Chapter 1
Basics in Lipoprotein Metabolism

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Abbreviations

APOA1	Apolipoprotein A-I
ApoB	Apolipoprotein B
APOE	Apolipoprotein E
CHD	Coronary heart disease
CMs	Chylomicrons
CVD	Cardiovascular disease
FH	Familial hyperlipidemia
FXR	Farsenoid X Receptor
HA	Hypoalphalipoproteinemia
HDL	High-density lipoprotein
HMG-CoA reductase	hydroxymethylglutaryl-coenzyme A reductase
IDL	Intermediate-density lipoprotein
LCAT	Lecithin-cholesterol acetyltransferase
CETP	Cholesterol ester transfer protein
LDL	Low-density lipoprotein
LDL-C	Low density lipoprotein- cholesterol
LDLR	Low density lipoprotein receptor
LRP	Lipoprotein receptor protein
LXR	Liver X ReceptorLp(a)—Lipoprotein(a)
LPL	Lipoprotein lipase
PPAR-alpha	Peroxisome proliferator-activated receptor alpha

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PPARs	Peroxisome proliferator-activated receptors
RBC	Red blood cells
RXR	Retinoic Acid Receptor
TG	Triglycerides
VLDL	Very-low-density lipoprotein

Overview of Lipoproteins

Structure of Human Plasma Lipoproteins Plasma lipoproteins contain a hydrophobic nonpolar lipid core of cholesteryl esters and triaclyglycerols and are illustrated in Fig. 1.1. They are surrounded on the surface by a more polar, hydrophilic coat of apolipoproteins, phospholipids, and unesterified cholesterol. The relative amount of core lipid to protein determines the size and density of the lipoprotein particles. Larger lipoproteins contain more core lipid and are less dense than the smaller lipoproteins. Lipoprotein transport in the plasma is made possible by apolipoproteins. These are amphipathic molecules that solubilize the nonpolar lipids. Apolipoproteins also have active roles in the metabolism of the lipoproteins and act as ligands for lipoprotein receptors and cofactors for lipolytic enzymes and lipid transferases. The apolipoproteins are named based on an alphabetical nomenclature, starting with A, B, C, and so forth. More than 12 apolipoproteins have been described. Among these, apo B and apo A-I have a paramount role. Elevated levels of the apo B–containing lipoproteins and low levels of the apo A-I–containing lipoproteins are associated with CHD.

Apo B–Containing Lipoproteins

These include chylomicrons, chylomicron remnants, VLDL, VLDL remnants (also known as IDL), LDL and Lipoprotein(a) (Lp[a]). Lp(a) consists of a molecule of

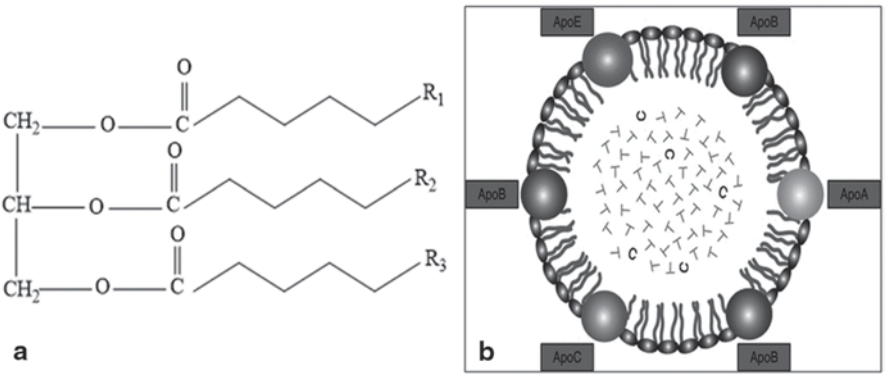

Fig. 1.1 Plasma lipoproteins. **a** Triacylglycerol. **b** Chylomicron with triacylglycerol and cholesterol ester core surrounded by a phospholipid and apolipoprotein membrane

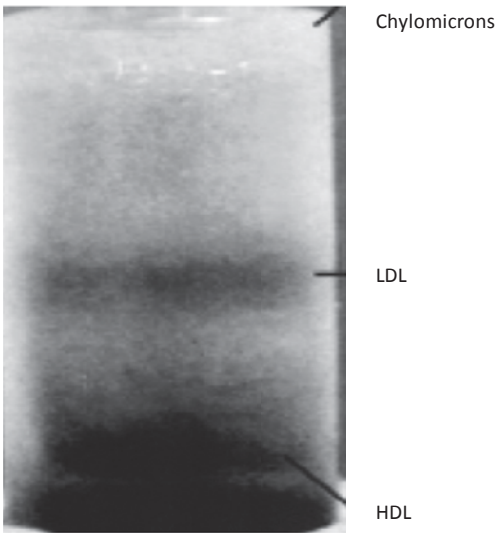

Fig. 1.2 Separation of plasma lipoproteins by density: This is an illustration of a plasma sample obtained after centrifugation demonstrating the separation of the plasma into distinct bands based on density. This sample reveals increased chylomicrons. Note that Chylomicrons are less dense, float and have a whitish appearance giving them the chyle description

LDL connected through a disulfide bridge on apo B to apo (a), a protein homologous to plasminogen [1].

ApoB containing lipoproteins are further classified based upon differences in the size and/or density. Generally VLDL and LDL are divided into large, intermediate, and small lipoprotein subclasses. An example of separation of lipoprotein by density is illustrated in Fig. 1.2 with centrifuged serum obtained from a hyperchylomicronemic patient postprandially. ApoB containing lipoproteins are lipid rich, and have an important role in carrying cholesterol and triglycerides in the circulation.

ApoA containing lipoproteins

These proteins form HDL particles and are also found on chylomicrons. They are critical components of reverse cholesterol and are excellent initial acceptors of cholesterol from peripheral tissues. The majority of HDL lipoproteins contain both AI and A-II. HDL lipoproteins are far more complex than LDL and VLDL, very heterogeneous and have a greater density due to enrichment with proteins. Recent studies suggest that these lipoproteins contain combinations of over 100 proteins [2–4] that are unified by having ApoA-1 as their major backbone.

ApoC and ApoE containing lipoproteins

These are "conductor" lipoproteins that can orchestrate the efficiency of lipoprotein metabolism. These two lipoprotein classes are being constantly exchanged between HDL and VLDL/LDL particles after meals [5]. Their capacity to move between particles regulates the rate of fatty acid, cholesterol and phospholipid turnover [6].

Table 1.1 Classification of plasma lipoproteins by density

Fraction	Density (g/mL)	Composition
Very low density lipoproteins/ chylomicrons	1.006	Apo B48 (Chylomicrons), ApoB100(VLDL), Apo E, Apo A-I and Apo CIII makes most of these proteins
Low density lipoproteins	1.006–1.06	Apo B-100 and Apo E defines the majority of these proteins
High density lipoproteins	1.06–1.21	Proteins with multiple amphipathic helical domains: Apo A-I and Apo A-II make 90% of these proteins HDL is characterized by an increase in surface phospholipid to cholesterol surface ratio, making it an excellent cholesterol acceptor
Non-lipidated plasma proteins	1.21	Albumin is a major component of this fraction

Table 1.2 Types and functions of apolipoproteins

Apo A-I	HDL structural protein, it activates LCAT and participates in reverse cholesterol transport
Apo A-II	Forms HDL and activates hepatic lipase
Apo A-IV	Activates LCAT. Also involved in triglyceride metabolism
Apo B-48	Structural component of chylomicrons. Binds to LDL receptor
Apo B-100	Structural component of all lipoproteins except HDL and chylomicrons. Binds to LDL receptor
Apo C-I	Inhibits lipoprotein binding to LDL receptor. Activates LCAT
Apo C-II	Activates lipoprotein lipase
Apo C-III	Inhibits lipoprotein lipase. Antagonizes Apo-E, inhibiting liver VLDL uptake
Apo-E	LDL and LRP receptor ligand, and is essential component of reverse cholesterol transport and triacylglycerol clearance

ApoC lipoproteins regulate lipoprotein lipase activity. ApoE lipoproteins determine the ability to efflux cholesterol in the periphery and the fate of cholesterol rich particles secondary to its affinity to the LDL receptor [7] (Table 1.1, Table 1.2).

Lipoprotein Metabolism

Chylomicron Metabolism

The average daily intake of lipids in the US is 81 grams, of which more than 90% is triacylglycerol (also known as triglycerides) [8]. The remaining dietary lipids consist of cholesterol, cholesterol esters, phospholipids and fatty acids. Triacylglycerols

are not soluble in the blood and therefore do not circulate free in the serum but are transported as chylomicrons and VLDL particles [9]. Following ingestion, TG, cholesterol esters and phospholipids are digested by lingual lipase and gastric lipase in the stomach. TG is further hydrolyzed by pancreatic lipase to a mixture of 2-monoacylglycerol and free fatty acids, while cholesterol esters are processed by cholesterol esterase to cholesterol and free fatty acids. These products are packed with bile salts and fat soluble vitamins into mixed micelles which are then taken up by the mucosal cells (enterocytes) of the intestinal villi. Within the enterocytes TG are reformed through re-acylation of the 2-monoacylglycerols by monoacylglycerol acyltransferase and diacylglycerol acyltransferase, while cholesterol is esterified with fatty acids by cholesterol acyltransferase [10]. The reformed TG and cholesterol esters are packaged as chylomicrons (lipid droplets surrounded by a phospholipid layer, unesterfied cholesterol and additional apolipoprotein B-48 and apolipoprotein A1), released into the lymphatic vessels where they are transported from the thoracic duct to the bloodstream. Each chylomicron particle contains a single molecule of apoB48 and has a hydrophobic core consisting mainly of triglyceride with a small amount of cholesteryl esters. The ratio of triglycerides to cholesterol in chylomicrons is 8:1 or greater. This differentiates them from VLDL, IDL and chylomicron remnants which have much lower triglyceride to cholesterol ratio. Approximately 80–90 % of chylomicrons are triglycerides, and 55 % of VLDL are triglycerides [9, 10]. It takes approximately 10–12 h to clear the blood of chylomicrons after a meal. Peak lipidemia is reached in approximately 3–5 h and persists for another 6–8 h [9].

Once in plasma, the apo B48 on the chylomicron surface activates lipoprotein lipase (LPL) that is present at the endothelial surface of capillaries in most tissues of the body. Activity of LPL results in hydrolysis of the triacylglycerol in chylomicrons. As a result of LPL activity, free fatty acids are released to peripheral tissues, either as a source of energy or, in the case of adipose tissue, for storage after being re-esterified into triacylglycerol. As chylomicrons lose triacylglycerols, their particle size decreases and become relatively cholesterol enriched. A second fate of circulating TG is their transfer to HDL particles. Cholesterol ester transfer protein (CETP) mediates transfer of TG to HDL in exchange for cholesteryl esters from HDLs, as shown in Fig. 1.3. In the case of chylomicrons, these metabolic processes result in the formation of smaller, cholesterol-enriched particles (known as chylomicron remnants) that are rapidly cleared by the liver and only rarely accumulate in significant amounts in plasma. Chylomicron remnants are responsible for transporting dietary cholesterol and very efficient at taking cholesterol from HDL and RBCs to the liver [11]. Thus, they are essential components of the reverse cholesterol transport pathway to the liver. Defective clearance with an increase in circulating chylomicron remnants, as seen in individuals with abnormal apo E genotypes is considered atherogenic.

VLDL Metabolism

The initial step in VLDL synthesis involves synthesis of Apo B-100 on ribosomes attached to the endoplasmic reticulum. An enzyme called “microsomal triacylglycerol

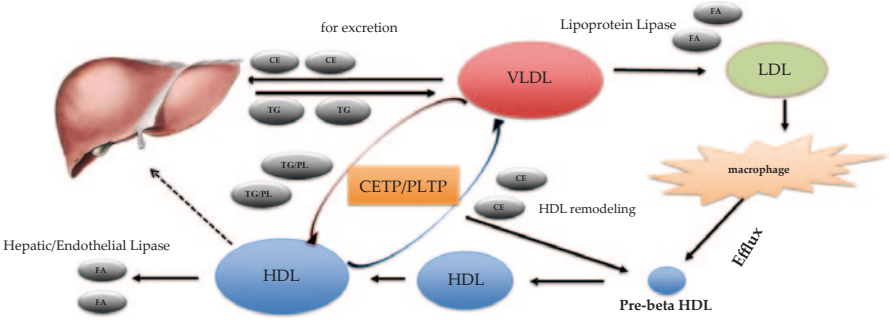

Fig. 1.3 Reverse cholesterol transport. This figure illustrates that the main mechanism for cholesterol transport back to the liver is through VLDL particles as a function of CETP in exchange for triglycerides. HDL can return cholesterol (*dashed line*), but this pathway contributes to less than 30% of the cholesterol ester pool returned to the liver [14]

transfer protein (MTTP or MTP)" assembles triacylglycerols and cholesterol with apolipoprotein B, E and a phospholipid. The next step involves transportation of fully lipidated VLDLs to the Golgi vesicles, where glycosylation proceeds, before they are transported to the plasma membrane and released into the space of Disse. Nascent VLDLs isolated from the Golgi apparatus contain newly synthesized apo E and apo C lipoproteins. They contain more phospholipids and much less unesterified cholesterol than plasma VLDLs. VLDL is secreted from the liver into the plasma. Triacylglycerols make up 50–60% of VLDL's weight. Triacylglycerols are the major fat to be transported from the liver into the bloodstream. VLDL also carries a lesser amount of cholesteryl esters in its core. It contains a number of apolipoproteins, but apo B-100 is necessary for its secretion from the liver. The circulatory half-life of VLDL particles is 30–60 min in humans. The contribution of VLDL cholesterol to the total cholesterol level is estimated by dividing the total triacylglycerol level by 5, because the average ratio of triacylglycerol to cholesterol on VLDL is 5–1. VLDL is metabolized in adipose tissue capillaries where Apo C-II on VLDL activates lipoprotein lipase (LPL) on adipose tissue capillaries. LPL is secreted into the interstitium by adipocytes and myocytes. It requires transport to the capillary lumen by Glycosylphosphatidylinositol anchored high density lipoprotein binding protein 1 (GPIHBP1) [12]. LPL breaks down VLDL into fatty acids and glycerol. Fatty acids are taken up by the adipocytes for storage or for β oxidation in muscle.

During lipoprotein mediated lipolysis, VLDL is remodeled by the activities of cholesterol ester transfer protein (CETP) and phospholipid transfer protein (PLTP). CETP exchanges cholesterol esters between VLDL and HDL for triacylglycerols. PLTP facilitates the transfer of phospholipids from VLDL to HDL [13]. By this process, HDL unloads its cholesterol content to VLDL and LDL particles to be returned to the liver, primarily on VLDL particles as demonstrated in Fig. 1.3 [14] and acquires phospholipids that are essential for its capacity to accept cholesterol from cells and bind to steroidogenic cells. Recent studies confirm that HDL particles

(together with albumin) act as a "shuttle" to move cholesterol to other lipoproteins that have a greater capacity to carry and transport cholesterol (such as LDL) [15]. The remnant VLDL particle is called Intermediate density lipoprotein (IDL). IDL has Apo E which can bind to the LDL or LRP receptor and is taken up by the liver. IDL can also be acted upon by hepatic lipase which removes the remaining triacylglycerol leaving behind an Apo B containing triacylglycerol depleted particle known as LDL. IDL contains equal amounts of triacylglycerol and cholesterol.

The main mechanism that regulates VLDL secretion and uptake in the liver is under control of the Farsenoid X receptor (FXR) and SREBP1c transcription factors [16]. Very low density lipoprotein (VLDL) is synthesized in the liver in response to the ingestion of excess calories in the form of carbohydrates. Kinetic studies suggest that de novo lipogenesis contributes to only 3–5 % of VLDL production. However, high carbohydrate diet can increase VLDL synthesis up to 30 % and is an important mechanism for obesity induced dyslipidemia [17].Increased insulin and glucose following high carbohydrate diets stimulate SREBP1c transcription factor inducing the activity of several lipogenic enzymes (acetyl-CoA carboxylase, ATP citrate lyase, fatty acid synthase, and stearoyl-CoA desaturase). This process results in the synthesis of fatty acids that get conjugated to a glycerol backbone forming triacylglycerol. To export these fatty acids out of the liver, they are packaged with lipoproteins in the form of VLDL as described above. This is one of the mechanisms that explain the increased triglycerides (increased VLDL production) in metabolic syndrome. Clearance of hepatic triglycerides is regulated by the FXR system. Activation of hepatic FXR lowers plasma free fatty acid (FFA) and TG, likely resulting from (i) repression of hepatic TG and fatty acid (FA) synthesis as a result of SHP-dependent inhibition of SREBP-1c; (ii) induction of apoC-II and repression of apoC-III and ANGTPL3 in the liver, resulting in enhanced lipoprotein lipase (LPL) activity; (iii) induction of VLDL receptor (VLDLR) and human syndecan-1 (hSyndecan-1), thus promoting clearance of TG-rich lipoproteins; and (iv) induction of human PPARα and FA β-oxidation [18]. The use of FXR agonists in non-alcoholic fatty liver disease is discussed in Chap. 9.

LDL Metabolism

LDL is triacylglycerol depleted with only one apo B-100 particle. LDL particles have a great capacity to carry cholesterol that can facilitate cholesterol delivery to tissues for example: (1) cholesterol can be taken up by the liver via the LDL receptor for storage or repackaging (2) cholesterol can supplied to the gonads or adrenal glands with cholesterol for steroidogenesis or (3) donated for cell membrane biosynthesis. By weight LDL is approximately 50 % phospholipid and 50 % cholesteryl esters, unesterified cholesterol, and triacylglycerol. Thus, LDL carries more cholesterol per particle than other plasma lipoproteins. VLDL, IDL, and LDL particles each contain 1 molecule of apo B-100 per particle. As VLDL is converted into IDL and then LDL, the apo B-100 molecule remains with the lipoprotein particle until

it is removed from the blood. Total apo B in plasma therefore reflects the apo B on VLDL, IDL, and LDL. The LDL receptor is an essential mechanism for clearing both triglycerides and cholesterols from circulation. The liver is the main organ responsible for clearing LDL and remnant lipoproteins. Two apolipoproteins have important roles in regulating this process: Apo E and CIII. Underproduction of Apo E, or production of Apo E variants with decreased activity or levels (such as E4 genotype) delays clearance of both triglyceride and cholesterol enriched lipoproteins (chylomicron or VLDL remnants). Defects in cholesterol rich particle clearance are considered atherogenic. On one hand, overproduction of ApoE (as with the use of LXR agonists [19]) stimulates VLDL liver production and inhibits LPL mediated lipolysis. In this situation, clearance of cholesterol in TG rich lipoproteins is not impaired, and the ensuing hypertriglyceridemia is not considered atherogenic given that cholesterol from these particles is efficiently cleared by the liver LDL receptor. This mechanism might explain why isolated hypertriglyceridemia observed in familial hypertriglyceridemia syndromes is not associated with increased risk for atherosclerosis [20]. On the other hand, overproduction of both Apo E and Apo CIII (which is common after saturated fat or carbohydrate ingestion [5], and in diabetes/metabolic syndrome [21]) delays cholesterol clearance through competitive inhibition of CIII on Apo E mediated -VLDL receptor particle uptake [21]. Increased Apo CIII expression has been associated with both hypertriglyceridemia, small dense LDL formation [22] and atherosclerosis [23]. More recently, loss-of-function gene mutations of Apo CIII were associated with lower CHD risk [24, 25]. Small dense LDL particles interact more avidly with proteoglycans in the vascular wall and are more susceptible to oxidation [26], and thus considered an important contributor to atherosclerosis development.

HDL Metabolism

The HDL pathway is initiated by secretion of a nascent, disc-shaped apo A-I containing particle by hepatocytes and enterocytes, known as nascent HDL. The half-life of Apo A1 is about 4–5 days. Apo A-I in this nascent HDL particle activates the adenosine triphosphate (ATP)–binding cassette (ABC) protein, ABCA1, on the surface of peripheral cells such as macrophages. Once activated, the ABCA 1 protein transports unesterified cholesterol from the cell onto the nascent HDL particle. On the surface of HDL particle, the cholesterol is esterified by lecithin-cholesterol acyl transferase (LCAT) and its cofactor, apo A-I. As it circulates, nascent HDL particles are transformed into a mature, spherical HDL particle that contains cholesteryl ester in its core. The resulting cholesterol ester (CE) changes the shape of the HDL particle and is transferred via CETP to very low density (VLDL) and low density (LDL) lipoproteins after which it is finally taken up by the hepatic apo B, E receptors. The esterification of UC may be associated with remodeling of HDL sub-populations and with the formation of larger HDL particles that associate with less coronary artery disease (CAD). In physiological states, HDL particles are constantly shifting

between larger and smaller particles that facilitate the transport of cholesterol, triacylglycerol and phospholipids between the different lipoproteins and are important to the first step of reverse cholesterol transport. Obesity can accelerate HDL catabolism. TG enriched HDL in obesogenic states are susceptible to digestion by hepatic lipase resulting in smaller HDL particles that can get cleared by the kidney faster than larger HDL particles. An illustration of reverse cholesterol transport is provided in Fig. 1.3. Controversies on whether increasing HDL cholesterol represents improvements in reverse cholesterol transport are discussed in Chap. 5.

Phospholipid Metabolism

There are two major groups of phospholipids: glycerophospholipids with the glycerol backbone (such as phosphatidyl choline, ethanolamine or serine-PC, PE, PS), and sphingophospholipids with a sphingosine backbone (such as sphingomylin-SM and ceramide). An illustration of phospholipids is presented in Fig. 1.4. Phospholipids are the major structural components of the phospholipid bilayer of cell membranes. They are also very essential components of the lipoprotein surfaces. The majority of circulating phospholipids are on lipoproteins where they participate in cholesterol transport from and to tissues. Phospholipids have distinct roles in the process that leads to cardiovascular disease depending whether they circulate on HDL or LDL particles, and if they are oxidized or not. The major lipid constituents of HDL are phospholipids: HDL-PC, and HDL sphingomyelin (HDL-SM), followed by cholesterol and cholesteryl ester. There are three major mechanisms for phospholipid incorporation or assembly into lipoproteins. The first mechanism involves lipidation of ApoB containing particles in the liver by the activity of PLTP, where PC and SM are incorporated into the nascent VLDL particle. The second mechanism

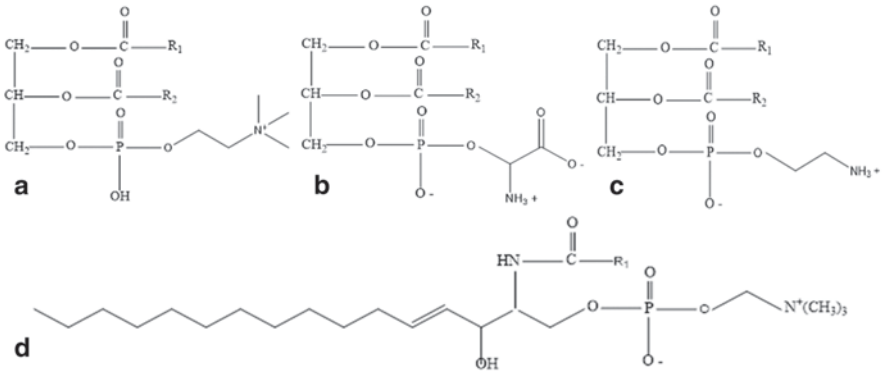

Fig.1.4 Phosphoplipids. **a** Phosphatidylcholine. **b** Phosphatidylserine. **c** Phosphatidylethanolamine. **d** Sphingomyelin. Phospholipids are amphiphilic molecules that have a polar head and a non-polar tail. These properties allow phospholipids to form the lipid bilayer portion of cell membranes and spontaneously form small vesicles in water

involves extracellular lipidation of nascent HDL as a function of ABCA-1 efflux of phospholipids. Recently, Sorci-thomas showed that the composition of nascent HDL is very similar to that of lipid rafts of plasma membranes and are formed by the activity of ABCA-1 [27]. The third mechanism involves the activity of phospholipid transfer protein (PLTP) in the plasma. PLTP transfers surface phospholipids from apoB containing particles to HDL during VLDL and chylomicron hydrolysis by lipoprotein lipase (LPL).

HDL-SM is considered atheroprotective and positively correlates with cholesterol efflux [28]. In contrast, LDL phospholipids are susceptible to oxidation and can be involved in promoting atherosclerosis. For example, LDL extracted from human atherosclerotic lesions is highly enriched with SM compared with plasma LDL, and SM carried into the arterial wall (associated with LDL) is acted upon by sphingomyelinase, increasing lesion ceramide levels, promoting LDL aggregation and foam cell formation [29]. More recent lipidomic studies suggest that certain species of shingolipids better define high risk CVD patients. Specifically, decreases in the long chain SM sphingolipids with an increase in the long chain ceramide sphingolipids characterize the plasma lipidomic profiles of high risk CVD.

Fatty Acids

Fatty acids have a hydrophilic carboxylic acid head and hydrophobic hydrocarbon tail. Fatty acids can be saturated or unsaturated (Fig. 1.5). Omega-3 fatty acids (also called ω-3 fatty acids or *n*-3 fatty acids) are polyunsaturated fatty acids (PUFAs) with a double bond (C=C) at the third carbon atom from the methyl (omega) end of the carbon chain. Fatty acids can be part of the cell membrane (part of phospholipids), or an energy source for the cell or a building block for more complex lipids. Fatty acid transportation and storage involve packaging fatty acids intro triglycerides (o triacylglycerols). Fatty acids can circulate unesterified, or bound to albumin. The regulation of fatty acid metabolism is coordinated in the liver, and their fate

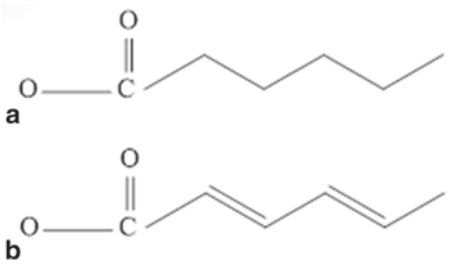

Fig. 1.5 Fatty Acids. **a** Saturated fatty acid (*Hydrocarbon chain only contains single bonds*). **b** Unsaturated fatty acid (*Hydrocarbon chain with single and double bonds*). Fatty acids have a hydrophilic carboxylic acid head and hydrophobic hydrocarbon tail. Fatty acids can be part of the cell membrane, an energy source for the cell or a building block for more complex lipids

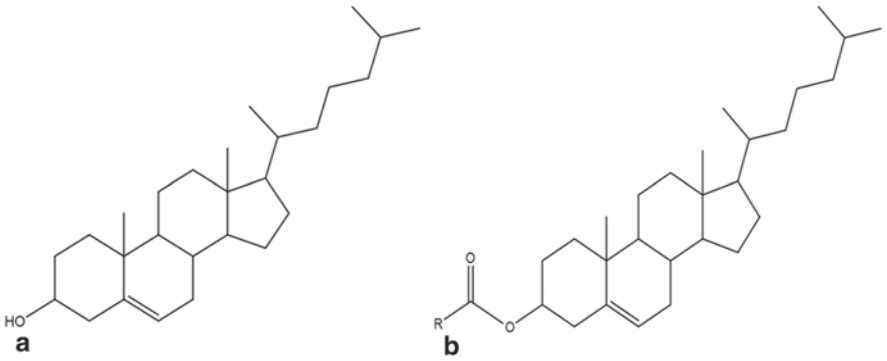

Fig.1.6 Cholesterol. **a** Cholesterol. **b** Cholesterol ester. Cholesterol is composed of a rigid ring structure, short hydrocarbon chain and polar hydroxyl group. Due to cholesterols stable structure cells are unable to degrade it. Therefore, it is either stored in the cell as a cholesterol ester or effluxed as free cholesterol

is dependent on lipase activities. In the muscle, lipoprotein lipase favors uptake of fatty acids for beta oxidation to generate energy. Each fatty acid molecule can liberate 9 Kcals of energy. Beta oxidation takes place in the mitochondria. In the adipose tissue, lipoprotein lipase favors storage of fatty acids in adipose cells where after uptake they are packaged into triacylglycerol droplets. In the liver, hepatic lipase regulates fatty acid liver uptake. Saturated fatty acid ingestion increases both LDL and HDL cholesterol. In contrast, ingestion of polyunsaturated fatty acids inhibits lipogenesis reducing triacylglycerol levels. Fatty acids regulate liver cholesterol and triacylglycerol by mechanisms that involve the liver sortlin 1 receptors and ERK signaling [30].

Cholesterol Metabolism

Cholesterol is composed of a rigid ring structure, short hydrocarbon chain and polar hydroxyl group and illustrated in Fig. 1.6. Cholesterol can circulate in plasma or can be packaged with lipoproteins in the form of free cholesterol or cholesterol esters. There is strong evidence from human and animals studies that link abnormal cholesterol metabolism to the development of atherosclerosis. Nikolai N. Anichkov first demonstrated the role of cholesterol in the development of atherosclerosis [31]. His classic experiments in 1913 involved feeding rabbits cholesterol-rich diets where he documented atherosclerotic lesions in the aorta. These experiments paved the way to our current understanding of the role of cholesterol in cardiovascular disease. Both human epidemiologic and genetic studies, together with the more recent statin intervention studies demonstrate a consistent strong association between non-HDL cholesterol levels and cardiovascular risk.

a) Sources of Cholesterol for the Humans There are two sources of cholesterol for the human body, de novo synthesis and diet. Biosynthesis of cholesterol accounts

for the majority of serum cholesterol (60–80 %) even when subjects are on a high cholesterol diet [32]. Intestinal absorption of cholesterol is the principal mechanism that regulates the contribution of dietary cholesterol to total cholesterol levels, with reduced cholesterol absorption at times of increased cholesterol consumption [32]. However, animal cholesterol intake efficiently shuts down liver synthesis of cholesterol and ultimately, cholesterol levels increase after prolonged cholesterol feeding. In contrast, ingestion of plant sterols can significantly decrease the amount of cholesterol absorbed and increase liver synthesis of cholesterol [33]. Niemann–Pick C1-Like 1 (NPC1L1) mediates intestinal cholesterol absorption and biliary cholesterol re-absorption [34]. This transporter is also the target of ezetimibe, an inhibitor of dietary cholesterol uptake which has been approved for the treatment of hypercholesterolemia. In contrast to NPC1L1, the heterodimer of ATP-binding cassette (ABC) transporters G5 (ABCG5) and G8 (ABCG8) has been shown to inhibit the absorption of cholesterol and plant sterols from the diet by mediating the efflux of these sterols from enterocytes back into gut lumen, and by promoting efficient secretion of cholesterol and plant sterols from hepatocytes into bile [35]. Sitosterolemia, a rare autosomal recessive disorder, is characterized by markedly elevated plasma levels of plant sterols and modest increases in plasma cholesterol, which is attributable to the hyper absorption of these sterols from the small intestine and reduced excretion into the bile [35].Our body synthesizes around 700 mg of cholesterol per day. The endoplasmic reticulum and cytoplasm are involved in the cholesterol biosynthesis. Although, any nucleated cell can synthesize cholesterol, the majority of blood cholesterol comes from the liver. Thus regulating the capacity of the liver to produce or catabolize cholesterol is critical to determining cholesterol levels in the blood. One example is the effect of statins on cholesterol metabolism. Statins can inhibit the production of cholesterol in the liver. This leads to the clearing of cholesterol from the body by up-regulating the liver LDL receptor.

b) Cellular Cholesterol Homeostasis There are three major mechanisms to control cellular cholesterol content. These are (1) de novo biosynthesis, (2) cholesterol uptake and esterification and (3) cholesterol efflux.

b.1.Cholesterol Biosynthesis The cholesterol biosynthesis is initiated when two molecules of acetyl-CoA condense to form acetoacetyl-CoA. This reaction is catalyzed by cytosolic thiolase. Acetoacetyl-CoA then condenses with another molecule of acetyl-CoA to form HMG-CoA; this reaction is catalyzed by HMG-CoA synthase. HMG-CoA is reduced to mevalonate by HMG-CoA reductase. This is the principal regulatory step in the pathway of cholesterol biosynthesis and the target of statins. The next stage is the formation of isoprenoid units from mevalonate by decarboxylation. Six isoprenoid units gather to form squalene. This in turn folds into lanosterol which is then converted into cholesterol in the membrane of the endoplasmic reticulum. Brown and Goldstein demonstrated an important mechanism by which the cell regulates its cholesterol content [36] through a cholesterol sensor system known as the sterol-regulatory-element-binding protein (SREBP-2) cleavage-activating protein (SCAP). When cholesterol levels are high, SREBP-2/SCAP is retained in the ER by binding to ISIG, a resident ER protein. When cholesterol

is low, the SREBP-SCAP complex exits from the ER, and SREBP undergoes two proteolytic cleavages. This releases the cytosolic domain of SREBP, which is then translocated into the nucleus regulating the transcription of many genes, including the LDL receptor and HMG-CoA reductase, the rate-limiting enzyme in cholesterol synthesis. Thus, this system regulates both the synthesis of cholesterol and its uptake by lipoproteins

b.2 Cellular Cholesterol Uptake and Esterification The major mechanism of cellular cholesterol uptake is through endocytic uptake of lipoproteins such as low-density lipoprotein (LDL) and hydrolysis of their cholesterol by cholesterol ester hydrolases [37]. Accumulation of free cholesterols in cells can lead to cell death [38], since cells cannot digest the cholesterol nucleus. To maintain a critical level of free cholesterol in the cell, it is stored in cells as cholesterol ester through the function of Acyl-Co A cholesterol acyl transferase (ACAT), forming lipid droplets. When there is a need for free cholesterol, cholesterol esters are hydrolyzed by neutral cholesterol ester hydrolases. The cholesterol released from the droplets can be used for cell membranes and, in steroidogenic cells, for steroid hormone synthesis. The cycle of cholesterol esterification and hydrolysis may provide an important buffering mechanism for maintaining cholesterol levels in cells. The activity of ACAT is regulated by cholesterol levels [38]. In the setting that favors the development of atherosclerosis, macrophages ingest modified LDL through scavenger receptors, a process that is independent of the LDL receptor and thus not subject to the cholesterol feedback mechanisms. Cholesterol esters build up forming foam cells. To prevent cell death, the macrophage lysosomes forms autophagosomes [39]. Cholesterol ester is hydrolyzed by the activities of lysosomal acid lipase to generate free cholesterol. These cellular cholesterol pools are dependent on the ABCA-1 transporter for cholesterol efflux as discussed below. Studies of Niemann-Pick disease type C (NPC), an inherited lysosomal storage disorder that leads to accumulation of cholesterol, have shown that a luminal protein (NPC2) and a transmembrane protein (NPC1) in late endosomes are required for efflux of cholesterol from these organelles [40].

b.3 Cholesterol Efflux and Excretion At the peripheral level (macrophages, that are of relevance to atherosclerosis), there are two major mechanisms for cholesterol efflux out of the cell to be incorporate into lipoproteins for liver excretion: First, there is an active mechanism that relies on the ABCA-1 transporter that usually gets activated after cholesterol loading of cells via Liver X Receptor (LXR) signaling. Second, there is a passive mechanism that relies on the cholesterol/phospholipid gradient between the cholesterol donor and acceptor. In the active process, the most avid cholesterol acceptor is lipid poor, Apo A-I. In the passive pathways, larger HDL particles with a large surface phospholipid to cholesterol ratio are the primary cholesterol acceptors. In addition to HDL, albumin, RBCs and other plasma proteins can accept cellular cholesterol and participate in reverse cholesterol transport [15, 41]. After cholesterol returns to the liver (chylomicrons in the fed state, VLDL and HDL in the fasting state), SREBP-1c is activated to assist with cholesterol storage, efflux or liver elimination in bile. This pathway links cholesterol and fatty

acid metabolism, perhaps as a means for the cell to achieve the appropriate ratio of cholesterol to other lipids and thereby maintain cellular membrane integrity. When insulin levels are high, SREBP-1c is transcribed at extremely high levels, and the resultant nuclear SREBP-1c activates genes necessary to produce fatty acids, which are incorporated into triglycerides. LXR-regulated genes include cholesterol 7α-hydroxylase (CYP7A1; [42]) which facilitates bile acid formation for elimination, the ATP-binding cassette transporter-1 (ABCA1; [43]) which is important for cholesterol efflux, and sterol regulatory element binding protein 1c (SREBP-1c, [44]), which governs the expression of stearoyl CoA desaturase (SCD-1) leading to the production of oleic acid needed for cholesterol esterification. Bile acids, the end products of hepatic cholesterol catabolism, are important for lipid digestion and absorption from the intestinal lumen, serve as signaling molecules, and also represent the principal means of eliminating cholesterol from the body. Importantly, in order to maintain whole body cholesterol homeostasis, approximately 5% of the bile acids secreted from the gall bladder into the duodenum are not reabsorbed and thus are excreted in the feces. FXR is a key sensor for bile acids and has a central role in maintaining bile acid homeostasis, as it regulates all aspects of bile acid metabolism, including bile acid synthesis, conjugation, secretion, absorption and refilling of the gall bladder [18]. More recently, a trans-intestinal mechanism has been described where cholesterol can be excreted directly through the intestine bypassing the liver [45].

Summary

Lipid metabolism is an integrated process through which peripheral tissues exchange cholesterol, fatty acids and phospholipids, and is mainly orchestrated in the liver. At times of high energy needs, fatty acids are oxidized in the muscle to produce energy. At times of excess calories, lipids are packaged and stored in the adipose tissue. Genetic defects in the LDL receptor or defects in particle clearance in obesity confer an increased risk for atherosclerosis, by favoring increases in circulating lipid species. The increased exposure to cholesterol or oxidized phospholipids favors inflammation in the artery wall, ultimately leading to wall thickening, plaque formation and rupture. Understanding the molecular mechanisms that facilitate lipid metabolism is critical to designing appropriate therapies to address the CVD risk.

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