Cholestasis and hypercholesterolemia in SCD1-deficient mice fed a low-fat, high-carbohydrate diet[S]

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Abstract **Stearoyl-coenzyme A desaturase 1-deficient ($SCD1^{-/-}$) mice have impaired MUFA synthesis. When maintained on a very low-fat (VLF) diet, $SCD1^{-/-}$ mice developed severe hypercholesterolemia, characterized by an increase in apolipoprotein B (apoB)-containing lipoproteins and the appearance of lipoprotein X. The rate of LDL clearance was decreased in VLF $SCD1^{-/-}$ mice relative to VLF $SCD1^{+/+}$ mice, indicating that reduced apoB-containing lipoprotein clearance contributed to the hypercholesterolemia. Additionally, HDL-cholesterol was dramatically reduced in these mice. The presence of increased plasma bile acids, bilirubin, and aminotransferases in the VLF $SCD1^{-/-}$ mice is indicative of cholestasis. Supplementation of the VLF diet with MUFA- and PUFA-rich canola oil, but not saturated fat-rich hydrogenated coconut oil, prevented these plasma phenotypes. However, dietary oleate was not as effective as canola oil in reducing LDL-cholesterol, signifying a role for dietary PUFA deficiency in the development of this phenotype. These results indicate that the lack of SCD1 results in an increased requirement for dietary unsaturated fat to compensate for impaired MUFA synthesis and to prevent hypercholesterolemia and hepatic dysfunction. Therefore, endogenous MUFA synthesis is essential during dietary unsaturated fat insufficiency and influences the dietary requirement of PUFA.**—Flowers, M. T., A. K. Groen, A. Tebon Oler, M. P. Keller, Y. Choi, K. L. Schueler, O. C. Richards, H. Lan, M. Miyazaki, F. Kuipers, C. M. Kendziorski, J. M. Ntambi, and A. D. Attie. **Cholestasis and hypercholesterolemia in SCD1-deficient mice fed a low-fat, high-carbohydrate diet.** ***J. Lipid Res.*** **2006.** 47: **2668–2680.**

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Although dietary saturated fat is generally regarded as hypercholesterolemic, the roles of de novo synthesized saturated fatty acids and MUFAs in the regulation of plasma cholesterol levels have not been thoroughly investigated (1–3). Stearoyl-coenzyme A desaturase 1 (SCD1) is a central enzyme in lipid metabolism that catalyzes the desaturation of palmitoyl-CoA and stearoyl-CoA to palmitoleoyl-CoA and oleoyl-CoA, respectively (4). The dietary requirement for MUFA, if any, is confounded by the capacity for endogenous MUFA synthesis. Furthermore, under certain metabolic conditions, both dietary and endogenously synthesized MUFAs have been hypothesized to influence the dietary requirement for certain PUFAs (5). SCD1-deficient ($SCD1^{-/-}$) mice have impaired MUFA synthesis and are a useful animal model to study the influence of de novo synthesized MUFAs on lipoprotein metabolism and the requirement for dietary unsaturated fat.

Four SCD isoforms (SCD1–SCD4), encoded by different genes, have been identified in the mouse (4). However, SCD1 is the most abundant isoform expressed in lipogenic tissues, such as liver and adipose tissue (4). SCD1 gene expression is regulated by both hormones (insulin, leptin) and by a variety of nutrients such as cholesterol, glucose, fructose, and PUFAs (4, 6–11). This transcriptional regulation involves several transcription factors, including carbohydrate response element binding protein, sterol-regulatory element binding protein-1c (SREBP-1c), and liver X receptor, highlighting the importance of SCD1 activity for the adaptation to different metabolic demands (12, 13). SCD1 activity influences the fatty acid composi-

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Abbreviations: apoB, apolipoprotein B; FPLC, fast-protein liquid chromatography; IDL, intermediate density lipoprotein; SCD1, stearoyl-coenzyme A desaturase 1; SREBP-1c, sterol-regulatory element binding protein-1c; VLF, very low-fat.

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[S] The online version of this article (available at http://www.jlr.org) contains supplemental data in the form of 4 tables and 2 figures.

tion of cellular and circulating triglycerides, cholesteryl esters, phospholipids, and free fatty acids (14, 15). SCD1 activity is also essential for the normal posttranslational processing of SREBP-1c and the upregulation of SREBP-1c target genes in response to a fat-free, high-carbohydrate diet (10, 15). This indicates that dietary induction of SCD1 not only affects fatty acid composition but also augments the expression of other lipogenic genes.

Previous studies using SCD1$^{-/-}$ mice have identified several interesting metabolic phenotypes, including resistance to diet-induced obesity, decreased lipogenesis, increased insulin sensitivity, fatty acid oxidation, and basal thermogenesis (4). It is important to note that most of these phenotypes, with the exception of impaired lipogenesis, have been identified using chow or high-fat diets containing ample unsaturated fat. Short-term feeding of a semipurified low-fat, high-carbohydrate diet has been shown to increase hepatic SCD1 expression, MUFA production, and triglyceride synthesis in wild-type (SCD1$^{+/+}$), but not SCD1$^{-/-}$, mice (10, 15). However, the chronic effect of this feeding regimen in SCD1$^{-/-}$ mice has not been adequately explored.

We report here that SCD1$^{-/-}$ mice fed a very low-fat (VLF) diet for 10 days develop severe hypercholesterolemia and hepatic dysfunction. These phenotypes are prevented by a combination of dietary MUFAs and PUFAs, indicating that lack of SCD1 results in an increased requirement for dietary unsaturated fat to compensate for impaired MUFA synthesis. These results also highlight the intricate balance between dietary and de novo unsaturated fat and indicate that upregulation of SCD1 during dietary unsaturated fat insufficiency may be a protective mechanism against the pathologies described in this study.

MATERIALS AND METHODS

Animals

SV129 SCD1$^{-/-}$ mice were backcrossed to C57BL/6 mice for at least five generations. Genetic purity was ascertained by marker-assisted genotyping. Both breeder mice and offspring were fed a standard rodent chow diet (PMI 5008 Formulab; PMI Nutrition International, Richmond, IN) and housed in a controlled environment with 12 h light and dark cycles. Male SCD1$^{-/-}$ mice were crossed with female heterozygous (SCD1$^{+/-}$) mice to produce SCD1$^{+/-}$ and SCD1$^{-/-}$ littermates. SCD1$^{+/+}$ mice were produced by crossing male and female C57BL/6 SCD1$^{+/+}$ mice. Protocols for animal experiments were approved by the Animal Care Research Committee of the University of Wisconsin-Madison. For the experiments described, male or female animals 7–12 weeks old were used. For diet studies, mice were maintained on chow until 10 weeks of age before dietary intervention.

Diet composition

Diets TD03045 (VLF), TD03138 (VLF-coconut), and TD04422 (VLF-canola) were obtained from Harlan Teklad (Madison, WI). The VLF-oleate diet was prepared using TD99252 basal mix supplemented with corn oil and triolein (99% pure; Sigma). These semipurified diets all contain sucrose (50%, w/w) as the primary carbohydrate source and corn oil (1%, w/w) as the invariant basal fat source. VLF-coconut, VLF-canola, and VLF-oleate diets differ primarily in the fat source used to supplement the VLF diet. The starch content of the fat-supplemented VLF-coconut, VLF-canola, and VLF-oleate diets was reduced to compensate for the added fat. See Supplementary Table I for more explicit diet composition information. Fat composition and nutrient content are expressed as percentage of weight of diet and were provided by the manufacturer.

Plasma and bile lipid analysis

Plasma from animals fasted for 4 h in the early light cycle was used for lipid analysis. Animals were euthanized by CO_2 asphyxiation, and blood was collected into EDTA (4 mM final concentration). Fresh plasma (100 μl) was fractionated on a Superose 6HR 10/30 fast-protein liquid chromatography (FPLC) column (Amersham Biosciences), and 500 μl fractions were collected. FPLC fractions or whole plasma were assayed for total cholesterol and triglyceride using the Infinity Cholesterol reagent and Infinity Triglyceride reagent (Thermo Electron Corp.). Plasma concentrations of free cholesterol were determined using the Free Cholesterol C kit (Wako, Richmond, VA). Plasma cholesteryl ester levels were calculated as the difference between total and free cholesterol. Plasma phospholipid levels were determined by the method of Rouser, Fkeischer, and Yamamoto (16). Total and direct bilirubin were measured using Infinity Total Bilirubin and Infinity Direct Bilirubin kits (Thermo Electron Corp.). Plasma bile acids were measured by a colorimetric enzyme cycling assay (Bioquant). Bile was collected by aspiration of the gallbladder or upon gallbladder cannulation and analyzed for cholesterol and phospholipids as described (17). Briefly, biliary cholesterol was measured by an enzymatic assay, using the cholesterol oxidase reaction coupled to fluorimetric determination of hydrogen peroxide. Choline-containing biliary phospholipids were determined by a similar approach using the oxidation of choline to generate hydrogen peroxide. The choline was liberated by enzymatic treatment with phospholipase D and choline oxidase. Total bile salt concentrations were measured using the 3α-hydroxysteroid dehydrogenase method (18). Biliary bile acid and phospholipid fatty acid compositions were determined by capillary gas chromatography after their conversion into methyl ester-trimethylsilyl ether derivatives or methyl esters, respectively, exactly as described (19, 20). To determine bile flow, the gallbladder of the mice was cannulated under isoflurane anesthesia after distal ligation of the common bile duct. Bile was collected in 10 min fractions, and flow was determined gravimetrically.

Hepatic lipid analysis

For hepatic lipid analysis, 100 mg of liver was homogenized, and an aliquot representing ~5 mg of tissue was lipid-extracted (21), dried under N_2, and resuspended in 10 μl of $CHCl_3$. After the addition of 90 μl of 10% Triton X-100 in isopropanol, the sample was vortexed for 10 s and assayed with Wako Triglyceride, Free Cholesterol C, or Cholesterol CII reagent according to the manufacturer's instructions, except that samples were vortexed to clarity before absorbance measurement. Hepatic cholesteryl ester levels were calculated as the difference between total and free cholesterol. Protein determination was by the method of Lowry et al. (22), and lipid data are expressed as μg lipid/mg protein. Plasma glucose and liver glycogen were measured as described previously (10).

Plasma lipoprotein analysis

Fresh plasma or FPLC-fractionated plasma lipoproteins were precipitated using PMH-L-Liposorb (Calbiochem) and resolved by SDS-PAGE. Gels were stained with SYPRO Ruby (Molecular

Probes), and proteins were imaged by Fluorimager quantitation (ImageQuant; Molecular Dynamics). For detection of lipoprotein X, fresh plasma was stained with Sudan black (0.02%, v/v) and subjected to agarose gel electrophoresis as described elsewhere (17, 23), except for the substitution of a Tris-Tricine buffer for barbital (24). Hepatic triglyceride secretion rates were determined by measuring the temporal increase in plasma triglyceride after inhibition of lipoprotein lipase by intraperitoneal injection of poloxamer 407 (P-407; BASF Corp.), as described by Millar et al. (25). Briefly, mice were fasted for 4 h and then administered P-407 at 2 g/kg. Blood was collected by retro-orbital puncture immediately before injection and at 30, 60, and 120 min after injection. The triglyceride and cholesterol secretion rates were calculated as the difference in plasma triglyceride and cholesterol over the 2 h time interval and are expressed as μmol/kg/h.

LDL clearance

LDL (d = 1.019–1.063 g/ml) was isolated from LDL receptor-deficient donors by density gradient ultracentrifugation, dialyzed to PBS, and labeled with [^{125}I]NaI (Perkin-Elmer Life Sciences). [^{125}I]NaI was added to IODO-Gen precoated tubes (Pierce Biotechnology), allowed to activate for 6 min, and then transferred to a new IODO-Gen tube containing LDL and allowed to react for 15 min according to the manufacturer's instructions. Unincorporated ^{125}I was removed by dialysis to PBS, and the specific activity of ^{125}I-LDL was determined by the amount of ^{125}I precipitable by 10% TCA. A typical ^{125}I-LDL preparation yielded >90% TCA-precipitable counts, and the major protein components of the isolated LDL were apolipoprotein B-100 (apoB-100), apoB-48, and apoE. After a 4 h fast, mice were delivered a tail vein injection of ^{125}I-LDL and bled by retro-orbital puncture at the indicated time. TCA-precipitable counts remaining in the plasma were measured by liquid scintillation counting and normalized to the amount of radioactivity present at 2 min after injection.

LCAT activity

Plasma LCAT activity was determined using a proteoliposome substrate as described (26) containing [4-^{14}C]cholesterol (American Radiolabeled Chemicals), human apoA-I (Calbiochem), and egg phosphatidylcholine (Sigma). Proteoliposome substrate was incubated with 15 μl of test plasma at 37°C for 1 h and immediately lipid-extracted. The ratio of [^{14}C]cholesteryl ester to [^{14}C]free cholesterol was determined by TLC and subsequent quantitation using an Instant Imager (Packard Instrument Co.). Plasma LCAT cholesterol esterification rates are expressed as nmol/ml/h.

Collection and preprocessing of gene expression data

Total RNA was extracted from liver using RNAzol reagent (Tel-Test) and purified with RNeasy mini columns (Qiagen) before being subjected to microarray studies using Affymetrix Mouse 430 2.0 microarray chips. A total of 20 arrays were used to study female SCD1$^{+/+}$ and SCD1$^{-/-}$ mice on chow or the VLF diet (2 strains × 2 diets × 5 replicates = 20 arrays). The complete MIAME formatted data set is deposited as Gene Expression Omnibus accession GSE3889, which may be accessed at http://www.ncbi.nlm.nih.gov/geo. Expression measurements were background-adjusted, normalized, and summarized with RMA (27) implemented in R, a publicly available statistical analysis environment (28).

Statistical methods

Comparison for each of the clinical variables was done by one-way ANOVA, and two-way ANOVA was used for comparisons containing both genotype and diet variables. Variables with unequal variance were log-transformed before analysis. Significant interactions were analyzed with Bonferroni posttests, and Bonferroni corrected P values < 0.05 were considered significant. The expression levels of genes involved in lipoprotein, bile, and cholesterol metabolism were compared by two-way ANOVA and further by within-genotype and within-diet t-tests. The false-discovery rate was estimated by calculating q-values (29) based on the P values from the t-tests. This calculation was done twice to yield two estimated false-discovery rates, once within genotype and a second time within diet.

RESULTS

A VLF diet causes an abnormal plasma lipid profile in SCD1$^{-/-}$ mice

We first studied plasma lipid profiles in animals maintained on either a chow diet or a VLF diet for 10 days. Surprisingly, total plasma cholesterol levels in the VLF SCD1$^{-/-}$ mice increased by ~250% relative to chow SCD1$^{-/-}$ mice (**Table 1**). In contrast, plasma cholesterol levels in VLF SCD1$^{+/-}$ mice were increased by only ~20% relative to chow SCD1$^{+/-}$ mice (Table 1). No significant increase was elicited by the VLF diet in SCD1$^{+/+}$ mice. In chow-fed animals, SCD1 deficiency decreased plasma cholesterol levels, primarily as a result of differences in the HDL fraction (Table 1, **Fig. 1A**). The absence of this ab-

TABLE 1. Plasma cholesterol and triglyceride concentrations

Animal and Measurement	Male, Chow	Male, VLF	Female, Chow	Female, VLF
Triglyceride (mg/dl)				
SCD1$^{+/+}$	80.0 ± 15.4 (12)	93.7 ± 24.5 (6)	55.9 ± 12.2 (11)	94.7 ± 6.0^{a} (5)
SCD1$^{+/-}$	85.7 ± 14.3 (11)	92.1 ± 15.8 (8)	56.1 ± 7.6 (8)	90.3 ± 21.5 (4)
SCD1$^{-/-}$	78.0 ± 23.3 (7)	50.0 ± 15.3^{b,c} (5)	60.7 ± 12.5 (14)	53.6 ± 15.9^{b,c} (9)
Cholesterol (mg/dl)				
SCD1$^{+/+}$	99.1 ± 13.3 (12)	108.6 ± 8.7 (5)	87.4 ± 12.2 (17)	89.0 ± 10.7 (16)
SCD1$^{+/-}$	90.9 ± 6.8 (14)	108.4 ± 13.3^{a} (16)	76.1 ± 8.0 (7)	90.0 ± 7.3 (7)
SCD1$^{-/-}$	81.2 ± 7.6^{b} (10)	200.2 ± 21.1^{a,b,c}(11)	69.8 ± 8.9^{b} (21)	162.7 ± 29.8^{a,b,c} (18)

SCD1, stearoyl-coenzyme A desaturase 1; VLF, very low-fat. Plasma from male and female SCD1$^{+/+}$, SCD1$^{+/-}$, and SCD1$^{-/-}$ mice maintained on chow or the VLF diet were analyzed for total plasma cholesterol and triglyceride. Data represent means ± SD. The number of animals in each subgroup is indicated in parentheses.

[a] $P < 0.05$, diet effect (same genotype).

[b] $P < 0.05$, genotype effect (same diet) versus SCD1$^{+/+}$.

[c] $P < 0.05$, genotype effect (same diet) versus SCD1$^{+/-}$.

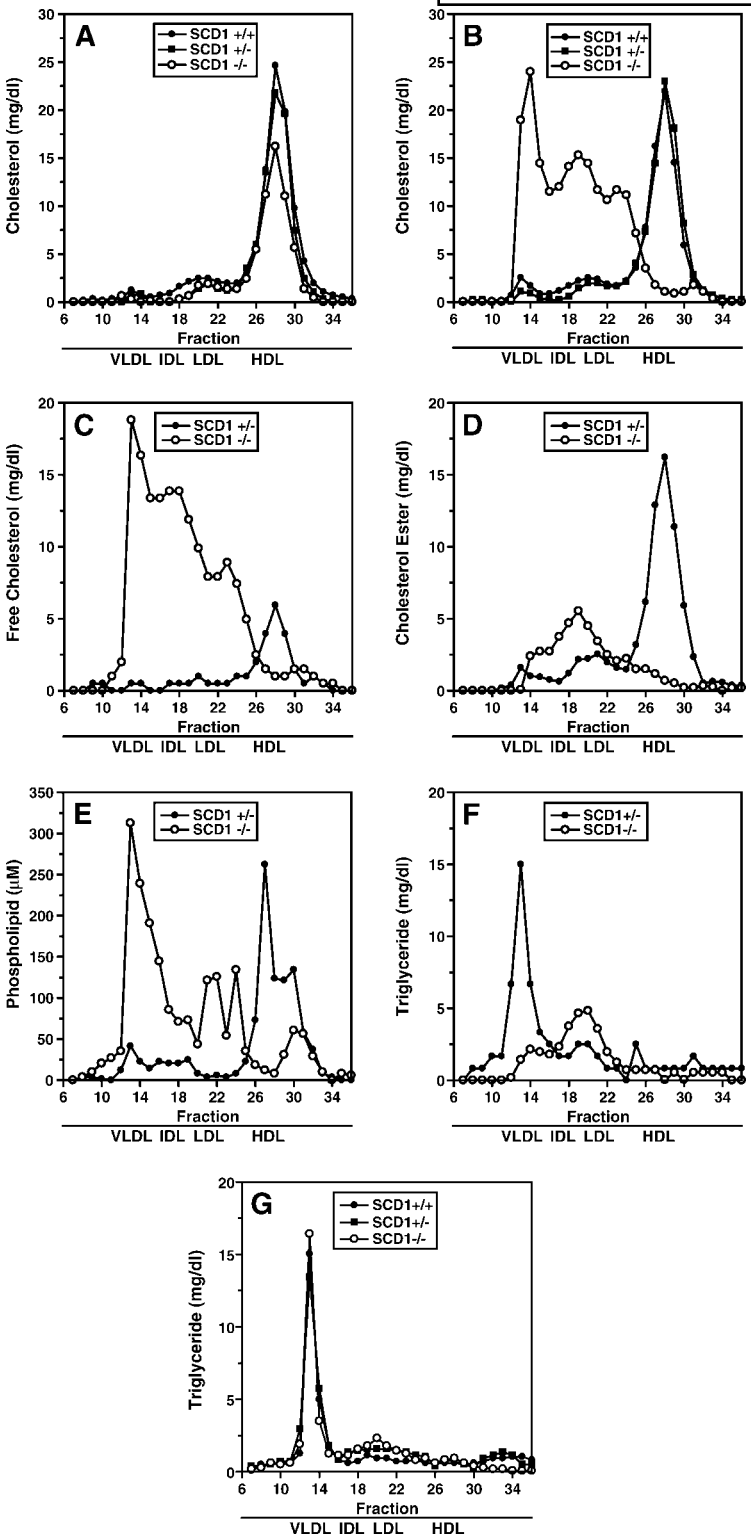

Fig. 1. Fast-protein liquid chromatography (FPLC) lipid analysis in chow-fed and very low-fat (VLF)-fed stearoyl-coenzyme A desaturase 1 (SCD1) mice. Plasma from $SCD1^{+/+}$, $SCD1^{+/-}$, and $SCD1^{-/-}$ animals maintained on chow or the VLF diet was fractionated by FPLC. Fractions 7–36 were analyzed for total cholesterol content. Profiles of male mice on the chow diet (A) and male mice on the VLF diet (B) are shown. Profiles of female mice show a similar pattern as those of male mice and are not shown. Plasma from male $SCD1^{+/-}$ and $SCD1^{-/-}$ mice fed the VLF diet was subjected to FPLC fractionation, and fractions 7–36 were analyzed for free cholesterol (C), cholesteryl ester (D), phospholipid (E), and triglyceride (F). FPLC triglycerides for chow-fed mice are shown in G. The location of fractions corresponding to VLDL, intermediate density lipoprotein (IDL), LDL, and HDL are indicated.

normal phenotype in VLF $SCD1^{+/-}$ mice indicates that $SCD1^{+/-}$ mice retained sufficient SCD activity to maintain normal cholesterol homeostasis. Therefore, the combination of complete SCD1 deficiency and VLF diet treatment is required to elicit hypercholesterolemia.

FPLC analysis of plasma lipids in the VLF $SCD1^{-/-}$ mice revealed a severe loss of HDL-cholesterol and a marked increase in the cholesterol content of the VLDL, intermediate density lipoprotein (IDL), and LDL fractions (Fig. 1B). This abnormal pattern of cholesterol distribution was not observed in $SCD1^{+/+}$ or $SCD1^{+/-}$ mice fed the VLF diet. The majority of the increased plasma cholesterol in the VLF $SCD1^{-/-}$ mice was free cholesterol (**Table 2**, Fig. 1C). Although total plasma cholesteryl ester levels were not increased by the VLF diet in $SCD1^{+/+}$ or $SCD1^{-/-}$ mice (Table 2), the VLF diet in $SCD1^{-/-}$ mice caused

TABLE 2. Plasma, liver, and biliary metabolite concentrations

Lipid	Chow, SCD1$^{+/+}$	Chow, SCD1$^{-/-}$	VLF, SCD1$^{+/+}$	VLF, SCD1$^{-/-}$
Plasma				
Free cholesterol (mg/dl)	28.6 ± 3.5	23.4 ± 2.5	26.7 ± 0.9	136.7 ± 31.7^{a,b}
Cholesteryl ester (mg/dl)	69.1 ± 12.7	53.5 ± 3.2	68.2 ± 6.3	54.4 ± 8.2
Phospholipid (mM)	2.07 ± 0.31	1.70 ± 0.14	2.12 ± 0.17	4.06 ± 0.66^{a,b}
Glucose (mg/dl)	136.1 ± 9.9	137.1 ± 19.6	159.8 ± 10.7	51.1 ± 7.6^{a,b}
Alkaline phosphatase (U/l)	212.0 ± 22.5	105.7 ± 5.1	157.9 ± 23.6	295.3 ± 126.6^{b}
Alanine aminotransferase (U/l)	29.0 ± 4.0	56.3 ± 27.6	23.4 ± 3.9	3532.0 ± 3890.3^{a,b}
Aspartate aminotransferase (U/l)	199.7 ± 69.0	210.3 ± 33.6	195.9 ± 54.7	3813.0 ± 3756.4^{a,b}
Liver				
Triglyceride (μg/mg protein)	56.8 ± 11.4	38.6 ± 5.50	136.8 ± 44.4^{b}	24.4 ± 6.84^{a}
Free cholesterol (μg/mg protein)	7.53 ± 0.43	6.41 ± 0.41	6.52 ± 1.17	13.55 ± 2.35^{a,b}
Cholesteryl ester (μg/mg protein)	1.84 ± 0.78	0.96 ± 0.25	8.17 ± 0.62^{b}	5.36 ± 0.7^{a,b}
Glycogen (mg/g tissue)	25.4 ± 10.5	19.1 ± 5.1	41.5 ± 9.4	6.6 ± 1.6^{a,b}
Biliary lipids				
Bile acids (mM)	132.8 ± 24.0	128.6 ± 19.6	147.9 ± 36.9	145.6 ± 67.1
Phospholipid (mM)	27.6 ± 7.6	32.1 ± 2.8	15.6 ± 9.4	7.5 ± 5.5^{b}
Cholesterol (mM)	3.49 ± 0.61	3.99 ± 0.22	2.26 ± 0.82	1.68 ± 0.83^{b}

Female SCD1$^{+/+}$ and SCD1$^{-/-}$ mice maintained on chow or the VLF diet were analyzed for plasma free cholesterol, cholesteryl ester, phospholipids, glucose, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, liver triglyceride, cholesteryl ester, free cholesterol, glycogen, and biliary lipids. Data represent means ± SD for four to five animals per group.

[a] $P < 0.05$, genotype effect (same diet).

[b] $P < 0.05$, diet effect (same genotype).

a redistribution of cholesteryl ester away from HDL into fractions corresponding to IDL and LDL (Fig. 1D). The plasma phospholipid in the VLF SCD1$^{-/-}$ mice was also redistributed away from HDL and into the VLDL, IDL, and LDL fractions (Table 2, Fig. 1E).

Analysis of the apolipoprotein composition of plasma lipoproteins revealed increased plasma apoB and apoE, but decreased apoA-I, in the VLF SCD1$^{-/-}$ mice (**Fig. 2A, B**). The increases in plasma apoB and apoE occurred in both large and small particles (Fig. 2C). The free cholesterol- and phospholipid-enriched VLDL-sized fraction in the VLF SCD1$^{-/-}$ mice is reminiscent of lipoprotein X, an abnormal VLDL-sized lipoprotein that is largely devoid of apolipoproteins and triglycerides (17, 23). Agarose gel electrophoresis of plasma from VLF SCD1$^{-/-}$ mice indicated the presence of a slow-mobility band consistent with the mobility of lipoprotein X (Fig. 2D) (17, 23). This indicates that the hypercholesterolemia in the VLF SCD1$^{-/-}$ mice is at least partly attributable to a metabolic disturbance leading to lipoprotein X formation.

Impaired LDL clearance contributes to hypercholesterolemia in the VLF SCD1$^{-/-}$ mice

To assess the rate of LDL clearance, SCD1$^{+/+}$ and SCD1$^{-/-}$ mice maintained on chow or the VLF diet were injected with ^{125}I-LDL, and the clearance was monitored over a 21 h time course. We observed no difference in the rate of LDL clearance between SCD1$^{+/+}$ and SCD1$^{-/-}$ mice maintained on a chow diet (**Fig. 3A**). However, the rate of LDL clearance was reduced by 50% in the VLF SCD1$^{-/-}$ mice (0.33 vs. 0.68 pools/h; Fig. 3B), indicating that the increased apoB in the plasma of VLF SCD1$^{-/-}$ mice may be attributed to a slower clearance rate of apoB-containing lipoproteins. The hepatic mRNA level of the LDL receptor (*Ldlr*) was also reduced by ~50% in the VLF SCD1$^{-/-}$ mice relative to the other groups (**Table 3**). Thus, the hypercholesterolemia in the VLF SCD1$^{-/-}$ mice is caused by at least two mechanisms: lipoprotein X formation and decreased LDL clearance.

In contrast to other previously reported mouse strains (10, 14, 30, 31), SCD1 deficiency in chow-fed C57BL/6 mice did not affect fasting plasma triglyceride levels (Table 1, Fig. 1G). However, loss of SCD1 decreased plasma triglyceride levels in the mice on the VLF diet by ~40% relative to SCD1$^{+/+}$ and SCD1$^{+/-}$ mice (Table 1, Fig. 1F). To estimate the rate of VLDL production, we measured the rate of hepatic triglyceride secretion in SCD1$^{+/+}$ and SCD1$^{-/-}$ mice on both chow and VLF diets after intraperitoneal injection of the lipoprotein lipase inhibitor P-407. The triglyceride secretion rate did not differ between chow-fed mice, but it decreased in VLF SCD1$^{-/-}$ mice relative to both chow SCD1$^{-/-}$ and VLF SCD1$^{+/+}$ mice (Fig. 3C). This suggests that the decreased plasma triglyceride of the VLF SCD1$^{-/-}$ mice is attributable to decreased triglyceride secretion. The lipid composition of the secreted VLDL particles is greatly influenced by the neutral lipid composition of the liver (32). Although the rates of cholesterol secretion were not affected by genotype or diet (Fig. 3D), the ratio of cholesteryl ester to triglyceride was increased in the livers of VLF SCD1$^{-/-}$ mice, as discussed below. Thus, the secretion of a greater number of cholesterol-rich and triglyceride-poor apoB-containing lipoprotein particles may also contribute to the plasma lipoprotein phenotype observed in the VLF SCD1$^{-/-}$ mice. However, the hepatic mRNA levels of microsomal triglyceride transfer protein (*Mttp*) and *Apob* were not increased in VLF SCD1$^{-/-}$ mice (Table 3).

SCD1$^{-/-}$ mice have reduced hepatic triglycerides and glycogen on the VLF diet

SCD1$^{-/-}$ mice have been shown previously to have an inability to upregulate hepatic triglyceride synthesis in

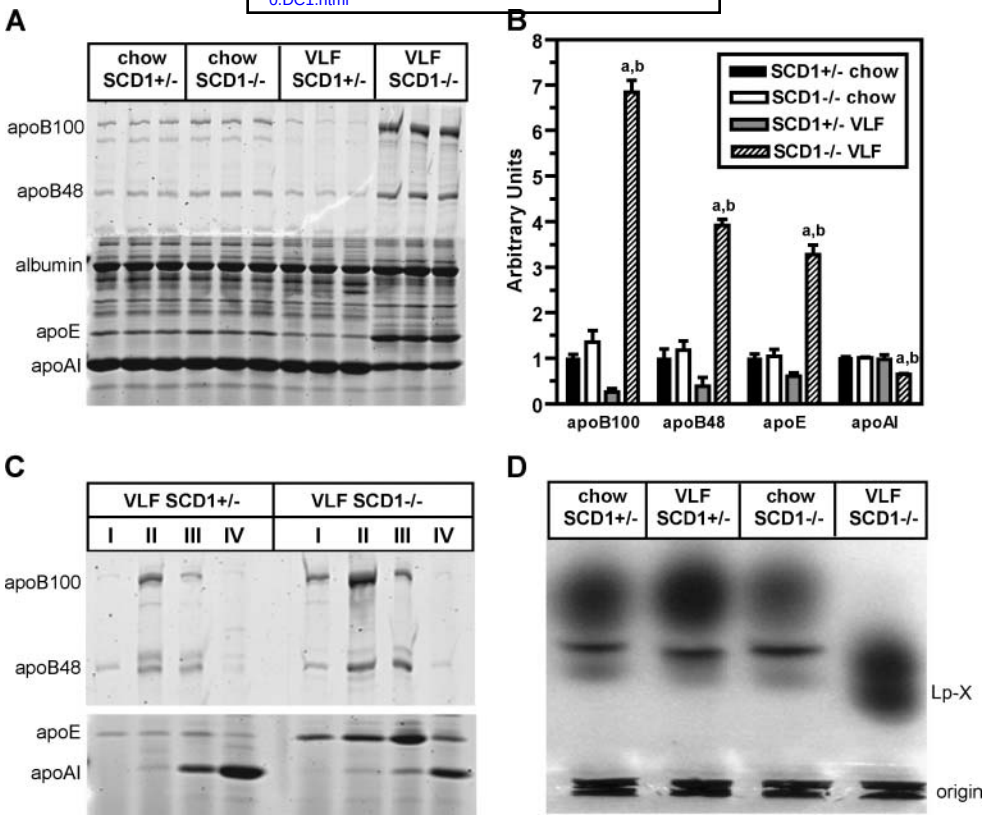

Fig. 2. Analysis of plasma lipoproteins. A: Plasma lipoproteins from whole plasma of male SCD1$^{+/-}$ and SCD1$^{-/-}$ mice maintained on chow or the VLF diet were isolated and analyzed by SDS-PAGE. Each lane represents one animal. ApoB-100, apolipoprotein B-100. B: Fluorimager quantitation of proteins analyzed in A, normalized to chow SCD1$^{+/-}$ values. Data represent means ± SD for three animals per group. [a] Genotype effect (same diet); [b] diet effect (same genotype). [a,b] $P < 0.05$. C: Lipoproteins from VLF SCD1$^{+/-}$ and VLF SCD1$^{-/-}$ mice were isolated from pooled FPLC fractions corresponding to fractions 11–15 (I), 16–21 (II), 22–25 (III), and 26–34 (IV) and analyzed by SDS-PAGE. Distribution patterns for chow SCD1$^{+/-}$ and SCD1$^{-/-}$ animals were indistinguishable and are omitted for clarity. See Fig. 1 for FPLC cholesterol levels corresponding to these fraction pools. D: Plasma was analyzed by agarose gel electrophoresis. The low-mobility band in the VLF SCD1$^{-/-}$ column is consistent with the mobility of lipoprotein X (Lp-X).

response to a high-sucrose, fat-free diet (15). Consistent with these previous observations, the hepatic triglyceride content of SCD1$^{+/+}$ mice fed the VLF diet increased by 240%, but failed to increase in SCD1$^{-/-}$ mice, relative to chow-fed animals. Although hepatic cholesteryl ester levels increased in both SCD1$^{+/+}$ and SCD1$^{-/-}$ mice on the VLF diet, the levels in the SCD1$^{-/-}$ mice did not attain those observed in the VLF SCD1$^{+/+}$ animals (Table 2). However, the VLF SCD1$^{-/-}$ mice also had a large increase in hepatic free cholesterol (Table 2). Despite the high carbohydrate content of the VLF diet, VLF SCD1$^{-/-}$ mice developed hypoglycemia and reduced hepatic glycogen stores (Table 2), similar to previous results observed in fructose-fed SV129 SCD1$^{-/-}$ mice (10). These diminished energy stores indicate a state of energy imbalance that may contribute to the development of the VLF diet-induced phenotypes.

VLF SCD1$^{-/-}$ mice develop hepatic dysfunction consistent with cholestasis

The hepatic uptake and canalicular secretion of many lipophilic molecules is an important function of the liver. The plasma from VLF SCD1$^{-/-}$ mice was yellowish in color, characteristic of the hyperbilirubinemia seen in jaundiced individuals. Indeed, VLF SCD1$^{-/-}$ mice had ~6-fold increased plasma bilirubin (**Table 4**). A majority of this increased bilirubin was conjugated (data not shown), indicating that the hepatic conjugation of bilirubin to glucuronide precedes the accumulation of bilirubin in the plasma. Additionally, plasma levels of bile acids were increased ~50-fold in the VLF SCD1$^{-/-}$ mice (Table 4). We also detected a dramatic increase in serum activities of alanine aminotransferase and aspartate aminotransferase in the VLF SCD1$^{-/-}$ mice, whereas alkaline phosphatase was only modestly increased (Table 2).

The high levels of plasma free cholesterol, phospholipids, bile acids, and conjugated bilirubin, as well as the presence of lipoprotein X and the loss of HDL-cholesterol, are all consistent with cholestasis, a condition in which bile formation or bile flow into the small intestine is impaired (17, 23). A reduction in plasma LCAT activity may also potentially explain the loss of HDL in the VLF SCD1$^{-/-}$ mice. Using a synthetic proteoliposome substrate, we measured a 40% decrease in LCAT activity in plasma from VLF SCD1$^{-/-}$ mice relative to VLF SCD1$^{+/-}$ mice (29.8 ±

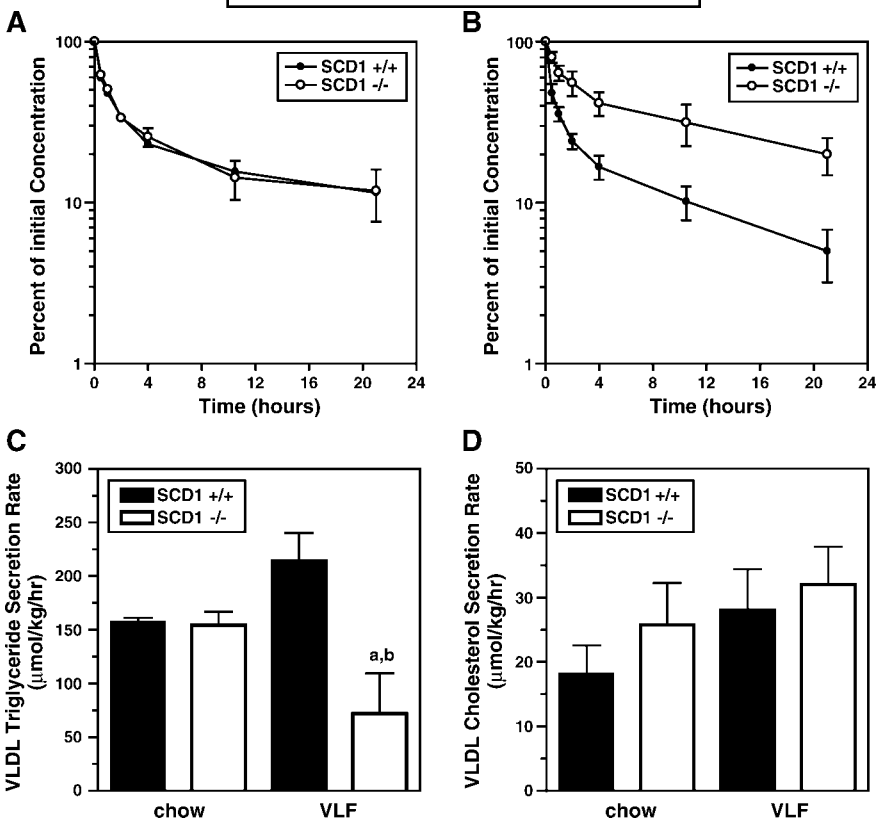

Fig. 3. LDL clearance is impaired in VLF SCD1$^{-/-}$ mice. Male SCD1$^{+/+}$ and SCD1$^{-/-}$ mice maintained on a chow (A) or a VLF (B) diet were injected via the tail vein with ^{125}I-LDL. At the indicated times, blood samples were collected by retro-orbital puncture, and the amount of TCA-precipitable radioactivity was measured. Data shown are means ± SD for three to six mice per group and are plotted as the percentage of radioactivity remaining in the plasma relative to the radioactivity present at 2 min after injection of ^{125}I-LDL. VLDL triglyceride (C) and cholesterol (D) secretion rates were determined in SCD1$^{+/+}$ and SCD1$^{-/-}$ mice maintained on the chow or the VLF diet and are expressed as μmol lipid/kg body weight/h. [a] Genotype effect (same diet); [b] diet effect (same genotype). [a,b] $P < 0.05$.

12.8 vs. 49.1 ± 6.7 nmol/ml/h, respectively; $P < 0.05$), whereas LCAT activity in chow SCD1$^{+/-}$ and chow SCD1$^{-/-}$ mice did not differ significantly (42.4 ± 6.8 and 43.0 ± 8.3 nmol/ml/h, respectively). The low plasma LCAT activity in VLF SCD1$^{-/-}$ mice is apparently caused by a 30% decrease in activity elicited by the VLF versus the chow diet in SCD1$^{-/-}$ mice. We also measured a similar reduction in hepatic *Lcat* mRNA abundance in VLF SCD1$^{-/-}$ mice relative to other groups (Table 3). Additionally, hepatic expression of scavenger receptor class B type I (*Scarb1*), which is involved in HDL uptake, and phospholipid transfer protein (*Pltp*), which is involved in HDL biogenesis, were increased and decreased, respectively, in VLF SCD1$^{-/-}$ mice relative to VLF SCD1$^{+/+}$ mice. On the other hand, the expression of *Apoa1* and *Abca1* were unchanged and increased, respectively, in VLF SCD1$^{-/-}$ mice (Table 3).

Although the plasma phenotypes are symptomatic of cholestasis, neither bile flow nor bile salt secretion was impaired in VLF SCD1$^{-/-}$ mice (see supplementary data). These phenotypes of the VLF SCD1$^{-/-}$ mice closely resemble those of ferrochelatase-deficient mice (23, 33). However, the abundance of hepatic ferrochelatase (*Fech*) mRNA was decreased by only ~30% in VLF SCD1$^{-/-}$ mice relative to other groups (Table 3); thus, it is unlikely to be the cause of the cholestasis in VLF SCD1$^{-/-}$ mice. Gallbladder lipid concentrations did not differ between chow-fed animals (Table 2). The VLF diet caused a decrease in biliary cholesterol and phospholipids in both SCD1$^{+/+}$ and SCD1$^{-/-}$ mice; however, the biliary lipid-lowering effect of the VLF diet was greater in the SCD1$^{-/-}$ mice and did not reach statistical significance in SCD1$^{+/+}$ mice (Table 2). In addition, the phospholipid fatty acid composition of the SCD1$^{-/-}$ mice on both diets reflects a deficiency in MUFAs relative to SCD1$^{+/+}$ on both diets (see supplementary data). Gallbladder total bile acid concentrations were unaffected by diet in either genotype, but the composition of the bile acid pool was altered in the VLF SCD1$^{-/-}$ mice (Table 2 and supplementary data).

The hepatic mRNA level of the bile salt export protein (*Abcb11*) was reduced, whereas the expression of the canalicular phospholipid transporter *Abcb4* was increased slightly in the VLF SCD1$^{-/-}$ mice (Table 3). A potential intrahepatic accumulation of bile salts would lead to the hepatic downregulation of bile acid synthesis via a farnesoid X receptor response (34). Indeed, *Cyp7a1*, *Cyp7b1*, *Cyp8b1*, and *Cyp27a1* were especially downregulated in the

TABLE 3. Expression of genes involved in lipoprotein and lipid metabolism

Affymetrix Identifier	Gene Symbol	Chow, SCD1$^{+/+}$	Chow, SCD1$^{-/-}$	VLF, SCD1$^{+/+}$	VLF, SCD1$^{-/-}$	Genotype P (q), Chow	Genotype P (q), VLF	Diet P (q), SCD1$^{+/+}$	Diet P (q), SCD1$^{-/-}$
Lipoprotein metabolism									
1421821_at	*Ldlr**	1.00	1.03	1.21	0.53	0.7024 (0.2617)	0.0019 (0.0029)	0.1440 (0.0405)	0.0040 (0.0031)
1455593_at	*ApoB**	1.00	1.02	1.07	0.83	0.6175 (0.2453)	0.0015 (0.0024)	0.2496 (0.0609)	0.0024 (0.0019)
1419400_at	*Mttp (MTP)**	1.00	1.14	1.30	1.11	0.0596 (0.0487)	0.1530 (0.0874)	0.0340 (0.0140)	0.4436 (0.0976)
1417043_at	*Lcat**	1.00	1.06	0.90	0.58	0.3318 (0.1583)	<0.0001 (0.0002)	0.0833 (0.0276)	<0.0001 (<0.0001)
1416050_a_at	*Scarb1 (SR-BI)*	1.00	1.28	1.04	1.62	0.0385 (0.0328)	0.0047 (0.0055)	0.6767 (0.1307)	0.0709 (0.0254)
1456424_s_at	*Pltp**	1.00	0.80	2.50	1.10	0.1903 (0.1044)	0.0025 (0.0036)	0.0014 (0.0014)	0.0367 (0.0145)
1421840_at	*Abca1*	1.00	1.21	1.12	1.53	0.0295 (0.0262)	0.0023 (0.0035)	0.1781 (0.0477)	0.0052 (0.0038)
1438840_x_at	*Apoa1*	1.00	0.97	0.95	1.00	0.2770 (0.1392)	0.1254 (0.0758)	0.1101 (0.0322)	0.3179 (0.0759)
1418699_s_at	*Fech (fch)**	1.00	1.08	0.99	0.72	0.0742 (0.0552)	<0.0001 (0.0002)	0.7589 (0.1417)	<0.0001 (<0.0001)
Biliary lipid homeostasis									
1449817_at	*Abcb11 (BSEP)**	1.00	0.91	0.86	0.48	0.1451 (0.0846)	0.0079 (0.0087)	0.0297 (0.0127)	0.0056 (0.0038)
1449818_at	*Abcb4 (MDR2)**	1.00	0.96	0.77	1.24	0.7939 (0.2830)	<0.0001 (0.0002)	0.0511 (0.0189)	0.0014 (0.0014)
1424898_at	*Slc10a1 (Ntcp)**	1.00	1.20	1.32	0.37	0.0860 (0.0578)	0.0002 (0.0004)	0.0238 (0.0108)	0.0002 (0.0002)
1438743_at	*Cyp7a1**	1.00	1.11	0.30	0.06	0.7701 (0.2817)	0.0012 (0.0022)	0.0115 (0.0063)	0.0001 (0.0001)
1421074_at	*Cyp7b1**	1.00	0.82	0.43	0.15	0.3933 (0.1745)	<0.0001 (0.0002)	0.0054 (0.0038)	<0.0001 (<0.0001)
1449309_at	*Cyp8b1**	1.00	1.05	0.58	0.04	0.9540 (0.3258)	0.0002 (0.0004)	0.1406 (0.0400)	0.0001 (0.0002)
1417590_at	*Cyp27a1**	1.00	1.01	0.62	0.43	0.7919 (0.2830)	0.0013 (0.0022)	0.0016 (0.0014)	<0.0001 (<0.0001)
1449854_at	*Nr0b2 (SHP)*	1.00	1.28	1.94	1.77	0.3869 (0.1735)	0.4595 (0.1916)	0.0001 (0.0002)	0.2065 (0.0531)
1420379_at	*Slco1a1 (OATP1)*	1.00	0.81	0.34	0.07	0.4130 (0.1794)	0.0002 (0.0005)	0.0018 (0.0015)	0.0062 (0.0040)
1420405_at	*Slco1a4 (OATP2)*	1.00	1.06	0.87	0.66	0.8293 (0.2931)	0.0214 (0.0199)	0.4033 (0.0914)	0.0377 (0.0147)
1449394_at	*Slco1b2 (OATP4)**	1.00	0.99	0.94	0.21	0.9687 (0.3258)	0.0001 (0.0004)	0.5629 (0.1161)	0.0002 (0.0002)
1433933_s_at	*Slco2b1 (OATP9)**	1.00	1.18	0.95	0.42	0.0133 (0.0129)	<0.0001 (0.0002)	0.5084 (0.1066)	<0.0001 (<0.0001)

Gene expression analysis was performed for chow- and VLF-fed SCD1$^{+/+}$ and SCD1$^{-/-}$ mice using Affymetrix Mouse 430 2.0 microarrays. Results are means of five mice per group, and changes in expression are given as normal ratios relative to chow-fed SCD1$^{+/+}$ mice. Genes with an interaction $P < 0.05$ as determined by two-way ANOVA are denoted by asterisks in the Gene Symbol column. t-tests were performed to calculate P values. The q-values for genotype effects (same diet) and diet effects (same genotype) were calculated as described previously (29).

TABLE 4. Effect of diet and genotype on plasma cholesterol, bilirubin, bile acids, body weight, and food intake

Diet	SCD1 Genotype	Cholesterol	Bilirubin	Bile Acids	Percentage Initial Body Weight	Food Intake
		mg/dl		*μM*		*g/day/g body weight*
Chow	+/+	87.4 ± 12.2	0.31 ± 0.04	14.3 ± 21.2	100.4 ± 1.3	0.130 ± 0.034
	−/−	69.8 ± 8.9[a]	0.33 ± 0.10	4.7 ± 1.3	98.8 ± 1.2	0.266 ± 0.026[a]
VLF	+/+	89.0 ± 10.7	0.41 ± 0.10	3.4 ± 0.6	102.2 ± 3.1	0.154 ± 0.007
	−/−	162.7 ± 29.8[a,b]	2.42 ± 0.79[a,b]	185.5 ± 5.6[a,b]	75.4 ± 4.4[a,b]	0.167 ± 0.023
VLF-coconut	+/+	116.8 ± 26.8	0.54 ± 0.20	31.2 ± 32.4	100.5 ± 2.9	0.153 ± 0.016
	−/−	344.3 ± 33.2[a,b,c]	2.24 ± 0.11[a,b]	186.5 ± 13.1[a,b]	85.2 ± 1.1[a,b,c]	0.198 ± 0.069
VLF-canola	+/+	100.7 ± 2.8	0.59 ± 0.11	5.63 ± 2.0	97.5 ± 3.0	0.163 ± 0.008
	−/−	103.7 ± 11.9[b,c]	0.51 ± 0.10[c]	4.92 ± 1.6[c]	99.8 ± 2.0[c]	0.193 ± 0.011
VLF-oleate	+/+	99.6 ± 5.2	0.41 ± 0.07	4.92 ± 0.40	100.4 ± 6.2	NM
	−/−	181.9 ± 18.7[a,b]	0.39 ± 0.06[c]	19.95 ± 18.1[c]	92.9 ± 1.8[c]	NM

Total plasma cholesterol levels were measured in female SCD1$^{+/+}$ and SCD1$^{-/-}$ mice maintained on the VLF diet supplemented with different fat sources. For comparison, chow and VLF values from Table 1 are shown. Plasma total bilirubin and bile acids were measured in female SCD1$^{+/+}$ and SCD1$^{-/-}$ plasma from animals on chow or the indicated diet. Body weights of female SCD1$^{+/+}$ and SCD1$^{-/-}$ mice were monitored after 9 days on the diet and are expressed as percentage of initial body weight on the chow diet before dietary intervention. Data represent means ± SD for three to six animals per group. NM, not measured.

[a] $P < 0.05$, genotype effect (same diet).

[b] $P < 0.05$, diet effect (same genotype) versus chow.

[c] $P < 0.05$, diet effect (same genotype) versus VLF.

VLF SCD1$^{-/-}$ mice relative to the other groups (Table 3). The expression of the taurocholic acid transport polypeptide (*Slc10a1*) and several members of the organic anion-transporting polypeptide family (*Slco1a1*, *Slco1a4*, *Slco1b2*, *Slco2b1*) was also decreased in the VLF SCD1$^{-/-}$ mice, predicting impaired uptake of bile acids from the blood (Table 3). In SCD1$^{+/+}$ mice, the VLF diet caused an intermediate downregulation of *Cyp7a1*, *Cyp7b1*, *Cyp8b1*, *Cyp27a1*, and *Slco1a1* relative to the VLF diet-induced effect in SCD1$^{-/-}$ mice (Table 3). In both SCD1$^{+/+}$ and SCD1$^{-/-}$ mice, the VLF diet caused a comparable increase in the expression of SHP (*Nr0b2*) (Table 3). Together, these data suggest that the VLF diet elicited a farnesoid X receptor response in both genotypes, but the VLF diet-induced effects were more severe in SCD1$^{-/-}$ mice. In addition, the intrahepatic accumulation of free cholesterol in VLF SCD1$^{-/-}$ mice (Table 2) was associated with the reduced expression of several genes involved in cholesterol synthesis (see supplementary data).

Supplementation of the VLF diet with unsaturated fat prevents hepatic dysfunction and hypercholesterolemia

To address whether a deficiency of dietary fat is responsible for the VLF diet-induced phenotypes, we supplemented the VLF diet with saturated fat (VLF-coconut) or unsaturated fat (VLF-canola). The VLF-coconut diet failed to elicit a normal lipoprotein profile in SCD1$^{-/-}$ mice and exacerbated the cholesterol accumulation in the VLDL, IDL, and LDL fractions (Table 4, **Fig. 4A**). Conversely, the VLF-canola diet prevented the hypercholesterolemia and the loss of HDL-cholesterol in the SCD1$^{-/-}$ mice (Table 4, Fig. 4B). These results indicate that the unsaturated fat content of the diet is a critical determinant of this phenotype. The VLF-canola, but not VLF-coconut, diet was also able to prevent the increase in the plasma bilirubin and bile acids (Table 4).

These VLF and VLF-coconut diet-induced phenotypes were also associated with a progressive loss of body weight in SCD1$^{-/-}$ mice (Table 4). Although SCD1$^{-/-}$ mice are hyperphagic relative to SCD1$^{+/+}$ mice on a chow diet, the two strains consume similar amounts of food on the VLF diet (Table 4). This suggests that a potential contributor to the loss of body weight is caloric deficit. However, SCD1$^{-/-}$ mice eat similar amounts of the VLF-canola and VLF-coconut diets despite the loss of body weight selectively in the VLF-coconut group (Table 4). These observations suggests that the VLF and VLF-coconut diet effects are not attributable to caloric deficiency but instead are caused by the deficiency of nutrient(s) found in the chow and VLF-canola diets.

Because the primary effect of SCD1 deficiency is the impairment of MUFA synthesis, we hypothesized that supplementing the VLF diet with MUFA-rich triolein (VLF-oleate) instead of the MUFA- and PUFA-rich canola oil would also prevent the onset of the VLF-induced phenotypes in the SCD1$^{-/-}$ mice. Dietary oleate ameliorated many of the aforementioned VLF diet-induced phenotypes but was unable to prevent the increase in LDL-cholesterol as effectively as the VLF-canola diet (Table 4, Fig. 4C). Therefore, MUFA supplementation provides incomplete protection relative to MUFA/PUFA supplementation, suggesting that PUFA deficiency is also involved in the development of the VLF diet-induced phenotypes.

VLF diet-induced phenotypes are reversed by dietary unsaturated fat supplementation

The previous observations suggest that a balance exists between dietary and de novo MUFA synthesis that influences several facets of metabolism. To more directly implicate dietary unsaturated fat as the critical determinant of the VLF-induced phenotypes, we treated SCD1$^{-/-}$ mice with the VLF diet for 10 days and then switched the animals to the VLF-canola diet. The VLF-canola diet reversed the VLF diet-induced changes in body weight, plasma bile acids, and plasma glucose to levels comparable to those observed before VLF treatment (**Fig. 5A–C**). Additionally, the VLF-canola diet ameliorated the substantial increase in plasma free cholesterol (Fig. 5D). However, total plasma

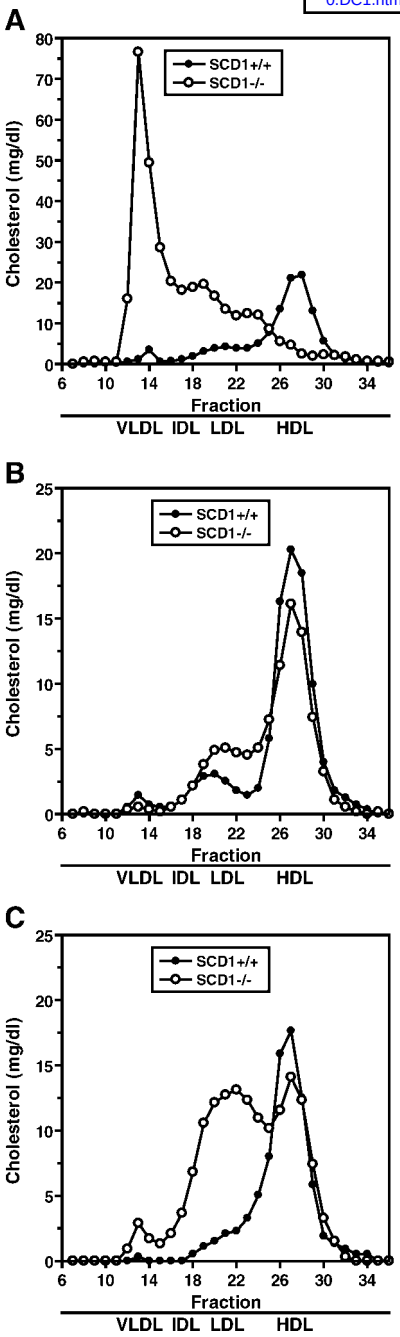

Fig. 4. Phenotypes of the VLF SCD1$^{-/-}$ mice are ameliorated by dietary unsaturated fat. Plasma from the female SCD1$^{+/+}$ and SCD1$^{-/-}$ mice described in Table 4 were fractionated by FPLC. Fractions 7–36 were analyzed for total cholesterol content. The locations of fractions corresponding to VLDL, IDL, LDL, and HDL are indicated. A: VLF-coconut. B: VLF-canola. C: VLF-oleate.

cholesterol levels were not restored to the baseline levels observed before VLF treatment. The incomplete rescue of hypercholesterolemia is similar to the phenotypes observed in the VLF-oleate SCD1$^{-/-}$ mice (Table 4, Fig. 4C). This suggests that depletion of PUFA stores elicited by the VLF diet could not be completely prevented by the concomitant presence of dietary MUFAs, nor could it be completely restored by the VLF-canola diet, in the time frame studied.

DISCUSSION

On a VLF diet, the contribution of dietary fat is limited and the normal physiological response is to increase the hepatic production of fatty acids from dietary carbohydrate. SCD1 activity augments lipogenesis from dietary carbohydrate in two ways. First, SCD1 controls the relative amounts of saturated fatty acids and MUFAs synthesized via de novo lipogenesis (15). During high-carbohydrate feeding, SCD1 is one of several lipogenic enzymes to be induced, resulting in increased MUFA production. Studies in SCD1$^{-/-}$ mice have also shown that SCD1 is required for the upregulation of lipogenic gene expression on high-carbohydrate diets (10, 15). Hence, SCD1 deficiency not only impairs MUFA synthesis but also interferes with the compensatory increase in fatty acid synthesis elicited by lipogenic diets. Under standard chow conditions, lipogenic induction is relatively low and dietary unsaturated fat is sufficient to sustain SCD1$^{-/-}$ mice. However, the VLF dietary regimen leads to severe hepatic dysfunction and hypercholesterolemia in the SCD1$^{-/-}$ mice.

We found that the low level of unsaturated fat in the VLF diet is a key determinant of the phenotypes that develop in the VLF SCD1$^{-/-}$ mice. The accumulation of plasma cholesterol, bile acids, and bilirubin, as well as the severe loss of body weight, were prevented by dietary unsaturated fat, but not by saturated fat. We were surprised to observe that the use of two different unsaturated fat sources revealed distinct forms of hypercholesterolemia present in the VLF SCD1$^{-/-}$ mouse. Both the canola oil- and oleate-supplemented diets prevented the massive accumulation of free cholesterol residing in the VLDL-sized lipoprotein X particles and the loss of HDL-cholesterol. However, the selective ability of the VLF-oleate diet to elicit an increase in LDL-cholesterol suggests that PUFA availability is also a determinant of the hypercholesterolemia in the VLF SCD1$^{-/-}$ mouse. Although SCD1 catalyzes the synthesis of MUFAs, these results indicate that under certain dietary conditions SCD1 deficiency results in an increased requirement for dietary PUFAs.

The superior hypocholesterolemic properties of the VLF-canola diet relative to the VLF-oleate diet suggest that the de novo synthesis of MUFAs represents a novel mechanism of cholesterol homeostasis that may involve PUFAs. The predominant PUFA in the VLF-canola diet is linoleic acid (18:2); however, at this time, we cannot exclude the possibility that linolenic acid (18:3) plays a role, because it is also present in the canola oil. The products of SCD1, palmitoleate and oleate, could spare the oxidation of PUFA in times of dietary essential fatty acid deficiency, as proposed by Cunnane (5). Indeed, hepatic SCD1 expression is repressed by dietary PUFAs (8), exemplifying a reciprocal relationship between endogenous MUFA production and essential fatty acid status. Although the VLF-canola diet was able to largely prevent the VLF diet-induced hypercholesterolemia in SCD1$^{-/-}$ mice switched directly from chow to the VLF-canola diet (Table 4), the VLF-canola diet was unable to completely reverse this hypercholesterolemia in SCD1$^{-/-}$ mice previously fed the VLF

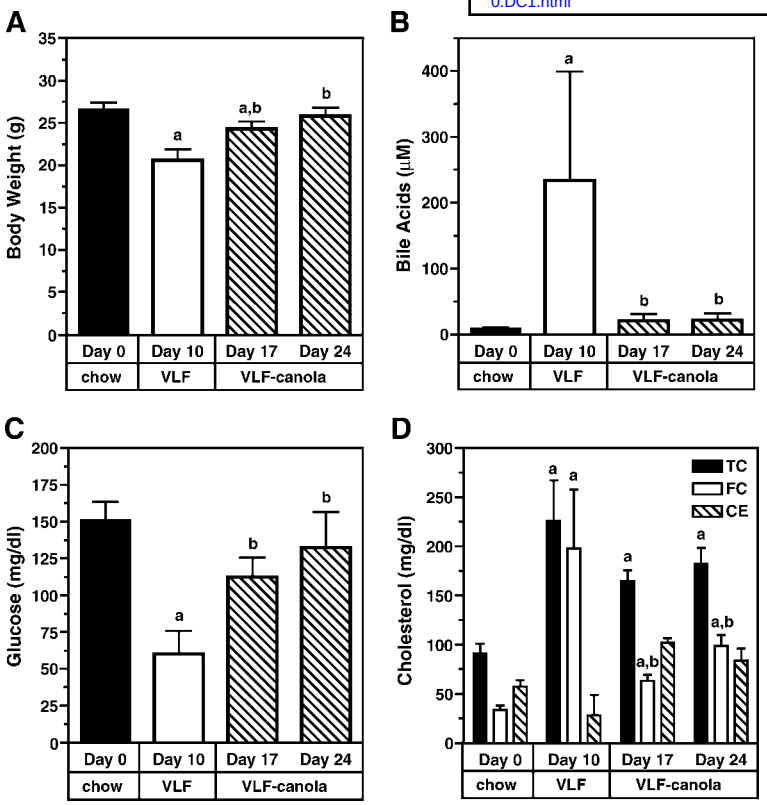

Fig. 5. Reversal of VLF-induced phenotypes by the VLF-canola diet. SCD1$^{-/-}$ mice were fed the VLF diet for 10 days and then subsequently switched to the VLF-canola diet for 14 days. Plasma samples were analyzed at day 0 (chow), day 10 (VLF), day 17 (7 days on VLF-canola), and day 24 (14 days on VLF-canola). A: Body weight. B: Plasma bile acids. C: Plasma glucose. D: Plasma cholesterol. Values for total cholesterol (TC), free cholesterol (FC), and cholesteryl ester (CE) are shown. Data represent means ± SD for five mice. [a] $P < 0.05$ versus day 0 (chow); [b] $P < 0.05$ versus day 10 (VLF).

diet (Fig. 5D). The loss of body weight elicited by the VLF diet in SCD1$^{-/-}$ mice is likely associated with a depletion of adipose PUFA stores, and this loss of body weight did not occur in mice switched directly from chow to the VLF-canola diet (Table 4, Fig. 5A). We speculate that the diminished body stores of PUFAs in SCD1$^{-/-}$ mice fed the VLF diet further increase the dietary requirement of PUFAs to prevent hypercholesterolemia.

Serum bile acid levels are increased in both ferrochelatase-deficient mice and ATP-dependent aminophospholipid translocase (ATP8B1) mutant mice without an impairment in biliary bile acid secretion (23, 35). Bloks et al. (23) hypothesized that altered membrane fluidity in ferrochelatase-deficient mice affects the ability of biliary bile acids to stimulate the canalicular secretion of phospholipid and cholesterol into bile, resulting in the formation of lipoprotein X. Perturbations in membrane lipid composition have also been suggested to be the cause of the disrupted bile salt homeostasis in mice with a mutation in ATP8B1 (35). We speculate that a common phenomenon may affect the VLF SCD1$^{-/-}$ mice as well. The lipid changes of the VLF SCD1$^{-/-}$ mice include a more highly saturated fatty acyl-CoA composition (our unpublished data) and an increase in hepatic free cholesterol (Table 2), but they also likely include altered membrane phospholipid composition.

Membrane lipid composition is important for membrane fluidity, membrane trafficking, signal transduction, and membrane protein function (36). Changes in the lipid environment of the cell may impair the normal activity of canalicular ABC transporters, resulting in an increased steady-state level of biliary components in the hepatocytes and plasma. The accumulation of various biliary components in the plasma of VLF SCD1$^{-/-}$ mice may also be attributable to impaired bile flow resulting from the development of an extrahepatic occlusion of the bile duct, or to an intrahepatic dysfunction in the transport of biliary components across the hepatic canalicular membrane. However, we observed no evidence of macroscopic precipitates or gallstones in the gallbladders of VLF SCD1$^{-/-}$ mice. We also observed no impairment of bile flow or biliary bile acid secretion (see supplementary data). Interestingly, plasma bile acids, but not bilirubin, were increased during the bile flow experiments in SCD1$^{-/-}$ mice fed the VLF diet for 8 days (data not shown). Thus, the increase in plasma bilirubin may be secondary to bile acid accumulation and occur at a late stage of cholestasis.

Although decreased abundance of the bile salt export protein *Abcb11* (Table 3) in VLF SCD1$^{-/-}$ mice may contribute to the development of this phenotype, loss of *Abcb11* in mice results in only mild, nonprogressive cholestasis (37), suggesting that another mechanism is responsible for the cholestasis in the VLF SCD1$^{-/-}$ mice. Together, these results suggest that the kinetics of ABCB11, and possibly other ABC transporters, have changed, because the same rate of bile salt export requires a higher concentration of intracellular bile acids. For example, an increased K_m of ABCB11 would be consistent with the accumulation of plasma bile acids concomitant with normal biliary bile acid secretion. The ability of the unsaturated fat-supplemented diets to rescue these phenotypes may be attributable to a restoration of adequate canalicular ABC transporter activity through the normal-

ization of canalicular plasma membrane composition. This hypothesis is difficult to test, for there is currently no in vitro system or cell line that properly mimics the canalicular membrane, nor is there a technique for isolating intact canalicular membranes without collapsing the phospholipid bilayer asymmetry. Alternatively, the altered biliary lipid composition in the VLF SCD1$^{-/-}$ mice could be the result of decreased secretion of phosphatidylcholine and cholesterol into bile, attributable to a deficiency of unsaturated fat and a limited availability of certain phosphatidylcholine species.

The loss of HDL-cholesterol in the VLF SCD1$^{-/-}$ mice has been observed in other models of murine cholestasis, but the mechanism is unknown (17, 23). In ferrochelatase-deficient mice, this loss is not attributable to decreased LCAT activity or *ApoaI* mRNA levels (23). Although lipoprotein X has also been observed in LCAT-deficient mice, the presence of many cholestatic symptoms in the VLF SCD1$^{-/-}$ mice indicates that the reduction in LCAT activity in VLF SCD1$^{-/-}$ mice may be a secondary consequence of cholestasis (17, 38, 39). *Apoa1* mRNA levels were not decreased in VLF SCD1$^{-/-}$ mice, suggesting a mechanism other than the farnesoid X receptor-mediated repression of apoA-I transcription for the loss of HDL-cholesterol (40). Bile duct ligation also results in the disappearance of HDL-cholesterol irrespective of the presence of lipoprotein X (17). Because the liver is a primary regulator of HDL biogenesis (41, 42), it is possible that altered intracellular lipid availability or lipid trafficking in the cholestatic liver impairs the ability to lipidate apoA-I.

Why is there so much free cholesterol in the plasma and liver of VLF SCD1$^{-/-}$ mice, and why did it not get esterified in the cells by ACAT or in the plasma by LCAT? Using a synthetic proteoliposome substrate, we observed a 30–40% reduction in LCAT activity in the plasma of VLF SCD1$^{-/-}$ mice relative to chow SCD1$^{-/-}$ mice and VLF SCD1$^{+/-}$ mice. This reduction in LCAT activity is likely insufficient to explain the complete loss of HDL-cholesterol, because LCAT$^{+/-}$ animals retain half-normal levels of HDL-cholesterol (43). In addition to the cholestasis-related changes in lipoprotein metabolism, the expansion of the plasma and liver free cholesterol pool may be attributable to the limited availability of donor unsaturated fatty acyl-CoA and phosphatidylcholine for the LCAT and ACAT reactions. Therefore, we speculate that VLF SCD1$^{-/-}$ mice have a defect in making fatty acids and/or cholesterol available for esterification in both the plasma and cellular compartments.

The effect of SCD1 deficiency on plasma lipids depends on the mouse strain. Although chow-fed asebia (ab^J/ab^J) mice have a severe depletion of plasma triglycerides relative to control mice (14, 31), mice with a targeted deletion of SCD1 have a more modest reduction in plasma triglycerides (10, 14, 30). Recently, antisense oligonucleotide-mediated inhibition of SCD1 in C57BL/6 mice was reported to not affect plasma triglyceride levels (44). In accordance with our current observations in C57BL/6 mice, we have also observed no influence of SCD1 deficiency on plasma triglycerides in mice on a BTBR genetic background (our unpublished data). Future studies on how these genetic variables influence the penetrance of the plasma triglyceride phenotype in SCD1$^{-/-}$ mice are warranted. The effect of SCD1 deficiency on plasma cholesterol levels also requires further investigation. Although one study in chow-fed ab^J/ab^J mice reported increased plasma HDL-cholesterol levels relative to control mice (31), other studies, including the results reported here, have reported either no effect or a decrease in plasma HDL-cholesterol levels in chow-fed SCD1$^{-/-}$ mice (14, 30).

In summary, we identified a novel genotype-diet interaction between SCD1 deficiency and dietary unsaturated fat restriction that elicits hypercholesterolemia and hepatic dysfunction. The superior hypocholesterolemic properties of dietary PUFAs relative to dietary MUFAs suggests that endogenous MUFA production affects the dietary requirement of PUFAs. Although treatment of SCD1$^{+/+}$ mice with an essential fatty acid-deficient diet for 8 weeks does not cause hypercholesterolemia or cholestasis (20), the effect of an essential fatty acid-deficient diet in SCD1$^{-/-}$ mice has not been explored previously. Future research will be necessary to further elucidate the mechanism of hepatic dysfunction and dyslipidemia observed in this model as well as to identify the PUFA species essential for the prevention of this phenotype.

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