Lipid Transport & Storage

BIOMEDICAL IMPORTANCE

Fat absorbed from the diet and lipids synthesized by the liver and adipose tissue must be transported between the various tissues and organs for utilization and storage. Since lipids are insoluble in water, the problem of how to transport them in the aqueous blood plasma is solved by associating nonpolar lipids (triacylglycerol and cholesteryl esters) with amphipathic lipids (phospholipids and cholesterol) and proteins to make **water-miscible lipoproteins**.

In a meal-eating omnivore such as the human, excess calories are ingested in the **anabolic phase** of the feeding cycle, followed by a period of **negative caloric balance when the organism draws upon its carbohydrate and fat stores**. Lipoproteins mediate this cycle by transporting lipids from the intestines as chylomicrons—and from the liver as **very low density lipoproteins (VLDL)**— to most tissues for oxidation and to adipose tissue for storage. Lipid is mobilized from adipose tissue as free fatty acids (FFAs) bound to serum albumin. Abnormalities of lipoprotein metabolism cause various **hypo**- or **hyperlipoproteinemias**. The most common of these is in **diabetes mellitus**, where insulin deficiency causes excessive mobilization of FFA and underutilization of chylomicrons and VLDL, leading to **hypertriacylglycerolemia**. Most other pathologic conditions affecting lipid transport are due primarily to inherited defects, some of which cause hypercholesterolemia and premature **atherosclerosis**. **Obesity**—particularly abdominal obesity—is a risk factor for increased mortality, hypertension, type 2 diabetes mellitus, hyperlipidemia, hyperglycemia, and various endocrine dysfunctions.

LIPIDS ARE TRANSPORTED IN THE PLASMA AS LIPOPROTEINS

Four Major Lipid Classes Are Present in Lipoproteins

Plasma lipids consist of **triacylglycerols** (16%), **phospholipids** (30%), **cholesterol** (14%), and **cholesteryl esters** (36%) and a much smaller fraction of unesterified long-chain fatty acids (free fatty acids or FFA) (4%). This latter fraction, the **FFA**, is metabolically the most active of the plasma lipids.

Four Major Groups of Plasma Lipoproteins Have Been Identified

Since fat is less dense than water, **the density of a lipoprotein decreases as the proportion of lipid to protein increases** (Table 1). Four major groups of lipoproteins have been identified that are important physiologically and in clinical diagnosis. These

are (1) **chylomicrons**, derived from intestinal absorption of triacylglycerol and other lipids; (2) **very low density lipoproteins** (VLDL), derived from the liver for the export of triacylglycerol; (3) **low-density lipoproteins** (LDL), representing a final stage in the catabolism of VLDL; and (4) **high-density lipoproteins** (HDL), involved in cholesterol transport and also in VLDL and chylomicron metabolism. Triacylglycerol is the predominant lipid in chylomicrons and VLDL, whereas cholesterol and phospholipid are the predominant lipids in LDL and HDL, respectively (Table 1). Lipoproteins may also be classified according to their electrophoretic properties into **α-** (HDL), **β**- (LDL), and **pre-β** (VLDL)-**lipoproteins**.

Table 1 Composition of the Lipoproteins in Plasma of Humans

				Composition			
Lipoprotein	**Source**	**Diameter (nm)**	**Density (g/mL)**	**Protein (%)**	**Lipid (%)**	**Main Lipid Components**	**Apolipoproteins**
Chylomicrons	Intestine	90-1000	<0.95	1-2	98-99	Triacylglycerol	A-I, A-II, A-IV,[a] B-48, C-I, C-II, C-III, E
Chylomicron remnants	Chylomicrons	45-150	<1.006	6-8	92-94	Triacylglycerol, phospholipids, cholesterol	B-48, E
VLDL	Liver (intestine)	30-90	0.95-1.006	7-10	90-93	Triacylglycerol	B-100, C-I, C-II, C-III
IDL	VLDL	25-35	1.006-1.019	11	89	Triacylglycerol, cholesterol	B-100, E
LDL	VLDL	20-25	1.019-1.063	21	79	Cholesterol	B-100
HDL	Liver, intestine, VLDL, chylomicrons					Phospholipids, cholesterol	A-I, A-II, A-IV, C-I, C-II, C-III, D,[b] E
HDL_1		20-25	1.019-1.063	32	68		
HDL_2		10-20	1.063-1.125	33	67		
HDL_3		5-10	1.125-1.210	57	43		
Preβ-HDL[c]		<5	>1.210				A-I
Albumin/free fatty acids	Adipose tissue		>1.281	99	1	Free fatty acids	

Lipoproteins Consist of a Nonpolar Core & a Single Surface Layer of Amphipathic Lipids

The **nonpolar lipid core** consists of mainly **triacylglycerol** and **cholesteryl ester** and is surrounded by a **single surface layer** of **amphipathic phospholipid** and **cholesterol** molecules (Figure 1). These are oriented so that their polar groups face outward to the aqueous medium, as in the cell membrane. **The protein moiety of a lipoprotein is known as an apolipoprotein or apoprotein**, constituting nearly 70% of some HDL and as little as 1% of chylomicrons.

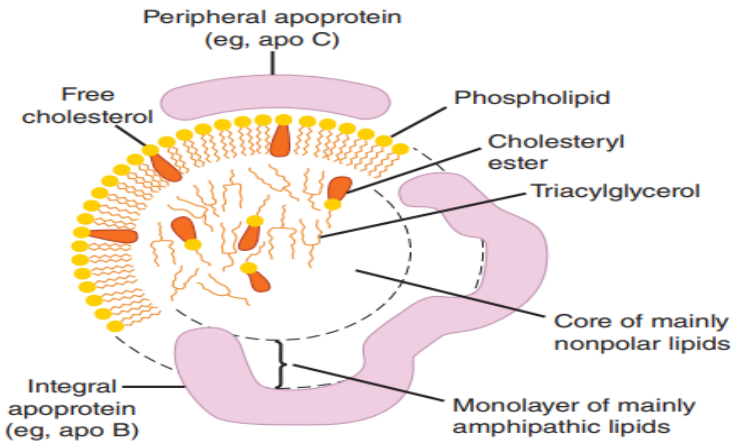

Figure 1 Generalized structure of a plasma lipoprotein. The similarities with the structure of the plasma membrane are to be noted. Small amounts of cholesteryl ester and triacylglycerol are found in the surface layer and a little free cholesterol in the core.

The Distribution of Apolipoproteins Characterizes the Lipoprotein

One or more apolipoproteins (proteins or polypeptides) are present in each lipoprotein. They are usually abbreviated as apo followed by the letter A, B, C, etc (Table 1). Some apolipoproteins are **integral** and cannot be removed (eg, apo B), whereas others are bound to the surface and are free to transfer to other lipoproteins, eg, apos C and E). The major apolipoproteins of HDL (α-lipoprotein) are **apoAs** (Table 1). The main apolipoprotein of LDL (β-lipoprotein) is **apo B (B-100)**, which is found also in VLDL. Chylomicrons contain a truncated form of **apo B (B-48) that is synthesized in the intestine**, while **B-100 is synthesized in the liver.** Apo B-100 is one of the longest single polypeptide chains known, having 4536 amino acids and a molecular mass of 550,000 Da. Apo B-48 (48% of B-100) is formed after transcription of the apoB-100 gene by the introduction of a stop signal into the mRNA transcript by an RNA editing enzyme. **Apos C-I, C-II, and C-III** are smaller polypeptides (molecular mass 7000- 9000 Da) **freely transferable** between several different lipoproteins. **Apo E**, found in VLDL, HDL, chylomicrons, and chylomicron remnants, is also **freely transferable**; it accounts for 5% to 10% of total VLDL apolipoproteins in normal subjects.

Apolipoproteins carry out several roles: (1) they can form **part of the structure** of the lipoprotein, for example, apo B; (2) they are **enzyme cofactors**, for example, C-II for lipoprotein lipase, A-I for lecithin:cholesterol acyltransferase, or **enzyme inhibitors**, for example, apo A-II and apo C-III for lipoprotein lipase, apo C-I for cholesteryl ester transfer protein; and (3) they act as **ligands** for interaction with lipoprotein receptors in tissues, for example, apo B-100 and apo E for the LDL receptor, apo E for the LDL-receptor-related protein-1 (LRP-1), which has been identified as the remnant receptor,

and apo A-I for the HDL receptor. The functions of apo A-IV and apo D, however, are not yet clearly defined, although apo D is believed to be an important factor in human neurodegenerative disorders.

FREE FATTY ACIDS ARE RAPIDLY METABOLIZED

The **FFAs** (also termed nonesterified fatty acids [NEFAs] or unesterified fatty acids) arise in the plasma from the **breakdown of triacylglycerol in adipose tissue or as a result of the action of lipoprotein lipase on the plasma triacylglycerols**. They are found **in combination with albumin**, a very effective solubilizer, in concentrations varying between 0.1 and 2.0 µeq/mL of plasma. Levels are low in the fully fed condition and rise to 0.7 to 0.8 µeq/mL in the starved state. In uncontrolled **diabetes mellitus**, the level may rise to as much as 2 µeq/mL.

FFAs are removed from the blood extremely rapidly and **oxidized** (fulfilling 25%-50% of energy requirements in starvation) or **esterified** to form triacylglycerol in the tissues. In starvation, esterified lipids from the circulation or in the tissues are oxidized as well, particularly in heart and skeletal muscle cells, where considerable stores of lipid are to be found.

The FFA uptake by tissues is related directly to the plasma-FFA concentration, which in turn is determined by the rate of lipolysis in adipose tissue. After dissociation of the fatty acid-albumin complex at the plasma membrane, fatty acids bind to a **membrane fatty acid transport protein** that acts as a transmembrane cotransporter with Na^+. On entering the cytosol, FFAs are bound by intracellular **fatty-acid-binding proteins**. The role of these proteins in intracellular transport is thought to be similar to that of serum albumin in extracellular transport of long-chain fatty acids.

TRIACYLGLYCEROL IS TRANSPORTED FROM THE INTESTINES IN CHYLOMICRONS & FROM THE LIVER IN VERY LOW DENSITY LIPOPROTEINS

By definition, **chylomicrons** are found in **chyle** formed only by the lymphatic system **draining the intestine**. They are responsible for the transport of all dietary lipids into the circulation. Small quantities of VLDL are also to be found in chyle; however, most **VLDL in the plasma** are of hepatic origin. **VLDL are the vehicles of transport of triacylglycerol from the liver to the extrahepatic tissues**.

There are striking similarities in the mechanisms of formation of chylomicrons by intestinal cells and of VLDL by hepatic parenchymal cells (Figure 2), perhaps because—apart from the mammary gland—**the intestine and liver are the only tissues** from which particulate lipid is secreted. Newly secreted or “nascent” chylomicrons and VLDL contain

only a small amount of apolipoproteins C and E, and the full complement is acquired from HDL in the circulation (Figures 3 and 4). Apo B, however, is an integral part of the lipoprotein particles, it is incorporated into the particles during their assembly inside the cells and is essential for chylomicron and VLDL formation. In **abetalipoproteinemia** (a rare disease), lipoproteins containing apo B are not formed and lipid droplets accumulate in the intestine and liver.

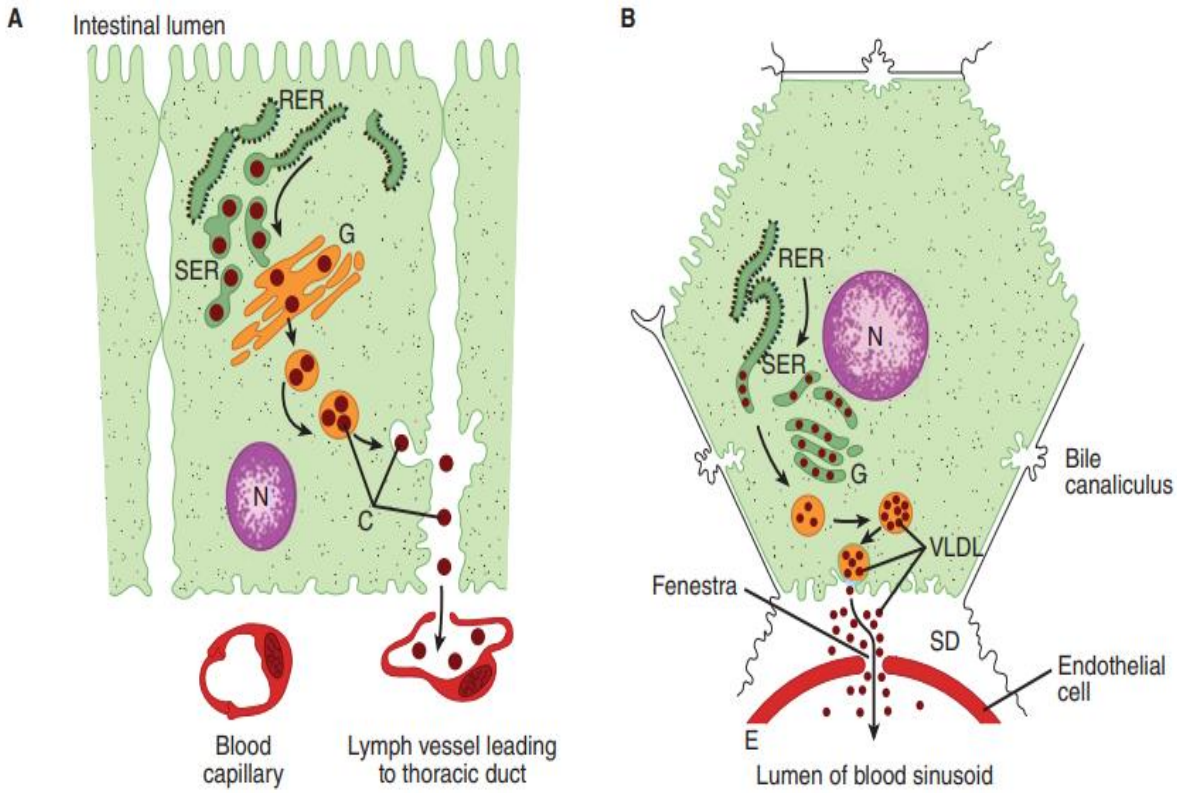

Figure 2 The formation and secretion of (A) chylomicrons by an intestinal cell and (B) very low density lipoproteins by a hepatic cell. (C, chylomicrons; E, endothelium; G, Golgi apparatus; N, nucleus; RER, rough endoplasmic reticulum; SD, space of Disse, containing blood plasma; SER, smooth endoplasmic reticulum; VLDL, very low density lipoproteins.) Apolipoprotein B, synthesized in the RER, is incorporated into particles with triacylglycerol, cholesterol, and phospholipids in the SER. After the addition of carbohydrate residues in G, they are released from the cell by reverse pinocytosis. Chylomicrons pass into the lymphatic system. VLDL are secreted into the space of Disse and then into the hepatic sinusoids through fenestrae in the endothelial lining.

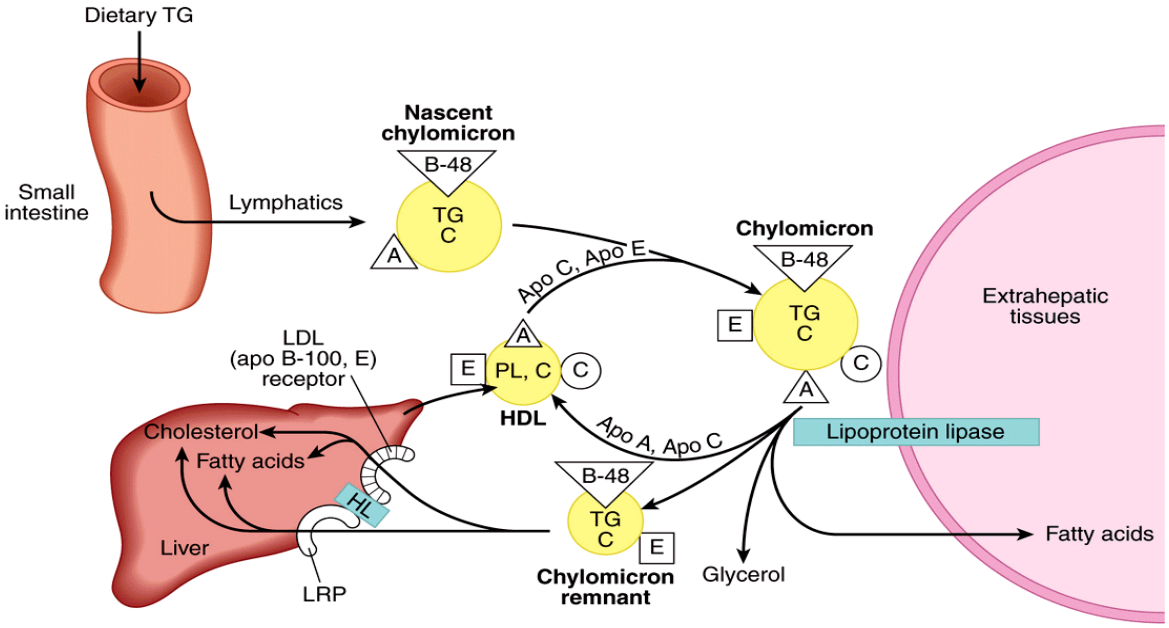

Figure 3 Metabolic fate of chylomicrons. (A, apolipoprotein A; B-48, apolipoprotein B-48; C, apolipoprotein C; C, cholesterol and cholesteryl ester; E, apolipoprotein E; HDL, high-density lipoprotein; HL, hepatic lipase; LRP, LDL-receptor-related protein; PL, phospholipid; TG, triacylglycerol.) Only the predominant lipids are shown.

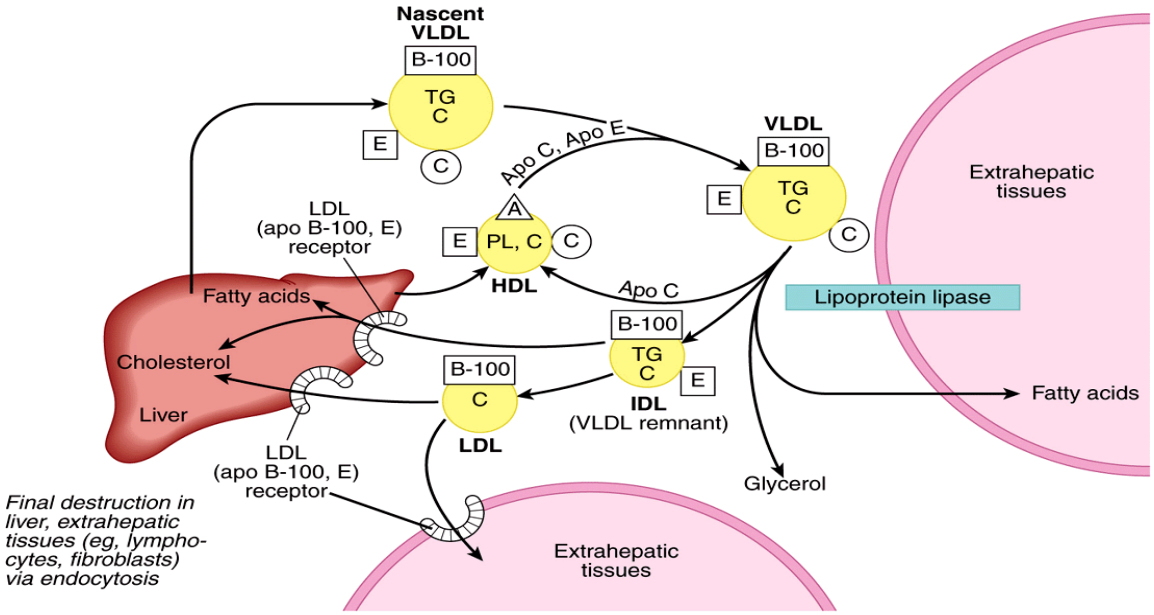

Figure 4 Metabolic fate of very low density lipoproteins (VLDL) and production of low-density lipoproteins (LDL). (A, apolipoprotein A; B-100, apolipoprotein B-100; C, apolipoprotein C; C, cholesterol and cholesteryl ester; E, apolipoprotein E; HDL, high-density lipoprotein; IDL, intermediate-density lipoprotein; PL, phospholipid; TG,

triacylglycerol.) Only the predominant lipids are shown. It is possible that some IDL is also metabolized via the low density lipoprotein receptor-related protein-1 (LRP-1.)

CHYLOMICRONS & VERY LOW DENSITY LIPOPROTEINS ARE RAPIDLY CATABOLIZED

The clearance of chylomicrons from the blood is rapid, the half-time of disappearance being under 1 hour in humans. Larger particles are catabolized more quickly than smaller ones. Fatty acids originating from chylomicron triacylglycerol are delivered mainly to adipose tissue, heart, and muscle (80%), while ~20% goes to the liver. However, **the liver does not metabolize native chylomicrons or VLDL significantly**; thus, the fatty acids in the liver must be secondary to their metabolism in extrahepatic tissues.

Triacylglycerols of Chylomicrons & VLDL Are Hydrolyzed by Lipoprotein Lipase to Form Remnant Lipoproteins

Lipoprotein lipase is located on the **walls of blood capillaries**, anchored to the endothelium by negatively charged proteoglycan chains of heparan sulfate. It has been found in heart, adipose tissue, spleen, lung, renal medulla, aorta, diaphragm, and lactating mammary gland, **although it is not active in adult liver**. It is not normally found in blood; however, following injection of **heparin**, lipoprotein lipase is released from its heparan sulfate binding sites into the circulation. **Hepatic lipase** is bound to the sinusoidal surface of liver cells and is also released by heparin. This enzyme, however, does not react readily with chylomicrons or VLDL but is involved in chylomicron remnant and HDL metabolism.

Both **phospholipids** and **apo C-II** are required as cofactors for lipoprotein lipase activity, while **apo A-II and apo C-III** act as inhibitors. Hydrolysis takes place while the lipoproteins are attached to the enzyme on the endothelium. Triacylglycerol is hydrolyzed progressively through a diacylglycerol to a monoacylglycerol and finally to FFA plus glycerol. Some of the released FFA return to the circulation, attached to albumin, but the bulk is transported into the tissue (Figures 3 and 4). **Heart lipoprotein lipase** has a low Km for triacylglycerol, about one-tenth of that for the enzyme in adipose tissue. This enables the delivery of fatty acids from triacylglycerol to be **redirected from adipose tissue to the heart in the starved state** when the plasma triacylglycerol decreases. A similar redirection to the mammary gland occurs during lactation, allowing uptake of lipoprotein triacylglycerol fatty acid for **milk fat** synthesis. The **VLDL receptor** plays an important part in the delivery of fatty acids from VLDL triacylglycerol to adipocytes by binding VLDL and bringing it into close contact with

lipoprotein lipase. In adipose tissue, **insulin** enhances lipoprotein lipase synthesis in adipocytes and its translocation to the luminal surface of the capillary endothelium.

Reaction with lipoprotein lipase results in the loss of 70% to 90% of the triacylglycerol of chylomicrons and in the **loss of apo C (which returns to HDL) but not apo E, which is retained.**

The resulting **chylomicron remnant** is about half the diameter of the parent chylomicron and is relatively enriched in cholesterol and cholesteryl esters because of the loss of triacylglycerol (Figure 3). Similar changes occur to VLDL, with the formation of **VLDL remnants** (also called **intermediate-density lipoprotein (IDL)** (Figure 4).

The Liver Is Responsible for the Uptake of Remnant Lipoproteins

Chylomicron remnants are taken up by the liver by receptor-mediated endocytosis, and the cholesteryl esters and triacylglycerols are hydrolyzed and metabolized. Uptake is mediated by **apo E** (Figure 3), via two apo E-dependent receptors, the **LDL (apo B-100, E) receptor** and **LRP-1 (LDL receptor-related protein-1)**. Hepatic lipase has a dual role: (1) it acts as a ligand to facilitate remnant uptake and (2) it hydrolyzes remnant triacylglycerol and phospholipid.

After metabolism to IDL, VLDL may be taken up by the liver directly via the LDL (apo B-100, E) receptor, or it may be converted to LDL. **Only one** molecule of apo B-100 is present in each of these lipoprotein particles, and this is conserved during the transformations. Thus, each LDL particle is derived from a single precursor VLDL particle (Figure 4). In humans, a relatively large proportion of IDL forms LDL, accounting for the increased concentrations of LDL in humans compared with many other mammals.

LDL IS METABOLIZED VIA THE LDL RECEPTOR

The liver and many extrahepatic tissues express the **LDL (apo B-100, E) receptor**. It is so designated because it is **specific for apo B-100** but not B-48, which lacks the carboxyl terminal domain of B-100 containing the LDL receptor ligand, and it **also takes up lipoproteins rich in apo E**. Approximately 30% of LDL is degraded in extrahepatic tissues and 70% in the liver. A positive correlation exists between the incidence of **atherosclerosis** and the plasma concentration of LDL cholesterol. The LDL (apoB-100, E) receptor is defective in **familial hypercholesterolemia**, a genetic condition which blood LDL cholesterol levels are increased, causing premature atherosclerosis.

HDL TAKES PART IN BOTH LIPOPROTEIN TRIACYLGLYCEROL & CHOLESTEROL METABOLISM

HDL is synthesized and secreted from both liver and intestine (Figure 5). However, apo C and apo E are synthesized in the liver and transferred from liver HDL to intestinal HDL when the latter enters the plasma. A major function of HDL is to act as a repository for the apo C and apo E required in the metabolism of chylomicrons and VLDL. Nascent HDL consists of discoid phospholipid bilayers containing apo A and free cholesterol. These lipoproteins are similar to the particles found in the plasma of patients with a deficiency of the plasma enzyme **lecithin:cholesterol acyltransferase (LCAT)** and in the plasma of patients with **obstructive jaundice**. LCAT—and the LCAT activator apo A-I—bind to the discoidal particles, and the surface phospholipid and free cholesterol are converted into cholesteryl esters and lysolecithin. The nonpolar cholesteryl esters move into the hydrophobic interior of the bilayer, whereas lysolecithin is transferred to plasma albumin. Thus, a nonpolar core is generated, forming a spherical, pseudomicellar HDL covered by a surface film of polar lipids and apolipoproteins. This aids the removal of excess unesterified cholesterol from lipoproteins and tissues as described below. The **class B scavenger receptor B1 (SR-B1)** has been identified as an **HDL receptor with a dual role in HDL metabolism**. In the liver and in steroidogenic tissues, it binds HDL via apo A-I, and cholesteryl ester is selectively delivered to the cells, although the particle itself, including apo A-I, is not taken up. In the tissues, on the other hand, SR-B1 mediates the acceptance of cholesterol effluxed from the cells by HDL, which then transports it to the liver for excretion via the bile (either as cholesterol or after conversion to bile acids) in the process known as **reverse cholesterol transport** (Figure 5). **HDL_3** , generated from discoidal HDL by the action of LCAT, accepts cholesterol from the tissues via the SR-B1 and the cholesterol is then esterified by LCAT, increasing the size of the particles to form the less dense **HDL_2** . HDL_3 is then reformed, either after selective delivery of cholesteryl ester to the liver via the SR-B1 or by hydrolysis of HDL_2 phospholipid and triacylglycerol by hepatic lipase and endothelial lipase. This interchange of HDL_2 and HDL_3 is called the **HDL cycle** (Figure 5). Free apo A-I is released by these processes and forms **preβ-HDL** after associating with a minimum amount of phospholipid and cholesterol. Surplus apo A-I is destroyed in the kidney. A second important mechanism for reverse cholesterol transport involves the **ATP-binding cassette transporters A1 (ABCA1) and G1 (ABCG1)**. These transporters are members of a family of transporter proteins that couple the hydrolysis of ATP to the binding of a substrate, enabling it to be transported across the membrane. ABCG1 mediates the transport of cholesterol from cells to HDL, while ABCA1 preferentially promotes efflux to poorly lipidated particles such as preβ-HDL or apo A-1, which are then converted to

HDL_3 via discoidal HDL (Figure 5). Preβ-HDL is the **most potent** form of HDL **inducing cholesterol efflux from the tissues**.

HDL concentrations vary reciprocally with plasma triacylglycerol concentrations and directly with the activity of lipoprotein lipase. This may be due to surplus surface constituents, eg, phospholipid and apo A-I, being released during hydrolysis of chylomicrons and VLDL and contributing toward the formation of preβ-HDL and discoidal HDL. HDL_2 concentrations are **inversely related to the incidence of atherosclerosis**, possibly because they reflect the efficiency of reverse cholesterol transport. **HDL_c (HDL_1)** is found in the blood of diet-induced hypercholesterolemic animals. **It is rich in cholesterol, and its sole apolipoprotein is apo E**. It appears that all plasma lipoproteins are interrelated components of one or more metabolic cycles that together are responsible for the complex process of plasma lipid transport.

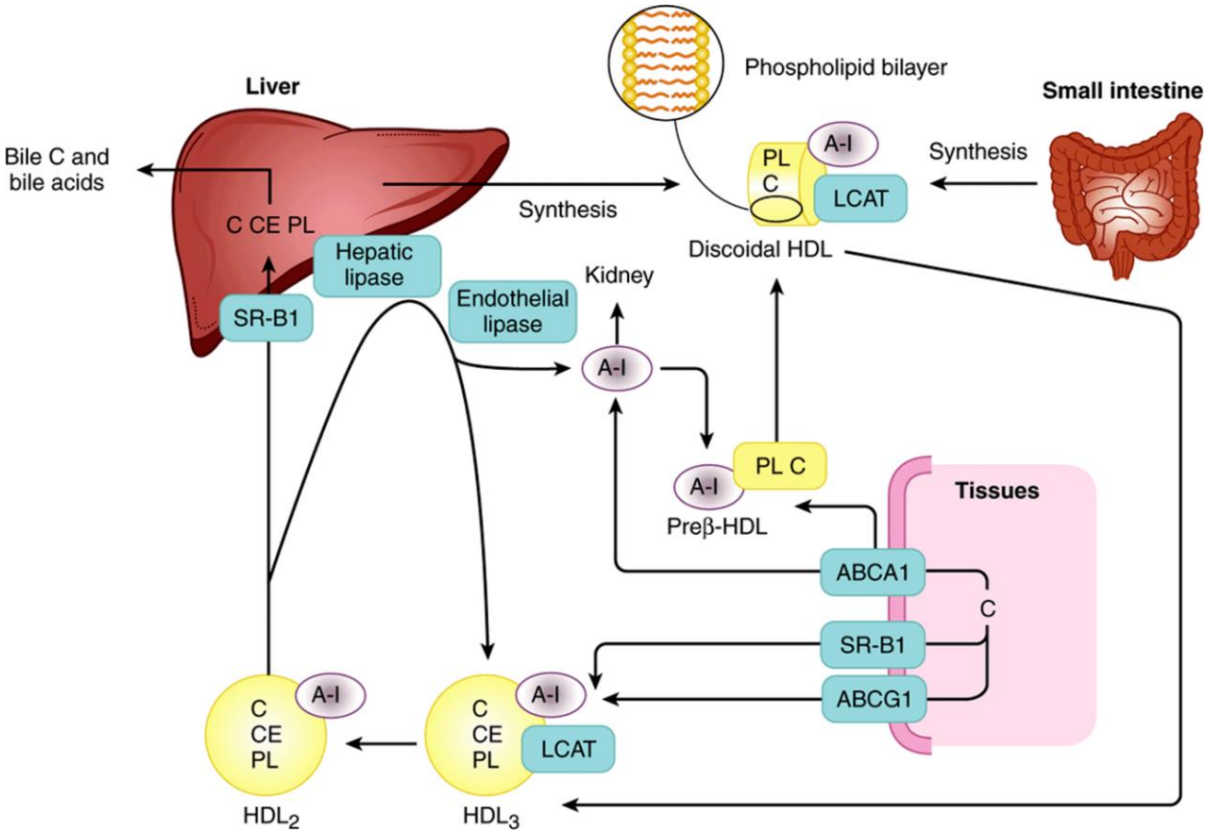

Figure 5 Metabolism of high-density lipoprotein (HDL) in reverse cholesterol transport. (A-I, apolipoprotein A-I; ABCA 1, ATP-binding cassette transporter A1; ABCG1, ATP-binding cassette transporter G1; C, cholesterol; CE, cholesteryl ester; LCAT, lecithin:cholesterol acyltransferase; PL, phospholipid; SR-B1, scavenger receptor B1.) PreβHDL, HDL_2 , HDL_3 —see Table 1. Surplus surface constituents from the action of lipoprotein lipase on chylomicrons and VLDL are another source of preβ-HDL. Hepatic

lipase activity is increased by androgens and decreased by estrogens, which may account for higher concentrations of plasma HDL_2 in women.

THE LIVER PLAYS A CENTRAL ROLE IN LIPID TRANSPORT & METABOLISM

The liver carries out the following major functions in lipid metabolism:

1. It facilitates the digestion and absorption of lipids by the production of **bile**, which contains cholesterol and bile salts synthesized within the liver de novo or after uptake of lipoprotein cholesterol.
2. It actively **synthesizes and oxidizes fatty acids** and also synthesizes triacylglycerols and phospholipids.
3. It converts **fatty acids to ketone bodies (ketogenesis)**.
4. It plays an integral part in the synthesis and metabolism of plasma lipoproteins.

Hepatic VLDL Secretion Is Related to Dietary & Hormonal Status

The cellular events involved in VLDL formation and secretion have been described above (Figure 2) and are shown in Figure 6. Hepatic VLDL assembly requires the synthesis of apoB100 and a source of triacylglycerol. ApoB100 is synthesized on polyribosomes and translocated to the lumen of the endoplasmic reticulum as it is formed. As the protein enters the lumen it is lipidated with phospholipid the aid of the **microsomal triglyceride transfer protein (MTP)**, which also facilitates the transfer of triacylglycerol across the ER membrane, and apoB-containing **VLDL2** (or precursor VLDL) particles are formed. The triacylglycerol (TG) is derived from lipolysis of cytosolic TG lipid droplets and reesterification in a pathway requiring phospholipid derivatives and diacylglycerol acyl transferases. TG not used for VLDL1 formation is recycled to the cytosolic droplets. After assembly in the ER, VLDL2 are carried in COPII vesicles to the golgi, where they fuse with TG-rich lipid droplets to produce **VLDL1**. Phosphatidic acid produced by the action of phospholipase D when activated by a small GTP binding protein called **ADP-ribosylation factor-1 (ARF-1)** is needed for the formation of the TG-rich particles and/or VLDL2. Although some VLDL2 particles may be secreted without fusion, most particles which leave the cell are in the form of VLDL1. These nascent VLDL then acquire apolipoproteins C and E from HDL in the circulation to become mature VLDL.

Triacylglycerol for VLDL formation is synthesized from FFA. The fatty acids used are derived from two possible sources: (1) de novo synthesis within the liver from **acetyl-CoA** derived mainly from carbohydrate (perhaps not so important in humans) and (2) uptake of **FFA** from the circulation. The first source is predominant in the well-fed condition, when fatty acid synthesis is high and the level of circulating FFAs is low. As

triacylglycerol does not normally accumulate in the liver in these conditions, it must be inferred that it is transported from the liver in VLDL as rapidly as it is synthesized. FFAs from the circulation are the main source during starvation, the feeding of high-fat diets, or in diabetes mellitus, when hepatic lipogenesis is inhibited. Factors that enhance both the synthesis of triacylglycerol and the secretion of VLDL by the liver include (1) the fed state rather than the starved state; (2) the feeding of diets high in carbohydrate (particularly if they contain sucrose or fructose), leading to high rates of lipogenesis and esterification of fatty acids; (3) high levels of circulating FFA; (4) ingestion of ethanol; and (5) the presence of high concentrations of insulin and low concentrations of glucagon, which enhance fatty acid synthesis and esterification and inhibit their oxidation.

Insulin suppresses hepatic VLDL secretion both by **inhibiting** apo 100 synthesis and by **inhibiting** the conversion of the smaller VLDL2 into VLDL1 by fusion with bulk TG. Some other factors which are known to inhibit or prevent VLDL assembly in the liver include the antibiotic brefeldin A, which inhibits the action of ARF-1; the sulfonylurea hypoglycemic drug, tolbutamide, dietary ω3 fatty acids, and orotic acid, an intermediate in the synthesis of pyrimidines decrease the rate of TG lipolysis; and a defect in the MTP gene. Glucose, on the other hand, enhances VLDL production by promoting TG lipolysis. The regulation of VLDL formation in the liver is complex and involves interactions between hormonal and dietary factors that are not yet fully understood.

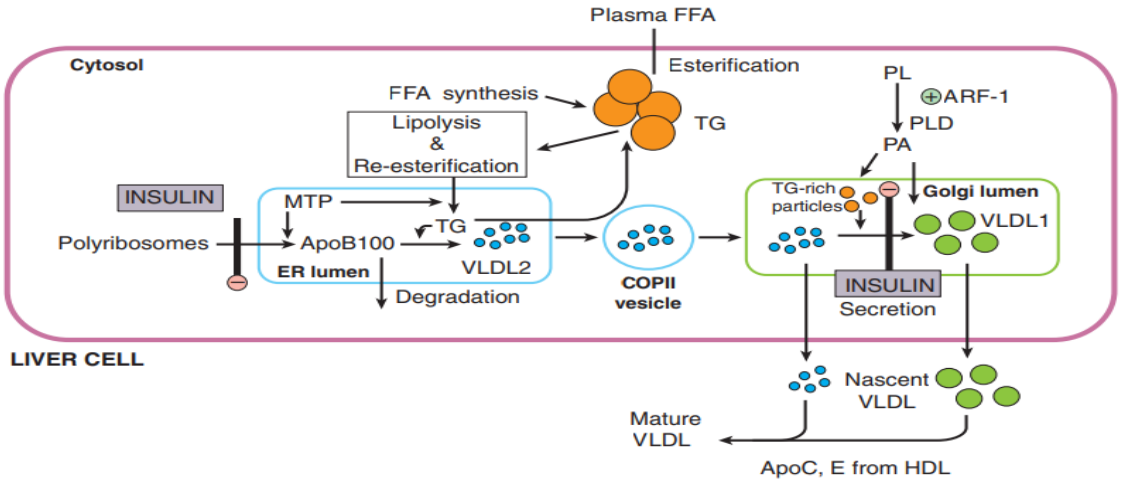

Figure 6 The assembly of very low density lipoprotein (VLDL) in the liver (Apo, apolipoprotein; ARF-1, ADP-ribosylation factor-1; FFA, free fatty acids; HDL, high-density lipoproteins; MTP, microsomal triacylglycerol transfer protein; PA, phosphatidic acid; PL, phospholipid; PLD, phospholipase D; TG, triacylglycerol.) The pathways indicated form a basis for events depicted in Figure 2. Apo B-100 is synthesized on polyribosomes and is lipidated with PL by MTP as it enters the ER lumen. Any excess is degraded in

proteasomes. TG derived from lipolysis of cytosolic lipid droplets followed by resynthesis is transferred into the ER lumen with the aid of MTP and interacts with apoB-100 forming VLDL2. Excess TG is recycled to the cytosolic lipid droplets.VLDL2 are translocated to the golgi in COPII vesicles where they fuse with TG-rich particles to form VLDL1. PA is produced by activation of PLD by ARF-1 and is incorporated into the TG-rich VLDL1 and/or VLDL2. Both VLDL1 and VLDL2 may be secreted into the blood. **Insulin inhibits VLDL secretion by inhibiting apoB-100 synthesis and the formation of VLDL1 from VLDL2.**

ADIPOSE TISSUE IS THE MAIN STORE OF TRIACYLGLYCEROL IN THE BODY

Triacylglycerols are stored in adipose tissue in large lipid droplets and are continually undergoing lipolysis (hydrolysis) and reesterification. These two processes are entirely different pathways involving different reactants and enzymes. This allows the processes of esterification or lipolysis to be regulated separately by many nutritional, metabolic, and hormonal factors. The **balance** between these two processes determines the magnitude of the FFA pool in adipose tissue, which in turn determines the level of FFA circulating in the plasma. Since the latter has most profound effects upon the metabolism of other tissues, particularly liver and muscle, the factors operating in adipose tissue that regulate the outflow of FFA exert an influence far beyond the tissue itself. Moreover, since the discovery in the last 20 years that adipose tissue secretes hormones such as leptin and adiponectin, known as adipokines, its role as an endocrine organ has been recognized. **Leptin**, regulates energy homeostasis by stimulating energy use and limiting food intake. If it is lacking, food intake may be uncontrolled, causing obesity. **Adiponectin** modulates glucose and lipid metabolism in muscle and liver, and enhances the sensitivity of tissues to insulin.

The Provision of Glycerol-3-Phosphate Regulates Esterification: Lipolysis Is Controlled by Hormone-Sensitive Lipase

Triacylglycerol is synthesized from acyl-CoA and glycerol-3-phosphate. Since the enzyme **glycerol kinase** is not expressed in adipose tissue, glycerol cannot be utilized for the provision of glycerol-3-phosphate, which must be supplied from glucose via glycolysis (Figure 7).

Triacylglycerol undergoes hydrolysis by a **hormone-sensitive lipase** to form FFA and glycerol. **This lipase is distinct from lipoprotein lipase**, which catalyzes lipoprotein triacylglycerol hydrolysis before its uptake into extrahepatic tissues. Since the glycerol cannot be utilized, it enters the blood and is taken up and transported to tissues such as the liver and kidney, which possess an active glycerol kinase. The FFA formed by lipolysis

can be reconverted in adipose tissue to acyl-CoA by **acyl-CoA synthetase** and reesterified with glycerol-3-phosphate to form triacylglycerol. Thus, **there is a continuous cycle of lipolysis and reesterification within the tissue** (Figure 7). However, when the rate of reesterification is not sufficient to match the rate of lipolysis, FFA accumulate and diffuse into the plasma, where they bind to albumin and raise the concentration of plasma-free fatty acids.

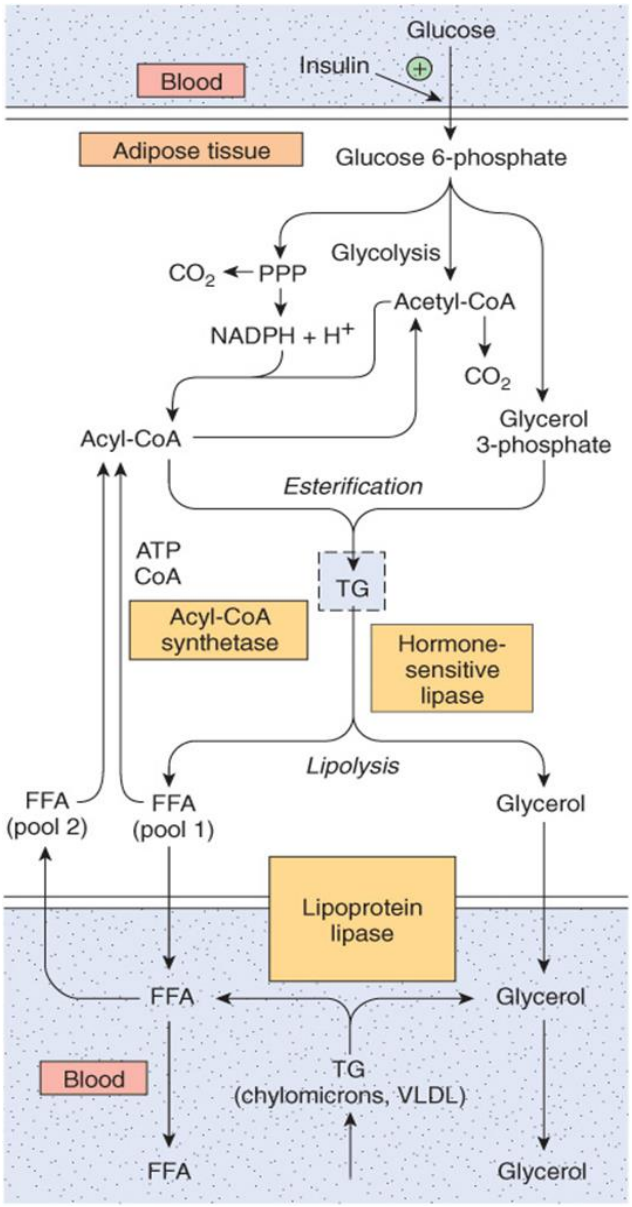

Figure 7 Triacylglycerol metabolism in adipose tissue. Hormone-sensitive lipase is activated by ACTH, TSH, glucagon, epinephrine, norepinephrine, and vasopressin and inhibited by insulin, prostaglandin E1 , and nicotinic acid. (FFA, free fatty acids; PPP, pentose phosphate pathway; TG, triacylglycerol; VLDL, very low density lipoprotein.)

Increased Glucose Metabolism Reduces the Output of FFA

When the utilization of glucose by adipose tissue is increased, **the FFA outflow decreases**. However, the release of glycerol continues, demonstrating that the effect of glucose is not mediated by reducing the rate of lipolysis. The effect is due to the provision of glycerol-3-phosphate, which enhances esterification of FFA. Glucose can take several pathways in adipose tissue, including oxidation to CO_2 via the citric acid cycle, oxidation in the pentose phosphate pathway, conversion to long-chain fatty acids, and formation of acylglycerol via glycerol 3-phosphate (Figure 7). When glucose utilization is high, a larger proportion of the uptake is oxidized to CO_2 and converted to fatty acids. However, as total glucose utilization decreases, the greater proportion of the glucose is directed to the formation of glycerol 3-phosphate for the esterification of acylCoA, which helps to minimize the efflux of FFA.

HORMONES REGULATE FAT MOBILIZATION

Adipose Tissue Lipolysis Is Inhibited by Insulin

The rate of release of FFA from adipose tissue is affected by many **hormones** that **influence either the rate of esterification or the rate of lipolysis**. Insulin inhibits the release of FFA from adipose tissue, which is followed by a fall in circulating plasma free fatty acids. Insulin also enhances lipogenesis and the synthesis of acylglycerol and increases the oxidation of glucose to CO_2 via the pentose phosphate pathway. All of **these effects are dependent on the presence of glucose** and can be explained, to a large extent, on the basis of the ability of insulin to enhance the uptake of glucose into adipose cells via the GLUT 4 transporter. In addition, **insulin increases the activity of the enzymes** pyruvate dehydrogenase, acetyl-CoA carboxylase, and glycerol phosphate acyltransferase, reinforcing the effects of increased glucose uptake on the enhancement of fatty acid and acylglycerol synthesis. These three enzymes are regulated in a coordinate manner by phosphorylation-dephosphorylation mechanisms.

Another principal action of **insulin in adipose tissue is to inhibit the activity of hormone-sensitive lipase, reducing the release not only of FFA but also of glycerol**. Adipose tissue is much more sensitive to insulin than many other tissues, which points to adipose tissue as a major site of insulin action in vivo.

Several Hormones Promote Lipolysis

Other hormones **accelerate the release of FFA from adipose tissue** and raise the plasma-free fatty acid concentration by **increasing the rate of lipolysis of the triacylglycerol stores** (Figure 8). These include **epinephrine**, **norepinephrine**, **glucagon**,

adrenocorticotropic hormone (ACTH), **α-** and **β-melanocyte-stimulating hormones (MSH)**, **thyroid-stimulating hormone (TSH)**, **growth hormone (GH)**, and **vasopressin**. Many of these activate hormone-sensitive lipase. For an optimal effect, most of these lipolytic processes require the presence of glucocorticoids and thyroid hormones. These hormones act in a **facilitatory** or **permissive** capacity with respect to other lipolytic endocrine factors.

The hormones that act rapidly in promoting lipolysis, ie, catecholamines (epinephrine and nor-epinephrine), do so by stimulating the activity of **adenylyl cyclase**, the enzyme that converts ATP to cAMP. The mechanism is analogous to that responsible for hormonal stimulation of glycogenolysis. cAMP, by stimulating **cAMP-dependent protein kinase**, activates hormone-sensitive lipase. Thus, processes which destroy or preserve cAMP influence lipolysis. cAMP is degraded to 5′-AMP by the enzyme **cyclic 3′,5′-nucleotide phosphodiesterase**. This enzyme is inhibited by methylxanthines such as **caffeine** and **theophylline**. **Insulin** antagonizes the effect of the lipolytic hormones. Lipolysis appears to be more sensitive to changes in concentration of insulin than are glucose utilization and esterification. The **antilipolytic effects** of insulin, nicotinic acid, and prostaglandin E_1 are accounted for by inhibition of the synthesis of cAMP at the adenylyl cyclase site, acting through a G_i protein. Insulin also stimulates phosphodiesterase and the lipase phosphatase that **inactivates hormone-sensitive lipase**. The effect of **growth hormone** in promoting lipolysis is dependent on synthesis of proteins involved in the formation of cAMP. **Glucocorticoids** promote lipolysis via synthesis of new lipase protein by a cAMP-independent pathway, which may be inhibited by insulin, and also by promoting transcription of genes involved in the cAMP signal cascade. These findings help to explain the role of the pituitary gland and the adrenal cortex in enhancing fat mobilization. The sympathetic nervous system, through liberation of norepinephrine in adipose tissue, plays a central role in the mobilization of FFA. Thus, the increased lipolysis caused by many of the factors described above can be reduced or abolished by denervation of adipose tissue or by ganglionic blockade.

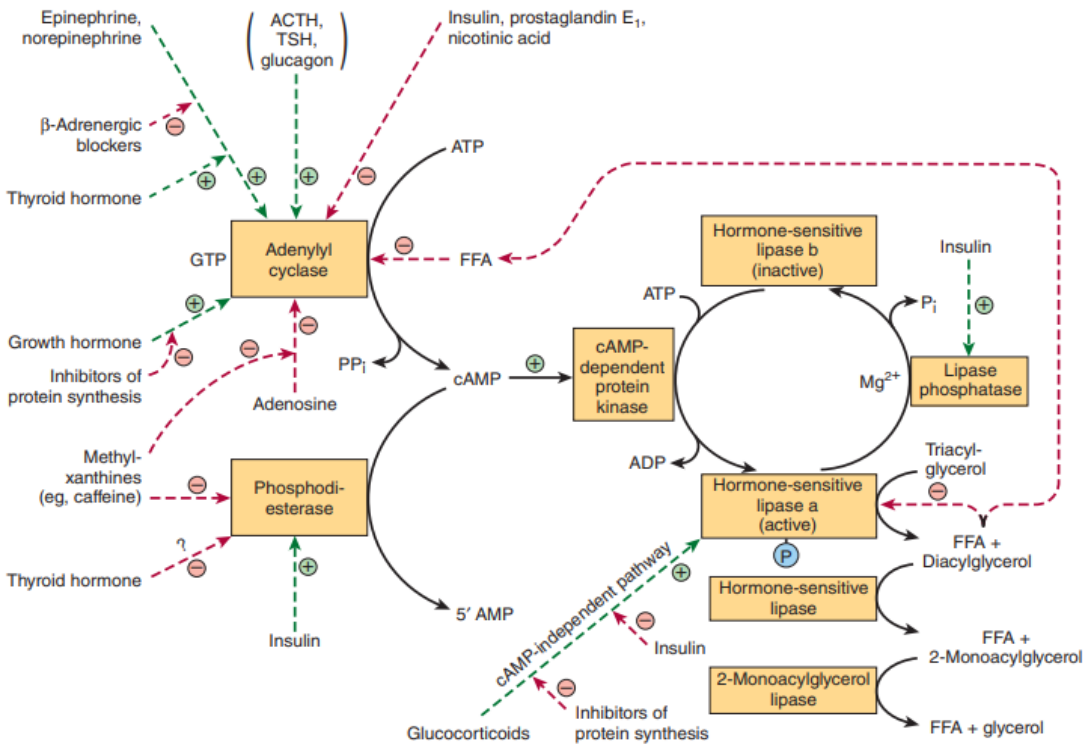

Figure 8 Control of adipose tissue lipolysis. (FFA, free fatty acids; TSH, thyroid-stimulating hormone.) Note the cascade sequence of reactions affording amplification at each step. The lipolytic stimulus is "switched off" by removal of the stimulating hormone; the action of lipase phosphatase; the inhibition of the lipase and adenylyl cyclase by high concentrations of FFA; the inhibition of adenylyl cyclase by adenosine; and the removal of cAMP by the action of phosphodiesterase. ACTH, TSH, and glucagon may not activate adenylyl cyclase in vivo since the concentration of each hormone required in vitro is much higher than is found in the circulation. Positive and negative regulatory effects are represented by broken lines and substrate flow by solid line.

Perilipin Regulates the Balance Between Triacylglycerol Storage and Lipolysis in Adipocytes

Perilipin, a protein involved in the formation of lipid droplets in adipocytes, inhibits lipolysis in basal conditions by preventing access of the lipase enzymes to the stored triacylglycerols. On stimulation with hormones which promote triacylglycerol degradation, however, the protein becomes phosphorylated and changes its conformation, exposing the lipid droplet surface to hormone-sensitive lipase and thus promoting lipolysis. **Perilipin, therefore, enables the storage and breakdown of triacylglycerol to be coordinated according to the metabolic needs of the body**.

Human Adipose Tissue May Not Be an Important Site of Lipogenesis

In adipose tissue, there is no significant incorporation of glucose or pyruvate into long-chain fatty acids, ATP-citrate lyase, a key enzyme in lipogenesis, does not appear to be present, and other lipogenic enzymes—for example, glucose-6-phosphate dehydrogenase and the malic enzyme—do not undergo adaptive changes. Indeed, it has been suggested that in humans there is a **"carbohydrate excess syndrome"** due to a unique limitation in ability to dispose of excess carbohydrate by lipogenesis. In birds, lipogenesis is confined to the liver, where it is particularly important in providing lipids for egg formation, stimulated by estrogens.

SUMMARY

■ Since nonpolar lipids are insoluble in water, for transport between the tissues in the aqueous blood plasma they are **combined with** amphipathic lipids and proteins to make water-miscible lipoproteins.

■ Four major groups of lipoproteins are recognized. Chylomicrons transport lipids resulting from digestion and absorption. Very low density lipoproteins (VLDL) transport triacylglycerol from the liver. Low-density lipoproteins (LDL) deliver cholesterol to the tissues, and high-density lipoproteins (HDL) remove cholesterol from the tissues and return it to the liver for excretion in the process known as **reverse cholesterol transport**.

■ Chylomicrons and VLDL are metabolized by hydrolysis of their triacylglycerol, and lipoprotein remnants are left in the circulation. These are taken up by liver, but some of the remnants (IDL), resulting from VLDL form LDL, which is taken up by the liver and other tissues via the LDL receptor.

■ **Apolipoproteins** constitute **the protein moiety of lipoproteins**. They act as enzyme activators (eg, apo C-II and apo A-I) or as ligands for cell receptors (eg, apo A-I, apo E, and apo B-100).

■ Triacylglycerol is the main storage lipid in adipose tissue. Upon mobilization, FFA and glycerol are released. FFAs are an important fuel source.