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CHAPTER 20

Dynamics of lipoprotein transport in the human circulatory system

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1. Overview

1.1. Functions of the major lipoproteins

Plasma lipoproteins are soluble complexes of lipids with specialized proteins (apolipoproteins). Their function is to deliver lipids from the tissues where they are synthesized (mainly the liver and intestine) to those that utilize or store them. The apolipoproteins solubilize and stabilize the insoluble lipids of the lipoprotein particles, and prevent the formation of aggregates. Many apolipoproteins have additional functions in plasma lipid metabolism. Some are ligands for cell surface receptors, and determine the tissue-specific delivery of lipids. Some are cofactors for plasma lipases. Others regulate lipid reactions in the plasma, as competitive inhibitors of lipid uptake or metabolism (see table 3 in Chapter 18). It is the protein composition of lipoproteins that in large part specifies their metabolism in the plasma compartment. Conversely, the apolipoprotein content of lipoprotein particles alters during recirculation, as changes in the lipid composition of the particles modify the affinity of apolipoproteins for their surface. The interaction of these processes largely specifies the delivery of lipids to different tissues. Lipids delivered via plasma lipoprotein particles, in addition to neutral acylglycerols, phospholipids, and free and esterified cholesterol, include fat-soluble vitamins and antioxidants. Lipid binding to apolipoproteins is in most cases via the hydrophobic faces of amphipathic helical domains (J.A. Gazzara, 1997). Apolipoproteins have little tertiary structure, which gives them flexibility on the surface of the lipoprotein, as the diameter of the particle responds to the loading or unloading of lipids. Amino acid sequences functional in receptor binding or enzyme activation usually include clusters of charged residues.

While blood plasma contains the highest levels of lipoprotein particles, most lipoproteins, with the exception of the largest, triacylglycerol-rich particles, can cross the vascular bed, though their concentrations in the extracellular space are significantly lower. Interstitial lipoprotein particles interact directly with the surface of peripheral cells, delivering and receiving lipids. This recirculation is completed when interstitial fluid is collected into the main trunk lymph ducts, and returned to the plasma.

1.2. 'Forward' lipid transport

Functionally there are two main classes of lipoproteins. The first consists of particles whose main role is to deliver lipids (mainly triacylglycerols) from the liver or intestine to peripheral, extrahepatic tissues. These particles contain apolipoprotein B (apo B) together with a changing admixture of other lipids and proteins. In chylomicrons, which are secreted from the small intestine, this triacylglycerol originates from dietary long-chain fatty acids. These are re-esterified in the intestinal mucosa before being incorporated into lipoproteins. They contain a single molecule of a truncated form of apo B (apo B48). After loss of most of their triacylglycerol during recirculation in the plasma compartment, the chylomicron 'remnants' are cleared by the liver. Very low-density lipoproteins (VLDLs) secreted from the liver, contain one molecule of the full-length form of apo B (apo B100). Following the loss of most of their triacylglycerol to peripheral tissues, some VLDLs are returned to the liver, endocytosed and catabolized. Others remain in the circulation as intermediate density lipoprotein particles (IDLs). These still contain significant amounts of triacylglycerol and most of their original content of cholesteryl ester and free cholesterol, together with apolipoproteins B and E. After further modification by plasma lipases, most of the apo B100 particles remain in the circulation in the form of low-density lipoproteins (LDLs). After a plasma half-life of about 2 days, LDL are endocytosed, mainly by the liver. Their protein is degraded; their sterol content can be recycled into newly secreted lipoprotein particles, or degraded to bile acids.

Functionally, VLDL (density < 1.006 g/ml), IDL (density 1.006–1.019 g/ml) and LDL (density 1.019–1.063 g/ml) particles represent a continuum of decreasing size and increasing density created by the lipolysis of triacylglycerol. The traditional density limits of these fractions, shown in fig. 1 of Chapter 18, reflect this continuum. The density of each fraction depends mainly on its weight ratio of triacylglycerol (density 0.91 g/ml) to protein (density 1.33 g/ml). In terms of apolipoprotein composition, VLDL particles contain apo B100 and apo Cs with or without apo E, IDLs contain apo B100 and apo E but not apo Cs, and LDLs contain only apo B100.

1.3. 'Reverse' lipid transport

The second major class of lipoprotein particles carries lipids (mainly free and esterified cholesterol) from peripheral tissues to the liver. These high-density lipoprotein particles (HDLs) contain 1–4 molecules of apolipoprotein A1 (apo A1), together with other apolipoproteins that specify the metabolism and delivery of these lipids. HDL-dependent lipid transport is often defined as *Reverse cholesterol transport (RCT)* (C.J. Fielding, 1995).

Several sources of cellular cholesterol contribute to RCT. A part reflects peripheral sterol synthesis, despite the downregulation of this pathway by cholesterol in circulating plasma lipoproteins, mainly LDL. A second part represents the recycling of lipoprotein cholesteryl esters, mainly in LDL, internalized via endocytosis at peripheral LDL receptors; these are also highly downregulated, under physiological conditions, by LDL. Probably the major part of RCT responds to the selective cellular uptake of

preformed lipoprotein free cholesterol, independent of LDL receptors. This enters recycling endosomes returning to the cell surface. Cholesterol from all these sources transfers to HDL for further metabolism, including esterification, outside the cell. Some free cholesterol transfers within the circulation to HDL from other plasma lipoproteins.

Part of the HDL cholesteryl ester formed is transferred to apo B lipoproteins prior to their uptake by the liver. The remainder, mostly cholesteryl ester, is selectively internalized (that is, without the rest of the lipoprotein particle) from HDL by hepatocytes, and by steroidogenic tissues.

HDLs accumulate lipids from the peripheral tissues, and return them to the liver. Newly formed HDLs have high density and little lipid. Their density decreases as they accumulate lipid in the circulation. The classical subfractions of HDL [HDL-3 (density 1.12–1.219 g/ml), HDL-2 (density 1.063–1.12 g/ml), HDL-1 (density < 1.063 g/ml)] reflect this functional and structural continuum.

2. *Lipoprotein triglyceride and lipolysis*

2.1. Initial events

The structure of newly synthesized intestinal apo B-containing lipoprotein particles (chylomicra) is described in Chapter 19. Each consists of a triacylglycerol core containing a small proportion of cholesteryl esters, stabilized by a surface film made up mainly of phospholipid, some free cholesterol, and one molecule of apo B. Triacylglycerol and cholesteryl and retinyl esters in chylomicrons are derived almost entirely from dietary cholesterol, vitamin A and unesterified fatty acids; chylomicron phospholipids and free cholesterol are made in the enterocyte. Editing of full-length apo B transcripts (see Chapter 19) generates apo B48, which contains only the terminal 2152 residues of full-length apo B100. Since apo B does not exchange between lipoprotein particles during recirculation, apo B48 is an effective marker for chylomicron particles, and dietary triacylglycerol (E. Campos, 1992). Mice in which the editing enzyme was knocked out, when fed the same triacylglycerol load as control mice, were significantly less efficient in secreting chylomicron particles [1]. Dietary triacylglycerol accumulated in the intestinal mucosal cells. This finding indicates that apo B editing may have evolved along with dietary fat consumption to optimize the synthesis of large triacylglycerol-rich particles.

Chylomicrons are co-secreted with apo A1 (the intestine is the major source of this apolipoprotein in human subjects). This apo A1 is lost spontaneously to HDL as soon as chylomicrons reach the circulation. The transfer is independent of triacylglycerol lipolysis. At the same time, apo E and apo C proteins move to the surface of chylomicrons from reservoirs within the plasma population of large spherical HDL particles.

VLDLs secreted from the liver include a single molecule of full-length apo B100 containing 4536 amino acids (Chapter 19). The triacylglycerol-rich core of VLDLs contains significant levels of hepatic cholesteryl esters. Studies with isolated rat livers indicate that the incorporation of cholesteryl esters into VLDLs is necessary for their

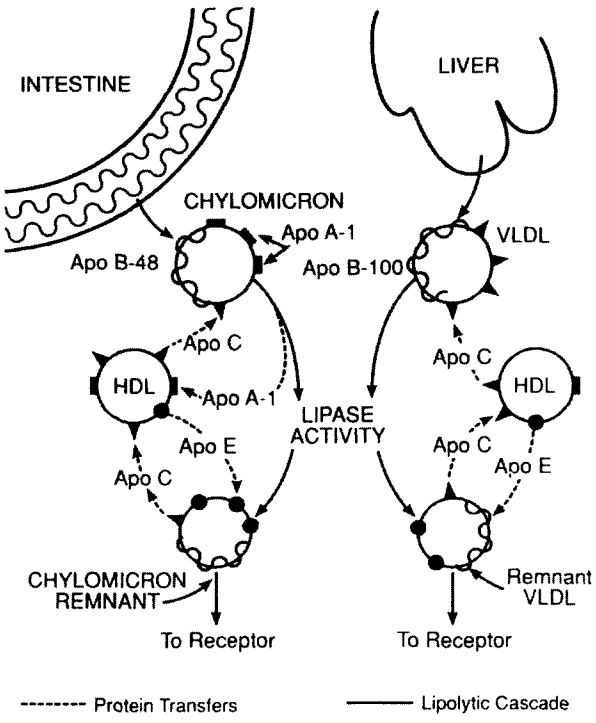

Fig. 1. Transfer of plasma apolipoproteins to newly secreted chylomicrons and VLDL. These small proteins play a critical role in optimizing the reaction rate of these triacylglycerol-rich particles with peripheral lipases and receptor proteins.

successful secretion into the perfusate. The phospholipid moiety of newly synthesized VLDL is enriched in phosphatidylethanolamine, in comparison with circulating VLDL. Newly synthesized plasma VLDLs contain apo C apoproteins but little apo E. As in the case of chylomicrons, enrichment of VLDL with apo E and additional apo Cs takes place in the plasma compartment.

These preliminary events in the circulatory system occupy about 5 min. In the case of both chylomicrons and VLDLs, the product is a triacylglycerol-rich apo B-particle functional to deliver triacylglycerol fatty acids to the peripheral tissues (Fig. 1). The purpose of this time lag is probably to allow these lipoproteins to distribute through the plasma compartment, prior to the inception of hydrolysis. A chylomicron or VLDL fully activated for lipolysis contains 10–20 molecules of apo C2, the cofactor of lipoprotein lipase (LPL). Titration of apo C2 content vs the rate of lipolysis indicates that 2–3 apo C2 molecules per chylomicron or VLDL are needed for maximal activity. Apo C2 and other apo Cs leave VLDL and chylomicrons as lipolysis proceeds, the triacylglycerol core shrinks, and surface phospholipid and proteins are transferred away to other lipoproteins, particularly HDL. Because apo C2 is present in initial excess, lipolysis rates are maintained until a major part (~80%) of initial triacylglycerol content of the particles has been lost.

2.2. The structure and activation of lipoprotein lipase (LPL)

LPL hydrolyzes the 1(3)-ester linkages of triacylglycerol of chylomicrons and VLDLs whose surface contains apo C2. The primary product of LPL-mediated lipolysis is 2-monoacylglycerol. After spontaneous isomerization of this lipid, LPL has activity against the 1-monoacylglycerol formed. Limited further lipolysis by plasma and platelet monoacylglycerol hydrolases also takes place. Monoacylglycerol is also readily internalized by vascular cells. As a result the end-products of LPL-mediated triacylglycerol hydrolysis are unesterified fatty acids, monoacylglycerol and glycerol. Fatty acids originating from LPL activity are cleared by adipose tissue and re-esterified under postprandial conditions and stored. Under fasting conditions, hormone-sensitive lipase promotes the release of unesterified fatty acids from adipocyte triacylglycerol back into the circulation (Chapter 10). Fatty acids from LPL-mediated lipolysis in muscle tissue are mainly catabolized to two-carbon subunits as part of oxidative metabolism.

LPL gene transcription is stimulated by sterol response element binding protein-1 (SREBP-1) (Chapter 15) and by Sp-1, and inhibited by Sp-3. The regulation of LPL expression is tissue-specific. During fasting, adipocyte LPL expression is reduced, while expression in muscle cells is increased. Postprandially, expression is upregulated in adipocytes, and decreased in muscle cells. Tissue-specific expression of LPL is mediated by the transcription factor PPARγ via its PPAR/RXR heterodimer [2]. This mechanism is related to the need to supply fatty acids to muscle for oxidative metabolism under conditions of scarcity, and to direct excess fatty acids to adipose tissue for storage postprandially, conditions where circulating glucose levels provide alternative substrate for muscle cells.

The secreted human LPL protein has 448 amino acids. It is functional as a dimer. LPL is a member of a triacylglycerol lipase protein family (Chapter 10) others of which include hepatic lipase, which like LPL is released into the plasma by heparin, and pancreatic lipase. Pancreatic lipase and several related fungal lipases have been crystallized. LPL is ~30% homologous in primary sequence to pancreatic lipase, whose X-ray coordinates have been used to model LPL structure. Other information on structure–function relationships in LPL has been obtained from the site-directed mutagenesis of key amino acids of receptor- and heparin-binding sites that are absent from pancreatic lipase (Fig. 2). LPL is a serine hydrolase whose active site triad is made up of the S_{132}, D_{156} and H_{241} residues. Consistent with other lipases in this family, the primary sequence of LPL predicts a polypeptide 'lid' (residues 239–264) which opens when LPL binds to its lipoprotein substrate. A short sequence of hydrophobic amino acids in the C-terminus (residues 387–394) has also been implicated in LPL binding to triacylglycerol-rich lipoproteins. Other data implicate residues 415–438 in both substrate interaction and dimer stability. Heparin binding by LPL was thought earlier to be mediated mainly via five basic residues in two adjacent clusters (R_{279}, K_{280}, R_{282}, K_{296}, R_{297}). Additional basic residues (K_{403}, R_{405}, K_{407}) were recently implicated (R.A. Sendak, 1998) The involvement of additional sequences at the C-terminus (residues 390–393, 439–448) has also been described (Y. Ma, 1994). These, while not directly heparin-binding, amplify binding by the other domains.

LPL is present within intracellular pools in adipocytes and muscle cells, but the

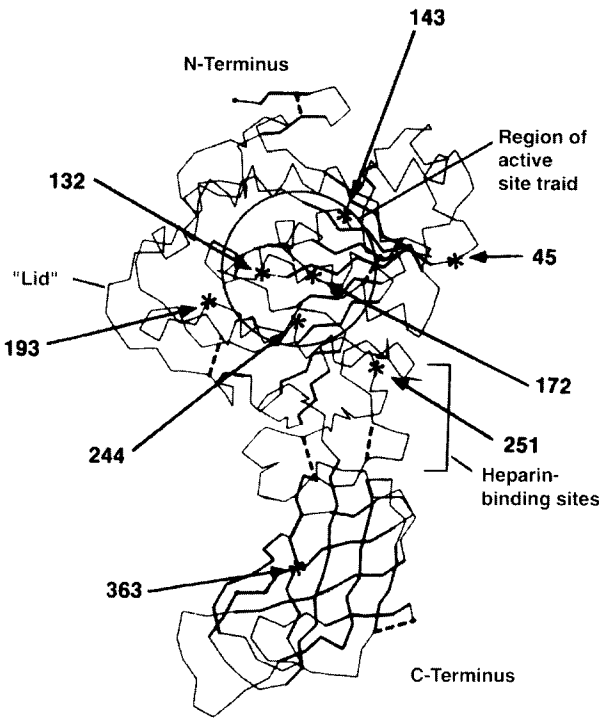

Fig. 2. Structure–function relationships in LPL. Because of the overall sequence similarity between LPL and pancreatic lipase, structural features and the locations of selected amino acids in LPL (which has not been crystallized) have been superimposed on the three dimensional structure of pancreatic lipase. (Modified from Faustinella et al. (1991) J. Biol. Chem. 266, 9481–9485, with permission).

functional fraction of LPL is at the vascular endothelial surface, where it is bound by heparin-like glycosaminoglycans. The products of the reaction of chylomicrons and VLDL with endothelial LPL, lipoprotein remnants, continue to circulate in the plasma compartment (see below). Small amounts of LPL are also present in the circulation, especially postprandially. LPL binds to several members of the LDL receptor protein family (specifically, LDL receptor-like proteins-1 and -2, and VLDL receptor protein) to induce receptor-mediated lipoprotein catabolism [3]. Some triacylglycerol clearance may occur via this endocytic route, but the predominant role of receptor binding by LPL is likely to lie in the endocytosis and degradation of LPL itself. Consistent with this, mice in which genes encoding the VLDL receptor, or both VLDL and LDL receptor proteins, were knocked out, had normal levels of plasma triacylglycerol. The receptor-binding domain in the LPL primary sequence lies within the C-terminal region of the protein. It includes K_{407}, but is distinct from the lipid-binding domain, which includes W_{393} and W_{394}.

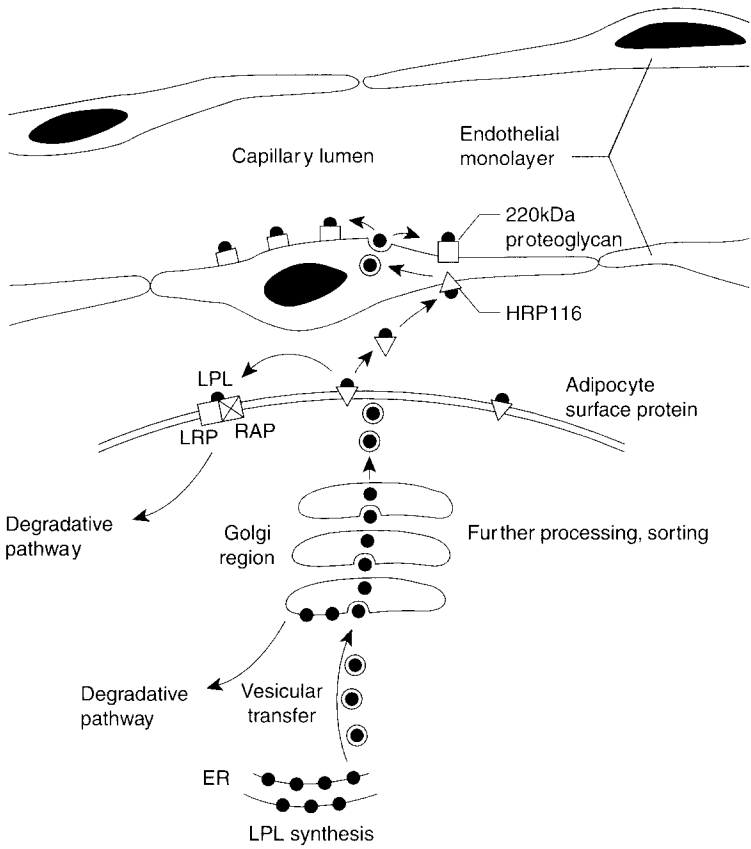

Fig. 3. Synthesis, secretion and transport of LPL from the adipocyte to the vascular endothelial surface. ER, endoplasmic reticulum. Degradative pathways from the Golgi compartment and cell surface are illustrated. RAP, receptor-associated protein; LRP, LDL-receptor-like protein. The suggested roles of HRP (heparin-released protein)-116, and 220 kDa proteoglycan are also shown.

2.3. Transport of LPL to its endothelial site

LPL, synthesized in adipocytes and myocytes, is transported out of the parenchymal cells, through the pericyte layer, and across the endothelium, before binding to functional sites on the vascular endothelial surface (Fig. 3). Mature LPL contains several polysaccharide chains, which are required for effective LPL secretion. Unesterified fatty acids and lysophosphatidylcholine increase the rate of LPL secretion from adipocytes. Adipocytes can degrade secreted LPL. Two distinct pathways are involved. One requires the 39 kDa receptor-associated protein (RAP) which binds to the LDL receptor-related protein [4]. LPL is also internalized via a proteoglycan-dependent pathway.

Subsequent stages of the activation of newly secreted LPL involve its transendothelial migration to specific binding site on the capillary vascular surface (Fig. 3). Transcytosis was recently shown to involve both the VLDL receptor protein, and proteoglycans

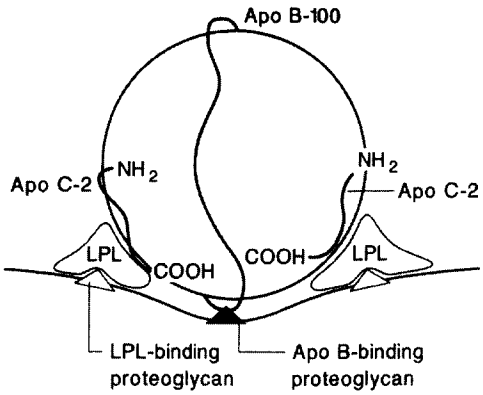

Fig. 4. Model to show interactions between the endothelial cell surface, triacylglycerol-rich lipoproteins, apo C2 and LPL. Two LPL molecules are shown reacting with the same VLDL particle. These are representative of the multiple LPLs probably reactive with each triacylglycerol-rich lipoprotein particle.

[3]. Earlier, a 116-kDa LPL-binding protein, released by heparin, had been implicated in LPL binding to endothelial cells. Microsequencing of peptides from this protein indicated it to be a fragment of apo B. The exact role of this fragment in the migration of LPL remains to be established. LPL is bound to the endothelial vascular surface via a 220 kDa proteoglycan whose functional site is probably a highly sulfated decasaccharide [5].

2.4. Structure of the LPL–substrate complex at the vascular surface

It seems likely that LPL and its large triacylglycerol-rich lipoprotein substrates both establish multiple interactions with each other and with the capillary wall to anchor the enzyme–substrate complex to the vascular surface. Components of such a multi-protein functional complex would include LPL itself, apo C2 and apo B on the VLDL or chylomicron, the 220 kDa proteoglycan, and possibly VLDL receptor protein or another member of this family (Fig. 4).

Apo B100 has a length sufficient to make only a single circumference of VLDL. This was estimated from electron microscopic studies of apo B in the smaller lipolysis product, LDL [6]. It follows that contact between apo B and the endothelial cell must be restricted to a relatively small fraction of the primary sequence. The same considerations apply to the interaction of the larger chylomicron particles, which are stabilized by the shorter apo B variant, apo B48. Since triacylglycerols in VLDL and chylomicrons are competitive substrates for LPL in mixtures of these lipoproteins, the same LPL binding sites must accommodate either lipoprotein particle.

Kinetic data suggest that several molecules of LPL simultaneously catabolize the triacylglycerol of each VLDL or chylomicron particle. The turnover number of LPL under physiological conditions is about 10 s^{-1}. For a chylomicron containing 3×10^5 molecules of triacylglycerol, catabolism of 50% of this lipid by a single LPL molecule would take about 3 h, yet the measured $t_{1/2}$ is 10–15 min. These data suggest that several molecules of LPL become attached to the circumference of each chylomicron

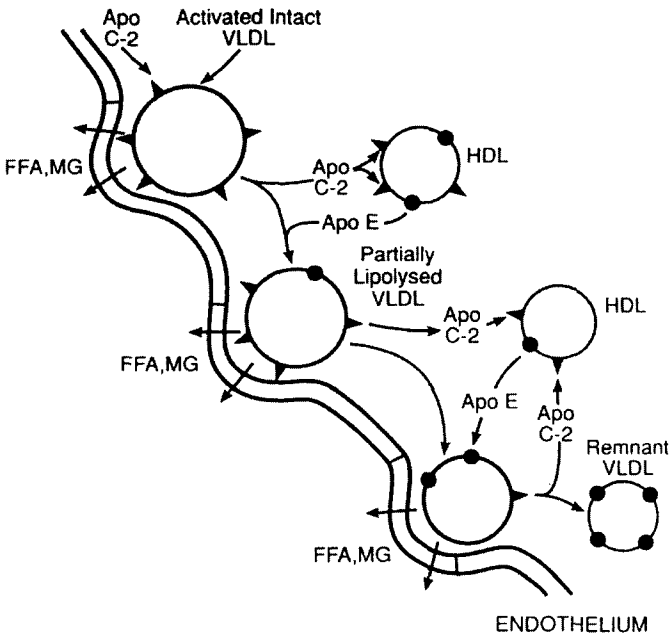

Fig. 5. Mechanism of remnant lipoprotein formation at the endothelial surface. Apo B is not illustrated. FFA, free fatty acid; MG, monoacylglycerol; apo C2, closed triangles; closed circles, apo E. This model reflects the appearance of partially lipolyzed lipoprotein particles in the circulation during LPL-mediated lipolysis of triacylglycerol-rich lipoproteins.

or VLDL particle during lipolysis, with each LPL activated by one molecule of apo C2. In Fig. 4, apo B is illustrated binding directly to the endothelial cell surface, while individual proteoglycan anchors bind LPL to the endothelium. Each apo C2 would link one LPL to the surface of the lipoprotein. If this model were correct, LPL binding sites on the capillary endothelium must be fluid yet highly organized, and able to adapt rapidly to substrates with different diameters and apo C2 contents.

2.5. Kinetics of the LPL reaction and the role of albumin

As VLDL and chylomicrons pass down their delipidation cascade, partially catabolized intermediates formed as a result of LPL activity are detected in the circulation (Fig. 5). This observation makes it likely that lipolysis does not result from a single binding event. Rather, there must be repeated dissociation and rebinding, during which lipoprotein triacylglycerol is catabolized, apo C2 is gradually lost, and LPL catalytic rate is decreased while remnant end-products are formed.

There has been considerable discussion of mechanisms by which triacylglycerol-rich lipoproteins could be reversibly displaced from the endothelial surface. The most likely would involve the transient accumulation of lysogenic lipolysis products at the lipoprotein surface within the LPL-binding surface microdomain. After dissociation of the lipoprotein particle, these lipids would diffuse away, leaving the partially lipolyzed

particle once more competent to bind to the lipase site. There are three candidate lipids for such a role: unesterified fatty acids, monoacylglycerols, and lysophosphatidylcholine.

Only a portion of the fatty acids generated by LPL are cleared locally. The rest remain in the circulation, after transfer from the surface of the substrate lipoprotein to albumin, part of which remains bound to the lipoprotein surface. Under physiological conditions, fatty acids are largely converted to their sodium and potassium salts, and can act as detergents. Monoacylglycerols are effective lysogens. Even at concentrations of 1–2 μM, they inhibit LPL activity in the isolated perfused rat heart. Monoacylglycerols do not bind to albumin, but are taken up rapidly by cells. LPL also generates lysophospholipids. These are effective lysogens but unlike monoacylglycerols, they form stable complexes with albumin. Although further research is needed, monoacylglycerols seem the most likely contributors to the transient displacement of triacylglycerol-rich lipoproteins from the vascular surface.

LPL has an important role in directing VLDL triacylglycerol to muscle tissues during fasting, and VLDL and chylomicron triacylglycerol to adipose tissue postprandially. In addition to the transcriptional regulation described above, there is evidence of posttranslational and kinetic differences between LPL sites in adipose and muscle tissues that contribute to the distribution of lipolysis products between tissues.

In adipocytes, fasting is associated with the synthesis of LPL molecules whose *N*-linked polysaccharide chains retain an unmodified high-mannose structure. In the fed state, these chains are modified by mannose trimming, and the addition of glucose, hexosamine and sialic acid units. The high mannose form of LPL has low specific activity and is retained within the adipocyte. The modified form is actively secreted. Insulin levels are an important determinant of LPL processing.

The apparent K_m of endothelial LPL in adipose tissue is relatively high, compared to that in muscle tissues such as the heart (C.J. Fielding, 1976). This means that the hydrolysis of lipoprotein triacylglycerol by LPL in adipose tissue remains proportional to substrate concentration. In contrast, LPL at the surface of muscle capillaries is saturated, even at the low circulating levels of triacylglycerol-rich lipoproteins characteristic of the fasting state.

2.6. Later metabolism of chylomicron and VLDL triacylglycerol

Chylomicrons recirculate until about 80% of initial triacylglycerol content has been catabolized in peripheral tissues. The chylomicron remnant is then endocytosed by hepatic receptors (Chapter 21). Chylomicron remnants retain almost the whole of their original cholesteryl and retinyl ester content. This is cleared by the liver along with remnant triacylglycerol.

The metabolism of VLDL remnants is more complex. In humans some VLDL remnants (IDL) are cleared by the liver via the LDL receptor; but a significant proportion (estimated at 50–70%) is further modified within the circulation to generate LDL. Essentially the whole of circulating LDL is formed in this way. Comparison of the composition of IDL with that of LDL indicates that this conversion involves the loss of 80–90% of IDL triacylglycerol, some phospholipid, and the dissociation of

remaining apo E. In contrast, IDL free cholesterol content is the same as that in VLDL while cholesteryl ester is increased in LDL, as a result of the activity of cholesteryl ester transfer protein (CETP) that exchanges apo B-associated triacylglycerol for apo A1-associated cholesteryl esters (see Section 4.3).

It was formerly considered that the loss of lipids from IDL was mediated mainly via the activity of hepatic lipase, a triacylglycerol hydrolase with a role in the generation of small HDL (see Section 3). Recent data make this explanation less likely. Mice in which hepatic lipase was inactivated, and mice and rabbits overexpressing hepatic lipase, had similar, normal levels of circulating total and remnant triacylgycerol, even postprandially. In contrast, IDL is an optimal substrate for CETP. While VLDL and IDL contain similar numbers of cholesteryl ester molecules per apo B, LDLs have about 50% more cholesteryl ester molecules per apo B than either. These data suggest that in normal metabolism, the conversion of IDL to LDL is driven mainly by CETP, and that the role of hepatic lipase is to hydrolyze triacylglycerol on HDL, not on IDL. If this model is correct, the loss of apo E which is part of the IDL-to-LDL conversion is probably passive, and reflects the changing surface lipid composition of IDL.

2.7. Congenital deficiencies of lipoprotein triacylglycerol metabolism

The functional pools of LPL and hepatic lipase are quantitatively released into plasma by heparin (post-heparin plasma). Genetic deficiency of LPL is associated with a massive increase in the circulating levels of chylomicrons, and an absence of LPL activity from post-heparin plasma. However, there is less increase in VLDL levels than would be predicted from the role of LPL in VLDL catabolism. An alternative, low-capacity pathway probably exists for the clearance of intact VLDL particles by the liver. Numerous mutations within the human LPL gene have now been identified. Their effects on LPL function were discussed in Section 2.2. Congenital hepatic lipase deficiency is associated with increased levels of plasma triacylglycerol compared to controls.

Because of the dominating role of apo C2 as cofactor for LPL activity, the effects of congenital apo C2 deficiency in human plasma mimic those of LPL deficiency. Mice overexpressing or deficient in LPL have been developed. Their plasma lipoprotein patterns resemble those of the corresponding human genetic deficiency, and confirm the roles of LPL and hepatic lipase in plasma lipid metabolism described above. Mice with muscle-specific overexpression of LPL developed insulin resistance along with the expected increase in muscle triacylglycerol levels [7]. This effect was associated with a decrease in insulin-stimulated glucose uptake. These findings show the power of transgenic mouse models in studying complex metabolic diseases.

3. HDL and plasma cholesterol metabolism

3.1. The origin of HDL

Unlike apo B-containing lipoproteins, HDL-containing apo A1 are formed in the extracellular space. This process involves the association of lipid-poor apo A1 with

cell-derived phospholipids and cholesterol. The association of apo A1 and phospholipid is thermodynamically favorable; phospholipid-free apo A1 has not been detected in biological fluids. Nevertheless isolated, lipid-free apo A1 is often used as a convenient surrogate for lipid-poor apo A1 in the analysis of lipid transfers from the cell surface.

Newly synthesized apo A1 made by the liver and (particularly in humans) by the small intestine is recovered loosely associated with the surface of lymphatic triacylglycerol-rich lipoproteins. The apo A1, probably in association with small amounts of phospholipid, dissociates spontaneously after entering the plasma compartment, in a reaction independent of lipolysis. Lipid-poor apo A1 can also be generated via the action of lipid transfer proteins and/or hepatic lipase (Sections 4.2 and 4.3), when these reduce the core size of mature, spherical HDL.

Both lipid-poor and lipid-free apo A1 demonstrate preβ-migration when plasma is fractionated by nondenaturing agarose gel electrophoresis. Under these conditions, the bulk of HDL, made up of spherical lipid-rich particles, has more rapid, α-migration, while LDL migrates more slowly in a β-position. This technique has proven to be useful for discriminating 'early' or lipid poor HDL from mature, lipid-rich particles in the RCT pathway. The major preβ-HDL of human plasma ($preβ_1$-HDL) has a molecular weight of about 70 kDa An increase in preβ-HDL levels has been correlated with an impairment of RCT and an increased risk of coronary artery disease in human patients.

There is little information yet on the physical structure of prebeta-HDL, although at least two inter-convertible forms, containing one and two molecules of apo A1, may be present in plasma.

3.2. Role of the ABCA1 transporter in HDL genesis

Studies in vitro have shown that the prebeta-HDL population includes avid acceptors of cell-derived cholesterol and phospholipids. These lipoprotein complexes are precursors of mature HDL. In human Tangier Disease, there is an almost complete deficiency of mature HDL. The low levels of apo A1 present (1–2% of normal) have preβ-mobility. Tangier Disease patients also have localized patches of orange, lipid-laden macrophages, classically in the tonsils. LDL levels are very low. Cultured Tangier Disease fibroblasts lack significant ability to transfer either phospholipid or free cholesterol to lipid-free apo A1, though transfer of cellular lipids to mature HDL is almost normal (G. Rogler, 1995). These data have led to the conclusion that Tangier Disease patients inherit a defect in the ability of peripheral cells to build normal mature HDL from lipid-poor, apo A1-containing precursors.

Genetic analysis of Tangier Disease families recently led to the identification of one of the key factors for HDL assembly. The DNA of these patients was found to contain deletions or other defects in the ABCA1 gene. ABCA1 is an ATP-binding cassette (ABC) transporter protein closely related to the multidrug resistance transporter, to several hepatic bile acid transporters, and other transporter proteins active in the transmembrane movement of small amphipathic solutes [8].

ABCA1 mRNA is widely expressed among tissues and in cultured cells. It has been studied most intensively in fibroblasts and macrophages, where its activity has been linked to the efflux of cholesterol and phospholipids to extracellular apo A1.

The regulation of ABCA1 expression is complex, and incompletely understood. At least three classes of mRNA transcripts have been identified, corresponding to different transcriptional start sites. These are under the regulation of different promoter sequences. In macrophages and hepatocytes, ABCA1 mRNA levels are strongly upregulated by oxysterols and retinoic acid via a LXR/RXR tandem transcription site (see Chapter 16) [9]. In a few rodent transformed macrophage cell lines, ABCA1 expression is regulated by cAMP but human cells generally are unaffected (A.E. Bortnick, 2000). ABCA1 mRNA levels are also upregulated by cholesterol itself. The molecular mechanism of the response to cholesterol has not yet been clarified. It may involve oxysterols generated intracellularly (X. Fu, 2001).

Despite the stimulus that the identification of ABCA1 has given to HDL studies, a number of key questions on its role in HDL formation remain unresolved. The first is the mechanism by which ABCA1 promotes lipid efflux, and the identity of the lipids transported. In ABCA1$^{-/-}$ mouse embryos, a defect was identified in the catabolism of apoptotic cell bodies. At the same time, ABCA1$^{-/-}$ cells were found to be defective in annexin V binding, an assay for exofacial phosphatidylserine in apoptotic cells (Y. Hamon, 2000). At the present time, the primary substrate for ABCA1 has not been identified. Phospholipid leaving the cell surface under the influence of ABCA1 is almost entirely phosphatidylcholine. One current hypothesis is that ABCA1 could modify the phospholipid composition, and possibly charge, of the exofacial leaflet of the membrane bilayer, thereby secondarily reducing the activation energy for efflux of phosphatidylcholine (Fig. 6). Another hypothesis is that ABCA1 directly transports phosphatidylcholine, and possibly free cholesterol. This possibility is consistent with the loss of both free cholesterol and phospholipid efflux from Tangier cells, and the restoration of both activities in cells transfected with ABCA1 cDNA. However, several recent reports suggest that ABCA1 might not play a direct role in cholesterol transport. These studies each showed that phospholipid efflux by ABCA1 was regulated independently of free cholesterol efflux [10]. These data appear more consistent with a two-step process: (1) addition of phospholipid; followed by (2) addition of FC.

3.3. The role of caveolae in HDL genesis

Another area of active investigation is the origin of the cellular cholesterol transferred to preβ-migrating (lipid-poor) HDL. There is general agreement that unesterified cholesterol transferred to lipid-poor apo A1 originates mainly from the plasma membrane. Caveolae (see Chapter 1) are microdomains of the cell surface implicated in cholesterol homeostasis and transport as well as signal transduction. These functions are probably related, because unesterified cholesterol levels in caveolae regulate the efficiency of signal transmission. In primary cells including fibroblasts, smooth muscle cells and endothelial cells, caveolae are implicated as the direct precursors of cholesterol in lipid-poor HDL. While caveolae contain several proteins involved in cellular cholesterol homeostasis, such as the scavenger receptor BI (see Section 4.4) there is no convincing evidence at present that the ABCA1 transporter is located there, consistent with the phospholipids and unesterified cholesterol on apo A1 originating from different membrane microdomains.

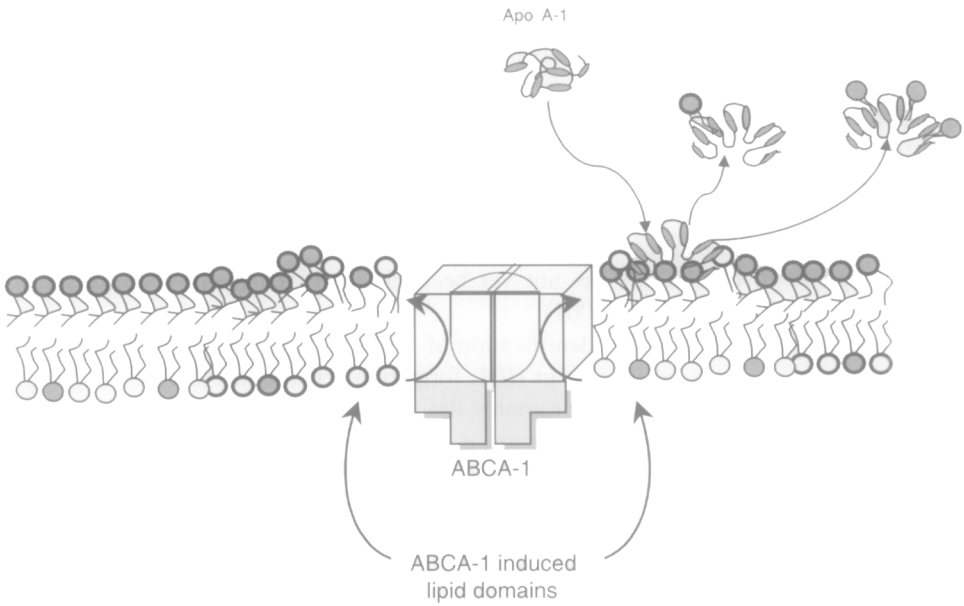

Fig. 6. The ABCA1 transporter and the formation of lipid-poor HDL reactive as acceptors of cell-derived unesterified cholesterol. The figure illustrates the role proposed for ABCA1 in the distribution of phospholipids between the exo- and cyto-facial leaflets of the membrane bilayer. The exofacial leaflet is rich in phosphatidylcholine, substrate for apo A1 at ABCA1 transporter sites. Closed circles, phospholipid; open circles, free cholesterol. Modified from G. Chimini (2002) by permission.

3.4. The role of LCAT in HDL genesis

Further growth and maturation of apo A1-HDL depend on the activity of lecithin : cholesterol acyltransferase (LCAT):

Unesterified cholesterol + phosphatidylcholine

$\longrightarrow$ cholesteryl ester + lysophosphatidylcholine

Though present in lymph, LCAT is active mainly in the plasma compartment. It had been thought until recently that the primary substrates of LCAT were phospholipid-rich, discoidal apo A1-containing particles which support maximal acyltransferase rates, and accumulate in the plasma of LCAT-deficient subjects. Recently, it was reported that LCAT could be directly reactive with lipid-poor HDL [11]. This could indicate that more than one pathway can convert lipid-poor to mature HDL.

LCAT consumes unesterified cholesterol and phospholipids to produce insoluble cholesteryl ester (Fig. 7). This is retained in the HDL core, while the water-soluble lysophosphatidylcholine formed at the same time is transferred away to albumin. In this way, LCAT maintains concentration gradients of cholesterol and phosphatidylcholine between cell and lipoprotein surfaces and the growing HDL particle. The later stages of HDL genesis probably depend entirely on diffusion of lipids from the surface of other lipoprotein particles that is independent of ABCA1 activity.

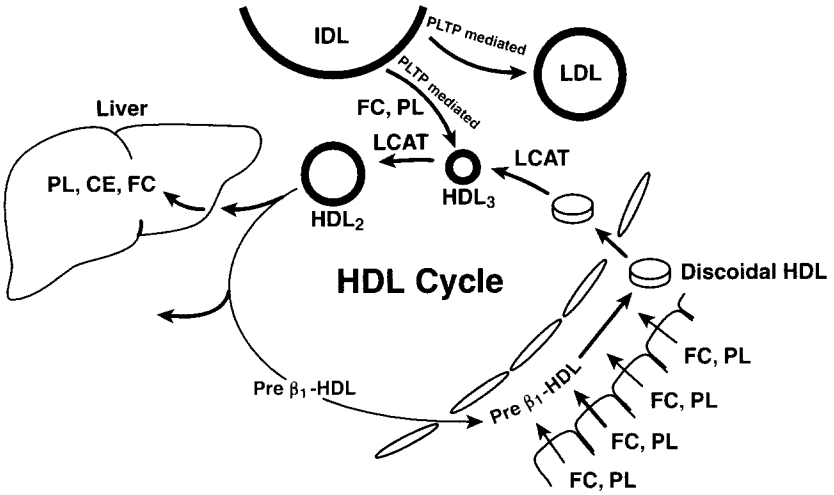

Fig. 7. The HDL cycle, showing: lipid-poor, prebeta-migrating (preβ-1) particles incorporating cell- and lipoprotein-derived unesterified cholesterol and phospholipid; the formation of discoidal HDL; the role of the LCAT reaction in generating spheroidal, alpha-migrating HDL; and the generation of a new cycle of lipid-poor particles at the surface of liver cells. The transfer of excess phospholipid from VLDL and IDL as a result of lipolysis, is catalyzed by phospholipid transfer protein (PLTP).

3.5. Regeneration of prebeta-migrating HDL

Lipid-poor, preβ-migrating HDL are formed when the size of the central lipid core of α-migrating HDL, which is mainly cholesteryl ester, is decreased, or when HDL surface lipids are increased (Fig. 7). The surface area occupied by HDL protein, cholesterol and phospholipids then exceeds the surface area of the core. Apo A1 dissociates from the particle in the form of lipid-poor, preβ-migrating HDL. Once released, these prebeta HDL are available as acceptors of additional cell-derived lipids. They may be sufficiently primed with phospholipid to participate in a new cycle of cholesterol efflux. Preβ-HDL particles in plasma are sphingomyelin-rich, while particles newly formed as the result of ABCA1 transporter activity contain mainly phosphatidylcholine molecules. This finding is consistent with the hypothesis that once formed, preβ-HDL can recycle via LCAT, losing phosphatidylcholine to transesterification but retaining sphingomyelin, which is not a LCAT substrate.

Three pathways have been identified for preβ-HDL formation from alpha-HDL:

(1) phospholipid transfer protein (PLTP) activity;
(2) exchange of HDL cholesteryl esters for triacylglycerol in VLDL and LDL catalyzed by cholesteryl ester transfer protein (Section 4.3) concomitant with hepatic lipase-mediated lipolysis of HDL triacylglycerol.
(3) selective uptake of cholesteryl ester from HDL catalyzed by the cell-surface scavenger receptor SR-BI.

It is not known if the preβ-HDL formed by these different pathways have the same kinetic properties as acceptors of cell-derived lipids, though it seems likely. Because triacylglycerol molecules have a similar volume to that of cholesteryl esters, CETP

alone would seem unlikely to promote preβ-HDL formation. The relative contribution of the different pathways towards the recycling of apo A1 is likely to differ significantly under different physiological conditions.

Several other plasma apolipoproteins (particularly apo A4 and apo E) have marked sequence similarity to apo A1. Lipid-poor HDL with these proteins in place of apo A1 have been identified. Their concentration is much lower than those of apo A1 particles. Also, it is not clear if apo A4 and apo E particles, two of those identified, can recycle between lipid-rich and lipid-poor populations in the way described for apo A1. As a result, apo A1 is likely to play the predominant role in transporting peripheral cell cholesterol through the plasma compartment to the liver, at least in normal metabolism.

3.6. Regulation of gene expression and structure of apo A1

The apo A1 gene codes for a 287-aa preproprotein. Following the loss of its leader sequence, and the removal of a 6-aa pro-sequence in plasma, mature apo A1 circulates as a 243-aa polypeptide. Apo A1 gene transcription rates are not highly regulated, compared to those of ABCA1 and other catalytic factors of the HDL cycle. Sp1, a 'housekeeping' transcription factor, plays a major role in regulating apo A1 transcription rates. PPARs which regulate phospholipid efflux to apo A1, are reported to have little effect on the expression of apo A1 itself. Regulation of the cholesterol transporting activities of apo A1 in plasma is probably determined for the most part by its distribution within its three structural forms, i.e. the amorphous (lipid-poor), discoidal and spheroidal HDL species.

Apo A1, like other phospholipid-binding plasma apolipoproteins, is largely made up a series of amphipathic helical segments, typically 22 amino acids in length [12] (Chapter 18). These are separated by helix-breaking proline or glycine residues. Synthetic amphipathic helical segments whose primary sequence is unrelated to that of native apo A1 can be effective mimics of native apolipoprotein in binding phospholipid, promoting cholesterol efflux from cells, and activating the formation of cholesteryl esters by the LCAT reaction. In spite of this, some repeats in native apo A1 are clearly of more significance than others. This has been seen in experiments where the position of repeats within the primary sequence was systematically varied, though the amino acid sequence of each repeat was unchanged (M. Sorci-Thomas, 1997). The biological activity of such mutant apo A1 species varied widely. These data indicate that apo A1 retains significant tertiary structure. Some generalizations are now possible. The central ('hinge') region of the polypeptide (residues 143–164) appears to be of particular significance in promoting the LCAT reaction [13]. The same domain is important in promoting cellular cholesterol efflux, in the presence or absence of LCAT activity. A C-terminal domain has been implicated in phospholipid binding.

Most of the information on apo A1 function has been obtained from synthetic discoidal recombinants of apo A1 and pure phospholipids, with a molar ratio of 1 : 200 to 1 : 500. The size and molecular properties of these particles, produced by sonication or dialysis from detergent solution, are quite similar to those of discoidal lipoproteins found in the plasma of LCAT-deficient human subjects. The particles have been shown to consist of a planar phospholipid bilayer. The edges of the bilayer are sealed from the

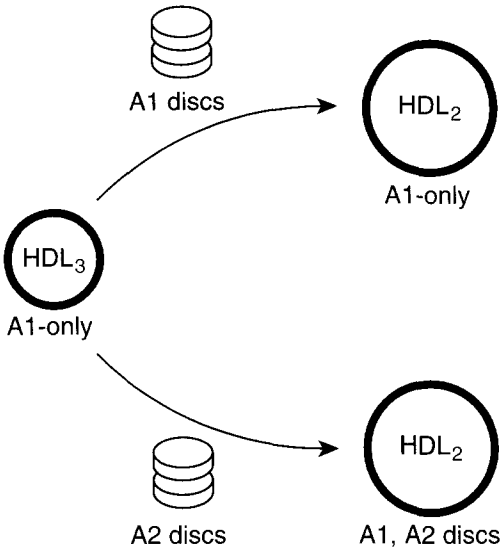

Fig. 8. HDL fusion and the formation of apo A1, apo A2 HDL. The role proposed for PLTP in the promotion of fusion is illustrated, together with the formation of apo A1, apo A2 products from alpha-HDL.

aqueous medium by apo A1. It was thought earlier that a 'picket fence' model in which the repeats were at right angles to the lipid bilayer, accurately reflected the structure of these particles (Chapter 18). A more recent 'belt' model has the repeats aligned circumferentially parallel to the bilayer [14]. The balance of evidence still suggests that discoidal HDL are the normal intermediate of the conversion of lipid-poor, prebeta HDL to mature, spherical particles. The presence of discoidal HDL in lymph is consistent with this interpretation. In any case, the end products of the action of LCAT on apo A1 complexes rich in cholesterol and phospholipid are alpha-migrating, spherical HDL particles rich in cholesteryl esters.

Most alpha HDL particles, unlike preβ- and discoidal HDL, include apo A2 as well as apo A1. Evidence recently obtained suggests that these are a product of the fusion of apo A1-only and apo A2-only HDL particles [15] (Fig. 8). This fusion could be mediated locally by the lysophosphatidylcholine formed in the LCAT reaction. In LCAT deficient plasma, apo A1 and apo A2 form distinct populations of HDL particles. Apo A2 has been considered an inhibitor of the LCAT reaction, and thus indirectly, of reverse cholesterol transport. Apo A2 might thus limit the size reached by spherical HDL. Mice transgenic for apo A2 were atherosclerosis-prone compared to normal animals of the same strain, but this effect has not been seen in mice of all genetic backgrounds.

The formation of α-HDL is accompanied by large changes in the conformation of apo A1. This was made clear by studies with monoclonal antibodies, as well as a variety of sensitive physical techniques. The unique properties of apo A1 in lipid binding and the promotion of reverse cholesterol transport reflect this elasticity.

3.7. Structure and properties of LCAT

Plasma LCAT originates mainly from hepatocytes. Hepatic levels of LCAT mRNA are determined mainly by the interplay of Sp1 and Sp3 promoter binding sites. The rate of the LCAT reaction in plasma is regulated for the most part not by changes in circulating LCAT protein levels, but by differences in its catalytic rate with the different HDL particles. Postprandially, LCAT rates are increased as unesterified cholesterol and phospholipid are transferred to HDL from triacylglycerol-rich lipoproteins; the level of LCAT protein in the circulation is unchanged.

There is enough LCAT in plasma for only about 1% of HDL particles to contain one molecule of enzyme. Either LCAT must move rapidly between HDL particles or, more likely, its substrates and products must be transferred effectively from a metabolically active HDL subfraction containing LCAT to other HDL particles. The spontaneous transfer of free cholesterol and lysophosphatidylcholine in plasma is rapid. That of phosphatidylcholine and cholesteryl esters is much slower. These transfers are stimulated by dedicated plasma lipid transfer proteins (Sections 4.2 and 4.3).

LCAT is a 416-amino acid serine hydrolase [16]. It has only limited sequence homology (<5%) to other lipases (LPL, hepatic lipase, pancreatic lipase). The amino acid residues which make up its active site triad have been identified. Several carbohydrate chains modify the reaction rate and substrate specificity of the enzyme. LCAT has not been crystallized. Efforts have been made to explain its three-dimensional structure using the coordinates obtained from X-ray diffraction analysis of triacylglycerol lipases. LCAT, like these lipases, probably has a mobile 'lid' responsive to the lipid interface of HDL. A helical domain, adjacent to the active site serine residue and partly homologous to a sequence in apo E, may be involved in lipid binding. To date, these insights have been insufficient to explain the unique selectively of LCAT for unesterified cholesterol, rather than the hydroxyl group of water, as acyl acceptor. In the complete absence of cholesterol, LCAT is an efficient phospholipase.

Apo A1 is required for both the acyltransferase and phospholipase activities of LCAT. Three arginine residues within the 143–164 repeat of apo A1 (R_{149}, R_{153}, and R_{160}) are essential for its activation by apo A1, suggesting a possible role for salt-bridges between these residues and either negatively charged amino acids in LCAT, and/or phosphate groups within phosphatidylcholine [17]. In reaction with LDL, LCAT catalyzes phosphatidylcholine acyl exchange. LCAT can also hydrolyze short-chain lipid esters. This reaction is independent of the presence of apo A1, consistent with the view that the apoprotein may be needed to align the enzyme and its substrates at a phospholipid–water interface.

3.8. Congenital deficiencies of LCAT and HDL

Two variants of LCAT deficiency are recognized. In the first, LCAT synthesizes no cholesteryl esters in plasma. Cholesterol accumulates as droplets in peripheral tissues. Apo-E-rich particles accumulate in LCAT deficient plasma, indicating this alternative cholesterol transport pathway, though upregulated, cannot fully substitute for that catalyzed by LCAT. Only lipid-poor and discoidal HDL particles are present under

these conditions. In the second type of LCAT deficiency (Fish-Eye Disease) LCAT can transesterify cholesterol from VLDL and LDL, but not from exogenous HDL. LCAT deficiency and Fish-Eye Disease are the result of different mutations in the primary sequence of the LCAT protein (J.A. Kuivenhoven, 1997).

Normal HDL are absent from plasma in congenital apo A1 deficiency, and also in ABCA1 deficiency (Tangier Disease). In apo A1 deficiency, no HDL is present. In Tangier Disease, HDL present is all in the form of prebeta-HDL. Whether prebeta-HDL in Tangier Disease have the same composition as those in normal plasma has apparently not been reported.

Epidemological studies consistently show that low HDL cholesterol is correlated with an increased risk of atherosclerotic vascular disease [18]. The relationship is usually stronger than that between the same disease and LDL. The evidence that heart disease is systematically increased in LCAT deficiency, apo A1 deficiency and Tangier Disease is equivocal at best, in spite of the fact that LCAT, apo A1 and ABCA1 play key roles in regulating cellular cholesterol content. This paradox has several possible explanations. The first is that these HDL deficiency diseases are also characterized by low levels of circulating LDL (25–50% of normal). This reduces the delivery of cholesterol to peripheral cells to partially offset reduced reverse cholesterol transport. A second possibility is that HDL cholesterol concentrations may not reflect the rate of reverse cholesterol transport. For example, mice transgenic for SR-BI (Section 4.4) increase cholesterol clearance to bile but decrease HDL cholesterol levels (K.F. Kozarsky, 1997). Other pathways, such as that involving apo E, may be able to assume part of the function of apo A1. A third possibility is that it is not HDL cholesterol as such, but a metabolically active subfraction of HDL, that is antiatherogenic. Changes in its composition could be less extreme than those of HDL cholesterol levels. Trace HDL proteins that could play such a role are the antioxidant proteins paraoxonase and platelet activating factor which are responsible for the protective role of HDL in neutralizing oxidized phospholipids in LDL (M. Navab, 2001).

4. Reactions linking the metabolism of apo A1 and apo B lipoproteins

4.1. Metabolic implications

Triacylglycerol carried by apo B lipoproteins is mainly catabolized in peripheral (that is, non-hepatic) tissues. Its fatty acids are used for oxidative metabolism or storage. In contrast, very little cholesterol is needed for growth or repair in peripheral tissues. Nevertheless there is a continuous 'forward' delivery of cholesterol to peripheral cells. Two main reasons can be suggested. Cholesteryl ester is needed for triacylglycerol-rich particles to be successfully secreted from the liver. Second, the recycling of free cholesterol between the liver and peripheral cells suppresses local cholesterol synthesis and the expression of lipoprotein receptors. These receptors would otherwise promote the futile uptake up large amounts of lipoprotein cholesteryl ester.

Despite the different roles of the apo A1- and apo B-lipoproteins systems, exchange reactions in plasma, catalyzed by lipid transfer proteins, have been identified. These

promote the movement of lipids between the major transport pathways. Transfer proteins are ATP-independent. Their reactions (i) are reversible; and (ii) proceed only down preexisting concentration gradients.

4.2. Phospholipid transfer protein (PLTP)

PLTP is a 476 amino acid protein showing ~20% sequence similarity to several other lipid-binding proteins, which include cholesteryl ester transfer protein and bacterial permeability inducing protein. Short, highly hydrophobic sequences in conserved regions of the primary sequence may represent the strands of a hydrophobic basket or cleft involved in lipid binding. The expression of PLTP is PPAR-gamma dependent, and may involve the LXR/RXR orphan receptor heterodimeric complex, the same factors that regulate ABCA1 expression, and phospholipid efflux from cells.

In plasma, PLTP catalyzes the transfer of phospholipids, particularly phosphatidylcholine, between lipoprotein classes [19]. The generation of excess surface phosphatidylcholine as a result of triacylglycerol lipolysis, and the consumption of phosphatidylcholine by LCAT, both ensure that a phospholipid gradient is maintained from VLDL and LDL to HDL. PLTP activity is reported to be present in all mammalian plasmas. Human genetic PLTP deficiency has not yet been unequivocally identified.

PLTP activity is needed for maximal LCAT activity because the rate of transfer of phospholipids from cells to plasma via ABCA1 transporter activity, and the spontaneous transfer of phospholipids from other lipoproteins, are both much slower than that of cholesterol. Without PLTP, reverse cholesterol transport might otherwise be limited. PLTP also plays a major role in generating prebeta-HDL, the major acceptor of cellular cholesterol. An additional role for PLTP recently identified is in the secretion of apo B lipoproteins from the liver [20]. Finally, PLTP can promote phospholipid efflux from the surface of fibroblast monolayers to preformed HDL, though not to lipid-free apo A1.

4.3. Cholesteryl ester transfer protein (CETP)

CETP is a 476-amino acid plasma protein structurally related to PLTP (Section 4.2). Like PLTP, CETP expression in hepatocytes is PPAR-dependent [21]. The C-terminus of CETP, a domain absent in PLTP, plays a key role in the transfer of both triacylglycerols and cholesteryl esters. Neither the tertiary structure nor the detailed mechanism of CETP-mediated lipid transfer are yet fully established. A model of CETP tertiary structure based on X-ray coordinates established for bacterial permeability-increasing protein has been described. Like LCAT, the activity of CETP is regulated more by the composition of substrate lipoproteins than by the circulating level of CETP protein. For example, increased CETP activity observed postprandially appears to be almost completely the consequence of increased triacylglycerol/cholesteryl ester ratios in triacylglycerol-rich dietary lipoproteins.

Like PLTP, the CETP reaction transfers lipids down a preexisting concentration gradient maintained by LCAT. CETP normally promotes transfer of CE to VLDL and LDL, at a rate typically ~50% that of LCAT. This means that much of the

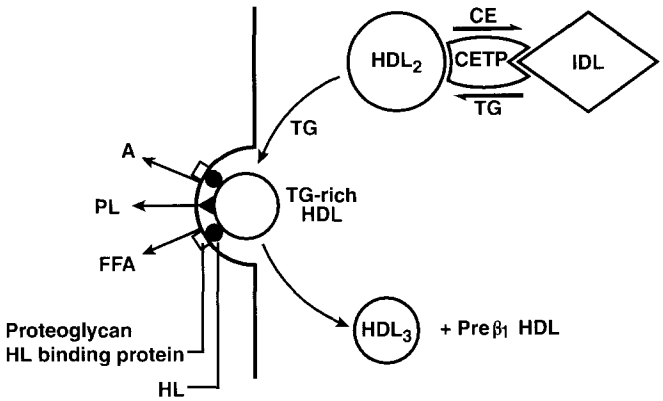

Fig. 9. Remodelling of HDL by hepatic lipase (HL). The hydrolysis of triacylglycerol (TG) transferred from VLDL and IDL via the activity of cholesteryl ester transfer protein (CETP) is shown, together with the displacement of lipid-poor (prebeta$_1$) HDL from the diminished surface of the spherical HDL particle. FFA, free fatty acids.

cholesteryl ester generated by LCAT is cleared directly from HDL, not from LDL after CETP-mediated lipid transfer (Section 4.4).

The net effect of CETP activity is to reduce HDL CE and increase LDL CE. In normal plasma, CE transfer is complemented by a similar and opposite transfer of triacylglycerol from VLDL and LDL to HDL. Under conditions where there is no cholesteryl ester concentration gradient between lipoproteins (for example, if VLDL secreted from the liver contains as much cholesteryl ester as HDL) CETP can still catalyze the unproductive exchange of cholesteryl esters between lipoprotein particles.

There has been considerable debate whether CETP should be considered a 'proatherogenic', pathologically neutral, or 'antiatherogenic' factor. The activities of CETP and hepatic lipase contribute to the recycling of lipid-poor apo A1, and the formation of prebeta-HDL (Fig. 9). Whether or not increased CETP activity leads to an increase in circulating LDL cholesterol levels depends on the capacity of hepatic LDL receptors. A study of Japanese subjects expressing partial CETP deficiency did not suggest any increased resistance to atherosclerosis. On the other hand, reduced CETP levels in hemodialysis patients have been linked to an increased incidence of heart disease (although other relevant activities, including LCAT, were also reduced). Several groups of thiol reagents have been described which inhibit CETP activity. As of the time of writing, CETP inhibitors have not been shown to reduce atherosclerosis in human populations, though beneficial effects in rabbits have been reported.

4.4. Scavenger receptor BI (SR-BI)

SR-BI (the human protein is also known as CL-A1) is a 409-amino acid transmembrane protein member of the scavenger B-family. Its primary sequence contains no consensus ATP binding site. As a result, lipid transfers mediated by SR-BI, like those supported by CETP and PLTP, are driven by established concentration gradients. (Fig. 10). SR-BI

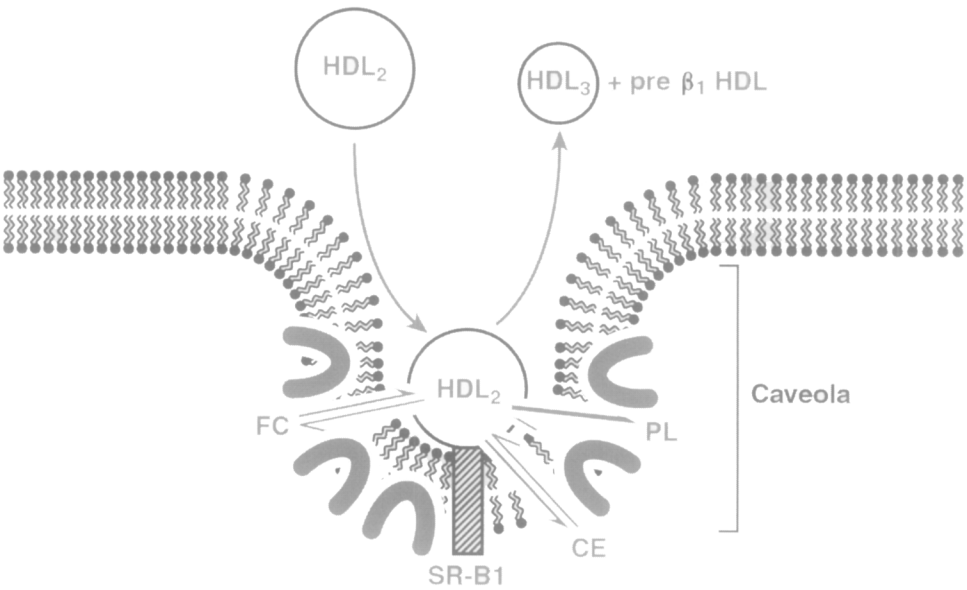

Fig. 10. Lipid transfers catalyzed by SR-BI. SR-BI is shown localized to caveolae. The selective transfers of cholesteryl ester (CE), free cholesterol (FC) and phospholipids (PL) are shown between HDL and the cell surface. The model suggests that SR-BI acts to promote facilitated (that is, protein-mediated) diffusion of lipoprotein lipids down their physiological concentration gradients, under conditions where FC efflux is driven by the LCAT reaction.

catalyzes the selective uptake of lipids, particularly CE, from lipoprotein particles, particularly HDL, though it is active with a wide array of lipids and lipoproteins. SR-BI also promotes efflux of unesterified cholesterol from cells [22]. Transcriptional regulation of SR-BI expression by transcription factor SF-1, by sterol regulatory element binding protein, and by Sp proteins 1 and 3 has been described. While there are significant species differences in the tissue specificity of SR-BI expression, SR-BI is usually expressed at high levels in tissues forming steroid hormones (such as adrenal and gonadal cells). Expression is high in mouse liver but low in human liver. This pattern is the inverse of that seen with CETP and reflects the specialization of humans and mice as 'LDL' and 'HDL' animals respectively. SR-BI has been localized to caveolae, the cholesterol-rich microdomains that are abundant in many peripheral cells.

The selective uptake from HDL requires SR-BI binding. Two positively charged residues (R_{402}, R_{418}) are important for efficient uptake of cholesteryl esters into the cell. The mechanism by which SR-BI promotes transport of unesterified cholesterol out of the plasma membrane is not yet clear. From a study of mutant SR-BI species and anti-SR-BI antibodies, it was suggested that HDL binding was essential for cholesterol efflux. In contrast, kinetic studies suggested that much of the effect of SR-BI on cholesterol efflux was indirect, possibly the result of induced local modifications in the distribution of lipids within the membrane bilayer. It is unclear to what extent cholesterol efflux from caveolae depends on the presence of SR-BI. CD-36, a second scavenger protein, also found in caveolae, has a weaker effect.

SR-BI may facilitate uptake of unesterified cholesterol from the intestinal lumen. ABCA1 has been implicated in the transport of unesterified cholesterol out of the intestine into lymph. While outside the scope of this chapter, these findings indicate that mechanisms parallel to those described in this chapter may be in place to regulate cholesterol transport to plasma from other extracellular spaces.

4.5. Animal models of human plasma cholesterol metabolism

The availability of technology to over-express or delete individual genes in mice has had a wide impact in this field. The effects of modulating the levels of a single enzyme or transport protein can be studied in vivo against the background of interacting factors. Many of these studies were initiated to estimate the role of each gene product in promoting or inhibiting atherogenesis. A major problem of the transgenic/knockout approach has been that in several respects plasma cholesterol metabolism and transport in mice differ significantly from that in humans. As a result, despite successful efforts to create mouse models of human lipid diseases, the quantitative role played by individual factors has sometimes been difficult to establish.

Identification of ABCA1 as the defective protein in human Tangier Disease led rapidly to the production of ABCA1$^{-/-}$ mice [23]. The plasma lipoprotein pattern in these animals, in particular the almost complete absence of HDL, mirrors that of human ABCA1 deficiency. In the developing mouse fetus, disposal of apoptotic cell bodies was inhibited. Cellular cholesterol accumulation in ABCA1$^{-/-}$ mice, particularly in the lungs, was dramatic. These abnormalities have not been reported in human Tangier patients.

Apo A1$^{-/-}$ mice showed decreased HDL cholesterol levels (about 25% of normal). Apo E levels in HDL were increased, and probably as a result, atherosclerosis susceptibility was not increased. Plasma LCAT activity was almost completely inhibited in these animals. This suggests that other apolipoproteins are unable to replace apo A1 in vivo as LCAT cofactors. In mice transgenic for human apo A1, the human protein displaced mouse apo A1 almost completely from HDL. These animals were protected against diet-induced atherosclerosis.

LCAT$^{-/-}$ knockout mice have a marked phenotypic resemblance to LCAT deficient human subjects. Discoidal and lipid-poor HDL species accumulate in the plasma, consistent with the identification of these as precursors of mature, alpha-migrating HDL. LCAT transgenic mice were not protected from atherosclerosis, in contrast to LCAT transgenic rabbits [24].

PLTP$^{-/-}$ mice had reduced HDL levels, consistent with the role proposed for this transfer protein in supplying phospholipids to the LCAT reaction, but atherosclerosis susceptibility was not increased [20]. This may be linked to a concomitant reduction in the secretion of apo B lipoproteins (Section 4.2). Overexpression of PLTP in mice was associated with atherosclerosis resistance, and the appearance of increased concentrations of prebeta-HDL, consistent with the role predicted for these particles in the early steps of reverse cholesterol transport.

CETP is absent from mouse plasma. The human CETP gene was expressed in mice in a number of independent studies. Its effects were complex. In one study, the expression

of human CETP together with human LCAT, reduced atherosclerosis susceptibility. In a second study, overexpression of human CETP alone in mice led to increased levels of cholesteryl ester in apo B lipoproteins, and induction of atherosclerosis. The molecular basis of these differences is still not clear.

SR-B1 deficiency was associated with reduction in the bilary cholesterol content, and an increase in circulating HDL levels. Intestinal cholesterol absorption was not increased in SR-BI$^{-/-}$ mice. Mice transgenic for human SR-BI showed significantly lower plasma HDL levels. Paradoxically, atherosclerosis susceptibility was reduced [25].

5. *Summary and future directions*

Since the last edition of this volume, there have been many advances in understanding plasma lipid metabolism. Major developments in basic mechanisms include the identification of ABCA1 and SR-BI. In the field of triacylglycerol transport, the roles of new members of the LDL receptor family have been established, and new insights into the regulation and transport of LPL identified. The efflux of cholesterol from cells to plasma lipoproteins, previously considered the result of passive diffusion, is now recognized to be highly regulated by cell membrane proteins, and on a par with influx as a key determinant of cellular cholesterol homeostasis. The availability of mice overexpressing or deficient in almost all known factors of plasma lipid transport has provided key insights into regulatory pathways.

In other areas, much remains to be done. Knowledge of the tertiary structure of key proteins of plasma lipid metabolism remains very incomplete. Our understanding of the transcriptional regulation of newly identified lipid transport proteins is, not surprisingly, still in its infancy. Efforts to generate mouse models better reflective of human vascular biochemistry and pathology continue. Plasma lipoprotein metabolism has shown itself again to be much more complex, and much more highly regulated than previously thought, and still a target for further intensive research.

Abbreviations

ABCA1	ATP-binding cassette transporter A1
Apo-	apolipoprotein
CETP	cholesteryl ester transfer protein
HDL	high-density lipoprotein
IDL	intermediate-density lipoprotein
LCAT	lecithin : cholesterol acyltransferase
LDL	low-density lipoprotein
LPL	lipoprotein lipase;
PLPT	phospholipid transfer protein
RCT	reverse cholesterol transport
SR-BI	scavenger receptor BI;
VLDL	very low-density lipoprotein

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