Abstract: Research into lipoprotein metabolism has developed because understanding lipoprotein metabolism has important clinical indications. Lipoproteins are risk factors for cardiovascular disease. Recent advances include the identification of factors in the synthesis and secretion of triglyceride rich lipoproteins, chylomicrons (CM) and very low density lipoproteins (VLDL). These included the identification of microsomal transfer protein, the cotranslational targeting of apoproteinB (apoB) for degradation regulated by the availability of lipids, and the characterization of transport vesicles transporting primordial apoB containing particles to the Golgi. The lipase maturation factor 1, glycosylphosphatidylinositol-anchored high density lipoprotein binding protein 1 and an angiopoietin-like protein play a role in lipoprotein lipase (LPL)-mediated hydrolysis of secreted CMs and VLDL so that the right amount of fatty acid is delivered to the right tissue at the right time. Expression of the low density lipoprotein (LDL) receptor is regulated at both transcriptional and post-transcriptional level. Proprotein convertase subtilisin/kexin type 9 (PCSK9) has a pivotal role in the degradation of LDL receptor. Plasma remnant lipoproteins bind to specific receptors in the liver, the LDL receptor, VLDL receptor and LDL receptor-like proteins prior to removal from the plasma. Reverse cholesterol transport occurs when lipid free apoAI recruits cholesterol and phospholipid to assemble high density lipoprotein (HDL) particles. The discovery of ABC transporters (ABCA1 and ABCG1) and scavenger receptor class B type I (SR-BI) provided further information on the biogenesis of HDL. In humans HDL-cholesterol can be returned to the liver either by direct uptake by SR-BI or through cholesteryl ester transfer protein exchange of cholesteryl ester for triglycerides in apoB lipoproteins, followed by hepatic uptake of apoB containing particles. Cholesterol content in cells is regulated by several transcription factors, including the liver X receptor and sterol regulatory element binding protein. This review summarizes recent advances in knowledge of the molecular mechanisms regulating lipoprotein metabolism.

Keywords: ABCA1; angiopoietin-like proteins; apoC; apoAI; apolipoprotein E (apoE); cholesterol ester transfer protein; chylomicron; LDL receptor; lecithin cholesterol acyl transferase; lipoprotein(a); lipoprotein lipase; microsomal transfer protein; prorperpin convertase subtilisin/kexin type 9; SR-BI; phospholipid transfer protein; very low density lipoproteins (VLDL).

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Background

Cardiovascular disease (CVD) is a major cause of worldwide mortality. The main clinical entities of CVD are coronary artery disease (CAD), ischemic stroke, and peripheral arterial disease (PAD). The risk factors for CVD are multifactorial and include age, male gender, tobacco smoking, lack of physical activity, dietary habits, blood pressure, and dyslipidemias.

The term dyslipidemias, elevated or decreased levels of lipoproteins, covers a broad spectrum of lipid abnormalities. Dyslipidemias may be secondary and related to other diseases or primary and due to the interaction between a genetic predisposition and environmental factors. Observational studies indicated a continuous positive relationship between coronary disease risk and blood cholesterol concentrations [1]. Further, research from experimental animals, laboratory investigations, epidemiology, and genetic forms of hypercholesterolemia indicate that elevated total cholesterol and low density lipoprotein (LDL) cholesterol is a risk factor for ischemic heart disease [2]. The Prospective Study Collaboration reports that the relations between cholesterol and CVD holds true across age and blood pressure categories. Interestingly, total/HDL-cholesterol ratio is more informative in this meta-analysis than high density lipoprotein (HDL), non-HDL or total cholesterol. The analysis, however, reported no clear association between cholesterol and stroke. Stroke is a heterogeneous condition and various causes of ischemic stroke may have different associations with cholesterol [3, 4]. By contrast, meta-analysis of randomized trials of few years of statin therapy have shown that reduction of LDL cholesterol by about 1.5 mmol/L reduces by about a third the incidence not only of ischemic heart disease but also of ischemic stroke, independently of age, blood pressure or prerandomization lipid concentrations. The benefits of treatment were significant in the first year, but remained in subsequent years. The reduction in ischemic stroke in randomized trials of statins suggests a need for more analysis of blood lipids and stroke subtypes [5]. Further meta-analysis of randomized trials comparing more versus less intensive statin therapy suggested that for each 1 mmol/L reduction in LDL cholesterol the annual rate of major vascular events was reduced by about a fifth. Absolute reduction in cardiac mortality produced by lowering LDL cholesterol with statin therapy in a given population depended chiefly on the absolute risk of death due to coronary exclusion. After adjustments for other risk factors, the risk ratio for upper versus lower tertiles of HDL-cholesterol was similar in participants allocated more intensive as well as those allocated less intensive statin therapy, suggesting that the risk reduction was similar in patients with higher HDL concentrations [6].

Hypertriglyceridemia is a heterogeneous disorder with an unclear association with atherosclerosis and ischemic heart disease. It is difficult to distinguish the effects of triglyceride on CVD risk from that of low HDL as HDL is inversely correlated to triglycerides. Most studies that reported an association between fasting plasma triglycerides and CVD risk found that after adjustment for other risk factors (especially HDL) triglyceride was no longer an independent risk factor [7]. A meta-analysis of studies carried out between 1996 and 2007 reported a positive association between triglyceride levels and stroke [8]. The practice of measuring triglycerides in a fasting condition to assess a relationship between cardiovascular risk and plasma triglyceride has been questioned as humans are in post-prandial state for a major part of the day. Mora et al. [9] suggest that HDL-cholesterol, triglycerides, total/HDL-cholesterol ratio and apolipoprotein AI (apoAI) are better predictors of CVD when measured in a non-fasting state. In the Copenhagen City Heart Study stepwise increasing levels of non-fasting cholesterol and non-fasting triglycerides were similarly associated with stepwise increasing risk of myocardial infarction, with non-fasting triglycerides being the best predictor in women and non-fasting cholesterol the best predictor in men. Surprisingly, only increasing levels of triglyceride were associated with total mortality whereas increasing cholesterol levels were not [10]. Using the same study cohort the authors found that stepwise increasing levels of non-fasting triglycerides were associated with risk of ischemic stroke in men and women [11, 12]. Increased levels of non-fasting triglyceride indicate the presence of increased levels of remnants from chylomicrons (CM) and very low density lipoproteins (VLDL). These cholesterol containing triglyceride rich lipoproteins can penetrate the arterial endothelium and may get trapped within the subendothelial space leading to the development of atherosclerosis [13]. Several studies have raised the possibility that remnant particles may be associated with CVD risk [14, 15]. In meta-analyses of the effects of Gly188Glu and Asn291Ser substitutions in lipoprotein lipase Wittrup et al. [16] reported that in carriers of both substitutions post-heparin lipoprotein lipase (LPL)
activity was decreased with an increase in plasma triglycerides. Accordingly, the risk of ischemic heart disease was increased in heterozygous carriers. In a further prospective study, over 20 years, relatives in familial combined hyperlipidemia families were at increased risk of CVD mortality. Among familial hypertriglyceridemia families the relative risk was higher but did not reach statistical significance, probably because of smaller sample size. Baseline triglyceride levels predicted subsequent CVD mortality in both families and the risk remained statistically significant after adjustment for total cholesterol [17]. Other studies suggest that LPL variants are associated with a differential susceptibility to CVD [18, 19].

Fibrates are agonists of the peroxisome proliferator-activator receptors (PPARs) and are selective for α receptors. They have been shown to raise HDL-cholesterol, lower total cholesterol and triglyceride concentrations and decrease LDL concentrations [20]. Several large scale trials of fibrate therapy have been completed, results, however, have been conflicting about the presence and magnitude of any cardiovascular protective effects. Subgroup analysis of fibrate trials suggest that for patients with type II diabetes mellitus or the metabolic syndrome, fibrates may exert a greater cardio protective effect than the general population [21]. The Action to Control Cardiovascular Disease in Diabetes (ACCORD) trial investigated the incremental effect of fenofibrate in patients with type 2 diabetes mellitus (T2DM) who were already being treated with simvastatin. The authors concluded that fenofibrate and simvastatin did not reduce the rate of fatal cardiovascular events, non-fatal stroke as compared with simvastatin alone [22]. In the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) trial treatment with fenofibrate did not significantly reduce the risk of first myocardial infarction (MI) or coronary heart disease (CHD) death, though it reduced non-fatal MI and revascularizations [23].

A large meta-analysis including more than 45,000 individuals suggested that therapy with fibrates can reduce the risk of coronary events [24]. Analysis of the ACCORD study suggested that patients with a high triglyceride level and low serum HDL-cholesterol level were a subset for which the greatest reduction in cardiovascular events was achieved. This was confirmed in a further meta-analysis which suggested that the greatest benefits of fibrates in reducing the risk of vascular events was most pronounced in patients with both high triglycerides and reduced HDL-cholesterol [25]. The Third Report of the National Cholesterol Education Program (NCEP) Expert Panel (Adult Treatment Panel III) recommends that in persons with high serum triglycerides, elevated remnant lipoproteins should be reduced in addition to lowering LDL [26].

As there is a need for strict maintenance of tissue cholesterol and triglyceride levels the body relies on a complex homeostatic network to modulate the availability of cholesterol and triglyceride to tissues. In recent years there have been great advances in the diagnosis and treatment of abnormalities of lipid homeostasis. There is mounting interest in optimizing dyslipidemia management through individualization of additional treatment. Further, understanding hyperlipidemias becomes relevant with the worldwide epidemic of obesity, metabolic syndrome, and T2DM.

A more complete understanding of the molecular mechanisms of lipoprotein metabolism should give an improved understanding of hyperlipidemias in humans and open new avenues for individualization of treatment of dyslipidemias and other metabolic disorders leading to dyslipidemias. This article will give an overview of recent studies and advancement in our knowledge of the metabolism of lipoproteins.

Normal lipoprotein metabolism

Triglycerides and cholesteryl esters are transported in the form of lipoproteins. Triglyceride and cholesteryl esters, fat soluble vitamins comprise the core of the lipoproteins and are enveloped by a layer of phospholipids, free cholesterol and proteins. The proteins [apoproteins (apo) or apolipoproteins] are critical regulators of lipid transport and lipid metabolism by mediating interactions with receptors, enzymes and lipid transport proteins. An overview of lipid metabolism is given in Figure 1.

Assembly and secretion of apoproteinB
(apoB) containing lipoproteins

Chylomicrons

Chylomicron assembly

Triacylglycerol is the predominant fat in the diet, contributing 90%–95% of the total energy derived from dietary fat. Dietary fats also include phospholipids (predominantly phosphatidylcholine), cholesterol and fat soluble vitamins. The activity of pancreatic lipase on triacylglycerols generates 2-monocacylglycerols and free fatty acids. Free fatty acids are absorbed from the intestinal lumen into the enterocyte for the biosynthesis of neutral fats. A protein independent diffusion model and protein dependent mechanisms have been proposed for the transport of free fatty acids across the apical membrane.
Table 1 Apolipoproteins.

<table>
<thead>
<tr>
<th>Apolipoprotein</th>
<th>Molecular weight</th>
<th>Lipoproteins</th>
<th>Metabolic functions</th>
<th>Synthesis</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>ApoAI</td>
<td>28,016</td>
<td>HDL, chylomicrons</td>
<td>Structural component of HDL, LCAT activator</td>
<td>Liver, intestine</td>
<td>[27]</td>
</tr>
<tr>
<td>ApoAII</td>
<td>17,414</td>
<td>HDL, chylomicrons</td>
<td>Involved in chylomicron assembly and secretion</td>
<td>Liver</td>
<td>[28, 29]</td>
</tr>
<tr>
<td>ApoAIV</td>
<td>46,465</td>
<td>HDL, chylomicrons</td>
<td>Effects on plasma triglyceride concentrations are complex and variable. Activator of intravascular hydrolysis by LPL. Modulates hepatic triglyceride metabolism</td>
<td>Predominantly in the liver</td>
<td>[30, 31]</td>
</tr>
<tr>
<td>ApoAV</td>
<td>HDL, VLDL, chylomicrons</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ApoB48</td>
<td>264,000</td>
<td>chylomicrons</td>
<td>Necessary for assembly and secretion of chylomicrons from the small intestine</td>
<td>Intestine</td>
<td></td>
</tr>
<tr>
<td>ApoB100</td>
<td>540,000</td>
<td>VLDL, IDL, LDL</td>
<td>Necessary for assembly and secretion of VLDL from liver. Structural protein of VLDL, IDL and LDL. Ligand for LDL receptor</td>
<td>Liver</td>
<td></td>
</tr>
<tr>
<td>ApoCI</td>
<td>6630</td>
<td>Chylomicrons, VLDL, IDL, HDL</td>
<td>ApoCI inhibits lipoprotein binding to its receptors. Potent inhibitor of cholesteryl ester transfer protein.</td>
<td>Liver</td>
<td>[32, 33]</td>
</tr>
<tr>
<td>ApoCII</td>
<td>8900</td>
<td>Chylomicrons, VLDL, IDL, HDL</td>
<td>Activator of lipoprotein lipase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ApoCIII</td>
<td>8800</td>
<td>Chylomicrons, VLDL, IDL, HDL</td>
<td>Inhibits lipoprotein lipase; increases VLDL secretion. ApoCIII can also stimulate several processes involved in atherogenesis and vascular inflammation. Interferes with remnant lipoprotein clearance.</td>
<td>Synthesized in the liver and to a lesser extent in the intestine</td>
<td>[32, 34–36]</td>
</tr>
<tr>
<td>ApoE</td>
<td>34,145</td>
<td>Chylomicrons, VLDL, IDL, HDL</td>
<td>LDL receptor ligand for LDL and chylomicron remnants. Ligand for LRP. Role in reverse cholesterol transport.</td>
<td>Predominantly in the liver</td>
<td>[37, 38]</td>
</tr>
<tr>
<td>Apo(a)</td>
<td>250,000–800,000</td>
<td>Lp(a)</td>
<td></td>
<td>Liver</td>
<td></td>
</tr>
</tbody>
</table>

of the enterocyte. Bile salt micelles facilitate the transfer of cholesterol across the brush border membrane. Studies with genetically modified animal models have identified Niemann-Pick C1-like 1 (NPC1L1) protein as a cholesterol uptake transporter and ATP-binding cassette (ABC) proteins ABCG5 and ABCG8 as cholesterol efflux transporters. NPC1L1 and ABCG5/G8 are almost exclusively on the apical membranes of enterocytes in the intestine and hepatocytes in the liver. This would allow for transport of intracellular sterols back into the small intestine for fecal excretion [39]. Endoplasmic reticulum (ER) membrane localized enzymes, acyl-CoA:cholesterol acyltransferase (ACAT) catalyze the esterification of intracellular cholesterol; it has been suggested that esterification of cholesterol is important for entry of cholesterol into nascent CMs [40]. Fatty acids and monoacyl glycerol are utilized to resynthesize diacyl glycerol and triacylglycerol by acyl-CoA:monoacylglycerol acyltransferase and acyl-CoA:diacylglycerol acyltransferase in the ER [41].

After ingestion of a meal, dietary fat are absorbed into the cells of the small intestine and incorporated into the core of nascent CMs. The human intestine is equipped to efficiently absorb dietary fat predominantly in the form of triacylglycerol and to form CMs and deliver lipids in this form to peripheral tissues. The multistep assembly of CMs within the enterocyte includes cellular lipid re-esterification, translocation of cellular lipid pools, synthesis and post-translational modification of apoproteinB (apoB), and finally packaging lipid, and apoB into CMs. Each of these lipoproteins contains 1 molecule of apoB [42].

Apolipoproteins and chylomicron synthesis
Apoprotein B48 (apoB48) is found on CMs. In humans, apoB48 found in the small intestine is a truncated form of apoB100 and is necessary for the intestinal secretion of these lipoproteins. ApoB100 is found mainly in the liver.
ApoB100 has 4536 amino acids. ApoB48 results from a post-transcriptional modification of the apoB mRNA, through the action of the APOB mRNA editing complex (APOBEC1) which forms a stop codon at approximately 48% of the full length coding sequence [43]. The truncated form of ApoB100, ApoB48 lacks the C-terminus which contains the domain recognized by the LDL receptor. Unlike exchangeable apolipoproteins, apoB remains associated with lipoproteins from assembly/secretion to lipoprotein remnant clearance. Given that the critical role that apoB plays in lipoprotein transport, it is not surprising that regulation of the secretion of apoB containing lipoproteins is complex. Microsomal triglyceride transfer protein (MTP) activity is required for apoB containing lipoprotein assembly and secretion. Early studies suggested that a significant amount of newly synthesized apoB is degraded. Unlike most secreted proteins, apoB levels are primarily regulated through degradation. Studies of apoB degradation indicated that the turnover of the protein was rapidly and negatively regulated by triglyceride synthesis. It was found that when conditions were not favorable for apoB assembly with lipids, apoB was degraded. Studies in several laboratories have demonstrated several points along the intracellular processing of apoB where it can be targeted for lipoprotein assembly and secretion or degradation [44] (Figure 2).

ApoB like all secreted proteins is synthesized at the surface of the ER. Once the ribosome is targeted and docked securely at the aqueous translocation channel the nascent chain is cotranslationally translocated across the ER membrane. The signal sequence which spans amino acids 1–27 of apoB contacts the ER membrane, and translocation of apoB occurs through the translocon, a proteinaceous channel in the ER membrane, formed mainly by the Sec61 protein. Entry of the N-terminus into the translocon causes reinitiation of the translation which is coordinated with translocation until the full length protein enters the ER lumen for further processing and transport [45]. MTP is a heterodimeric protein complex possessing lipid transfer activity, which functions in the small intestine and liver to transfer lipids to assist in the formation of primordial apoB lipoproteins. It has been shown to have three domains: 1) an apoB binding domain; 2) a lipid transfer domain; and 3) a membrane association domain [46]. The initial incorporation of lipids into apoB by MTP may prevent apoB from degradation. The absence of either a lipid substrate or MTP may result in the improper folding of apoB and consequently its degradation [47]. However, it has been suggested that both enterocytes and hepatocytes may have different methods of apoB stabilization to achieve their specific metabolic functions. It has been demonstrated in Caco-2 cells that mature apoB proteins accumulate in the

Figure 1  Pathways of triglyceride rich lipoprotein metabolism. Intestinal enterocytes absorb dietary lipids and package most of it into chylomicrons. Endothelial bound LPL hydrolyzes triglycerides in CM to generate chylomicron remnants which are rapidly cleared by the liver. Intrahepatic lipids are repackaged and secreted as VLDLs which are substrates for LPL. Remnants formed by hydrolysis of VLDL are either taken up by the liver or converted to LDL. The hepatic LDL receptor is responsible for clearing LDL from human plasma.
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 cis-Golgi network. The nascent CMs are larger (250 nm or more) in diameter while protein vesicles are smaller (60–80 nm) [53], and it is unlikely that nascent CMs can move across the ER because of physical constraints. This suggests that prechylomicron transport vesicles (PCTVs) may require specialized transport machinery for their export from the ER to the cis-Golgi. In addition, the intermittent nature of PCTV formation suggests that PCTV budding may require proteins that differ from those used for protein vesicles. It has been suggested that an isoform of protein kinase C(ζ) (PKC) and vesicle-associated membrane protein 7 (VAMP7) play a role in the formation of PCTVs and delivery to the cis-Golgi [54, 55]. Characterization of PCTVs showed that various proteins including liver fatty acid binding proteins collocate with apoB48 [56]. CD36, described as a fatty acid translocase has been demonstrated to be part of the PCTVs budding complex [57]. COPII coats are assembled from three components, i.e., Sar1b, Sec23/24 and Sec 13/31 complexes [58] and are needed to form lipid vesicles that can fuse with the cis-Golgi complex. The Sar1b protein rallies the COPII-complex to form a shell around the vesicles transporting CMs to the cis-Golgi [59]. It has been suggested that COPII proteins are required for fusion of PCTVs with the cis-Golgi, but that, in the intestine, membrane bound lipid particles can bud from the ER in a COPII independent manner [53]. N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) proteins facilitate the targeting, docking and fusion of transport vesicles with their target membranes. SNARE proteins are type II integral membrane proteins which are present on transport vesicles and their target membranes. The PCTV uses VAMP7 and syntaxin 5 with rBet1 and vti1a as the cis-Golgi SNARE proteins involved in the SNARE fusion complex. The lipid composition of CMs changes in the Golgi and it is likely that they acquire apoAI in this compartment [60]. MTP is found within the Golgi apparatus and takes part in triglyceride transfer within the Golgi apparatus [61]. Fully assembled lipoproteins are released by exocytosis of secretory vesicles from the basolateral membranes of enterocytes.

In humans, apoAIV is a 46kd plasma glycoprotein synthesized predominantly by the intestine. ApoAIV enters the circulation on the surface of nascent CMs. It has been suggested that apoAIV enhances particle expansion and increases triglyceride secretion [62]. In the circulation, apoAIV is displaced from CMs by HDL-associated apoC, and the exchange facilitates the activation of LPL by its cofactor apoCII [63].

In humans, severe hyperchylomicronemia has been associated with mutations in the apolipoprotein AV (APOAV) gene [64]. ApoAV circulates at very low...
concentrations in the plasma in association with triglyceride rich lipoproteins and HDL. Experimental data suggest that apoAV enhances the catabolism of triglyceride rich lipoproteins by stimulating LPL. One hypothesis is that ApoAV binds to CMs or VLDL and to endothelial proteoglycans and LPL and stabilizes the endothelial lipolytic system [30]. The question as to whether ApoAV is a key determinant of triglycerides levels in humans remains conjectural. APOA5 is part of the APOA1/A4/C3/A5 gene cluster located in chromosome 11. Studies in cell culture and animal models have identified several transcription factors including PPAR-α, liver X receptor-α (LXR-α), hepatocyte nuclear factor 4-α (HNF4-α) and upstream stimulatory factor (USF1) that contribute to the regulation of APOA5 expression [65, 66]. In a recent study, Lee et al. [67] identified cyclic AMP responsive element binding protein H (CREB-H) as a regulator of several proteins affecting lipolysis, including apoAV, apoCII and apoCIII. Three isoforms of PPARs (α, β, γ) have been described. PPARs are major regulators of lipid and glucose metabolism, allowing adaptation to changing nutritional environment. PPARα potentiates fatty acid metabolism in the liver and PPARγ induces adipocyte differentiation [68].

Catabolism of chylomicrons

In the lymph and blood, CMs acquire apoCI, apoCII, apoCIII and apoE (Table 1). After gaining apoCII the activator of LPL, chylomicron interacts with the enzyme and triglyceride hydrolysis is initiated. LPL mediated triglyceride hydrolysis is accompanied by a reduction in the core volume of the CMs and transfer of phospholipid, free cholesterol, apoCII and apoCIII back to HDL. As the chylomicron circulates, the core triglyceride undergoes hydrolysis by endothelial bound LPL with entry of fatty acids into muscle for energy production and adipocytes for storage. In the two-step model, the remaining relatively triglyceride-depleted chylomicron ‘remnant’ particles which are enriched in cholesteryl ester (from both dietary- and HDL-derived cholesteryl ester) and apoE enriched can interact with receptors on hepatocytes and be removed from the circulation [69]. All CMs usually disappear from the circulation within 12–14 h after a meal [70].

Lipoprotein lipase and chylomicron metabolism

LPL is synthesized by a number of cells and tissues. The major sites of LPL synthesis are the skeletal and cardiac muscle and adipose tissue. LPL must transfer from its cell of origin to the luminal surface of capillary endothelial cells that are exposed to large triglyceride rich lipoproteins in the blood. In humans, LPL and hepatic lipase (HL) have been analyzed in plasma samples, collected after the injection of heparin, a procedure used to release LPL and HL from cellular binding. LPL is a member of the lipase family which includes HL and pancreatic lipase. All three proteins are encoded by genes that share structural similarities [71]. Due to its central role in lipid metabolism, understanding the mechanisms controlling LPL expression becomes important. Changes in LPL expression are mainly through the action of hormones such as insulin, glucocorticoids and adrenaline [72, 73]. Published data on the points of control of LPL show its considerable complexity. LPL is regulated in a tissue/cell-specific manner, and both transcriptional and post-transcriptional control mechanisms have been identified in the regulation of LPL gene expression [74]. This is physiologically important because LPL directs fatty acid utilization according to the metabolic demands of each individual tissue. LPL generates fatty acids that are either used for fuel in muscle or stored in the form of triglycerides in adipose tissue. For example, fasting decreases LPL activity in adipose tissue but increases activity in cardiac tissue [73].

LPL is glycosylated and exists as a dimer and is engaged in a number of different molecular interactions as part of its functions. Several effectors of LPL have been identified that modulate the function of LPL in vivo. ApoCII is an essential cofactor of LPL activity and angiopoietin-like proteins (ANGPTL) 3 and 4 have been shown to inhibit LPL activity. The interaction between ANGPTL4 and LPL results in the conversion of enzyme from catalytically active dimers to inactive monomers The ANGPTL protein family currently has six members [75]. ANGPTL3 is expressed almost exclusively in the liver an organ that expresses little or no LPL in adults, and is presumed to function as a circulating inhibitor of LPL. ANGPTL4 is expressed in multiple tissues, with the highest level in mice in the adipose tissue. ANGPTL4 expression in adipose tissue is induced by fasting, suggesting it inhibits LPL in adipose tissue to reroute fat from adipose tissue to other tissues including muscle during fasting [76].

Recently two new proteins that are involved in post-translationally regulating LPL activity have been identified. The lipase maturation factor 1 (LMF1) plays an essential role in the formation of catalytically active LPL from newly synthesized polypeptides. The glycosylphosphatidylinositol-anchored high density lipoprotein binding protein 1 (GPIHBPI) is critically involved in LPL-mediated hydrolysis of TG rich lipoproteins [77]. GPIHBPI provides an important platform for LPL-mediated hydrolysis of chylomicron triglycerides. Davies et al. [78] showed that GPIHBPI was effective in transporting

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LPL from the basolateral to the apical surface of endothelial cells. Recently, it has been suggested that LPL and GPIHBP1 move bidirectionally across the cell surface, however, the understanding of the molecular processes of the transport remains incomplete [79]. In mice, GPIHBP1 was expressed highly on the capillaries of lipolytic tissues such as adipose tissue, heart and skeletal muscle. These findings suggest that GPIHBP1 is an important platform for the LPL-mediated processing of CMs in capillaries and that the differences in GPIHBP1 expression could play a role in regulating the delivery of lipid nutrients to different tissues. The high affinity binding of LPL to GPIHBP1 implies that GPIHBP1 can tear LPL from endothelial heparin sulphate proteoglycans (HSPG) [80]. Levels of expression of GPIHBP1 in liver are low, which likely contributes to poor hepatic clearance of CMs until triglyceride hydrolysis transforms them into remnant lipoproteins [81].

**Cellular membrane receptors and chylomicron metabolism**

The mechanism of hepatic clearance of chylomicron remnants remains controversial. The particles first may be sequestered in the liver perisinusoidal space (space of Disse) where they may undergo further processing by HL and LPL, both of which are detectable in the space of Disse. A group of hepatic lipoprotein receptors then endocytose the lipoproteins leading to their lysosomal catabolism. Multiple receptors clearing triglyceride rich remnants are thought to exist. Several authors have implicated HSPGs as receptors for lipoprotein remnants [82, 83]. Each HSPG consists of a polypeptide strand called the core protein, onto which highly negative carbohydrate polymers called heparin sulphate are assembled. The carbohydrate side chains of hepatic HSPGs exhibit structures of high flexibility and negative charge that can increase ligand affinity and direct lipoprotein remnants to the liver. The syndecan-1 HSPG has been suggested as a major HSPG receptor for remnant lipoproteins in the liver [84]. Several lipid binding proteins such as LPL [85], HL and apoE [86] can bind to HSPGs and facilitate hepatic remnant clearance. The highly sulphated HSPGs in the liver result in negative charges that can bind LPL and apoE. It is postulated that apoE interacts initially with HSPG prior to transfer to receptors for internalization [86].

Early studies suggested that the metabolism of chylomicron remnants is severely impaired in patients with familial hypercholesterolemia (FH) who lacked functioning LDL receptors. CMs require four LDL receptors for binding compared with one receptor per LDL particle [87]. Other studies suggest that chylomicron remnant clearance is not impaired in FH subjects [88]. Using a lipid emulsion mimicking the composition of CMs, and measuring metabolism using a stable isotope breath test, Watts et al. [89] suggest that the metabolism of CM remnants is not impaired in patients with FH. The role of LDL receptors in the metabolism of chylomicron remnants remains controversial. Experiments with mice and in vitro cell culture studies suggest that uptake with LDL receptors occur rapidly (t1/2 ~10 mins) while the second LDL receptor independent pathway occurs less rapidly (t1/2 ~60 mins) [83, 90].

It has been suggested by other authors that in the absence of LDL receptors other receptors such as the low density lipoprotein receptor-related proteins (LRP) may contribute to chylomicron remnant uptake, though LDL receptors account for the majority of chylomicron remnant uptake under normal physiological circumstances [91]. The LRP is larger than and structurally similar to other members of the LDL receptor gene family. Whereas the LDL receptor appears to function solely in lipoprotein metabolism, the LRP appears to have several known ligands. It has been suggested that LRP may function as a multifunctional scavenger receptor, with a major function in the removal of proteinase and proteinase inhibitor complexes [92]. A role for LRP in lipoprotein metabolism has been further suggested by the fact that LRP binds not only chylomicron remnants but also lipases [93]. Studies in mice suggest that in an apoE deficient model, in which LRP1 (the largest member of the low density lipoprotein receptor family) mediated clearance of lipoprotein particles is impaired, further LRP1 dysfunction drives the system to compensate by upregulation of LDR receptor expression [94]. Binding studies suggest that apoAV interacts with two members of the LDL receptor family, LRP and mosaic type-1 receptor, SorLA. Association of apoAV with LRP or SorLA resulted in enhanced binding of human CMs to receptor covered chips, leading to the hypothesis that apoAV may influence lipid homeostasis by enhancing receptor-mediated endocytosis of CMs [95, 96].

**Very low density lipoproteins (VLDL)**

**VLDL assembly**

Intestinal CMs and liver-derived VLDL represent the two classes of triglyceride rich lipoproteins responsible for the transfer of lipids to other cells of the body. CMs mediate the transport of dietary lipids whereas VLDL delivers endogeneous lipids to peripheral tissues. The production
of VLDLs needs to be synchronized with their secretion to avoid adverse consequences such as hepatic steatosis and higher concentrations of VLDLs in the blood. There are three main sources that supply free fatty acids to the liver: 1) free fatty acids from adipocytes; 2) chylomicron remnants; and 3) the intestine via the portal vein [97]. Mobilized lipid storage pool in the liver, de novo synthesis of fatty acids and phospholipids contribute to hepatic VLDL synthesis. Phosphatidate phosphatase-1 converts phosphatidate to diacylglycerol and plays a role in the biosynthesis of phospholipids and triacyl glycerol [98].

Apolipoproteins and VLDL synthesis

ApoB100 is a large hydrophobic protein which is the main structural component of VLDL and LDL. Models of the secondary structure of apoB100 contain three α helical domains (1%–22%, 48%–56% and 89%–100% of full length of apoB100) and two long β-sheet domains (22%–48% and 56%–89%) (NH₂-βα₁-β₁-α₁-β₂-α₂-COOH) [99]. Other studies have indicated that the first 17% of the N-terminal end of apoB100 has the LPL binding domain, that the domain between 66%–83% of the full length apoB100 is the LDL receptor binding domain, and the sequences between 1% and 5.8% and between 9% and 16% are the binding sites for MTP [100].

If MTP and lipid availability is adequate, nascent apoB100, like apoB48 and other secretory proteins is translocated efficiently across the ER membrane. There it can be assembled with lipids into VLDL in the ER lumen and secreted into the medium. If instead, there is inadequate lipid or MTP, cotranslational translocation of apoB100 is inefficient and newly synthesized apoB100 can be degraded. It is believed that the complex structure of apoB100 plays a role in its post-translational degradation, which is a predominant way in which the secretion of apoB lipoproteins is controlled. In contrast to other secretory proteins, the stepwise translocation of apoB results in the transient exposure of large domains to the cytoplasm as assayed by exogenous proteases. It has been suggested that transient intermediates of nascent apoB are paused during their passage across the bilayer of ER. Both pause transfer sequences [101] and the presence of β-sheet domains [100] have been indicated as influencing the translocation efficiency of apoB. Both the incomplete nascent apoB polypeptides and full length glycosylated apoB protein have been shown to be degraded by the ubiquitin-proteasome system in the cytosol. The proteasome is a multicatalytic machine that destroys ER-associated degradation (ERAD) substrates. Proteasomes located at the cytosolic surface of the ER can ingest ubiquitinylated apoB as it emerges in a retrograde manner from the translocon. It is still unclear how full length glycosylated apoB, which is a large protein, can cross ER membrane into the cytosol [102]. In addition, apoB interacts with an ensemble of molecular chaperones during and after translocation. Molecular cytosolic chaperones such as Hsp70 and Hsp90 participate to varying degrees in the degradation of almost every ERAD substrate examined and it has been suggested that both are required for the interaction of apoB with the proteasomal pathway [103]. In contrast, the Hsp110 chaperone protects apoB from degradation [104]. Further studies implicate the ER chaperone glucose-regulated protein/binding immunoglobulin protein (Grp78/BiP) in ERAD of apoB100 [105]. It has been suggested that cotranslational degradation of apoB may be initiated by strong binding of BiP to the N-terminus followed by association of the AAA-ATPase, p97 with the C-terminus [106].

ERAD is not the only mechanism used to regulate apoB. Two other processes have been suggested. Fisher et al. [107] demonstrated that the post-translational degradation of apoB100 also occurs by a previously unknown mechanism, in a compartment separate from the ER, known as post-ER presecretory proteolysis (PERPP). PERPP stimulation induces aggregation of apoB containing lipoproteins which are then sorted to degradation through the autophagy pathway. During autophagy damaged organelles, cytosolic proteins and protein aggregates are engulfed by double-membraned vesicles (autophagosomes) which deliver their cargo for degradation to the lysosomes [108]. It has been suggested that ERAD destroys apoB when triglycerides are limiting but PERPP occurs when triglycerides are normal. Dietary polyunsaturated fatty acids (particularly the n-3 fatty acids enriched in fish oils) lower VLDL levels. The stimulation of PERPP by major polyunsaturated fatty acids appears selective for apoB incorporated into VLDL particles [109]. The second process has been demonstrated to occur in vitro. Williams et al. [110] identified an unstirred water layer around the plasma membrane from which apoB containing lipoproteins are taken up via the LDL receptor. In addition, it has been suggested that the LDL receptor is involved in hepatic VLDL assembly and that it promotes post-translational degradation thus interfering with secretion of VLDL from the liver. This suggests a gatekeeping function for the receptor [111, 112].

The current model of VLDL (and chylomicron) assembly is in two steps. In the first step newly synthesized apoB is lipided during its translocation across the ER into the lumen yielding the primordial apoB particle. In the second step bulk transfer of core lipids from ER luminal
lipid droplets to the primordial apoB particle is thought to take place post-translationally. Hepatic VLDL assembly and secretion is also influenced by de novo biosynthesis of phospholipids and triacylglycerol [113]. Lipid droplets were long considered simple storage lipid depots, but are now thought of as dynamic cellular organelles [114]. Various cytosolic lipid droplet-associated proteins, (perilipin2, CideB and CGI-58) contribute to lipid metabolism, but the exact mechanism remains to be elucidated [115].

It is suggested that both CMs and VLDL utilize different vesicles and attendant mechanisms to be transported from ER to the cis-Golgi. Unlike CMs, VLDLs depart from the ER in a COPII dependent fashion [116]. Furthermore, it is suggested that VLDL exits the ER as a poorly lipidated LDL-sized particle, and thus smaller vesicles are able to accommodate immature VLDL [117]. Although it is established that the first step of VLDL assembly occurs in the ER, the location of additional steps in VLDL maturation is less clear. Several studies have suggested that ER is the final site of VLDL maturation to VLDL2 (Sf 20–100) or VLDL1 (Sf >100) [118], whereas others have implicated the Golgi complex as a second site of maturation [119]. The VLDL transport vesicle fuses with liver cis-Golgi to deliver VLDL to the Golgi lumen. The components of the SNARE complex that play a role in docking and fusion of the VLDL transport vesicles have been identified as Sec22b (vesicle SNARE), syntaxin 5, rBet1 and Gos28 (target membrane SNARE) [120]. The ER to Golgi movement of VLDL is prerequisite for their ultimate secretion from hepatocytes (Figure 3).

Although other functions have been characterized for the VLDL-associated apolipoproteins apoE, apoCIII and apoV, studies have implicated these apolipoproteins in the assembly and secretion of the fully lipidated VLDL, independent of their role in lipoprotein clearance [35, 63, 121]. Experimental evidence suggests that apoE may facilitate the early stage of VLDL assembly in the ER and apoCIII plays a role in the post-ER state of the assembly process. The mechanism by which apoAV can attenuate the production or secretion of VLDL is still unclear [122].

Catabolism of VLDL

Once in the plasma VLDL is hydrolyzed by LPL to generate smaller denser particles and subsequently intermediate density lipoproteins (IDL). During lipolysis these remnants become enriched with HDL-derived apoE, a high affinity ligand for LDL receptor. IDL particles can undergo further catabolism by LPL to become LDL with loss of apoE. The sole remaining protein apoB100 binds to LDL receptors.

Studies with transgenic mice suggest that by binding to the LDL receptor, apoE and apoB100 containing lipids are cleared from the plasma [123].

LDL receptor and apolipoproteins

Plasma apoE originates primarily from the liver and, to a small but functionally significant extent, macrophages. ApoE contains 299-amino acid residues. The three isoforms, apoE2, apoE3 and apoE4 differ only at positions 112
and/or 158. ApoE3 contains a cysteine at position 112 and an arginine at 158, while apoE2 contains cysteine at both sites and apoE4 contains arginine at these sites. ApoE3 is considered the wild type isoform in humans because of its high allelic frequency and lack of human disease phenotype [124]. It was proposed that apoE contains two domains an N-terminal domain (a four-helix bundle) and C-terminal domain linked by a flexible hinge region. The N-terminal is responsible for LDL receptor binding but in isolation binds to lipids. The C-terminal possesses the major lipoprotein binding sites. X-ray crystallographic structure revealed a salt bridge between Arg158 and Asp154 in apoE3 that is absent in apoE2. Further in apoE2 an alternative salt bridge forms between Arg150 and Asp154 effectively eliminating the availability of Arg150 for interaction with the LDL receptor. Lipid association is required for biologically active apoE to bind to the LDL receptor. It is generally thought that the protein undergoes a lipid binding-induced conformational change, though the ultimate conformation adopted by apoE remains unsolved [37]. Studies with point mutants suggested that the receptor binding region lies in the vicinity of residues 136–150. However, residues outside the receptor binding region can also affect receptor binding activity. Mutagenesis studies suggested that Arg172 contributed to receptor binding activity [125]. The major role for apoE is to function as a ligand for cell surface receptors. As a player in lipoprotein metabolism, apoE binds to LDL receptor, LR1P1 and the VLDL receptor [126].

The two physiological ligands of LDL receptor apoE and apoB100 bind to the ligand binding domain of the LDL receptor which consists of seven repeats of approximately 40 amino acid cysteine rich tandem repeats. The ligand binding domain is located at the N-terminus of the protein and is followed by the epidermal growth factor (EGF) precursor domain. The EGF precursor domain contains two cysteine rich EGF domains of 40 amino acids (EGF-A and EGF-B) separated from a third EGF-repeat (EGF-C) by a 280-amino acid β-propeller domain. Immediately downstream of the EGF precursor homology is a threonine and serine rich region to which multiple O-linked sugars are attached which is followed by the transmembrane domain and a relatively short cytoplasmic tail that contains all the sequences required for receptor clustering in clathrin-coated pits and for the internalization of the receptor [127, 128].

LDL receptors are gathered in clathrin-coated pits. Receptor bound LDL remained on the surface of cells for <10 min and once in the cell the protein component of LDL was digested to amino acids within 60 min. Lysoosomal digestion of LDL releases amino acids and the cholesterol generated within the lysosome suppresses 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA) activity, the enzyme which catalyzes the rate limiting step in cholesterol production. Expression of LDL receptor is regulated at both transcriptional and post-transcriptional levels which influence synthesis, LDL receptor stability, endocytosis and trafficking. LXRs are nuclear receptors that are activated in response to cellular cholesterol levels. They inhibit cholesterol uptake by inducing the expression of the inducible degrader of the LDL receptor (IDOL) an E3 ubiquitin ligase that mediates ubiquitination and degradation of the LDL receptor [129]. The IDOL pathway appears to be active in macrophages, adrenals, intestine and liver [130]. IDOL contains two distinct domains: a C-terminal RING E3 ligase domain and an N-terminal FERM (Band 4.1, ezrin, -radixin-moeisin) domain which is responsible for target recognition [131]. Receptor bound LDL is internalized by endocytosis. Rudenko et al. [132] crystallized the three dimensional structure of the extra-cellular domain at an acidic pH. At neutral pH the ligand binding repeats are predicted to be extended away from the EGF domains and accessible to lipoproteins. When the pH falls as occurs in the endosome, the ligand binding domain forms a physical association with the EGF precursor homology domain. The acid dependent conformational change in the LDL receptor releases the lipoprotein from the ligand binding domain and signals the receptor to return to the cell surface. When cell cholesterol increases the production of LDL receptors is reduced. In mammals the cholesterol content of the cell membranes is controlled by a family of membrane bound transcription factors designated as sterol-regulated transcription proteins (SREBPs). In cholesterol-depleted cells the SREBPs are escorted by SREBP cleavage activating proteins (SCAP) in budding vesicles to the Golgi complex where they are processed by two proteases (site-1 protease and site-2 protease) to release a fragment that enters the nucleus and activates transcription of multiple target genes including those encoding HMG-CoA reductase and all other enzymes of cholesterol biosynthesis as well as the LDL receptor. SREBPs are weak activators of gene expression by themselves and function in a synergistic manner with more generic transcriptional co-regulatory factors. When LDL-derived cholesterol enters the cells, SCAP senses the excess cholesterol through its membranous sterol sensing domain, changing its conformation so that SCAP/SREBP complex is no longer incorporated into ER transport vesicles [133, 134]. SREBP-1c and SREBP-2 are the predominant isoforms of SREBP in liver and most other intact tissues. SREBP-1c favors the fatty acid biosynthethic pathway and SREBP-2 favors cholesterolgenesis [135]. Using IDOL-null
coding RNAs that are important post-transcriptional regulators of gene expression. By binding to the 3′ untranslated region of the protein-coding mRNA transcripts they can reduce translation from these transcripts and in some cases lead to their degradation. The genetic locus of SREBP2 and SREBP1 contain highly conserved miRNAs that regulate cholesterol export and fatty acid oxidation. Two miRNAs, miR-33a and miR-33b are encoded within introns of SREBP2 and SREBP1, respectively. miR-33 reduces cholesterol export by directly targeting the transcripts of the ABCA1 protein and reduces β-oxidation by directly targeting transcripts for the β-subunit of the mitochondrial trifunctional protein (hydroxacyl-coenzyme A dehydrogenase/3-ketoacyl-coenzyme A thiolase/enoyl-coenzyme A hydratase β-subunit), the liver-specific isoform carnitine palmityltransferase 1A and the carnitine O-octanoyltransferase [137].

Propertin convertase subtilisin/kexin type 9 (PCSK9) promotes degradation of LDL. PCSK9 is mainly expressed in liver, with lower levels of expression in the kidney, intestine and brain [138] and is present in human plasma [139]. PCSK9 is a 692-amino acid glycoprotein that contains a 22-residue signal sequence followed by a prodomain (residues 31–152) and a catalytic domain (residues 153–454) that shares structural homology with the proteinase K family of subtilisin-like serine proteases. The cysteine and histidine rich C-terminal domain consists of residues 455–692 which are three tightly packed modules named M1, M2 and M3. PCSK9 undergoes autocatalytic cleavage in the ER that severs the covalent attachment of the prodomain with the catalytic domain. Nevertheless, the prodomain remains tightly associated with the catalytic domain of PCSK9 moves through the secretory pathway [140]. PCSK9 gene transcription is under the control of SREBP1P and a highly conserved hepatocyte nuclear factor 1 binding site has been identified as a sequence motif for PCSK9 transcription. When cholesterol is depleted SREBP1 is activated increasing the expression of LDL receptor and PCSK9 genes at the same time [141]. In cultured cells the addition of recombinant PCSK9 to the medium results in LDL receptor degradation, providing evidence that PCSK9 can promote the degradation of LDL receptor by acting on the cell surface [142]. The proteolytic activity of PCSK9 is not required for PCSK9 action on the LDL receptor [143]. PCSK9 binds specifically to the EGF-A domain. Study of the crystal structure of the complex between PCKS9 and EGF-A domain suggests binding site for EGF-A domain lies in the catalytic domain of PCKS9 [144]. Binding of PCSK9 to EGF-A was calcium dependent and increased dramatically with reduction in pH from 7 to 5.2. The data suggest a model in which the binding of PCKS9 to EGF-A interferes with an acid dependent conformational change (i.e., at the acidic pH of endosomes) required for receptor cycling. As a consequence the LDL receptor is rerouted from the endosome to the lysosome where it is degraded [145]. A second binding site between the positively charged C-terminal domain of PCSK9 and the negatively charged ligand binding site of the LDL receptor with a much greater binding at pH 5.4 has been identified. This constitutes a second step driving the LDL receptor towards degradation [146, 147]. Association of PCSK9 with LDL particles in plasma lowers the ability of PCSK9 to bind to cell surface LDL receptors, blunting PCSK9-mediated LDL receptor degradation. An N-terminal region of the PCSK9 prodomain (amino acid residues 31–52) was required for binding to LDL in vitro [148]. An endogenous inhibitor annexin A2 interacts with the C-terminal of PCSK9 preventing PCSK9 from interacting with LDL receptor [149]. It is likely that there are other key tissue-specific protein partners which would allow for different levels of LDL receptor in tissues. The mechanism by which an LDL receptor with bound PCSK9 is excluded from the recycling tubules of the sorting endosome and is rerouted to the lysosomes is not determined. LDL receptors that bind PCSK9 at the cell membrane are internalized by the clathrin dependent endocytic pathway, but fail to enter the recycling pathway. The PCSK9-LDL receptor pathway is routed to the lysosomes via a pathway that does not require ubiquitination of the cytoplasmic tail of the receptor and does not involve the proteosomal or autophagy systems [150]. Two propertin convertases, furin and PCS/6A, cleave PCSK9 at the Arg198-Gln209 peptide bond, though it is controversial whether cleavage affects PCSK9 function [139, 151]. In addition a further protein, the LDL receptor adaptor protein may affect LDL receptor stability or recycling [152].

Yamamoto et al. [153] were the first to clone and characterize VLDL receptor cDNAs from rabbit heart and human macrophage cells [154]. VLDL receptor mRNA are highly abundant in heart, muscle, adipose tissue and brain and are barely detectable in the liver where LDL receptors are expressed abundantly, suggesting that this receptor may be responsible for the entry of triglyceride rich lipoproteins into muscle and fat. In contrast to the LDL receptor the VLDL receptor binds apoE2 as well as apoE3 particles and its expression is not down regulated by intracellular lipoproteins. In vitro and in vivo studies have shown that the VLDL receptor binds VLDL but not...
Lipoprotein(a)

Lipoprotein(a) [Lp(a)] consists of an LDL-like lipoprotein bound to a plasminogen-like glycoprotein, apo(a) which is disulfide linked to apoB. Levels of Lp(a) may vary thousand-fold among individuals and levels are partly determined by polymorphisms in the LPA gene coding for the apo(a) moiety of Lp(a). Apo(a) is composed of multiple kringle containing domains and a serine protease domain. LPA gene which encodes apo(a) is thought to have been generated by duplication of the neighboring plasminogen gene. Kringle motifs are disulfide linked to apoB. Analysis of secreted proteins identified 80% of recycled triglyceride rich lipoprotein proteins in the HDL fraction [159]. Other studies suggest that at least a portion of the apoE is present in the Golgi apparatus and is rerouted through the secretory pathway, and that apoE deficient VLDL may serve as an acceptor [160]. Recycling of apoE may be mediated by Golgi-derived secretory vesicles or apoE may be resecreted from peripheral endosomal compartments. In human hepatoma cells, triglyceride rich lipoprotein derived apoE colocalizes with apoAI in endosomes. The authors suggest that apoAI may be targeted to endosomes containing apoE/cholesterol complexes to promote recycling and efflux. Further, these findings may indicate that apoE recycling provides an efficient mechanism to re-supply plasma with apoE containing HDL particles [161]. Dis-similar recycling of apoE isoforms could contribute to the development of apoE4-associated diseases [162]. Studies suggest that apoE directs the intracellular routing of internalized remnant lipoproteins, with the smaller VLDL particles directed to the perinuclear regions of the mouse macrophage whereas the larger remnants remain closer to the plasma membrane [160].
reviewed epidemiologic evidence linking Lp(a) with CVD and venous thromboembolism. The increased risk of CHD associated with high Lp(a) was independent of other established risk factors for CHD [174–177]. Risk estimation however, may be higher in subjects with elevated Lp(a) and other cardiovascular risk factors [178].

Pathways regulating the synthesis and degradation of Lp(a) are not well understood. Early studies suggest that human hepatocytes secrete the apo(a)-apoB100 complex [179]. Other studies suggest that apo(a) is synthesized by hepatocytes and associates with LDL after secretion to form Lp(a) in the sinusoids of the liver. Lp(a) assembly involves an initial non-covalent interaction between amino acids 680–780 of apoB100 and kringle 4 types 5–8 followed by formation of a disulfide bond [180]. Cys4326 of APOB has been shown to be required for the covalent link between apo(a) and apoB100 [181]. It has been suggested that the liver is the major organ of clearance of Lp(a) and that apo(a) moiety plays a role in the plasma clearance of Lp(a). ApoE and the receptors that mediate the binding and uptake of apoB100 containing lipoproteins have been proposed as having a role in Lp(a) catabolism. However, the nature of the major receptor(s) that mediate the plasma clearance and tissue uptake of Lp(a) remains to be determined [182]. Clearance of Lp(a) is not well understood, but the LDL receptor or apo(a) size does not appear to be the main determinant of clearance [183]. A putative physiological role of Lp(a) remains to be identified. Apo(a) has potent lysine binding domains, similar to those on plasminogen and binds to damaged endothelial cells. In vitro studies have identified domains in apo(a) that mediate inhibitory effects on fibrin clot lysis [184]. The mechanisms by which Lp(a) increases CHD risk is not well defined but may include a prothrombotic effect due to the similarity of apo(a) to the fibrinolytic enzyme plasminogen, and the atherogenic effect mediated by the preferential binding of oxidized phospholipids by Lp(a) as well Lp(a) deposition in the arterial wall [185, 186]. Animal models suggest that the proteolytic breakdown products of apo(a) retain anti-angiogenic and anti-tumoral properties, which would suggest a beneficial effect of this protein [187].

High density lipoprotein (HDL)

Biosynthesis of HDL and reverse cholesterol transport

Cholesterol is an important constituent of cell membranes and is a precursor of steroid hormones. Cholesterol in the body is transported to the liver and converted to bile acids that are extensively used in the enterohepatic circulation. HDL particles mediate the transport of cholesterol from peripheral tissues to the liver in a process termed reverse cholesterol transport [188]. Excess accumulated cholesterol is toxic to cells. As in humans, cells other than those in steroidogenic tissues and the liver cannot metabolize cholesterol, reverse cholesterol transport may reduce intracellularly accumulated cholesterol and contribute to cholesterol homeostasis at the cellular and whole body level. About 70% of the total HDL protein is apoAI, although it contains a variety of other proteins. The liver and intestine synthesize and secrete apoAI into the circulation as a lipid free or poorly lipidated protein [189]. Thus lipid free apoAI is released from the cells and interacts with cellular ABCA1 for assembly of HDL particles. HDLs are heterogeneous in size and composition [27]. Mass spectrometric analysis of HDL confirmed the presence of several apolipoproteins [AI, AII, AIV, B, (a), C1, CII, CIV, D, E, F, H, J, LI and M] as well as proteins associated with inflammation, clotting, complement regulation as well as proteolytic enzymes. As the plasma abundance of most of these proteins is insufficient to allow one copy per HDL molecule, it is likely that specific proteins are bound to distinct HDL particles. This heterogeneity may translate into distinct functionality of HDL subclasses [190, 191]. ApoAI is the other major apolipoprotein in HDL. Early studies suggested that the proportion of apoAI and apoAII apolipoproteins varied in different density subfractions of HDL: particles containing apoAI and apoAII in nearly constant ratio and particles containing no apoAII [192]. In mice approximately 30% of steady state plasma HDL is contributed by the intestine [193].

ABCA1 and HDL assembly

Different steps that control the delivery and disposal of cholesterol are regulated by membrane transporters of the ABC superfamily. The human genome contains 48 distinct ABC transporters that are grouped into seven subclasses of single structural units that form active heterodimers or homodimers. Four members of this family have been shown to have a major impact on lipoprotein metabolism: ABCA1 is a 2261-amino acid integral membrane whole transporter protein that mediates the export of cholesterol and phospholipids to lipid poor lipoprotein; ABCG1 a homodimeric half transporter that mediates cellular
transport cholesterol transport to lipidated lipoprotein particles and ABCG5 and ABCG8 form heterodimers that restrict intestinal absorption and promote biliary excretion of sterols [194].

In intestinal cells, ABCA1 has been shown to regulate the efflux of cholesterol from the basolateral but not the apical membrane [195]. ABCA1 is expressed only on the basolateral surface of the hepatocytes [196]. Studies suggest that dietary/biliary and newly synthesized cholesterol contribute to the origins of intestinal HDL-cholesterol [197].

The most critical step of ‘reverse cholesterol transport’ is the release of cholesterol from cells, and the reduction of cellular cholesterol. The ACAT reaction and cholesterol release are active systems to protect the cells from membrane toxic excess accumulation of free cholesterol. The same source of cholesterol is the preferred substrate for ACAT- and ABCA1-mediated lipid secretion. Cholesterol enrichment in HDL can be regulated by the modulation of cellular factors. Both ACAT and PKC activities are involved in regulating the rate of mobilization of ABCA1-mediated cholesterol release by apoAI. PKC inhibitors and activators modulate both cholesterol content in the HDL generated by the apolipoprotein-cell interaction and the change in ACAT accessible cholesterol pool in certain cells under certain conditions [198].

The generation of new HDL particles is mediated by the interaction of helical apolipoproteins with cellular ABCA1. The efficiency of reverse cholesterol transport depends on the specific ability of apoAI to promote cholesterol efflux, bind lipids, activate lecithin cholesterol acyl transferase (LCAT) and form mature HDL that interacts with specific receptors and lipid transfer proteins. As HDL particle shape can affect the activities of HDL remodeling and activities of LCAT and cholesterol ester transfer protein (CETP) understanding apoAI spatial arrangement on disc and sphere HDL is important. Early studies suggested that free apolipoproteins, apoAI, apoAII and apoE can interact directly with macrophages preloaded with cholesteryl ester in culture to form HDL-like particles in the medium [199]. Other exchangeable apolipoproteins including apoCIII, apoCIII and apoE have been shown to stimulate ABCA1-mediated lipid efflux [200]. On the basis of studies using synthetic peptide mimics of apolipoproteins it appears that the key structural motif necessary for an apolipoprotein to function as a lipid acceptor is the presence amphipathic α-helices [201]. Studies in mice suggest that in addition to its role in clearing TG rich lipoproteins apoE participates in the biogenesis of apoE containing HDL particles with the participation of ABCA1 [202]. It is likely, however, that the concentration of lipid poor apolipoprotein in the extracellular fluid is more relevant for ABCA1 efflux (Figure 4).

### Apolipoprotein AI and HDL assembly

Human apoAI is a 243-amino acid protein, the first 43 residues are encoded by exon3 and the 44–243 region by exon4. Residues 44–243, are composed of eight 22 amino acid and two-11 amino acid repeats that are predicted to form amphipathic α-helices. It has been suggested that α-helix formation especially in the C-terminal region provides the energy source necessary for the high affinity binding of apoAI to lipids [203]. Each of the 10 helical repeats has one face enriched in hydrophobic amino acids and the opposing face enriched in negatively charged amino acids. The area between the hydrophilic and hydrophobic faces of the helix is enriched in positively charged amino acids, usually lysine and arginine. This structure is thought to be suited to lipid binding. The N-terminus residues (1–187) form a globular domain, while the C-terminus (188–243) consists largely of a series of amphipathic α-helices [204]. Several studies including an analysis of the crystal structure of apoAI suggest that apoAI consists of two helical domains: a four-helix antiparallel bundle formed by the N-terminal three quarters of apoAI and a two-helix bundle adopted by the C-terminal end [205]. The central region corresponding to residues 121–186 is important for the activation of the enzyme LCAT [206]. In contrast to the crystal structure which suggests 80% helical content, hydrogen exchange and mass spectrometry indicate a helical content of approximately 50% [207]. It is likely that apoAI structure is dynamic (molten globular) and its relative instability may explain the ability of apoAI to unfold and remodel during HDL formation and maturation. However, in true solution the first 98 residues of the N-terminus are probably not folded into the helical structure apparent in the crystal form [208]. Saito et al. [38] proposed a two-step mechanism for lipid binding of apoAI: an initial lipid binding step occurs through the flexible carboxyl-terminal domain followed by a major conformational reorganization of the amino-terminal helix bundle, converting the hydrophobic helix-helix interactions to helix-lipid interactions. In humans the monomeric form of apoAI has been hypothesized as the predominant form of apoAI in plasma and that ABCA1-mediated lipid release is reduced when apoAI is in a self-associated state [209].

Although it is known that the interaction of apoAI with functional ABCA1 results in the lipidation of apoAI and the formation of HDL the mechanism of this interaction is not resolved. Some studies have pointed to the
importance of apoAI/ABCA1 interactions whereas others have postulated apoAI/lipid interactions for the overall binding process. Other studies suggest two binding sites for apoAI are created at the surface of cells containing functional ABCA1. Cross linking studies suggest that apart from ABCA1, apoAI does not bind to any other cellular protein, and that the other binding site for apoAI is a lipid site [210]. It has been suggested that the presence of a functional ABCA1 site leads to the formation of a major lipid containing site for the binding and lipidation of apoAI. The structural requirements for apoAI to associate with plasma membrane are unknown, though studies with deletion mutants suggest that the C-terminal is required for a productive complex with ABCA1 [211]. Apolipoprotein M (apoM) a 26 kDa protein has been hypothesized to function catalytically to transfer lipid onto nascent HDL during or after their formation by ABCA1 [212].

It is now accepted that ABCA1 is expressed in multiple cells and tissues, in the liver, macrophages, brain and various other tissues [213, 214]. The ABCA1 protein contains two transmembrane domains of six $\alpha$-helices and two intracellular nucleotide binding domains. Its ABC consists of Walker A and Walker B motifs [215]. To maintain lipid homeostasis ABCA1 is tightly regulated both transcriptionally and post-translationally. Expression of ABCA1 is regulated primarily by the LXR/retinoid X receptor (RXR) system. The LXRs act as cholesterol sensors and induce genes that protect the cell from cholesterol overload [216]. The cloning and sequencing of the human ABCA1 gene [217] identified a cholesterol response element upstream of the transcriptional site of the human ABCA1 gene [218]. Chinetti et al. [219] have reported that PPAR$\alpha$ and PPAR$\gamma$ agonists induce ABCA1 mRNA expression and apoAI-mediated cholesterol efflux in normal

Figure 6 Biosynthesis of HDL.
Biosynthesis of HDL begins with the interaction of lipid poor apoAI with ABCA1. Lipidated apoAI is the major acceptor for SR-BI and ABCG1 pathways. CETP promotes the transfer of cholesteryl esters from HDL to VLDL and LDL. PLTP transfers phospholipid between VLDL and HDL and remodels HDL into larger and smaller particles with the dissociation of lipid poor or lipid free apoAI. Solid arrows indicate cholesterol/cholesteryl ester transfer.
macrophages. Cyclic AMP induces the ABCA1 gene transcription in certain types of cells especially in macrophage cell lines [220]. Calpain-mediated proteolysis is a further regulatory factor for ABCA1. ApoAI interacts with ABCA1 before endocytosis inhibiting calpain-mediated proteolysis. Consequently, ABCA1 is recycled to the cell surface without degradation. ApoAI therefore increases cell surface ABCA1 [221]. In one study, prevention of phosphorylation of ABCA1 threonine and serine residues by PKC2-enhanced apoAI binding was observed [222]. Several intracellular proteins have been examined to determine whether they modulate ABCA1 activity. Of particular interest is caveolin-1, the main structural protein of caveolae, the cholesterol rich invaginated microdomains at the surface of most peripheral cells. However, the effects of caveolin-1 on cholesterol efflux are still controversial. The overall effect is likely to be cell specific. Overexpression of caveolin-1 in certain cells results in the enhancement of cholesterol efflux [223, 224].

Previous work suggested that a significant basal level of phospholipidation of apoAI occurs in the absence of ABCA1 with both intracellular and peri-cellular lipidation of apoAI [225]. ABCA1 was localized to both the plasma membrane and endocytic compartments which cycle between plasma membrane and other endocytic compartments [226]. Models suggest that ABCA1 and apolipoprotein containing vesicles endocytose to intracellular lipid deposits where ABCA1 pumps lipids into vesicle lumen for release by endocytosis termed retro endocytosis [194]. The relative contribution of cell surface versus intracellular events in cholesterol efflux warrant further investigation [227]. Recent work suggests that HDL biogenesis takes place on the cell surface rather than on endosomes [228]. A generally accepted model for the mechanism by which apoAI/ABCA1 interaction creates nascent HDL particles does not exist, though several models have been proposed. One model suggests that membrane phospholipid translocation via ABCA1 induces bending of the membrane bilayer to create high curvature sites to which apoAI can bind and solubilize membrane phospholipid and cholesterol to create nascent HDL particles [229]. The identification of substrate specificity of ABCA1 and whether phospholipid and cholesterol efflux are coupled will require additional studies, but it is probable that the overall transport activity of ABCA1 may be modulated by the availability of the substrate in the plasma membrane [230].

Nascent HDL particles are heterogeneous with respect to size and lipid content. The major nascent HDL particles comprise of discoidal particles containing two, three or four apoAI molecules per particle. It has been suggested that the stability of the apoAI N-terminal helix bundle domain and the hydrophobicity of the C-terminal domain are important determinants of both nascent HDL particle size and their rate of formation [231]. These discoidal particles are the major progenitors of the spherical HDL particles that form the major fraction of circulating HDL in human plasma. In addition to the HDL particles that each contain several apoAI molecules, some of the apoAI in human plasma is present in a monomeric form. This species is called preβ1-HDL or lipid poor apoAI. These preβ1-HDL particles have been shown to react with ABCA1 leading to their conversion into larger discoidal particles [232].

In the double belt model of discoidal HDL particles the apoAI molecules are packed around the edge of the disc in an antiparallel orientation with their amphipathic α-helical segments interacting with the phospholipid acyl chains with an overall horseshoe shape [233]. This structure is consistent with crystal structures derived from N- or C-terminal truncated apoAI molecules [234, 235]. Most of the effort to define lipitated apoAI structure has been directed at reconstituted/recombinant HDL which can be reproducibly prepared in homogeneous size and composition. Different models have been suggested for two apoAI bound to recombinant HDL. There is considerable agreement between the different models, as all models suggest that the central region of the bound apoAI assumes an antiparallel double belt with the amphipathic helix 5 of each strand adjacent to the same region of the second molecule of apoAI. Differences between the models appear to involve interaction of the N- and C-terminal regions of the two monomers: 1) the solar flares model allows for regions of the apoAI to be more solvent accessible; and 2) belt buckle model in which the N- and C-terminal are closely associated but fold back to the central region of the apoAI belt [236]. Wu et al. [237] using small angle neutron scattering suggest an anti-parallel double superhelix wrapped around an ellipsoidal lipid phase. It has been suggested that α-helices in apoAI in the lipid free and lipid-associated state, probably unfold and refold. This dynamic behavior and structural flexibility of apoAI facilitates the remodeling of HDL particles, which is essential for their maturation and metabolism [238]. Recently, a battery of molecular tools have been used to refine the conformation of apoAI on HDL and a variety of physical chemical methods have been used to study reconstituted HDL and the results have been consistent with the double belt model. Molecular dynamic simulations are consistent with salt bridge pairing and the ‘sticky’ N-terminal hypothesis. Both the crystal and molecular dynamic simulation models suggest that pairwise helix repeats are uniquely designed to create a gap that exposes
both acyl chains and unesterified cholesterol, resulting in a LCAT presentation tunnel [239]. Sorci-Thomas et al. [240] characterized apoAI containing nascent HDL particles from ABCA1 expressing human embryonic kidney cells. They suggest that the smaller nascent HDL particles carried two molecules of apoAI and that larger cholesterol rich nascent HDL particles contained three apoAI molecules. The discoidal nascent HDL particles are the progenitors of spherical HDL2 and HDL3 particles that form the two major subpopulations of circulating HDL in human plasma. HDL2 is large, light, cholesteryl ester rich and HDL3 is small, dense cholesteryl ester poor and protein rich [241]. In plasma nascent HDL is modified by LCAT to generate cholesteryl ester rich particles that are larger and more spherical. LCAT reacts with unesterified cholesterol in HDL transferring the 2-acyl group of lecithin or phosphatidylethanolamine to the free hydroxyl residue of cholesterol to generate cholesteryl esters [242]. Chemically and physically defined reconstituted spherical HDL particles can be formed by incubating nascent HDL with LCAT. Spherical HDL particles are the more mature form of HDL and contain a large amount of neutral lipids, predominantly cholesteryl ester. During maturation, cholesteryl ester molecules formed by LCAT at the HDL surface partition into the particle core. Concurrently, the particle changes from the more elliptical nascent HDL to a spherical form. The spheroidal HDL is the main form of HDL responsible for cholesterol transport to the liver [243].

Despite spherical HDL being the most abundant circulating form of HDL, relatively few studies have experimentally addressed its structure in detail because of its size and compositional heterogeneity. Using cross linking chemistry and mass spectrometry Silva et al. [244] determined that the general structural organization of apoAI was similar overall between discs and spheres. Other studies suggest a conformational difference between apoAI on spheres versus discs. Using small angle neutron scattering Wu et al. [245] suggest that the protein component of spherical HDL is a hollow structure that cradles the non-spherical compact lipid core, and that the lipid component is only partially surrounded by the protein wrapper. They suggest that a model in which three apoAI chains are arranged as a combination of a helical dimer and folded back hairpin was more consistent with cross linking and mass spectrometry analysis. Other investigators have looked at computational models as a means of studying the structure and dynamics of spherical HDL structure [246, 247]. Molecular dynamic structure modeling to investigate the dynamics of HDL synthesis suggests that incremental twisting of the apoAI double helix structure incorporates increasing concentrations of phospholipid in the form of minimal surface patches of lipid bilayer [248]. During lipid metabolism apoAI moves between HDL and triglyceride rich lipoproteins [249].

**Cholesteryl ester transfer protein (CETP) and phospholipid transfer protein**

CETP is a 476-amino acid protein that mediates the transfer of neutral lipids including cholesteryl ester from HDL to VLDL and LDL in exchange for triglycerides, with subsequent uptake of LDL by hepatic LDL receptors. This pathway is commonly called the indirect reverse cholesterol transport pathway. CETP is secreted primarily by the liver and adipose tissue and circulates primarily associated with HDL [250]. Triglyceride enrichment of HDL enhances the clearance of apoAI from plasma. HDL mediated hydrolysis of HDL results in decreased HDL particle size. Accelerated clearance of HDL and apoAI ensues with decreased plasma levels of HDL and apoAI [251, 252]. CETP may consequently exert both pro-atherogenic and anti-atherogenic actions. Lipid transfer inhibitor protein, also known as apolipoprotein F (apoF), modulates CETP function by preferentially blocking CETP activity on LDL. By selectively blocking lipid transfer to and from LDL, apoF enhances the net flux of cholesteryl ester from HDL to VLDL. As VLDL has a relatively short plasma lifetime, this flux is proposed to facilitate the clearance of HDL-derived cholesteryl ester, thus promoting reverse cholesterol transport. ApoF does not bind to CETP, but rather inhibits its activity by preventing CETP binding to the lipoprotein surface [253]. ApoF exists in two forms, one active and the other inactive. Active apoF is bound to LDL and it has been suggested that the conversion of apoF to its active form depends on LDL composition [254]. Electron microscopy studies suggest that the N-terminal domain of CETP penetrates into HDL and that the C-terminal end penetrates into LDL and VLDL. Thus apoAI and apoB or other proteins are not essential. Electron microscopy show virtually no CETP complexes bridging two HDLs, LDLs or VLDLs, implying CETP domain specificity for HDL (N-terminal) and LDL and VLDL (C-terminal) [255]. The co-existence of ternary complexes of LDL-CETP-LDL and HDL-CETP-VLDL and lipid transfer are consistent with the mechanistic model of cholesterol ester transfer through a tunnel within CETP, though at this point the continuous hydrophobic tunnel between the distal portions of the N- and C-terminal domains of CETP is hypothetical. Changes in hydrophobicity along the central cavity are suggested as one of the dynamic forces which favor a cholesteryl ester N- to C-terminus transfer [255]. Both CETP structure
ABCG1

ABCA1 is a key regulator of cholesterol and phospholipid transport to lipid free apolipoproteins [264] forming nascent HDL but is unlikely to be involved in cholesterol transport to mature HDL. ABCG1 has been identified as the more likely mediator of cholesterol transport to HDL but not to lipid poor apoAI [265]. Gelissen et al. [266] showed that a range of phospholipids containing acceptors other than HDL subclasses are efficient in mediating the export of cholesterol via ABCG1. Acceptors for ABCG1-mediated cholesterol transport can be generated from incubation of cells with lipid free apoAI through the action of ABCA1 alone. This implies a potential synergistic relationship between ABCA1 and ABCG1 in peripheral cholesterol export, where ABCA1 lipidates lipid poor apoAI to generate nascent HDL which in turn serve as substrates for ABCG1-mediated cholesterol export. Although no human phenotype has been associated with ABCG1 ablation, targeted disruption of ABCG1 in mice on a high fat diet led to extensive lipid accumulation in tissue macrophages whereas overexpression of ABCG1 protected against diet induced lipid deposition. No change in plasma HDL was found in these mice, suggesting that the most important action of ABCG1 may be in tissue macrophages [267]. Wang et al. [268] found that LXR agonist treatment of mouse macrophages increased the mass of cholesterol released from cells to HDL₂, this increased cholesterol mass efflux was markedly reduced in ABCG1 deficient macrophages. In addition LXR activation induces the redistribution of ABCG1 from intracellular sites to the plasma membrane in human macrophages. Several N-terminal variants of ABCG1 have been proposed based on different translational sites. Inhibition of protein kinase A resulted in an increase in cholesterol export from cells with selective stabilization of an ABCG1 isoform [269]. ABCG1 is expressed in several tissues including brain, spleen, lung, placenta, macrophages and the liver [270]. It has been proposed that ABCG1 may not serve as a direct transporter of membrane cholesterol to acceptors but rather play a role by enriching the cell membrane with cholesterol that can be incorporated into a variety of acceptor particles, i.e., ABCG1 effluxes cellular cholesterol by a process that is not dependent upon interaction with an extracellular protein [271]. However, macrophages may have special mechanisms to handle the massive amounts of cholesterol that they can take up through scavenger receptors or phagocytosis. An alternative hypothesis is that the primary function of ABCG1 may be to control intracellular sterol homeostasis. ABCG1 present in endosomes and recycling endosomes suggests a mechanism by which ABCG1 transfers sterols to the inner leaflet of these vesicles before their fusion to the plasma membrane. This would result in redistribution of these sterols to the outer leaflet of the plasma membrane such that they can desorb in a non-specific manner to multiple lipid acceptors [272].

Concentrations of HDL with apoE are low in humans [273]. Matsuura et al. [274] showed that HDL-apoE from CETP deficient humans can effectively accept unesterified cholesterol from macrophages. HDL from CETP deficient patients had markedly increased content of apoE and LCAT. HDL-apoE in the presence of LCAT promoted HDL particle expansion, and this cholesterol efflux was ABCG1 mediated.
dependent. In addition, removal of the apoE resulted in a loss of ability of HDL to promote cholesteryl ester accumulation suggesting that apoE may play a key role. Other studies suggest that apoE has an important role as an LCAT activator in mouse apoB lipoproteins [275]. In an x-ray crystal model of apoE bound to phospholipids, it has been suggested that apoE molecule was folded into half in a belt-like manner around a spheroidal phospholipid molecule, forming a circular horseshoe two helices thick and one helix wide but not a complete belt [276].

Scavenger receptor BI (SR-BI)

ABCA1, ABCG1 and scavenger receptor BI (SR-BI) make up the known proteins that play a role in cholesterol efflux. As far as the mechanism of efflux and the preferred acceptors ABCA1 remains unique. ABCA1 uses lipid free and lipid poor exchangeable apolipoproteins as its preferred acceptors. In contrast, ABCG1 and SR-BI use HDL and not lipid free apolipoproteins as cholesterol acceptors. SR-BI is an 82 kDa membrane glycoprotein containing a large extra-cellular domain and two transmembrane domains with short cytoplasmic amino and carboxy terminal domains [277]. The tissue distribution of SR-BI which is predominantly expressed in liver, adrenal gland and ovary is compatible with it playing a role in HDL-derived cholesteryl ester transport to the liver and steriodogenic tissues [278]. Although the liver and steriodogenic tissues are the sites of greatest SR-BI expression, SR-BI is expressed in other sites throughout the body. SR-BI is expressed in the intestine, lung, endothelial cells and macrophages. SR-BI is a multiligand receptor, however, it does not bind a wide array of polyanionic molecules that are ligands of other scavenger receptors. There are now many members of a superfamly of scavenger receptors that have diverse structures, expression patterns and functions [279]. The promoter region of the SR-BI gene contains consensus DNA sequences that bind several transcription factors including steriodogenic factor (SF-1) [280], SREBP-1 [281], LXR [282] and liver receptor homolog 1 [283]. Stimulation of PPAR-α increases SR-BI mRNA levels and protein in macrophages [284]. Taken together the data suggest that activation of multiple signaling pathways may participate in modulating SR-BI expression. The ability of SR-BI to mediate cholesterol efflux was established by studies in SR-BI-transfected cultured cell lines [285]. Direct evidence for the role of SR-BI in HDL metabolism in vivo has come from studies in which the levels of SR-BI in mice have been manipulated. SR-BI knockout mice have, relative to wild type control animals, a 2.5-fold increase in total cholesterol levels, mostly due to increased cholesterol in HDL particles. These HDL particles are larger and more heterogeneous in size than those in wild type animals, and they are enriched in apoE [286]. Increased SR-BI expression also enhanced cholesterol efflux to exogenously added apoE [287]. In other studies lipid free apoE only partially competes with the binding of HDL to SR-BI, whereas the lipidated apoE competes fully [288]. SR-BI-mediated transfer of unesterified cholesterol between cells and HDL is bidirectional and sensitive to HDL phospholipid content and composition. SR-BI-mediated uptake should be greatest for free cholesterol and cholesteryl ester but significant catabolism of HDL phospholipid and triglyceride can occur through SR-BI-mediated uptake [289, 290]. Based on studies with mutant forms of SR-BI and apoAI, it has been suggested that SR-BI-mediated transport of lipids between cells and lipoproteins involves two sequential steps: 1) lipoprotein binding; and 2) binding-dependent yet distinct SR-BI-mediated lipid transfer [291, 292]. Other studies suggest that transfection mediated SR-BI overexpression by COS cells can alter the lipid organization of cell membranes and that the proposed binding independent SR-BI-mediated efflux might be a consequence of lipid organization [293, 294]. Pagler et al. [295] suggest that retroendocytosis of HDL is mediated by SR-BI and that resorption of HDL particle is accomplished by an efflux of cellular cholesterol.

The potential structural and/or functional interaction between ABCA1 and SR-BI in regulating cholesterol efflux has been explored. Using a murine macrophage cell line Chen et al. [296] showed that the expression of SR-BI expression reduced ABCA1-mediated efflux of cholesterol but not phospholipid. The authors suggest that SR-BI expression produced a reuptake pathway in which cholesterol released from the cells to lipoproteins by ABCA1 is incorporated back into the cell by SR-BI-mediated influx. Another possibility is that SR-BI reorganizes membrane lipids, sequestering cholesterol and making it unavailable for ABCA1 efflux. In a further study co-expression of SR-BI with ABCG1 inhibited the ABCG1-mediated net cholesterol efflux to HDL [297]. Other studies suggest that ABCG1 and SR-BI in hepatocytes do not contribute to cholesterol efflux to apoAI or the accompanying nascent HDL formation. In contrast, ABCA1 in hepatocytes is essential for nascent HDL formation [298]. In human macrophage foam cells cholesterol efflux stimulated by LXR agonist requires SR-BI and ABCA1 but not ABCG1 [299]. It is likely that the contributions of ABCA1, ABCG1 and SR-BI to cholesterol efflux differ in a tissue- and species-specific manner [300].

As SR-BI-mediated transport does not appear to be dependent on ATP, it is likely that net flux of lipids is
driven by a concentration gradient between cells and extracellular donor/acceptor particles. It is likely that SR-BI mediates efflux to lipid poor HDL from macrophages loaded with non-metabolizable cholesterol and from the plasma membrane of hepatocytes and steroidogenic cells down a concentration gradient determined by intracellular cholesterol metabolism.

In vitro studies have shown that in addition to HDL, SR-BI binds apoB containing lipoproteins [301]. Studies with mice suggest that, at least in rodents, SR-BI may have other roles in post-prandial lipid metabolism. It has been suggested that SR-BI may have a role in facilitating chylomicron remnant metabolism or function as an initial recognition site for chylomicron remnants [302] and may facilitate the metabolism of VLDLs [303]. Recently, it has been shown that HDL-SR-BI complex serves to direct signal initiation by SR-BI that leads to activation of diverse kinase cascades that may contribute to the cardiovascular benefits of the lipoprotein [304].

Other factors in HDL remodeling

Remodeling of HDL particles by plasma factors is an important part of HDL metabolism. Endothelial lipase is synthesized primarily by the vascular endothelial cells and to a lesser extent by macrophages and smooth muscle cells. In humans’ plasma endothelial cell mass was inversely correlated with HDL-cholesterol levels [305]. HL can hydrolyze triglycerides in all lipoproteins but is predominant in the conversion IDL to LDL, and can also convert post-prandial triglyceride rich HDL into post-absorptive triglyceride poor HDL [306].

Hormones and lipid metabolism

In the forward transport system triglyceride rich lipoproteins from the liver (VLDL) and intestine (CM) deliver fatty acids to peripheral tissues. LPL plays a role in VLDL fatty acid release and in its subsequent conversion to LDL. Conversely in the reverse transport system HDL transports excess cholesterol from extrahepatic cells, e.g., macrophages to the liver where it can be recycled or catabolized to bile acids. Lipid (and carbohydrate) homeostasis in higher organisms is under the control of an integrated system that has the capacity to rapidly respond to metabolic changes. Regulation of lipoprotein production is an interplay between systemic free fatty acid delivery, hormonal and nutritional factors. A detailed discussion of the role of hormones on lipid metabolism is beyond the scope of this review and a brief overview of the subject will be given.

Lipid metabolism is endogenously controlled by pancreatic, pituitary and adrenal hormones. Insulin is the primary regulator of blood glucose concentration. Insulin promotes the storage of substrates in fat, liver and muscle by stimulating lipogenesis, glycogen and protein synthesis and inhibiting lipolysis, glycogenolysis and protein breakdown [307]. Insulin appears to be one of the key players ‘directing’ the flow of fatty acids to or from tissues for utilization and storage. Glucagon decreases hepatic triglyceride synthesis and secretion [308]. Glucagon has been shown to decrease hepatic lipoprotein secretion and inhibit particle clearance but does not regulate intestinal lipoprotein metabolism in humans [309]. The role of glucagon and the glucagon receptor in regulating lipoprotein metabolism is, however, still not clear.

In the liver, insulin has been shown to acutely inhibit the synthesis of VLDL particles in healthy individuals by a mechanism that involves an increase in apoB degradation and decreased expression of MTP, thus leading to a suppression of VLDL release. Studies suggest that insulin signaling through FoxO1 plays an important role in regulating hepatic MTP expression and VLDL production [310]. Insulin favors the degradation of apoB in a pre-secretory compartment. Insulin-mediated suppression involves the activation of phosphatidylinositol-3-kinase by the insulin receptor transduced through insulin receptor substrates; a process that correlates with the translocation of activated phosphatidylinositol-3-kinase to intracellular membranes [311]. Insulin-induced suppression of intestinal lipoprotein secretion is both direct and indirect through suppression of circulating free fatty acids [312].

Thyroid hormones can influence lipid metabolism by: 1) inducing HMG CoA reductase; 2) controlling SREBP-2 which regulates LDL receptor expression; 3) increasing CETP activity; and 4) upregulating the synthesis of apoAV [313–316]. ANGPTL3 gene is negatively regulated by triiodothyronine leading to the speculation that triiodothyronine can lower plasma triglycerides by suppressing the ANGPTL3 gene thus activating LPL which plays a central role in lipid metabolism [317]. Thyroid hormone interaction with the nuclear thyroid hormone receptor accounts for several of the lipid reduction effects by thyroid hormones, though recent work suggests that non-classic mechanism involving cytosolic second messengers including calcium and phosphoinositide-3-kinase, could contribute to the effect of thyroid hormones on lipid homeostasis [318].

Hydrolysis of the triacylglycerol reserves in adipocytes provides fatty acids during times of energy demand.
such as fasting and exercise. Abnormalities of adipose tissue lipolysis are associated with the development of metabolic diseases. Catecholamines stimulate lipolysis in humans. Catecholamines stimulate adenyl cyclase leading to increased cyclic AMP concentrations and cyclic AMP dependent phosphorylation of hormone sensitive lipase, one of the key enzymes controlling lipolysis [319]. Glucocorticoids may also participate in the stimulation of lipolysis in adipose tissue by stimulating the lipase desnutrin [320]. In the fed state insulin inhibits lipolysis by activation of phosphodiesterase that reduces cyclic AMP levels [321].

Several hormones have been implicated in the transition from fasted to fed state. Intestinal lipid absorption virtually ceases in the post-absorptive state, whereas secretion of hepatic VLDL persists. In the fasted state the majority of triglycerides in VLDL were from circulating free fatty acid from adipose tissue lipolysis [322]. Post-prandially the majority of the chylomicron triglycerides are sequestered by extrahepatic tissues (mainly adipose) following hydrolysis by the insulin-stimulated LPL [323]. Intestinally derived hormones glucagon-like peptide-1 and gastric inhibitory polypeptide enhance glucose stimulated insulin secretion by pancreatic β cells [324]. During fasting states growth hormone increases lipolysis and free fatty acid levels [325]. Studies suggest that monosaccharides can acutely enhance intestinal lipoprotein particle production [326]. The transition from fed to fasted state involves nutrient and hormonal signals to intestine, liver and adipose tissue. During the post-prandial state this ensures efficient absorption, transport and storage of lipids and in the fasted state this ensures subsequent redistribution and utilization of stored lipids.

Clinical implications of lipoprotein metabolism

Recent progress in the understanding of lipoprotein metabolism has fostered understanding of the genetic basis of dyslipidemias. Severe dyslipidemias can be monogenic disorders with a Mendelian inheritance and present in childhood. More commonly they develop in later life and are an interplay between genetic variants and environmental variants such as poor quality of life and imbalanced caloric intake or certain medications. The current review focuses on the molecular basis of normal lipoprotein metabolism but will be incomplete without a brief account of the more recent advances in the inherited form of dyslipidemias.

Early work on the LDL receptor leads to new ways of thinking on cholesterol metabolism. FH inherited as an autosomal dominant disorder, was found to be caused by a genetic defect in the LDL receptor [133]. Several mutations in the LDL receptor binding domain of apoB100 have been described that are associated with hypercholesterolemia with an autosomal codominant inheritance pattern [327]. In contrast, many non-sense, frameshift and splicing mutations in the APOB gene causing the formation of truncated apoB can cause familial hypobetalipoproteinemia [328]. In 2003 mutations in the PCSK9 gene was identified in two French families with an autosomal dominant form of FH [138]. Statins currently form the standard of care in hypercholesterolemia. Combination therapy with statins is well established and ezetimibe is often used as an additional LDL cholesterol lowering agent. Two cases of homozygous ‘loss of function’ mutations in PCSK9 have been described. The carriers, who lacked PCSK9 were healthy and fertile suggesting that pharmacologic inhibition of PCSK9 may be safe [329]. In some cases FH is inherited by mutations in the LDL receptor adapter protein1, a protein that interacts with the cytoplasmic tail of the LDL receptor [330]. Newer more potent methods of LDL cholesterol reduction are currently being tested. Mimetic peptides and monoclonal antibodies that specifically target PCSK9 are in development as are inhibitors of CETP and MTP. Antisense oligonucleotides to specifically silence or reduce the expression of apoB as a means of lowering VLDL are under investigation [331]. Statins activate the SREBP pathway and lower the formation of LXR ligands with subsequent decreased ABCA1 expression [332]. As there is cooperativity and cross talk between the different lipoprotein metabolism pathways it is likely that future research will focus on combination therapy with the newer agents, as more effective methods of lowering LDL cholesterol.

As a therapeutic approach to increasing HDL researchers have focused on injections of reconstituted HDL, apoAI mimetics or full length apoAI. ABCA1 has been identified as defective in Tangier disease patients with HDL deficiency [333].

A few individuals with hypertriglyceridemia have rare monogenic disorders with loss of function mutation in LPL, APOCII, APOAV, LMF1 or GPIHBP1 genes. Most patients with hypertriglyceridemia, however, have a complex genetic etiology. Genome wide association studies have identified several common genetic variants associated with hypertriglyceridemia at genome wide significance levels including APOAV, LPL and APOB genes [334]. As the genetic basis for hypertriglyceridemia becomes available
this might be used to predict the individual’s response to diet and drug therapy.

Future directions

Multiple genes and complex pathways are involved in lipoprotein metabolism and cholesterol and lipid homeostasis. These include pathways of intestinal chylomicon secretion and liver VLDL secretion and cholesterol homeostasis with HDL providing a driving force for reverse cholesterol transport. Hormonal and nutritional factors further regulate lipoprotein metabolism.

There is growing evidence using genome wide association studies that several common genetic variants of genes are associated with lipid traits in humans. Understanding the mechanisms of lipoprotein metabolism is the basis for exploring the pathophysiology of dyslipidemias and has important clinical applications. The central question is how this explosion of knowledge may benefit human health. It is likely that newer drugs can be used in combination with statins to reduce LDL cholesterol and further decrease CVD risk. Novel compounds under investigation are those that inhibit VLDL production, those that increase LDL receptor expression, or those that modulate LDL cholesterol content. Despite our increased molecular understanding of lipoprotein metabolism much, however, remains to be learned. As knowledge of lipoprotein metabolism increases, this will in future, open possibilities for targeted individualized treatment for dyslipidemias.

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