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Phospholipid Remodeling in Physiology and Disease

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Abstract

Phospholipids are major constituents of biological membranes. The fatty acyl chain composition of phospholipids determines the biophysical properties of membranes and thereby affects their impact on biological processes. The composition of fatty acyl chains is also actively regulated through a deacylation and reacylation pathway called Lands' cycle. Recent studies of mouse genetic models have demonstrated that lysophosphatidylcholine acyltransferases (LPCATs), which catalyze the incorporation of fatty acyl chains into the *sn*-2 site of phosphatidylcholine, play important roles in pathophysiology. Two LPCAT family members, LPCAT1 and LPCAT3, have been particularly well studied. LPCAT1 is crucial for proper lung function due to its role in pulmonary surfactant biosynthesis. LPCAT3 maintains systemic lipid homeostasis by regulating lipid absorption in intestine, lipoprotein secretion, and de novo lipogenesis in liver. Mounting evidence also suggests that changes in LPCAT activity may be potentially involved in pathological conditions, including nonalcoholic fatty liver disease, atherosclerosis, viral infections, and cancer. Pharmacological manipulation of LPCAT activity and membrane phospholipid composition may provide new therapeutic options for these conditions.

Keywords

lysophosphatidylcholine acyltransferase; phospholipid remodeling; lipid metabolism; intestinal homeostasis; lipoprotein production; lipogenesis; cholesterol metabolism; surfactant biosynthesis

1. INTRODUCTION

Phospholipids are composed of two hydrophobic fatty acyl chains and one hydrophilic head group. Along with cholesterol, they are the major constituents of biological membranes. Phospholipid bilayers fulfill important structural functions by segregating cellular contents from the surrounding environment, forming subcellular organelles and providing platforms for a variety of cellular processes. Phospholipids are also substrates for the generation of bioactive molecules involved in signal transduction, such as eicosanoids, lysophosphatidylcholine (LPC), lysophosphatidic acid (LPA), and diacylglycerol (1–3). The major structural phospholipids in mammalian membranes are glycerophospholipids,

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including phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylinositol (PI), and phosphatidic acid (PA). Among these glycerophospholipids, PC is the most abundant in mammalian cell membranes and subcellular organelles, accounting for ~40–50% of total phospholipids (2).

The fatty acyl moieties of membrane phospholipids exhibit considerable diversity in chain length and degree of saturation (double versus single bonds). These two parameters determine the biophysical properties of cell membranes, including their fluidity, curvature, and subdomain architecture. These factors in turn influence membrane-associated cellular processes, such as vesical trafficking, signal transduction, and molecular transport (1, 4). Although in vitro studies have greatly advanced our understanding of how phospholipids affect cellular processes, it is largely unknown how changes in phospholipid composition impact physiology, due to the difficulty of introducing specific changes in membrane composition in living organisms, especially higher organisms such as mammals. In mammalian cells, phospholipid composition is largely maintained through a remodeling process of deacylation and reacylation—a pathway referred to as Lands' cycle (5). Due to the substrate specificity of the enzymes in this pathway, saturated and monosaturated fatty acyl chains are preferably linked at the *sn*-1 position and polyunsaturated fatty acids at the *sn*-2 position. The discovery of the lysophopholipid acyltransferase (LPLAT) family of phospholipid remodeling enzymes that catalyze the reacylation of lysophopholipids at the *sn*-2 position and thus modulate the fatty acyl composition of phospholipids has led to a better understanding of how phospholipid remodeling contributes to physiology in vivo.

Over the last five years, studies using genetic models have demonstrated that lysophophatidylcholine acyltransferases (LPCATs) play important roles in lipid metabolism and homeostasis by regulating the abundance of different PC species in multiple cell and tissue types. In this review, we focus on the biochemistry and function of PC remodeling and its links to mammalian physiology. We give special attention to newly appreciated roles for LPCATs in liver, intestine, and lung and their potential involvement in the pathogenesis of human diseases.

2. PHOSPHATIDYLCHOLINE METABOLISM

2.1. Phosphatidylcholine Biosynthesis (The Kennedy Pathway)

In mammals, the primary route for de novo PC synthesis is through the CDP-choline pathway, also known as the Kennedy pathway, which was first described by Kennedy & Weiss in 1956 (6) (Figure 1). The Kennedy pathway involves three enzymatic reactions: phosphorylation of choline by choline kinase, formation of CDP-choline from phosphocholine and CTP catalyzed by CTP:phosphocholine cytidylyltransferase (CT), and the replacement of cytidine monophosphate by diacylglycerol (DAG) to produce PC catalyzed by CDP-choline:1,2-diacylglycerol cholinephosphotransferase. In addition to the Kennedy pathway, the liver has a unique pathway for PC synthesis via three sequential methylations of the ethanolamine moiety of PE catalyzed by PE methyltransferase, which contributes to ~30% of hepatic PC synthesis (7).

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2.2. Phospholipid Remodeling (Lands' Cycle)

As mentioned above, the fatty acyl chains in phospholipids are highly diverse and asymmetrically distributed. This distribution cannot be fully explained by the de novo synthesis pathway, as the enzymes in the Kennedy pathway have little fatty acyl–coenzyme A substrate specificity. The composition and asymmetrical distribution of fatty acyl chains in individual phospholipids are modified after their de novo synthesis by a remodeling process known as Lands' cycle. In 1958, Lands first described the rapid turnover of *sn*-2 fatty acyl moiety of glycerophospholipids (5). He and colleagues proposed that membrane phospholipids are metabolically active and undergo a series of deacylation and reacylation reactions, which result in the incorporation of polyunsaturated fatty acids at the *sn*-2 position of phospholipids (8–10). Upon de novo synthesis, the fatty acyl chains at the *sn*-2 position of phospholipids are hydrolyzed by phospholipases A2 (PLA2s) to generate 1-acyl lysophopholipids, which are reacylated by LPLAT to incorporate another fatty acid to the *sn*-2 position and form a new phospholipid species.

3. LYSOPHOSPHATIDYLCHOLINE ACYLTRANSFERASES

Several PLA2s were identified and characterized in late 1980s (11, 12); however, LPLATs were not identified until the late 1990s (13), and the first LPCAT was cloned in the 2000s. In 2006, two groups independently reported the cloning of the first LPCAT, *Lpcat1*, originally named *Agpat9* or acyltransferase-like 2 (AT-like 2) (14, 15). In early 2007, Shimizu and colleagues (16) cloned a second *Lpcat* (*Lpcat2*, also called lysoPAFAT, *Agpat11*, or AT-like 1), which possesses both acetyl-CoA:lysoPAF acetyltransferase activity that generates platelet-activating factor (PAF), and acyl-CoA:lysoPC acyltransferase activity that generates 1-*O*-alkyl-PC. The two other mammalian LPCATs, LPCAT3 and LPCAT4, were cloned by several groups in 2008 (17–19).

LPCATs belong to two different families based on their amino acid sequences. LPCAT1 and LPCAT2 are members of acylglycerophosphate acyltransferase family, which contains four conserved domains designated as LPA acyltransferase motifs 1–4 (20, 21) and an endoplasmic reticulum (ER) localization sequence (22). On the other hand, LPCAT3 and LPCAT4 (also called MBOAT5 and MBOAT2, respectively) belong to the membrane-bound *O*-acyltransferase (MBOAT) family. They contain MBOAT motifs but lack the LPA acyltransferase motifs (17, 23). LPCAT3 and LPCAT4 are also ER membrane proteins.

LPCATs display distinct tissue distributions, enzymatic activities, and substrate preferences (Table 1). Multiple studies have demonstrated that LPCAT1 is primarily expressed in lung alveolar type II cells where it catalyzes the generation of the dipalmitoyl-PC (DPPC) component of pulmonary surfactant (14, 15, 24). LPCAT2 is highly expressed in inflammatory cells, including resident macrophages and casein-induced neutrophils; it is also present in skin, colon, spleen, and brain (16). In contrast, LPCAT3 is more widely expressed. It is abundant in testis, kidney, and metabolic tissues including liver, intestine, and adipose (17, 18, 25). The expression of LPCAT4 is selectively expressed in epididymis, brain, testis, and ovary (17). In addition to their primary lysoPC acyltransferase activity, each LPCAT possesses other enzymatic activities. For example, LPCAT1 and LPCAT2 have been demonstrated to have lysoPAF acetyltransferase activity that catalyzes the

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incorporation of acetyl to the *sn*-2 site of lysoPAF in the biosynthesis of PAF (16, 26). LPCAT1 also display acyltransferase activity with LPG and LPA as acyl acceptors (14). LPCAT3 exhibits activities for lysoPE and lysoPS as substrates, and LPCAT4 possesses modest activity toward lysoPE as a substrate (17).

Most importantly, each LPCAT exhibits different acyl-CoA preferences. LPCAT1 prefers palmitoyl-CoA (16:0-acyl-CoA) as an acyl donor to synthesize dipalmitoyl PC [DPPC (14, 15)], but it prefers linoleoyl-CoA (18:2-acyl-CoA) and linoleoyl-CoA (18:3-acyl-CoA) as substrates for its LPGAT activity. LPCAT2 shows the highest activity in the presence of acetyl-CoA or arachidonoyl-CoA (20:4-acyl-CoA) (16). In contrast, LPCAT3 and LPCAT4 prefer polyunsaturated fatty acyl CoAs (18:2-acyl-CoA or 20:4-acyl-CoA) and oleoyl-CoA (18:1-acyl-CoA) as substrates, respectively (17, 25). Thus, the different substrate preferences and tissue expression patterns of LPCATs contribute to the tissue-selective remodeling of membrane PC species. As discussed below, LPCAT1 is highly expressed in lung, and catalyzes the production of DPPC, a major component of lung surfactant. In contrast, LPCAT3 is highly expressed in liver and intestine and catalyzes the production of arachidonoyl and linoleoyl PC.

4. LPCATS IN LIPID METABOLISM

Previous studies have documented that both de novo PC biosynthetic pathways are required for lipoprotein production and secretion. This is not surprising, as PC is the major phospholipid component of all plasma lipoproteins (reviewed extensively in 27–29). However, recent studies have demonstrated that both the quantity of PC and the fatty acyl chain composition of PC are important regulators of lipoprotein secretion and lipid metabolism in liver and intestine (Figures 2 and 3).

4.1. Lpcat3 Regulates Very Low-Density Lipoprotein Secretion

Among phospholipid remodeling enzymes, the role of Lpcat3 in lipid metabolism is by far the best characterized. The initial suggestion that Lpcat3 may function in lipid metabolism came from the observations that the gene encoding Lpcat3 is a direct transcriptional target of LXR (25, 30) and PPARs alpha and gamma (18, 31), two lipid-activated nuclear receptors that play important roles in regulating lipid homeostasis (32–34). Furthermore, Lpcat3 is highly expressed in metabolic tissues, including liver, intestine, and adipose tissue. In liver, Lpcat3 is the most abundant Lpcat and accounts for more than 90% of the total lysoPC acyltransferase activity (18, 35, 36).

We and others have shown that acute knockdown of *Lpcat3* in mouse liver with a specific shRNA-expressing adenovirus increases plasma triglyceride levels accompanied with reduced hepatic triglyceride (25, 35). This is likely caused by the accumulation of lysoPC, which in turn increases microsomal triglyceride transfer protein (MTP) expression and facilitates apoB-containing very low-density lipoprotein (VLDL) assembly and secretion (35). Conversely, mice acutely over-expressing human *LPCAT3* in liver for several days show reduced VLDL secretion and lowered hepatic triglyceride levels, perhaps due to the fact that, at reduced levels, lysoPC no longer suppresses fatty acid β-oxidation in hepatocytes (37). These mice also displayed beneficial lipoprotein profiles with increased

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levels of protective ApoE-rich high-density lipoprotein (HDL) in plasma. The effect of long-term activation or overexpression of *LPCAT3* remains to be determined.

Surprisingly, permanently deleting *Lpcat3* in mouse hepatocytes results in metabolic phenotypes that differ from those described above for the acute knockdown. These differences are likely due to the more extensive changes in membrane phospholipids expected with the complete absence of hepatic *Lpcat3* that begins prenatally. Mice lacking *Lpcat3* in hepatocytes show reduced plasma triglycerides and hepatic steatosis and secrete lipid-poor VLDL (36, 38). In contrast to the acute knockdown of *Lpcat3*, lysoPC does not accumulate to an appreciable degree in the livers of *Lpcat3* knockout mice, most likely because the excess lysoPC is channeled into the increased biosynthesis of saturated and monounsaturated PCs. Earlier studies demonstrated that mice lacking genes involved in the de novo PC synthesis (e.g., *Pemt* and *CT-α*) exhibited poor VLDL lipidation and impaired VLDL secretion, as evidenced by reduced ApoB protein in plasma (39–42). In contrast, ApoB levels in plasma are not altered in *Lpcat3*-deficient mice, indicating that they retain the ability to secrete ApoB (38). Rather, the small size of plasma VLDL particles, together with the reduced triglyceride-rich ApoB-containing particles in the Golgi fraction of *Lpcat3*-deficient livers, revealed that Lpcat3 impacts the second step of VLDL assembly—the bulk triglyceride addition to lipid-poor ApoB particles and the generation of mature VLDL.

Mechanistically, these phenotypes can be traced to decreases in ER membrane mobility and curvature caused by loss of linoleoyl and arachidonoyl phospholipids. Membranes containing higher amounts of these polyunsaturated phospholipids are more fluid and dynamic. Biophysical studies have suggested that greater lipid transport is generally observed with more fluid and highly curved membrane surfaces (43). Interestingly, proteomic studies have identified LPCAT3 as a component of the VLDL transport vesicle, indicating that LPCAT3 travels with primordial VLDL particles as they bud from the ER and move to the Golgi (44). Therefore, it is likely that Lpcat3 modifies the linoleoyl- and arachidonoyl-PC composition of both membranes and lipoprotein particles during VLDL assembly, thereby generating a local membrane environment that facilitates lipid transport and bulk lipidation. Another study has suggested that high levels of polyunsaturated phospholipids that accumulate in membranes as a result of Lpcat3 activity enable triglyceride to locally cluster in high density and that this clustering promotes efficient triglyceride transfer (36).

The induction of *Lpcat3* expression in liver also appears to be an important contributor to the pharmacological effects of LXR agonists. LXR activation promotes VLDL secretion. This effect was previously believed to be due, at least in part, to the induction of SREBP-1c–dependent lipogenesis and increased expression of phospholipid transfer protein (PLTP). This process transfers phospholipids into nascent VLDL, allowing particle expansion (45, 46). Studies with *Lpcat3*-deficient mice suggest that as an LXR target gene, *Lpcat3* also mediates some effects of LXR on VLDL secretion. Mice lacking *Lpcat3* in liver secrete less VLDL in response to synthetic LXR agonist treatment compared to controls (38).

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4.2. Role of Lpcat3 in Hepatic SREBP-1c Processing and Lipogenesis

LXR activation strongly promotes lipogenesis in liver. This effect has been primarily attributed to its role in the transcriptional activation of SREBP-1c and its downstream lipogenic target genes, including *Fasn* and *Scd-1* (47–49). We recently demonstrated that LXR activation also promotes SREBP-1c posttranslational processing through the induction of *Lpcat3* expression (50). We showed that the incorporation of polyunsaturated fatty acids into phospholipids in ER membranes by Lpcat3 facilitates SREBP-1c processing and thereby enhances lipogenesis. Conversely, *Lpcat3* deficiency in mouse hepatocytes reduces polyunsaturated phospholipid levels in ER membranes, reduces nuclear SREBP-1c levels, and blunts the lipogenic response to LXR agonist treatment.

The mechanism by which ER membrane phospholipid composition regulates SREBP-1c processing is not yet clear. It has been shown that the effect of Lpcat3 and membrane fatty acyl chain composition on SREBP-1c processing is SCAP dependent and therefore presumably involves the transport of SREBP-1c from ER to Golgi. We hypothesize that the flexible polyunsaturated fatty acyl chains in the local environment of the SREBP-1c/SCAP/Insig complex increase membrane dynamics and facilitate the release of the SREBP-1c/SCAP complex from the ER. In agreement with this model, prior studies reported that processing of the SREBP ortholog in *Drosophila* S2 cells could be inhibited by saturated PE, the predominant phospholipid in *Drosophila* membranes (51). Another study showed that SREBP-1 activity is also affected by changes in total cellular levels of phospholipids (52). Reducing the total cellular phospholipid levels by inhibiting enzymes involved in phospholipid de novo biosynthesis pathway activates SREBP-1 processing by disrupting COPII-dependent ER–Golgi transport and causing the mislocalization of site 1 protease (S1P) and S2P to ER.

Lpcat3 appears to mediate lipogenesis both in physiological contexts such as feeding, as well as in pathological conditions such as obesity, in which SREBP-1c activity and lipogenesis are known to be enhanced (53, 54). Mass spectrometry analysis revealed that polyunsaturated phospholipid levels are selectively increased in the ER of wild-type mice during feeding and in obese mice at baseline. Furthermore, these changes in membrane composition are at least in part dependent on Lpcat3 activity. Inhibition of Lpcat3 activity by adenovirus encoding shRNA against *Lpcat3* in obese mice reduces SREBP-1c processing, blunts lipogenesis, and ameliorates the development of fatty liver. These findings suggest that pharmacologic inhibition of LPCAT3 may be of potential therapeutic benefit in the setting of fatty liver disease.

4.3. Lpcat3 and Lipid Absorption in Intestine

It has long been recognized that PC in the intestinal lumen facilitates lipid absorption. Studies in rats with biliary fistulas showed that inclusion of PC or lysoPC in the infusate restored the lymphatic output of both triglyceride and phospholipid (55). Luminal PC secreted from liver along with bile acids promotes the solubilization and hydrolysis of dietary fat in the intestinal lumen (56). However, mice deficient in multidrug-resistant protein 2 (Mdr2), a PC-specific flippase that secretes PC into bile acid, show reduced chylomicron production without a defect in fat absorption even though they lack the

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majority of luminal PC (57), suggesting that PC functions as more than a surfactant. PC accounts for >70% of the total phospholipids in chylomicrons (58). Therefore, adequate PC levels are important for chylomicron production within enterocytes. Like triglyceride, luminal PC is hydrolyzed into lysoPC in the lumen and then reacylated in enterocytes through lysoPC acyltransferase activity (59). Interestingly, infusion of choline in bile-diverted rats only partially restored chylomicron output (55), suggesting that the CDP-choline pathway may not be as critical as the Lpcat reacylation pathway in lipid absorption.

As in the liver, *Lpcat3* is the most highly expressed *Lpcat* in intestine and accounts for 80–90% of total lysoPC acyltransferase activity (36, 60). Recent studies have revealed that Lpcat3 in the small intestine plays a particularly important role in plasma lipid metabolism (60–62). Global *Lpcat3* knockout mice are born normal but rapidly develop hypoglycemia and die shortly after birth on P2 (36, 38). Similarly, conditional knockout of intestinal *Lpcat3* using *Vilin-Cre* results in hypoglycemia and death during lactation, suggesting that intestinal Lpcat3 is important for postnatal survival (61).

We and others have revealed that Lpcat3 activity is also a critical determinant of lipid absorption in intestine (60, 61) (Figure 3). Mice lacking Lpcat3 in intestine ($Lpcat3^{Vil\text{-}Cre}$ mice) have reduced serum triglyceride and cholesterol levels on chow diet. When challenged with a bolus of lipid, $Lpcat3^{Vil\text{-}Cre}$ mice exhibit a severe defect in fatty acid uptake into enterocytes, have reduced serum triglyceride output, and secrete smaller chylomicrons. In contrast to the liver, where loss of Lpcat3 mainly affects the lipidation rather than the secretion of VLDL, *Lpcat3* deficiency in intestine reduces ApoB-48 levels in chylomicrons and promotes ApoB-48 accumulation in intestine. These findings point to impaired chylomicron secretion in addition to defective chylomicron lipidation.

Mechanistic studies showed that loss of Lpcat3 in intestine results in a selective defect in the incorporation of linoleate and arachidonate into membrane phospholipids. These changes, possibly together with an increase in saturated and monosaturated PC, lead to a marked decrease in membrane fluidity, thus impairing passive fatty acid transport across the apical membrane of enterocytes. Li et al. (60) reported that the expression of CD36 and FATP4, two putative fatty acid transporters, was reduced in enterocytes of *Lpcat3* global knockout mice and suggested that this may contribute to the impaired fatty acid uptake. However, most studies argue against an essential role for these proteins in dietary lipid uptake. It has long been debated whether fatty acids are transported across the enterocyte apical membrane via passive diffusion or by carrier-mediated processes (63). In vitro studies utilizing Caco2 cells suggest that a passive transport dominates, especially when the fatty acid concentration is high. Such studies have shown that the rate of fatty acid uptake is linear, protease resistant, and temperature independent (64, 65). In agreement with these observations, deletion of either CD36 or FATP4 in mouse intestine does not appear to dramatically alter fatty acid uptake (66, 67).

Furthermore, our lab showed that acute administration of polyunsaturated PC increases passive fatty acid uptake in the *Lpcat3*-deficient intestine, demonstrating that altered membrane composition per se is the proximal cause of the defect. These observations are consistent with the hypothesis that the increased abundance of polyunsaturated PC (both AA

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and LA) in the apical membrane facilitates the flip-flop of fatty acids into the bilayer (68, 69). Therefore, our studies favor a biophysical model in which passive transport of fatty acids across a permissive enterocyte membrane predominates in the context of bolus lipid challenge in vivo. Whether *Lpcat3* deficiency-induced changes in membrane fluidity and dynamics also contribute to the impaired chylomicron transport and secretion remains to be investigated.

In addition to defective triglyceride absorption, $Lpcat3^{Vil\text{-}Cre}$ mice also have lower serum cholesterol levels (60–62), likely due to reduced chylomicron and HDL production from the intestine. The expression of NPC1L1, a protein critical for intestinal cholesterol absorption (70), was reported to be reduced in the *Lpcat3*-deficient intestine. Studies have differed as to whether cholesterol absorption is substantially reduced in the knockout mice (60, 62). One study also reported a decrease in HDL-cholesterol levels in $Lpcat3^{Vil\text{-}Cre}$ mice, which the authors attributed to decreased expression of ABCA1 and reduced ApoA-I secretion (62). Intestinal ABCA1 activity has been shown to produce approximately 30% of the total plasma HDL pool in mice (71). However, it remains unclear how loss of Lpcat3 and the subsequent changes in membrane phospholipid composition affect expression of genes involved in cholesterol transport.

Interestingly, our studies also revealed an unexpected function for intestinal Lpcat3 in supporting the survival of mice on a lipid-rich diet (61). Loss of intestinal Lpcat3 renders mice unable to survive a high-fat diet due to the combined effect of defective lipid absorption and dramatically reduced food intake. Mice lacking *Lpcat3* in intestine stop eating once switched to a high-fat diet or Western diet. They continue to resist eating and rapidly lose body weight, even though they exhibit signs of starvation. The suppression of food intake appears to be dependent on the amount of fat present in the diet. There is no difference in food consumption between $Lpcat3^{Vil\text{-}Cre}$ mice and controls on chow diet, and they are able to adapt to a 30% fat diet after several days. These observations suggest that the inability to process dietary fat triggers one or more signals that inhibit food intake in $Lpcat3^{Vil\text{-}Cre}$ mice.

GLP-1 and PYY are two such factors that have been demonstrated to control appetite (72, 73), and both are highly induced upon high-fat diet feeding in $Lpcat3^{Vil\text{-}Cre}$ mice (61). These hormones are secreted from enteroendocrine L cells in the distal small intestine, most likely in response to luminal fatty acids. We postulate that the reduced fatty acid uptake in the duodenum and jejunum of $Lpcat3^{Vil\text{-}Cre}$ mice results in more fatty acids reaching the ileum, where they trigger the secretion of gut hormones. Our data suggest that the excessive GLP-1 secretion indeed contributes to anorexia in $Lpcat3^{Vil\text{-}Cre}$ mice, as administration of a GLP-1 receptor antagonist partially rescues food intake. High-fat feeding also induces the biosynthesis of intestinal oleoylethanolamide (OEA), a lipid messenger that inhibits food intake by activating the paraventricular nuclei in the hypothalamus and peripheral sensory fibers (74, 75). Interestingly, intestinal and serum OEA levels are hyperinduced in high-fat diet–fed $Lpcat3^{Vil\text{-}Cre}$ mice, suggesting that they may also contribute to reduced food intake.

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4.4. Lpcat3 and Adipogenesis

Lpcat3 is the most highly expressed LPCAT in adipose tissue. Recent in vitro studies in preadipocyte cell lines suggested that Lpcat3 may be involved in adipogenesis. Using C3H10T1/2 cells, a mesenchymal stem cell line capable of differentiating to adipocyte-like cells, Eto et al. (76) showed that the expression of Lpcat3 and lysophopholipid acyltransferase activity are increased during the differentiation of adipocytes. Consistent with this change in gene expression, levels of arachidonoyl phospholipids, including PC and PE, were increased in adipocytes. Arachidonic acid in phospholipids is a substrate for the biosynthesis of eicosanoids, some of which have been proposed to act as endogenous ligands for PPARγ (77). Eto and colleagues suggest that Lpcat3-mediated arachidonic acid incorporation into phospholipids may promote production of endogenous lipid ligands for PPARγ, although this hypothesis remains to be tested directly.

Another study showed that knockdown of Lpcat3 in 3T3-L1 preadipocytes impairs their adipogenesis and differentiation (78). Lpcat3 inhibition was found to reduce levels of polyunsaturated phospholipids, such as linoleoyl and arachidonoyl phospholipids, and decrease the expression of adipogenesis-related genes, such as SREBPs, PPARγ, and C/EBPs, perhaps through effects on the Wnt/β-catenin pathway. While these cellular studies indicate that loss of Lpcat3 expression can impair adipogenesis, the in vivo physiological roles of Lpcat3 in adipose tissue and its potential impact on systemic metabolism remain to be determined.

4.5. Role of LPCAT1 and LPCAT2 in Lipid Droplet Formation

Lipid droplets (LDs) are intracellular organelles that store neutral lipids for use as an energy source in membrane synthesis and in production of signaling lipids (79). LDs consist of a neutral lipid core surrounded by a monolayer of phospholipids and proteins. As is true for other intracellular membranes, PC is the major phospholipid component of LD membranes, comprising 50–60% of the total phospholipids (80, 81). It has been shown that PC functions as a surfactant to limit coalescence and size of LD. Moreover, during LD expansion, new PC is synthesized via recruitment of carboxyltransferase (CT) (82). Inhibiting PC biosynthesis increases the size of LDs, presumably because larger droplets require less phospholipid to cover their surface area compared to smaller droplets (82, 83).

Recent studies have demonstrated that LPCAT1 and LPCAT2 can also localize to the surface of LDs in a variety of mammalian cells (84–86). Interestingly, LDs have been shown to possess lysoPC acyltransferase activity, which correlates with LPCAT1 and LPCAT2 expression (84). Similar to PC de novo synthesis, eliminating *LPCAT1* or *LPCAT2* expression alters LD morphology and increases LD size (84, 86, 87). However, the PC de novo synthesis and remodeling pathways appear to affect LD metabolism through different mechanisms. Knockdown of LPCAT1 or LPCAT2 increases LD size without changing the neutral lipid pool, suggesting that the increase in LD size likely results from an adjustment of the surface-to-volume ratio rather than the formation of more neutral lipids (86). In contrast, reducing CT expression results in increased LDs with a higher triglyceride content, possibly by switching lipid metabolism from PC to triglyceride biosynthesis (86, 88).

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What is the function of LPCAT activity on LDs? Studies suggest that LPCATs either provide the PC required for the fission of LDs through acylation of lysoPC, or they change the composition of PC species on the LD surface monolayer, which thereby changes the biophysical properties of the monolayer and reduces the tendency of LDs to coalesce (84, 86). Recently, M'barek et al. (87) reported that lysoPC may be involved in the determination of LD size through regulating LD budding from ER in the absence of LPCAT1 activity. They showed that lysoPC in the ER reduces the ER membrane tension and facilitates LD budding. They hypothesized that inhibition of LPCAT1 likely increases the accumulation of lysoPC in the ER, which in turn should favor LD budding and result in smaller LDs. However, they observed larger LDs in the absence of LPCAT1. They reasoned that lysoPC is rapidly recycled to lysoPA and PA by the action of lysophopholipase D. Indeed, knockdown of LPCAT1 together with pharmacological inhibition of lysophopholipase D resulted in smaller LDs. These data suggested that lysoPC in ER membranes may be involved in regulating LD metabolism. However, inhibition of two other ER-localized LPCATs, LPCAT3 and LPCAT4, has no effect on LD size, indicating that LPCAT activity on LDs and not on the ER is important for LD remodeling (86).

LDs play critical cellular and physiological roles in a variety of biological processes, such as lipid storage, fatty acid trafficking, and the activation of transcription factors (79). Whether and how LPCAT1/2-mediated LD remodeling may affect any of these processes has not be well studied. Dupont et al. (89) reported that LDs contribute to autophagic initiation by acting as a cellular store for neutral lipids. They demonstrated that neutral lipids in LDs are mobilized into phospholipids necessary for autophagosome membrane formation and growth. Consequently, inhibition of LPCAT2 reduces autophagosome formation, identifying LPCAT2 as an LD-dependent regulator of autophagy (89). In a recent study, Delmas and colleagues (90) demonstrated that LPCAT2-mediated LD production contributes to chemotherapy resistance in colorectal cancer. They showed that LD content in colorectal cancer cells positively correlates with LPCAT2 expression. Overexpression of LPCAT2 promotes LD formation and chemotherapy resistance, most likely by blocking chemotherapy-induced caspase activation, ER stress, calreticulin protein membrane translocation, and subsequent cell death. These findings indicate that targeting LPCAT2-mediated LD formation may be a therapeutic approach for restoring chemotherapy sensitivity in colorectal cancer cells.

5. LPCAT3 IN INTESTINAL STEM CELL HOMEOSTASIS

Recent work from our laboratory has uncovered an unexpected role of phospholipid remodeling in modulating intestinal stem cell (ISC) proliferation and intestinal homeostasis (91). As mentioned above, loss of Lpcat3 in intestine results in severe lipid malabsorption, accompanied by mucosal hypertrophy in the duodenum and jejunum with profound lengthening of villi and longer small intestines (61). Further studies demonstrated that mucosal hypertrophy is not just a compensatory response to malabsorption but also a direct consequence of *Lpcat3* deletion in intestinal epithelium that is driven by changes in membrane phospholipid composition. We showed that deletion of *Lpcat3* in adult mice promotes stem cell and progenitor cell proliferation, as evidenced by increased crypt height and stem/progenitor cell numbers in the crypts of *Lpcat3*-deficient mice. Ex vivo analysis of

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intestinal organoid cultures demonstrated that loss of Lpcat3 increased organoid growth and the self-renewal of ISCs. Lpcat3 deficiency in the intestine increased the saturation of fatty acyl chains in membrane phospholipids. Moreover, supplementation of polyunsaturated PC rescued organoid growth and self-renewal, confirming that hyperproliferation of ISC and progenitor cell in the absence of Lpcat3 is a consequence of altered membrane composition.

Gene expression profiling revealed that loss of Lpcat3 activates the cholesterol biosynthesis pathway in enterocytes, an effect likely mediated by increased nuclear SREBP2 protein levels. Accordingly, the levels of free cholesterol were increased in *Lpcat3*-deficient crypts. Furthermore, cholesterol itself appears to act as a mitogen for ISCs. Increasing cellular cholesterol content, either by overexpression of SREBP2 or providing excess exogenous cholesterol, was sufficient to drive intestinal stem cell proliferation. Conversely, pharmacologic inhibition of cholesterol synthesis normalized crypt hyperproliferation in *Lpcat3*-deficient organoids and mice, suggesting that cholesterol biosynthesis mediates the effect of Lpcat3 deficiency on ISC proliferation.

ISCs have been shown to be the cells of origin for the intestinal tumors in mice carrying mutations in tumor suppressor gene adenomatous polyposis coli (*Apc*) (92, 93). In agreement with these observations, loss of LPCAT3 or overexpression of SREBP2 markedly promotes intestinal tumor formation in Apc^{min} mice and leads to poor survival. Moreover, suppression of cholesterol biosynthesis decreases tumor initiation and growth, implicating enhanced cholesterol synthesis as a major contributor to tumorigenesis in *Lpcat3*-deficient mice.

Previous studies have suggested links between phospholipid metabolism and intestinal tumori-genesis. Polymorphisms in the secretory phospholipase A2 (*Pla2g2a*) gene, which encodes an enzyme catalyzing the deacylation of *sn*-2 fatty acids in Lands' cycle, influences the incidence of intestinal tumors in Apc^{min} mice (94, 95). Loss of *Pla2g2a* increases Apc^{min}-induced tumor number (96), whereas overexpression of *Pla2g2a* reduces tumor multiplicity and size (97). In contrast, deletion of another phospholipase A2 member, cytosolic phospholipase A2 (*Pla2g4*), has been reported to suppress Apc^{min}-induced tumorigenesis (98). Similarly, cholesterol consumption has long been associated with increased gastrointestinal cancer risk in epidemiological studies (99). However, the mechanisms underlying the effect of phospholipid remodeling and cholesterol on intestinal tumorigenesis have remained enigmatic. Our studies demonstrated that phospholipid remodeling and cholesterol availability are important for maintaining intestinal homeostasis and may contribute to tumorigenesis by modulating ISC and progenitor cell function.

How Lpcat3-dependent phospholipid remodeling activates cholesterol biosynthesis in the intestine remains to be determined. Our studies suggest that Lpcat3 deficiency likely promotes the processing of the SREBP2 precursor from. As mentioned above, the processing of SREBP1, but not SREBP2, is regulated by ER phospholipid composition in the liver (50). In contrast, LPCAT3 deficiency selectively affects the activation of the SREBP2 pathway in the intestine. Thus, phospholipid remodeling likely affects the SREBP1 and SREBP2 pathways in a tissue-specific manner. Several signaling pathways, such as the PI3K/AKT/mTOR and p53 pathways, have been shown to regulate SREBP activity in cancer

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cells, potentially linking cell proliferation with lipid biosynthesis (100–102). Interestingly, prior studies have demonstrated that membrane phospholipid composition can modulate the activity of cellular signaling pathways, including the AKT pathway (103). In the future it will be important to determine whether Lpcat3 modulates any of these signaling pathways and whether they may mediate effects of Lpcat3 on sterol synthesis.

6. LPCAT1 IN PULMONARY SURFACTANT HOMEOSTASIS

Pulmonary surfactant is essential for the proper function of lung. Pulmonary surfactant, composed of 90% lipid and 10% protein, functions to lower the surface tension at the air–liquid interface for efficient gas exchange and to prevent alveolar collapse and small airway closure (104). Deficiency in surfactant production is known to be involved in the pathogenesis of several pulmonary diseases, including neonatal respiratory distress syndrome (105) and acute respiratory distress syndrome (106). DPPC constitutes ~50% of surfactant lipid and is primarily responsible for the surface tension–lowering property of surfactant (107). Although some of the DPPC can be synthesized de novo through the Kennedy pathway (108), ~55–75% of DPPC is produced via the remodeling pathway (109). Lpcat1, the most abundant Lpcat in alveolar type II cells, is responsible for the biosynthesis of DPPC (14, 15). Consistent with increased production of surfactant in the fetal lung toward the end of gestation (110), expression of Lpcat1 rises significantly in late stages of embryonic development and is induced by glucocorticoids and keratinocyte growth factor (14), both of which are known to regulate phospholipid biogenesis in alveolar type II cells (111).

More importantly, mice bearing a hypomorphic allele of *Lpcat1* generated by gene trapping ($Lpcat1^{GT/GT}$) exhibit perinatal mortality due to respiratory failure, with signs of respiratory distress such as atelectasis and hyaline membranes (24). Expression of *Lpcat1* and lysoPC acyltransferase activity are reduced in newborn $Lpcat1^{GT/GT}$ mice and are directly correlated with saturated PC content and survival. These findings demonstrate that Lpcat1 activity in the lung is required for the biosynthesis of saturated PCs that are essential for successfully transitioning to air breathing at birth.

In addition to its essential role in DPPC biosynthesis, Lpcat1 appears to be involved in the transport of DPPC in alveolar type II cells. Shannon and colleagues (112) showed that Lpcat1 directly interacts with START domain–containing protein 10 (StarD10), a phospholipid binding protein, to initiate the trafficking of DPPC from the ER to the cytoplasmic lamellar body for storage prior to secretion, suggesting that Lpcat1 likely performs functions beyond its acyltransferase activity in modulating surfactant homeostasis in lung.

7. LPCATS IN PATHOLOGICAL CONDITIONS

Recent studies have shown that LPCATs not only play important roles in lipid metabolism and homeostasis but also contribute to several pathological conditions, including nonalcoholic fatty liver disease (NAFLD), hepatitis C virus (HCV) infection, atherosclerosis, and cancer.

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7.1. LPCATs in Nonalcoholic Fatty Liver Disease

NAFLD encompasses a spectrum of progressive diseases that occur in the absence of excessive alcohol consumption, ranging from simple steatosis to nonalcoholic steatohepatitis (NASH), liver fibrosis, and cirrhosis (113). It is generally accepted that the pathogenesis of NAFLD is initiated by increased flux of free fatty acids (FFAs) in the liver resulting from an imbalance between FFA formation (circulating FFAs, de novo lipogenesis, and dietary uptake) and utilization (oxidation and triglyceride synthesis) that promotes hepatic lipotoxicity and ER stress (113). Persistent lipotoxicity and ER stress induce hepatocellular injury, inflammation, the activation of stellate cells, and ultimately progression into NASH and fibrosis.

Phospholipid metabolism has long been shown to affect the development of NAFLD. Mice lacking *CT-α* or *Pemt* develop NAFLD, probably due to decreased PC/PE ratios and impaired VLDL secretion (114, 115). Recent studies demonstrated that LPCATs may also contribute to the pathogenesis of NAFLD. Attempts to identify diagnostic markers of NAFLD have found that serum lysoPC levels are decreased in NASH patients (116–118). Similar decreases in serum lysoPC levels were observed in a mouse NASH model induced by a methionine- and choline-deficient (MCD) diet (119). In agreement with reduced lysoPC levels, the expression of *Lpcat* was increased in the livers of MCD diet–fed mice, suggesting that LPCATs may influence the development of NAFLD by modulating the levels of their substrates.

Lipidomics analysis in liver tissues showed that levels of polyunsaturated PC, including linoleoyl and arachidonoyl PC, are decreased in steatosis and NASH patients compared to controls (120, 121). Moreover, matrix-assisted laser desorption ionization–imaging mass spectrometry (MALDIIMS) analysis revealed distinct distributions of several PC species in control, steatotic, and NASH livers (120). It is well recognized that different zones exist in liver lobules due to proximity to the blood flow. These are defined as zone 1 (periportal), zone 2 (midzonal), and zone 3 (pericentral) (122). Previous studies have demonstrated that hepatocytes from different zones show metabolic heterogeneity, leading to zonation of metabolic processes (123). MALDI-IMS showed that in normal obese livers, polyunsaturated PC 34:2 and PC 36:4 display an azonal and zone 1–enriched distribution, respectively, while monounsaturated PC 34:1 shows limited zone 1 accumulation (120). In contrast, PC 34:2 distribution is zone 3 dominant in steatotic livers, whereas PC 36:4 and PC 34:1 display similar distributions as in normal obese livers. Interestingly, the zonal distribution of phospholipids is largely lost in NASH specimens, suggesting that phospholipid zonation may be involved in the pathogenesis of NASH. Similarly, Hall et al. (124) observed the loss of zonal distribution in both diet-induced NASH mouse models and human patients, despite the fact that they reported slightly different zonal distributions for some PC species in normal and steatotic livers. How the zonal distribution of PCs is regulated and whether the change in lipid zonation is a cause or effect of NAFLD are not clear. LPCAT2 has been shown to be primarily localized in zone 3 in liver (124) and can be induced by proinflammatory cytokines (119, 125), suggesting that LPCAT2 may contribute to the zonal distribution of some PC species. Given that LPCAT3 is the most abundant hepatic LPCAT that catalyzes the biosynthesis of polyunsaturated PC and given its important

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roles in regulating lipid metabolism, it would be interesting to determine if LPCAT3 may be involved in the regulation of PC zonal distribution and the pathogenesis of NAFLD.

7.2. LPCAT1 in Hepatitis C Virus Infection

HCV infection is known to alter lipid metabolism in the host liver to promote viral replication and propagation (126). HCV infection increases lipogenesis, reduces β-oxidation of fatty acids, and leads to hepatic steatosis in both human patients and animal models (127). Inhibition of lipogenesis has been shown to effectively suppress HCV infection, highlighting the importance of cellular lipid constituents for HCV replication (128, 129). Furthermore, HCV co-opts the hepatic VLDL assembly, maturation, and secretion pathway to produce infectious HCV virions, which are released from hepatocytes as hybrid lipo-viro-particles with viral proteins attached to the surface of VLDL (130, 131). As major neutral lipid storage organelles and the triglyceride source of VLDL assembly, LDs have been shown to play a crucial role in HCV infection (126). HCV-encoded core and nonstructural protein 5A proteins directly bind to LDs (132, 133), which not only provide a platform for HCV assembly and secretion via the VLDL pathway but also regulate HCV infection through modulating the interferon response to HCV and the stability of HCV proteins (126).

Given the important roles of phospholipids in LDs and VLDL metabolism, it is conceivable that phospholipids may have roles in HCV infection. In a recent study, Beilstein et al. (134) demonstrated that HCV infection in primary human hepatocytes and Huh7.5.1 cells inhibits gene expression of *LPCAT1*. LPCAT1 depletion increases the production of HCV particles with high infectivity, likely through remodeling LD metabolism and modulating VLDL secretion. In agreement with previous studies, they found that inhibition of LPCAT1 increases the size of LDs and triglyceride storage. However, in contrast to a previous report that showed reduced secretion of ApoB-containing lipoprotein particles in Huh7 cells (86), Beilstein and colleagues found that knockdown of LPCAT1 increases the secretion of triglyceride-rich lipoprotein particles. Nevertheless, this study supports the notion that HCV hijacks the LD metabolism of host cells for the benefit of HCV morphogenesis.

7.3. Lpcat3 in Endoplasmic Reticulum Stress and Inflammation in Liver

ER is a crucial organelle for protein folding and maturation, lipid biosynthesis, and calcium and redox homeostasis (135). Perturbations of ER homeostasis induce ER stress, which triggers the activation of unfolded protein response (UPR). Chronic UPR can initiate inflammatory responses and contribute to the pathogenesis of metabolic diseases, such as obesity, type 2 diabetes, liver diseases, and atherosclerosis (136, 137). Increased levels of saturated fatty acids induce ER stress and the UPR, which likely involve changes in ER membrane composition (138). Knockdown of stearoyl-CoA desaturase 1 (SCD1) in mammalian cells increases the amount of saturated fatty acids in phospholipids and induces UPR (139).

Our studies have shown that Lpcat3 is involved in the regulation of ER homeostasis through the modulation of membrane phospholipid composition (25). As a target of LXRs, Lpcat3 mediates the function of LXR activation in suppressing ER stress induced by saturated fatty acids in vitro and hepatic lipid accumulation in *ob*/*ob* mice in vivo. LXR activation increases

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the expression of *Lpcat3*, which drives the incorporation of polyunsaturated fatty acids into phospholipids, thereby reducing ER membrane saturation. Conversely, adenovirus-mediated acute knockdown of *Lpcat3* in liver exacerbates ER stress. Furthermore, inhibition of Lpcat3 activity also increases hepatic inflammation by regulating the activation of inflammatory kinase c-Src through altering the composition of membrane microdomains. Lpcat3 activity also impacts the availability of free arachidonic acid for the production of lipid inflammatory mediator prostaglandin E2, which also contributes to inflammation. Interestingly, we did not observe basal ER stress and inflammation in *Lpcat3* liver-knockout mice, likely due to compensatory responses in membrane composition that prevent induction of the ER stress response in the setting of chronic *Lpcat3* deletion (38).

7.4. LPCAT3 in Atherosclerosis

Atherosclerosis is a chronic inflammatory disease initiated by subendothelial retention of cholesterol-rich, ApoB-containing lipoproteins in arteries (140, 141). Modification of lipoprotein particles induces local inflammation, activates endothelial cells, and leads to the infiltration of monocytes. These monocytes differentiate into tissue macrophages, which phagocytose the modified lipoproteins through scavenger receptors and become lipid-laden foam cells (142, 143). Foam cells secrete proinflammatory mediators, including cytokines, chemokines, and reactive oxygen species, which contribute to unresolved inflammation and the progression of lesions into more advanced plaques.

LPCAT3 may be involved in atherogenesis by affecting several aspects of this processes. LPCAT3 increases the content of polyunsaturated fatty acid–containing phospholipids, which are prone to be oxidized to produce Ox-PL. Moreover, LPCAT3 was shown to mediate the effect of LXR on arachidonic acid distribution and the release of bioactive lipid mediator eicosanoids in macrophages (144). Both Ox-PL and eicosanoids have been shown to play proatherogenic roles in atherosclerosis (145, 146). LPCAT3 has also been shown to modulate the polarization of macrophages (147), which are known to play central roles in atherosclerosis (143). A recent study showed that the expression of LPCAT3 is decreased with the progression of atherosclerosis accompanied with decreased arachidonoyl-PC and increased lysoPC in the lesions (148), suggesting that LPCAT3 may have some roles in atherosclerosis progression. Further studies using in vivo models are needed to better understand the roles of LPCAT3 in atherosclerosis.

7.5. LPCATs in Cancer

Uncontrolled cell proliferation in cancer requires an adequate supply of energy and cellular building blocks, including phospholipids. In addition to their roles as biomass components, phospholipids play regulatory roles as signaling molecules that engage specific receptors and transcription factors (1, 149). Recent studies revealed that phospholipid composition and the expression of LPCATs are altered in tumors from a variety of tissues, including liver, colon, prostate, and breast. As discussed above, loss of Lpcat3 in mouse intestine reduces the composition of polyunsaturated phospholipids and promotes tumor initiation and growth in Apc^{min} mice (91). In addition, other LPCATs have also been demonstrated to contribute to tumor development in various tissues.

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Upregulation of LPCAT1 has been observed in multiple tumors, including clear renal cell carcinoma (150), oral squamous cell carcinoma (151), hepatoma (152), as well as esophageal (153), gastric (154), breast (155), colorectal (156), and prostate cancers (157–159). LPCAT1 expression correlates with the prognosis and survival in several tumors (150, 155, 157, 158) and may be used as a diagnostic marker in others (153, 157). Overexpression of LPCAT1 has been shown to increase cell proliferation, migration, and metastasis in several cancer cell lines (150, 152, 156). However, the underlying mechanisms are not clear. Consistent with LPCAT1 enzymatic activity, the levels of saturated phospholipids are increased in these tumors (150, 152, 154). Whether changes of these PC levels affect the activity of any signaling pathways that control the behaviors of cancer cells has not been studied. LPCAT1 may also contribute to tumor growth through its lysoPAF acetyltransferase activity to produce PAF, a lipid mediator that plays important roles in cell proliferation (151, 159).

As described above, LPCAT2 affects chemoresistance in colorectal cancer by modulating LD metabolism (90). Interestingly, LPCAT4 was also shown to be upregulated in colorectal cancer and is responsible for the elevated 16:0/16:1 PC levels in tumors (160). Whether and how 16:0/16:1 PC species drive colorectal cancer cell proliferation have yet to be investigated. LPCAT2 was also reported to be overexpressed in cervical and breast cancers (161) and was identified as a susceptibility gene in aggressive prostate cancer in animal models and genome-wide association studies in human patients (162). Because the possible involvement of LPCATs and phospholipid metabolism in cancer has only recently emerged, future mechanistic studies are needed to better understand how LPCATs and phospholipid remodeling influence cancer initiation and progression.

8. CONCLUSION

The development of genetic mouse models has enabled great progress in characterizing functions of LPCATs in physiology. Indeed, the roles of LPCAT1 in lung surfactant homeostasis and LPCAT3 in lipid metabolism are now well appreciated. Recent studies also suggest that loss or amplification of LPCATs results in development of several pathological conditions. How LPCATs and phospholipid remodeling specifically cause disease remains to be fully elucidated. Translating recent findings obtained in animal models to human pathophysiology will also be important. Further basic and translational research will be needed to develop novel strategies for manipulating LPCAT activities as potential therapeutics for these diseases.

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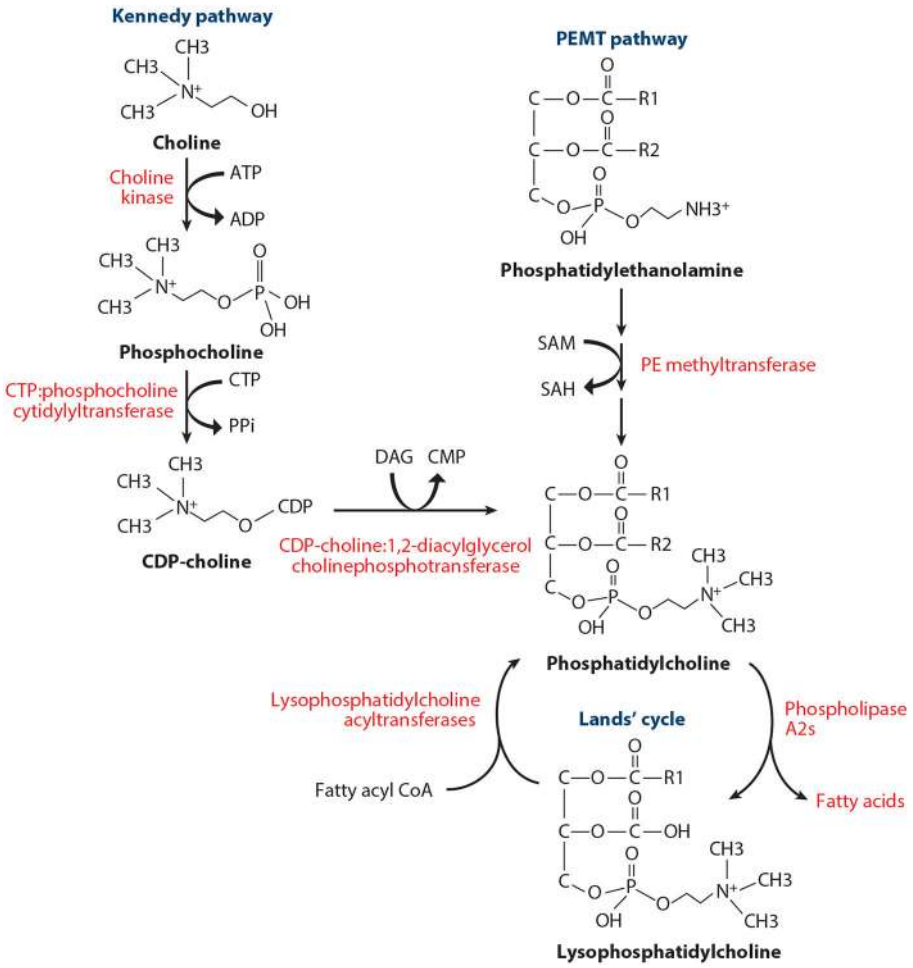

Figure 1.

PC metabolism in mammalian cells. PC is synthesized de novo through the Kennedy pathway. Choline is first phosphorylated by choline kinase to generate phosphocholine, followed by the formation of CDP-choline catalyzed by CT. Finally, CDP-choline is converted into PC by CPT. In liver, PC can also be generated through the PEMT pathway in which PE is sequentially methylated by PEMT. De novo synthesized PC undergoes remodeling in a process called Lands' cycle, which determines the acyl chain linked to PC species at the *sn*-2 position. Fatty acyl chains at *sn*-2 site of PC are hydrolyzed by PLA2s. The resulting lysoPC is reacylated by LPCATs. LPCATs catalyze the incorporation of another fatty acyl chain into the *sn*-2 site of lysoPC to produce a new PC species. Abbreviations: CMP, cytidine monophosphate; CPT, CDP-choline:1,2-diacylglycerol cholinephosphotransferase; CT, CTP:phosphocholine cytidylyltransferase; DAG, diacylglycerol; LPCAT, lysophosphatidylcholine acyltransferase; PC, phosphatidylcholine; PEMT, phosphatidylethanolamine *N*-methyltransferase; SAH, *S*-adenosyl homocysteine; SAM, *S*-adenosyl methionine.

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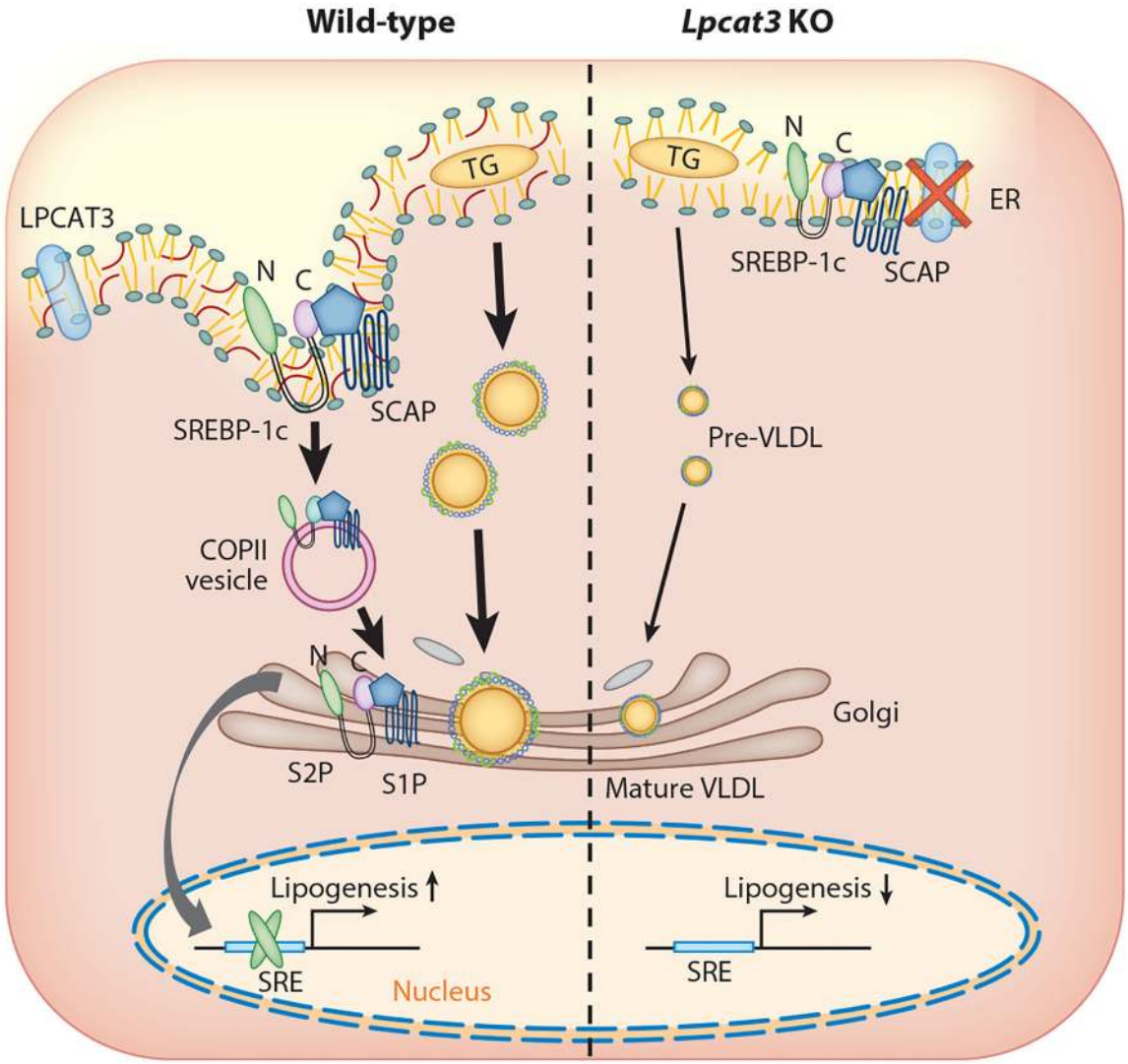

Figure 2.

Roles of Lpcat3 in VLDL secretion and SREBP-1c–mediated lipogenesis in liver. In wild-type mice, *Lpcat3* catalyzes the incorporation of polyunsaturated fatty acids into phospholipids. Polyunsaturated phospholipids facilitate SREBP-1c transport and processing, thereby promoting lipogenesis. Increased abundance of polyunsaturated phospholipids in endoplasmic reticulum creates a dynamic membrane environment that facilitates the transfer of triglyceride to pre-VLDL, leading to the efficient lipidation of VLDL. In contrast, loss of *Lpcat3* in liver reduces membrane arachidonoyl phospholipids and decreases membrane mobility and curvature, which impacts the bulk triglyceride addition to lipid-poor ApoB particles and thus produces smaller VLDL particles. Similarly, reduced membrane mobility in *Lpcat3*-deficient liver impairs SREBP-1c transport and processing, leading to reduced lipogenesis. Abbreviations: C, C terminus; COPII, coat protein complex II; ER, endoplasmic reticulum; KO, knockout; Lpcat, lysophosphatidylcholine acyltransferase; SCAP, sterol regulatory element-binding protein cleavage-activating protein; SRE, SREBP response element; TG, triglyceride; VLDL, very low-density lipoprotein.

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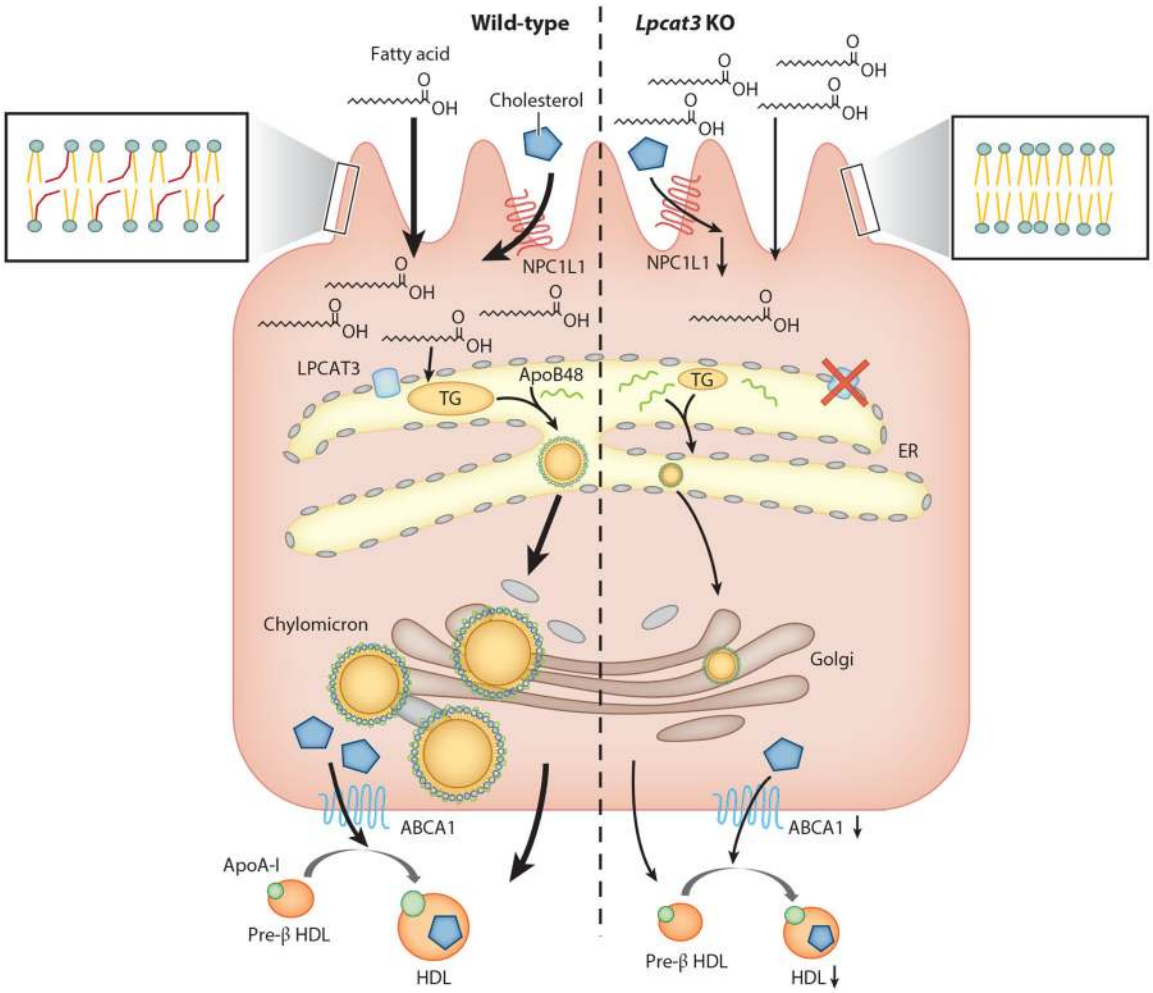

Figure 3.

Lpcat3 and phospholipid remodeling in lipid absorption in small intestine. Loss of LPCAT3 in intestine reduces polyunsaturated phospholipid content and membrane fluidity, impairs passive fatty acid transport across the apical membrane of enterocytes and decreases chylomicron assembly and secretion. In wild-type mice, *Lpcat3* activity increases polyunsaturated phospholipid levels and membrane fluidity, which is essential for efficient fatty acid transport into enterocytes for TG synthesis and chylomicron assembly, when challenged with a bolus of lipids. *Lpcat3* deficiency reduces the expression of NPC1L1 and ABCA1 in the enterocytes, which leads to decreased cholesterol absorption and cholesterol transfer to pre-β HDL to produce HDL. Abbreviations: ER, endoplasmic reticulum; HDL, high-density lipoprotein; KO, knockout; Lpcat, lysophosphatidylcholine acyltransferase; TG, triglyceride.

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Table 1

Properties of various lysophosphatidylcholine acyltransferases (LPCATs)

Family	Enzyme	Other symbols	Enzymatic activity	Substrate preference	Product	Tissue distribution	Cellular localization
AGPAT	LPCAT1	AGPAT9 AYTL2	LysoPC acyltransferase LysoPAF acetyltransferase LysoPG acyltransferase	16:0-CoA Acetyl-CoA 18:2-CoA 18:3-CoA	DPPC PAF PG	Lung (alveolar type II cells) ≫ spleen > brain	ER Lipid droplet
	LPCAT2	lysoPAFAT AGPAT11 AYTL1	LysoPAF acetyltransferase LysoPC acyltransferase	Acetyl-CoA 20:4-CoA	PAF PC	Macrophage ≫ neutrophil ≫ skin > brain, heart, stomach, colon, spleen	ER Lipid droplet
MBOAT	LPCAT3	MBOAT5	LysoPC acyltransferase LysoPE acyltransferase LysoPS acyltransferase	20:4-CoA 18:2-CoA 20:4-CoA 18:2-CoA 20:4-CoA 18:2-CoA	PC PE PS	Testis, liver, small intestine, adipose tissue, kidney	ER
	LPCAT4	MBOAT2	LysoPC acyltransferase LysoPE acyltransferase	18:1-CoA 18:1-CoA	PC PE	Epididymis, brain, testis, ovary	ER

Abbreviations: DPPC, dipalmitoyl-PC; ER, endoplasmic reticulum; MBOAT, membrane-bound *O*-acyltransferase family; PAF, platelet-activating factor; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine.