A lipogenic diet in mice with a disruption of the stearoyl-CoA desaturase 1 gene reveals a stringent requirement of endogenous monounsaturated fatty acids for triglyceride synthesis

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Abstract **Stearoyl-CoA desaturase (SCD) catalyzes the de novo biosynthesis of oleate and palmitoleate, which are the major fatty acids found in triglycerides, cholesteryl esters, and phospholipids. A high carbohydrate (lipogenic) diet induces lipogenic gene expression by sterol regulatory element binding protein 1 (SREBP-1c)-mediated gene transcription, leading to an increase in the synthesis of triglycerides. The lipogenic diet fed to mice with a null mutation in the SCD1 gene (SCD−/−) fails to induce the synthesis of triglycerides in liver, despite the induction of expression of SREBP-1 and its target genes, fatty acid synthase and glycerol-3-phosphate acyltransferase. The lipogenic diet led to a decrease in the levels of triglyceride, but an increase in the level of cholesteryl esters of saturated fatty acids. Feeding a lipogenic diet supplemented with high levels of oleate to the SCD−/− mice resulted in incorporation of oleate in the liver of SCD−/− mice, but failed to restore triglycerides to the levels in the normal mouse. Triglyceride synthesis, as measured by the incorporation of [^{3}H]glycerol, was dramatically reduced in the liver of SCD−/− mouse fed a lipogenic diet compared with the normal mouse. These observations demonstrate that induction of triglyceride synthesis is highly dependent on SCD1 gene expression.—** Miyazaki, M., Y-C. Kim, and J. M. Ntambi. **A lipogenic diet in mice with a disruption of the stearoyl-CoA desaturase 1 gene reveals a stringent requirement of endogenous monounsaturated fatty acids for triglyceride synthesis. *J. Lipid Res.* 2001.** 42: **1018–1024.**

Supplementary key words triglycerides • cholesteryl esters • gene expression • SREBP

Stearoyl-CoA desaturase (SCD) is a key enzyme involved in the biosynthesis of unsaturated fatty acids, as well as the regulation of this process. It catalyzes the Δ^9-desaturation of long-chain fatty acids, leading to biosynthesis of palmitoleic (C16:1) and oleic (C18:1) acids as major products (1). Palmitoleic and oleic acids are the major monounsaturated fatty acids of triglycerides, cholesteryl esters, and membrane phospholipids. When fasted animals are subsequently fed a high carbohydrate (lipogenic) diet, there is a dramatic increase in the levels of SCD and several other enzymes involved in fatty acid and triglyceride synthesis such as acetyl-CoA carboxylase, fatty acid synthase (FAS), and glycerol-3-phosphate acyltransferase (GPAT) (2–3). The coordinate induction of these enzymes is due to increased sterol regulatory element binding protein 1 (SREBP-1)-stimulated gene transcription (4–8). The induction of lipogenic gene transcription is followed by a prompt increase in fatty acids and triglycerides to the levels above those observed in animals fed normally (2). The majority of the excess triglycerides are incorporated and secreted by the liver as VLDL-TG for transport to other tissues (9). A high level of VLDL-TG in plasma is a risk factor for cardiovascular disease (10) and, therefore, identifying a control point in the pathway of triglyceride biosynthesis can be a selective target for treating the condition.

Several isoforms of SCD exist in the mouse genome. SCD1 and SCD2, which are products of different genes, are the most well characterized (3, 11, 12). Most organs of different mouse strains express SCD1 and SCD2 with the exception of liver, which expresses mainly the SCD1 isoform. SCD2 is constitutively expressed in the brain (11), and is expressed at high levels in livers of mice that overexpress the truncated nuclear form of SREBP-1a (7). SCD3 is expressed mainly in the skin (12). Several in vivo studies have shown that SCD1 gene expression is highly regulated by dietary changes, hormonal factors, developmental processes, and peroxisomal proliferators (12), implicating the role of SCD in several physiological processes.

Abbreviations: FAS, fatty acid synthase; GPAT, glycerol-3-phosphate acyltransferase; SCD, stearoyl-CoA desaturase; SREBP-1, sterol regulatory element binding protein 1.

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Recent studies using the asebia mutant mouse model have begun to address the role of SCD1 gene expression and its products, the monounsaturated fatty acids, in physiological processes (13–15). The asebia mouse mutation (ab), an autosomal recessive trait characterized by hypoplastic sebaceous glands, arose more than 30 years ago in a colony of BALB/c Crg/Ga mice (15, 16). A similar mutation, designated ab^{j}, arose spontaneously in the BALB/cJ inbred strain at the Jackson Laboratory (Bar Harbor, ME), and was found to be allelic to asebia (16, 17). Using positional and high resolution genetic mapping, the ab^{j} mutation was localized to chromosome 19 and has recently been determined to be in the SCD1 gene (14). Further characterization of the asebia mice showed that they express the SCD2 mRNA isoform in various tissues, but lack the SCD1 gene function (13, 14). In addition to the presence of hypoplastic sebaceous glands, the asebia mice appear hairless with a short sparse hair coat and dry scaly skin (14). The mice also suffer from corneal opacities and hypoplastic meibomian glands, implicating the SCD1 in normal ocular barrier function. In addition, we recently showed that the livers of asebia mice are deficient in hepatic triglycerides and cholesteryl esters (13), suggesting that the SCD1 gene plays an important role in cholesterol and lipoprotein metabolism. The mechanisms leading to these various phenotypes are not yet known, but are postulated to involve deficiencies in the de novo biosynthesis of sufficient monounsaturated fatty acids, mainly oleate and palmitoleate, that serve as the major substrates for the biosynthesis of neutral lipids.

In the present study, we fed a lipogenic diet to mice, and examined the role of SCD1 gene expression in the induction of de novo synthesis of hepatic triglycerides. Normally, a lipogenic diet induces SCD1 gene expression and other lipogenic genes, resulting in increased levels of monounsaturated fatty acids and triglycerides in liver. This feeding regimen allowed us to analyze the fate of endogenously synthesized monounsaturated fatty acids without interference from exogenous sources. We showed that a lipogenic diet failed to induce triglyceride synthesis in a SCD−/− mouse, despite the induction of expression of SREBP-1 and two of its target genes, FAS and GPAT. Feeding a lipogenic diet supplemented with high levels of triolein to the SCD−/− mice resulted in incorporation of oleate into the liver of SCD−/− mice, but failed to restore triglycerides to the levels found in the normal mouse. Further, the rate of triglyceride synthesis, as measured by the incorporation of [^{3}H]glycerol, was dramatically reduced in the livers of SCD−/− mice. Taken together, our results show that the expression of SCD1 is important for the de novo synthesis of monounsaturated fatty acids that act as the main substrates for the dietary induction of triglyceride biosynthesis in liver. Because triglycerides are a major source of stored energy, and most of the hepatic triglycerides are secreted in the form of VLDL for transport to other tissues, the dietary regulation of SCD gene expression may play a larger role in energy and lipoprotein metabolism than was previously thought.

MATERIALS AND METHODS

Animals and diets

Asebia homozygous (SCD−/−) and heterozygous (SCD+/−) mice, originally obtained from the Jackson Laboratory (Bar Harbor, ME), were reared and housed in a pathogen-free animal facility of the department of Biochemistry of the University of Wisconsin–Madison, operating at a 12-h light/12-h dark cycle. The breeding of the animals was in accordance with the protocols approved by the Animal Care Research Committee of the University of Wisconsin–Madison. In this study, the heterozygous mice are considered to have the normal phenotype (13, 14). At 16 weeks of age, the mice were fed, ad libitum for 2 days either chow diet (5008 test diet; PMI Nutrition International Inc., Richmond, IN), high carbohydrate diet (TD 99252, Harlan Teklad, Madison WI), or high carbohydrate diet supplemented with triolein (50% of total fat). The high carbohydrate diet contained, by weight, 21% casein, 14% maltodextrin, 55% sucrose, 5% cellulose, 3% mineral mix (AIN-93G-MX), and 1% vitamin mix (AIN-93-VX).

Materials

Radioactive [^{32}P]dCTP (3,000 Ci/mmol) was obtained from DuPont Co. (Wilmington, DE). TLC plates (TLC Silica Gel G60) were from Merck (Darmstadt, Germany). [1,2,3-^{3}H]-glycerol was obtained from American Radiolabeled Chemicals Inc. (St. Louis, MO). The cDNA probes for FAS and GPAT were obtained from Dr. H. Sul, University of California, Berkeley. All other chemicals were purchased from Sigma (St. Louis, MO).

Lipid analysis

Total lipids were extracted from liver according to the method of Bligh and Dyer (18), and were separated by silca gel TLC using petroleum ether–diethyl ether–acetic acid (80:30:1) as the developing solvent. The lipids were visualized by cupric sulfate in 8% phosphoric acid. The lipids were scraped, methylated, and analyzed by gas-liquid chromatography as previously described (13). Triheptadecanoylglcerol, heptadecanoyl cholesterol, and 1,2-dihepatadecanoyl-L-α-phosphatidylcholine (Sigma) were added prior to lipid extraction as internal standards for the quantitation of triglycerides, cholesteryl esters, and phospholipids, respectively.

Isolation and analysis of RNA

Total RNA was isolated from livers using the acid guanidinium-phenol-chloroform extraction method (19). Twenty micrograms of total RNA were separated by 1.0% agarose/2.2 M formaldehyde gel electrophoresis, and transferred onto nylon membrane. The membrane was hybridized with ^{32}P-labeled FAS (2), GPAT (20), SREBP-1c (21), and SCD1 cDNA probes (3). pAL15 cDNA probe (19) was used as a control for equal loading of RNA.

In vivo assay for triglyceride synthesis using [^{3}H]glycerol

[1,2,3-^{3}H]glycerol was dissolved in 0.9% NaCl at a concentration of 50 μCi/0.15 ml. Mice that had been fed high carbohydrate diet or chow diet were injected intraperitoneally with 50 μCi of [^{3}H]glycerol 15 min before being sacrificed (22). Hepatic lipids were extracted using Bligh and Dyer's method and separated by TLC using hexane–ether–acetic acid (90:30:1, v/v) as developing solvent. Each lipid extract was scraped off the plate, and the radioactivity was measured using a liquid scintillation counter.

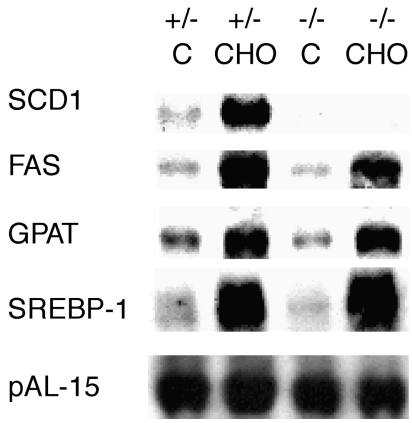

Fig. 1. Northern blot analysis for the expression of SCD1, SREBP-1, FAS, and GPAT mRNA from livers of SCD1−/− and heterozygous (+/−) mice fed a chow (C) or lipogenic diet (CHO). Total RNA (20 μg) pooled from four mice of each group was subjected to Northern analysis followed by hybridization with labeled probes specific for SCD1, SREBP-1, FAS, and GPAT. A cDNA probe for pAL15 (19) was used to confirm equal loading of RNA.

RESULTS

Figure 1 shows a Northern blot of total RNA isolated from the livers of SCD−/− and SCD+/− mice that were fed a chow diet or a lipogenic diet for 2 days and analyzed the expression of SCD1, SREBP-1, FAS, and GPAT mRNAs. There were no noticeable alterations in food intake between the SCD−/− and the wild-type controls. The expression of these genes is induced by a lipogenic diet, resulting in increased levels of fatty acids and triglycerides (2–4). As shown previously (13), the SCD1 mRNA is not detectable in liver of SCD1−/− mice, whereas SCD+/− mice that are phenotypically indistinguishable from normal mice (14) express the 4.9-kb SCD1 mRNA. The SREBP-1, FAS, and GPAT mRNA were detectable in SCD−/− and SCD+/− animals on a chow diet. Upon feeding with a lipogenic diet, the SCD1 mRNA expression was induced 12-fold only in the SCD+/− mouse, whereas the SREBP-1, FAS, and GPAT mRNAs were induced 9.5-, 22-, and 6.5-fold, respectively, in both the SCD−/− and SCD+/− mouse. pAL 15 mRNA expression used as a loading control did not change in both groups of animals on either chow diet or lipogenic diet.

Figure 2 shows TLC analysis of lipids extracted from liver of SCD−/− and SCD+/− mice fed a chow diet or a lipogenic diet. The levels of cholesteryl esters and triglycerides of SCD−/− mice on a chow diet were lower than those of SCD+/− mice, consistent with our previous results (13). When the mice were fed a lipogenic diet, there was a marked increase in triglyceride levels in livers of SCD +/− mice, whereas SCD−/− mice showed a marked reduction in the triglyceride levels. The cholesteryl ester fraction was increased in both SCD+/− and SCD−/− mice. **Table 1** shows that the triglyceride content in liver of SCD+/− mice increased by 2.3-fold, whereas the triglyceride content in the SCD1−/− mice decreased by 59% compared with the animals on a chow diet. The cholesteryl ester fraction was increased by 4.1- and 5.0-fold, respectively, in the livers of the SCD+/− and SCD−/− mice.

Table 2 shows the relative percentage compositions of the major fatty acids measured in the total lipid fraction, as well as in the triglyceride, cholesteryl ester, and phospholipid fractions of livers of SCD+/− and SCD−/− mice fed a lipogenic or chow diet. In the total lipid fraction, the SCD1+/− livers from mice fed a lipogenic diet compared with a chow diet had a 54% increase in the relative amount of 16:1, and a 45% increase in 18:1. In the tri-

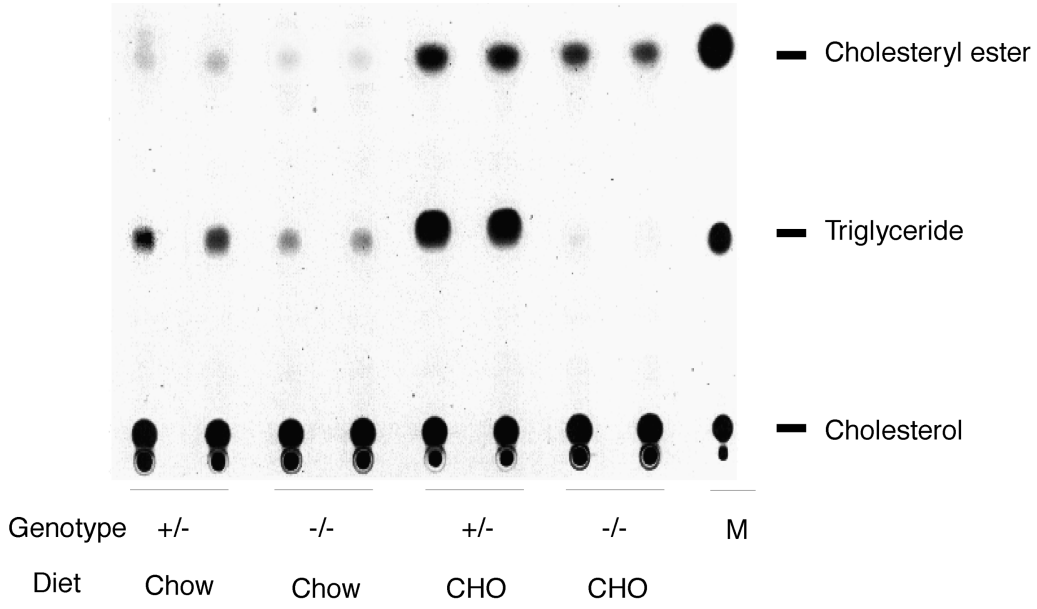

Fig. 2. TLC of lipid extracts from livers of SCD1−/− and heterozygous (SCD+/−) mice fed chow or lipogenic diet for 2 days. Total lipids were extracted from livers of heterozygous and SCD1−/− mice. Lipid extracts were pooled and analyzed by TLC. Equivalent amounts of lipid extract (from 2.5 mg of tissue homogenate) were loaded in each lane. Each lane represents lipids from livers of three mice.

TABLE 1. Phenotypic differences in neutral lipid content between SCD−/− and SCD+/− mice

Lipids	Chow Diet		CHO Diet	
	+/−	−/−	+/−	−/−
	mg/g liver			
Triglycerides	6.0 ± 0.2	3.6 ± 0.7[a]	13.5 ± 1.3[b]	1.5 ± 0.3[c,d]
Cholesteryl esters	1.1 ± 0.0	0.4 ± 0.1[a]	4.5 ± 0.5[b]	2.0 ± 0.4[c,d]
Phospholipids	22.5 ± 0.9	23.3 ± 0.5	19.8 ± 0.4	22.9 ± 1.1

Values are means ± SD (n = 4). Female mice were fed chow diet and lipogenic (CHO) diet for 2 days. Liver triglycerides, cholesteryl esters, and phospholipids were measured as described under Materials and Methods.

[a] $P < 0.01$ between chow diet-fed SCD+/− and chow diet-fed SCD−/− mice.

[b] $P < 0.01$ between chow diet-fed SCD+/− and CHO diet-fed SCD+/− mice.

[c] $P < 0.01$ between chow diet-fed SCD−/− and CHO diet-fed SCD−/− mice.

[d] $P < 0.01$ between CHO diet-fed SCD+/− and CHO diet-fed SCD−/− mice.

glyceride fraction, the relative amount of 16:1 increased by 42%, whereas that of 18:1 increased by 26%. In the liver cholesteryl ester fraction, the relative amount of 16:1 and 18:1 increased by 28% and 15%, respectively. The phospholipid fraction showed a 44% and 38% increase, respectively, in 16:1 and 18:1. In the total lipid fraction, the SCD1−/− livers from mice fed a lipogenic diet compared with the SCD+/− mice had a 100% decrease in the relative amount of 16:1 and a 63% decrease in 18:1. In the hepatic triglyceride fraction, the relative amount of 16:1 decreased by 76%, whereas that of 18:1 decreased by 70%. The liver cholesteryl ester fraction of SCD1−/− mice showed a 67% decrease of 16:1 and a 69% decrease of 18:1, whereas the phospholipid fraction showed a 78% decrease in 16:1 and a 55% decrease in 18:1.

Figure 3 shows the content (mg/g of liver) of the monounsaturated and saturated fatty acids measured in the triglyceride, cholesteryl ester, and phospholipid fractions in the livers of SCD+/− and SCD−/− mice fed a lipogenic diet or chow diet. In the triglyceride fraction of the SCD+/− mouse on a lipogenic diet, the amount of monounsaturated fatty acids increased by 3.1-fold, whereas that of saturated fatty acids increased by 1.9-fold when compared with the chow diet. The liver of SCD−/− mice showed a 97% reduction in the monounsaturated fatty acids compared with the SCD+/− mice. The triglyceride fraction of the SCD1−/− mice showed a 36% decrease in the contents of saturated fatty acids (16:0 and 18:0) compared with the liver of animals on a chow diet. The cholesteryl ester fraction showed a greater than 8.2-fold increase in the content of saturated fatty acids in both the SCD−/− and SCD+/− mice. These data indicate that the increased levels of cholesteryl ester observed in the SCD−/− mouse in response to a lipogenic diet (Fig. 2, Table 1) is most likely due to incorporation of saturated fatty acids, mainly 18:0 and 16:0, in this fraction. The phospholipid fraction also incorporated saturated fatty acids, but to a lesser extent than the cholesteryl ester fraction. Because very low levels of saturated fatty acids were incorporated in the triglyceride fraction, the results would suggest

TABLE 2. Fatty acid composition of livers from SCD+/− and SCD−/− mice fed lipogenic diet or chow diet

Lipids	16:0	16:1	18:0	18:1	18:2	20:4	22:6
				%			
Total lipids							
+/−							
Chow	28.5	0.6	17.6	16.2	14.8	14.9	7.4
CHO	28.5	1.3[a]	14.2[a]	29.3[a]	9.6[a]	11.8	5.3
−/−							
Chow	30.3	0.2	20.4	10.3	17.0	13.3	8.5
CHO	31.5[c]	0.0[c]	23.1[b,c]	10.7[c]	14.3[b,c]	12.0	8.4[c]
Triglycerides							
+/−							
Chow	21.2	1.0	5.3	46.2	17.0	2.3	5.9
CHO	18.4[a]	1.7[a]	3.7[a]	62.6[a]	8.5[a]	1.2[a]	3.1
−/−							
Chow	26.9	0.4	10.0	25.8	23.8	2.1	9.9
CHO	29.7[b,c]	0.4[c]	26.6[b,c]	18.7[b,c]	12.5[b,c]	2.6[c]	6.3[c]
Cholesteryl esters							
+/−							
Chow	24.3	4.4	6.6	54.4	8.8	0.8	0.0
CHO	16.9[a]	6.1[a]	5.7	64.3[a]	4.6[a]	2.4[a]	0.0
−/−							
Chow	16.9	3.2	25.4	32.3	18.7	3.5	0.0
CHO	37.4[b,c]	2.0[b,c]	32.4[b,c]	19.8[b,c]	6.1[b]	2.2	0.0
Phospholipids							
+/−							
Chow	22.3	0.5	15.7	11.9	14.4	19.4	13.3
CHO	23.0	0.9[a]	14.4	19.2[a]	10.2[a]	18.9	11.5
−/−							
Chow	23.7	0.1	19.3	6.6	15.7	18.6	14.4
CHO	27.1[b,c]	0.2[c]	18.7[c]	8.6[c]	14.5[c]	15.2	13.8

Liver lipids from individual mice of each group were extracted, and major classes of lipids were separated on TLC. The lipid fractions were methyl-esterified and quantified by gas-liquid chromatography as described under Materials and Methods. Standard errors of the mean were all less than 20% of the mean and are omitted for clarity.

[a] $P < 0.01$ between chow diet-fed SCD+/− and chow diet-fed SCD−/− mice.

[b] $P < 0.01$ between chow diet-fed SCD+/− and CHO diet-fed SCD+/− mice.

[c] $P < 0.01$ between chow diet-fed SCD−/− and CHO diet-fed SCD−/− mice.

that the triglycerides synthesized in response to lipogenic diet contain mainly monounsaturated fatty acids.

To determine whether dietary oleate could substitute for the endogenously synthesized oleate and restore the hepatic triglycerides of the SCD−/− mice to the levels observed in the heterozygous mice, we supplemented the lipogenic diets with high levels of 18:1 (50% of total fat) as triolein, and fed it to the mice for 2 days. There were no noticeable alterations in food intake between the SCD−/− and the wild-type controls. Total liver extracts were prepared, and the lipid fractions were analyzed by TLC. The fatty acid composition of the liver was analyzed by gas-liquid chromatography. **Table 3** shows that feeding diets supplemented with triolein to the SCD−/− mice resulted in an increase in the levels of 18:1 in the liver. However, as shown (**Fig. 4**), there was no recovery in the levels of triglycerides in the SCD−/− mice to the levels observed in the SCD+/− mice fed a lipogenic diet or even to the levels of the SCD−/− mice fed a chow diet. This observation suggests that the increase in the levels of triglycerides in liver in response to a lipogenic diet is largely dependent on the presence of endogenously synthesized oleate.

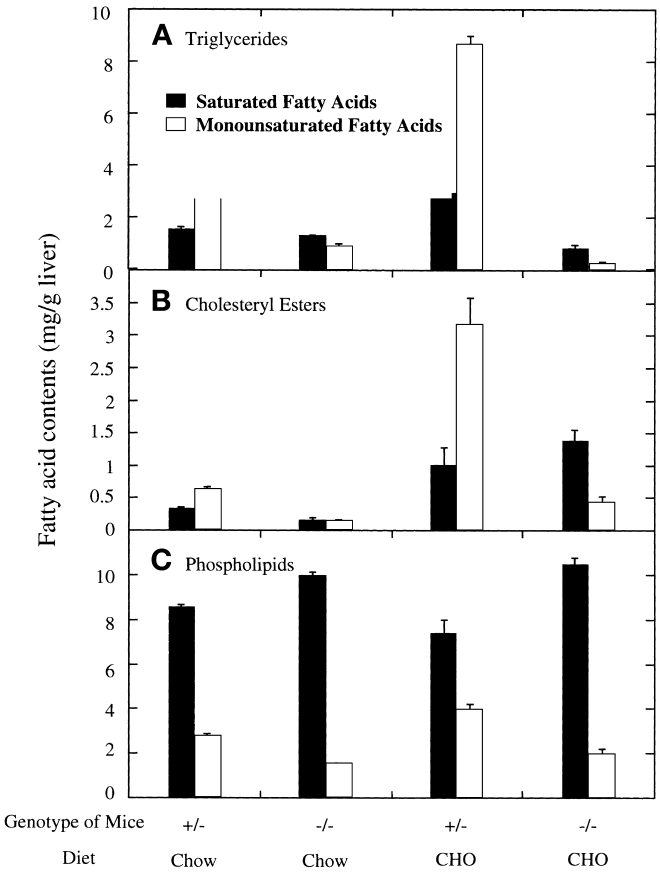

Fig. 3. The contents of saturated and monounsaturated fatty acids in triglyceride, cholesteryl ester, and phosholipid fractions of SCD−/− and SCD+/− mice fed a chow diet or a lipogenic diet. Triheptadecanoylglycerol, heptadecanoyl cholesteryl, and 1,2-dihepatadecanoyl-L-α-phosphatidylcholine were added prior to lipid extraction as internal standards for the quantitation of triglycerides, cholesteryl esters, and phospholipids, respectively. Lipid extracts were pooled and analyzed by TLC, methyl esterified, and quantitated by gas-liquid chromatography.

To establish that the low levels of triglycerides in the SCD−/− mice are due to lower triglyceride synthesis rates, we used [^{3}H]glycerol as a precursor of lipid synthesis to directly measure newly synthesized triglyceride in livers of SCD+/− and SCD−/− mice fed a lipogenic diet. **Figure 5** shows that the triglyceride synthetic rate was 7-fold higher in the liver of the heterozygous mice, but only 1.25-fold in the liver of the SCD−/− mice. This figure also shows that although triglyceride synthesis was reduced, that of the phospholipid fraction was increased in both the SCD−/− and SCD+/− mice.

DISCUSSION

It is well known that feeding a lipogenic diet to rodents induces SCD1 gene expression and several other lipogenic genes, leading to high levels of monounsaturated fatty acids and triglycerides (2–4). This feeding regimen allows for the analysis of the fate of de novo synthesized

TABLE 3. Fatty acid composition of livers from high oleate diet-fed SCD−/− mice

Lipids	16:0	16:1	18:0	18:1	18:2	20:4	22:6
				%			
Total lipids							
Chow	30.3	0.2	20.4	10.3	17.0	13.3	8.5
CHO	31.5	0.0	23.1	10.7	14.3	12.0	8.4
CHO+18:1	32.3	0.7[a,b]	24.3[a]	24.2[a,b]	6.8[a,b]	6.9[a,b]	4.8[a,b]
Triglycerides							
Chow	26.9	0.4	10.0	25.8	23.8	2.1	9.9
CHO	29.7	0.4	26.6	18.7	12.5	2.6	6.3
CHO+18:1	32.5[a]	0.7[a,b]	26.8[a]	35.4[a,b]	2.5[a,b]	0.7[a,b]	1.0[a,b]

Liver lipids from individual mice of each group were extracted, and major classes of lipids were separated on TLC. The lipid fractions were methyl-esterified and quantified by gas-liquid chromatography as described under Materials and Methods. Standard errors of the mean were all less than 15% of the mean and are omitted for clarity.

[a] $P < 0.01$ between chow diet-fed SCD−/− and high oleate (CHO+18:1) diet-fed SCD−/− mice.

[b] $P < 0.01$ between CHO diet-fed SCD−/− and CHO+18:1 diet-fed SCD−/− mice.

fatty acids without interference from exogenous sources. Using this feeding regimen, we showed that a lipogenic diet fails to induce triglyceride synthesis in SCD−/− mice, despite the induction of expression of SREBP-1, as well as two of its target genes, FAS and GPAT, involved in saturated fatty acid and triglyceride synthesis, respectively. The triglyceride levels, instead, were decreased. We have established that the lower hepatic triglyceride levels are due to a lower rate of triglyceride synthesis in the mutant mice. Because the SCD+/− and the SCD−/− mice consumed equivalent amounts of food, the decrease in the triglyceride levels in the mutant mouse could be due to increased β-oxidation. We also demonstrated that dietary monounsaturated fatty acids could not substitute for the

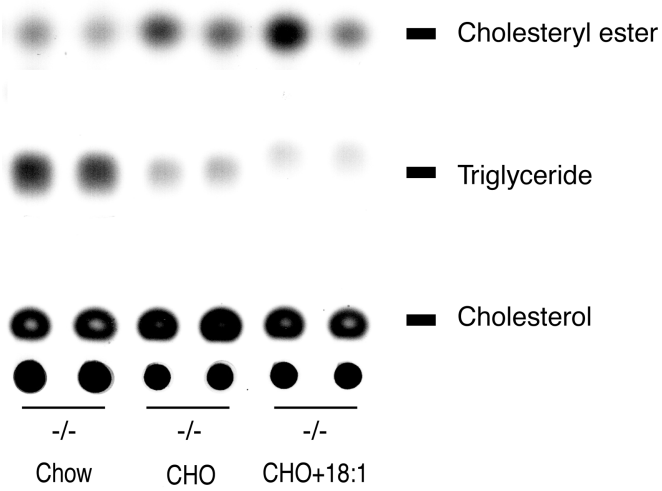

Fig. 4. TLC of lipid extracts from livers of SCD−/− mice fed high oleate (CHO+18:1) diet for 2 days. Total lipids were extracted from livers of SCD−/− mice. Lipid extracts were pooled and analyzed by TLC as described in Materials and Methods. Equivalent amounts of lipid extract (from 2.5 mg of tissue homogenate) were loaded in each lane. Each lane represents lipids from liver of one mouse.

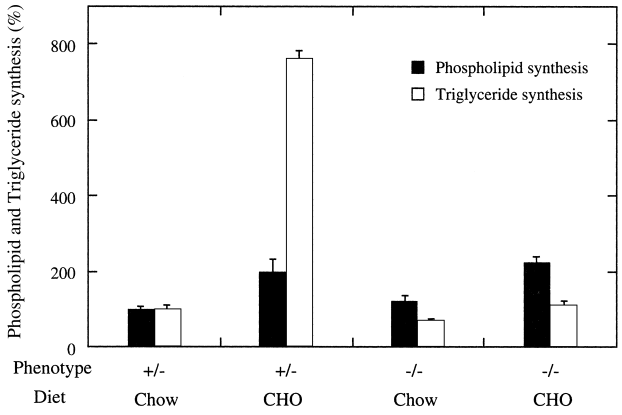

Fig. 5. The rate of glycerolipid synthesis in liver. Mice fed chow diet or lipogenic diet for 2 days were intraperitoneally injected with 50 μCi of [^{3}H]glycerol 15 min before being sacrificed. After extraction of hepatic lipids, phospholipids and triglycerides were separated, and the radioactivity was determined. Data are expressed as a percentage of the values of chow diet-fed heterozygous mice.

ones endogenously synthesized by SCD as substrates for adequate triglyceride synthesis in liver.

The consequences of regulating the SCD1 gene expression by diet may be relevant to lipoprotein metabolism. The majority of de novo synthesis of fatty acids in liver is directed toward triglyceride synthesis and secretion (7). The deficiency in triglyceride levels that we observed could have been due to increased synthesis or secretion of VLDL-TG. However, the lipoprotein profile previously performed on plasma of the SCD−/− showed that these mice have very low levels of triglycerides in the VLDL, LDL, and HDL lipoprotein fractions (13). The current results, therefore, suggest that in the absence of cellular monounsaturated fatty acids due to SCD1 deficiency, the levels of hepatic triglycerides are reduced, leading to lower levels of plasma VLDL-TG.

The use of a lipogenic diet supplemented with high levels of triolein enabled us to test the possibility that the liver could utilize dietary monounsaturated fatty acids instead of those generated in vivo by SCD for de novo synthesis of triglycerides. Dietary oleate or palmitoleate from the small intestine would reach the liver mainly as glycerol esters of chylomicron remnants. The triglycerides are then cleaved into free glycerol and free oleate and palmitoleate in the lysosomes. Because the SCD−/− mice possess normal activity of GPAT (13), this enzyme would then use the freed oleate and palmitoleate as substrates for triglyceride synthesis. We found that feeding a lipogenic diet rich in monounsaturated fatty acids did not correct the deficiency in triglyceride levels in the SCD−/− mice, despite an increase in the levels of 16:1 and 18:1 in the liver. This observation suggests that although the monounsaturated fatty acids reach the liver, they do not necessarily become fatty acyl-CoA substrates at the site of de novo synthesis of liver triglycerides. It is possible that dietary monounsaturated fatty acids and those synthesized endogenously constitute different pools and end up in different environments of the endoplasmic reticulum or cellular compartments where they may be utilized for different purposes. A possible physiological explanation for the requirement of dietary induction of SCD expression is to produce more readily accessible substrates within the vicinity of GPAT to aid in the efficient esterification of glycerol 3-phosphate for triglyceride synthesis.

Feeding a lipogenic diet to the SCD−/− mice led to further accumulation of saturated fatty acids in liver. We found that the saturated fatty acids made endogenously were incorporated into the cholesteryl ester and, to a lesser extent, in the phospholipid fraction, resulting in an increase in the levels of these lipids in liver of both the SCD−/− and SCD+/− mice. However, the saturated fatty acids failed to elevate the levels of the triglyceride fraction in the SCD−/− mice. This observation thus suggests that the endogenously synthesized oleate or palmitoleate, which arise from SCD activity in the endoplasmic reticulum, are critical substrates for the synthesis of triglycerides by GPAT. The results also indicate a more stringent requirement of monounsaturated fatty acids as substrates of diacylglycerol acyltransferase than ACAT. The diacylglycerol acyltransferase step may be important in selecting monounsaturated fatty acids over saturated fatty acids, providing a mechanism to funnel saturated fatty acids into metabolic pools.

The mouse and rat genomes contain several SCD genes, two of which (SCD1 and SCD2) are well characterized (3, 11). These two genes, which are products of two separate genes, are highly homologous at both the nucleotide and amino acid sequence levels, but exhibit divergent tissue-specific expression. Most organs of different mouse strains express SCD1 and SCD2, with the exception of liver, which expresses mainly the SCD1 isoform. In liver, a lipogenic diet and insulin induce SCD1 gene expression, but SCD2 is silent under both conditions (3, 23). The insulin induction of SCD1 gene expression is thought to be mediated by the truncated nuclear form of the SREBP-1c. Liver SCD2 becomes expressed at higher levels only in livers of mice that overexpress SREBP-1 (7). Thus, the transcription of both SCD1 and SCD2 can be regulated by SREBP-1c. We have shown previously that SCD2 mRNA is present in the SCD−/− mice (13), and although expressed at low levels, would be expected to be induced by SREBP-1-mediated gene transcription. However, despite the induction of SREBP-1 by a lipogenic diet and the expression of SCD2 in liver of the SCD−/− mice, the levels of triglycerides remained low, suggesting that SCD2 could not compensate for the SCD1 deficiency. These results point to SCD1 gene expression as a key control point in the induction of triglyceride synthesis in liver.

In conclusion, the present work demonstrates that dietary induction of triglyceride synthesis in mouse liver is highly dependent on the expression of the SCD1 gene. SCD2 cannot compensate for the deficiency. We propose that the synthesis of triglycerides requires endogenously synthesized monounsaturated fatty acids by SCD1 as critical substrates. Dietary monounsaturated fatty acids can get into the liver, but cannot be utilized to synthesize enough triglycerides. Because most of the hepatic triglycerides are

packaged and incorporated into VLDL-TG and transported to tissues such as adipose tissue for storage, the induction of SCD1 gene expression can have a wide range of effects on lipoprotein metabolism in normal and disease states. For instance, high SCD activity has been correlated with high levels of triglycerides in plasma (24). Hypertriglyceridemia is a risk factor for cardiovascular disease, and high SCD expression may be a major cause of this condition in humans. Thus, the regulation of SCD may have broad implications for its potential use as a target in the treatment of human hypertriglyceridemia.

We thank Dr. Alan Attie for useful discussions and critical review of the manuscript. This work was supported, in part, by a grant from the American Heart Association and Xenon Genetics.

Manuscript received 18 January 2001 and in revised form 12 March 2001.

REFERENCES

1. Enoch, H. G., and P. Strittmatter. 1978. Role of tyrosyl and arginyl residues in rat liver microsomal stearylcoenzyme A desaturase. *Biochemistry.* **17:** 4927–4932.
2. Sul, H. S., and D. Wang. 1998. Nutritional and hormonal regulation of enzymes in fat synthesis: studies of fatty acid synthase and mitochondrial glycerol-3-phosphate acyltransferase gene transcription. *Annu. Rev. Nutr.* **18:** 331–351.
3. Ntambi, J. M., S. A. Buhrow, K. H. Kaestner, R. J. Christy, E. Sibley, T. J. Kelly, Jr., and M. D. Lane. 1988. Differentiation-induced gene expression in 3T3–L1 preadipocytes. Characterization of a differentially expressed gene encoding stearoyl-CoA desaturase. *J. Biol. Chem.* **263:** 17291–17300.
4. Horton, J. D., Y. Bashmakov, I. Shimomura, and H. Shimano. 1998. Regulation of sterol regulatory element binding proteins in livers of fasted and refed mice. *Proc. Natl. Acad. Sci. USA.* **95:** 5987–5992.
5. Shimano, H., N. Yahagi, M. Amemiya-Kudo, A. H. Hasty, J. Osuga, Y. Tamura, F. Shionoiri, Y. Iizuka, K. Ohashi, K. Harada, T. Gotoda, S. Ishibashi, and N. Yamada. 1999. Sterol regulatory element-binding protein-1 as a key transcription factor for nutritional induction of lipogenic enzyme genes. *J. Biol. Chem.* **274:** 35832–35839.
6. Shimano, H., I. Shimomura, R. E. Hammer, J. Herz, J. L. Goldstein, M. S. Brown, and J. D. Horton. 1997. Elevated levels of SREBP-2 and cholesterol synthesis in livers of mice homozygous for a targeted disruption of the SREBP-1 gene. *J. Clin. Invest.* **100:** 2115–2124.
7. Shimomura, I., H. Shimano, B. S. Korn, Y. Bashmakov, and J. D. Horton. 1998. Nuclear sterol regulatory element-binding proteins activate genes responsible for the entire program of unsaturated fatty acid biosynthesis in transgenic mouse liver. *J. Biol. Chem.* **273:** 35299–35306.
8. Shimano, H., J. D. Horton, R. E. Hammer, I. Shimomura, M. S. Brown, and J. L. Goldstein. 1996. Overproduction of cholesterol and fatty acids causes massive liver enlargement in transgenic mice expressing truncated SREBP-1a. *J. Clin. Invest.* **98:** 1575–1584.
9. Taghibiglou, C., A. Carpentier, S. C. Van Iderstine, B. Chen, D. Rudy, A. Aiton, G. F. Lewis, and K. Adeli. 2000. Mechanisms of hepatic very low density lipoprotein overproduction in insulin resistance. Evidence for enhanced lipoprotein assembly, reduced intracellular ApoB degradation, and increased microsomal triglyceride transfer protein in a fructose-fed hamster model. *J. Biol. Chem.* **275:** 8416–8425.
10. Taskinen, M. R. 1995. Insulin resistance and lipoprotein metabolism. *Curr. Opin. Lipidol.* **6:** 153–160.
11. Kaestner, K. H., J. M. Ntambi, T. J. Kelly, Jr., and M. D. Lane. 1989. Differentiation-induced gene expression in 3T3–L1 preadipocytes. A second differentially expressed gene encoding stearoyl-CoA desaturase. *J. Biol. Chem.* **264:** 14755–14761.
12. Ntambi, J. M. 1999. Regulation of stearoyl-CoA desaturase by polyunsaturated fatty acids and cholesterol. *J. Lipid Res.* **40:** 1549–1558.
13. Miyazaki, M., Y. C. Kim, M. P. Gray-Keller, A. D. Attie, and J. M. Ntambi. 2000. The biosynthesis of hepatic cholesterol esters and triglycerides is impaired in mice with a disruption of the gene for stearoyl-CoA desaturase 1. *J. Biol. Chem.* **275:** 30132–30138.
14. Zheng, Y., K. J. Eilertsen, L. Ge, L. Zhang, J. P. Sundberg, S. M. Prouty, K. S. Stenn, and S. Parimoo. 1999. Scd1 is expressed in sebaceous glands and is disrupted in the asebia mouse. *Nat. Genet.* **23:** 268–270.
15. Josefowicz, W. J., and M. H. Hardy. 1978. The expression of the gene asebia in the laboratory mouse. 2. Hair folicles. *Genet. Res.* **31:** 145–155.
16. Gates, H. A., and M. Karasek. 1965. Hereditary absence of sebaceous glands in the mouse. *Science.* **148:** 1471–1473.
17. Sundberg, J. P., D. Boggess, B. A. Sundberg, K. Eilertsen, S. Parimoo, M. Filippi, and K. Stenn. 2000. Asebia-2J (Scd1(ab2J)): a new allele and a model for scarring alopecia. *Am. J. Pathol.* **156:** 2067–2075.
18. Bligh, E. G., and W. J. Dyer. 1959. A rapid method of total lipid extraction and purification. *Can. J. Biol. Chem. Physiol.* **37:** 911–917.
19. Bernlohr, D. A., M. A. Bolanowski, T. J. Kelly, Jr., and M. D. Lane. 1985. Evidence for an increase in transcription of specific mRNAs during differentiation of 3T3–L1 preadipocytes. *J. Biol. Chem.* **260:** 5563–6667.
20. Yet, S. F., S. Lee, Y. T. Hahm, and H. S. Sul. 1993. Expression and identification of p90 as the murine mitochondrial glycerol-3-phosphate acyltransferase. *Biochemistry.* **32:** 9486–9491.
21. Kim, J. B., P. Sarraf, M. Wright, K. M. Yao, E. Mueller, G. Solanes, B. B. Lowell, and B. M. Spiegelman. 1998. Nutritional and insulin regulation of fatty acid synthetase and leptin gene expression through ADD1/SREBP1. *J. Clin. Invest.* **101:** 1–9.
22. Kudo, N., and Y. Kawashima. 1997. Fish oil-feeding prevents perfluorooctanoic acid-induced fatty liver in mice. *Toxicol. Appl. Pharmacol.* **145:** 285–293.
23. Parimoo, S., Y. Zheng, K. Eilertsen, L. Ge, S. Prouty, J. Sundberg, and K. Stenn. 1999. Identification of a novel SCD gene and expression of the SCD gene family in mouse skin. *J. Investig. Dermatol. Symp. Proc.* **4:** 320–322.
24. Legrand, P., D. Catheline, M. C. Fichot, and P. Lemarchal. 1997. Inhibiting delta 9-desaturase activity impairs triacylglycerol secretion in cultured chicken hepatocytes. *J. Nutr.* **127:** 249–256.