol. 1 = cholesterol desmolase (involved in side chain cleavage);  $2 = 3\beta$ -hydroxysteroid dehydrogenase;  $3 = 17\alpha$ -hydroxylase; 4 = 21-hydroxylase;  $5 = 11\beta$ -hydroxylase.

In the pathway of cortisol synthesis, the 17-hydroxylation of progesterone yields  $17-\alpha$ -hydroxyprogesterone, which, along with progesterone, is transported to the smooth endoplasmic reticulum. There the membrane-bound P450<sub>C21</sub> (21- $\alpha$ -hydrox-ylase) enzyme catalyzes the hydroxylation of C21 of 17- $\alpha$ -hydroxyprogesterone to form 11-deoxycortisol (and of progesterone to form deoxycorticosterone [DOC], a precursor of the mineralocorticoid, aldosterone; see Fig. 34.23).

The final step in cortisol synthesis requires transport of 11-deoxycortisol back to the inner membrane of the mitochondria, where  $P450_{C11}$  (11- $\beta$ -hydroxylase) receives electrons from electron transport protein intermediates (adrenodoxin, which when oxidized is reduced by adrenodoxin reductase). The enzyme then transfers these reducing equivalents by way of oxygen to 11-deoxycortisol for hydroxylation at C11 to form cortisol. The rate of biosynthesis of cortisol and other adrenal steroids is dependent on stimulation of the adrenal cortical cells by adrenocorticotropic hormone (ACTH).

#### **B.** Synthesis of Aldosterone

The synthesis of the potent mineralocorticoid aldosterone in the zona glomerulosa of the adrenal cortex also begins with the conversion of cholesterol to progesterone (see Figs. 34.23 and 34.24). Progesterone is then hydroxylated at C21, a reaction catalyzed by  $P450_{C21}$ , to yield DOC. The  $P450_{C11}$  enzyme system then catalyzes the reactions that convert DOC to corticosterone. The terminal steps in aldosterone synthesis, catalyzed by the P450 aldosterone system, involve the oxidation of corticosterone to 18-hydroxycorticosterone, which is oxidized to aldosterone.

The primary stimulus for aldosterone production is the octapeptide angiotensin II, although hyperkalemia (greater than normal levels of potassium in the blood) or hyponatremia (less than normal levels of sodium in the blood) may directly stimulate aldosterone synthesis as well. ACTH has a permissive action in aldosterone production. It allows cells to respond optimally to their primary stimulus, angiotensin II.

#### C. Synthesis of the Adrenal Androgens

Adrenal androgen biosynthesis proceeds from cleavage of the 2-carbon side chain of 17-hydroxypregnenolone at C17 to form the 19-carbon adrenal androgen dehydroepiandrosterone (DHEA) and its sulfate derivative (DHEAS) in the zona reticulosum of the adrenal cortex (see Fig. 34.23). These compounds, which are weak androgens, represent a significant percentage of the total steroid production by the normal adrenal cortex, and are the major androgens synthesized in the adrenal gland.

Androstenedione, another weak adrenal androgen, is produced when the 2-carbon side chain is cleaved from  $17\alpha$ -hydroxyprogesterone by the C17-C20 lyase activity of P450<sub>C17</sub>. This androgen is converted to testosterone primarily in extraadrenal tissues. Although the adrenal cortex makes very little estrogen, the weak adrenal androgens may be converted to estrogens in the peripheral tissues, particularly in adipose tissue (Fig. 34.25).

## D. Synthesis of Testosterone

Luteinizing hormone (LH) from the anterior pituitary stimulates the synthesis of testosterone and other androgens by the Leydig cells of the human testicle. In many ways, the pathways leading to androgen synthesis in the testicle are similar to those described for the adrenal cortex. In the human testicle, the predominant pathway leading to testosterone synthesis is through pregnenolone to 17- $\alpha$ -hydroxypregnenolone to DHEA (the  $\Delta^{5}$  pathway), and then from DHEA to androstenedione, and from androstenedione to testosterone (see Fig. 34.23). As for all steroids, the rate-limiting step in testosterone production is the conversion of cholesterol to pregnenolone. LH controls the rate of side-chain cleavage from cholesterol at carbon 21 to form pregnenolone, and thus regulates the rate of

Congenital adrenal hyperplasia (CAH) is a group of diseases caused by a genetically determined deficiency in a variety of enzymes required for cortisol synthesis. The most common deficiency is that of  $21-\alpha$  hydroxylase, the activity of which is necessary to convert progesterone to 11-deoxycorticosterone and  $17-\alpha$  hydroxy progesterone to 11-deoxycortisol. Thus, this deficiency reduces both aldosterone and cortisol production, without affecting androgen production. If the enzyme deficiency is severe, the precursors for aldosterone and cortisol production are shunted to androgen synthesis, producing an overabundance of androgens, which leads to prenatal masculinization in females and postnatal virilization of males. Another enzyme deficiency in this group of diseases is that of  $11-\beta$  hydroxylase, which results in the accumulation of 11-deoxycorticosterone. An excess of this mineralocorticoid leads to hypertension (through binding of 11deoxycorticosterone to the aldosterone receptor). In this form of CAH, 11-deoxycortisol also accumulates, but its biologic activity is minimal, and no specific clinical signs and symptoms result. The androgen pathway is unaffected, and the increased ACTH levels may increase the levels of adrenal androgens in the blood. A third possible enzyme deficiency is that of 17- $\alpha$  hydroxylase. A defect in 17- $\alpha$  hydroxylase leads to aldosterone excess and hypertension; however, because adrenal androgen synthesis requires this enzyme, no virilization occurs in these patients.

Hyperplasia or tumors of the adrenal cortex that produce excess aldosterone result in a condition known as primary aldosteronism, which is characterized by enhanced sodium and water retention, resulting in hypertension.

Although aldosterone is the major mineralocorticoid in humans, excessive production of a weaker mineralocorticoid, DOC, which occurs in patients with a deficiency of the 11-hydroxylase (the P450<sub>C11</sub> enzyme), may lead to clinical signs and symptoms of mineralocorticoid excess even though aldosterone secretion is suppressed in these patients.

Androstenedione can be purchased at health food stores under the name Andros. It is touted to improve athletic performance through its ability to be converted to testosterone. Its use has been banned by most major sports, although in 1998 it was a legal supplement in baseball. During that year, the drug received a lot of publicity, as the supplement had been used by a player who broke the major league home run record.



Fig. 34.25. Adrenal androgens. These weak androgens are converted to testosterone or estrogens in other tissues.



## Biologically, the most potent circulating androgen is testosterone. Approx-

imately 50% of the testosterone in the blood in a normal woman is produced equally in the ovaries and in the adrenal cortices. The remaining half is derived from ovarian and adrenal androstenedione, which, after secretion into the blood, is converted to testosterone in adipose tissue, muscle, liver, and skin. The adrenal cortex, however, is the major source of the relatively weak androgen dehydroepiandrosterone (DHEA). The serum concentration of its stable metabolite, DHEAS, is used as a measure of adrenal androgen production in hyperandrogenic patients with diffuse excessive growth of secondary sexual hair, e.g., facial hair as well as that in the axillae, the suprapubic area, the chest, and the upper extremities.



The results of the blood tests on Vera Leizd showed that her level of testosterone was normal but that her dehydroepiandrosterone sulfate serum (DHEAS) level was significantly elevated. Which tissue was the most likely source of the androgens that caused Vera's hirsutism (a male pattern of secondary sexual hair growth)?



Ergosterol is the provitamin of vitamin D<sub>2</sub>, which differs from 7-dehydrocholesterol and vitamin  $D_3$ , respectively, only by having a double bond between C22 and C23 and a methyl group at C24. Vitamin  $D_2$  is the constituent in many commercial vitamin preparations and in irradiated milk and bread. The antirachitic potencies of  $D_2$  and  $D_3$  in humans are equal, but both must be converted to 25-(OH)-cholecalciferol and eventually to the active form calcitriol  $(1,25-(OH)_2D_3)$  for biologic activity.



Rickets is a disorder of young children caused by a deficiency of vitamin D. Low levels of calcium and phosphorus in the blood are associated with skeletal deformities in these patients.

testosterone synthesis. In its target cells, the double bond in ring A of testosterone is reduced through the action of  $5-\alpha$  reductase, forming the active hormone dihydrotestosterone (DHT).

## E. Synthesis of Estrogens and Progesterone

Ovarian production of estrogens, progestins (compounds related to progesterone), and androgens requires the activity of the cytochrome P450 family of oxidative enzymes used for the synthesis of other steroid hormones. Ovarian estrogens are C18 steroids with a phenolic hydroxyl group at C3 and either a hydroxyl group (estradiol) or a ketone group (estrone) at C17. Although the major steroid-producing compartments of the ovary (the granulosa cell, the theca cell, the stromal cell, and the cells of the corpus luteum) have all of the enzyme systems required for the synthesis of multiple steroids, the granulosa cells secrete primarily estrogens, the thecal and stromal cells secrete primarily androgens, and the cells of the corpus luteum secrete primarily progesterone.

The ovarian granulosa cell, in response to stimulation by follicle-stimulating hormone (FSH) from the anterior pituitary gland and through the catalytic activity of P450 aromatase, converts testosterone to estradiol, the predominant and most potent of the ovarian estrogens (see Fig. 34.23). Similarly, androstenedione is converted to estrone in the ovary, although the major site of estrone production from androstenedione occurs in extraovarian tissues, principally skeletal muscle and adipose tissue.

## **XI. VITAMIN D SYNTHESIS**

Vitamin D is unique in that it can be either obtained from the diet (as vitamin  $D_2$  or  $D_3$ ) or synthesized from a cholesterol precursor, a process that requires reactions in the skin, liver, and intestine. The calciferols, including several forms of vitamin D, are a family of steroids that affect calcium homeostasis (Fig. 34.26). Cholecalciferol (vitamin D<sub>3</sub>) requires ultraviolet light for its production from 7-dehydrocholesterol present in cutaneous tissues (skin) in animals and from ergosterol in plants. This irradiation cleaves the carbon–carbon bond at C9–C10 to open the B ring to form cholecalciferol, an inactive precursor of 1,25-(OH)<sub>2</sub>-cholecalciferol (calcitriol). Calcitriol is the most potent biologically active form of vitamin D (see Fig. 34.26).

The formation of calcitriol from cholecalciferol begins in the liver and ends in the kidney, where the pathway is regulated. In this activation process, carbon 25 of vitamin D<sub>2</sub> or D<sub>3</sub> is hydroxylated in the microsomes of the liver to form 25-hydroxycholecalciferol (calcidiol). Calcidiol circulates to the kidney bound to vitamin D-binding globulin (transcalciferin). In the proximal convoluted tubule of the kidney, a mixed function oxidase, which requires molecular O2 and NADPH as cofactors, hydroxylates carbon 1 on the A ring to form calcitriol. This step is tightly regulated and is the rate-limiting step in the production of the active hormone.

1,25-(OH)<sub>2</sub>D<sub>3</sub> (calcitriol) is approximately 100 times more potent than 25- $(OH)D_3$  in its actions, yet 25- $(OH)D_3$  is present in the blood in a concentration that may be 100 times greater, which suggests that it may play some role in calcium and phosphorus homeostasis.

The biologically active forms of vitamin D are sterol hormones and, like other steroids, diffuse passively through the plasma membrane. In the intestine, bone, and kidney, the sterol then moves into the nucleus and binds to specific vitamin  $D_3$ receptors. This complex activates genes that encode proteins mediating the action of active vitamin D<sub>3</sub>. In the intestinal mucosal cell, for example, transcription of genes encoding calcium-transporting proteins is activated. These proteins are capable of carrying Ca<sup>2+</sup> (and phosphorus) absorbed from the gut lumen across the cell, making it available for eventual passage into the circulation.

#### **CLINICAL COMMENTS**

Ann Jeina is typical of patients with essentially normal serum triacylglycerol levels and elevated serum total cholesterol levels that are repeatedly in the upper 1% of the general population (e.g., 325–500 mg/dL). When similar lipid abnormalities are present in other family members in a pattern

of autosomal dominant inheritance and no secondary causes for these lipid alterations (e.g., hypothyroidism) are present, the entity referred to as "familial hypercholesterolemia (FH), type IIA" is the most likely cause of this hereditary dyslipidemia.

FH is a genetic disorder caused by an abnormality in one or more alleles responsible for the formation or the functional integrity of high-affinity LDL receptors on the plasma membrane of cells that normally initiate the internalization of circulating LDL and other blood lipoproteins. Heterozygotes for FH (1 in 500 of the population) have roughly one half of the normal complement or functional capacity of such receptors, whereas homozygotes (1 in 1 million of the population) have essentially no functional LDL receptors. The rare patient with the homozygous form of FH has a more extreme elevation of serum total and LDL cholesterol than does the heterozygote and, as a result, has a more profound predisposition to premature coronary artery disease.

Chronic hypercholesterolemia not only may cause the deposition of lipid within vascular tissues leading to atherosclerosis but also may cause the deposition of lipid within the skin and eye. When this occurs in the medial aspect of the upper and lower eyelids, it is referred to as xanthelasma. Similar deposits known as xanthomas may occur in the iris of the eye (arcus lipidalis) as well as the tendons of the hands ("knucklepads") and Achilles tendons.

Although therapy aimed at inserting competent LDL receptor genes into the cells of patients with homozygous FH is undergoing clinical trials, the current approach in the heterozygote is to attempt to increase the rate of synthesis of LDL receptors in cells pharmacologically.

Ann Jeina was treated with cholestyramine, a resin that binds some of the bile salts in the intestine, causing these resin-bound salts to be carried into the feces rather than recycled to the liver. The liver must now synthesize more bile salts, which lowers the intrahepatic free cholesterol pool. As a result, hepatic LDL receptor synthesis is induced, and more circulating LDL is taken up by the liver.

HMG-CoA reductase inhibitors, such as pravastatin, also stimulate the synthesis of additional LDL receptors but do so by inhibiting HMG-CoA reductase, the ratelimiting enzyme for cholesterol synthesis. The subsequent decline in the intracellular free cholesterol pool also stimulates the synthesis of additional LDL receptors. These additional receptors reduce circulating LDL-cholesterol levels by increasing receptor-mediated endocytosis of LDL particles.

A combination of strict dietary and dual pharmacologic therapy, aimed at decreasing the cholesterol levels of the body, is usually quite effective in cor-

Ann Jeina was treated with a statin (pravastatin) and cholestyramine, a bile acid sequestrant. With the introduction of the cholesterol absorption blocker ezetimibe, the use of cholestyramine with its high level of gastrointestinal side effects may decline. Ezetimibe reduces the percentage of absorption of free cholesterol present in the lumen of the gut and hence the amount of cholesterol available to the enterocyte to package into chylomicrons. This, in turn, reduces the amount of cholesterol returning to the liver in chylomicron remnants. The net result is a reduction in the cholesterol pool in hepatocytes. The latter induces the synthesis of an increased number of LDL receptors by the liver cells. As a consequence, the capacity of the liver to increase hepatic uptake of LDL from the circulation leads to a decrease in serum LDL levels.



**Fig. 34.26.** Synthesis of active vitamin D.  $(1, 25-di (OH)_2D_3)$  is produced from 7-dehydrocholesterol, a precursor of cholesterol. In the skin, ultraviolet (UV) light produces cholecalciferol, which is hydroxylated at the 25-position in the liver and the 1-position in the kidney to form the active hormone.



recting the lipid abnormality and, hopefully, the associated risk of atherosclerotic cardiovascular disease in patients with heterozygous familial hypercholes-terolemia.

Low-density lipoprotein cholesterol is the primary target of cholesterollowering therapy because both epidemiologic and experimental evidence strongly suggest a benefit of lowering serum LDL cholesterol in the prevention of atherosclerotic cardiovascular disease. Similar evidence for raising subnormal levels of serum HDL cholesterol is less conclusive but adequate to support such efforts, particularly in high-risk patients, such as **Ivan Applebod**, who have multiple cardiovascular risk factors. The first-line therapy in this attempt is nonpharmacologic and includes such measures as increasing aerobic exercise, weight loss in overweight patients, avoidance of excessive alcohol intake, reducing the intake of refined sugars, and cessation of smoking. If these efforts fail, drug therapy to raise serum HDL cholesterol levels must be considered.

So far, Mr. Applebod has failed in his attempts to diet and exercise. His LDL cholesterol level is 231 mg/dL. According to Table 34.1, he is a candidate for more stringent dietary therapy and for drug treatment. He could be given an HMG CoA reductase inhibitor such as pravastatin and, perhaps, a bile salt–binding resin such as cholestyramine. Other lipid-lowering drugs such as the fibric acid derivatives and ezetimibe, which also decrease triacylglycerol levels and potentially increase HDL levels, should be considered (Table 34.5).

**Vera Leizd** was born with a normal female genotype and phenotype, had normal female sexual development, spontaneous onset of puberty, and regular, although somewhat scanty, menses until the age of 20. At that point, she developed secondary amenorrhea (cessation of menses) and evidence of male hormone excess with early virilization (masculinization).

The differential diagnosis included an ovarian versus an adrenocortical source of the excess androgenic steroids. A screening test to determine whether

Table 34.5. Mechanism(s)	of Action and Efficacy of	Lipid-Lowering Agents

			Percentage change in serum lipid level (monotherapy)		
Agent	Mechanism of Action	Total cholesterol	LDL-cholesterol	HDL cholesterol	Triacylglycerols
Statins	Inhibits HMG-CoA reductase activity	↓15–60%	↓ 20–60%	↑5–15%	↓10–40%
Bile acid resins	Increase fecal excretion of bile salts	↓15–20%	↓10–25%	↑3–5%	Variable, depending on pretreatment level of triacylglycerols (may increase)
Niacin	Activates LPL; reduces hepatic production of VLDL; reduces catabolism of HDL	↓22-25%	↓10–25%	15–35%	↓ 20–50%
Fibrates	Antagonizes PPAR-α causing an increase in LPL activity, a decrease in apoprotein C-III production, and an increase in apoprotein A-I production.	J12–15%	Variable, depending on pretreatment levels of other lipids	↑5–15%	↓20–50%
Ezetimibe	Reduces intestinal absorption of free cholesterol from the gut lumen	↓10–15%	↓ 15–20%	↑1–3%	↓ 5–8% if triacylglycerols are high pretreatment

Abbreviations: LPL, lipoprotein lipase; LDL, low-density lipoprotein; HDL, high-density lipoprotein; triacylglycerols, triglycerides; PPAR, peroxisome proliferators-activated receptor (the Table is adapted from Circulation 2002; 106:3145–3457).

the adrenal cortex or the ovary is the source of excess male hormone involves the measurement of the concentration of dehydroepiandrosterone sulfate (DHEAS) in the patient's plasma, because the adrenal cortex makes most of the DHEA, and the ovary makes little or none. Vera's plasma DHEAS level was moderately elevated, identifying her adrenal cortices as the likely source of her virilizing syndrome.

If the excess production of androgens is not the result of an adrenal tumor, but the result of a defect in the pathway for cortisol production, the simple treatment is to administer glucocorticoids by mouth. The rationale for such treatment can be better understood by reviewing Fig. 34.23. If Vera Leizd has a genetically determined partial deficiency in the P450<sub>C11</sub> enzyme system needed to convert 11deoxycortisol to cortisol, her blood cortisol levels would fall. By virtue of the normal positive feedback mechanism, a subnormal level of cortisol in the blood would induce the anterior pituitary to make more ACTH. The latter would not only stimulate the cortisol pathway to increase cortisol synthesis to normal but, in the process, would also induce increased production of adrenal androgens such as DHEA and DHEAS. The increased levels of the adrenal androgens (although relatively weak androgens) would cause varying degrees of virilization, depending on the severity of the enzyme deficiency. The administration of a glucocorticoid by mouth would suppress the high level of secretion of ACTH from the anterior pituitary gland that occurs in response to the reduced levels of cortisol secreted from the adrenal cortex. Treatment with prednisone (a synthetic glucocorticoid), therefore, will prevent the ACTH-induced overproduction of adrenal androgens. However, when ACTH secretion returns to normal, endogenous cortisol synthesis falls below normal. The administered prednisone brings the net glucocorticoid activity in the blood back to physiologic levels. Vera's adrenal androgen levels in the blood returned to normal after several weeks of therapy with prednisone (a synthetic glucocorticoid). As a result, her menses eventually resumed, and her virilizing features slowly resolved.

Because Vera's symptoms began in adult life, her genetically determined adrenal hyperplasia is referred to as a "nonclassic" or "atypical" form of the disorder. A more severe enzyme deficiency leads to the "classic" disease, which is associated with excessive fetal adrenal androgen production in utero and, therefore, manifests itself at birth, often with ambiguous external genitalia and virilizing features in the female neonate.

#### **BIOCHEMICAL COMMENTS**

Defects in the LDL receptor gene are responsible for the elevated blood levels of LDL, and thus of cholesterol, in FH. Over 300 mutations have been found in the LDL receptor gene, affecting all stages in the production and functioning of the receptor.

The LDL receptor gene, which contains 18 exons and is 45 kilobases (kb) in length, is located on the short arm of chromosome 19. The exons share sequences for the C9 component of complement (a blood protein involved in the immune response), and the N-linked oligosaccharide domain is homologous to the genes for the precursor of EGF and also for three proteases of the blood clotting system, Factors IX and X and protein C (see Chapter 45). The LDL receptor gene encodes a glycoprotein that contains 839 amino acids.

Heterozygotes for FH have one normal and one mutant allele. Their cells produce approximately half the normal amount of receptor and take up LDL at about half the normal rate. Homozygotes have two mutant alleles, which may either be identical or differ. They produce very little functional receptor.

The genetic mutations are mainly deletions, but insertions or duplications also occur, as well as missense and nonsense point mutations (see Fig. 34.20). Four classes of mutations have been identified. The first class involves "null" alleles that either direct the synthesis of no protein at all or a protein that cannot be precipitated by antibodies to the LDL receptor. In the second class, the alleles encode proteins, but they cannot be transported to the cell surface. The third class of mutant alleles encodes proteins that reach the cull surface but cannot bind LDL normally. Finally, the fourth class encodes proteins that reach the surface and bind LDL but fail to cluster and internalize the LDL particles. The result of each of these mutations is that blood levels of LDL are elevated because cells cannot take up these particles at a normal rate.

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## **REVIEW QUESTIONS—CHAPTER 34**

- 1. Which of the following steps in the biosynthesis of cholesterol is the committed rate-limiting step?
  - (A) The condensation of acetoacetyl-CoA with a molecule of acetyl-CoA to yield β-hydroxy-β methylglutaryl-CoA (HMG-CoA)
  - (B) The reduction of HMG-CoA to mevalonate
  - (C) The conversion of mevalonate to two activated isoprenes
  - (D) The formation of farnesyl pyrophosphate
  - (E) Condensation of six activated isoprene units to form squalene
- 2. Considering the final steps in cholesterol biosynthesis, when squalene is eventually converted to lanosterol, which of the following statements is correct?
  - (A) All of the sterols have three fused rings (the steroid nucleus) and are alcohols with a hydroxyl group at C-3.
  - (B) The action of squalene monooxygenase oxidizes carbon 14 of the squalene chain, forming an epoxide.
  - (C) Squalene monooxygenase is considered a mixed function oxidase because it catalyzes a reaction in which only one of the oxygen atoms of  $O_2$  is incorporated into the organic substrate.
  - (D) Squalene monooxygenase uses reduced flavin nucleotides (e.g., FAD(2H)) as the cosubstrate in the reaction.
  - (E) Squalene is joined at carbons 1 and 30 to form the fused ring structure of sterols.
- 3. Of the major risk factors for the development of atherosclerotic cardiovascular disease (ASCVD) such as sedentary lifestyle, obesity, cigarette smoking, diabetes mellitus, hypertension, and hyperlipidemia, which one, if present, is the only risk factor in a given patient without a history of having had a myocardial infarction that requires that the therapeutic goal for the serum LDL cholesterol level be < 100mg/dL?
  - (A) Obesity
  - (B) Cigarette smoking
  - (C) Diabetes mellitus
  - (D) Hypertension
  - (E) Sedentary lifestyle

- 4. Which one of the following apoproteins acts as a cofactor activator of the enzyme lipoprotein lipase (LPL)?
  - (A) ApoC-III
  - (B) ApoC-II
  - (C) ApoB-100
  - (D) ApoB-48
  - (E) ApoE
- 5. Which one of the following sequences places the lipoproteins in the order of most dense to least dense?
  - (A) HDL/VLDL/chylomicrons/LDL
  - (B) HDL/LDL/VLDL/chylomicrons
  - (C) LDL/chylomicrons/HDL/VLDL
  - (D) VLDL/chylomicrons/LDL/HDL
  - (E) LDL/chylomicrons/VLDL/HDL