Review Article

Shunts, channels and lipoprotein endosomal traffic: a new model of cholesterol homeostasis in the hepatocyte

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Abstract

The liver directs cholesterol metabolism in the organism. All the major fluxes of cholesterol within the body involve the liver: dietary cholesterol is directed to the liver; cholesterol from peripheral cells goes to the liver; the liver is a major site of cholesterol synthesis for the organism; cholesterol is secreted from the liver within the bile, within apoB lipoproteins and translocated to nascent HDL. The conventional model of cholesterol homeostasis posits that cholesterol from any source enters a common, rapidly exchangeable pool within the cell, which is in equilibrium with a regulatory pool. Increased influx of cholesterol leads rapidly to decreased synthesis of cholesterol. This model was developed based on in vitro studies in the fibroblast and validated only for LDL particles. The challenges the liver must meet in vivo to achieve cholesterol homeostasis are far more complex. Our model posits that the cholesterol derived from three different lipoproteins endosomes has three different fates: LDL-derived cholesterol is largely recycled within VLDL with most of the cholesterol shunted through the hepatocyte without entering the exchangeable pool of cholesterol; high density lipoprotein-derived CE is transcytosed into bile; and chylomicron remnant-derived cholesterol primarily enters the regulatory pool within the hepatocyte. These endosomal channels represent distinct physiological pathways and hepatic homeostasis represents the net result of the outcomes of these distinct channels. Our model takes into account the distinct physiological challenges the hepatocyte must meet, underlie the pathophysiology of many of the apoB dyslipoproteinemias and account for the sustained effectiveness of therapeutic agents such as statins.

Keywords: ACAT2, cholesterol, hepatocyte, HMGCR, LDLR

Cholesterol, an amphipathic four-ringed lipid molecule, was discovered in gallstones in 1784. The word-cholesterol- is derived from the ancient Greek: "chole" meaning bile and "stereos" meaning solid. Since then, cholesterol has been studied extensively, and successfully, with 13 Nobel Prizes awarded for describing its synthetic and transport pathways. Cholesterol is a critical component of biologic membranes providing stability but also fluidity in the plasma membrane of cells with no cell wall. Cholesterol is also a precursor to many biologically essential products such as steroid hormones, bile acids, vitamins, and co-factors. Some have suggested that the evolution of animals was dependent on the presence of cholesterol[1] and it is found in all eukaryotes: yeast[2], C. elegans[3], zebrafish[4], moths[5], and vertebrates. Because of its unique

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amphipathic nature, cholesterol must be transported through the blood packaged within lipoprotein particles. This transport is achieved by different lipoprotein particles that transport the dietary cholesterol from the intestine to the liver and from the periphery to the liver. In the early 1960s, the risk of atherosclerotic coronary artery disease was positively related to the plasma levels of total cholesterol and then low density lipoprotein-cholesterol (LDL-C). For this reason, the regulation of cholesterol transport and synthesis have been major directions of research. Clinical outcomes have improved substantially with the development and implementation of statins and our biologic understanding with the recognition and elucidation of clathrin-dependent receptor-mediated endocytosis of lipoprotein particles.

Current model of cholesterol homeostasis within the liver

The current cholesterol homeostatic models are deeply entrenched in the literature. Brown, Goldstein, their colleagues and others constructed and explicated an explanatory model with several key steps. Embedded in the ER membrane, sterol-cleavage activated protein (SCAP) binds sterol response element binding protein (SREBP1 or SREBP2) and serves as a chaperone to both SREBPs. For the present discussion of cholesterol metabolism, we will restrict our discussion to SREBP2. With lower cellular cholesterol, the SCAP/SREBP2 complex traffics through the secretory pathway where SREBP2 interacts with two separate proteases in the Golgi apparatus: Site 1 protease (S1P) and Site 2 protease (S2P). S1P cleaves SREBP2 removing the domain that SCAP binds to, releasing a membrane bound truncated SREBP2. Subsequent S2P interaction and cleavage releases a soluble truncation form of SREBP2 that is a transcription factor. This soluble form migrates to the nucleus and interacts with specific DNA sequences termed sterol response elements (SRE). Genes that possess SRE include, but are not limited to, the low density lipoprotein receptor (LDLR) and 3-hydroxyl, 3-methylglutaryl coenzyme A reductase (HMGCR). Binding of SREBP2 to the SRE of the LDLR gene and HMGCR gene promotes transcription of the mRNAs, ultimately resulting in increased LDLR protein on the cell surface and increased HMGCR protein in the ER membrane. Increased LDLR on the cell surface results in increased binding and uptake of LDL particles from the extracellular milieu, thus increasing intracellular cholesterol. HMGCR is the rate-limiting enzyme of cholesterol biosynthesis. An increase in HMGCR results in an increase of de novo synthesized cholesterol. This two-punch combination-increased uptake and increased synthesis-increases the cellular content of cholesterol.

When cholesterol content tends to become excessive, mechanisms exist to reduce cellular cholesterol levels and restore cholesterol homeostasis. LDL is taken up by receptor mediated endocytosis in clathrin coated pits. LDLR and bound LDL are internalized within endosomes; LDLR is recycled back to the plasma membrane and LDL is degraded in lysosomes, with LDL-derived cholesterol trafficked out of the lysosome by Niemann-Pick Type C protein 1 and 2 (NPC1, NPC2). LDL-derived cholesterol is trafficked to the ER membrane, where it interacts with SCAP, strengthening SCAP’s interaction with the insulin induced gene 1 (INSIG1) preventing the trafficking of the SCAP/SREBP2 complex to the Golgi apparatus. Thus, no cleavage of SREBP2 occurs, no trafficking of SREBP2 to the nucleus takes place, and no transcriptional activation of SREBP2-regulated genes results. HMGCR and LDLR gene transcription are shut down, closing the cycle.

This is the core of the conventional model of cholesterol homeostasis within cells. If the mass of cholesterol within the cell increases, then endogenous synthesis of cholesterol decreases as does the synthesis of the LDLR, preventing exogenous uptake of LDL-derived cholesterol. If the mass of cholesterol within the cell decreases, endogenous synthesis of cholesterol and exogenous uptake of cholesterol is increased.

Acute regulation of both LDLR and HMGCR also occurs. Inducible degrader of LDLR (IDOL) acts as an E3 ubiquitin ligase specific for LDLR and promotes the proteolytic degradation of LDLR independent of SREBP2. Ubiquitination of HMGCR also occurs although the mechanism may involve one of many identified effectors. Likewise, gp78 is the E3 ubiquitin ligase for INSIG1. Ubiquitination of squalene synthase, another key regulatory enzyme of de novo cholesterol biosynthesis, occurs mediated by MARCH6. Ubiquitination of HMGCR and INSIG1 is dependent on the presence and binding of an oxysterol, such as 25-hydroxycholesterol, to each protein. This adds a level of complexity in that oxysterols may be key in regulating the over-accumulation of cholesterol in cells (for more information, please see reviews).

Lever X receptor (LXR) forms a heterodimeric transcription factor and, in response to binding of oxysterols, binds LXR response elements (LXRE) promoting the transcription of a number of genes including ATP binding cassette transporter A1 and G1 (ABCA1, ABCG1), stearoyl Co-A desaturase, apolipoprotein E, SREBP1c, fatty acid synthase (FAS), and
**Cholesterol influx into the hepatocyte**

Numerous lipoprotein receptors exist on the surface of the hepatocyte: LDLR, VLDLR, LRP1, LRP5/6, apoER2, scavenger receptors SR-BI, and P2Y13, as well as potentially other yet unidentified scavenger receptors. The most important is the LDLR responsible for uptake of LDL in the liver. The VLDLR, though predominantly expressed in neurons and adipose tissue, may play a role in uptake of VLDL and in TAG metabolism in the liver. LRP1 is a type I transmembrane protein receptor with over 50 known ligands including protease inhibitor complexes (e.g., uPR), transfer proteins (e.g., lactoferrin), and signaling proteins (e.g., transforming growth factor beta). Importantly, LRP1 may be the primary receptor for apoE containing lipoproteins including chylomicron remnants (CR). Chylomicrons are produced in the intestine, released into the portal circulation, where they are acted upon by lipoprotein lipase primarily in adipose tissue and skeletal muscle, hydrolyzing the TAG-rich core of the chylomicrons and releasing the fatty acids to be taken up and utilized by the adjacent cells. The residual lipoprotein particle, CR, is TAG-depleted but retains its complement of cholesterol and cholesteryl ester. The liver takes up almost all of the CR suggesting that LRP1 plays a role in the uptake of the majority of CR. LRP5/6 and apoER2 (LRP8) are predominantly expressed in nervous tissue and play a major role in signaling in the Wnt pathway. However, a role of these receptors in liver hepatocyte lipoprotein metabolism cannot be ruled out. SR-BI is a scavenger receptor that mediates selective uptake of HDL-derived cholesteryl ester (and also partially LDL-derived cholesteryl ester), but may also serve as a vehicle for efflux of free cholesterol to lipoprotein acceptors. Knockout models and genetic variants in human populations have demonstrated that SR-BI plays a major role determining plasma HDL-levels and in the reverse cholesterol transport pathway (ie. return of cholesterol from the periphery to the liver). Therefore, SR-BI is a major contributor to the cholesterol pool of the hepatocyte. Ecto-F1-ATPase, expressed at the basolateral membrane of hepatocytes, binds HDL. Subsequent activation of the purinergic receptor P2Y13 results in clathrin-mediated endocytosis of HDL. P2Y13 plays a role in HDL uptake and in sterol transport into bile. In addition to the defined lipoprotein receptors, heparan sulfate proteoglycans are glycoproteins on the surface of cells that bind lipoproteins, and assist in their uptake by the hepatocyte.

The uptake of lipoproteins in the hepatocyte has...
primarily been assessed by knockout mouse models. KO of the LDLR in fibroblasts virtually abolishes LDL uptake (>95% inhibition); however, KO of LDLR in the hepatocyte does not abolish LDL uptake but results in a decrease from 50 to 70%[70-74]. Heparan sulfate proteoglycans account for some but not all of the residual LDL uptake, suggesting other receptors, previously mentioned or unknown, may also be contributing[14,41,69,75-76]. Multiple KOs have also been attempted demonstrating a partial overlap of specificity and compensation of different receptors. For example, a recent study demonstrated that hepatic uptake of VLDL in the LRP1/LDLR/VLDLR triple KO is also mediated by heparan sulfate proteoglycans and SR-BI. It is for these reasons (number of different receptors and overlap of lipoprotein receptor specificity) that the hepatocyte cannot easily be compared with simpler cell models such as fibroblasts. Likewise, the mouse model system is not a perfect model of human lipoprotein metabolism (hence the attempt to derive “humanized” mouse models), and conclusions drawn from mouse KOs must be assessed in this light.

**Cholesterol synthesis**

The enzyme pathway that converts acetyl CoA into the 27 carbon, 4-ring structure that is cholesterol has been described in detail. The rate limiting enzymes for cholesterol biosynthesis are HMGCR and squalene synthase (also called farnesyl diphosphate farnesyl-transferase)[6,28-29]. Most of the literature has focused on HMGCR (as the majority of regulation does occur here) and so we will focus our attention here. HMGCR is regulated by insulin/glucagon and by cellular energy levels (by AMP-dependent protein kinase). At a transcriptional level, HMGCR is activated by SREBP2. At a post-translational level, HMGCR is targeted for proteolytic degradation in acute response to high cellular cholesterol levels. The liver produces about 75% of the total body cholesterol. This demonstrates that dietary cholesterol plays a significant but lesser role in total body cholesterol and that the regulation of cholesterol synthesis in the liver is the most important metabolic target. For this reason, statins which target the liver have been an effective treatment for hypercholesterolemia[6].

**Hepatic cholesterol influx and efflux**

The liver is the central organ for cholesterol metabolism and homeostasis. On the influx side, the liver is the major site for cholesterol synthesis in the organism. The liver is also the major site to which cholesterol is delivered to the liver within CR, VLDL, LDL and HDL particles in amounts that are substantially larger than the capacity of the liver to secrete cholesterol within bile or as bile acids. The most obvious physiologic role of VLDL is to remove excess TAG from the liver and deliver it to adipose tissue and skeletal muscle. However, VLDL particles contain substantial amounts of co-secreted cholesterol or CE plus substantial amounts of cholesterol transferred from HDL particles by cholesteryl ester transfer protein.

We ingest between 500 mg and 1 g of cholesterol per day. We secrete between 500 mg and 1 g of cholesterol per day either as bile acids or cholesterol dissolved within the bile. At least 3-4 g of cholesterol per day return to the liver within CR, VLDL, LDL and HDL particles[77]. If the only routes of cholesterol out of the liver were dissolved in bile acids or broken down to bile acids, cholesterol would accumulate progressively, and soon unacceptably, within the hepatocyte. Secretion within VLDL particles or transfer to HDL particles are the only options to maintain the balance. Thus, microsomal triglyceride transfer protein (MTP) co-translationally loads the apoB-100 with CE as well as TAG to generate a VLDL particle[78-81]. Thus, secretion of VLDL can offload CE as well as TAG from the liver. Most of the cholesterol secreted from the hepatocyte within VLDL particles plus all of the cholesterol transferred from HDL particles to either VLDL or LDL particles returns to the liver. The VLDL secretion pathway is, therefore, largely a futile cycle without physiologic purpose so far as net movement of cholesterol in and out of the liver. The hepatocyte produces the highest level of any cell type of the ATP binding cassette transporter ABCA1 which, with the help of apoA-I, generates nascent HDL particles, which can remove cholesterol from the hepatocyte.

**Regulatory intracellular cholesterol pool**

The cytosolic enzyme, acylCoA:cholesterol acyltransferase (ACAT or stearoylCoA-O-acyltransferase (SOAT)), converts the amphipathic free cholesterol, the biologically active form of cholesterol, to the hydrophobic CE, the biologically inactive form of cholesterol, which must then be stored within a lipid droplet to sequester its hydrophobicity[7,12]. The storage capacity of lipid droplets is substantial in many cell types (for example macrophages that become foam cells). CE hydrolases (CEH) are also present to provide ready access to stored CE, if necessary[7].

The regulatory pool represents a pool of cholesterol
that serves as a reservoir for cellular needs, but, more importantly, as the driver of the mechanisms to regulate the total cellular cholesterol content of the cell[6,9,15]. If there is too little cholesterol, mechanisms are activated to rectify the shortfall. If there is too much, then alternate mechanisms are activated to rectify the excess. Regulation of ACAT/CEH activity can control the regulatory pathways of cholesterol within the cell. The actual physical site of the regulatory pool is likely the ER membrane. The SCAP/SREBP2 complex is in the ER membrane. HMGCR is found in the ER membrane. VLDL is synthesized in the ER lumen. It is safe to say that the ER membrane is where the action is occurring.

However, the ER membrane is characterized by very low levels of cholesterol. This is advantageous for regulation of cholesterol by SCAP/SREBP, HMGCR and ACAT, as cholesterol levels can be maintained at a low threshold that when exceeded can be promptly detected and reacted to[82-84]. However, the plasma membranes, not the ER membranes, are the location of the bulk of free cholesterol in the cell. Furthermore, all the major physical pools of cholesterol (plasma membrane, mitochondria, lipid droplet, TGN, ER membrane) are either in close contact or have access to transport mechanisms that allow rapid equilibrium or functional accumulation. Moreover, cholesterol can be removed from the regulatory pool and stored as CE or released from this compartment to re-enter the regulatory pool. This is the conventional model of cholesterol homeostasis.

**Hepatocyte cholesterol homeostasis**

Unlike the simple model of cholesterol homeostasis described in fibroblasts[6], the liver model of homeostasis is much more complicated. There are multiple mechanisms of influx of cholesterol and efflux of cholesterol as well as complex regulation of endogenous synthesis. Furthermore, the LDLR is regulated by a protein called proprotein convertase subtilisin/kexin type 9 (PCSK9)[13,40,47-48,85]. PCSK9, which is synthesized and secreted by hepatocytes, binds the LDLR on its ligand binding domain, leading to the internalization of the LDLR:PCSK9 complex and their subsequent degradation in the lysosome, rather than allowing LDLR’s recycling back to the plasma membrane. Paradoxically, PCSK9 is regulated by SREBP2, which can lead to a confounding effect of low cellular cholesterol levels leading to upregulation of HMGCR, LDLR (increasing cellular cholesterol levels through endogenous and exogenous pathways) and upregulation of PCSK9 (decreasing cell surface levels of LDLR). Hepatocytes also express two types of ACAT (in contrast to fibroblasts): ACAT1 (SOAT1) and ACAT2 (SOAT2)[12]. These proteins are independently regulated and serve two separate physiologic functions. ACAT1, expressed at nominal levels and unregulated, is responsible for the cytosolic pool of CE generation stored in the lipid droplets, which is intimately connected to the regulatory pool. ACAT2, which is strongly inducible, produces CE dedicated to VLDL secretion[86]. The source of cholesterol for ACAT2 is not known, since it is unlikely that ACAT1 and ACAT2 share a substrate pool. In addition to these hepatocyte specific features of cholesterol homeostasis, a number of observations in hepatocytes have argued against the cholesterol homeostatic mechanism described above.

1) LDLR is not downregulated upon LDL uptake in hepatocytes: Even in patients with hypercholesterolemia (high plasma LDL-C), the LDLR is still expressed on the surface of hepatocytes[70-74]. Under high LDL-C conditions, LDL uptake is continuous and each hepatocyte should have amassed a large surplus of intracellular cholesterol[87]. According to the conventional model of cholesterol homeostasis, LDL-derived cholesterol that is taken up by hepatocytes should enter the regulatory pool, should inactivate SREBP2, and over time, should shut down synthesis of the LDLR as it does in fibroblasts[6]. However, this does not occur and this suggests that the hepatic LDLR is regulated in a different fashion by cholesterol and other factors[86,88-99]. On the other hand, uptake of CR and the accompanying cholesterol results in a significant down-regulation of the LDLR.

2) HMGCR activity and endogenous cholesterol synthesis is not downregulated by uptake of LDL. LDL-derived cholesterol should bind SCAP preventing SREBP trafficking and processing, leading to a downregulation of HMGCR expression, especially under chronic high LDL-C conditions. However, endogenous synthesis of cholesterol and HMGCR activity remain elevated in high LDL-C conditions[71]. On the other hand, and again in contrast to LDL-uptake of CR-derived cholesterol results in a significant repression of endogenous cholesterol synthesis.

3) If LDL-derived cholesterol is not affecting LDLR or HMGCR expression, then LDL-derived cholesterol is not entering the regulatory pool[72,100]. On the other hand, CR-derived cholesterol significantly downregulates LDLR and HMGCR expression and clearly enters the regulatory pathway. Accordingly, we postulate that in hepatocytes LDL-derived cholesterol and CR-derived cholesterol go to different intracellular locations.

4) LDL uptake occurs in the absence of LDLR: Familial hypercholesterolemia (FH) is characterized by...
defective functioning LDLR. Nevertheless, LDL is still taken up by hepatocytes\(^{95,101-102}\). LDL can be taken up by receptors other than the LDLR (LRP1, LRP5/6, Sort1)\(^{14,48,51,57,61,76,103-109}\). In addition, several researchers have postulated the occurrence of a low affinity, unsaturable binding site mediating uptake of LDL\(^{102,110}\). It was postulated that this binding site may account for up to 30% of total uptake (in the presence of LDLR) and a higher proportion under high plasma cholesterol conditions. However, it is not known what the fate of the LDL-derived cholesterol would be when LDL is taken up by these different pathways.

5) PCSK9 is the main regulator of hepatic LDR expression levels, not SREBP2. In the fibroblast, the SCAP/SREBP2 mechanism is the primary regulator of LDR levels notwithstanding IDOL’s function. In addition, there are acute mechanisms to regulate cholesterol levels (discussed above). However, in the hepatocyte, PCSK9 expression is the primary regulator of LDR cell surface expression\(^{13,40,47,85}\). Therefore, the coincident upregulation of PCSK9 may have the most profound effect on LDR but not HMGCR.

6) VLDL secretion is a major outlet for cholesterol from hepatocytes: Lipid availability is a prerequisite for VLDL secretion. Both TAG and CE are essential components and inhibition of either is sufficient to inhibit VLDL secretion\(^{78,80,111-116}\). Furthermore, experiments with an ACAT2-specific inhibitor resulted in a diminishment of VLDL secretion\(^{117-120}\), but, interestingly, increase in ABCA1 expression and efflux of cholesterol to HDL\(^{121}\) and fecal excretion of cholesterol\(^{122}\). Accordingly, we posit that VLDL secretion is a major outlet for cholesterol from the hepatocyte and can be enhanced under conditions of excess hepatocyte cholesterol.

7) LDL-derived cholesterol is a substrate for ACAT2: There is evidence that CR-derived cholesterol is preferentially esterified by ACAT1 through its interaction with the regulatory pool whereas LDL-derived cholesterol is preferentially esterified by ACAT2\(^{86}\). Importantly, when an ACAT inhibitor is added to LDL-treated cells, the LDL-derived cholesterol does invoke a regulatory effect on LDR and HMGCR (similar to CR-derived cholesterol) suggesting that esterification of LDL-derived cholesterol is an essential step in its redirection from the regulatory pool. These observations imply different physical intracellular locations of the active sites of ACAT1 (ER-cytosolic facing) and ACAT2 (ER-lumen facing). They also suggest separate intracellular trafficking itineraries of LDL-derived cholesterol and CR-derived cholesterol.

8) LDL-derived cholesterol is preferentially resecreted within VLDL: Since ACAT2 provides the substrate CE for VLDL secretion and LDL-derived cholesterol is a preferential substrate for ACAT2, then it is postulated that LDL-derived cholesterol is preferentially shunted into an ACAT2 accessible pool for secretion within VLDL. Experimental observations have demonstrated this in a primary hamster hepatocyte model but need to be confirmed in other models\(^{86}\).

Taken together, these observations provide evidence for a “shunt” pathway in which LDL-derived cholesterol does not enter the regulatory cholesterol pool but instead bypasses it by being esterified by ACAT2-dependent fashion. The newly formed CE then becomes associated with newly synthesized apoB100 and is secreted with VLDL particles. By this metabolic route, LDL-derived cholesterol cycles through hepatocytes without ever entering the regulatory pool (Fig. 1). Because it does not enter the regulatory pool, synthesis of LDR and HMGCR is not downregulated.

**Metabolic rationale for the shunt pathway**

Why would a shunt pathway exist? One possibility is that the biologic challenge of a high plasma LDL is a relatively modern development. Previously in our evolutionary history, we must have eaten animal products rarely to the point that our bodies conserved cholesterol, a point reinforced by the very efficient recycling of bile acids and cholesterol in bile. Recently, we farmed animals and began consuming larger amounts of cholesterol containing animal products: milk, eggs, and meat. Accordingly, cholesterol and fatty acid intake increased. Increased delivery of dietary fatty acids to the liver leads to not only to increased TAG synthesis but also to increased synthesis of cholesterol. As the amount of cholesterol accumulated in our bodies, so did the cholesterol found in LDL. Instead of LDL delivering cholesterol to peripheral tissues (forward cholesterol transport), the vast majority of LDL was taken back up by the liver\(^{74}\). Our biology simply did not evolve to deal with accumulating plasma LDL, with the liver left to deal with most of the cholesterol burden.

Why would a hepatocyte recycle LDL-derived cholesterol without allowing that cholesterol to interact with the regulatory pool? The answer may lie in the unique position that the liver plays in total body cholesterol homeostasis. Peripheral cells have a limited capacity to take up LDL particles. The hepatocyte does not. Even without any LDL receptors, as in patients with homozygous familial hypercholesterolemia, LDL particles will be removed by the liver by non-specific internalization. In compensation for this unfavorable position, the hepatocyte has tools to deal with cholesterol. Since it cannot limit its intake of choles-
terol, the hepatocyte transforms biologically active cholesterol to biologically inert CE by ACAT. Also, hepatocytes transform free cholesterol to CE and then export it within VLDL. Moreover, the hepatocyte expresses the highest level of apoA-I and ABCA1 promoting cholesterol release to HDL. The hepatocyte secretes cholesterol and bile acids (derived from cholesterol) directly into the bile. In this way, one could envision a substantial capacity to survive excess cellular cholesterol levels.

The biologic irony is that the organ is protected at the cost of the organism. The increased secretion of VLDL particles by the liver leads to increased numbers of LDL particles accumulating in the plasma compartment and the increased number of LDL particles drives the atherosclerotic process within the arterial wall. The net result is that the liver is protected but at a potentially fatal cost to the organism.

**HDL-bile acid channel**

Many researchers demonstrated that SR-BI mediates selective uptake of CE from HDL (reviewed in[62,123]). SR-BI on the basolateral membrane of hepatocytes

**Fig. 1  Endosomal transport channels and regulation of lipoprotein-derived cholesterol in hepatocytes.** In many models, it was thought that cholesterol from all sources would enter a common regulatory pool before subsequent trafficking and regulatory steps. We present evidence here that supports a model where there is independent uptake, trafficking and regulation of cholesterol taken up from LDL, chylomicron remnants (CR) and HDL. HDL-derived cholesterol is taken up by SR-BI or ecto-F1-ATPase/P2Y13 (right side), directed to the apical surface of the plasma membrane and released into the bile. CR-derived cholesterol (from the diet; middle section) is directed to the ER membrane to interact with SCAP (so-called “regulatory” pool) to prevent release of SREBP2, thereby preventing upregulation of transcription of LDLR and HMGCR (among others). Excess cholesterol in the ER membrane can be esterified to CE by ACAT1 and stored within a cytosolic lipid droplet (LD). LDL-derived cholesterol is directed to a subdomain of the ER where the cholesterol is esterified by ACAT2. This CE is directed toward the lumen of the ER to interact with apoB-100 forming a precursor VLDL particle. Upon sufficient lipidation, the VLDL is secreted. Since the LDL-derived cholesterol bypasses SCAP (or other elements of the regulatory pool), we have termed this a shunt pathway. ABCA1/ABCG1 mediate cholesterol efflux to form HDL and reduce the cholesterol load in hepatocytes. Interestingly, cholesterol efflux and HDL biogenesis and then reuptake of HDL by SR-BI or P2Y13 may not represent a futile cycle if that cholesterol is redirected to bile acid secretion. All of these pathways represent channels with independent effectors mediating trafficking and regulation, with independent effects on intracellular cholesterol homeostasis.
internalizes only CE (not the whole particle) and that CE must be hydrolyzed to free cholesterol. Then, the cholesterol is preferentially trafficked to the apical surface for secretion into bile\(^{[124-126]}\) (Fig. 1). We have only a hint as to the mechanism of transport of cholesterol via transcytosis\(^{[127]}\); however, it is accepted in the literature as a channel specific for HDL-derived CE. In addition, HDL-apoA-I binds to the ecto-F1-ATPase expressed at the basolateral membrane of hepatocytes and stimulates the hydrolysis of extracellular ATP to ADP\(^{[63,128]}\). The extracellular ADP generated then selectively activates the P2Y13 purinergic receptor, resulting in cytoskeleton reorganization and subsequent clathrin-dependent endocytosis of whole HDL particles. P2Y13-knockout mice displayed impaired biliary cholesterol secretions\(^{[64,67-68]}\) and were prone to atherosclerosis on apoE-KO background\(^{[65]}\), consistent with the role of P2Y13 in HDL endocytosis by hepatocytes. Conversely, overexpression of P2Y13 in mice is atheroprotective\(^{[66]}\). These observations support the work of Robins and Fasulo\(^{[129]}\) describing that HDL, but not other lipoproteins, provide a vehicle for sterol transport to bile. Together, they represent a channel as an independent trafficking itinerary for HDL-derived cholesterol.

**Endosomal transport channels for lipoprotein cholesterol**

The evidence we have reviewed points to specific endosomal transport channels within hepatocytes for the different lipoprotein particles that are taken up by hepatocytes (Fig. 1). CR-derived cholesterol enters the regulatory pool of cholesterol (causing the down-regulation of synthesis of cholesterol and the LDLR). This is also true of cholesterol delivered via VLDL, β-VLDL, chylomicron, or through non-lipoprotein means. Therefore, all these lipoproteins, besides HDL and LDL, deliver their cholesterol to the plasma membrane/regulatory pool. LDL-derived cholesterol preferentially enters the VLDL secretory pathway, not the regulatory pool (therefore the cholesterol within this endosome has little effect on cholesterol and LDLR synthesis). HDL-derived cholesterol is preferentially trafficked to the apical surface for secretion into bile (Fig. 1). Importantly, when one of these channels is blocked or inhibited, another channel is turned on\(^{[86,121-122]}\). This suggests that there can be overlap or compensation under conditions where one channel is blocked. Therefore, we postulate the existence of lipoprotein-specific channels that direct cholesterol to a specific location with differential outcomes (Fig. 1). However, simply by the fact that we can identify the lipoprotein-derived cholesterol specific channels suggests that these channels constrain the direction and flow and incoming cholesterol.

**Discussion**

The hepatocyte is the epicenter of whole body cholesterol homeostasis and, accordingly, faces unique and evolving metabolic changes. The endosomal transport model posits that the cholesterol derived from three different lipoproteins endosomes has three different fates: LDL-derived cholesterol is largely recycled into VLDL, HDL-derived CE is transcytosed into bile, and CR-derived cholesterol enters the regulatory pool. These channels represent distinct physiologic fates for cholesterol and create a new model of cholesterol homeostasis within the hepatocyte. These channels may have great physiologic relevance, as in human subjects with high plasma LDL-C, where one would expect that these pathways play a major role in determining plasma LDL-C levels and hepatocyte cholesterol levels.

**References**

1. Brown AJ, Galea AM. Cholesterol as an evolutionary response to living with oxygen[J]. *Evolution*, 2010, 64(7): 2179–2183.

2. Souza CM, Schwabe TM, Pichler H, et al. A stable yeast strain efficiently producing cholesterol instead of ergosterol is functional for tryptophan uptake, but not weak organic acid resistance[J]. *Metab Eng*, 2011, 13(5): 555–569.

3. Matyash V, Geier C, Henske A, et al. Distribution and transport of cholesterol in Caenorhabditis elegans[J]. *Mol Biol Cell*, 2001, 12(6): 1725–1736.

4. Anderson JL, Carten JD, Farber SA. Using fluorescent lipids in live zebrafish larvae: From imaging whole animal physiology to subcellular lipid trafficking[J]. *Methods Cell Biol*, 2016, 133: 165–178.

5. Yun HK, Jouni ZE, Wells MA. Characterization of cholesterol transport from midgut to fat body in Manduca sexta larvae[J]. *Insect Biochem Mol Biol*, 2002, 32(9): 1151–1158.

6. Brown MS, Goldstein JL. Cholesterol feedback: from Schoenheimer’s bottle to Scap’s MELADL[J]. *J Lipid Res*, 2009, 50(Suppl): S15–S27.

7. Ghosh S. Early steps in reverse cholesterol transport: cholesteryl ester hydrolysis and other hydrolyses[J]. *Curr Opin Endocrinol Diabetes Obes*, 2012, 19(2): 136–141.

8. Goedeke L, Fernández-Hernando C. Regulation of cholesterol homeostasis[J]. *Cell Mol Life Sci*, 2012, 69(6): 915–930.

9. Goldstein JL, Brown MS. The LDL receptor[J]. *Arterioscler*
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Thromb Vasc Biol, 2009, 29(4): 431–438.

[10] Liu M, Chung S, Shelnos GS, et al. Hepatic ABCA1 and VLDL triglyceride production[J]. Biochim Biophys Acta, 2012, 1821(5): 770–777.

[11] Maxfield FR, van Meer G. Cholesterol, the central lipid of mammalian cells[J]. Curr Opin Cell Biol, 2010, 22(4): 422–429.

[12] Rogers MA, Liu J, Song BL, et al. Acyl-CoA: cholesterol acyltransferases (ACATs/SOATs): Enzymes with multiple sterols as substrates and as activators[J]. J Steroid Biochem Mol Biol, 2015, 150: 102–107.

[13] Strong A, Patel K, Rader DJ. Sortilin and lipoprotein metabolism: making sense out of complexity[J]. Cold Spring Harb Perspect Biol, 2015, 3(7): a004754.

[14] Hartman IZ, Liu P, Zehmer JK, et al. Sterol-induced dislocation of 3-hydroxy-3-methylglutaryl coenzyme A reductase from endoplasmic reticulum membranes into the cytosol through a subcellular compartment resembling lipid droplets[J]. J Biol Chem, 2010, 285(25): 19288–19298.

[15] van der Wulp MY, Verkade HJ, Groen AK. Regulation of cholesterol homeostasis[J]. Mol Cell Endocrinol, 2013, 368(1-2): 1–16.

[16] Ye J, DeBose-Boyd RA. Regulation of cholesterol and fatty acid synthesis[J]. Cold Spring Harb Perspect Biol, 2011, 3(7): a004754.

[17] Zhang L, Reue K, Fong LG, et al. Feedback regulation of cholesterol uptake by the LXR-IDOL-LDLR axis[J]. Arterioscler Thromb Vasc Biol, 2012, 32(11): 2541–2546.

[18] Hampton RY. Cholesterol homeostasis: ESCAPEs from the ER[J]. Curr Biol, 2000, 10(8): R298–R301.

[19] Rawson RB. The site-2 protease[J]. Biochim Biophys Acta, 2013, 1828(12): 2801–2807.

[20] Gong Y, Lee JN, Lee PC, et al. Sterol-regulated ubiquitination and degradation of Insig-1 creates a convergent mechanism for feedback control of cholesterol synthesis and uptake[J]. Cell Metab, 2006, 3(1): 15–24.

[21] Radhakrishnan A, Ikeda Y, Kwon HJ, et al. Sterol-regulated transport of SREBPs from endoplasmic reticulum to Golgi: oxysterols block transport by binding to Insig[J]. Proc Natl Acad Sci U S A, 2007, 104(16): 6511–6518.

[22] Zelcer N, Hong C, Boyadjian R, et al. LXRs regulate cholesterol uptake through Idol-dependent ubiquitination of the LDL receptor[J]. Science, 2009, 325(5936): 100–104.

[23] Hong C, Marshall SM, McDaniel AL, et al. The LXR-Idol axis differentially regulates plasma LDL levels in primates and mice[J]. Cell Metab, 2014, 20(5): 910–918.

[24] Hartman IZ, Liu J, Zehmer JK, et al. Sterol-induced dislocation of 3-hydroxy-3-methylglutaryl coenzyme A reductase from endoplasmic reticulum membranes into the cytosol through a subcellular compartment resembling lipid droplets[J]. J Biol Chem, 2010, 285(25): 19288–19298.

[25] Morris LL, Hartman IZ, Jun DJ, et al. Sequential actions of the AAA-ATPase valosin-containing protein (VCP)/p97 and the proteasome 19 S regulatory particle in sterol-accelerated, endoplasmic reticulum (ER)-associated degradation of 3-hydroxy-3-methylglutaryl-coenzyme A reductase[J]. J Biol Chem, 2014, 289(27): 19053–19066.

[26] Song BL, Sever N, DeBose-Boyd RA. Gp78, a membrane-anchored ubiquitin ligase, associates with Insig-1 and couples sterol-regulated ubiquitination to degradation of HMG CoA reductase[J]. Mol Cell, 2005, 19(6): 829–840.

[27] Tsai YC, Leichner GS, Pearce MM, et al. Differential regulation of HMG-CoA reductase and Insig-1 by enzymes of the ubiquitin-proteasome system[J]. Mol Biol Cell, 2012, 23(23): 4484–4494.

[28] Sharpe LJ, Brown AJ. Controlling cholesterol synthesis beyond 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCr)[J]. J Biol Chem, 2013, 288(26): 18707–18715.

[29] Do R, Kiss RS, Gaudet D, et al. Squalene synthase: a critical enzyme in the cholesterol biosynthesis pathway[J]. Clin Genet, 2009, 75(1): 19–29.

[30] Loregger A, Cook EC, Nelson JK, et al. A MARCH6 and IDOL E3 ubiquitin ligase circuit uncouples cholesterol synthesis from lipoprotein uptake in hepatocytes[J]. Mol Cell Biol, 2015, 35(2): 285–294.

[31] Zelcer N, Sharpe LJ, Loregger A, et al. The E3 ubiquitin ligase MARCH6 degrades squalene monooxygenase and affects 3-hydroxy-3-methyl-glutaroyl coenzyme A reductase and the cholesterol synthesis pathway[J]. Mol Cell Biol, 2014, 34(7): 1262–1270.

[32] Luu W, Sharpe LJ, Capell-Hattam I, et al. Oxysterols: Old Tale, New Twists[J]. Annu Rev Pharmacol Toxicol, 2016, 56: 447–467.

[33] Mutembezi V, Guillemot-Legris O, Muccioli GG. Oxysterols: From cholesterol metabolites to key mediators[J]. Prog Lipid Res, 2016, 64: 152–169.

[34] Lee SD, Tontonoz P. Liver X receptors at the intersection of lipid metabolism and atherogenesis[J]. Circ Res, 2007, 104(16): 6511–6518.
[41] Zhong LY, Cayabyab FS, Tang CK, et al. Sortilin: A novel regulator in lipid metabolism and atherogenesis[J]. *Clin Chim Acta*, 2016, 460: 11–17.

[42] Kim K, Utoh R, Ohashi K, et al. Fabrication of functional 3D hepatic tissues with polarized hepatocytes by stacking endothelial cell sheets in vitro[J]. *J Tissue Eng Regen Med*, 2015.

[43] Homola L, Fu D, Sengupta P, et al. LKB1/AMPK and PKA revealed by selective gene knockout studies[J]. *Am J Pathol*, 2013, 57(4): 1366–1379.

[44] Le Vee M, Jouan E, Noel G, et al. Polarized location of SLC transporter-related protein 1: unique tissue-specific functions revealed by selective gene knockout studies[J]. *Am J Pathol*, 2015, 56(11): 2133–2142.

[45] Levy G, Bonzé D, Heinz S, et al. Long-term culture and expansion of primary human hepatocytes[J]. *Nat Biotechnol*, 2015, 33(12): 1264–1271.

[46] Gao Y, Shen W, Lu B, et al. Uptregulation of hepatic VLDLR via PPARα is required for the triglyceride-lowering effect of fenofibrate[J]. *J Lipid Res*, 2014, 55(8): 1622–1633.

[47] Roubtsova A, Chamberland A, Marcinkiewicz J, et al. PCSK9 deficiency unmasks a sex- and tissue-specific subcellular distribution of the LDL and VLDL receptors in mice[J]. *J Lipid Res*, 2015, 56(11): 785–791.

[48] Roubtsova A, Munkonda MN, Awan Z, et al. Circulating proprotein convertase subtilisin/kexin 9 (PCSK9) regulates VLDLR protein and triglyceride accumulation in visceral adipose tissue[J]. *Arterioscler Thromb Vasc Biol*, 2011, 31(4): 2066–2073.

[49] Ho J, Choe SS, Shin KC, et al. Endoplasmic reticulum stress induces hepatic steatosis via increased expression of the hepatic very low-density lipoprotein receptor[J]. *Hepatology*, 2013, 57(4): 1366–1377.

[50] Gonias SL, Campana WM. LDL receptor-related protein-1: a regulator of inflammation in atherosclerosis, cancer, and injury to the nervous system[J]. *Am J Pathol*, 2014, 184(1): 18–27.

[51] Lillis AP, Van Duyn LB, Murphy-Ullrich JE, et al. LDL receptor-related protein 1: unique tissue-specific functions revealed by selective gene knockout studies[J]. *Physiol Rev*, 2008, 88(3): 877–918.

[52] May P. The low-density lipoprotein receptor-related protein 1 in inflammation[J]. *Curr Opin Lipidol*, 2013, 24(2): 134–137.

[53] Ma CI, Martin C, Ma Z, et al. Engagement protein GULP is regulator of transforming growth factor-β response in ovarian cells[J]. *J Biol Chem*, 2012, 287(24): 20636–20651.

[54] Muratoglu SC, Belgrave S, Lillis AP, et al. Macrophage LRP1 suppresses neo-intima formation during vascular remodeling by modulating the TGF-β signaling pathway[J]. *PLoS One*, 2011, 6(12): e28846.

[55] Borrell-Pages M, Carolina Romero J, Badimon L. LRP5 and plasma cholesterol levels modulate the canonical Wnt pathway in peripheral blood leukocytes[J]. *Immunol Cell Biol*, 2015, 93(7): 653–661.

[56] Kysenius K, Muggalla P, Määtlik K, et al. PCSK9 regulates neuronal apoptosis by adjusting ApoER2 levels and signaling[J]. *Cell Mol Life Sci*, 2012, 69(11): 1903–1916.

[57] Joiner DM, Ke J, Zhong Z, et al. LRP5 and LRP6 in development and disease[J]. *Trends Endocrinol Metab*, 2013, 24(1): 31–39.

[58] MacDonald BT, He X. Frizzled and LRP5/6 receptors for Wnt/beta-catenin signaling[J]. *Cold Spring Harb Perspect Biol*, 2012, 4(12): a007880.

[59] Bock HH, May P. Canonical and Non-canonical Reelin Signaling[J]. *Front Cell Neurosci*, 2016, 10: 166.

[60] Ranaivoson FM, Daake Sv, Comolotti D. Structural Insights into Reelin Function: Present and Future[J]. *Front Cell Neurosci*, 2016, 10: 137.

[61] Saddar S, Carriere V, Lee WR, et al. Scavenger receptor class B type I is a plasma membrane cholesterol sensor[J]. *Circ Res*, 2013, 112(1): 140–151.

[62] Shen WJ, Hu J, Hu Z, et al. Scavenger receptor class B type I (SR-BI): a versatile receptor with multiple functions and actions[J]. *Metabolism*, 2014, 63(7): 875–886.

[63] Martinez LO, Jacquet S, Esteve JP, et al. Ectopic beta-chain of ATP synthase is an apolipoprotein A-I receptor in hepatic HDL endocytosis[J]. *Nature*, 2003, 421(6918): 75–79.

[64] Lichtenstein L, Serhan N, Annema W, et al. Lack of P2Y13 in mice fed a high cholesterol diet results in decreased hepatic cholesterol content, biliary lipid secretion and reverse cholesterol transport[J]. *Nat Metab (Lond)*, 2013, 10(1): 67.

[65] Lichtenstein L, Serhan N, Espinosa-Delgado S, et al. Increased atherosclerosis in P2Y13/apolipoprotein E double-knockout mice: contribution of P2Y13 to reverse cholesterol transport[J]. *Cardiovasc Res*, 2015, 106(2): 314–323.

[66] Goffinet M, Tardy C, Boubekeur N, et al. P2Y13 receptor regulates HDL metabolism and atherosclerosis in vivo[J]. *PLoS One*, 2014, 9(4): e95807.

[67] Blom D, Yamin TT, Champy MF, et al. Altered lipoprotein metabolism in P2Y(13) knockout mice[J]. *Biochim Biophys Acta*, 2010, 1801(12): 1349–1360.

[68] Fabre AC, Vantourout P, Champagne E, et al. Cell surface adenylyl kinase activity regulates the F(1)-ATPase/P2Y(13)-mediated HDL endocytosis pathway on human hepatocytes[J]. *J Cell Mol Life Sci*, 2006, 63(7): 2829–2837.

[69] Hu L, van der Hoogt CC, Espirito Santo SM, et al. The hepatic uptake of VLDL in lrp-ldlr-/-vldlr-/- mice is regulated by LPL activity and involves proteoglycans and SR-BI[J]. *J Lipid Res*, 2008, 49(7): 1553–1561.

[70] Pangburn SH, Newton RS, Chang CM, et al. Receptor-mediated catabolism of homologous low density lipoproteins in cultured pig hepatocytes[J]. *J Biol Chem*, 1981, 256(7): 3340–3347.

[71] Cohen LH, Princen HM, Kwekkeboom J, et al. Regulation of cholesterol metabolism in the liver in vivo and in vitro[J].
Novel aspects of hepatocyte cholesterol homeostasis: is the low-density lipoprotein pathway a regulatory or a shunt pathway[J]. Arterioscler Thromb Vasc Biol, 2013, 33(11): 2481–2490.

[87] Zhang Y, Ma KL, Ruan XZ, et al. Dysregulation of the Low-Density Lipoprotein Receptor Pathway Is Involved in Lipid Disorder-Mediated Organ Injury[J]. Int J Biol Sci, 2016, 12(5): 569–579.

[88] Ai D, Chen C, Han S, et al. Regulation of hepatic LDL receptors by mTORC1 and PCSK9 in mice[J]. J Clin Invest, 2012, 122(4): 1262–1270.

[89] Davis W Jr, Boyd JT, Ile KE, et al. Human ATP-binding cassette transporter-2 (ABCA2) positively regulates low-density lipoprotein receptor expression and negatively regulates cholesterol esterification in Chinese hamster ovary cells [J]. Biochim Biophys Acta, 2004, 1683(1-3): 89–100.

[90] Di Croce L, Bruscalupi G, Trentalance A. Independent behavior of rat liver LDL receptor and HMGCa reductase under estrogen treatment[J]. Biochem Biophys Res Commun, 1996, 224(2): 345–350.

[91] Lee YJ, Han DH, Pak YK, et al. Circadian regulation of low density lipoprotein receptor promoter activity by CLOCK/BMAL1, Hes1 and Hes6[J]. Exp Mol Med, 2012, 44(11): 642–652.

[92] Liu J, Ma KL, Zhang Y, et al. Activation of mTORC1 disrupted LDL receptor pathway: a potential new mechanism for the progression of non-alcoholic fatty liver disease[J]. Int J Biochem Cell Biol, 2015, 61: 8–19.

[93] Lorbek G, Perše M, Horvat S, et al. Sex differences in the hepatic cholesterol sensing mechanisms in mice[J]. Molecules, 2013, 18(9): 11067–11085.

[94] Osono Y, Woollett LA, Herz J, et al. Role of the low density lipoprotein receptor in the flux of cholesterol through the plasma and across the tissues of the mouse[J]. J Clin Invest, 1995, 95(3): 1124–1132.

[95] Truong TQ, Auger A, Denizeau F, et al. Analysis of low-density lipoprotein catabolism by primary cultures of hepatic cells from normal and low-density lipoprotein receptor knockout mice[J]. Biochim Biophys Acta, 2000, 1484(2-3): 307–315.

[96] Goedeke L, Rotllan N, Canfrán-Duque A, et al. MicroRNA-148a regulates LDL receptor and ABCA1 expression to control circulating lipoprotein levels[J]. Nat Med, 2015, 21 (11): 1280–1289.

[97] Ma KL, Ruan XZ, Powis SH, et al. Sirolimus modifies cholesterol homeostasis in hepatic cells: a potential molecular mechanism for sirolimus-associated dyslipidemia[J]. Transplantation, 2007, 84(8): 1029–1036.

[98] Ma KL, Ruan XZ, Powis SH, et al. Inflammatory stress exacerbates lipid accumulation in hepatic cells and fatty livers of apolipoprotein E knockout mice[J]. Hepatology, 2008, 48 (3): 770–781.

[99] Zhao L, Chen Y, Tang R, et al. Inflammatory stress...
exacerbates hepatic cholesterol accumulation via increasing cholesterol uptake and de novo synthesis[J]. J Gastroenterol Hepatol, 2011, 26(5): 875–883.

[100] Wang MD, Franklin V, Sundaram M, et al. Differential regulation of ATP binding cassette protein A1 expression and ApoA-I lipiddation by Niemann-Pick type C1 in murine hepatocytes and macrophages[J]. J Biol Chem, 2007, 282 (31): 22525–22533.

[101] Dichek HL, Johnson SM, Akeefe H, et al. Hepatic lipase overexpression lowers remnant and LDL levels by a noncatalytic mechanism in LDL receptor-deficient mice[J]. J Lipid Res, 2001, 42(2): 201–210.

[102] Harders-Spengel K, Wood CB, Thompson GR, et al. Difference in saturable binding of low density lipoprotein to liver membranes from normocholesterolemic subjects and patients with heterozygous familial hypercholesterolemia[J]. Proc Natl Acad Sci U S A, 1982, 79(20): 6355–6359.

[103] Karavia EA, Papachristou NI, Sakellaropoulos GC, et al. Scavenger receptor class B type I regulates plasma apolipoprotein e levels and dietary lipid deposition to the liver[J]. Biochemistry, 2015, 54(36): 5605–5616.

[104] Kartz GA, Holme RL, Nicholson K, et al. SR-BI/CD36 chimeric receptors define extracellular subdomains of SR-BI critical for cholesterol transport[J]. Biochemistry, 2014, 53 (39): 6173–6182.

[105] Kim DH, Inagaki Y, Suzuki T, et al. A new low density lipoprotein receptor related protein, LRPS, is expressed in hepatocytes and adrenal cortex, and recognizes apolipoprotein [J]. J Biochem, 1998, 124(6): 1072–1076.

[106] Rein-Fischboeck L, Krautbauer S, Eisinger K, et al. Hepatic scavenger receptor BI is associated with type 2 diabetes but unrelated to human and murine non-alcoholic fatty liver disease[J]. J Lipid Res, 2015, 467(2): 377–382.

[107] Strong A, Ding Q, Edmondson AC, et al. Hepatic sortilin regulates both apolipoprotein B secretion and LDL catabolism [J]. J Clin Invest, 2012, 122(8): 2807–2816.

[108] Ye ZJ, Go GW, Singh R, et al. LRP6 protein regulates low density lipoprotein (LDL) receptor-mediated LDL uptake[J]. J Biol Chem, 2012, 287(2): 1335–1344.

[109] Scott CC, Vossio S, Vacca F, et al. Wnt directs the endosomal flux of LDL-derived cholesterol and lipid droplet homeostasis [J]. EMBO Rep, 2015, 16(6): 741–752.

[110] Wu GY, Wu CH, Rifici VA, et al. Activity and regulation of low density lipoprotein receptors in a human hepatoblastoma cell line[J]. Hepatology, 1984, 4(6): 1190–1194.

[111] Olofsson SO, Borén J. Apolipoprotein B secretory regulation by degradation[J]. Arterioscler Thromb Vasc Biol, 2012, 32 (6): 1334–1338.

[112] Yao Z, Zhou H, Figeys D, et al. Microsome-associated lumenal lipid droplets in the regulation of lipoprotein secretion [J]. Curr Opin Lipidol, 2013, 24(2): 160–170.

[113] Zhang Z, Cianflone K, Sniderman AD. Role of cholesterol ester mass in regulation of secretion of ApoB100 lipoprotein particles by hamster hepatocytes and effects of statins on that relationship[J]. Arterioscler Thromb Vasc Biol, 1999, 19(3): 743–752.

[114] Sahoo D, Trischuk TC, Chan T, et al. ABCA1-dependent lipid efflux to apolipoprotein A-I mediates HDL particle formation and decreases VLDL secretion from murine hepatocytes[J]. J Lipid Res, 2004, 45(6): 1122–1131.

[115] Twisk J, Gillian-Daniel DL, Tebon A, et al. The role of the LDL receptor in apolipoprotein B secretion[J]. J Clin Invest, 2000, 105(4): 521–532.

[116] Temel RE, Hou L, Rudel LL, et al. ACAT2 stimulates cholesteryl ester secretion in apoB-containing lipoproteins[J]. J Lipid Res, 2007, 48(7): 1618–1627.

[117] Alger HM, Brown JM, Sawyer JK, et al. Inhibition of acyl-coenzyme A:cholesterol acyltransferase 2 (ACAT2) prevents dietary cholesterol-associated steatosis by enhancing hepatic triglyceride mobilization[J]. J Biol Chem, 2010, 285(19): 14267–14274.

[118] Melchior JT, Olson JD, Kelley KL, et al. Targeted knockdown of hepatic soat2 with antisense oligonucleotides stabilizes atherosclerotic plaque in ApoB100-only LDLr-/- mice[J]. Arterioscler Thromb Vasc Biol, 2015, 35(9): 1920–1927.

[119] Ohshiro T, Ohtawa M, Nagamitsu T, et al. New pyrropipene A derivatives, highly SOAT2-selective inhibitors, improve hypercholesterolemia and atherosclerosis in atherogenic mouse models[J]. J Pharmaco Exp Ther, 2015, 355(2): 299–307.

[120] Zhang J, Sawyer JK, Marshall SM, et al. Cholesterol esters (CE) derived from hepatic sterol O-acyltransferase 2 (SOAT2) are associated with more atherosclerosis than CE from intestinal SOAT2[J]. Circ Res, 2014, 115(10): 826–833.

[121] Pedrelli M, Davoodpour P, Degirolamo C, et al. Hepatic ACAT2 knock down increases ABCA1 and modifies HDL metabolism in mice[J]. PLoS One, 2014, 9(4): e93552.

[122] Marshall SM, Gromovsky AD, Kelley KL, et al. Acute sterol o-acyltransferase 2 (SOAT2) knockdown rapidly mobilizes hepatic cholesterol for fecal excretion[J]. PLoS One, 2014, 9(6): e98953.

[123] Meyer JM, Graf GA, van der Westhuyzen DR. New developments in selective cholesteryl ester uptake[J]. Curr Opin Lipidol, 2013, 24(5): 386–392.

[124] Harder CJ, Meng A, Rippstein P, et al. SR-BI undergoes cholesterol-stimulated transcytosis to the bile canaliculus in polarized WIF-B cells[J]. J Biol Chem, 2007, 282(2): 1445–1455.

[125] Ji Y, Wang N, Ramakrishnan R, et al. Hepatic scavenger receptor BI promotes rapid clearance of high density lipoprotein free cholesterol and its transport into bile[J]. J Biol Chem, 1999, 274(47): 33398–33402.

[126] Kozarsky KF, Donahee MH, Rigotti A, et al. Overexpression
of the HDL receptor SR-BI alters plasma HDL and bile cholesterol levels[J]. Nature, 1997, 387(6631): 414–417.

[127] Wang J, Bie J, Ghosh S. Intracellular cholesterol transport proteins enhance hydrolysis of HDL-CEs and facilitate elimination of cholesterol into bile[J]. J Lipid Res, 2016, 57 (9): 1712–1719.

[128] Rai AK, Spolaore B, Harris DA, et al. Ectopic F0F1 ATP synthase contains both nuclear and mitochondrially-encoded subunits[J]. J Bioenerg Biomembr, 2013, 45(6): 569–579.

[129] Robins SJ, Fasulo JM. High density lipoproteins, but not other lipoproteins, provide a vehicle for sterol transport to bile[J]. J Clin Invest, 1997, 99(3): 380–384.

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