Stearoyl-CoA Desaturase 1 Deficiency Increases CTP:Choline Cytidyltransferase Translocation into the Membrane and Enhances Phosphatidylcholine Synthesis in Liver*

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Stearoyl-CoA desaturase (SCD) is the rate-limiting enzyme in monounsaturated fatty acid synthesis. Previously, we showed that Scd1 deficiency reduces liver triglyceride accumulation and considerably decreases synthesis of very low density lipoprotein and its secretion in both lean and obese mice. In the present study, we found that Scd1 deficiency significantly modulates hepatic glycerophospholipid profile. The content of phosphatidylethanolamine (PC) was increased by 40% and the activities of CTP:choline cytidyltransferase (CCT), the rate-limiting enzyme in de novo PC synthesis, and choline phosphotransferase were increased by 64 and 53%, respectively, in liver of Scd1−/− mice. In contrast, the protein level of phosphatidylethanolamine N-methyltransferase, an enzyme involved in PC synthesis via methylation of phosphatidylethanolamine, was decreased by 80% in the liver of Scd1−/− mice. Membrane translocation of CCT is required for its activation. Immunoblot analyses demonstrated that twice as much CCT was associated with plasma membrane in livers of Scd1−/− compared with wild type mice, suggesting that Scd1 mutation leads to an increase in CCT membrane affinity. The incorporation of [3H]glycerol into PC was increased by 2.5-fold in Scd1−/− primary hepatocytes compared with those of wild type mice. Furthermore, mitochondrial glycerol-3-phosphate acyltransferase activity was reduced by 42% in liver of Scd1−/− mice; however, the activities of microsomal glycerol-3-phosphate acyltransferase, diacylglycerol acyltransferase, and ethanolamine phosphotransferase were not affected by Scd1 mutation. Our study revealed that SCD1 deficiency specifically increases CCT activity by promoting its translocation into membrane and enhances PC biosynthesis in liver.

The regulation of triacylglycerol (TAG) and phospholipid (PL) synthesis plays a critical role in disorders such as obesity, diabetes, and atherosclerosis. TAG is the major energy storage form, as well as a major component of secreted chylomicra and lipoproteins, mainly very low density lipoprotein (VLDL). PLs, the primary lipid component of cellular membranes, are essential for the synthesis and secretion of bile and lipoproteins, as well as providing a reservoir of signaling molecules like lysophosphatidic acid, phosphatidic acid, diacylglycerol, and the arachidonate- and eicosapentaenoate-derived eicosanoids (1).

Stearoyl-CoA desaturase 1 (SCD1), an enzyme that catalyzes the conversion of stearoyl-CoA to oleoyl-CoA (or palmitoyl-CoA to palmitoleoyl-CoA), has recently been shown to be an important control point of lipogenesis, leading many to believe that SCD inhibition might play a protective role against obesity and the metabolic syndrome (reviewed in Refs. 2, 3). The significance of SCD in TAG synthesis has been confirmed by studies in mouse models that have a disruption in the Scd1 gene. Mice lacking Scd1 have a 50% decrease in epididymal fat pad weights (4), are deficient in hepatic TAG and cholesteryl esters, and have reduced plasma VLDL secretion (5, 6). Scd1-deficient animals are resistant to diet-induced weight gain as well as high fat and high carbohydrate diet-induced liver steatosis (4, 7) and have increased insulin sensitivity in muscle and brown adipose tissue (8, 9). Furthermore, Scd1 has been found to be a major target gene of leptin, and the antisteatotic effects of this hormone in the liver were shown to be, to a large extent, mediated by repression of Scd1 (10). Scd1 deficiency also attenuates liver steatosis in peroxisome proliferator-activated receptor-α-deficient mice (11). In liver and skeletal muscle, Scd1 deficiency was shown to increase the rate of β-oxidation through activation of the AMP-activated protein kinase pathway (12, 13) and by up-regulating genes of fatty acid oxidation, e.g. carnitine palmitoyltransferase 1, acyl-CoA oxidase, and very long chain acyl-CoA dehydrogenase (4). SCD1 deficiency also acts to suppress lipid synthesis by reducing the expression of hepatic lipogenic genes, including sterol regulatory element-binding protein-1c, fatty acid synthase, acetyl-CoA carboxylase, and glycerol-3-phosphate acyltransferase (GPAT) (4, 5, 7).

Phosphatidylethanolamine (PE) is the primary PL of eukaryotic cellular membranes and has a crucial role in structural maintenance of the lipid bilayer. In mammals, PC is also the predominant phospholipid in plasma lipoproteins and bile and plays a critical role as a second messenger in signal transduction (14). PC is the major component of intracellular transport vesicles, and the synthesis of PC is intimately involved in the
regulation of vesicle transport (15). Ongoing PC synthesis is also required for global transcriptional regulation of lipid bio-
synthesis (reviewed in Ref. 15). In liver, PC is synthesized
through two routes, the CDP-choline pathway (the Kennedy
pathway) and the methylation of phosphatidylethanolamine (PE)
catalyzed by phosphatidylethanolamine N-methyltrans-
ferase (PEMT). Studies on Pemt+/− mice have suggested that
PEMT is involved in synthesizing PC that is exported into bile (16)
and plays a significant role in synthesis and secretion of
VLDL (17, 18).

The CDP-choline pathway is a three-step process: phospha-
tration of intracellular choline and conversion of phosphocho-
dein to CDP-choline, which reacts with 1,2 diacylglycerol (DAG)
to form PC and accounts for ~70% of hepatic PC biosynthesis
(19). The rate-limiting enzyme of this pathway is CTP-choline
cytidylyltransferase (CCT), which catalyzes the conversion of
phosphocholine to CDP-choline (19, 20). Although hepatic cells
express two isoforms of CCT, α and β, CCTα is the predominant
isoform in liver (20, 21). One well characterized mode of regu-
lation of CCT activity is through translocation of the protein
from an inactive, soluble form to an active, membrane-bound
form (20, 22). The insertion of CCT into membranes has been
shown to increase by the properties of membranes, including
loose packing of lipids in the membrane (23), curvature strain
(24), and changes in the fatty acid composition of PL (25, 26).
Because oleic acid is the most abundant monounsaturated fatty
acid in mammalian PL, we hypothesized that membrane per-
turbation that might occur as a consequence of Scd1 deficiency
would affect CCT translocation into membranes, and thus SCD
might play an important role in regulating PC biosynthesis.

To understand the role of SCD in PL synthesis, we investi-
gated the major steps of glycerophospholipid biosynthesis
in liver of Scd1−/− mice. We analyzed the activities of key en-
zymes involved in PL biosynthesis in liver and measured the
incorporation of [3H]glycerol into different glycerolipid classes
in Scd1−/− primary hepatocytes. Our study revealed that
Scd1 deficiency specifically increases CCT activity by promot-
ing its translocation into the membrane and thus enhances PC
biosynthesis in liver.

EXPERIMENTAL PROCEDURES

Animals—The generation of Scd1−/− mice has been previously de-
scribed (5). Twelve-week-old purebred homozygous (Scd1−/−) and
wild type mice on SV-129 background were used. Mice were housed in
a pathogen-free facility operating on a 12-h light/12-h dark cycle
and were fed a normal nonpurified diet (5008 test diet; PMI Nutrition
International Inc., Richmond, IN). The breeding of these animals was
in accordance with the protocols approved by the Animal Care Research
Committee of the University of Wisconsin-Madison. Mice were sacri-
ficed and livers were isolated, frozen in liquid nitrogen, and stored
at −80 °C.

Materials—CDP(methyl-14C)choline, CDP[2-14C]ethanolamine, [3H]-
glycerol, [methyl-14C]phosphocholine, [ß3Hglycerol-3-phosphate, [1-14C]-
oleyl-CoA, and α-[14F]dCTP were purchased from American Radiola-
beled Chemicals Inc. (St. Louis, MO) and PerkinElmer Life Sciences.
Anti-CTTo antibodies were purchased from Santa Cruz Biotechnology
(Santa Cruz, CA), and anti-PEMT antibodies were obtained from Dr. Jean
Vance (University of Alberta, Edmonton, Canada). Lipid standards were
from Nu-Chek Prep, Inc. (Newark, DE). All other chemicals were pur-
blished either from Sigma or Fisher Scientific (Pittsburgh, PA).

Measurement of Lipids—Liver lipids were extracted by the method
of Bligh and Dyer (27) and measured as described (28, 29). Briefly, the
lipids were separated into DAG, TAG, free fatty acids, and PL by thin
layer chromatography on silica gel plates (Merck) in heptane/isopro-
pyl ether/glacial acetic acid (60/40/4, v/v/v) with authentic standards.
Additionally, PL classes were separated in chloroform/methanol/glacial
acetic acid/water (50/37.5/3.5/2, v/v/v/v). The bands corresponding to
standards were scraped off the plate and transferred to screw cap glass
vials containing methylenedecanoic acid as an internal standard.
Fatty acids were then transmethylated in the presence of 14% boron
 trifluoride in methanol. The resulting methyl esters were extracted
with hexane and analyzed by gas-liquid chromatography. Total
contents were calculated from individual fatty acid content in each fraction.

Hepatocyte Isolation and Radiolabeling with [3H]Glycerol—Hepato-
cyes from wild type and Scd1−/− mice were isolated as described (30).
Briefly, mice were euthanized by CO2 asphyxiation, and the livers
were perfused first with Buffer A (10 mM Hepes, pH 7.4, 132 mM NaCl, 20 mM
NaOH, 6.7 mM KCl, 1 mM adenosine, 1.5 mM NaHCO3, 0.1 mM EGTA, 100
mM insulin) for 10 min (7 ml/min), followed by Buffer B (Buffer A without
EGTA, supplemented with 5 mM CaCl2 and 0.2 mg/ml collagenase B)
for an additional 10 min. Hepatocytes were released into Hanks’ buffer
(137 mM NaCl, 5.36 mM KCl, 1.26 mM CaCl2, 0.81 mM MgSO4, 4.19 mM
NaHCO3, 0.33 mM NaH2PO4, 0.44 mM KH2PO4, 5 mM glucose, 9.12 mM
Hepes, pH 7.4) containing 2% BSA and washed twice with ice-
cold low-glycerol-Dulbecco’s modified Eagle’s medium containing 10% fetal
bovine serum, 1% penicillin/streptomycin (5000 units/ml), and 100
unit insulin. Resuspended hepatocytes were plated in 6-well plates at 6.2 × 106
cells/well. The cells were incubated with [3H]glycerol (4.4 μCi, 25 μM final
concentration) in 2 ml of 10% fetal bovine serum, low-glucose-Dulbecco’s
modified Eagle’s medium as described (28). After 12 h, the cells were
washed three times with phosphate-buffered saline, and lipids were ex-
tracted and fractionated by TLC as described above. Incubations were
performed in triplicate and terminated by placing the culture plates on
ice. The [3H]-labeled spots were scraped into vials and counted in a liquid
scintillation counter.

Western Blot Analysis—For the measurement of total levels of PEMT
and CCTα, liver samples were homogenized in ice-cold 50 mM HEPES
buffer (pH 7.4) containing 150 mM NaCl, protease inhibitors, and 10%
glycerol and centrifuged at 3,000 × g for 10 min. For measurement of
membrane-associated CCTα, total membranes were isolated by centrif-
ugation at 100,000 × g for 1 h as described (17). Proteins were sepa-
rated on a 3% SDS-PAGE gel, transferred and immobilized on nitrocel-
lulose membrane, and probed with antibodies against PEMT and
CCTα. The proteins were visualized using ECL (Amersham Bio-
sciences) and quantified by densitometry. Glycerophosphate-3-phosphate
dehydrogenase protein level was used as a loading control.

Glycerol-3-phosphate Acyltransferase Activity—GPT activity was
assayed with 300 μM [3Hglycerol-3-phosphate and 80 μM palmitoyl-
CoA according to Muoio et al. (31) in samples containing both micro-
osomes and mitochondria. Microsomal GPT was estimated by subtract-
ing the N-ethylmaleimide-resistant activity (mitochondrial GPT) from
the total.

CTP-Phosphocholine Cytidlyltransferase (CCT), Choline Phospho-
transferase (CPT), and Ethanolamine Phosphotransferase Activities—
Livers were homogenized in 2 ml of buffer (50 mM Tris-HCl, pH 7.5, 150
mM NaCl, 1 mM dithiothreitol, 0.1 mM phenylmethylsulfonyl fluoride,
and centrifuged at 3,000 × g for 10 min. CCT activity was measured in
homogenates (50 μg of protein) by monitoring the conversion of [methyl-
14C]phosphocholine into CDP-choline (32). CPT and ethanolamine
phosphotransferase activities were measured in isolated microsomes by
monitoring incorporation of either CDP-[methyl-14C]choline into PC or
CDP-[2-14C]ethanolamine into PE as described (21).

Diacylglycerol Acyltransferase Activity and Gene Express-
ion—DGAT activity was determined as described (33). The incorpora-
tion of [1-14C]oleyl-CoA into triglyceride was measured by adding
exogenous diacylglycerol substrate in acetone (4 mM solution). Partially
purified membranes (100 μg of protein) were used as the enzyme
source. Reactions were carried out for 5 min at 37 °C, and the products
were analyzed as described (34). Total RNA was isolated from liver
using TRIzol reagent. Dgat1 and Dgat2 gene expression was analyzed
by Northern blotting. 20 μg of total RNA were fractionated on 1%
agarose-2 M formaldehyde gels and transferred to nylon membranes.
After UV cross-linking, the membrane was hybridized with cDNA
probes labeled with [32P]dCTP by a random primer labeling kit (Pro-
mega, Madison, WI). After washing, the membranes were exposed to
x-ray film at ~80 °C, and signals were quantified by densitometry. The
probes for Dgat1 and Dgat2 were obtained from Dr. Robert Farese, Jr.
(University of California, San Francisco). pAL15 mRNA was used as an
internal control (5).

Protein Content—The protein concentration was determined with
Bio-Rad protein assay using bovine serum albumin as a standard.

Statistical Analysis—Results were analyzed using the Student’s t
 test. A difference of p < 0.05 was considered significant. Values are
presented as means ± S.D. (n = 6 mice/group).

RESULTS

PC and PE Levels Are Increased in the Liver of Scd1−/−
Mice—We have previously shown that SCD1 deficiency reduces
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liver TAG content in both lean (7) and obese (10) mice. Consistently in the present study we found a 48% reduction in TAG content in the liver of Scd1−/− compared with wild type mice (Fig. 1A). The total content of liver free fatty acids was decreased by 34% in Scd1−/− mice, whereas DAG level was not significantly different between Scd1−/− and wild type mice (Fig. 1A). The contents of PC and PE were increased by 40 and 23%, respectively, whereas the contents of phosphatidylserine (PS) and phosphatidylinositol (PI) were decreased by 16 (p = 0.071) and 27%, respectively, in the liver of Scd1−/− mice compared with wild type mice (Fig. 1B). Because the increases in PC and PE levels were greater than the decreases in PI and PS, the total content of PLs tends to be higher in the liver of Scd1−/− mice (p = 0.27) (Fig. 1A).

SCD1 Deficiency Alters Phospholipid Fatty Acid Composition in the Liver—The FA composition of phospholipids is tightly regulated and believed to stabilize membrane fluidity and functions of intrinsic membrane proteins, including receptors and transporters (35). To determine changes in fatty acid composition of hepatic glycerophospholipids due to Scd1 deficiency, PC, PE, PS, and PI were separated and their fatty acid contents were determined. The relative amounts of palmitoleic (16:1) and oleic (18:1) acids were reduced by 67 and 85% in PC, by 50 and 68% in PE, by 27 and 57% in PS, and by 64 and 65% in PI in the liver of Scd1−/− compared with wild type mice. The relative amounts of their saturated precursors, palmitic (16:0) and stearic (18:0) acids, were unchanged or slightly increased due to SCD1 deficiency in most of the PL fractions (Table I). Only in the PS fraction, the 18:0 was increased by 83% and 16:0 was reduced by 20% in liver of Scd1−/− as compared with wild type mice (Table I). SCD1 deficiency also affected the relative amounts of polyunsaturated fatty acids in hepatic PLs. Linoleic (18:2) and arachidonic (20:4) acids were increased by 24 and 32%, respectively, in PC and by 17 and 36%, respectively, in PE in the liver of Scd1−/− compared with wild type mice (Table I). In the PI fraction, SCD1 deficiency increased 20:4 by 31%, whereas the relative amounts of docosahexaenoic acid (22:6) and 18:2 were reduced by 50 and 31%, respectively (Table I). In the PS fraction, 22:6 was increased by 20%, whereas 18:2 was reduced by 23% in liver of Scd1−/− mice compared with the controls (Table I). Differences in other major fatty acid species were not significant (data not shown).

**The Incorporation of [3H]Glycerol into PC and PE Is Increased in Scd1−/− Mice**—To measure the rate of lipid synthesis, we analyzed the incorporation of [3H]glycerol into the various glycerolipids in primary hepatocytes derived from livers of Scd1−/− and wild type mice. The incorporation of [3H]glycerol into TAG was decreased by 56%, whereas its incorporation into total PLs was increased by 78% in Scd1−/− compared with wild type hepatocytes (Fig. 2A). [3H]Glycerol incorporation into PC and PE was 2.5- and 1.4-fold higher, respectively, whereas incorporation into PI and PS was reduced by 21 and 38%, respectively, in Scd1−/− hepatocytes (Fig. 2B). [3H]Glycerol incorporation into hepatic DAG was not significantly different between wild type and Scd1−/− mice (Fig. 2A).

**Mitochondrial GPAT Activity Is Decreased in the Liver of Scd1−/− Mice**—The initial and committed step in the de novo synthesis of all cellular glycerolipids is the acylation of sn-glycerol-3-phosphate to form 1-acyl-sn-glycerol-3-phosphate by GPAT. Mammalian tissues contain two GPAT isoforms, one in the outer mitochondrial membrane (mitochondrial GPAT) and the other in the endoplasmic reticulum (microsomal GPAT) (1, 36). Previously, we have shown that GPAT mRNA level is...
Hepatocytes were incubated for 12 h with \([3H]\) glycerol, and glycerol was estimated by subtracting the \(N\)-ethylmaleimide-resistant activity (membrane-associated \(\text{CCT}\)) from the total as described under “Experimental Procedures.” Data are averages \(\pm\) S.D. of three independent experiments. *, \(p < 0.05\) versus wild type mice.

**FIG. 3.** The activities of microsomal (\(\text{mcGPAT}\)) and mitochondrial (\(\text{mtGPAT}\)) glycerol-3-phosphate acyltransferase in the liver of \(\text{Scd1}^{-/-}\) and wild type mice. \(\text{GPAT}\) activity was measured using \([3H]\) glycerol-3-phosphate as a substrate, and microsomal \(\text{GPAT}\) was estimated by subtracting the \(N\)-ethylmaleimide-resistant activity (mitochondrial \(\text{GPAT}\)) from the total as described under “Experimental Procedures.” *, \(p < 0.05\) versus wild type mice.

**FIG. 2.** The effect of \(\text{SCD1}\) deficiency on incorporation of \([3H]\) glycerol into glycerolipids. Incorporation of \([3H]\) glycerol into triglyceride (TAG), phospholipids (PL), and 1,2 diacylglycerol (DAG) (A) and into individual PL subclasses, phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), and phosphatidylinositol (PI) (B) in primary hepatocytes isolated from \(\text{Scd1}^{-/-}\) and wild type mice were incubated for 12 h with \([3H]\) glycerol, and glycerol incorporation was calculated as described under “Experimental Procedures.” Data are averages \(\pm\) S.D. of three independent experiments. *, \(p < 0.05\) versus wild type mice.

significantly reduced in the liver of \(\text{Scd1}^{-/-}\) mice (4). In the present study we found that mitochondrial \(\text{Gpat}\) activity is 42% lower in the liver of \(\text{Scd1}^{-/-}\), whereas the activity of microsomal \(\text{Gpat}\) does not differ between wild type and \(\text{Scd1}^{-/-}\) mice (Fig. 3).

**CDP-Choline Pathway of PC Synthesis Is Up-regulated, whereas PEMT Pathway Is Down-regulated in the Liver of \(\text{Scd1}^{-/-}\) Mice—**We hypothesized that the increase in PC content and higher incorporation of \([3H]\) glycerol into PC in \(\text{Scd1}^{-/-}\) liver results from an increased rate of PC biosynthesis. There are two pathways of PC biosynthesis in liver, the CDP-choline pathway and PE methylation by PEMT (19). CCT, the rate-limiting enzyme in the CDP-choline pathway, undergoes post-transcriptional regulation by its translocation from an inactive, soluble form to an active, membrane-bound form (20). To determine whether disruption of the \(\text{Scd1}\) gene resulted in increased CCT protein level and/or enhanced its membrane association, immunoblot analysis was performed on liver homogenates and isolated membranes. CCT\(_{\alpha}\), the predominant isoform of CCT in liver (21), was analyzed. The amount of membrane-associated CCT\(_{\alpha}\) was increased by 1.6-fold, whereas the total protein level of CCT\(_{\alpha}\) measured in homogenates was reduced by 20% in the liver of \(\text{Scd1}^{-/-}\) compared with wild type mice (Fig. 4A). Glyceraldehyde-3-phosphate dehydrogenase used as a control was not different in homogenates and membranes. The analysis of CCT subcellular distribution revealed that the liver from \(\text{Scd1}^{-/-}\) mice had a \(>2\)-fold increase in the levels of CCT associated with total membranes than did liver of wild type mice (Fig. 4B). Consequently, the activity of CCT was 62% higher in liver of \(\text{Scd1}^{-/-}\) compared with wild type mice (Fig. 4C).

The activity of CPT, an enzyme catalyzing the final step in PC synthesis via the CDP-choline pathway, was increased by 49% in liver of \(\text{Scd1}^{-/-}\) mice (Fig. 5A). Interestingly, the protein level of PEMT, an enzyme involved in PC synthesis via the PE methylation pathway, was reduced by 80% in liver of \(\text{Scd1}^{-/-}\) compared with wild type mice (Fig. 5B). The activity of ethanolamine phosphotransferase, the enzyme involved in de novo synthesis of PE, was not affected by \(\text{Scd1}\) deficiency (Fig. 5C). Thus, the increase in PE accumulation in the liver of \(\text{Scd1}^{-/-}\) mice (Fig. 1B) appears to be because of its reduced catabolism rather than increased biosynthesis.

DAG, a precursor to PC and PE, is also the substrate for synthesis of TAG, and DAG availability acts as a branchpoint affecting the proportional synthesis of these three lipid fractions (1, 36). Therefore, we also analyzed the activity and gene expression of \(\text{Dgat}\), an enzyme catalyzing the final, committed step for TAG synthesis. \(\text{Dgat}\) mRNA level and its activity were not affected by \(\text{Scd1}\) deficiency (Fig. 6).

**DISCUSSION**

SCD, the rate-limiting enzyme in monounsaturated fatty acid synthesis, has recently been shown to be the critical control point regulating hepatic lipogenesis. \(\text{Scd1}^{-/-}\) mice accumu-
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mulate less TAG in liver and other tissues and have reduced plasma VLDL levels (5, 6, 10). In the present study, we have shown that SCD1 deficiency significantly modulates the hepatic profile of PLs and changes the rate of their biosynthesis.

Analysis of various PL classes revealed that the major PL affected by Scd1 mutation is PC. Increased activities of CCT (Fig. 4C) and CPT (Fig. 5A) and increased [3H]glycerol incorporation into PC (Fig. 2A) indicate increased de novo synthesis of PC in the liver of Scd1−/− mice. CCT catalyzes the rate-limiting step of the CDP-choline pathway for PC synthesis (19, 20), and translocation of this enzyme into cell membranes has been shown to be critical for its activation (20). The data presented herein showed that SCD1 deficiency increased CCT activity by enhancing its membrane translocation, because twice as much CCTα was associated with membrane in livers from Scd1−/− compared with wild type mice (Fig. 4B) even though SCD1 deficiency reduced the total content of hepatic CCTα (Fig. 4A).

The membrane affinity of CCT is regulated primarily through compositional changes in membrane lipid and through physical properties of the membrane (20, 37). Our study has shown that SCD1 deficiency leads to decreased content of monounsaturated FA and increased content of polyunsaturated FA (mainly 20:4) in liver PLs, whereas the relative amount of saturated FA in hepatic PLs was not significantly altered due to Scd1 mutation (Table I). The changes in PL fatty acid composition in liver of Scd1−/− mice might be associated with reduced activity of mitochondrial GPAT (Fig. 3), because mitochondrial GPAT plays a key role in establishing initial asymmetric distribution of saturated and unsaturated FA in PL (1, 36) and its deficiency was shown to decrease 16:0 and increase 20:4 contents in hepatic PC and PE (38). Given that insertion of CCT into membrane was shown to be enhanced by polyunsaturated fatty acids (39), increased unsaturated PE level (20, 37), and by disadvantaged acyl chains of PL (25, 26) in vitro, alterations in PL fatty acid composition and its membrane organization due to Scd1 mutation might directly promote CCT membrane binding. Indeed, recent confocal microscopy studies have revealed that Scd expression is a very potent regulator of membrane domain structure (40). Overexpression of Scd in Chinese hamster ovary cells led to an increased content of monounsaturated FA in plasma membrane at the expense of saturated FA and consequently decreased Triton X-100-resistant domains in the plasma membrane (40). Our findings provide the first evidence to suggest that CCT membrane translocation is affected by membrane lipid composition in vivo and might be regulated by changes in SCD activity. It is also possible that the membrane binding of CCT in the Scd1−/− mice was caused by changes in the composition of the free fatty acyl-CoA pool due to Scd1 deficiency. The observation that SCD regulates PC biosynthesis and substantially changes membrane organization raises the possibility that many different cellular functions that are attributed to membrane properties, such as activities of nitric-oxide synthase (41) and protein kinase C (42), as well as vesicle transport (15) and functions of intrinsic membrane receptors (43), could be altered by changes in Scd expression.

CPT activity is mainly regulated by availability of its substrates, DAG and CDP-choline (19). Because CPT catalyzes CDP-choline synthesis, increased CPT activity due to Scd1 mutation leads to activation of CPT in liver of Scd1−/− mice (Fig. 5A). Because DGAT and CPT draw from the same pool of DAG for synthesis of TAG and PC, respectively, CPT activity might indirectly determine the rate of TAG synthesis as well (1, 44). Although DAG content (Fig. 1A) and [3H]glycerol incorporation into DAG (Fig. 2A) were not affected by SCD1 deficiency, the rate of TAG synthesis was decreased by 56% (Fig. 2A) despite normal DGAT activity (Fig. 6B) in the liver of Scd1−/− mice. The lower incorporation of glycerol into TAG in Scd1-deficient hepatocytes was accompanied by a 2.5-fold increase in glycerol incorporation into PC (Fig. 2B). Given that the affinity of CPT for DAG is severalfold higher than that of DGAT (1, 44), increased CPT activity in the liver of Scd1−/− mice might result in suppression of DAG conversion to TAG. Increased rate of PC synthesis has previously been shown to inhibit TAG synthesis through diversion of DAG in rat hepatocytes (44) and yeast (45). Taken together, these data suggest that increased PC biosynthesis via the CDP-choline pathway might be par-
liver of partially responsible for the decreased rate of TAG synthesis in liver of Scd1−/− mice were shown previously (2, 4, 12). * indicates steps activated by Scd1 deficiency; † indicates steps inhibited by Scd1 deficiency. Step 1, glycerol-3-phosphate acyltransferase (GPAT); step 2, monoyl-3-phosphate acyltransferase; step 3, phosphatidic acid phosphatase; step 4, diacylglycerol acyltransferase (DGAT); step 5, ethanolamine phosphotransferase; step 6, choline phosphotransferase (CPT); step 7, phosphatidylcholine N-methyltransferase (PEMT); step 8, CTP-choline cytidylyltransferase (CCT).

FIG. 7. Schematic representation of the effects of Scd1 gene deletion on glycerolipid synthesis in liver. Decreased de novo FA synthesis and increased rate of β-oxidation in Scd1−/− mice were shown previously (2, 4, 12). * indicates steps activated by Scd1 deficiency; † indicates steps inhibited by Scd1 deficiency. 1, glycerol-3-phosphate acyltransferase (GPAT); step 2, monoyl-3-phosphate acyltransferase; step 3, phosphatidic acid phosphatase; step 4, diacylglycerol acyltransferase (DGAT); step 5, ethanolamine phosphotransferase; step 6, choline phosphotransferase (CPT); step 7, phosphatidylcholine N-methyltransferase (PEMT); step 8, CTP-choline cytidylyltransferase (CCT).

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| EXPERIMENTAL PROCEDURE | RESULTS |
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velope pathway is down-regulated in Scd1−/− mice, leading to accumulation of PE. Increased PC synthesis via the CDP-choline pathway in the liver of Scd1−/− mice was coupled with decreased synthesis of TAG, even though DGAT gene expression and activity were not significantly different. Because DGAT and CPT draw from the same pool of DAG, the data suggest that, in addition to increased β-oxidation (4, 12) and decreased lipogenesis (2), increased PC biosynthesis could be responsible for reduced TAG synthesis in Scd1−/− mice.
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