Dietary cholesterol opposes PUFA-mediated repression of the stearoyl-CoA desaturase-1 gene by SREBP-1 independent mechanism

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Abstract **Stearoyl-CoA desaturase (SCD) catalyzes the rate-limiting step in the cellular synthesis of monounsaturated fatty acids, mainly oleate (18:1) and palmitoleate (16:1), which are the major monounsaturated fatty acids of membrane phospholipids, cholesteryl esters, waxes, and triglycerides. The mouse expresses three well-characterized SCD genes (SCD1, 2, and 3). SCD1 is the main isoform expressed in the liver of mice. Previous in vivo studies have shown that the transcriptional repression by n-3 and n-6 polyunsaturated fatty acids (PUFAs) and the induction by cholesterol of the SCD1 gene are dependent on the maturation of the sterol regulatory element-binding protein-1c (SREBP-1c). We studied the regulation of SREBP-1, SCD1, and other SREBP-1 target genes when a high cholesterol diet is combined with PUFA as n-6 PUFA rich soybean oil (SO), or n-3 PUFA rich fish oil (FO). While the PUFA/cholesterol (PUFA/CH) diets repressed the maturation of the SREBP-1, the SCD1 mRNA levels, and protein and enzyme activity were induced. Compared with PUFA diets, hepatic cholesterol ester and triglyceride were enriched with 16:1 and 18:1 monounsaturated fatty acids in mice fed PUFA/CH diets. Total plasma cholesterol levels were not altered but plasma triglycerides were reduced in SO/CH-fed mice compared with SO-fed mice. The mRNA for SREBP-1 was increased by the PUFA/CH diet but the mRNA levels of SREBP-1 target genes such as fatty acid synthase and LDL receptor were decreased, indicating that the main control of PUFA-mediated suppression of SREBP-1 target genes is the maturation of SREBP-1. This study demonstrates that cholesterol overrides the PUFA-mediated repression of the SCD1 gene and regulates SCD1 gene expression through a mechanism independent of SREBP-1 maturation.—**Kim, H-J., M. Miyazaki, and J. M. Ntambi. **Dietary cholesterol opposes PUFA-mediated repression of the stearoyl-CoA desaturase-1 gene by SREBP-1 independent mechanism. *J. Lipid Res.* 2002.** 43: **1750–1757.**

Supplementary key words hepatic gene expression • cholesterol supplemented PUFA diet • fatty acid composition

Stearoyl-CoA desaturase (SCD) is a microsomal fatty acid modifying enzyme that catalyzes the introduction of the *cis* 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). The cellular roles of monounsaturated fatty acids are diverse and crucial in a living organism. Oleic acid is the major monounsaturated fatty acid of membrane phospholipids, triglycerides, waxes, and cholesteryl esters (CE) (2). Monounsaturated fatty acids have also been implicated as mediators of signal transduction and cellular differentiation (3). Overall the expression of SCD can influence membrane fluidity, lipid metabolism, and adiposity. A high SCD activity and alteration in the balance between saturated and monounsaturated fatty acids are implicated in various disease states including cancer, diabetes, atherosclerosis, and obesity (4).

Several studies have shown that SCD1 gene expression is highly regulated by dietary factors. A high carbohydrate fat-free diet induces SCD1 mRNA in mouse liver, whereas the supplementation of polyunsaturated fatty acids (PUFAs) to this diet suppresses the transcription of the SCD1 gene (5). On the other hand, a high cholesterol diet induces SCD1 gene expression in liver, leading to an increase in the levels of monounsaturated fatty acids (16:1 and 18:1) in liver CE (6–7). Both the repression of the SCD1 gene by polyunsaturated fatty acids and its induction by cholesterol have been shown to be dependent on sterol regulatory element binding proteins (SREBPs) (4, 7–9). Three major SREBP isoforms, SREBP-1a, SREBP-1c, and SREBP-2, are encoded by two different genes and are

Abbreviations: CE, cholesteryl ester; FAS, fatty acid synthase; LXR, liver X receptor; PUFA, polyunsaturated fatty acid; SCD, stearoyl-CoA desaturase; SREBP, sterol regulatory element-binding protein.

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well characterized (10–14). These proteins are synthesized as precursors and inserted into the endoplasmic reticulum (ER) membrane where they are anchored through a two-pass membrane spanning domain with both the amino and carboxylic acid domains facing the cytosolic side of the membrane. In sterol deficient cells, proteolytic cleavage of the SREBPs by the SREBP specific proteases (S1P and S2P) occurs in the golgi and releases their N-terminal mature and active forms from the membrane. This enables them to enter the nucleus, where they bind to the sterol regulatory elements (SREs) and/or E-box sequences and activate genes involved in cholesterol, triglyceride, and fatty acid biosynthesis (15–17). In contrast, when cellular sterol levels are high, the proteolytic processing of SREBPs is inhibited, the nuclear levels of mature proteins are decreased, and transcription of target genes is reduced. Thus, both SREBP-1 gene expression and the processing of the SREBPs regulate target gene expression (9, 17).

A significant role for SREBPs in both lipogenesis and cholesterol metabolism has been established by numerous studies, through both genetic manipulations of SREBP levels and feeding experiments in mammals (18–24). Several expression studies of the individual isoforms demonstrate that SREBP-1a isoform, which is the dominant form in cell lines, is the regulator of genes encoding proteins involved in both lipogenesis and cholesterol biosynthesis (23). The SREBP-1c isoform in liver is a key regulator of early events in the liver's response to insulin and is a major determinant of lipogenic gene transcription, including SCD1 (24, 25). SREBP-2 preferentially regulates the genes of cholesterol metabolism, but also has an influence on regulation of genes of fatty acid synthesis.

PUFA-rich diets repress the transcription of lipogenic genes by suppressing SREBP-1 gene transcription or by reducing the maturation of SREBP-1 protein (8, 20–22). On the other hand, cholesterol rich diets induce SREBP-1 gene expression and maturation. It has been suggested that cholesterol induction of the SCD1 gene expression is indirect and is through a liver X receptor (LXRα)-mediated up regulation of SREBP-1 gene expression (7). This paper reported that LXRα, a member of the nuclear steroid hormone receptor superfamily, regulates transcription of SREBP-1c gene suggesting that LXRα has a crucial influence on cholesterol homeostasis.

Previous studies examined the independent effects of PUFA or cholesterol, but in the present study we examined the combined effects of cholesterol and PUFA on the modulation of SREBP-1 gene expression, precursor and mature SREBP-1 protein levels, SCD1 gene expression, and other SREBP-1 target genes. Our studies demonstrate that the repressive effect of dietary PUFAs on the SCD1 gene expression is overcome by dietary cholesterol resulting in increased SCD protein and activity. When mice were fed cholesterol supplemented PUFA diets, the hepatic cholesterol and triglyceride levels enriched with 18:1 and 16:1 monounsaturated fatty acids were increased compared with PUFA diets. The mature and active form of SREBP-1 protein was decreased, indicating that the PUFAs override the cholesterol-mediated induction of SREBP-1 maturation in mice fed PUFA/CH diets. We also show that the expression of several SREBP-1 target genes was decreased in SO/CH fed-mice, suggesting that the control of PUFA-mediated suppression of these genes is mainly through the maturation of SREBP-1. Our results indicate that cholesterol induces SCD1 gene expression through a mechanism independent of SREBP-1 maturation and implicates SCD as an important enzyme in cholesterol and triglyceride homeostasis.

MATERIALS AND METHODS

Materials

Radioactive [α-^{32}P]dCTP (3,000 Ci/mmol) was obtained from Dupont Corp. (Wilmington, DE). Thin layer chromatography plates (TLC Silica Gel G60) were from Merck (Darmstadt, Germany). Immobilon-P transfer membranes were from Millipore (Danvers, MA). ECL Western blot detection kit was from Amersham-Pharmacia Biotech, Inc. (Piscataway, NJ). All other chemicals were purchased from Sigma Chemical Co. (St Louis, MO).

Animals and diets

C57BL6/129J female mice were bred and maintained in animal room facilities of the department of Biochemistry of the University of Wisconsin-Madison. The breeding of these animals and all experimental procedures were in accordance with the protocols approved by the animal care research committee of the University of Wisconsin-Madison. Mice were maintained on a 12-h dark/light cycle and were fed semi-purified diet (TD 99252; Harlan Teklad, Madison, WI) containing 5% fat in the form of triolein, soybean oil, or fish oil with or without 2% cholesterol (w/w). The semi-purified diet (TD 99252) contained, by weight, 54% sucrose, 21% casein, 14% maltodextrin, 5.2% cellulose, 3.6% mineral mix (AIN-93G-MX), 1.1% vitamin mix (AIN-93G-MX), 0.32% L-cystine, and 0.26% choline bitartrate. Soybean oil (Harlan Teklad, Madison, WI) contained 22% oleic acid (18:1 n-9) and 54% linoleic acid (18:2 n-6) as the main fatty acids. The fish oil (Sigma Chemical Co.; St Louis, MO) contained about 25% n-3 fatty acids (eicosapentaenoic and docosahexaenoic acids). Animals were anesthetized by intraperitoneal injection of pentobarbital sodium (0.08 mg/g of body weight; Nembutal, Abbot, Chicago, IL) or sacrificed by CO_2 asphyxiation. A part of the liver of each mouse was immediately weighed and homogenized to obtain membrane fractions and nuclear extracts (19). The remaining liver was frozen in liquid nitrogen for Northern analysis and lipid analysis as described previously (26). Free cholesterol, total cholesterol, and triglyceride content of plasma were determined by enzymatic assays (Sigma Chemical Co., St Louis, MO and Wako Chemicals, Japan).

Isolation and analysis of RNA

Total RNA was isolated from livers using the acid guanidinium-phenol-chloroform extraction method (27). Twenty micrograms of total RNA was separated by 0.8% agarose/2.2 M formaldehyde gel electrophoresis and transferred onto nylon membrane. The DNA fragments for mouse SREBP-1, SREBP-2, LDL receptor were obtained by reverse transcriptase-polymerase chain reaction from first strand cDNA derived from mouse liver total RNA. The amplified products were subcloned into pGEM-T Easy vector (Promega, WI). Dr John Chiang at Northeastern Ohio University kindly provided the cDNA probe for rat cholesterol 7α-hydroxylase (Cyp7α).

Immunoblotting

Pooled liver membranes from three mice of each group were prepared as described by Heinemann et al. (28). The same amount of protein (40 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% non-fat milk in TBS buffer (pH 8.0) plus Tween at 4°C overnight, the transfer membrane was washed and incubated with rabbit anti-rat SCD as primary antibody and goat anti-rabbit IgG-HRP conjugate as the secondary antibody. Visualization of the SCD protein was performed with ECL Western blot detection system kit (Amersham-Pharmacia Biotech). For the immunoblotting of the SREBP-1 proteins, nuclear extracts and membrane fractions of mice livers were prepared according to the methods of Sheng et al. (19). Aliquots of nuclear extracts (30 μg) were mixed with SDS loading buffer, subjected to SDS/PAGE on an 8% gel, transferred, and immobilized on Immobilon-P transfer membranes. After blocking with 3% BSA in TBS buffer (pH 8.0) plus Tween 20 at 4°C overnight, the transfer membrane was washed and incubated with monoclonal anti-SREBP-1 (IgG-2A4) as primary antibody and anti-mouse IgG-HRP conjugate as the secondary antibody. Visualization of the SREBP-1 protein was performed with ECL Western blotting detection system kit (Amersham-Pharmacia Biotech). The bands were quantified by scanning with Canon Scanner (Canon Inc., Tokyo, Japan). Dr. Osamu Ezaki at the National Institute of Health and Nutrition in Japan kindly provided monoclonal antibody for SREBP-1.

SCD activity assay

Stearoyl-CoA desaturase activity was measured in liver microsomes essentially as described by Oshino et al (29). Tissues were homogenized in 10 vol 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 100 μg of protein homogenate with 30 μM of [1-^{14}C]stearoyl-CoA (60,000 dpm), 1 μM of NADH and 50 μM of Tris/HCl buffer (pH 7.4). Following incubation, fatty acids were extracted and then methylated with 5% hydrochloride/methanol. Saturated fatty acid and monounsaturated fatty acid methyl esters were separated by 10% $AgNO_3$-impregnated TLC using hexane-diethyl ether (9:1, v/v) as developing solution. The plates were sprayed with 0.2% 2′, 7′-dichlorofluorescein in 95% ethanol and the lipids were identified under UV light compared with authentic fatty acid standards. The fractions were scraped off the plate, and the radioactivity was measured using a liquid scintillation counter (26). The enzyme activity was expressed as nmol min^{-1} mg^{-1} protein.

Plasma lipoprotein analysis

Mice were fasted a minimum of 4 h and sacrificed by CO_2 asphyxiation and/or cervical dislocation. Blood was collected aseptically by direct cardiac puncture, EDTA was added to protect a clot, and centrifuged (13,000 *g*, for 5 min at 4°C) to collect plasma. Lipoproteins were fractionated on a Superose 6HR 10/30 FPLC column (Pharmacia). Plasma samples were diluted 1:1 with PBS, filtered (Cameo 3AS syringe filter, 0.22 μm), and the equivalent of 100 μl of plasma was injected onto a column that had been equilibrated with PBS containing 1 mM EDTA and 0.02% NaN_3. The flow rate was set constant at 0.3ml/min. Five-hundred microliter fractions were collected and used for total triglyceride and cholesterol measurements using Sigma Diagnostic Kits (Sigma Chemical Co.; St Louis, MO). Values reported are μg per fraction total triglycerides or cholesterol.

Lipid analysis

Total lipids were extracted from liver and plasma according to the method of Bligh and Dyer (30). Phospholipids, free cholesterol, triglycerides, and CE were separated by silica gel TLC using hexane-diethyl ether-acetic acid (90:20:1, v/v/v) as a developing solvent. Spots were visualized by 0.2% 2′, 7′-dichlorofluorescein in 95% ethanol. The spots corresponding to phospholipids, triglycerides, and CE were scraped and extracted. Fatty acid methyl esters (FAMEs) were prepared by heating with 5% HCl-methanol at 100°C for 1 h. The FAMEs were analyzed by gas-liquid chromatography using cholesterol heptadecanoate as internal standard (26). Liver CE and triglyceride contents were determined by enzymatic assays (Sigma Chemical Co., St Louis, MO and Wako Chemicals, Japan).

RESULTS

Figure 1A shows Northern blot analysis of mRNA expression of SCD1, fatty acid synthase (FAS), LDL receptor (LDLR), SREBP-1, SREBP-2, and Cyp7α in liver of mice fed soybean oil (SO), or SO diet supplemented with 2% cholesterol (SO/CH). Compared with the SO diet, which is control group in this experiment, the SO/CH diet increased the levels of SREBP-1 and SCD1 mRNA 6-fold and 2-fold respectively, but reduced FAS and LDLR mRNA levels. The mRNA levels of the Cyp7α gene, a known cholesterol target gene (7, 31), were increased in the SO/CH diet-fed mice. SREBP-2 mRNA level was not changed by cholesterol supplemented SO diet. Figure 1B shows the Western blot analysis using a monoclonal rabbit SCD antibody. The SCD protein level in SO/CH fed mice was higher than in SO-fed mice. As shown in Fig. 1C, the SCD enzyme activity was increased 3-fold by the SO/CH diet compared with the SO diet, which was consistent with the trends of the Northern and Western blots shown in Fig. 1A and 1B. Figure 1D shows the immunoblotting of precursor and mature SREBP-1 in liver. The SO/CH diet decreased the SREBP-1 mature form by more than 60% compared with SO diet, while the levels of the SREBP-1 precursor form were not dramatically decreased. SREBP-2 protein levels were not altered consistent with the SREBP-2 mRNA result shown in Fig. 1A (data not shown).

Table 1 shows liver and plasma parameters in mice fed SO and SO/CH diets. The CE content in liver of mice fed SO/CH diet was increased by 4.2-fold whereas the triglyceride content was slightly increased compared with the animals on the SO diet. Although plasma free cholesterol was reduced 38% in SO/CH group compared with SO group, the plasma total cholesterol content was similar between the two groups. Plasma triglycerides were reduced 45% in the SO/CH fed mice compared with SO fed mice.

The distribution of triglycerides and cholesterol among lipoproteins is shown in **Fig. 2**. In the SO/CH-fed mice, the lipoprotein profile showed an increase in the cholesterol content of the VLDL fraction with a corresponding decrease in the HDL fraction compared with SO fed mice (Fig. 2A). The triglyceride content was decreased more than 40% in the VLDL fraction of the SO/CH-fed mice (Fig. 2B). Both results agree with results shown in the Table 1.

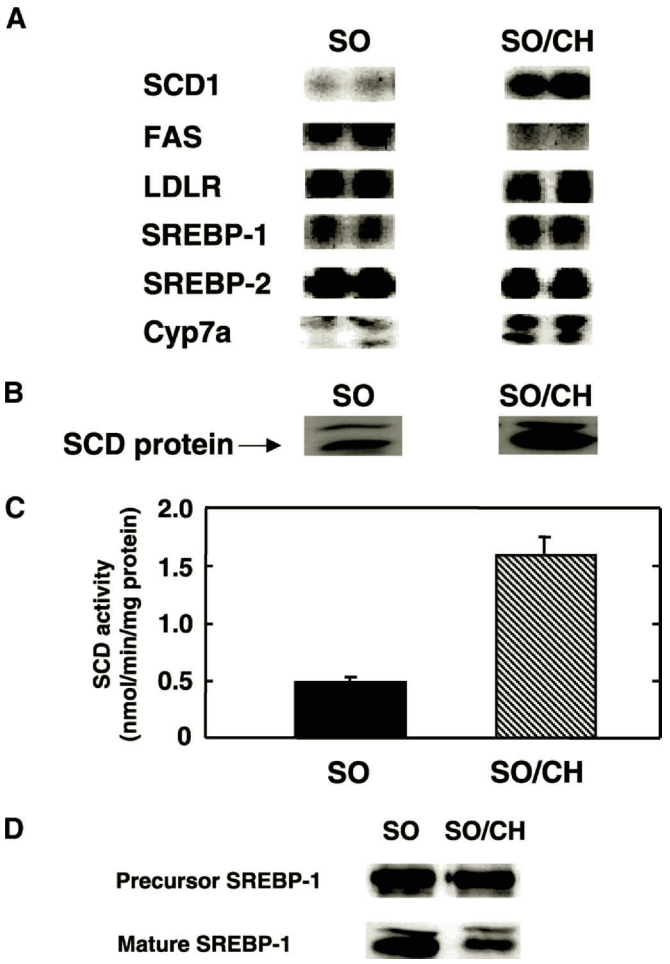

Fig. 1. Northern blot analysis for the expression of various mRNA genes involved in lipid metabolism (A), stearoyl-CoA desaturase (SCD) immunoblotting analysis (B), SCD enzyme activity (C), and immunoblotting analysis of sterol regulatory element-binding protein (SREBP)-1 (D) in livers of SO- and SO/CH-fed mice. A: Total RNA was isolated from mice fed SO and SO/CH. Twenty μg of total RNA were subjected to electrophoresis and followed by hybridization with labeled probes specific for stearoyl Co-A desaturase1 (SCD1), fatty acid synthase (FAS), low density lipoprotein receptor (LDLR), sterol regulatory element binding protein 1 (SREBP-1), SREBP2 and cholesterol-7α hydroxylase (Cyp7α) transcripts. A cDNA probe for pAL15 (29) was used to confirm equal loading. For SCD immunoblotting analysis (B), aliquots of membrane fraction (40 μg) from pooled livers of each group were subjected to 10% SDS polyacrylamide gel electrophoresis followed by detection with SCD antibody. For enzyme activity (C), aliquots of microsome fraction (100 μg) from livers of each mouse were incubated with a reaction mixture containing [1-^{14}C]stearoyl-CoA for 5 min. The products were saponified and acidified and the fatty acids were extracted and separated by TLC as described under Materials and Methods. Each value represents the mean ± SD (n = 5). D: Thirty microgram aliquots of membrane fractions (for SREBP-1 precursor protein) and nuclear extracts (for SREBP-1 mature form) were subjected to 8% SDS-polyacrylamide gel electrophoresis and transferred to Immobilon-P transfer membranes followed by detection with 5 μg/ml of mouse monoclonal antibody IgG 2A4 against amino acids 301–407 of human SREBP-1.

Figure 3A shows the relative percentage of the major fatty acids measured in the total liver lipid extracts of animals fed SO or SO/CH diets. The SO/CH fed animals had a decrease in the relative amount of palmitate (16:0), a 95% increase in palmitioleate (16:1) and a 30% increase in oleate (18:1). There were only minor changes in the other fatty acids between the two diet groups. The relative amount of palmitoleate (16:1) in the hepatic CE fraction of livers of the SO/CH mice increased by 3.1-fold while the relative amount of oleate (18:1) increased 60% compared with SO fed control group (data not shown).

TABLE 1. Comparison of mice fed 5% soybean oil diet with or without 2% (w/w) cholesterol

Parameter	Soybean Oil (SO)	Soybean Oil/Cholesterol (SO/CH)
Final body weight(g)	18.83 ± 0.95	23.17 ± 2.40[a]
Liver weight (g)	0.82 ± 0.08	1.30 ± 0.23[a]
Liver weight/ body weight (%)	4.34 ± 0.39	5.61 ± 0.78
Liver triglycerides (mg/g)	27.70 ± 9.44	50.78 ± 18.52
Liver cholesterol ester (mg/g)	10.55 ± 0.56	42.51 ± 4.21[b]
Plasma triglycerides (mg/dl)	46.97 ± 6.68	25.95 ± 3.99[a]
Plasma total cholesterol (mg/dl)	86.22 ± 5.76	84.94 ± 3.42
Plasma free cholesterol (mg/dl)	17.10 ± 3.08	10.68 ± 0.60

Female mice were fed SO or SO/CH diet for 2 weeks and sacrificed. Liver triglycerides and CE, plasma triglycerides, total cholesterol and free cholesterol were measured as described under Materials and Methods. Data are presented as mean ± SD (n = 3).

[a] $P < 0.05$ (Student's *t*-test); compared with SO diet-fed mice.

[b] $P < 0.001$ (Student's *t*-test); compared with SO diet-fed mice.

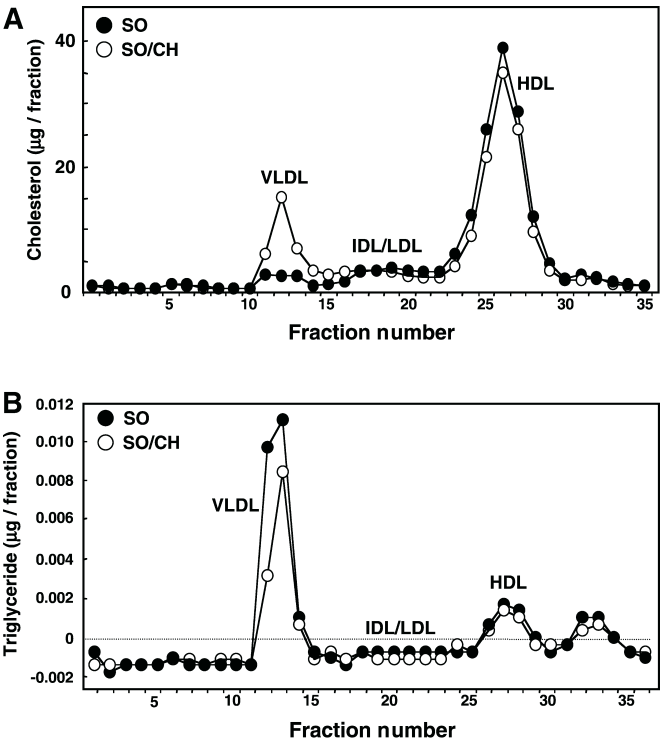

Fig. 2. The lipoprotein profile of plasma of SO- and SO/CH- fed mice Plasma lipoproteins were separated by fast performance liquid chromatography. The distributions of cholesterol (A) and triglyceride (B) among lipoproteins in each fraction were measured. The lipoprotein peaks for VLDL, IDL/LDL, and HDL are indicated. Closed circle, SO-fed mice; open circle, SO/CH-fed mice.

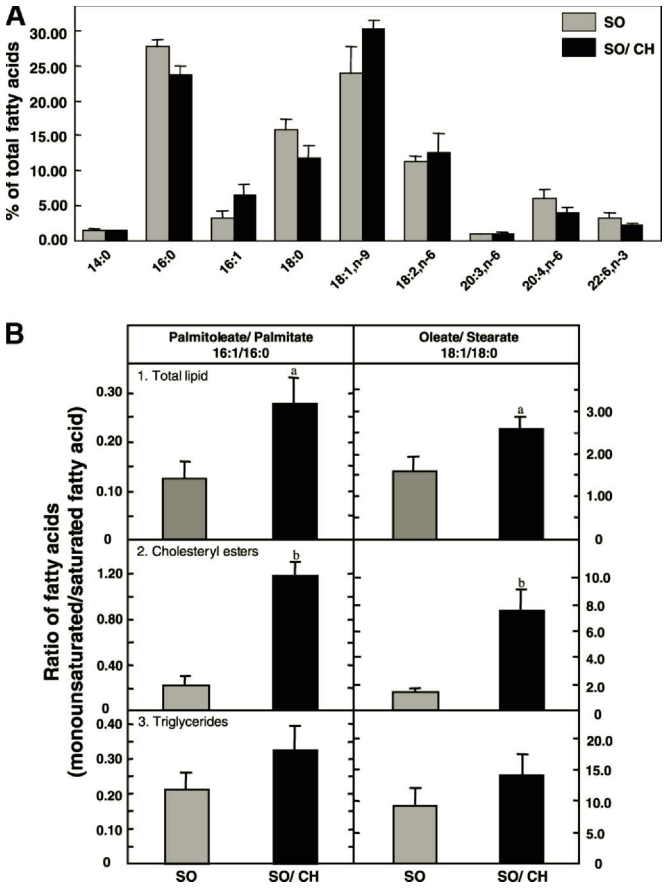

Fig. 3. Fatty acid composition in total lipid extracts (A) and the ratio of palmitoleate (16:1) to palmitate (16:0) and oleate (18:1) to stearate (18:0) in total lipid extracts, cholesteryl ester (CE), and triglyceride fractions (B) of livers from SO- and SO/CH-fed mice. Total lipids were extracted from liver and separated by silica gel TLC, methyl esterified, and analyzed by GLC as described under Materials and Methods. Data are presented as mean ± SD (n = 3). [a] $P < 0.05$ (Student's *t*-test); compared with SO diet-fed mice. [b] $P < 0.01$ (Student's *t*-test); compared with SO diet-fed mice.

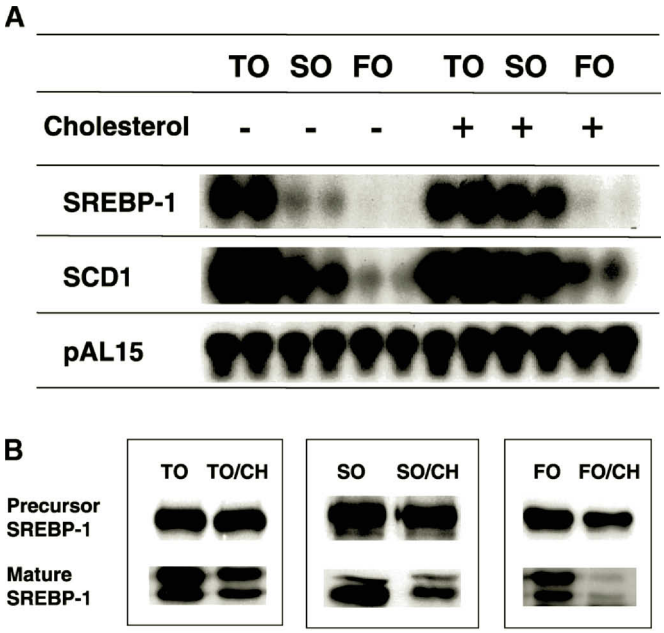

Fig. 4. Northern blot analysis for the expression of SCD1 and SREBP-1 (A), and immunoblotting analysis (B) of SREBP-1 in livers of TO-, SO-, FO- and TO/CH- SO/CH-, FO/CH-fed mice. A: Total RNA was isolated from mice liver. Twenty micrograms of total RNA were subjected to electrophoresis and followed by hybridization with labeled probes specific for SCD1 and SREBP-1 transcripts. A cDNA probe for pAL15 (29) was used to confirm equal loading. B: Thirty micrograms aliquots of membrane fractions (for SREBP-1 precursor protein) and nuclear extracts (for SREBP-1 mature form) were subjected to 8% SDS-polyacrylamide gel electrophoresis and transferred to Immobilon-P transfer membranes followed by detection with 5 μg/ml of mouse monoclonal antibody IgG 2A4 against amino acids 301–407 of human SREBP-1.

The relative increases of the 16:1 and 18:1 were accompanied by decreases in 16:0 and 18:0. Figure 3B shows the ratio of monounsaturated fatty acids to saturated fatty acids (desaturation index) measured in the total lipid, CE, and triglyceride fractions. In the total lipid fraction (panel 1), the 16:1/16:0 and 18:1/18:0 ratios were increased in the livers of the SO/CH-fed mice by 30% and 67% compared with SO fed mice, respectively. In the cholesterol ester fractions (panel 2), the ratios of 16:1/16:0 and 18:1/18:0 of the SO/CH-fed mice were significantly increased compared with control mice, 5.4- and 5.5-fold, respectively. The liver triglyceride fractions (panel 3) of the SO/CH-fed mice showed a 57% increase of the ratio of 16:1/16:0 and 55% increase of the ratio of 18:1/18:0 compare with respective control. The increase in the desaturation index indicates that the SO/CH diet induces SCD activity, resulting in increased monounsaturated fatty acid content in the cholesterol ester and triglyceride fractions. There were no significant changes in the fatty acid composition of the phospholipid fractions (data not shown).

Figure 4A shows a Northern blot analysis of mRNA expression of SCD1, SREBP-1 and pAL15 cDNA in liver of mice fed a 5% triolein (TO), soybean oil (SO), or fish oil (FO) diet with or without 2% cholesterol. The SO and FO diets decreased both the SREBP-1 and SCD1 mRNA levels compared with TO diet. As reported previously (20), the FO diet was very potent in suppressing the SREBP-1 and SCD1 mRNA levels. On the other hand, compared with the SO and FO diets, the SO/CH and FO/CH diets increased the levels of SREBP-1 and SCD1 mRNAs 3- and 6-fold respectively. There is a 3-fold increase of SCD-1 mRNA in FO/CH diet compared with FO diet and the increase in the SREBP-1 mRNA is clearly seen upon longer exposure of the autoradiograph. This indicates that cholesterol overcomes the suppressive effects of PUFA on SREBP-1 and SCD1 gene expression. The levels of pAL15 mRNA used as a loading control were not different at all treatments. Figure 4B shows the immunoblotting analysis for SREBP-1. While both the SO/CH and FO/CH diets induced the SREBP-1 mRNA compare with SO and FO diets (Fig. 4A), these cholesterol supplemented diets reduced the precursor and mature forms of SREBP-1. Specifically, the FO/CH diet showed a greater inhibitory effect on SREBP-1 maturation than the SO/CH diet.

TABLE 2. Fatty acid composition of liver from mice fed 5% fish oil diet with or without 2% (w/w) cholesterol

	16:0	16:1	18:0	18:1	18:2	20:3	20:4	20:5	22:6
Total lipid									
FO	27.46	3.02	13.97	27.49	7.92	0.34	2.61	4.02	7.86
FO/CH	23.64[b]	4.6[c]	10.31[c]	38.49[b]	6.43	0.26[a]	1.73[b]	4.01	5.74(1.37)
Cholesteryl ester									
FO	27.75	5.19	23.36	28.97	6.16	0.00	0.00	0.00	0.00
FO/CH	20.93[b]	11.50[a]	12.11[a]	46.37[b]	3.87[a]	0.00	0.00	0.00	0.00
Triglyceride									
FO	26.59	4.62	5.46	40.79	9.02(2.49)	0.26	1.35	1.86	4.51
FO/CH	22.85[b]	4.80	4.71	49.89[a]	6.36	0.19	0.54[b]	1.83(0.48)	3.73(1.45)
Phospholipid									
FO	28.20	1.39	20.48	12.20	7.87	0.65	5.79	6.88	12.59
FO/CH	26.58	1.40	21.75	13.87	7.68	0.62	5.33	8.70	10.20

Liver lipids from individual mice were extracted, and each fraction of lipids was separated using TLC. The lipid fractions were methyl-esterified and quantified by gas-liquid chromatography as described under Materials and Methods. Standard deviations of the mean were less than 20% of the mean except for a few values which were omitted for clarity. Numbers in parenthesis indicate standard deviations (SD) of more than 20% of the mean. Data are presented as mean ± SD (n = 3).

[a] $P < 0.05$ (Student's *t*-test); compared with fish oil (FO) diet-fed mice.

[b] $P < 0.01$ (Student's *t*-test); compared with FO diet-fed mice.

[c] $P < 0.001$ (Student's *t*-test); compared with FO diet-fed mice.

Table 2 shows the relative percentage of the major fatty acids measured in the total lipid, CE, triglyceride, and phospholipid fractions in liver of animals fed FO or FO/CH diets. In the total lipid fraction, the FO/CH group had a 52% increase in the relative amount of palmitoleate (16:1) and a 40% increase in oleate (18:1) compare with FO group. The relative amount of palmitoleate (16:1) in the CE fraction increased 22% while the relative amount of oleate (18:1) increased by 60%. The liver triglycerides of FO/CH-fed mice showed a significant increase of oleate (18:1) but there was no change in the amount of palmitoleate (16:1). There were no significant fatty acid composition changes in the phospholipid fractions (data not shown). Thus, the ratios of 16:1/16:0 (0.19 ± 0.03 vs. 0.11 ± 0.01) and 18:1/18:0 (3.76 ± 0.30 vs. 1.97 ± 0.16) were greater in the livers of the FO/CH-fed mice than FO-fed mice, indicating an increase in SCD enzyme activity by cholesterol supplementation.

DISCUSSION

It is well known that feeding a PUFA-rich diet to rodents represses several lipogenic genes including SCD1, resulting in reduced levels of monounsaturated fatty acids, triglycerides, and CEs in plasma and liver (4, 8, 20). On the other hand, a high cholesterol diet up-regulates SREBP-1c expression and indirectly induces SCD1 gene expression, leading to increased levels of CE of oleate (6, 7). We combined PUFA and cholesterol in experimental diets and determined their combined effects on lipogenic gene expression. Using this feeding regimen, we demonstrated that cholesterol overrides the PUFA-mediated suppression of the SCD1 and SREBP-1 genes. However, PUFA oppose the cholesterol-mediated induction of SREBP-1 protein maturation, leading to repression of other SREBP-1 target genes such as FAS and LDL receptor. The increase in SCD enzyme activity correlated with increased synthesis of oleic acid and its enhanced incorporation into CE and triglycerides. In this work, the overall effect of the PUFA/CH diet was to induce the SCD1 gene that provides the monounsaturated fatty acids used by acyl-CoA cholesterol acyltransferase (ACAT) for conversion of cellular cholesterol into CE for storage. This suggests that the SCD gene has a role as a control point in cholesterol homeostasis.

Studies over the last few years have revealed that cholesterol and fatty acids are co regulated by the SREBPs and that SREBP-1c is a key activator of lipogenic gene expression in response to insulin signaling in liver (25). Cholesterol has been shown to suppress cholesterologenic and lipogenic enzymes in several cultured cell lines through the inhibition of proteolytic processing of SREBP-1a (17, 32). However, unlike in vitro results, in vivo studies showed that high levels of dietary cholesterol induce SREBP-1c gene expression and protein maturation in mouse liver, resulting in the induction of mainly SCD1 as a target gene (7). The other SREBP-1 target genes such as FAS and acetyl-CoA carboxylase that were studied were either reduced or not altered (7). It is also known that PUFAs reduce the nuclear content of SREBP-1 via a two-phase mechanism (22, 33). The first phase is believed to be rapid and involves inhibition of the proteolytic release step. The second phase involves a reduction in the SREBP-1 mRNA that is subsequently followed by reduction in the amount of precursor SREBP-1 protein (20–22). The suppression of SREBP-1 gene expression by PUFAs and a corresponding reduction in the levels of the mature SREBP-1 protein results in decreased expression of genes encoding enzymes and proteins involved in fatty acid biosynthesis. Since cholesterol induces SREBP-1 mRNA expression but the SREBP-1 protein is repressed (Figs. 1 and 4), our results suggest that PUFAs exert their main inhibitory effects on the maturation of SREBP-1. Fish oil was more potent in repressing lipogenic gene expression than the soybean oil. However,

cholesterol added to the FO diet was able to overcome the strong suppressive effects of FO and induced SCD1 gene expression.

The mechanism of cholesterol-mediated induction of SREBP-1c expression has been reported to be through oxysterol nuclear receptor LXRα (7). In previous studies it was proposed that the cholesterol induction of SCD1 gene expression previously observed in liver (6) was indirect and was due to the induction of SREBP-1c (7). However, our results presented in this paper indicate that by combining cholesterol with PUFA (PUFA/CH diet), SCD1 gene expression, protein and activity are induced compared with PUFA diet alone, despite a reduction in levels of the mature SREBP-1 protein. These results suggest that cholesterol induction of SCD1 gene expression is independent of SREBP-1 maturation. The induction of the SREBP-1 and SCD1 mRNA expressions by the PUFA/CH diets demonstrates that cholesterol overrides the PUFA-mediated suppression of SCD1 and SREBP-1 gene expression.

The Cyp7α gene, a well-known LXRα target gene (7, 31), was induced by the PUFA/CH diet (Fig. 1A). Cholesteryl and LXRα-target genes such as the ABC transporters and cholesterol ester transfer protein (CEPT) have all been implicated in different aspects of cholesterol homeostasis (7, 34–36). Feeding cholesterol to LXRα knockout mice did not result in the induction of SCD1 gene expression or SREBP-1 gene expression by cholesterol feeding (7, 34). Studies by Liang et al (37) indicated that feeding an LXRα agonist T090137 to SREBP-1c knockout mice partially induced SCD1 gene expression, suggesting that SCD-1 itself is a direct target of LXRα. T090137 induces SREBP-1c expression through LXRα (7) and thus a much higher induction of SCD1 gene was observed with the T090137 in the wild-type mouse. These studies would strongly suggest that SCD1 is both a target of SREBP-1c and LXRα.

We propose that when PUFAs are combined with cholesterol, the cholesterol opposes the PUFA-mediated reduction in SCD1 gene expression, whereas the PUFAs override the cholesterol-mediated induction of SREBP-1 maturation. The strong inhibitory effect of PUFA on SREBP-1 maturation results in suppression of SREBP-1 target genes such as FAS. The benefit of up-regulating SCD1 by cholesterol would be to increase oleoyl-CoA, the preferred substrate for ACAT-mediated cholesterol esterification (4, 6, 26) and DGAT for triglyceride synthesis (26). Thus under condition of high cellular cholesterol, induction of SCD1 gene expression would indirectly protect the cell from the harmful effects of free cholesterol by converting it to CE by ACAT. In addition, the increase of monounsaturated fatty acid synthesis under these conditions would promote a more appropriate ratio of cholesterol to other lipids to maintain cell membrane integrity (38), although this concept has not been rigorously tested.

In conclusion, the present work demonstrates that cholesterol induces SCD1 gene expression through an SREBP-independent mechanism. PUFA repression of SREBP-1 maturation leads to decreased expression of lipogenic genes, including SCD1. However, when cholesterol levels increase, cholesterol overcomes the suppressive effects of PUFA and induces SCD1 gene expression to provide the monounsaturated fatty acids, mainly oleate, for esterification to free cholesterol for storage in liver. Thus, the SCD1 gene expression may be another important checkpoint in the process of cholesterol homeostasis and lipoprotein metabolism.

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