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2.3.2 Lipoprotein metabolism

Erez F. Scapa, Keishi Kanno and David E. Cohen

Introduction

Lipoproteins are macromolecular aggregates of lipids and proteins that function to transport otherwise insoluble lipid molecules through the plasma. This chapter will discuss the structure and function of lipoproteins. Emphasis will be placed on the transport of triglycerides and cholesterol, which constitute the principal lipids carried by lipoprotein particles.

Triglycerides, which consist of three fatty acids esterified to a glycerol molecule, are insoluble in water [1]. Triglycerides are either absorbed from the diet following a meal or assembled by the liver. Lipoproteins transport triglycerides to muscles, which utilize the fatty acids as a key source of energy. Triglycerides are also transported to adipose tissue, where the fatty acids are taken up by adipocytes, reassembled and stored for later use by the body.

Cholesterol is a critical regulator of membrane structure and function. Its concentration in membranes preserves bilayer fluidity and governs the formation of microdomains. Microdomains facilitate the association of plasma membrane proteins that participate in critical cell functions, including signal transduction and receptor–ligand binding. In addition to its role in membrane biology, cholesterol is the substrate for bile salt and steroid hormone biosynthesis (see also Chapter 2.3.6) [2]. Oxidized cholesterol molecules (i.e. oxysterols) serve as ligands for nuclear hormone receptors, which regulate cellular lipid metabolism [3]. Although cholesterol is absorbed in substantial amounts by the intestine, there does not appear to be a dietary cholesterol requirement. This is because virtually all cells in the body synthesize cholesterol molecules.

An important determinant of the physical state of cholesterol is whether the hydroxyl group is esterified to a long-chain fatty acid. For cholesterol to reside in membranes, this hydroxyl group must be unesterified. Molecules of esterified cholesterol (i.e. cholesteryl esters) are too insoluble even to be accommodated within membrane bilayers in more than trace quantities.

The liver is the only organ capable of degrading cholesterol and eliminating it from the body. As a result, excess cholesterol

must be transported by lipoproteins through the plasma to the liver. This process is commonly referred to as reverse cholesterol transport.

Lipoprotein structure

Overview

Although different types of lipoprotein particles circulate in the plasma, their structures are similar (Fig. 1), reflecting common physicochemical mechanisms for transporting water-insoluble lipids. Lipoproteins are assembled from polar and neutral lipids, as well as specific proteins, which are referred to as apolipoproteins or apolipoproteins. Apolipoproteins are amphiphilic proteins capable of interacting with both lipids and the surrounding

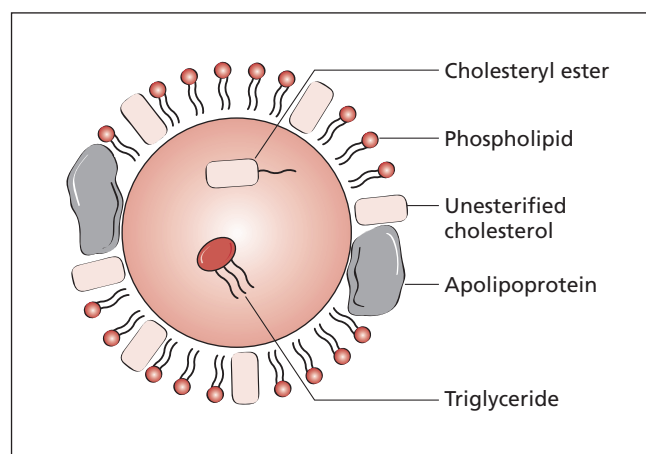


Fig. 1 Lipoprotein structure. The surface coat of lipoprotein particles comprises the polar lipids, unesterified cholesterol and phospholipids, as well as apolipoproteins. The non-polar lipids, cholesteryl esters and triglycerides, are contained in the core. Unless otherwise indicated in subsequent figures, phospholipid and unesterified cholesterol molecules are not shown for clarity.

aqueous environment of the plasma. The principal lipid components of lipoproteins include the non-polar lipids, triglycerides and cholesteryl esters, and the polar lipids, phospholipids and unesterified cholesterol [1]. The hydrophobic core of a lipoprotein particle contains non-polar lipids. The amphiphilic coat comprises polar lipids and apolipoproteins, which create a stable emulsion of the core. The phospholipids orient themselves as a monolayer surrounding the hydrophobic core, and the apolipoproteins align themselves at the lipid–plasma interface [4]. Because it is a polar lipid, unesterified cholesterol resides within the phospholipid monolayer of the surface coat of the lipoprotein.

The lipoproteins present in plasma are: chylomicrons (CM), very-low-density lipoproteins (VLDL), low-density lipoproteins (LDL), intermediate-density lipoproteins (IDL) and high-density lipoproteins (HDL). The characteristics of lipoproteins [4], which are categorized by density using ultracentrifugation or by electrophoretic mobility using agarose gels, are listed in Table 1. Lipoprotein size increases in proportion to the triglyceride and cholesteryl ester contents of the core, whereas the percentage of phospholipids comprising the coat decreases as a result of the corresponding decrease in surface-to-volume ratio. The density of lipoproteins is proportional to their protein contents, and inversely proportional to their lipid contents, whereas mobility on agarose gels depends upon both size and charge [4].

Apolipoproteins

Apolipoproteins are commonly identified using the abbreviation ‘apo’ followed by a capital letter identifying the particular protein (e.g. apoA-I). From a structural standpoint, an important common feature of these proteins is their amphiphilicity (i.e. their capacity to interact with both lipids and water). This property is primarily attributable to α -helical secondary structural elements with distinct hydrophobic and hydrophilic surfaces. These allow apolipoproteins to orient themselves at the lipid–water interface of lipoproteins and stabilize the particles. In general, apolipoproteins associate reversibly with lipids and,

Table 1 Characteristics of plasma lipoproteins.^a

| | CM | VLDL | IDL | LDL | HDL |
|--|---------|--------------|-------------|-------------|--------------------------|
| Density (g/mL) | < 0.95 | 0.95–1.006 | 1.006–1.019 | 1.019–1.063 | 1.063–1.210 |
| Diameter (nm) | 75–1200 | 30–80 | 25–35 | 18–25 | 5–12 |
| Total lipid (% wt) | 98 | 90 | 82 | 75 | 67 |
| Composition (% dry weight) | | | | | |
| Protein | 2 | 10 | 18 | 25 | 33 |
| Triglycerides | 83 | 50 | 31 | 9 | 8 |
| Unesterified cholesterol plus cholesteryl esters | 8 | 22 | 29 | 45 | 30 |
| Phospholipids (% wt lipid) | 7 | 18 | 22 | 21 | 29 |
| Electrophoretic mobility ^b | None | Pre- β | β | β | α or pre- β |

^aAdapted from ref. 4 with permission.

^bElectrophoretic mobility of lipoprotein particles is designated relative to migration of plasma α - and β -globulins.

thereby, may exchange among lipoprotein particles in plasma. The exceptions are apoB-48 and apoB-100. Hydrophobic β -sheet elements contained in apoB-48 and apoB-100 embed within the lipid aggregate of lipoprotein particles, so that these apolipoproteins remain tightly associated with their respective lipoprotein particles and do not exchange [5].

Triglyceride delivery to muscle and adipose tissue: metabolism of apoB-containing lipoproteins

Overview

The apoB-containing lipoproteins include CM, VLDL, IDL and LDL. Their primary function is the delivery of dietary and endogenous triglycerides to muscle and fat tissue. As will be discussed later, a secondary function of apoB-containing lipoproteins is to assist with reverse cholesterol transport.

ApoB

A single apoB gene is transcribed principally in the intestine and liver. Other than its tissue-specific expression, regulation of apoB expression is largely posttranscriptional. As depicted in Figure 2, a key point in the posttranscriptional regulation of apoB metabolism occurs when mRNA transcripts from the apoB gene undergo an editing process [6]. A cytosine within the mRNA coding sequence is converted to a uracil. As a result, a codon that is normally translated into a glutamine is instead converted to a stop codon, resulting in a truncated apoB protein. The editing process is the result of tissue-specific expression of proteins collectively known as the apoB editing complex. The catalytic protein apobec-1 is responsible for deaminating cytosine [6]. In humans, apoB editing occurs in the intestine and not the liver. As a result, the edited apoB transcript in the intestine is translated into a 2152-amino-acid (aa) protein. Owing to the absence of apobec-1 expression in liver, the full-length 4536-aa protein is translated. Because intestinal apoB is 48% of the length in aa of the apoB expressed in liver, the proteins are referred to as apoB-48 and apoB-100 respectively.

Assembly and secretion of apoB-containing lipoproteins

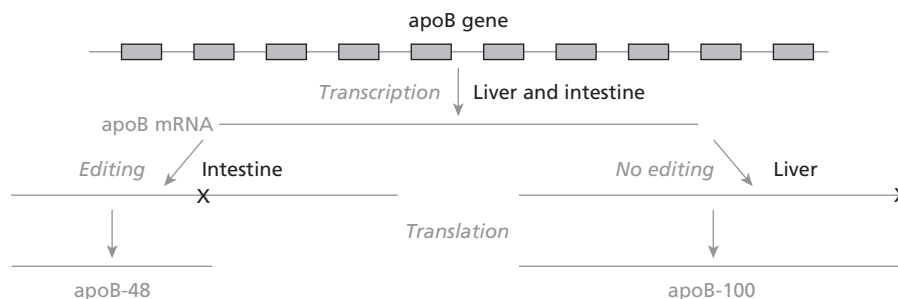
ApoB-48 and apoB-100 undergo posttranslational modification in the endoplasmic reticulum. Disulphide bond formation appears to occur cotranslationally and requires the presence of protein disulphide isomerase (PDI), which is a luminal protein in the endoplasmic reticulum that mediates protein folding [7].

The secretion of apoB-containing lipoproteins depends critically upon the availability of triglycerides and the presence of microsomal transfer protein (MTP). MTP is a heterodimeric protein consisting of M and P subunits. The P subunit of MTP is PDI. The M subunit of MTP catalyses intermembrane transfer of triglycerides and other lipids *in vitro*. Whereas the precise role of MTP in lipoprotein assembly and secretion is incompletely understood, it appears that MTP–apoB binding and MTP lipid transfer activity are required for particle formation. Figure 3 illustrates a schematic model of how MTP participates in the assembly of apoB-containing lipoproteins, a process commonly referred to as lipidation. Consistent with its critical role in the assembly of CM and VLDL, mutations in MTP result in abetalipoproteinaemia, which is characterized by hypolipidaemia and absence in plasma of apoB-containing lipoproteins [8]. During lipoprotein assembly within the hepatocyte or enterocyte, a single apoB-100 or apoB-48 becomes embedded in a VLDL or CM particle respectively [9].

Once assembled, secretion of CM and VLDL occurs by exocytosis. CM particles first enter the lymph and subsequently the blood via the thoracic duct. In contrast, VLDL particles are secreted from the liver directly into the bloodstream. CM particles are generally larger and more variable in size than VLDL [10]. Whereas particle assembly occurs by a similar mechanism, the cellular pathways for secretion of CM and VLDL differ. Anderson's disease (also known as CM retention disease) represents a failure of exocytosis of CM from enterocytes in the setting of normal VLDL secretion [10]. Moreover, CM secretion can be inhibited pharmacologically without affecting VLDL secretion [10].

When the intracellular supply of triglyceride molecules is reduced, degradation of apoB-48 and apoB-100 occurs by both

Fig. 2 Editing of apoB mRNA. The apoB gene, with exons represented by squares and introns by lines, is transcribed in both the intestine and the liver. In the intestine, but not the liver, a protein complex containing apobec-1 modifies a single nucleotide in the apoB mRNA. As a result, the codon containing this nucleotide is converted to a premature stop codon, as indicated by the position of the 'X'. The protein that is translated in the intestine (apoB-48) is only 48% as long as the full-length protein that is translated in the liver (apoB-100).



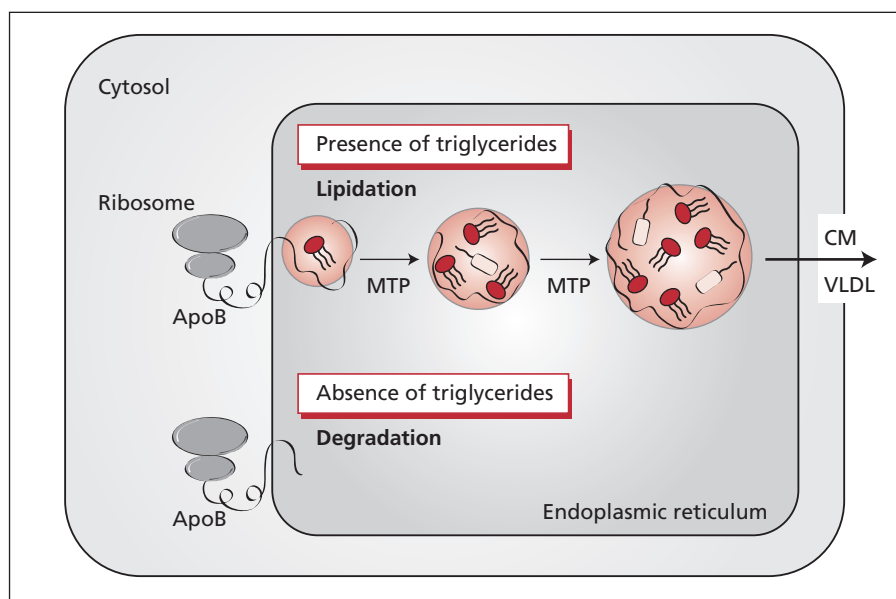


Fig. 3 Assembly and secretion of CM and VLDL particles. CM and VLDL particles are assembled and secreted by similar mechanisms in the enterocyte and hepatocyte respectively. The apoB protein (i.e. apoB-48 or apoB-100) is translated by ribosomes and enters the lumen of the endoplasmic reticulum. If triglycerides are available, the apoB protein is lipidated by the action of microsomal triglyceride transfer protein (MTP) in two distinct steps, accumulating triglyceride as well as cholesteryl ester molecules. The resulting CM or VLDL particle is secreted by exocytosis into the lymphatics by enterocytes or into the plasma by hepatocytes. In the absence of triglycerides, the apoB protein is degraded.

proteasomal and non-proteasomal pathways [11]. Similarly, the genetic absence of MTP results in rapid intracellular degradation of apoB-48 and apoB-100 [12]. In either case, lipoprotein secretion is reduced. Constitutive translation of apoB-48 allows for the prompt and highly efficient absorption of large quantities of triglycerides from the diet following a meal [10]. During fasting, apoB-48 is degraded in the intestine, whereas, in liver, continued lipidation of apoB-100 with endogenous triglycerides ensures that the metabolic demands of muscle tissue are met. When insulin levels are low, fatty acids are released from adipose tissue through the action of hormone-sensitive lipase and delivered to the liver via plasma. Among the metabolic fates of these fatty acids is incorporation into VLDL particles for delivery to muscle tissue. In the setting of insulin resistance, an inappropriately high flux of fatty acids from adipose tissue to the liver serves to increase VLDL secretion [13].

Intravascular metabolism of apoB-containing lipoproteins

As depicted in Figure 4, the delivery of triglycerides by apoB-containing lipoproteins to muscle and adipose tissue is determined to a large extent by tissue-specific expression and activity of lipoprotein lipase (LPL). LPL is abundantly expressed only on the vascular surface endothelia that line the capillary beds of cardiac, skeletal muscle and adipose tissues [14]. This enzyme hydrolyses triglycerides in the cores of CM and VLDL to form monoglycerides and fatty acids. Fatty acids that are taken up by muscle cells are consumed by oxidative metabolism as a direct source of energy. Within adipose tissue, fatty acids are re-esterified and stored as triglyceride droplets.

LPL is synthesized in parenchymal cells of muscle and adipose tissues. Within each tissue, the LPL expression level depends on

metabolic demand for fatty acids and can vary severalfold. Once translated, the protein is exocytosed and translocated across both the intercellular space and the endothelial cell. LPL is then anchored to the vascular surface of the endothelial cell by electrostatic interactions with charged sulphated proteoglycans on the plasma membrane. When infused intravascularly, heparin displaces LPL by competing for binding to these proteoglycans.

The relative expression of LPL in muscle vs. adipose tissue plays a key role in the preferential trafficking of triglycerides to muscle tissue during fasting and to adipose tissue in the postprandial state [14]. This is achieved principally by posttranscriptional mechanisms. In the fed state, insulin increases LPL mRNA levels in adipocytes, apparently by increasing mRNA stability. In the fasted state, altered glycosylation patterns of LPL in adipocytes lead to cellular retention of the enzyme and decreased expression on the endothelial surface.

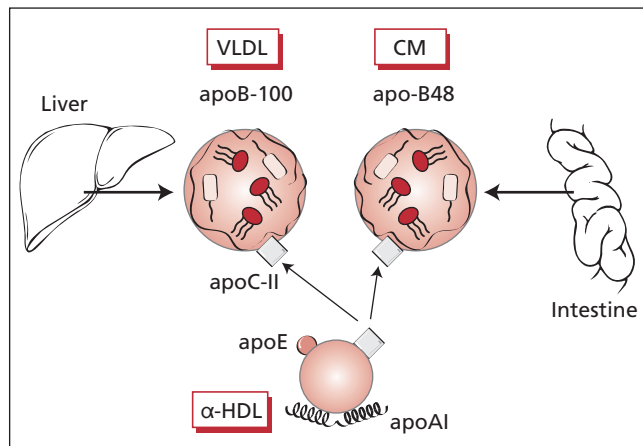
Before encountering LPL, triglyceride-rich CM and VLDL particles must first acquire apoC-II (Fig. 4a), which is required for LPL activity (Fig. 4b). In contrast to apoB molecules, which remain embedded in CM and VLDL, apoC-II is an exchangeable apolipoprotein. ApoC-II molecules are acquired by CM and VLDL by exchange from HDL particles, which serve as a reservoir for exchangeable apoproteins in addition to their functions in cholesterol transport which are described below [15,16]. Within approximately 5 min following lipoprotein secretion, CM and VLDL acquire the content of apoC-II that is required for optimal LPL activity [16]. This lag period appears to be important for allowing widespread vascular distribution of apoB-containing lipoproteins to occur prior to hydrolysis [16]. Consistent with a central role in promoting lipoprotein-LPL interactions, patients with genetic defects in apoC-II manifest high plasma triglyceride concentrations [17].

Owing to the large sizes of triglyceride-rich lipoproteins, a single particle interacts with multiple LPL molecules [16]. Each LPL is bound and activated by one molecule of apoC-II (Fig. 4b). VLDL and CM also undergo a series of binding and

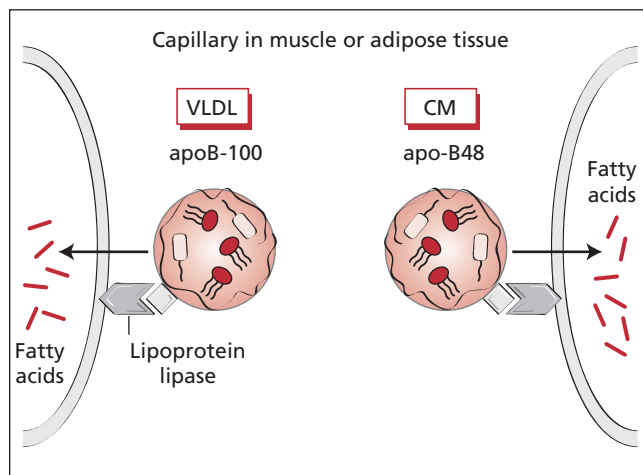
detachment events during catabolism. Because several molecules of LPL simultaneously catabolize triglycerides from VLDL or CM, hydrolysis occurs at a rapid rate so that half the core triglycerides are consumed in approximately 10–15 min.

Whereas apoC-II promotes LPL-mediated hydrolysis of triglycerides in the cores of CM and VLDL, apoC-III functions to inhibit LPL activity [16]. ApoC-III appears to be secreted in association with CM and VLDL particles, and concentrations of apoC-III in plasma correlate directly with plasma triglyceride concentrations [15]. The function of apoC-III may be to help promote the widespread distribution of these triglyceride-rich particles throughout the body.

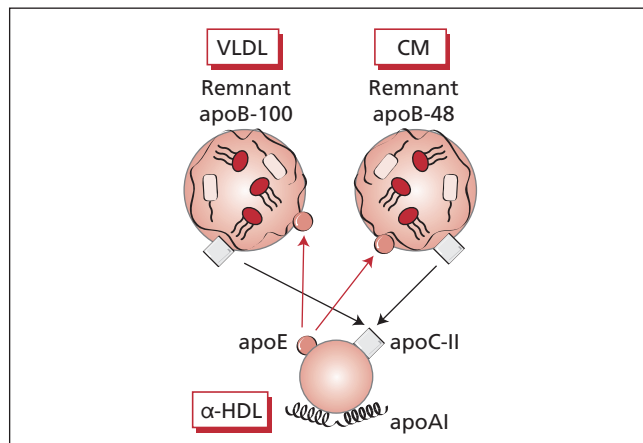
The activity of LPL reduces the triglyceride contents of CM and VLDL particles, which become relatively enriched in cholesteryl esters. Lipolysis of apoB-containing particles continues until about 80% of the initial triglyceride content has been removed from CM and about 50% from VLDL [16]. At this point, apoC-II is transferred to HDL, and HDL-associated apoE is acquired (Fig. 4c) [18]. The resulting CM and VLDL particles that contain apoE but lack apoC-II are referred to as 'remnants' [19].



(a)



(b)



(c)

Removal of apoB-containing lipoproteins from the circulation

ApoE plays a central role in CM and VLDL remnant uptake by receptor-mediated pathways. It is expressed in most tissues, but most prominently in liver and brain. In humans, apoE exists as three isoforms, apoE-2, apoE-3 and apoE-4 [20]. These isoforms differ in primary structure as a result of amino acid substitutions that influence the physical-chemical properties of the molecule by altering intramolecular disulphide bond formation [20]. Epidemiologically, inheritance of apoE-4 has been correlated with increased risk of developing Alzheimer's disease [21].

As illustrated in Figure 5, the liver efficiently removes CM remnants through receptor-mediated clearance [22]. Once the activity of LPL has sufficiently reduced particle size, CM remnants enter the space of Disse via endothelial fenestrations [19]. Within the space of Disse, CM remnants interact with heparan sulphate proteoglycan (HSPG) molecules. This represents the first of three steps in the process of CM uptake and is referred to as sequestration. Sequestration accounts for the rapid disappearance

Fig. 4 (opposite) Metabolism of apoB-containing lipoproteins. (a) Following secretion, CM and VLDL particles are activated for lipolysis when they encounter HDL particles in the plasma and acquire the exchangeable apolipoprotein apoC-II. (b) When CM and VLDL circulate into capillaries of muscle or fat tissue, apoC-II promotes binding of the particle to lipoprotein lipase, which is bound to the surface of endothelial cells. Lipoprotein lipase mediates hydrolysis of triglycerides, but not cholesteryl esters, from the core of the lipoprotein particle. The resulting fatty acids are taken up into muscle or fat tissue. (c) Upon completion of hydrolysis, CM and VLDL lose affinity for lipoprotein lipase. When an HDL particle is encountered, apoC-II is transferred back to HDL particles in exchange for apoE. The resulting particles are CM and VLDL remnants.

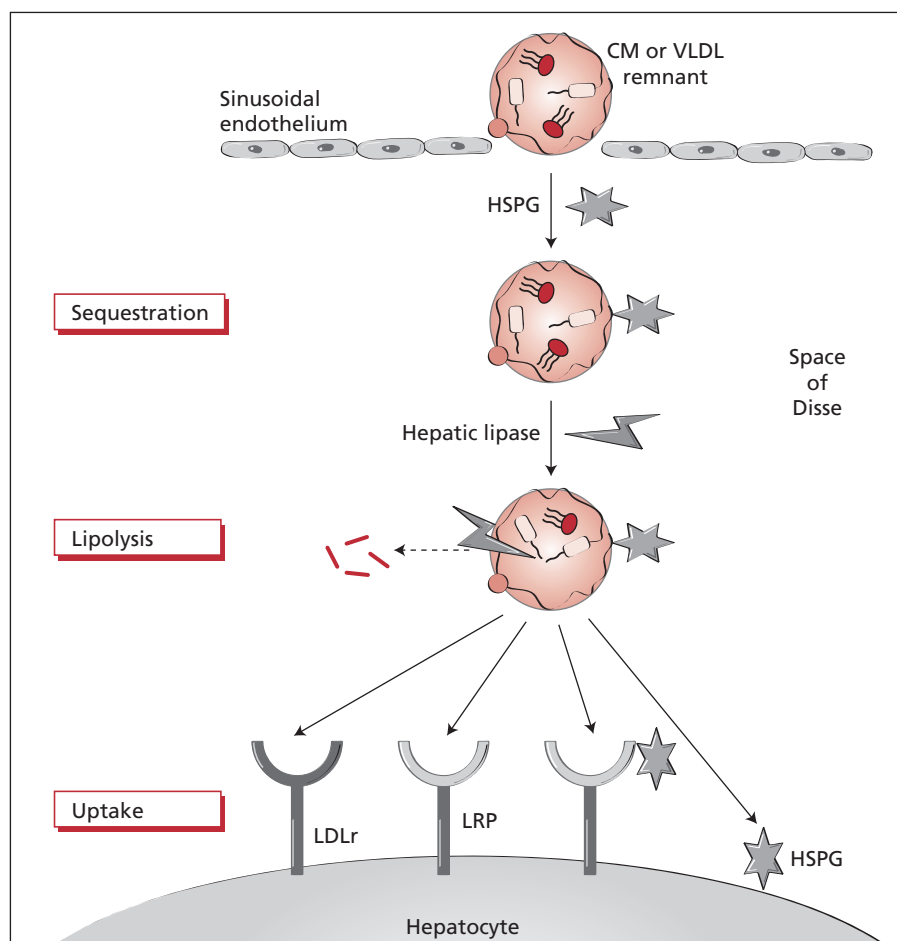


Fig. 5 Hepatic uptake of remnant particles. The activity of lipoprotein lipase results in remnant lipoprotein particles that are small enough in size to enter the space of Disse. Remnant lipoproteins are sequestered in the space of Disse by binding to high-molecular-weight heparan sulphate proteoglycan (HSPG) molecules. This is followed by binding of hepatic lipase, which promotes lipolysis of some residual triglycerides in the core of the remnant lipoproteins and the release of fatty acids (as indicated by the dashed arrow). Uptake of remnant lipoprotein particles into hepatocytes is mediated by the LDL receptor (LDLr), the LDL-related protein (LRP), a complex formed between LRP and HSPG or HSPG alone. Figure adapted from ref. 19, with permission.

of CM remnants from plasma. The second phase of CM clearance is a lipolytic processing step that occurs within the space of Disse. CM remnants bind to hepatic lipase, which is expressed by hepatocytes. It promotes CM remnant uptake by further modifying the lipid content of the particle. Finally, remnant particles are taken up into hepatocytes. The LDL receptor is a high-affinity receptor for apoE [23] that is expressed mainly in the liver, but also on a variety of cell types [22]. The LDL receptor-related protein (LRP) also plays a central role in the uptake of remnant particles through apoE-mediated interactions [19,20,24]. In addition, CM remnant uptake occurs through interactions with HSPG alone or in combination with LRP [19]. On account of these redundant mechanisms for CM remnant uptake, CM remnant particles do not tend to accumulate, even if one of the receptor pathways is disrupted [19].

About half of VLDL remnants are cleared directly by the liver, essentially as described for CM remnant particles. However, the remaining VLDL remnants are subjected to additional LPL-mediated triglyceride hydrolysis to form IDL particles, which are smaller remnant particles that may be cleared from the plasma by the same mechanisms [25]. A fraction of IDL is subject to further triglyceride hydrolysis by hepatic lipase. Presumably

because of the decrease in size that ensues, apoE dissociates from the particles, forming LDL. LDL are cholesteryl ester-rich particles that contain apoB-100, but no other apolipoproteins [16].

Plasma clearance of LDL particles occurs as the result of interactions with the LDL receptor [23]. Because the LDL receptor is the only receptor that effectively clears LDL particles from the plasma, mutations in the LDL receptor or proteins that support its function constitute the genetic basis for familial hypercholesterolaemia [23,26]. The LDL receptor is regulated in response to cellular cholesterol levels. When intracellular cholesterol levels are reduced, such as occurs during therapy with statin drugs, the LDL receptor is upregulated as a result of the proteolytic processing and activity of sterol response element binding protein 2 (SREBP-2) [27].

Reverse cholesterol transport: HDL metabolism

Overview

Whereas apoB-containing lipoproteins function primarily in

the delivery of triglycerides to muscle and adipose tissue, HDL particles are largely responsible for reverse cholesterol transport [28], whereby excess cholesterol molecules from tissues are removed and delivered to the liver for elimination via bile.

Apolipoproteins associated with HDL

ApoA-I is the major structural protein of HDL particles. It is the principal determinant of particle structure and receptor binding. ApoA-II is also a major apolipoprotein of HDL, but its precise role in the structure and metabolism of HDL is not well understood [29]. ApoA-I and apoA-II are both exchangeable apolipoproteins, and HDL particles may contain apoA-I alone or both apoA-I and apoA-II. As described above, other exchangeable apolipoproteins that are critical for apoB-containing particle metabolism variably associate with HDL particles.

Formation of HDL particles

The process of HDL formation begins when lipid-poor apoA-I secreted by the liver interacts with adenosine triphosphate (ATP) binding cassette protein A1 (ABCA1) on the plasma membrane of hepatocytes (Fig. 6) [30]. Although ABCA1 is expressed on the plasma membrane of a variety of cell types, its presence in hepatocytes regulates HDL formation [31]. In patients with Tangier disease, genetic inactivation of ABCA1 leads to a marked reduction in plasma HDL concentrations [32]. ABCA1 is required for cellular efflux of phospholipids and unesterified cholesterol in response to interactions between apoA-I in plasma and the hepatocyte plasma membrane [30,32]. The interaction of apoA-I and ABCA1 results in the formation of small pre- β -migrating HDL (pre- β -HDL) particles that are discoidal in shape [33].

Formation of pre- β -HDL is an essential step in reverse cholesterol transport, but these particles are not optimized for the removal of cholesterol from plasma membranes of extrahepatic tissues. Therefore, it is critical that pre- β -HDL undergo a maturation process to form spherical α -migrating HDL. α -HDL particles are referred to as HDL for simplicity and are highly efficient acceptors of excess cholesterol from cells. The maturation process is accomplished by the activity of lecithin-cholesterol acyltransferase (LCAT) [16]. LCAT is a circulating enzyme that is synthesized in liver. As shown in Figure 6b, it binds to HDL particles and catalyses the transfer of a fatty acid from phosphatidylcholine (also known as lecithin) to the hydroxyl group of unesterified cholesterol. The result is the formation of a cholesteryl ester and a lysophosphatidylcholine molecule [34]. The lysophosphatidylcholine is transferred to albumin in the plasma [16]. The newly formed cholesteryl ester spontaneously relocates from the surface of HDL to the core of the particle. As a result, LCAT transforms discoidal pre- β -HDL to spherical HDL particles.

HDL-mediated removal of cholesterol from cells

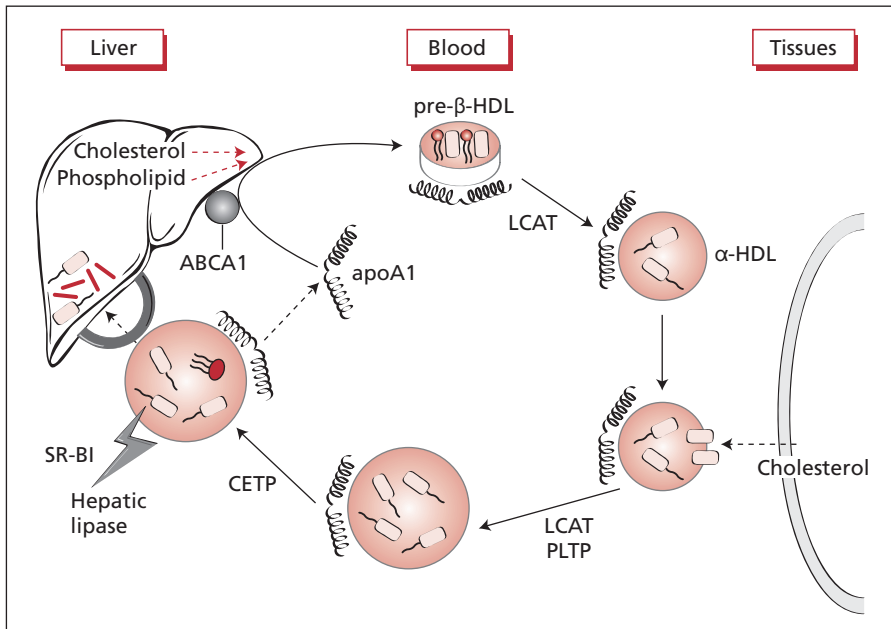
Efflux of unesterified cholesterol from plasma membranes to α -migrating HDL particles is the principal mechanism for cholesterol removal from cells in extrahepatic tissues (Fig. 6a and b) [35]. When the particle is in close proximity to a cell, unesterified cholesterol molecules within the plasma membrane desorb and traverse the aqueous plasma until they are incorporated into the phospholipid-rich surface coat of HDL. This is a process of passive diffusion that is controlled principally by the cholesterol concentration gradient between plasma membranes and HDL. Scavenger receptor class B type 1 (SR-BI) is expressed on a variety of cell types. *In vitro*, this receptor mediates the bidirectional flux of unesterified cholesterol between cells and HDL particles [30]. It has been proposed that HDL particles in the plasma may become tethered by SR-BI to cells [30] and, when the concentration gradient favours cellular efflux, this could promote the net movement of unesterified cholesterol to HDL [30]. Recent studies *in vitro* also suggest that ATP binding cassette proteins G1 and G4 may play significant roles in promoting cholesterol movement from cells to HDL particles [36].

Optimization of HDL-mediated cholesterol removal from cells

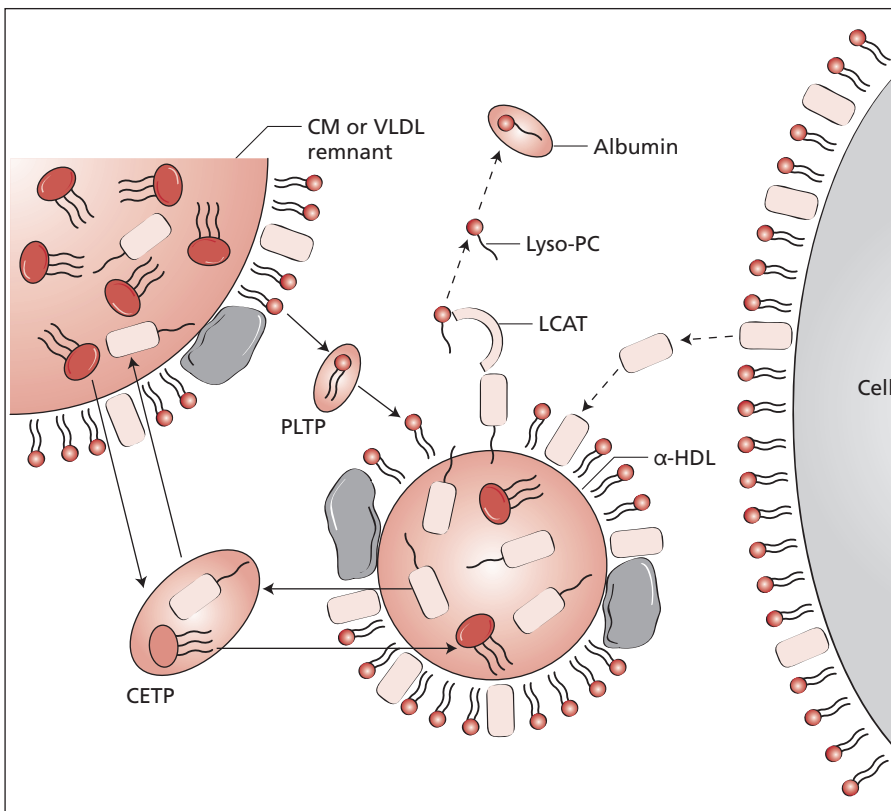
Ongoing cholesterol removal from cells to the surface coat of HDL requires a sustained concentration gradient [37]. This is achieved by the collective activities of three circulating plasma proteins (Fig. 6a and b). In addition to allowing HDL to mature from a discoidal to a spherical particle, LCAT activity allows unesterified cholesterol that is transferred to the surface of spherical HDL particles to be relocated to the core.

Whereas LCAT removes unesterified cholesterol from the surface of the particle, its enzymatic activity also consumes surface phosphatidylcholines, which are essential for HDL to increase in size and to accept additional cholesterol molecules from cells [16]. Phospholipid transfer protein (PLTP) is a plasma protein that replenishes the surface coat of HDL using phospholipids from remnant apoB-containing lipoprotein particles. The gradient for this transfer of phospholipids is established by the activity of LPL. As triglycerides contained within the cores of VLDL and CM are hydrolysed by LPL, their surface-to-volume ratio increases. PLTP removes excess surface phospholipids and transfers them to HDL particles [38]. This replaces phosphatidylcholine molecules that are hydrolysed by LCAT, allowing for particle growth. Based on studies in genetically engineered mice, PLTP activity constitutes the source of as much as 80% of HDL phospholipids [38].

Cholesterol ester transfer protein (CETP) promotes the transfer of cholesteryl esters from the cores of HDL particles to remnants of apoB-containing lipoproteins in exchange for triglyceride molecules from the cores of the remnant particles.



(a)



(b)

Fig. 6 Reverse cholesterol transport. (a) The process of reverse cholesterol transport begins when apoA-I is secreted from the liver. ApoA-I in plasma interacts with ATP binding cassette protein AI (ABCA1), which incorporates a small amount of phospholipid and unesterified cholesterol from hepatocyte plasma membranes to form a discoidal-shaped pre-β-HDL particle. On account of the activity of lecithin-cholesterol acyltransferase (LCAT) in plasma, pre-β-HDL particles mature to form spherical α-HDL. Spherical α-migrating HDL particles function to accept excess unesterified cholesterol from the plasma membranes of cells in a wide variety of tissues. The unesterified cholesterol is transferred from the cell to nearby HDL particles by diffusion through the plasma. As explained in (b), LCAT and phospholipid transfer protein (PLTP) increase the capacity of HDL to accept unesterified cholesterol molecules from cells by allowing for expansion of the core and the surface coat of the particle. Cholesteryl ester transfer protein (CETP) exchanges some of the cholesteryl ester molecules from the core of HDL for triglycerides from the core of remnant particles. Whereas remnant particles are taken up by a variety of receptors on the liver (Fig. 5), HDL particles interact with scavenger receptor class B type I (SR-BI), which mediates selective hepatic uptake of cholesteryl esters, but not apoA-I. This process is facilitated when hepatic lipase hydrolyses triglycerides from the core of the particle. The remaining apoA-I molecules may begin the cycle of reverse cholesterol transport again. In (a), solid arrows indicate metabolic events in HDL metabolism, whereas dashed arrows denote transfer of molecules. (b) LCAT, PLTP and CETP promote the removal of excess cholesterol from the plasma membranes of cells. LCAT removes a fatty acid from a phosphatidylcholine molecule in the surface coat of α- (or pre-β-)HDL and esterifies an unesterified cholesterol molecule on the surface of the particle. The resulting lysophosphatidylcholine (lyso-PC) becomes bound to albumin in the plasma, whereas the cholesteryl ester migrates spontaneously into the core of the lipoprotein particle. The unesterified cholesterol molecules that are consumed by LCAT are replaced by unesterified cholesterol from cells. HDL phospholipids that are consumed by LCAT action are replaced with excess phospholipids from remnant particles by the activity of PLTP. As described in (a), CETP increases the efficiency of cholesterol movement to the liver by exchanging cholesteryl ester molecules from the core of α-HDL for triglycerides from the core of remnant particles. In (b), solid arrows denote protein-mediated lipid transfer, whereas dashed arrows indicate that lipids move by diffusion through the plasma.

This transfer of cholesteryl esters allows HDL to continue to accept cholesterol from tissues. Moreover, it utilizes remnant particles, which have completed their function in triglyceride delivery and are destined for hepatic clearance, for the ancillary purpose of assisting with reverse cholesterol transport. The activity of CETP also enriches HDL with triglycerides, forming the more buoyant HDL particles. As described below, this helps to increase the efficiency with which HDL delivers cholesterol to the liver [39,40]. Considering that a fraction of VLDL remnants are ultimately transformed into LDL particles, the activity of CETP also increases plasma LDL cholesterol concentrations, while decreasing the plasma concentrations of HDL cholesterol.

Hepatic uptake of HDL cholesterol from plasma

In addition to the cholesterol that is delivered to the liver by remnant particles via CETP, HDL transports cholesterol directly to the liver. As illustrated in Figure 6a, SR-BI, which is highly expressed in liver, promotes the selective uptake of lipids, but not protein, from HDL particles [41].

The process of SR-BI-mediated selective lipid uptake into hepatocytes is facilitated by the activity of hepatic lipase. Hepatic lipase promotes hydrolysis of HDL triglycerides and phospholipids, and this optimizes the SR-BI-mediated hepatic uptake of cholesteryl esters [42]. When the HDL particle decreases in size, apoA-1 molecules dissociate and interact with ABCA1 to promote the formation of new pre- β -HDL particles [33].

Summary and conclusions

This chapter has described how the structures of lipoproteins are optimally suited for their functions in the transport of insoluble triglyceride and cholesterol molecules through the plasma. The primary role of apoB-containing lipoproteins is to deliver dietary triglycerides from the intestine and endogenous triglycerides from the liver to muscle or adipose tissue depending upon metabolic needs. HDL metabolism facilitates the transport of excess cellular cholesterol from extrahepatic tissues to the liver. Owing to the activity of CETP in plasma, remnants of apoB-containing lipoproteins also assist with reverse cholesterol transport. An appreciation of these principles should provide the foundation for understanding genetic and acquired disorders of lipoprotein metabolism, as well as the molecular and cellular mechanisms of lipid-lowering therapies.

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2.3.3 Protein and amino acid metabolism

Margaret E. Brosnan and John T. Brosnan

Introduction

The liver is a major organ of amino acid metabolism. It is responsible for the disposal of much of the dietary amino acid load; it is the only organ with a complete urea cycle; it is capable of synthesizing some amino acids; and it produces glucose from muscle-derived amino acids during starvation and in diabetes. Hepatic amino acid metabolism is finely regulated. There is evidence that the liver is largely responsible for maintaining circulating amino acid homeostasis. The liver also plays a critical role in the biosynthesis of key molecules from amino acids, e.g. creatine and glutathione. It also uses amino acids and glutathione in the conjugation of xenobiotics and toxic molecules to ensure their elimination from the body. In addition, there is good evidence that amino acids play a crucial regulatory role in controlling the turnover of hepatic proteins.

Amino acid pools and amino acid transport

Rapidly frozen rat liver contains a total of 20 $\mu\text{mol/g}$, which translates into an intracellular concentration of about 40 mM [1]. This figure ignores compartmentation between and within cells, however. Nevertheless, it gives an accurate picture of the magnitude of the hepatic intracellular amino acid pool. Given that the liver cell, like plasma, experiences an osmotic pressure of about 305 mOsM, it is apparent that free amino acids account for about 13% of all intracellular osmolytes. The amino acids with the highest hepatic concentrations include taurine (7.5 mM), aspartate (6.3 mM), glutamate (2.8 mM), glutamine (10.6 mM), glycine (4.0 mM) and alanine (4.7 mM). By also measuring plasma amino acids, intracellular/extracellular concentration ratios can be calculated (Table 1). Very high ratios (> 10) were identified for taurine, aspartate, glutamate, glutamine, glycine, alanine and histidine. No amino acid displayed a ratio significantly less than 1. These ratios are largely a result of the operation of hepatic amino acid transporters.

Hepatocytes communicate with their environment via their plasma membranes, either by signal transduction or via transport. Enormous progress has been made in the field of amino acid transport as our knowledge has advanced from kinetic to molecular characterization. Different amino acid transporters are responsible for the transport of groups of structurally similar amino acids. Transport of the three classes of amino acids (zwitterionic, cationic and anionic) is effected by a number of different transporters with different (though overlapping) specificities. Energetically, we can divide these transport systems into two classes: sodium-linked transporters (an example of secondary active transport) that employ the Na^+ electrochemical