Identification and Characterization of a Major Liver Lysophosphatidylcholine Acyltransferase*

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Phosphatidylcholine (PC) is synthesized through the Kennedy pathway, but more than 50% of PC is remodeled through the Lands cycle, i.e. the deacylation and reacylation of PC to attain the final and proper fatty acids within PC. The reacylation step is catalyzed by lysophosphatidylcholine acyltransferase (LPCAT), and we report here the identification of a novel LPCAT, which we named LPCAT3. LPCAT3 belongs to the membrane-bound O-acyltransferase (MBOAT) family and encodes a protein of 487 amino acids with a calculated molecular mass of 56 kDa. Membranes from HEK293 cells overexpressing LPCAT3 showed significantly increased LPCAT activity as assessed by thin layer chromatography analysis with substrate preference toward unsaturated fatty acids. LPCAT3 is localized within the endoplasmic reticulum and is primarily expressed in metabolic tissues including liver, adipose, and pancreas. In a human hepatoma Huh7 cells, RNA interference-mediated knockdown of LPCAT3 resulted in virtually complete loss of membrane LPCAT activity, suggesting that LPCAT3 is primarily responsible for hepatic LPCAT activity. Furthermore, peroxisome proliferator-activated receptor α agonists dose-dependently regulated LPCAT3 in liver in a peroxisome proliferator-activated receptor α-dependent fashion, implicating a role of LPCAT3 in lipid homeostasis. Our studies identify a long-sought enzyme that plays a critical role in PC remodeling in metabolic tissues and provide an invaluable tool for future investigations on how PC remodeling may potentially impact glucose and lipid homeostasis.

The biomembrane lipid bilayer serves as an important structure that compartmentalizes living cells and also forms organized intracellular membrane organelles for various physiological functions. The major and essential structural components of membrane lipid bilayers are phospholipids (PLs) including phosphatidylcholine (PC),2 phosphatidylserine (PS), and phosphatidylethanolamine (PE), among which PC is the most abundant in mammals. Besides the fundamental role in maintaining the membrane structure, some PLs such as phosphatidylinositol (PI) and diacylglycerol (DG) play important roles in signal transduction. The pathway of PL de novo synthesis has been pioneered and elegantly elucidated by Dr. Eugene Kennedy and colleagues (1).

PLs are known to be remodeled through their de novo synthesis and reacylation processes to acquire their appropriate fatty acid composition. This deacylation and reacylation process, defined as the Lands cycle, may be critical for proper membrane fluidity, vesicle trafficking, and other relevant biological functions (2–5). In pancreatic β cells, PL remodeling is believed to play an essential role in amplifying the signal for insulin secretion; and in hepatocytes, proper PL composition appears vital for very low-density lipoprotein assembly and secretion (6, 7). The saturated fatty acids found frequently at the sn-1 position of PC are believed to be derived from de novo biosynthesis, whereas the unsaturated fatty acids, usually found at the sn-2 position in PC, are esterified mainly through the remodeling process (5). Indeed, the two acyl-CoA:acylglycerol-3-phosphate acyltransferase (AGPAT) enzymes described in recent years do not appear to have specific substrate preference toward fatty acyl-CoAs at the sn-2 position within the de novo PC biosynthetic pathway (8, 9). In contrast, fatty acyl chain at the sn-2 position of PC is believed to be hydrolyzed by a specific phospholipid lipase A2 family member, which is then re-acylated by the lysophosphatidylcholine acyltransferases (LPCAT). LPCAT activity has been found to reside primarily in microsomal fractions of tissues and characterization of this activity using microsomes from various tissues has led to the hypothesis that multiple forms of enzymes may exist in different tissues with different substrate specificity of fatty acyl-CoAs (5, 10). Attempts to purify the enzyme(s) have not been successful historically largely due to its membrane protein nature and the fact that LPCAT activity was mostly lost during solubilization and/or further fractionation (5, 10). However, through genomic efforts, Chen and colleagues (11) and Nakanishi and colleagues (12) recently reported the cloning of a distinct LCAT1 that is specifically expressed in lung alveolar type II cells and favors saturated fatty acyl-CoAs as substrates. A second LPCAT (LPCAT2) was also reported that is also an acetyl-

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2 The abbreviations used are: PC, phosphatidylcholine; PPARα, peroxisome proliferator-activated receptor α; LPC, lysophosphatidylcholine; LPS, lysophosphatidylserine; LPA, lysophosphatidic acid; LPG, lysophosphatidylglycerol; LPE, lysophosphatidylethanolamine; LPI, lysophosphatidylinositol; PS, phosphatidylserine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PA, phosphatic acid; PI, phosphatidylinositol; MBOAT, membrane-bound O-acyltransferase; DGAT1, diacylglycerol acyltransferase 1; LPSAT, lysophosphatidylserine acyltransferase; PL, phospholipid; DG, diacylglycerol; AGPAT, acyl-CoA:acylglycerol-3-phosphate acyltransferase; LPCAT, lysophosphatidylcholine acyltransferase; ER, endoplasmic reticulum; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; siRNA, small interfering RNA; LPAAT, lysophosphatidic acid acyltransferase; LPAGAT, lysophosphatidylglycerol acyltransferase.
CoA:lyso platelet-activating factor acyltransferase (lyso-PAFAT) with relatively broad tissue distribution (13). LPCAT activity from liver microsomes indicated that the liver LPCAT prefers unsaturated fatty acyl-CoA as substrates but the identity of this enzyme has yet to be determined (14).

Here we report the identification and characterization of such an enzyme, which we named LPCAT3. LPCAT3 belongs to the MBOAT family and is localized to the ER and most abundantly expressed in liver, pancreas, and adipose. Importantly, LPCAT3 prefers unsaturated fatty acyl-CoAs as substrates and appears to be the only such enzyme in liver responsible for this function.

**EXPERIMENTAL PROCEDURES**

**Materials**—Various lysophospholipids and acyl-CoAs were purchased from Avanti Polar Lipids (Alabaster, AL). 14C-Labeled lysophospholipids, acyl-CoAs, and phospholipids were ordered from American Radiolabeled Chemicals (St. Louis, MO).

**Plasmid Construction**—A full-length human LPCAT3 coding cDNA (NCBI accession number NM_005768) was purchased from Invitrogen. The C-terminal FLAG-tagged LPCAT3 was generated by PCR and subcloned into the pCR8/GW/TOPO vector (Invitrogen). The sequences of the primers are as follows: 5’-TCTA-CCATGGCCGTCCTCAGCGGAGG-3’ (forward) and 5’-TCAC-TATGCATCTCATCCCTTGTAATCAGCAGCAGCTTCCATGCGTCCTCAGCGGAGG-3’ (reverse). The LPCAT3-FLAG fusion was transferred to the pcDNA3.2/V5/DEST vector using LR Clonase II (Invitrogen) following the manufacturer’s instructions. The human LPCAT1 (SC112001), LPCAT2 (SC100893), GPAM (SC106982), LPGAT1 (SC121130), and AGPAT2 (SC116102) cDNA were purchased from Origene Technologies (Rockville, MD). The sequences of all the constructs were confirmed by sequencing from both directions.

**Cell Culture, Transfection, and Membrane Protein Preparation**—Human 293, COS-7, and Huh7 cells were purchased from American Type Culture Collection (Manassas, VA) and cultured in Dulbecco’s modified Eagle’s/F-12 (3:1) medium supplemented with 5% fetal bovine serum in a humidified air atmosphere containing 5% CO2. For transfections, 5 × 105 COS-7 cells, or 2 × 105 Huh7 cells were seeded in 6-well plates (Falcon, Corning, NY). 1 μg of DNA was transfected using FuGENE 6 (Roche Diagnostics) in each experiment according to the manufacturer’s instructions. 48 h after transfection, cells were scraped into 150 μl of ice-cold buffer containing 20 mM Tris-HCl (pH 7.5), 250 mM sucrose, 1 mM EDTA, and a protease inhibitor mixture (Roche Diagnostics), and then sonicated three times for 30 s. After centrifugation for 10 min at 1,000 × g, the supernatant was centrifuged at 100,000 × g for 1 h. The pellet as the membrane fraction was resuspended in the same buffer and frozen at −80 °C until use. Protein concentrations were determined using a commercially prepared protein assay kit (Pierce) and bovine serum albumin as a standard. Expression of FLAG-tagged LPCAT3 was verified by Western blot analysis with a mouse anti-FLAG monoclonal antibody (Sigma).

**In Vitro Aciyltransferase Activity Assays**—Acytransferase activity was determined by measuring the incorporation of radiolabeled acyl-CoAs into phospholipids. The reaction mixture in a total volume of 100 μl contained 75 mM Tris-HCl (pH 7.5), 1 mg/ml fatty acid-free bovine serum albumin, 200 μM of each lysophospholipid, 20 μM [1-14C]acyl-CoA, and 5 μg of membrane proteins from Hek293 cells transfected with LPCAT1, LPCAT2, LPCAT3, AGPAT2, LPGAT1, GPAM, or empty vector. The reaction was started by the addition of the membrane proteins, incubated for 20 min at room temperature and was stopped by adding 1 ml of chloroform/methanol (2:1, v/v). The phospholipids were extracted by the method of Bligh and Dyer (15). The organic phase was air dried and separated by thin layer chromatography using chloroform/methanol/water (65:25:4) as the solvent followed by exposure to a PhosphorImager screen. The Prism4 software was used for non-linear regression, curve fit analysis to calculate apparent Km and Vmax.

**Confocal Microscopy**—COS-7 cells were transfected with LPCAT3-FLAG on glass-bottom culture dishes (MetTek, Ashland, MA). 48 h post-transfection, cells were incubated with 100 nm MitoTracker Orange CMTMRos (Invitrogen) for 10 min at 37 °C. The cells were fixed with 4% paraformaldehyde for 20 min at 37 °C and permeabilized with 0.2% Triton X-100 in phosphate-buffered saline. The samples were then incubated for 2 h at room temperature with a mouse anti-FLAG monoclonal antibody (Sigma) or a rabbit anti-calnexin polyclonal antibody (StressGen Biotechnologies, Victoria, Canada) in phosphate-buffered saline with 1% bovine serum albumin. After briefly washing with phosphate-buffered saline, the samples were incubated for 1 h at room temperature with Alexa Fluor 488 goat anti-Mouse SFX or Alexa Fluor 555 goat anti-rabbit SFX (Invitrogen). The cells were counterstained with rabbit SFX (Invitrogen). The cells were counterstained with a ×40 water immersion objective lens (numerical aperture = 1.2). LPCAT3-FLAG was monitored by excitation at 488 nm and emission with a 505–530-nm filter. Endoplasmic reticulum and mitochondria markers were monitored by excitation at 543 nm and emission with a 585–615-nm filter. For the detection of propidium iodide, the excitation was at 633 nm and emissions were taken with a long pass 650-nm filter.

**Reverse Transcription and Real-time PCR**—Poly(A)+ RNA was purchased from Clontech (Mountain View, CA) and the cDNA was synthesized from 500 ng of poly(A)+ RNA utilizing the Invitrogen SuperScriptTM III First-strand Synthesis System for reverse transcriptase-PCR. The TaqMan gene expression assays ordered from Applied Biosystems (Foster City, CA) were Hs00195039_A1 (human LPCAT3) and Hs99999905_A1 (human GAPDH). Real-Time PCR was performed on 7900HT Fast Real-time PCR System (Applied Biosystems) using TaqMan® Universal PCR Master Mix (Roche). Cycle threshold (Ct) values were obtained using Applied Biosystems SDS2.3 software; glycerol-aldehyde-3-phosphate dehydrogenase (GAPDH) was used as a reference gene. The data were normalized by setting the average spleen expression value to 1.

**siRNA Transfection**—200 pmol of human LPCAT3 siRNAs (siRNA ID numbers 122586 and 16819; Ambion) and control siRNA (silencer negative control 1; Ambion) were transfected using Lipofectamine 2000 (Invitrogen) according to the
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RESULTS

Genomic efforts in recent years have identified a family of glycerolipid acyltransferases and a family of membrane-bound O-acyltransferases (16–18). The biochemical roles for most of these proteins have not been defined. Some of these family members, however, have been identified as acyl-CoA:diacylglycerol acyltransferase (19), lysophosphatidic acid acyltransferase (LPAAT) (8, 9), lysophosphatidyglycerol acyltransferase (LPGAT) (20), and acyl-CoA:lysocardiolipin acyltransferase (21). We thus focused on screening the putative acyltransferases for LPCAT activity to identify the LPCAT in liver. Candidate cDNAs were subcloned into an expression vector and the cognate proteins were expressed in HEK293 cells. Membranes were then prepared and analyzed for LPCAT activity as described under “Experimental Procedures.” One of the candidates, the human membrane-bound O-acyltransferase 5 (MOBAT5) exhibited robust LPCAT activity and was further characterized. Fig. 1A shows the LPCAT activity of human MOBAT5 (named LPCAT3 later in the text), whereas human LPCAT1 and LPCAT2 were used as controls. Contrary to what was reported by Agarwal et al. (22), human LPCAT1 had ample LPCAT activity compared with vector and mitochondria acyl-CoA:glycerol-3-phosphate acyl-CoA acyltransferase (mGPAT/GPAM) as negative controls, consistent with the reports by Chen et al. (11) and Nakanishi et al. (12) using mouse LPCAT1 protein. LPCAT2 had very limited LPCAT activity in our experiments (its lyso-PAFAT activity was confirmed in a separate experiment, data not shown). Human MOBAT5 had virtually equivalent LPCAT activity to that of LPCAT1 and we therefore renamed it as LPCAT3. Because of the fact that we do not know the relative protein expression levels, the relative activity of each LPCAT could not be accurately assessed. Insertion of a FLAG tag at the carboxyl terminus of the protein slightly reduced, but largely retained LPCAT activity. In addition, LPCAT3 also had discernable lysophosphatidylserine acyltransferase (LPSAT) activity (Fig. 1A). To examine the sub-
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LPCAT3 does not contain HX4D, a sequence motif that is conserved among glycerol acyltransferase family members. The extreme carboxyl terminus as a potential ER retention signal contains 487 amino acids and contains the KKME motif at position 566 deduced from the cDNA sequence is shown in Fig. 2D.

We then conducted kinetic studies using 1-palmitoyl-LPC and different fatty acyl-CoAs as the substrates (Fig. 4A and Table 1). Increased fatty acyl-CoA concentrations resulted in increased LPCAT activity. The apparent \( K_m \) and \( V_{max} \) values were calculated and are listed in Table 1. Whereas the unsaturated fatty acyl-CoAs have higher apparent \( K_m \) values than those of saturated fatty acyl-CoAs, the apparent \( V_{max} \) values are in

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LPCAT3 is a specific LPCAT and LPSAT.

Figure 3. Substrate specificity of the recombinant human LPCAT3 expressed in HEK293 cells. A, acyl-CoA specificity of the recombinant human LPCAT3 toward different acyl-CoAs, including n-octanoyl-CoA (8:0), lauroyl-CoA (12:0), palmitoyl-CoA (16:0), stearoyl-CoA (18:0), oleoyl-CoA (18:1), linoleoyl-CoA (18:2), and arachidonyl-CoA (20:4), was analyzed by incubating 200 \( \mu \)M each of the [14C]acyl-CoAs in the presence of 5 \( \mu \)g of membrane proteins from HEK293 cells transfected with empty vector (vector) or LPCAT3 expression vector (LPCAT3), followed by lipid extraction and TLC analysis.

B, specificity of the recombinant human LPCAT3 toward various LPCs with different acyl group at the sn-1 position, including hexanoyl (6:0), myristoyl (14:0), palmitoyl (16:0), stearoyl (18:0), oleoyl (18:1), and arachidonyl (20:0) in the presence of 20 \( \mu \)M [14C]palmitoyl-CoA and 200 \( \mu \)M each of the LPCs, respectively. C, acyl-CoA specificity of the LPSAT activity of the recombinant human LPCAT3 toward different acyl-CoAs was analyzed similarly to A.

The substrate specificity of LPCAT3 against other lysophospholipids, lysophosphatic acid (LPA), lysophosphatidylglycerol (LPG), lysophosphatidylethanolamine (LPE), and lysophosphatidylinositol (LPI) were used as substrates along with [14C]-labeled palmitoyl-CoA. LPCAT3 did not demonstrate any detectable activity against these substrates, whereas AGPAT2 and LPGAT1 exhibited respective LPAAT and LPGAT activities against LPA or LPG as positive controls (Fig. 1B). Thus, LPCAT3 is a specific LPCAT and LPSAT.

The complete amino acid sequence of human LPCAT3 derived from the cDNA sequence is shown in Fig. 2A. The protein has 487 amino acids and contains the KKME motif at the extreme carboxyl terminus as a potential ER retention signal. In contrast to other lysophospholipid acyltransferases, LPCAT3 does not contain HX4D, a sequence motif that is conserved among glycerolipid acyltransferase family members (17).

The detailed alignment of the region with those of human diacylglycerol acyltransferase 1, cholesterol acyltransferase 1 and 2 (Fig. 2B) suggested multiple conserved amino acids including the invariable Asn-358 and His-374 in the MBOAT family, implicating potential roles of these amino acids in substrate binding or catalysis or maintaining the proper structure for such enzymatic activities. Examination of the LPCAT structure via hydrophathy plot (Fig. 2C) suggested the existence of seven transmembrane domains and a hypothetical membrane topology model (Fig. 2D). When expressed in HEK293 cells and examined using Western blotting analysis, the protein had an apparent molecular mass of about 56 kDa, consistent with the predicted molecular mass from the primary sequence (Fig. 2D).

The LPCAT3 substrate specificity was further examined in three separate experimental protocols. First, using 1-palmitoyl-LPC as a substrate, different fatty acyl-CoAs were tested as acyl donors in the same experiment. Among the saturated fatty acyl-CoAs, only lauroyl-CoA had comparable activity compared with unsaturated fatty acyl-CoAs. Oleoyl-CoA (18:1), linoleoyl-CoA (18:2), and arachidonyl-CoA (20:4) all demonstrated robust LPCAT activity (Fig. 3A). The specificity of LPC was evaluated using different fatty acyl chains at the sn-1 position of LPC. Among all the different substrates examined, 1-myristoyl-LPC (14:0) and 1-palmitoyl-LPC (16:0) had the most robust activity, whereas no detectable activity was observed using 1-hexanoyl (6:0) and 1-arachidonyl (20:0) LPC as substrates (Fig. 3B). Finally, using 1-palmitoyl-LPS as the substrate, different fatty acyl-CoAs were evaluated within the same experiment with their relative LPSAT activity. All unsaturated fatty acyl-CoAs exhibited greater activity than saturated fatty acyl-CoAs and increase in desaturation appeared to result in a further increase in LPSAT activity (Fig. 3C). In short, LPCAT3 favors unsaturated fatty acyl-CoAs as acyl donors compared with saturated fatty acyl-CoAs.

We then conducted kinetic studies using 1-palmitoyl-LPC and different fatty acyl-CoAs as the substrates (Fig. 4A and Table 1). Increased fatty acyl-CoA concentrations resulted in increased LPCAT activity. The apparent \( K_m \) and \( V_{max} \) values were calculated and are listed in Table 1. Whereas the unsaturated fatty acyl-CoAs have higher apparent \( K_m \) values than those of saturated fatty acyl-CoAs, the apparent \( V_{max} \) values are in...
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Kinetic studies of the recombinant human LPCAT3 expressed in HEK293 cells. A, LPCAT assays were performed by incubating increasing concentrations of palmitoyl-CoA (C16:0), stearoyl-CoA (C18:0), oleoyl-CoA (C18:1), linoleoyl-CoA (C18:2), and arachidonoyl-CoA (C20:4) with 20 \( \mu \)M palmitoyl-LPC for 20 min under the same assay condition. The mean relative LPCAT activity was determined. All enzyme activity data were derived from two independent experiments and are shown as mean ± S.E.

B, the LPCAT assays were performed by incubating increasing concentrations of 1-palmitoyl-LPC with 20 \( \mu \)M \([1-14C]\)arachidonyl-CoA and the \( K_m \) and \( V_{max} \) values were calculated and given in Table 1. B, the LPCAT assays were performed by incubating increasing concentrations of 1-palmitoyl-LPC with 20 \( \mu \)M \([1-14C]\)arachidonyl-CoA and the \( K_m \) and \( V_{max} \) values were calculated. All enzyme activity data were derived from two independent experiments and are shown as mean ± S.E.

To examine the relative contribution of human LPCAT3 to total cellular LPCAT activity, we used siRNA knockdown technology to effectively reduce LPCAT3 mRNA in human hepatoma Huh7 cells to below 15% of the control level with two different oligonucleotides (Fig. 7A). The reduced LPCAT3 expression resulted in more than 90% reduced membrane LPCAT activity compared with the control (Fig. 7B and C). This study suggested that LPCAT3 is the major enzyme contributing to LPCAT activity in Huh7 cells and possibly liver in vivo.

PPAR\(\alpha\) is a nuclear receptor that regulates fatty acid oxidation and target genes involved in lipoprotein metabolism (23).

| Acyl-CoA            | \( K_m \) (\( \mu \)M) | \( V_{max} \) (nmol/min/mg protein) | \( V_{max}/K_m \) |
|---------------------|--------------------------|-----------------------------------|------------------|
| Palmitoyl-CoA (16:0)| 41.29                    | 1,782                             | 43.2             |
| Stearoyl-CoA (18:0) | 35.65                    | 996                               | 27.5             |
| Oleoyl-CoA (18:1)   | 72.68                    | 4,698                             | 64.6             |
| Linoleoyl-CoA (18:2)| 201.4                    | 18,148                            | 90.1             |
| Arachidonoyl-CoA (20:4)| 71.56                  | 6,247                             | 87.3             |
| 1-Palmitoyl-LPC     | 72.19                    | 6,364                             | 88.2             |

FIGURE 4. Kinetic studies of the recombinant human LPCAT3 expressed in HEK293 cells. A, LPCAT assays were performed by incubating increasing concentrations of palmitoyl-CoA (C16:0), stearoyl-CoA (C18:0), oleoyl-CoA (C18:1), linoleoyl-CoA (C18:2), and arachidonoyl-CoA (C20:4) with 20 \( \mu \)M [1-14C]palmitoyl-LPC for 20 min under the same assay condition. The \( K_m \) and \( V_{max} \) values were calculated and given in Table 1. B, the LPCAT assays were performed by incubating increasing concentrations of 1-palmitoyl-LPC with 20 \( \mu \)M [1-14C]arachidonyl-CoA and the \( K_m \) and \( V_{max} \) values were calculated. All enzyme activity data were derived from two independent experiments and are shown as mean ± S.E.

TABLE 1
Kinetic parameters of LPCAT3

Kinetic data from Fig. 4 were used to calculate apparent \( K_m \) and \( V_{max} \).

| Acyl-CoA            | \( K_m \) (\( \mu \)M) | \( V_{max} \) (nmol/min/mg protein) | \( V_{max}/K_m \) |
|---------------------|--------------------------|-----------------------------------|------------------|
| Palmitoyl-CoA (16:0)| 41.29                    | 1,782                             | 43.2             |
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| 1-Palmitoyl-LPC     | 72.19                    | 6,364                             | 88.2             |
Long chain fatty acids are believed to be endogenous ligands for PPARα (23). Because of the predominant expression of LPCAT3 in the liver and its possible link to lipid metabolism, its regulation by PPARα agonists was examined. C57BL6 mice were treated with prototypic PPARα agonists for 7 days and liver LPCAT3 mRNA levels were examined. Fenofibrate, a PPARα agonist drug in clinical use, dose-dependently elevated liver LPCAT3 mRNA with maximum induction of 3-fold. A structurally divergent prototypic PPARα agonist, WY14643, also dose-dependently increased liver LPCAT3 mRNA by 3-fold (Fig. 8A). More importantly, this regulation was completely absent in PPARα null mice, indicating the PPARα agonist regulation of LPCAT3 by these two compounds was specifically mediated by PPARα (Fig. 8B).

**DISCUSSION**

We report here the identification and characterization of a novel LPCAT, which we named LPCAT3. We believe that LPCAT3 is the long-sought liver LPCAT based on the following observations. 1) Membranes from HEK293 cells overexpressing LPCAT3 in multiple tissues was measured using GAPDH as a reference gene and normalized to the spleen expression. Quantitative RT-PCR was performed as described under “Experimental Procedures.”

Long chain fatty acids are believed to be endogenous ligands for PPARα (23). Because of the predominant expression of LPCAT3 in the liver and its possible link to lipid metabolism, its regulation by PPARα agonists was examined. C57BL6 mice were treated with prototypic PPARα agonists for 7 days and liver LPCAT3 mRNA levels were examined. Fenofibrate, a PPARα agonist drug in clinical use, dose-dependently elevated liver LPCAT3 mRNA with maximum induction of 3-fold. A structurally divergent prototypic PPARα agonist, WY14643, also dose-dependently increased liver LPCAT3 mRNA by 3-fold (Fig. 8A). More importantly, this regulation was completely absent in PPARα null mice, indicating the PPARα agonist regulation of LPCAT3 by these two compounds was specifically mediated by PPARα (Fig. 8B).

DISCUSSION

We report here the identification and characterization of a novel LPCAT, which we named LPCAT3. We believe that LPCAT3 is the long-sought liver LPCAT based on the following observations. 1) Membranes from HEK293 cells overexpressing LPCAT3 have increased LPCAT activity. 2) LPCAT3 is localized within ER, consistent with the finding that LPCAT activity is largely microsomal. 3) LPCAT3 prefers unsaturated fatty acyl-CoAs as substrates, which is in agreement with the previous characterization of LPCAT activity using liver microsomes. 4) LPCAT3 is highly expressed in liver. 5) Reduction of LPCAT3 expression through siRNA in hepatoma Huh7 cells resulted in dramatic reduction of membrane LPCAT activity.

It has been known for years that PC undergoes a deacylation-reacylation remodeling process in many cell and tissue types primarily at the sn-2 position (24). It has also been estimated that more than 50% of PC is acquired from PC remodeling (2, 5,
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PL remodeling is probably a necessary step to attain the appropriate fatty acyl chains within PL to maintain the proper membrane structure for a variety of physiological functions because the de novo PL biosynthetic enzymes appear to have very limited substrate specificity (10). The reacylation of PL, catalyzed by lyso-PL acyltransferase(s), has been identified primarily within microsomal fractions (24). It has been hypothesized that different LPCATs may exist in different cell types or tissues based on the studies using microsomal preparations from different cells and tissues (5, 10). Indeed, Chen et al. (11) and Nakanishi et al. (12) reported the cloning of an LPCAT (LPCAT1) with expression specifically in alveolar type II cells in lung. This enzyme prefers saturated fatty acyl-CoA and is thought to be primarily responsible for dipalmitoyl-PC production in surfactants. Another LPCAT (LPCAT2), with weak activity in our assay system, is also a lyso-PAFAT with a speculated role in inflammatory cells (13). The LPCAT we identified (which we named LPCAT3) is expressed primarily in metabolic tissues (liver, pancreas, and adipose). LPCAT3 has comparable LPCAT activity compared with that of LPCAT1, but has opposite substrate specificity in that it prefers unsaturated fatty acids. Our work along with others convincingly proves the original hypothesis that different enzyme forms with differential substrate specificity exist and are likely to perform specific physiological functions in different cells or tissues. In addition, our siRNA knockdown experiment in Huh7 cells suggests that LPCAT3 is primarily responsible for the LPCAT activity detected in hepatocytes.

It is interesting to note that LPCAT3 has moderate activity toward LPS while not displaying any detectable activity against LPA, LPE, and LPI, especially because a recently identified yeast membrane-bound O-acyltransferase has relatively broad substrate activities against all the lyso-PLs (25–29). It is possible that specific assay conditions may have prevented us from detecting such activities. Alternatively, distinct forms of LPE and LPI acyltransferases in mammals may exist in contrast to the situation in yeast. Cloning of mammalian LPAAT1 and LPAAT2 were reported in recent years, whereas mammalian LPE and LPI acyltransferases have yet to be identified. Studies using rat liver microsomes suggest the existence of such activities and possibly a distinct LPEAT and/or LPIAT (3).

The exact physiological function of PC remodeling is not clear, although it appears that the remodeling process is necessary for cells to acquire the appropriate PC composition for specific functions. Our work thus provides an invaluable tool for future investigations in this area. Importantly, LPCAT3 has a very high level expression in liver, pancreas, and adipose tissue. It is known that in pancreatic β-cells, PC remodeling is an important step in generating necessary signals for optimal insulin release. The release of arachidonic acid and LPC may have important functions in this process (6). The role of LPCAT could be to build the initial appropriate PC substrates for the ensuing hydrolysis by phospholipase A2 or alternatively to reacylate LPC back to PC to switch off the insulin release. The investigation of the exact role of LPCAT in this process is warranted. In hepatocytes, PC content and composition may impact very-low density lipoprotein secretion (7, 30), thus the relevance of LPCAT3 in lipid homeostasis will be an interesting subject to explore in the future. In this regard, it is important to note that Yao and Ye (30) recently demonstrated that reduced PC biosynthesis in Huh7 cells resulting from reduced long chain fatty acyl-CoA synthetase 3 expression significantly impacted apolipoprotein B lipiddation and secretion.

PPARα is a nuclear receptor that primarily regulates fatty acid oxidation. PPARα also regulates apolipoproteins important for cholesterol and triglyceride homeostasis. The endogenous ligands for PPARα are believed to be long chain fatty acids. Our finding that LPCAT3 is specifically regulated by PPARα agonists in a PPARα-dependent manner further implicates a role for LPCAT3 in lipid metabolism. The exact role of LPCAT3 in a variety of physiological processes will have to await future in vitro and in vivo studies.

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FIGURE 8. PPARα agonists regulate LPCAT3 expression in liver. A, C57BL/6 mice were treated with various doses (mpk, mg/kg) of fenofibrate or WY14643 for 7 days (6 animals per group), and the liver mRNA expression level of LPCAT3 was examined by quantitative PCR analysis as described under “Experimental Procedures.” B, wild type C57BL/6 mice or PPARα-deficient mice (6 animals per group) were treated with 100 mg/kg fenofibrate or vehicle as described under “Experimental Procedures” and the LPCAT3 mRNA was assessed similarly to A. *, p < 0.05 and **, p < 0.001 versus the vehicle control. ns, nonsignificant.
REFERENCES

1. Kennedy, E. P. (1989) in Phosphatidylcholine Metabolism (Vance, D. E., ed) pp. 1–9, CRC Press, Boca Raton, FL.
2. Lands, W. E. (1960) J. Biol. Chem. 235, 2233–2237.
3. Lands, W. E., and Merkl, I. (1963) J. Biol. Chem. 238, 898–904.
4. Merkl, I., and Lands, W. E. (1963) J. Biol. Chem. 238, 905–906.
5. MacDonald, J. L., and Sprecher, H. (1991) Biochim. Biophys. Acta 1084, 105–121.
6. Bao, S., Bohrer, A., Ramanadham, S., Jin, W., Zhang, S., and Turk, J. (2006) J. Biol. Chem. 281, 187–198.
7. Tran, K., Sun, F., Cui, Z., Thorne-Tjomsland, G., St. Germain, C., Lapierre, L. R., McLeod, R. S., Jamieson, J. C., and Yao, Z. (2006) Biochim. Biophys. Acta 1761, 463–473.
8. Aguado, B., and Campbell, R. D. (1998) J. Biol. Chem. 273, 4096–4105.
9. Eberhardt, C., Gray, P. W., and Tjoelker, L. W. (1997) J. Biol. Chem. 272, 20299–20305.
10. Choy, P. C., Skrzypczak, M., Lee, D., and Jay, F. T. (1997) Biochim. Biophys. Acta 1348, 124–133.
11. Chen, X., Hyatt, B. A., Mucenski, M. L., Mason, R. J., and Shannon, J. M. (2006) Proc. Natl. Acad. Sci. U. S. A. 103, 11724–11729.
12. Nakajima, H., Shindou, H., Hishikawa, D., Harayama, T., Ogasawara, R., Suzuki, A., Taguchi, R., and Shimizu, T. (2006) J. Biol. Chem. 281, 20140–20147.
13. Shindou, H., Hishikawa, D., Nakajima, H., Harayama, T., Ishii, S., Taguchi, R., and Shimizu, T. (2007) J. Biol. Chem. 282, 6532–6539.
14. Lands, W. E., Inoue, M., Sugiuira, Y., and Okuyama, H. (1982) J. Biol. Chem. 257, 14968–14972.
15. Bligh, E. G., and Dyer, W. J. (1959) Can. J. Biochem. Physiol. 37, 911–917.
16. Hofmann, K. (2000) Trends Biochem. Sci. 25, 111–112.
17. Coleman, R. A., and Lee, D. P. (2004) Prog. Lipid Res. 43, 134–176.
18. Beigneux, A. P., Vergnes, L., Qiao, X., Quatela, S., Davis, R., Watkins, S. M., Coleman, R. A., Walzem, R. L., Philips, M., Reue, K., and Young, S. G. (2006) J. Lipid Res. 47, 734–744.
19. Cases, S., Smith, S. J., Zheng, Y. W., Myers, H. M., Lear, S. R., Sande, E., Novak, S., Collins, C., Welch, C. B., Lusis, A. J., Erickson, S. K., and Farese, R. V., Jr. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 13018–13023.
20. Yang, Y., Cao, J., and Shi, Y. (2004) J. Biol. Chem. 279, 55866–55874.
21. Cao, J., Liu, Y., Lockwood, J., Burn, P., and Shi, Y. (2004) J. Biol. Chem. 279, 31727–31734.
22. Agarwal, A. K., Sukumaran, S., Bartz, R., Barnes, R. L., and Garg, A. (2007) J. Endocrinol. 193, 445–457.
23. Staels, B., Dallongeville, J., Auwerx, J., Schoonjans, K., Leitersdorf, E., and Fruchtart, J. C. (1998) Circulation 98, 2088–2093.
24. Lands, W. E. M., and Crawford, C. G. (1976) in The Enzymes of Biological Membranes (Martonosi, A. N., ed) Vol. 2, pp. 3–85, Plenum Press, New York.
25. Jain, S., Stanford, N., Bhagwat, N., Seiler, B., Costanzo, M., Boone, C., and Oelkers, P. (2007) J. Biol. Chem. 282, 30562–30566.
26. Riekhof, W. R., Wu, J., Gijon, M. A., Zarini, S., Murphy, R. C., and Voelker, D. R. (2007) J. Biol. Chem. 282, 28344–28352.
27. Riekhof, W. R., Wu, J., Jones, J. L., and Voelker, D. R. (2007) J. Biol. Chem. 282, 38653–38661.
28. Bengherzal, M., Roubaty, C., Veepuri, V., Knudsen, J., and Conzelmann, A. (2007) J. Biol. Chem. 282, 30845–30855.
29. Tamaki, H., Shimada, A., Ito, Y., Ohyama, M., Takase, I., Miyashita, M., Miyagawa, H., Nozaki, H., Nakayama, R., and Kumagai, H. (2007) J. Biol. Chem. 282, 34288–34298.
30. Yao, H., and Ye, J. (2008) J. Biol. Chem. 283, 849–854.