The Biosynthesis of Hepatic Cholesterol Esters and Triglycerides Is Impaired in Mice with a Disruption of the Gene for Stearoyl-CoA Desaturase 1

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Stearoyl-CoA desaturase (SCD) is a microsomal enzyme required for the biosynthesis of oleate and palmitoleate, which are the major monounsaturated fatty acids of membrane phospholipids, triglycerides, and cholesterol esters. Two well characterized isoforms of SCD, SCD1 and SCD2, exist in the mouse. Most mouse tissues express SCD1 and 2 with the exception of the liver, which expresses mainly the SCD1 isoform. We found that asebia mice homozygous for a natural mutation of the gene for SCD1 (SCD−/−) are deficient in hepatic cholesterol esters and triglycerides despite the presence of normal activities of acyl-CoA:cholesterol acyltransferase and glycerol phosphate acyltransferase, the enzymes responsible for cholesterol ester and triglyceride synthesis, respectively, in the liver of these mice. Feeding diets supplemented with triolein or tri-palmitolein to the SCD−/− mice resulted in an increase in the levels of 16:1 and 18:1 in the liver but failed to restore the 18:1 and 16:1 levels of the cholesterol ester and triglycerides to the levels found in normal mice. The SCD−/− mouse had very low levels of triglycerides in the VLDL and LDL lipoprotein fractions compared with the normal animal. Transient transfection of an SCD1 expression vector into Chinese hamster ovary cells resulted in increased SCD activity and esterification of cholesterol to cholesterol esters. Taken together, our observations demonstrate that the oleoyl-CoA and palmitoleyl-CoA produced by SCD1 are necessary to synthesize enough cholesterol esters and triglycerides in the liver and suggest that regulation of SCD1 activity plays an important role in mechanisms of cellular cholesterol homeostasis.

Stearoyl-CoA desaturase (SCD) is a microsomal fatty acid modifying enzyme that catalyzes the introduction of the first double bond between carbons 9 and 10 of saturated fatty acyl-CoA substrates resulting in the production of monounsaturated fatty acids. The preferred substrates for SCD are palmitoyl- and stearoyl-CoA, which are converted to palmitoleoyl- and oleoyl-CoA, respectively (1). Overall, SCD expression affects the fatty acid composition of membrane phospholipids, triglycerides, and cholesterol esters. Effects on composition of phospholipids ultimately determine membrane fluidity, whereas the effects on the composition of cholesterol esters and triglycerides can affect lipoprotein metabolism and adiposity. Thus the regulation of SCD is of considerable physiological importance, and high SCD activity has been implicated in a wide range of disorders including diabetes, atherosclerosis, cancer, and obesity (2).

Two mouse isoforms of SCD, SCD1 and SCD2, which are products of different genes are currently known and are well characterized (2–4). Most organs of different mouse strains express SCD1 and 2 with the exception of the liver, which expresses mainly the SCD1 isoform. SCD2 is expressed at higher levels in livers of mice overexpressing the truncated nuclear form of the sterol regulatory element-binding protein-1a (5). Several studies have shown that the SCD1 gene expression is highly regulated by dietary changes, hormonal factors, developmental processes, temperature changes, metals, alcohol, peroxisomal proliferators, and phenolic compounds (6–8). Oleate, one of the most abundant fatty acids in the diet, is the end product of SCD. Why is it then, that SCD1 is such a highly regulated gene? A clue as to the physiological role of the SCD1 gene and its endogenous products (the monounsaturated fatty acids) has come from recent studies of the asebia (ab) mutant mouse strain (9). The asebia mouse arose from BALB/c mice and was first described by Gates and Karasek (10). They named this mouse strain “asebia” because they found no evidence of sebaceous glands and subsequently reported that the mouse had a mutation that was due to a single autosomal recessive gene with complete penetrance; mice heterozygous for asebia appeared normal (9–11). The recessive mutation was located on chromosome 19 (12) and has recently been determined to be in the SCD1 gene (9). Characterization of the asebia mice (ab1) indicated that the mice express the SCD2 gene isoform in various issues, but SCD1 mRNA expression is not detectable in several mouse tissues so far studied (9). The absence of SCD1 gene expression is due to an extensive deletion in the SCD1 gene in which the first four exons of the gene are missing (9). The asebia mice develop alopecia, reminiscent of some of the clinical scarring alopecias in humans (9). The mice appear hairless with a short, sparse hair coat and dry, slightly scaly skin (10), suggesting the importance of SCD1 expression to the normal development of sebaceous glands and hair growth. In addition, the mice suffer from corneal opacity and hypoplastic meibomian glands, implicating the importance of SCD1 expression in normal ocular barrier function (9). The
mechanisms leading to these phenotypes are not known but are dependent on SCD1 gene expression and suggest that regulation of SCD1 activity plays an important role in mechanisms of cellular cholesterol and lipoprotein homeostasis.

**EXPERIMENTAL PROCEDURES**

**Animals and Diets—**Aeasia homozygous (ab/ab) and heterozygous (+/ab or +/) and heterozygous (+/+) mice were obtained from the Jackson Laboratory (Bar Harbor, ME) and bred at the University of Wisconsin Animal Care Facility. In this study, comparisons are made between the homozygous (+/−) and the heterozygous (+/+ ) mice because the latter are indistinguishable from normal mice (9, 10). Mice were housed in a pathogen-free barrier facility operating a 12-h light/12-h dark cycle. At 3 weeks of age, these mice were fed ad libitum for 2 weeks or 2 months on laboratory chow diet or on a semi-purified diet containing 50% (% of total fatty acids) triolein or tripalmitolein. The semi-purified diet was purchased from Harlan Teklad (Madison, WI) and contained 18% total fatty acids, 0.3% L-methionine, 0.1% choline chloride, salt mix (AIN-76A) and vitamin mix (AIN-76A). The fatty acid composition of the experimental diets was determined by gas-liquid chromatography. The control diet contained 11% palmitic acid (16:0), 23% oleic acid (18:1n-9), 53% linoleic acid (18:2n-6), and 8% linolenic acid (18:3n-3). The high triolein diet contained 7% 16:0, 50% 18:1n-9, 35% 18:2n-6, and 5% 18:3n-3. The high tripalmitolein diet contained 6% 16:0, 49% palmitoleic acid (16:1n-7), 12% 18:1n-9, 27% 18:2n-6, and 4% 18:3n-3. Animals were anesthetized at about 10:00 a.m. by intraperitoneal injection of pentobarbital sodium (0.08 mg of Nembutal/kg of body weight; Abbott, North Chicago, IL). Liver was isolated immediately, weighed, and kept in liquid nitrogen. Blood samples were obtained from the abdominal vein.

**Materials—**Radioactive [3-2H]DCTP (3000 Ci/mmol) was obtained from DuPont. Thin layer chromatography plates (TLC Silica Gel G60) were from Merck. [1-14C]Stearyl-CoA, [3H]choleratide, and [1-14C]oleyl-CoA were purchased from American Radiolabeled Chemicals, Inc. (St. Louis, MO). Immobilon-P transfer membranes were from Millipore (Bedford, MA). ECL Western blot detection kit was from Amersham Pharmacia Biotech, Inc. LT-1 transfection reagent was from PanVera (Madison, WI). All other chemicals were purchased from Sigma. The antibody for rat liver microsome SCD was provided by Dr. Travis Knight at Iowa State University.

**Lipid Analysis—**Total lipids were extracted from liver and plasma according to the method of Bligh and Dyer (13), and phospholipids, free cholesterol, triglycerides and cholesterol esters were separated by gel TLC. Petroleum ether/diethyl ether/acetonic acid (80:30:1) was used as a developing solvent. Spots were visualized by 0.2% 2′,7′-dichlorofluorescein in 95% ethanol or by 10% cupric sulfate in 8% phosphoric acid. The phospholipid, cholesterol ester, and triglyceride spots were scraped, and 1 ml of 5% HCl-methanol was added and heated at 100 °C for 1 h. The methyl esters were analyzed by gas-liquid chromatography using cholesterol heptadecanoate as internal standard (14, 15). Free cholesterol, cholesterol ester, and triglycerides contents of liver and plasma were determined by enzymatic assays (Sigma and Wako Chemicals).

**Plasma Lipoprotein Analysis—**Mice were fasted a minimum of 4 h and sacrificed by CO2 asphyxiation and/or cervical dislocation. Blood was collected aseptically by direct cardiac puncture and centrifuged (13,000 × g, 5 min, 4 °C) to collect plasma. Lipoproteins were fractionated on a Superose 6HR 10/30 fast protein liquid chromatography column (Amersham Pharmacia Biotech). Plasma samples were diluted 1:1 with PBS and filtered (0.22 μm, 0.02 μm) and injected onto the column that had been equilibrated with PBS containing 1 mM EDTA and 0.02% NaN3. The equivalent of 100 μl of plasma was injected onto the column. The flow rate was set constant at 0.3 ml/min. 500-μl fractions were collected and used for total triglyceride measurements (Sigma). Values reported are for total triglyceride mass/fraction. The identities of the lipoproteins have been confirmed by using anti- ApoB immunoactivity for LDL and anti-Apo AI immunoactivity for HDL (not shown).

**Isolation and Analysis of RNA—**Total RNA was isolated from livers using the acid guanidinium-phenol-chloroform extraction method (16). 20 μg of total RNA was separated by 0.8% agarose/2.2 m formaldehyde gel electrophoresis and transferred onto nylon membrane. The membrane was hybridized with 32P-labeled cDNA probes synthesized using the divergent 5′- or 3′-untranslated regions specific for either the SCD1 (4) or the SCD2 (3) cDNAs. PalA15 probe (16, 17) was used as control for equal loading.

**SCD Activity Assay—**Stearyl-CoA desaturase activity was measured in liver microsomes essentially as described by Oshino et al. (18). Tissues were homogenized in volumes of buffer A (0.25 M sucrose/1 mM EDTA/10 mM Tris-HCl/1 mM phenylmethylsulfonyl fluoride, pH 7.4). The microsomal membrane fractions (100,000 × g pellet) were isolated by sequential centrifugation. Reactions were performed at 37 °C for 5 min with 400 μg of protein homogenate and 27 nM of [1-14C]stearyl-CoA (60,000 cpm), 1 μM of NADH, 50 μM of Tris/HCl buffer, pH 7.4. After the reaction, fatty acids were extracted and then methylated with 10% acetic chloride/methanol. Saturated fatty acid and monounsaturated fatty acid methyl esters were separated by 10% AgN03-impregnated TLC using hexane/diethyl ether:acetic acid (90:30:1) as developing solution. The plates were sprayed with 0.2% 2′,7′-dichlorofluorescein in 95% ethanol, and the lipids were identified under UV light. The fractions were scraped off the plate, and the radioactivity was measured using a liquid scintillation counter (21). The enzyme activity was expressed as nmol min−1 mg−1 protein.

**ACAT Activity Assay—**ACAT activity was measured essentially as described (19). Reactions were performed at 37 °C for 5 min with 250 μg/ml microsome protein homogenate and 20 μM [1-14C]oleoyl-CoA (18 μCi/μmol). Exogenous cholesterol (20 nmol) was added as complex of Triton WR1339:cholesterol (100:1) to the reaction mixture. Cholesterol esterified was extracted by TLC using hexane/diethyl ether:acetic acid (90:30:1) as developing solution. The plates were sprayed with 0.2% 2′,7′-dichlorofluorescein in 95% ethanol, and the lipid fractions were identified under UV light. The fractions were scraped off the plate, and the radioactivity was measured with liquid scintillation counter (21).

**GPAT Activity Assay—**GPAT activity was measured essentially as described by Thomas and Poznansky (25). The incubation mixture contained 75 μM Tris/HCl, pH 7.4, 4 μM MgCl2, 2 mg/ml of bovine serum albumin, 50 μM palmitoyl-CoA, 0.3 mM [1-14C]glycerol 3-phosphate (7.6 μCi/μmol), and 500 μg/ml microsome protein. The assay mixture was incubated at 37 °C for 10 min, and the reaction was terminated by adding 3 ml of chloroform:methanol (1:2) and 0.6 ml of 1% perchloric acid. After 5 min, the lipids were extracted according to Bligh and Dyer (13). The lower phase was washed three times with 2 ml of 1% perchloric acid. After drying, the radioactivity of lower phase was measured with liquid scintillation counter (21).

**Immunoblotting—**Pooled liver membranes from five mice of each group were prepared as described by Heinemann and Ozols (20). The same amount of protein (80 μg) from each fraction was subjected to 10% SDS-polyacrylamide gel electrophoresis and transferred to Immobilon-P transfer membranes at 4 °C. After blocking with 10% nonfat milk in TBS buffer, pH 8.0 overnight, the membranes were washed and incubated with rabbit anti-rat SCD as primary antibody (20) and goat anti-rabbit IgG-horseradish peroxidase conjugate as the secondary antibody. Visualization of the SCD protein was performed with ECL Western blot detection kit.

**Transient Transfections of CHO Cell—**CHO cells were transfected with pcDNA3–1 mammalian expression vector containing the wild type-mouse SCD1 cDNA (4) or the plant SCD cDNA in the antisense orientation. 10 μg of DNA was transfected with LT-1 transfection reagent into equivalent amounts of cells (1 × 106) plated on 6-cm dishes. Approximately 48 h after transfection, cells were washed twice with PBS, harvested by scraping, and homogenized in 20 mM Tris, 150 mM...
pooled livers of each group were subjected to 10% SDS-polyacrylamide immunoblot analysis, aliquots of membrane fraction (80 mg) from 2 and heterozygous (+/−) mice. Total RNA (20 mg) pooled from 5 mice of each group was subjected to Northern blot analysis followed by hybridization with labeled probes specific for SCD1 and SCD2 cDNA. A cDNA probe for pAL15 (16) was used to confirm equal loading. For enzyme activity, aliquots of microsome fraction (400 mg) from livers of each group were incubated with a reaction mixture containing [1-14C]stearoyl-CoA for 5 min. The products were saponified and acidified, and the fatty acids were extracted and separated by TLC as described under “Experimental Procedures.” Each value represents the mean ± S.D. (n = 5). For immunoblot analysis, aliquots of membrane fraction (80 mg) from pooled livers of each group were subjected to 10% SDS-polyacrylamide gel electrophoresis followed by detection with SCD antibody.

NaCl, and 0.1% Triton X-100 followed by 10 passes through a 25-gauge needle. Total membranes were isolated and used for immunoblotting and for SCD enzyme assay as described above. To measure cholesterol esterification, CHO cultures were incubated with [1-14C]cholesterol (5 µCi/ml) for 12 h. The cells were washed twice with PBS. Total lipids were then extracted, and the incorporation of [3H]cholesterol into cholesterol esters was measured by TLC followed by liquid scintillation counter (21).

RESULTS

Absence of SCD1 mRNA, Protein, and Enzyme Activity in the Liver of SCD−/− Mice—Northern blot analysis shows that SCD1 mRNA is not detectable in liver of SCD1−/− mice, whereas heterozygous mice (+/−), which are phenotypically indistinguishable from normal mice (9, 10), express the 4.9-kilobase SCD1 mRNA (Fig. 1A). SCD2 mRNA was expressed in the livers of both SCD1−/− and heterozygous mice at much lower level and was visualized only after a much longer exposure of the autoradiogram. There was no change in the pAL15 mRNA expression, used as a control for equal loading of the RNA. SCD enzyme activity, as measured by the rate of conversion of [1-14C]stearoyl-CoA to [1-14C]oleate (Fig. 1B), was high in the heterozygous mice but was decreased by greater than 90% in the total extracts of livers of the SCD1−/− mice. The low level of SCD activity remaining in the extracts of the SCD1−/− mice is probably due to expression of the SCD2 isoform. Immunoblot analysis (Fig. 1C) shows that SCD protein was not detectable in the liver microsomes of the SCD1−/− mice, confirming that the antibody is specific for the liver microsomal SCD1 isoform.

SCD1−/− Mice Have Low Levels of Hepatic Cholesterol Esters and Triglycerides of Oleate and Palmitoleate—TLC of lipids extracted from liver of SCD1−/− mice demonstrated markedly reduced cholesterol ester and triglyceride levels compared with the lipids extracted from liver of heterozygous mice (Fig. 2). Table I shows that the total cholesterol ester content in liver of SCD1−/− mice was decreased by 87%. The total plasma cholesterol increased by 35% in the SCD1−/− mice, whereas the liver free cholesterol levels remained the same in both groups of animals. The total liver triglyceride content decreased by 62% in the SCD−/− mice, whereas the plasma triglyceride content decreased by 67%. Plasma lipoproteins were separated by fast performance liquid chromatography, and the distribution of triglycerides among lipoproteins in the various density fractions of the mice are shown in Fig. 3. Lipoprotein profile showed a major difference in the distribution of triglycerides in the VLDL fraction of the SCD−/− and SCD+/− mice. The levels of triglycerides in the SCD+/− were 25 μg/dl in the VLDL, with very low levels in the LDL and HDL fractions. In contrast the SCD−/− had very low levels of triglycerides in the three lipoprotein fractions.

The major monounsaturated fatty acids of cholesterol esters and triglycerides are palmitoleate and oleate (5). To determine whether the livers of the SCD−/− mice contained less monounsaturated fatty acids in the cholesterol ester and triglyceride fractions, the lipids were separated by TLC, and the relative fatty acid composition in each fraction was determined. Table II shows the relative percentage of the major fatty acids measured in the cholesterol ester and triglyceride fractions. In the total lipid fraction, the SCD1−/− livers had a 50% decrease in the relative amount of palmitoleate (16:1) and a 43% decrease in oleate (18:1). The relative amount of palmitoleate (16:1) in hepatic cholesterol ester fraction of livers of the SCD1−/− mice decreased by 85%, whereas the relative amount of oleate (18:1) decreased by 60%. Liver triglycerides of
SCD1−/− mice showed an 85% decrease of palmitoleate (16:1) and an 83% decrease of oleate (18:1) compared with the heterozygous mice. The relative decreases in the monounsaturated fatty acids (16:1 and 18:1) were accompanied by significant increases in the relative percentages of the saturated fatty acids (16:0 and 18:0). Fig. 4 shows the ratio of monounsaturated fatty acids to saturated fatty acids (desaturation index) measured in the cholesterol ester and triglyceride fractions in the livers of the SCD1−/− and heterozygous mice. In the total lipid fraction (Fig. 4, A and D), the 16:1/16:0 and 18:1/18:0 ratios were decreased in the livers of the SCD1−/− compared with the heterozygous mice. In the cholesterol ester and triglyceride fractions (Fig. 4, B, C, E, and F), the ratios of 16:1/16:0 and 18:1/18:0 were decreased by greater than 90%. These data indicate that although the liver cholesterol ester and triglyceride levels are reduced as shown in Fig. 2 and Table I, the absolute monounsaturated fatty acid content in each fraction is dramatically reduced in the SCD1−/− mice with corresponding increases in the saturated fatty acids. There were only minor changes in the composition of other fatty acids. The changes measured in the relative amounts of monounsaturated and saturated fatty acids in the phospholipid fractions were much smaller but followed the trend of the cholesterol esters and triglycerides (data not shown).

Dietary 18:1 or 16:1 Did Not Alter Cholesterol Ester and Triglyceride Levels in the Livers of SCD−/− Mice—The cellular oleate or palmitoleate used for hepatic cholesterol ester and triglyceride synthesis, could be synthesized either de novo or by desaturation of exogenous palmitate or stearate indirectly from the diet. To determine whether dietary oleate or palmitoleate could substitute for the endogenously synthesized oleate or palmitoleate and restore the hepatic cholesterol esters and triglycerides of the SCD−/− mice to the levels observed in the heterozygous mice, we supplemented the semipurified mouse diets with high levels of 18:1 or 16:1 (50% of total fat) as triolein or tripalmitolein and then fed these diets to SCD−/− mice for 2 months. Total liver extracts were prepared, and the lipid fractions were analyzed by TLC, and the fatty acid composition of the liver was analyzed by gas-liquid chromatography. Cholesterol esters and triglycerides were also assayed enzymatically and quantitated as mg/g of liver. Feeding diets supplemented with triolein or tripalmitolein to the SCD−/− mice resulted in an increase in the levels of 16:1 and 18:1 in the liver (Table III) but as shown in Fig. 5A, there was no recovery in the synthesis of cholesterol esters and triglycerides in the SCD−/− mice to the levels observed in the heterozygous mice. Instead, as shown in Fig. 5B, there was an 85 and 60% reduction in the cholesterol esters and triglycerides, respectively, in the livers of SCD−/− mice fed with high 18:1 or 16:1. Taken together, these observations suggest that the synthesis of cholesterol esters and triglycerides in liver is dependent on the presence of endogenously synthesized palmitoleate and oleate.

Reduced Levels of Hepatic Cholesterol Esters and Triglycerides in the SCD−/− Mice Is Not Due to Lack of ACAT or GPAT—The low levels of cholesterol esters and triglycerides observed in the SCD−/− mice could have resulted from reduced levels of ACAT or GPAT, the enzymes that catalyze the synthesis of cholesterol esters and triglycerides, respectively. ACAT activity as measured by the rate of conversion of [1-14C]oleate to labeled cholesterol oleate (Fig. 6) was present at almost equal levels in the liver microsomes of both SCD−/− and heterozygous mice. Cholesterol as a substrate of esterification was not limiting because the free cholesterol levels in the liver of the SCD−/− and heterozygous mice were similar (Table I). GPAT activity, as measured by the esterification of palmitoyl-CoA to radioactive glycerol 3-phosphate, was also similar in both groups of animals (Fig. 6). These observations further confirm the requirement of endogenously synthesized oleate or palmitoleate as the limiting substrates for cholesterol ester and triglyceride synthesis in liver.

Cholesterol Esterification Is Enhanced by SCD1 Gene Overexpression—To confirm that the synthesis of cholesterol esters is dependent on the expression of the SCD1 gene, we transiently expressed wild type mouse SCD1 cDNA (4) in cultured CHO cells and measured cholesterol esterification by labeling

![Figure 3](http://www.jbc.org/)

**TABLE II**

Fatty acid composition of livers of SCD−/− and SCD+/+ mice

Female mice were fed control diets for 2 weeks and sacrificed at 5 weeks of age. Liver samples from each mouse were extracted, and the major classes of lipids were separated by TLC. The lipid fractions were methyl-esterified and quantitated by gas-liquid chromatography as described under “Experimental Procedures.” Only the major fatty acids are presented. Bold values denote a level of statistical significance of p < 0.05 between SCD−/− and SCD+/+ (Student’s t test).

<table>
<thead>
<tr>
<th>Genotype of mice</th>
<th>Fatty acid composition (%) of total</th>
<th>16:0</th>
<th>16:1-7</th>
<th>18:0</th>
<th>18:1-9</th>
<th>18:2-6</th>
<th>18:3-3</th>
<th>20:3-6</th>
<th>20:4-6</th>
<th>22:6-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total fatty acids</td>
<td></td>
<td>26.8</td>
<td>0.5</td>
<td>21.8</td>
<td>8.1</td>
<td>20.8</td>
<td>0.0</td>
<td>2.2</td>
<td>17.8</td>
<td>0.1</td>
</tr>
<tr>
<td>Cholesterol esters</td>
<td></td>
<td>23.4</td>
<td>0.4</td>
<td>5.6</td>
<td>34.4</td>
<td>29.6</td>
<td>1.3</td>
<td>1.4</td>
<td>3.0</td>
<td>1.1</td>
</tr>
<tr>
<td>Triglycerides</td>
<td></td>
<td>23.5</td>
<td>0.5</td>
<td>14.3</td>
<td>40.7</td>
<td>13.7</td>
<td>0.8</td>
<td>0.0</td>
<td>1.4</td>
<td>0.0</td>
</tr>
</tbody>
</table>

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the cultured cells with \(^{3}H\)cholesterol for 12 h, followed by TLC analysis of the lipid fraction and scintillation counting (21). The transfection of the SCD1 cDNA resulted in a 3–4 fold increase in SCD enzyme activity (Fig. 7A) and 5–7 fold increase in protein level (Fig. 7B) compared with the control cells that had been transfected with the plant SCD in the antisense orientation. The increase in SCD enzyme activity in the SCD1-transfected cells resulted in increased esterification of cholesterol to cholesterol esters at levels 2-fold higher than in control cells (Fig. 7C). These \textit{in vitro} results suggest that during the synthesis of cholesterol esters, the majority of cellular palmitoleate and oleate were required, because substrates of esterification must be made \textit{de novo} by the SCD1 gene.

**DISCUSSION**

Stearoyl-CoA desaturase has been implicated as a regulatory enzyme in lipogenesis, because stearoyl-CoA desaturation is the rate-limiting step in the overall \textit{de novo} synthesis of unsaturated fatty acids from acetyl-CoA in animal tissues (7). Why SCD is highly regulated and yet oleate, the major product of this enzyme is one of the most abundant fatty acid in the diet has not been well addressed. For instance, dietary oleate is well known for its hypotriglyceridemic effects and yet high SCD activity has been implicated in diabetes, obesity, and atherosclerosis in several animal models (7). By using the asebia mutant mouse strain that has a null mutation in the SCD1 gene as a model, we have begun to address the physiological role of SCD gene expression in normal and disease states. We found that the asebia mice have a deficiency in cholesterol esters and triglycerides in liver and demonstrated by expression of the SCD1 gene in CHO cells that the synthesis of cholesterol esters, is dependent on SCD1 gene expression. This observation suggests that endogenously synthesized monounsaturated fatty acids most likely serve as the main substrates for the synthesis of cholesterol esters. Our observations also indicate that endogenously synthesized monounsaturated fatty acids most likely serve as the main substrates for the synthesis of hepatic triglycerides. The alteration in the levels of monounsaturated fatty acids as a result of SCD1 gene expression can therefore have a wide range of effects on cholesterol and lipoprotein metabolism.

It has always been perceived that dietary intake is ultimately the major lipid source of monounsaturated fatty acids that get incorporated in triglycerides and cholesterol esters. The concept that SCD modulation in animal tissues differs from feeding animals diets rich in oleate or monounsaturated fatty acids has not been rigorously tested. Dietary oleate or palmitoleate from the small intestine would reach the liver mainly as cholesterol esters of chylomicron remnants. The cholesterol esters are then cleaved into free cholesterol and free oleate and palmitoleate in the lysosomes. ACAT or GPAT would then use the free oleate and palmitoleate as substrates for cholesterol ester or triglyceride synthesis. We tested the possibility that dietary oleate and palmitoleate would serve as substrates of ACAT or GPAT and correct the deficiency in cholesterol esters and triglycerides in the SCD1–/– mice by supplementing the semipurified diet with high levels of oleate or palmitoleate. After several weeks of feeding, our results indicated that dietary oleate or palmitoleate could not correct the deficiency in cholesterol ester and triglyceride levels in the SCD–/– mice despite an increase in the levels of 16:1 and 18:1 in the liver as well as normal activities of ACAT and GPAT. This observation may be explained if we consider that dietary oleate or palmitoleate does not necessarily increase the availability of nonesterified oleate or palmitoleate at the very site of cholesterol ester or triglyceride synthesis. The endogenously synthesized oleate or palmitoleate, which arise from SCD activity in the endoplasmic reticulum, would be better substrates for ACAT and GPAT. When we expressed the mouse SCD1

![FIG. 4. The ratio of palmitoleate (16:1) to palmitate (16:0) and oleate (18:1) to stearate in cholesterol ester and triglyceride fraction of SCD–/– and heterozygous (+/–) mice. Total lipids were extracted from livers of SCD–/– and heterozygous mice. Lipid extracts were pooled and analyzed by TLC, methyl esterified, and quantitated by gas-liquid chromatography. The values used for calculating the ratio of monounsaturated to saturated fatty acids are derived directly from the values presented in Table III.](http://www.jbc.org/)
cDNA in CHO cells there was increased expression of SCD protein and enzyme activity with a corresponding increase in esterification of cholesterol to cholesterol esters. Although this could be artifact of overexpression of the SCD1 cDNA, this in vitro result demonstrates that SCD1 gene expression is required to generate monounsaturated fatty acids as the preferred substrates for cholesterol ester synthesis. Because SCD, ACAT, and GPAT are endoplasmic reticulum membrane enzymes, a possible physiological explanation for the requirement of SCD expression is to produce more readily accessible substrate within the vicinity of ACAT and GPAT to aid in the efficient esterification of cholesterol and glycerol 3-phosphate for cholesterol ester and triglyceride synthesis, respectively.

The mouse and rat genomes contain two well characterized structural genes (SCD1 and SCD2) that are highly homologous at the nucleotide and amino acid level and encode the same functional protein (3, 4). The two SCD genes are products of different genes, but despite the fact that both genes are structurally similar, sharing ~87% nucleotide sequence identity in the coding regions, their 5’-flanking regions differ, resulting in divergent tissue-specific gene expression. As shown in the present investigation, despite the expression of the SCD2 in liver of SCD1−/− mice, the full esterification of cholesterol could not occur, suggesting that the SCD2 isoform could not compensate for the SCD1 deficiency. The physiological significance of having two or more mouse SCD isoforms expressed in the same tissue is not currently known but could be related to the substrate specificity of each SCD isoform or the means by which cells compartmentalize lipid biosynthesis for specific functions.
The absence of SCD protein in liver microsomes (Fig. 1C) but residual activity in the total extracts may suggest that SCD1 and SCD2 reside in different cellular compartments.

The consequences of the changes in expression of SCD are relevant to cholesterol and lipoprotein homeostasis. The majority of endogenous synthesis of fatty acids in liver is directed toward cholesterol ester and triglyceride synthesis and secretion (5). Oleic acid, the major product of SCD, is the preferred substrate for ACAT, the enzyme responsible for esterification of cholesterol (22). SCD activity was shown to increase significantly in liver of rats fed high cholesterol diets with a corresponding increase in cholesterol oleate content (23). Additionally, overexpression of sterol regulatory element-binding proteins-1a, 1c, and 2 in mouse liver has been shown to induce SCD1 mRNA and enzyme activity, resulting in the dramatic increase in cholesterol esters and triglycerides of oleate and palmitoleate (5). The increased esterification of cholesterol would prevent the toxic accumulation of free cholesterol in liver, and the increase in the availability of cholesterol esters and triglycerides would lead to their secretion by the liver in the form of VLDL (24). We observed a dramatic decrease in total cholesterol esters and triglycerides in liver and plasma of SCD −/− mice (Table I). Very low levels of VLDL particles with very low levels of triglycerides were present in the SCD −/− mice, suggesting that SCD1 deficiency affects VLDL synthesis, secretion, or possibly clearance from plasma.

In conclusion, the present work demonstrates that cholesterol ester and triglyceride synthesis in mouse liver is highly dependent on the expression of the SCD1 gene. We propose that dietary oleate or palmitoleate does not necessarily increase the availability of nonesterified oleate or palmitoleate at the very site of cholesterol ester and triglyceride synthesis in liver. Normally cholesterol esters are stored in liver hepatocytes or together with triglycerides can be packaged into VLDL and secreted in plasma for transport to other tissues. However, in the absence of cellular monounsaturated fatty acids because of SCD1 deficiency, the levels of hepatic cholesterol esters and triglycerides are reduced, leading to changes in cellular cholesterol levels and plasma lipoprotein profile. Thus, the SCD1 gene may be another checkpoint in the process of cholesterol homeostasis and lipoprotein metabolism and may have broad implications for its potential use as a target in human disease.

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REFERENCES

The Biosynthesis of Hepatic Cholesterol Esters and Triglycerides Is Impaired in Mice with a Disruption of the Gene for Stearoyl-CoA Desaturase 1
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