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LIFE AT THE MOLECULAR LEVEL

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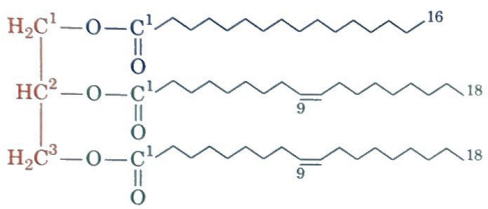
Most cells contain a wide variety of lipids, but many of these structurally distinct molecules are functionally similar. For example, most cells can tolerate variations in the lipid composition of their membranes, provided that membrane fluidity, which is largely a property of their component fatty acid chains, is maintained (Section 9-2B).

Even greater variation is exhibited in the cellular content of lipids stored as energy reserves. Triacylglycerol stores are built up and gradually depleted in response to changing physiological demands. The camel's hump is a well-known example of a fat depot that supplies energy (the catabolism of the fat also generates water). Other organisms that undergo dramatic changes in body fat content are hibernating mammals and birds that migrate long distances without refueling. Lipid metabolism in these cases is notable for the sheer amount of material that flows through the few relatively simple biosynthetic and degradative pathways.

In this chapter, we acknowledge the central function of lipids in energy metabolism by first examining the absorption and transport of their component fatty acids and the oxidation of these fatty acids to produce energy. The second part of this chapter examines the synthesis of fatty acids and other lipids, including glycerophospholipids, sphingolipids, and cholesterol.



1-Palmitoyl-2,3-dioleoyl-glycerol

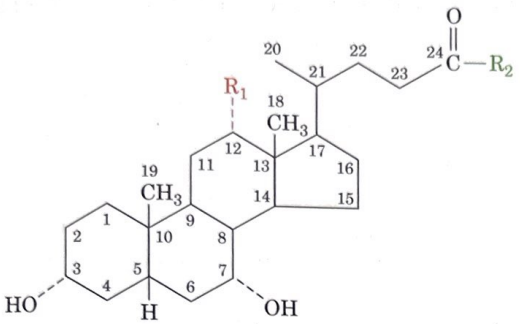
1 Lipid Digestion, Absorption, and Transport

Triacylglycerols (also called fats or triglycerides) constitute ~90% of the dietary lipid and are the major form of metabolic energy storage in humans. Triacylglycerols consist of glycerol triesters of fatty acids such as palmitic and oleic acids (*at left*; the names and structural formulas of some biologically common fatty acids are listed in Table 9-1). The mechanisms for digesting, absorbing, and transporting triacylglycerols from the intestine to the tissues must accommodate their inherent hydrophobicity.

A Digestion and Absorption

Since triacylglycerols are water insoluble, whereas digestive enzymes are water soluble, triacylglycerol digestion takes place at lipid–water interfaces. The rate of triacylglycerol digestion therefore depends on the surface area of the interface, which is greatly increased by the churning peristaltic movements of the intestine combined with the emulsifying action of **bile acids.** The bile acids (also called **bile salts**) are amphipathic detergent-like molecules that act to solubilize fat globules. Bile acids are cholesterol derivatives that are synthesized by the liver and secreted as glycine or **taurine** conjugates (Fig. 19-1) into the gallbladder for storage. From there, they are secreted into the small intestine, where lipid digestion and absorption mainly take place.

Lipases Act at the Lipid–Water Interface. Pancreatic **lipase (triacylglycerol lipase)** catalyzes the hydrolysis of triacylglycerols at their 1 and 3 positions to form sequentially **1,2-diacylglycerols** and **2-acylglycerols,** together with the Na^+ and K^+ salts of fatty acids (soaps). The enzymatic activity of pancreatic lipase greatly increases when it contacts the lipid–water interface, a phenomenon known as **interfacial activation.** Binding to the lipid–water interface requires mixed micelles of phosphatidylcholine and bile acids, as well as pancreatic **colipase,** a 90-residue protein that forms a 1:1 complex with lipase. The X-ray structures, determined by Christian Cambillau, of pancreatic lipase–colipase complexes reveal the structural basis of the



	$R_1 = OH$	$R_1 = H$
$R_2 = OH$	**Cholic acid**	**Chenodeoxycholic acid**
$R_2 = NH—CH_2—COOH$	**Glycocholic acid**	**Glycochenodeoxycholic acid**
$R_2 = NH—CH_2—CH_2—SO_3H$	**Taurocholic acid**	**Taurochenodeoxycholic acid**

Figure 19-1 Structures of the major bile acids and their glycine and taurine conjugates.

interfacial activation of lipase as well as how colipase and micelles help lipase bind to the lipid–water interface (Fig. 19-2).

The active site of the 449-residue pancreatic lipase, which is contained in the enzyme's N-terminal domain (residues 1–336), contains a catalytic triad that closely resembles that in serine proteases (Section 11-5B; recall that ester hydrolysis is mechanistically similar to peptide hydrolysis). In the

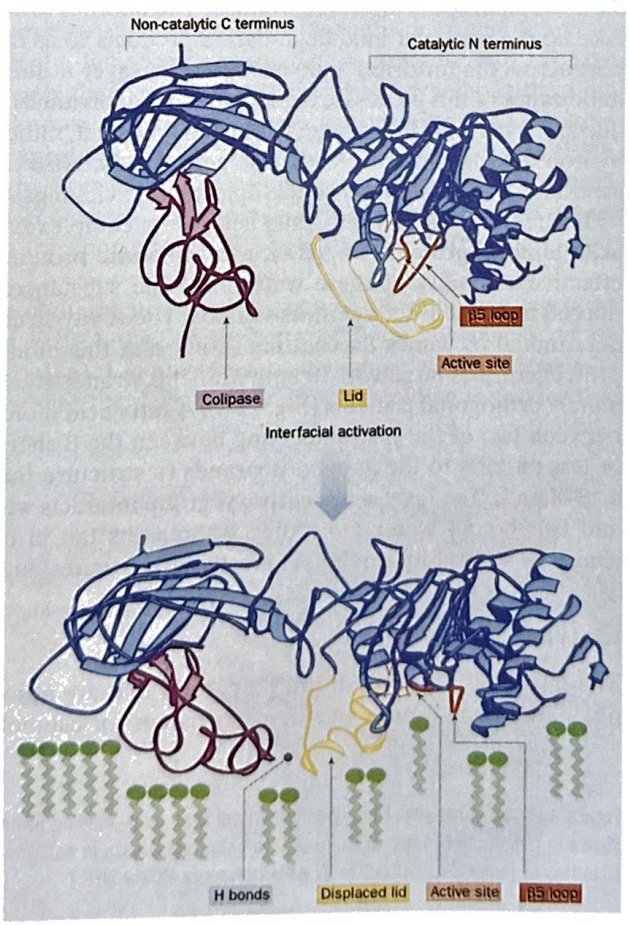
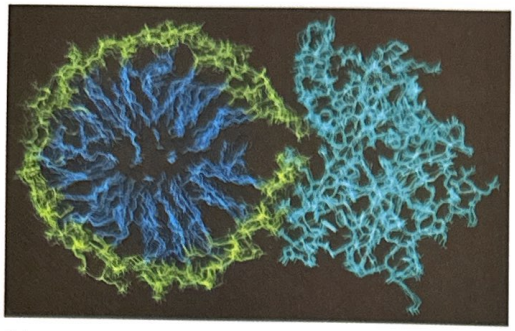
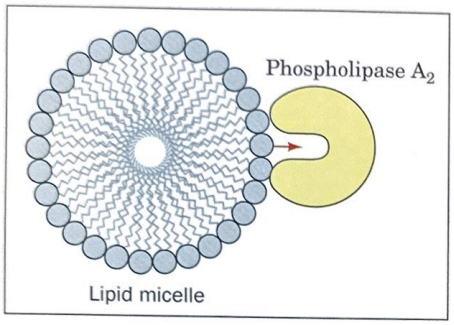


Figure 19-2 The mechanism of interfacial activation of triacylglycerol lipase. The enzyme is in complex with procolipase (the precursor of colipase; *magenta*). On binding to a phospholipid micelle (*green*), the 25-residue lid (*yellow*) covering the enzyme's active site (*tan*) changes conformation so as to expose its hydrophobic residues, thereby uncovering the active site. This causes the 10-residue β5 loop (*brown*) to move aside in a way that forms the enzyme's oxyanion hole. The procolipase also changes its conformation so as to hydrogen bond to the "open" lid, thereby stabilizing it in this conformation and, together with lipase, forming an extended hydrophobic surface. [From *Nature* **362**, 793 (1993). Reproduced with permission. PDBid 1LPA.]



(*a*)



(*b*)

Figure 19-3 Substrate binding to phospholipase A_2. (*a*) Hypothetical model of phospholipase A_2 in complex with a micelle of lysophosphatidylethanolamine as shown in cross section. The protein is drawn in cyan, the phospholipid head groups are yellow, and their hydrocarbon tails are blue. The calculated atomic motions of the assembly are indicated through a series of superimposed images taken at 5-ps intervals. [Courtesy of Raymond Salemme, E. I. du Pont de Nemours & Company.] (*b*) Schematic diagram of a phospholipid contained in a micelle entering the hydrophobic phospholipid channel in phospholipase A_2 (*red arrow*).

absence of lipid micelles, lipase's active site is covered by a 25-residue helical "lid." However, in the presence of the micelles, the lid undergoes a complex structural reorganization that exposes the active site. Simultaneously, a 10-residue loop, called the β5 loop, changes conformation in a way that forms the active enzyme's oxyanion hole and generates a hydrophobic surface near the entrance to the active site.

Colipase binds to the C-terminal domain of lipase (residues 337–449) such that the hydrophobic tips of its three loops extend from the complex. This creates a continuous hydrophobic plateau, extending >50 Å past the lipase active site, that presumably helps bind the complex to the lipid surface. Colipase also forms three hydrogen bonds to the opened lid, thereby stabilizing it in this conformation.

Other lipases, such as phospholipase A_2 (Fig. 9-6), also preferentially catalyze reactions at interfaces. Instead of changing its conformation, however, phospholipase A_2 contains a hydrophobic channel that provides the substrate with direct access from the phospholipid aggregate (micelle or membrane) surface to the bound enzyme's active site (Fig. 19-3). Hence, on leaving its micelle to bind to the enzyme, the substrate need not become solvated and then desolvated. In contrast, soluble and dispersed phospholipids must first surmount these significant kinetic barriers in order to bind to the enzyme.

Bile Acids and Fatty Acid–Binding Protein Facilitate the Intestinal Absorption of Lipids. The mixture of fatty acids and mono- and diacylglycerols produced by lipid digestion is absorbed by the cells lining the small intestine (the intestinal **mucosa**). *Bile acids not only aid lipid digestion; they are essential for the absorption of the digestion products.* The micelles formed by the bile acids take up the nonpolar lipid degradation products so as to permit their transport across the unstirred aqueous boundary layer at the intestinal wall. The importance of this process is demonstrated in individuals with obstructed bile ducts: They absorb little of their ingested lipids but, rather, eliminate them in hydrolyzed form in their feces. Bile acids are likewise required for the efficient intestinal absorption of the lipid-soluble vitamins A, D, E, and K.

Inside the intestinal cells, fatty acids form complexes with **intestinal fatty acid–binding protein (I-FABP),** a cytoplasmic protein that increases the effective solubility of these water-insoluble substances and also protects the cell from their detergent-like effects. The X-ray structure of rat I-FABP, determined by James Sacchettini, shows that this monomeric, 131-residue protein consists largely of 10 antiparallel β strands stacked in two approximately orthogonal β sheets (Fig. 19-4). A fatty acid molecule occupies a gap between two of the β strands, lying between the β sheets so that it is more or less parallel to the gapped β strands (a structure that has been dubbed a "β-clam"). The fatty acid's carboxyl group interacts with Arg 106, Gln 115, and two bound water molecules, whereas its tail in encased by the side chains of several hydrophobic, mostly aromatic residues.

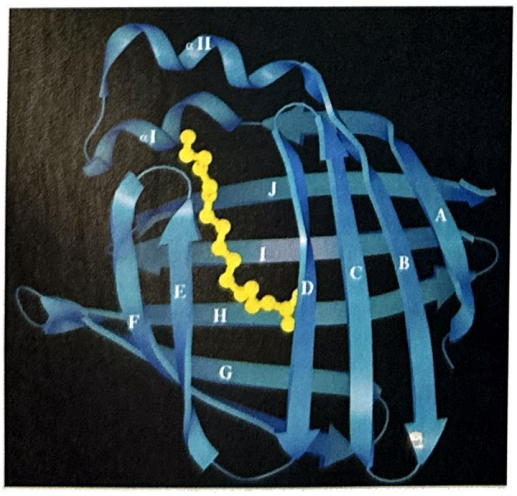


Figure 19-4 X-Ray structure of rat intestinal fatty acid–binding protein. The structure is shown in ribbon form (*blue*), in complex with palmitate, shown in ball-and-stick form (*yellow*). [Courtesy of James Sacchettini, Texas A&M University. PDBid 2IFB.]

B Lipid Transport

The fatty acid products of lipid digestion that are absorbed by the intestinal mucosa make their way to other tissues for catabolism or storage. But

Table 19-1 Characteristics of the Major Classes of Lipoproteins in Human Plasma.

	Chylomicrons	VLDL	IDL	LDL	HDL
Density ($g \cdot cm^{-3}$)	<0.95	<1.006	1.006–1.019	1.019–1.063	1.063–1.210
Particle diameter (Å)	750–12,000	300–800	250–350	180–250	50–120
Particle mass (kD)	400,000	10,000–80,000	5000–10,000	2300	175–360
% Protein[a]	1.5–2.5	5–10	15–20	20–25	40–55
% Phospholipids[a]	7–9	15–20	22	15–20	20–35
% Free cholesterol[a]	1–3	5–10	8	7–10	3–4
% Triacylglycerols[b]	84–89	50–65	22	7–10	3–5
% Cholesteryl esters[b]	3–5	10–15	30	35–40	12
Major apolipoproteins	A-I, A-II, B-48, C-I, C-II, C-III, E	B-100, C-I, C-II, C-III, E	B-100, C-I, C-II, C-III, E	B-100	A-I, A-II, C-I, C-II, C-III, D, E

[a]Surface components.

[b]Core lipids.

because they are only sparingly soluble in aqueous solution, lipids are transported by the circulation in complex with proteins.

Lipoproteins Are Complexes of Lipid and Protein. ***Lipoproteins*** *are globular micelle-like particles that consist of a nonpolar core of triacylglycerols and cholesteryl esters surrounded by an amphiphilic coating of protein, phospholipid, and cholesterol.* There are five classes of lipoproteins (Table 19-1), which vary in composition and physiological function.

Intestinal mucosal cells convert dietary fatty acids to triacylglycerols and package them, along with dietary cholesterol, into lipoproteins called **chylomicrons.** These particles are released into the intestinal lymph and are transported through the lymphatic vessels before draining into the large veins. The bloodstream then delivers chylomicrons throughout the body. Other lipoproteins known as **very low density lipoproteins (VLDL), intermediate density lipoproteins (IDL),** and **low density lipoproteins (LDL)** are synthesized by the liver to transport endogenous (internally produced) triacylglycerols and cholesterol from the liver to the tissues. **High density lipoproteins (HDL)** transport cholesterol and other lipids from the tissues back to the liver.

Each lipoprotein contains just enough protein, phospholipid, and cholesterol to form an ~20-Å-thick monolayer of these substances on the particle surface (Fig. 19-5). Lipoprotein densities increase with decreasing particle diameter because the density of their outer coating is greater than

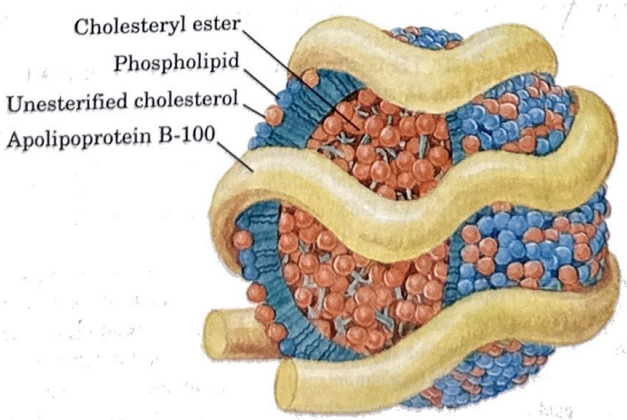


Figure 19-5 Diagram of LDL, the major cholesterol carrier of the bloodstream. This spheroidal particle consists of some 1500 cholesteryl ester molecules surrounded by an amphiphilic coat of ~800 phospholipid molecules, ~500 cholesterol molecules, and a single 4536-residue molecule of apolipoprotein B-100.

that of their inner core. Thus, the HDL, which are the most dense of the lipoproteins, are also the smallest.

Apolipoproteins Coat Lipoprotein Surfaces. The protein components of lipoproteins are known as **apolipoproteins** or just **apoproteins.** At least nine apolipoproteins are distributed in different amounts in the human lipoproteins (Table 19-1). For example, LDL contain **apolipoprotein B-100 (apoB-100).** This protein, a 4536-residue monomer (and thus one of the largest monomeric proteins known), has a hydrophobicity approaching that of integral membrane proteins. Each LDL particle contains only one molecule of apoB-100, which appears to cover at least half of the particle surface (Fig. 19-5).

Unlike apoB-100, the other apolipoproteins are water soluble and associate rather weakly with lipoproteins. These apolipoproteins also have a high helix content, which increases when they are incorporated into lipoproteins. Contact with a hydrophobic surface apparently favors formation of helices, which satisfy the hydrogen bonding potential of the protein's polar backbone groups. In addition, *the helices in apolipoproteins have hydrophilic and hydrophobic side chains on opposite sides of the helical cylinder, suggesting that lipoprotein α helices are amphipathic and float on phospholipid surfaces, much like logs on water.* The charged head groups of the lipids presumably bind to oppositely charged residues on the helix while the first few methylene groups of their fatty acyl chains associate with the nonpolar face of the helix.

Apolipoprotein A-I (apoA-I), which occurs in chylomicrons and HDL, is a 243-residue, 29-kD polypeptide. It consists largely of tandem 22-residue segments of similar sequence. The X-ray structure of a truncated apolipoprotein A-I (lacking residues 1–43) reveals that the polypeptide chain forms a pseudocontinuous α helix that is punctuated by kinks at Pro residues spaced about every 22 residues. Four monomers associate to form the structure shown in Fig. 19-6*a*. The size and twisted elliptical shape of this complex seem ideal for wrapping around an HDL particle. Figure 19-6*b* contains a helical wheel representation of a portion of apoA-I, illustrating the amphipathic nature of the helix.

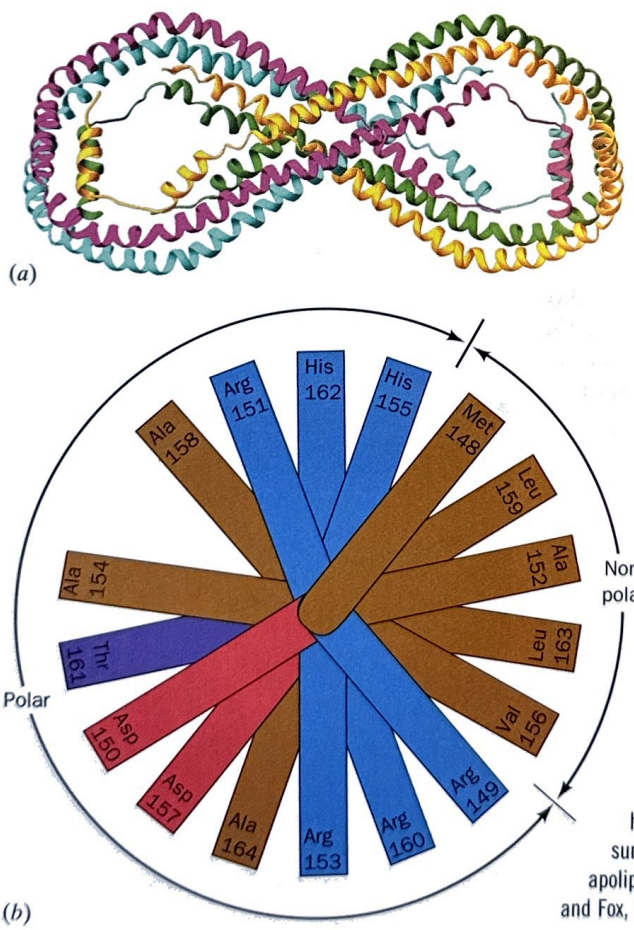


Figure 19-6 Structure of human apolipoprotein A-I. (*a*) The X-ray structure of this D_2-symmetric homotetramer with its four subunits, which lack their N-terminal 43 residues, drawn in different colors. The complex, which has a twisted ellipsoidal shape, is viewed along one of its twofold axes. [Based on an X-ray structure by David Borhani, Southern Research Institute, Birmingham, Alabama, and Christie Brouillette, University of Alabama Medical Center, Birmingham, Alabama. PDBid 1AV1.] (*b*) A helical wheel projection of the amphipathic α helix constituting residues 148 to 164 of apolipoprotein A-I (in a helical wheel representation, the side chain positions are projected down the helix axis onto a plane). Note the segregation of nonpolar and polar residues to different sides of the helix as well as the segregation of the basic residues to the outer edges of the polar surface, where they can interact with the anionic head groups of membrane lipids. Other apolipoprotein helices have similar polarity distributions. [After Kaiser, E.T., *in* Oxender, D.L. and Fox, C.F. (Eds.), *Protein Engineering, p.* 194, Liss (1987).]

Chylomicrons Are Delipidated in the Capillaries of Peripheral Tissues. Chylomicrons adhere to binding sites on the inner surface (endothelium) of the capillaries in skeletal muscle and adipose tissue. The chylomicron's component triacylglycerols are hydrolyzed through the action of the extracellular enzyme

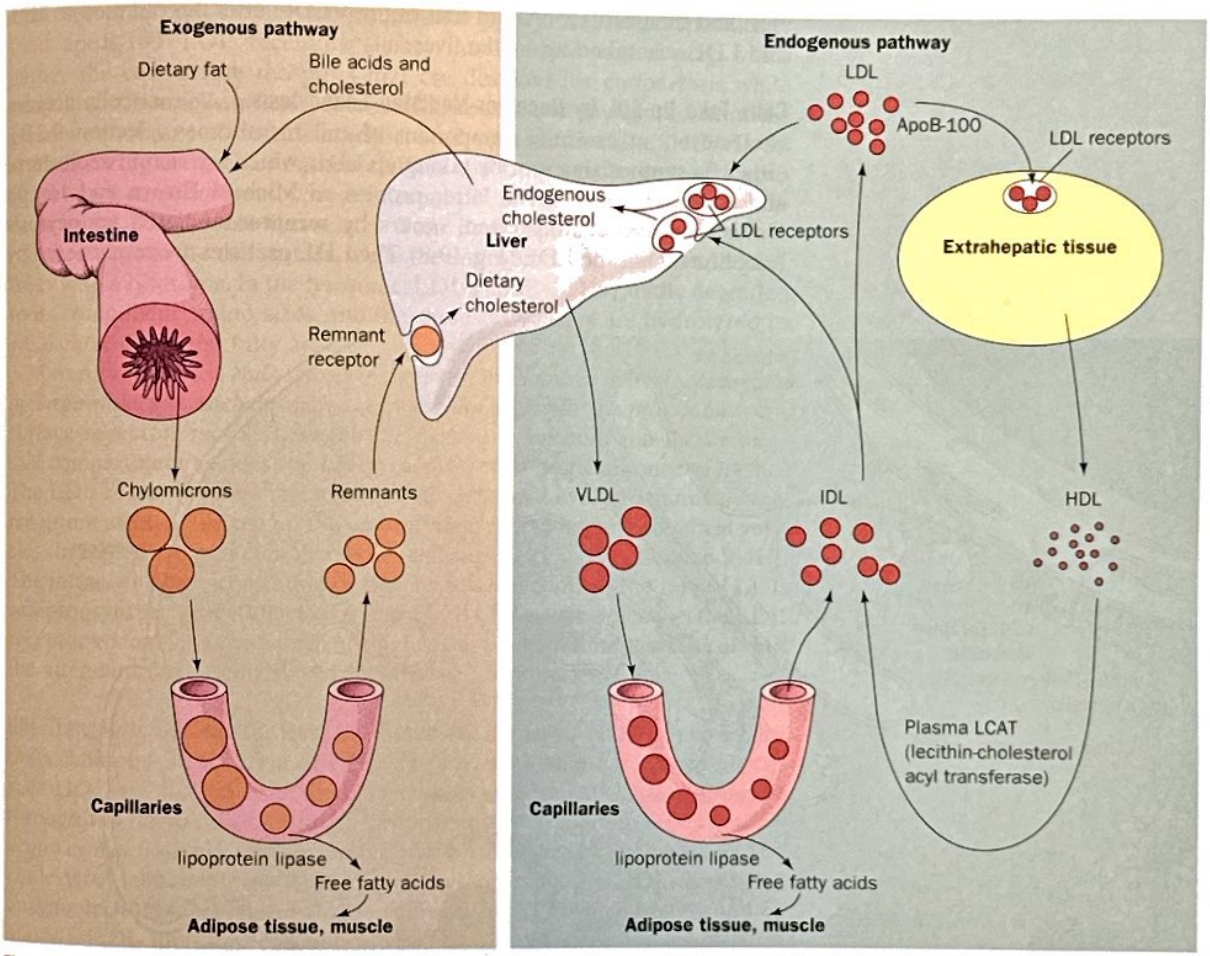


Figure 19-7 Model for plasma triacylglycerol and cholesterol transport in humans. [After Brown, M.S. and Goldstein, J.L., *in* Brunwald, E., Isselbacher, K.J., Petersdorf, R.G., Wilson, J.D., Martin, J.B., and Fauci, A.S. (Eds.), *Harrison's Principles of Internal Medicine* (11th ed.), *p.* 1652, McGraw-Hill (1987).] See the Animated Figures.

lipoprotein lipase. The tissues then take up the liberated monoacylglycerol and fatty acids. The chylomicrons shrink as their triacylglycerols are progressively hydrolyzed until they are reduced to cholesterol-enriched **chylomicron remnants.** The remnants dissociate from the capillary endothelium and re-enter the circulation to be taken up by the liver. *Chylomicrons therefore deliver dietary triacylglycerols to muscle and adipose tissue, and dietary cholesterol to the liver* (Fig. 19-7, *left*).

VLDL Are Gradually Degraded. Very low density lipoproteins (VLDL), which transport endogenous triacylglycerols and cholesterol, are also degraded by lipoprotein lipase in the capillaries of adipose tissue and muscle (Fig. 19-7, *right*). The released fatty acids are taken up by the cells and oxidized for energy or used to resynthesize triacylglycerols. The glycerol backbone of triacylglycerols is transported to the liver or kidneys and converted to the glycolytic intermediate dihydroxyacetone phosphate. Oxidation of this three-carbon unit, however, yields only a small fraction of the energy available from oxidizing the three fatty acyl chains of a triacylglycerol.

After giving up their triacylglycerols, the VLDL remnants, which have also lost some of their apolipoproteins, appear in the circulation first as

IDL and then as LDL. About half of the VLDL, after degradation to IDL and LDL, are taken up by the liver.

Cells Take Up LDL by Receptor-Mediated Endocytosis. Animal cells acquire cholesterol, an essential component of cell membranes (Section 9-2B), either by synthesizing it or by taking up LDL, which are rich in cholesterol and cholesteryl esters. The latter process, as Michael Brown and Joseph Goldstein have demonstrated, occurs by **receptor-mediated endocytosis** (engulfment) of the LDL (Fig. 19-8). The LDL particles are sequestered by

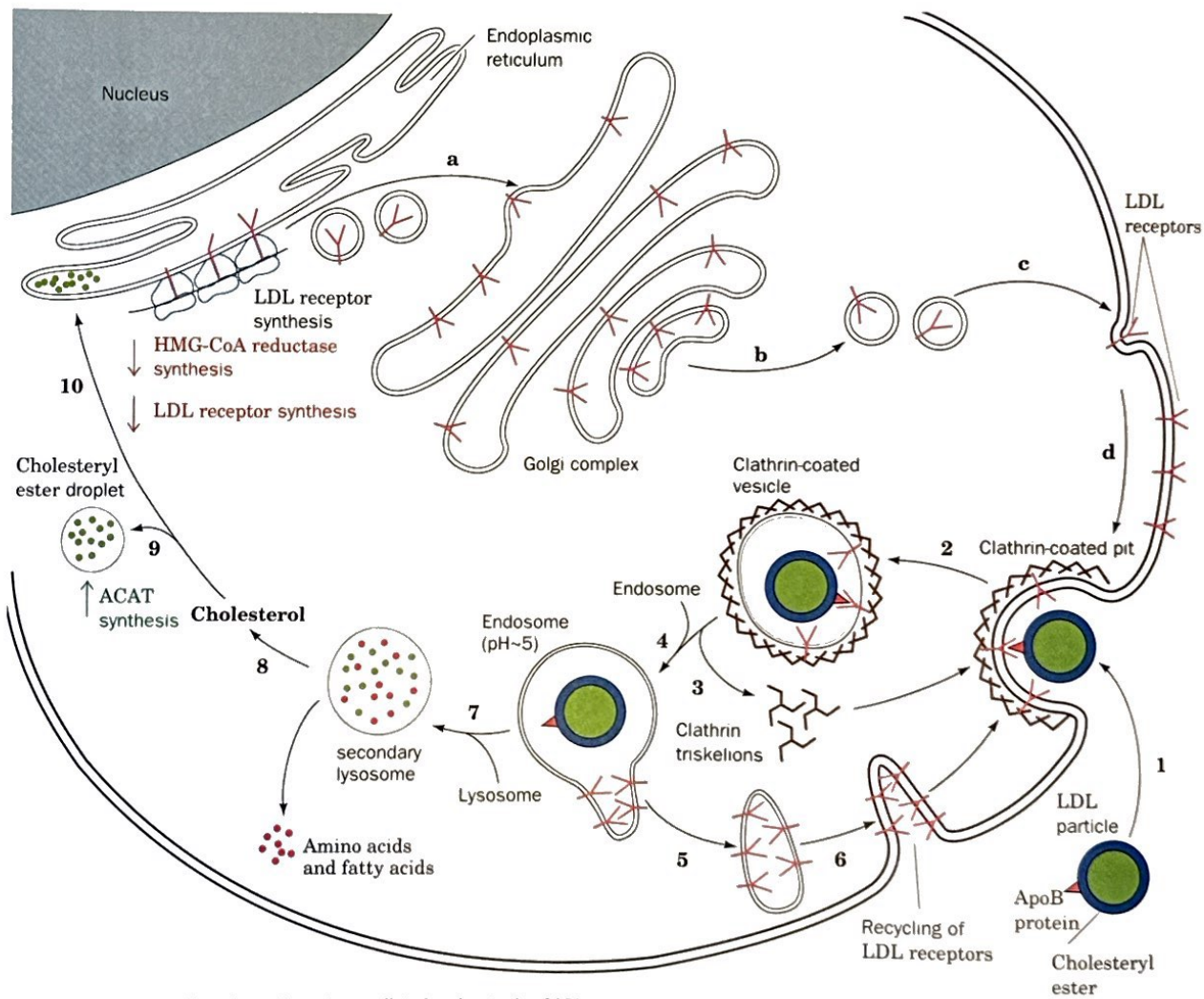


Figure 19-8 ***Key to Function.*** **Receptor-mediated endocytosis of LDL.** LDL receptor is synthesized on the endoplasmic reticulum (**a**), processed in the Golgi apparatus (**b**), and inserted into the plasma membrane (**c**), where it becomes a component of clathrin-coated pits (**d**). The apolipoprotein B-100 (apoB-100) component of LDL specifically binds to LDL receptors on clathrin-coated pits (**1**). These bud into the cell (**2**) to form clathrin-coated vesicles, whose clathrin coats depolymerize to form triskelions (**3**), which are recycled to the cell surface. The uncoated vesicles fuse with vesicles called endosomes (**4**), which have an internal pH of ~5.0. The acidity induces the LDL particle to dissociate from its receptor. The LDL accumulates in the vesicular portion of the endosome, whereas the LDL receptors concentrate in the membrane of an attached tubular structure, which then separates from the endosome (**5**) and subsequently recycles the LDL receptors to the plasma membrane (**6**). The vesicular portion of the endosome fuses with a lysosome (**7**), yielding a **secondary lysosome** wherein the apoB-100 component of the LDL and the cholesteryl esters are hydrolyzed. This releases cholesterol (**8**), which either is converted to cholesteryl esters through the action of **acyl-CoA:cholesterol acyltransferase (ACAT)** and sequestered in a droplet (**9**) or proceeds to the endoplasmic reticulum (**10**). Increased cholesterol concentration in the endoplasmic reticulum decreases the rate of synthesis of **HMG-CoA reductase** (the enzyme that catalyzes the rate-limiting step of cholesterol biosynthesis; Section 19-7B) and LDL receptors (*down arrows*), while increasing that of ACAT (*up arrow*). [After Brown, M.S. and Goldstein, J.L., *Curr. Top. Cell. Reg.* **26,** 7 (1985).] **See the Animated Figures.**

LDL receptors, cell-surface transmembrane glycoproteins that specifically bind apoB-100. LDL receptors cluster into **clathrin-coated pits,** which gather the cell-surface receptors that are destined for endocytosis while excluding other cell-surface proteins. The coated pits invaginate from the plasma membrane to form **clathrin-coated vesicles** (Section 9-4E). Then, after divesting themselves of their clathrin coats, the vesicles fuse with vesicles known as **endosomes,** whose internal pH is ~5.0. Under these conditions, the LDL particle dissociates from its receptor. The receptors are recycled back to the cell surface, while the endosome with its enclosed LDL fuses with a lysosome. In the lysosome, LDL's apoB-100 is rapidly degraded to its component amino acids, and the cholesteryl esters are hydrolyzed to yield cholesterol and fatty acids.

Receptor-mediated endocytosis is a general mechanism whereby cells take up large molecules, each through a corresponding specific receptor. Many cell-surface receptors recycle between the plasma membrane and the endosomal compartment as does the LDL receptor, even in the absence of ligand. The LDL receptor cycles in and out of the cell about every 10 minutes. New receptors are synthesized on the endoplasmic reticulum and travel via vesicles through the Golgi complex to the cell surface (Fig. 19-8; Section 9-4E). The intracellular concentration of free cholesterol controls the rate of LDL receptor synthesis (Sections 19-7A and 19-7B). However, defects in the LDL receptor system lead to abnormally high levels of circulating cholesterol with the attendant increased risk of heart disease (Section 19-7C).

HDL Transports Cholesterol from the Tissues to the Liver. HDL have essentially the opposite function of LDL: *They remove cholesterol from the tissues.* HDL are assembled in the plasma from components largely obtained through the degradation of other lipoproteins. *A circulating HDL particle acquires its cholesterol by extracting it from cell-surface membranes.* The cholesterol is then converted to cholesteryl esters by the HDL-associated enzyme **lecithin–cholesterol acyltransferase (LCAT),** which is activated by apoA-I. HDL therefore function as cholesterol scavengers.

The liver is the only organ capable of disposing of significant quantities of cholesterol (by its conversion to bile acids; Fig. 19-1). About half of the VLDL, after their degradation IDL and LDL, are taken up by the liver via LDL receptor-mediated endocytosis (Fig. 19-7, *right*). However, liver cells take up HDL by an entirely different mechanism: Rather than being engulfed and degraded, an HDL particle binds to a cell-surface receptor named **SR-BI** (for *s*cavenger *r*eceptor class *B* type *I*) and selectively transfers its component lipids to the cell. The lipid-depleted HDL particle then dissociates from the cell and re-enters the circulation.

2 Fatty Acid Oxidation

The triacylglycerols stored in adipocytes are mobilized in times of metabolic need by the action of **hormone-sensitive lipase** (Section 19-5). The free fatty acids are released into the bloodstream, where they bind to albumin, a soluble 66-kD monomeric protein. In the absence of albumin, the maximum solubility of fatty acids is $\sim 10^{-6}$ M; the effective solubility of fatty acids in complex with albumin is as high as 2 mM. Nevertheless, those rare individuals with **analbuminemia** (severely depressed levels of albumin) suffer no apparent adverse symptoms; evidently, their fatty acids are transported in complex with other serum proteins.

Fatty acids are catabolized by an oxidative process that releases free energy. The biochemical strategy of fatty acid oxidation was understood long