Inhibition of stearoyl CoA desaturase activity induces hypercholesterolemia in the cholesterol-fed hamster[1]

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Abstract **Reduction of stearoyl CoA desaturase (SCD) activity has been shown to induce resistance to diet-induced obesity in mice. In the present study, SCD was inhibited by feeding sterculic oil (SO) to male Golden Syrian Hamsters fed high-fat diets with or without added dietary cholesterol. In the absence of cholesterol, SO had little impact on adipose tissue mass or plasma lipoprotein concentrations. When cholesterol was included in the diet, inhibition of SCD resulted in reduced body weight, adipose tissue mass, and feed efficiency. These animals also exhibited a marked hypercholesterolemia, with an accumulation of free-cholesterol–rich particles within the LDL density range, and reduced hepatic cholesterol esterification. This was accompanied by a 20-fold increase in plasma alanine aminotransferase, which was suggestive of significant hepatic damage. Hepatic acetyl CoA carboxylase and fatty acid synthase mRNA concentrations were reduced by feeding cholesterol and SO, whereas lipoprotein lipase and SCD mRNA were increased. These changes were associated with decreased hepatic sterol regulatory element binding protein 1a and 1c mRNA concentrations. Thus, inhibition of SCD activity in the cholesterol-fed hamster results in a reduction in overall body weight and adipose tissue deposition. However, this also causes marked hypercholesterolemia and potential liver damage.**—Major, C. A., K. Ryan, A. J. Bennett, A. L. Lock, D. E. Bauman, and A. M. Salter. **Inhibition of stearoyl CoA desaturase activity induces hypercholesterolemia in the cholesterol-fed hamster.** ***J. Lipid Res.*** **2008.** 49: **1456–1465.**

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Stearoyl CoA desaturase (SCD, also known as Δ9 desaturase) catalyzes the introduction of a double bond in the Δ9 position, between carbons 9 and 10, of a variety of fatty acyl CoA substrates. The preferred substrate is stearic acid (C18:0), converted to oleic acid (C18:1) (1). It is, however, also responsible for the synthesis of palmitoleic acid (C16:1) from palmitic acid (C16:0) and *cis*9, *trans*11–conjugated linoleic acid from vaccenic acid (*trans*11 C18:1). As such, it plays a major role in regulating the fatty acid composition of tissues. The mouse expresses at least four different isoforms of the enzyme, which are the products of different genes and are differentially expressed in a tissue-specific manner (2). Of these, SCD1 is the most widely expressed and appears to be regulated in a complex fashion by a variety of endocrine, nutrient, and metabolic factors (3). Until recently, it was assumed that only one isoform is expressed in humans; however, a recent report suggests the presence of a second gene (4).

Extensive studies in the mouse have shown that SCD1 is highly regulated by both hormones and nutrients (3). Insulin, high-carbohydrate diets, cholesterol, and vitamin A have all been shown to increase SCD1 gene expression. Polyunsaturated fatty acids, thyroid hormone, and leptin all reduce SCD1 gene expression.

In recent years, the activity of SCD has attracted considerable interest because of a possible link in regulating adiposity. $SCD^{-/-}$ mice are resistant to diet-induced obesity (5). Furthermore, crossing of the leptin-deficient (ob/ob) mouse with the asebia mouse, which exhibits a specific naturally occurring mutation in SCD, protects animals from the obesity characteristic of the leptin deficiency (6). This protection from obesity is associated with reduced synthesis of triacylglycerol and cholesteryl ester in the liver (7). $SCD^{-/-}$ mice also exhibit lowered plasma triacylglycerol concentrations (7).

The effects of reducing SCD activity have thus far been limited to studies in SCD1 knock-out mice or in mice ex-

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hibiting a specific mutation in the gene. This extreme condition is unlikely to be mirrored in humans if SCD1 activity were to be modulated as a treatment for obesity. Furthermore, SCD1 knock-out mice suffer severe abnormalities of both skin and fur. The male Golden Syrian Hamster has been used extensively as a model of dietary-induced hyperlipidemia (8–12). In the present experiment, we have investigated the effects of moderately reduced SCD activity by feeding hamsters sterculic oil (SO), which serves as a source of the cyclopropenyl fatty acid, sterculic acid, which has been shown to be a direct inhibitor of SCD activity (13). SO was incorporated into diets enriched in dairy fat. Previous studies have shown that SCD knock-out mice are resistant to the obesity induced by such diets (5). We also investigated the impact of including cholesterol (0.2% w/w) in the diets, because previous work (10, 12) has shown that hamsters respond to such diets by increasing hepatic storage of cholesteryl ester, a process that may be inhibited by reduction of SCD activity (7). The effects of these diets on body weight, fat deposition, plasma lipoproteins, hepatic lipid concentrations, and hepatic expression of lipogenic genes were examined.

MATERIALS AND METHODS

Protocol of animal treatment

All procedures involving hamsters were subject to UK Home Office regulations, and animals were housed as previously described (14). Hamsters were anesthetized using sodium pentabarbitone (Sagatal, 1 ml/kg), and 3–4 ml blood was collected by cardiac puncture and placed into EDTA tubes. Although animals were not fasted, they were euthanized during the light phase (when food intake would be minimal), between the times of 09.00–12.00 h. Plasma was isolated by centrifugation and stored at 4°C until lipoprotein separation, which was commenced within 48 h of collection. Livers and perirenal and epididymal fat pads were removed, weighed, and snap-frozen in liquid nitrogen.

Diet formulation and feeding

Experimental diets consisted of RM3 chow supplemented with 20% (w/w) standard butter [source and fatty acid composition as previously reported (14)] with or without 0.2% (w/w) added cholesterol (Sigma Chemical Co., Poole, UK) or SO (0.5% w/w). This produced four groups (n = 8) consuming *1*) no cholesterol/no SO oil (control), *2*) 0.2% cholesterol/no SO (chol), *3*) no cholesterol/0.5% SO (SO), and *4*) 0.2% cholesterol/0.5% sterculic oil (chol + SO). SO was prepared from *Sterculia foetida* seeds as described by Corl et al. (15) and contained 55.9% sterculic acid [C19:1; 8-(2-octyl-1-cyclopropenyl) octanoic acid]. Animals were fed for 28 days, during which time they were provided free access to food and water; food was completely replaced every 2 to 4 days.

Cholesterol and triacylglycerol analysis

Plasma lipoproteins were separated from plasma (normally 1 ml) by sequential ultracentrifiugation as previously described (14). Chylomicrons were first isolated from plasma by spinning at 16,000 *g* for 20 min at 12°C. VLDL (d < 1.006 g/ml) and LDL (d = 1.02–1.06 g/ml) were then isolated by spinning at 130,000 *g* for 20 h. HDL (d > 1.060 g/ml) was collected as the subnatant after spinning up LDL. Total cholesterol and triacylglycerol in plasma and individual lipoprotein fractions were determined using diagnostic kits from Thermo-Trace (Infinity Cholesterol and Infinity Triacylglycerol Enzymatic kits, Alpha Laboratories, Eastleigh, UK). Plasma free cholesterol was determined using a diagnostic kit from Wako Chemicals (Free Cholesterol C, Alpha Laboratories). Hepatic cholesterol and cholesteryl esters were separated by TLC, extracted, and assayed enzymatically as previously described (14). Hepatic triacylglycerol was measured enzymatically, following extraction of lipids using a mixture of hexane and isopropyl alcohol.

Plasma alanine aminotransferase activity

Plasma alanine aminotransferase activity (ALT) was measured using a diagnostic kit from Thermo-Trace (Alpha Laboratories).

Fatty acid analysis

The frozen samples of liver and perirenal adipose tissue were pulverized at liquid nitrogen temperature. Total lipids were extracted and fatty acids methylated as described by Lock et al. (14). Fatty acid methyl esters (FAMEs) were analyzed by GC [Perkin Elmer (Norwal, CT), Clarus 500 with flame ionization detector] using a CP-Sil 88 capillary column (100 m × 0.25 mm i.d. with 0.2 μm film thickness; Varian, Walnut Creek, CA) according to methods described previously (14). FAME standards were used to identify sample FAME. Hepatic lipids were further separated by TLC and extracted. Fatty acid composition was then determined as described above.

Determination of mRNA levels

RNA was extracted from liver and perirenal adipose tissue using Trizol (Invitrogen, Paisley, UK) according to the manufacturer's instructions. Genomic DNA was digested with DNase, RNA purity, and yield determined, and RT-PCR performed as described previously (16). For the relative quantification of cDNA for ABCA1, acetyl CoA carboxylase (ACC), FAS, LPL, LDL receptor (LDLR), microsomal triglyceride transfer protein (MTP), proprotein convertase subtilisin kexin 9 (PCSK9), SCD, and sterol-regulatory element binding proteins 1a, 1c, and 2 (SREBP1a, SREBP1c, and SREBP2), quantitatative real-time PCR was performed using either a PRISM 7700 Sequence Detector (Applied Biosystems, Foster City, CA) or a Lightcycler 480 (Roche, Basel, Switzerland). Primer and probe sequences for ACC, FAS, and LPL were as described by Guo et al. (17). Sequences for primers for hamster SCD were obtained from Dr. Q. Guo (Merck and Co., Inc., personal communication). Hamster-specific ABCA1, MTP, PCSK9, SREBP1a, SREBP1c, and SREBP2 primers and probes were designed using Primer Express (Applied Biosystems). Where available, primer and probe sequences were checked against hamster-specific sequence data (Ensembl; Sanger Institute, Cambridge, UK), and primer products were confirmed to be of the predicted size. For TATA box binding protein and PCSK9, hamster sequences were not available. In these cases, primers were designed using mouse sequences, and the extended amplicon region was amplified by gradient PCR from hamster cDNA. Primer and probe sequences were then designed to the sequenced products. All probes were 5′-6 carboxyfluorescein- and 3′-6-carboxy-N,N,N‘,N’-tetramethylrhodamine-labeled. Previously unpublished primer and probe sequences are shown in **Table 1**. Real-time PCR was performed as previously described (16). Standard curves were used to check assay linearity and to determine sample gene expression in RNA equivalents using the C_T values. In addition to the genes of interest, the mRNA concentrations for three housekeeping genes, β-actin, GAPDH, and TATA box binding protein, were measured. Both β-actin and GAPDH were found to differ between treatment groups, and therefore relative expression of genes of interest was normalized to TATA box binding protein and expressed as arbitrary units.

TABLE 1. Probe and primer sequences for determination of mRNA concentration by quantitative real-time PCR

Gene	Forward Primer	Reverse Primer	Probe
TATA-box binding protein	tgccacaccagcctccgaga	tttacatccaagattcaccgtgga	tctgggatcgtaccccagctgcaaaatattgt
ABCA1	gggacacacaggcagcatc	aatgaagaaccccctcgccc	tcttgattttgtcactgctacactggcagg
MTP	gggtcctcttccgcctatactg	aataaagcatgtcgaggctgtatg	tgaacgtagtccccacgcagca
PCSK9	tgaactgtcaaggaaagggc	tgcattagctggctttttccaaata	tcagcggcatcctcacaggcct
SCD[a]	ccgagagacttcagggaacttg	ggtaggcaggtatgctccga	cagttgctcctggctgtggtgaagtct
SREBP1a	ctgtggaacaggcactgacaga	agctggagcatgtcttcgatgt	tctggacgccgcgctgctg
SREBP1c	gaagccatggattgcacgt	attcaaacaggccagggaagt	tgctccagctcatcaacaaccaagaca
SREBP2	cgttctgaggaaggccattg	agcaccatgttctcctgacgta	catcaagtatttgcagcaggtcaaccaca

[a] Sequences kindly supplied by Dr. Q. Kuo, Merck Research Laboratories, Rahway, NJ.

Statistical analysis

Data were analyzed using Genstat for Windows, release 8.1 (Lawes Agricultural Trust). Analysis was performed by two-way ANOVA with absence or presence of added dietary cholesterol as one factor and absence or presence of dietary SO as the other. Data not normally distributed was multiplied by 100 (to avoid negative numbers) and $\log_{10}$-transformed prior to analysis. Tables indicate standard error of differences of means (SED) and significances (P) for effect of dietary cholesterol (C), SO (S) and an interaction between the two (C × S). Differences were considered significant when $P < 0.05$.

RESULTS

Body mass, food intake, and tissue weights

Groups were not significantly different in body weight at the start of the trial. As can be seen in **Fig. 1A**, whereas animals that were fed control, SO, or chol diets gradually increased weight by about 8 g throughout the trial, those fed chol + SO lost approximately 12 g. As a result, the final body weight of those animals in the chol + SO group was approximately 15% lower than that of those on the other three diets (**Table 2**). Animals consuming diets containing added cholesterol consumed less food than those on diets without cholesterol. Cumulative feed intake suggests that this was primarily a result of reduced intake by animals in the chol + SO group, although there was no statistically significant interaction between cholesterol and SO consumption. Overall, animals fed chol + SO consumed approximately 450 kJ less than those in the control group, over the course of the whole experiment. When weight change per gram of food consumed was calculated, it was found that although the other groups all gained approximately 40 mg of body weight per gram consumed, those in the chol + SO group actually lost 7.5 mg per gram of food intake.

For animals in the chol group, liver weight was significantly higher than control both in absolute terms and as a percentage of body weight (**Table 3**). However, when cholesterol was fed together with SO, this effect was abolished. Perirenal adipose tissue weight was significantly lower (both in absolute and percentage terms) in the animals fed chol + SO than in the other three groups. Although the absolute weight of the epididymal adipose tissue depot was significantly reduced in the chol + SO animals, this was not significant when expressed as a percentage of total body weight.

Tissue fatty acid composition

Feeding cholesterol significantly increased oleic acid (C18:1) and decreased stearic acid (C18:0) content of liver but had no effect in perirenal adipose tissue (**Table 4**). As

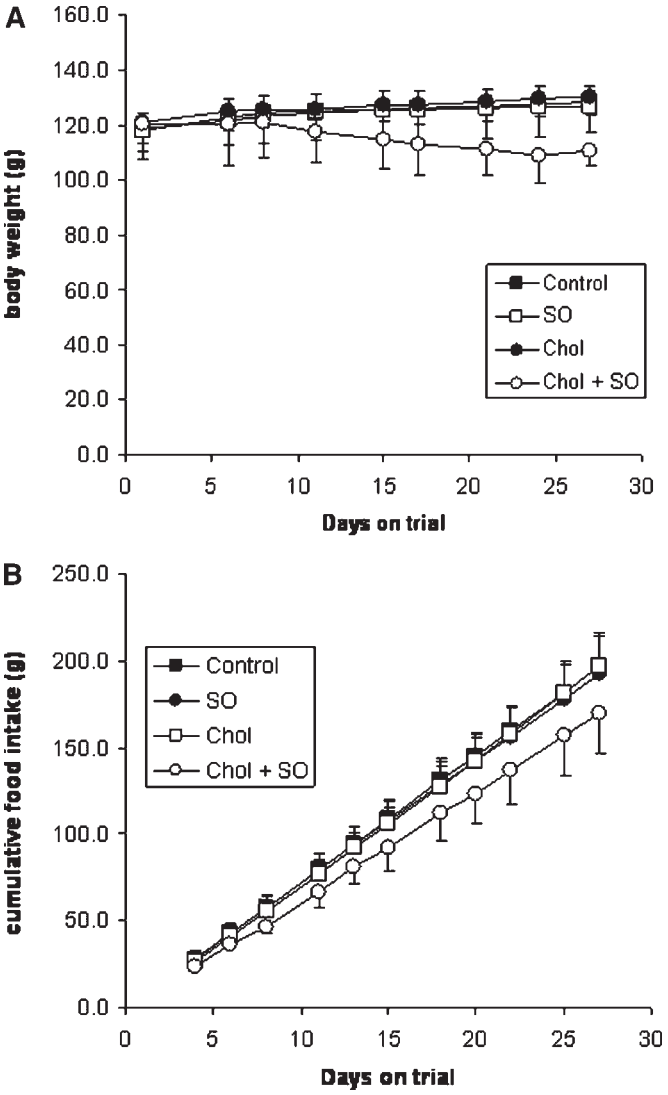

Fig. 1. Effect of feeding sterculic oil (SO) with or without dietary cholesterol (chol) on body weight (A) and feed intake (B) in male Golden Syrian Hamsters. Groups of hamsters (n = 8) were fed experimental diets consisting of chow supplemented with 20% butter with or without 0.2% (w/w) added cholesterol or SO (0.5% w/w). Animals were provided free access to food and water, and food was completely replaced every 2–4 days. Error bars indicate ± SD.

TABLE 2. Body weight and food intake of hamsters fed diets with or without 0.2% added dietary cholesterol and with or with out 0.5% added sterculic oil

	Sterculic Oil[b]	Cholesterol −	Cholesterol +	ANOVA[a]	SED	P
Final weight (g)	−	127.3	126.8	C	3.16	0.002
	+	129.5	108.5	S	3.16	0.016
				C × S	4.46	0.003
Weight change (g)	−	7.8	8.7	C	2.57	<0.001
	+	8.6	−11.9	S	2.57	<0.001
				C × S	3.63	<0.001
Total food (g)	−	197.2	192.7	C	7.20	0.034
	+	197.3	169.7	S	7.20	0.123
				C × S	10.18	0.120
Efficiency (g/g)[c]	−	0.036	0.048	C	0.015	<0.001
	+	0.046	−0.075	S	0.015	<0.001
				C × S	0.021	<0.001

SED, standard error of differences of means. Values are means for individual groups, n = 8.

[a] Data were analyzed by 2-way ANOVA with absence (−) or presence (+) of added dietary cholesterol as one factor and absence (−) or presence (+) of dietary sterculic oil as the second factor. *P* values and associated SEDs are for an effect of cholesterol (C), an effect of sterculic oil (S), or an interaction between the two (C × S).

[b] Sterculic oil contained 55.9% sterculic acid.

[c] Change in body weight (g) per g food consumed.

expected, SO markedly decreased both palmitoleic (C16:1) and oleic acid contents of liver and adipose tissue. This was accompanied by a significant increase in stearic acid, but no change in palmitic acid (C16:0) in both tissues. SO consumption resulted in a significant increase in the linoleic acid (C18:2) content of liver but not adipose tissue. No significant effect of diet was seen on arachadonic acid (C20:4)

TABLE 3. Liver and adipose tissue weights of hamsters fed diets with or without 0.2% added dietary cholesterol and with or without 0.5% added sterculic oil

	Sterculic Oil[b]	Cholesterol −	Cholesterol +	ANOVA[a]	SED	P
Liver (g)	−	5.56	6.94	C	0.23	0.015
	+	5.68	5.50	S	0.23	0.008
				C × S	0.33	0.002
Liver (% BW[c])	−	4.31	5.47	C	0.14	<0.001
	+	4.36	4.99	S	0.14	0.117
				C × S	0.19	0.057
Perirenal adipose (g)	−	2.04	2.05	C	0.12	0.007
	+	1.91	1.20	S	0.12	<0.001
				C × S	0.17	0.005
Perirenal adipose (% BW[c])	−	1.58	1.61	C	0.08	0.048
	+	1.48	1.09	S	0.08	<0.001
				C × S	0.12	0.018
Epididymal adipose (g)	−	2.07	2.04	C	0.16	0.021
	+	2.11	1.38	S	0.16	0.059
				C × S	0.22	0.036
Epididymal adipose (% BW[c])	−	1.60	1.60	C	0.11	0.108
	+	1.63	1.25	S	0.11	0.161
				C ×S	0.16	0.108

Values are means for individual groups, n = 8.

[a] Data were analyzed by 2-way ANOVA with absence (−) or presence (+) of added dietary cholesterol as one factor and absence (−) or presence (+) of dietary sterculic oil as the second factor. *P* values and associated SEDs are for an effect of cholesterol (C), an effect of sterculic oil (S), or an interaction between the two (C × S).

[b] Sterculic oil contained 55.9% sterculic acid.

[c] Tissue weight expressed as percent of final body weight (BW).

TABLE 4. Fatty acid composition of liver and perirenal adipose tissue lipid of hamsters fed diets with or without 0.2% added dietary cholesterol and with or without 0.5% added sterculic oil

		Sterculic Oil[b]	Cholesterol −	Cholesterol +	ANOVA[a]	SED	P
Liver	C16:0	−	28.9	27.9	C	0.7	0.008
		+	29.6	26.5	S	0.7	0.655
					C × S	1.0	0.172
	C16:1	−	2.0	2.4	C	0.2	0.194
		+	1.1	1.3	S	0.2	<0.001
					C × S	0.3	0.526
	C18:0	−	18.8	14.1	C	1.6	0.034
		+	24.3	22.0	S	1.6	<0.001
					C × S	2.2	0.463
	C18:1	−	26.8	34.3	C	1.9	0.003
		+	20.1	25.1	S	1.9	<0.001
					C × S	2.7	0.507
	C18:2	−	20.1	17.2	C	1.2	0.288
		+	21.2	21.6	S	1.2	0.023
					C × S	1.6	0.150
	C20:4	−	11.9	7.5	C	1.5	0.14
		+	11.4	11.4	S	1.5	0.25
					C × S	2.1	0.14
Perirenal adipose	C16:0	−	28.2	27.5	C	0.5	0.016
		+	30.2	28.2	S	0.5	0.013
					C × S	0.7	0.204
	C16:1	−	4.2	4.0	C	0.2	0.127
		+	2.7	2.3	S	0.2	<0.001
					C × S	0.3	0.561
	C18:0	−	4.4	5.0	C	0.5	0.671
		+	9.4	8.4	S	0.5	<0.001
					C × S	0.7	0.141
	C18:1	−	42.1	42.5	C	0.7	0.211
		+	36.6	38.0	S	0.7	<0.001
					C × S	1.0	0.414
	C18:2	−	15.2	15.5	C	0.5	0.205
		+	14.8	15.9	S	0.5	0.960
					C × S	0.7	0.432

Values are means for individual groups, n = 8. Fatty acid methyl esters, g/100 g.

[a] Data were analyzed by 2-way ANOVA with absence (−) or presence (+) of added dietary cholesterol as one factor and absence (−) or presence (+) of dietary sterculic oil as the second factor. *P* values and associated SEDs are for an effect of cholesterol (C), an effect of sterculic oil (S), or an interaction between the two (C × S).

[b] Sterculic oil contained 55.9% sterculic acid.

content of liver (this fatty acid was below the level of detection in adipose tissue), and there was no significant statistical interactions between the effects of cholesterol and SO consumption on the proportion of the fatty acids in either tissue.

Plasma and lipoprotein lipids

As expected, plasma total cholesterol was significantly increased in those animals consuming high-cholesterol diets (**Table 5**). However, this effect was significantly greater in those also consuming SO, such that average total cholesterol was over 50% higher in the chol + SO group than in the chol group. Although free cholesterol and esterified cholesterol were both increased in this group, proportionally, the most dramatic effect was on free cholesterol, which was approximately 135% higher than in the chol group. The overall result was reduction in the proportion of plasma cholesterol that was esterfied in animals fed chol + SO. No significant effects of feeding SO alone were seen on plasma cholesterol. Total plasma triacylglycerol was reduced

TABLE 5. Plasma total, free, and cholesteryl ester and total triacylglycerol of hamsters fed diets with or without 0.2% added dietary cholesterol and with or without 0.5% added sterculic oil

	Sterculic Oil[b]	Cholesterol −	Cholesterol +	ANOVA[a]	SED	P
Total chol[c] (mmol/l)	−	4.7 (2.67)	9.9 (2.99)	C	0.01	<0.001
	+	4.0 (2.61)	15.1 (3.18)	S	0.01	<0.001
				C × S	0.02	<0.001
Free chol[c] (mmol/l)	−	1.5 (2.17)	2.6 (2.42)	C	0.02	<0.001
	+	1.3 (2.10)	6.1 (2.78)	S	0.02	<0.001
				C × S	0.03	<0.001
Cholesteryl ester (mmol/l)	−	3.2 (2.51)	7.2 (2.86)	C	0.01	<0.001
	+	2.8 (2.44)	9.0 (2.95)	S	0.01	0.003
				C × S	0.02	<0.001
Cholesteryl ester (%)	−	68.5	73.3	C	1.1	0.067
	+	68.8	60.0	S	1.1	<0.001
				C × S	1.5	<0.001
TAG (mmol/l)	−	1.9	2.6	C	0.4	0.285
	+	1.5	1.5	S	0.4	0.054
				C × S	0.5	0.371

TAG, triacylglycerol. Values are means for individual groups, n = 8.
[a] Data were analyzed by 2-way ANOVA with absence (−) or presence (+) of added dietary cholesterol as one factor and absence (−) or presence (+) of dietary sterculic oil as the second factor. *P* values and associated SEDs are for an effect of cholesterol (C), an effect of sterculic oil (S), or an interaction between the two (C × S).
[b] Sterculic oil contained 55.9% sterculic acid.
[c] Not normally distributed, therefore statistics were performed with transformed data ($\log_{10} \times 100$) given in parentheses.

TABLE 6. Lipoprotein cholesterol and TAG concentrations of hamsters fed diets with or without 0.2% added dietary cholesterol and with or with out 0.5% added sterculic oil

	Sterculic Oil[b]	Cholesterol −	Cholesterol +	ANOVA[a]	SED	P
Chylo chol[c] (mmol/l)	−	0.19 (1.15)	0.80 (1.75)	C	0.17	<0.001
	+	0.24 (1.15)	1.21 (1.89)	S	0.17	0.677
				C × S	0.24	0.678
Chylo TAG[c] (mmol/l)	−	0.46 (1.50)	0.40 (1.50)	C	0.14	0.765
	+	0.26 (1.29)	0.35 (1.32)	S	0.14	0.125
				C × S	0.20	0.941
VLDL chol (mmol/l)	−	0.21	1.36	C	0.10	<0.001
	+	0.20	0.49	S	0.10	<0.001
				C × S	0.14	<0.001
VLDL TAG (mmol/l)	−	0.78	1.44	C	0.22	0.251
	+	0.62	0.47	S	0.22	0.014
				C × S	0.30	0.071
LDL chol[c] (mmol/l)	−	0.22 (1.36)	1.63 (2.20)	C	0.04	<0.001
	+	0.20 (1.30)	5.59 (2.73)	S	0.04	<0.001
				C × S	0.05	<0.001
HDL chol (mmol/l)	−	2.67	3.52	C	0.22	<0.001
	+	2.31	3.73	S	0.22	0.729
				C × S	0.31	0.205

Chylo, chylomicron. Values are means for individual groups, n = 8.
[a] Data were analyzed by 2-way ANOVA with absence (−) or presence (+) of added dietary cholesterol as one factor and absence (−) or presence (+) of dietary sterculic oil as the second factor. *P* values and associated SEDs are for an effect of cholesterol (C), an effect of sterculic oil (S), or an interaction between the two (C × S).
[b] Sterculic oil contained 55.9% sterculic acid.
[c] Not normally distributed, therefore statistics were performed with transformed data ($\log_{10} \times 100$) given in parentheses.

by feeding SO both in the absence and the presence of dietary cholesterol. No effect of dietary cholesterol was seen on plasma triacylglycerol.

Chylomicron cholesterol was increased by cholesterol feeding but was unaffected by SO (**Table 6**). No significant effect of either cholesterol or SO was seen on chylomicron triacylglycerol. Cholesterol feeding significantly increased VLDL cholesterol, but this effect was markedly attenuated when SO was also included in the diet. SO reduced VLDL triacylglycerol, but there was no significant effect of dietary cholesterol. LDL cholesterol was markedly increased by cholesterol feeding, and this effect was dramatically enhanced by including SO in the diet. Thus, LDL cholesterol was almost 250% higher in the chol + SO group compared with the chol group. HDL cholesterol was increased by cholesterol feeding but unaffected by SO.

Liver lipids

Consuming a cholesterol-enriched diet significantly increased the amount of free and esterfied cholesterol in the liver (**Table 7**). By contrast, dietary SO reduced hepatic concentration of both. As such, the amount of cholesteryl ester stored in the livers of the animals in the chol + SO group was only 42% of that in the chol group. There was a significant interaction in the effects of cholesterol and SO on hepatic triacylglycerol concentrations, with SO having no effect in animals not fed cholesterol but reducing it by 62% in those that were. The fatty acid composition of hepatic cholesteryl ester and triacylglycerol were also investigated (**Table 8**). The major fatty acid in both lipid fractions was palmitic acid (C16:0). In triacylglycerol, the relative amount of C16:0 was not influenced by diet. SO increased the relative amount of stearic acid (C18:0) and decreased the amount of oleic acid (C18:1) in hepatic triacylglycerol. The effect on the latter was greatest in cholesterol-fed animals. Palmitic acid content of cholesteryl ester was specifically reduced in the chol + SO animals. Cholesterol feeding produced a marked decrease in C18:0 and increase in C18:1 in cholesteryl ester, and this effect was at least partially reversed when SO was included in the diet.

Plasma ALT

No significant difference in plasma ALT levels was seen between control and SO-fed animals (Table 7). Although a modest increase was apparent in cholesterol-fed animals, levels in those fed chol + SO were raised 20-fold.

Effects on gene expression

ACC and FAS mRNA were specifically reduced in the livers of animals in the chol + SO group (**Table 9**). By contrast, hepatic ABCA1, LPL, and SCD mRNA were markedly increased in the chol + SO group. Highly significant correlations were seen between ABCA1 mRNA and LPL mRNA ($r^2 = 0.73$, $P < 0.001$) and SCD mRNA ($r^2 = 0.88$, $P < 0.001$). LDLR mRNA concentrations were reduced by cholesterol feeding but unaffected by SO. Hepatic PCSK9 mRNA was unaffected by diet. Hepatic SREBP1a, -1c, and -2 mRNA concentrations were also measured (Table 9), and both SREBP1a and -1c were shown to be specifically decreased in the chol + SO group. SREBP2 mRNA was reduced by cholesterol feeding but unaffected by SO. Hepatic

TABLE 7. Hepatic free and cholesteryl ester and TAG concentrations and plasma alanine aminotransferase activity of hamsters fed diets with or without 0.2% added dietary cholesterol and with or without 0.5% added sterculic oil

		Cholesterol		ANOVA[a]		
	Sterculic Oil[b]	−	+		SED	p
Free chol (μmol/liver)	−	21.4	36.7	C	2.0	<0.001
	+	16.3	33.0	S	2.0	0.035
				C × S	2.8	0.713
Cholesteryl ester[c] (μmol/liver)	−	8.8 (2.91)	157.9 (4.19)	C	0.06	<0.001
	+	2.7 (2.38)	66.4 (3.79)	S	0.06	<0.001
				C × S	0.09	0.308
TAG (μmol/liver)	−	5.62	9.22	C	0.49	0.199
	+	5.76	3.46	S	0.49	<0.001
				C × S	0.69	<0.001
Plasma ALT	−	50	114	C	49	<0.001
(i.u./l)	+	44	1,429	S	49	<0.001
				C × S	69	<0.001

ALT, plasma alanine aminotransferase. Values are means for individual groups, n = 8.

[a] Data were analyzed by 2-way ANOVA with absence (−) or presence (+) of added dietary cholesterol as one factor and absence (−) or presence (+) of dietary sterculic oil as the second factor. *P* values and associated SEDs are for an effect of cholesterol (C), an effect of sterculic oil (S) or an interaction between the two (C × S).

[b] Sterculic oil contained 55.9% sterculic acid.

[c] Not normally distributed, therefore statistics were performed with transformed data ($\log_{10} \times 100$) given in parentheses.

MTP mRNA levels were reduced by dietary cholesterol, and this effect was attenuated by SO.

DISCUSSION

A variety of evidence, largely arising from work on SCD1-deficient mice, suggests that SCD may have a role in regulating adiposity (2, 5, 6, 18). It has been suggested that inhibition of SCD activity could represent a potential target for the treatment of obesity. In the present study, we investigated the impact of decreasing SCD activity in Golden Syrian Hamsters by feeding SO. SO is known to be a potent inhibitor of SCD, in vivo and in vitro, and appears to directly inhibit SCD enzyme activity without affecting either SCD1 mRNA or protein levels (19, 20). In the present study, we saw decreases in the proportion of C16:1 and C18:1 in both adipose tissue and liver. The reduction in C16:1 probably best reflects the extent of inhibition of SCD, inasmuch as this is predominantly synthesized endogenously, with very little coming from the diet. In animals fed SO (with or without cholesterol), the ratio of C16:0/C16:1 was decreased by approximately 50%. Previous work using SCD antisense RNA in mice suggested that this level of inhibition was sufficient to have significant effects on body adiposity (18). Thus, the lack of effect of SO in animals not fed cholesterol appears to be a specific difference in response between these two species. Previous studies have been inconclusive as to the effect of cyclopropenoid fatty acids on the activity of other fatty acid desaturases. Although it was originally suggested that they had no effect on Δ6 desaturase activity (19), subsequent studies have questioned this (21, 22). In the present study, there was a small increase in hepatic C18:2 associated with SO feeding. However, there was no evidence of a concomitant decrease in C20:4 concentrations. Furthermore, no change in adipose tissue C18:2 was observed. It is possible that the change in C18:2 in the liver was an adaptive mechanism to maintain membrane fluidity as a result of the fall in C16:1 and C18:1 concentrations. Overall, our data suggest that if there are any effects of SO on other desaturases, they are very modest compared with the inhibition of SCD. Previous studies have described reduced growth rates with feeding cyclopropenoid fatty acids, although these have tended to be at doses greater than 0.5% (23–25).

TABLE 8. Fatty acid composition of hepatic TAG and cholesteryl ester of hamsters fed diets with or without 0.2% added dietary cholesterol and with or without 0.5% added sterculic oil

			Cholesterol		ANOVA[a]		
		Sterculic Oil[b]	−	+		SED	p
Liver TAG	C16:0	−	55.3	53.6	C	1.2	0.192
		+	54.7	53.3	S	1.2	0.714
					C × S	1.6	0.921
	C18:0	−	28.6	27.4	C	1.5	0.390
		+	31.6	35.5	S	1.5	0.001
					C × S	2.1	0.103
	C18:1	−	14.5	17.3	C	1.0	0.866
		+	12.5	10.0	S	1.0	<0.001
					C × S	1.4	0.014
Liver cholesteryl ester	C16:0	−	54.1	51.1	C	0.9	<0.001
		+	53.7	47.2	S	0.9	0.024
					C × S	1.2	0.055
	C18:0	−	34.1	23.1	C	1.0	<0.001
		+	35.1	34.8	S	1.0	<0.001
					C × S	1.4	<0.001
	C18:1	−	9.7	21.9	C	1.1	<0.001
		+	9.4	15.4	S	1.1	0.004
					C × S	1.6	0.015

Hepatic lipids were extracted and separated by TLC. TAG and cholesteryl ester were reextracted from the TLC plates, and fatty acids were methylated and analyzed by GC. Values are means for individual groups, n = 8.

[a] Data were analyzed by 2-way ANOVA with absence (−) or presence (+) of added dietary cholesterol as one factor and absence (−) or presence (+) of dietary sterculic oil as the second factor. *P* values and associated SEDs are for an effect of cholesterol (C), an effect of sterculic oil (S), or an interaction between the two (C × S).

[b] Sterculic oil contained 55.9% sterculic acid.

TABLE 9. Hepatic mRNA concentrations in liver of hamsters fed diets with or without 0.2% added dietary cholesterol and with or without 0.5% added sterculic oil

		Cholesterol		ANOVA[a]		
	Sterculic Oil[b]	−	+		SED	*P*
ACC	−	2.40	1.97	C	0.22	<0.001
	+	2.76	1.00	S	0.22	0.170
				C × S	0.31	0.005
FAS	−	2.59	1.43	C	0.18	<0.001
	+	2.93	0.65	S	0.18	0.232
				C × S	0.26	0.005
LPL	−	0.65	2.10	C	0.34	<0.001
	+	0.56	4.25	S	0.34	0.006
				C × S	0.48	0.003
SCD	−	0.58	1.89	C	0.31	<0.001
	+	0.61	3.52	S	0.31	0.012
				C × S	0.44	0.015
LDLR	−	1.13	0.86	C	0.19	0.008
	+	1.57	0.74	S	0.19	0.411
				C × S	0.27	0.151
SREBP1c	−	1.02	1.02	C	0.07	0.004
	+	1.22	0.77	S	0.07	0.745
				C × S	0.10	0.004
SREBP1a	−	0.96	1.02	C	0.06	0.073
	+	1.09	0.82	S	0.06	0.527
				C × S	0.08	0.005
SREBP2	−	1.11	0.95	C	0.07	0.005
	+	1.23	0.94	S	0.07	0.457
				C × S	0.10	0.423
ABCA1	−	0.61	1.08	C	0.07	<0.001
	+	0.67	1.35	S	0.07	0.029
				C × S	0.10	0.160
MTP	−	1.15	0.44	C	0.09	<0.001
	+	0.84	0.53	S	0.09	0.683
				C × S	0.12	0.005
PCSK9	−	1.10	0.80	C	0.22	0.117
	+	1.44	1.04	S	0.22	0.199
				C × S	0.31	0.813

ACC, CoA carboxylase; LDLR, LDL receptor; MTP, microsomal triglyceride transfer protein; PCSK9, proprotein convertase subtilisin kexin 9; SCD, stearoyl CoA desaturase; SREBP1a, SREBP1c, and SREBP2, sterol-regulatory element binding proteins 1a, 1c, and 2. First-strand cDNA was synthesized from an equal quantity of total mRNA and used to quantify individual mRNAs, which were then normalized to TATA box binding protein mRNA as internal standard. Values are means for individual groups, n = 8.

[a] Data were analyzed by 2-way ANOVA with absence (−) or presence (+) of added dietary cholesterol as one factor and absence (−) or presence (+) of dietary sterculic oil as the second factor. *P* values, and associated SEDs are for an effect of cholesterol (C), an effect of sterculic oil (S), or an interaction between the two (C × S).

[b] Sterculic oil contained 55.9% sterculic acid.

We found no such effects in animals fed SO without added cholesterol. There was also no evidence of hepatotoxicity, as determined by plasma ALT concentrations in these animals. This was in marked contrast to the situation in which SO was fed together with cholesterol and plasma ALT was increased 20-fold. Paradoxically, Flowers et al. (26) reported similar liver damage, and raised plasma free cholesterol concentrations in $SCD^{-/-}$ mice fed cholesterol-free, very low fat diets. This was also associated with changes in plasma bile acids and bilirubin, which the authors attributed to cholestasis, possibly as a result of altered membrane fatty acid composition. Interestingly, supplementing the very low fat diet with oleic acid–rich oil appeared to reverse the hepatotoxic effects. In the present experiment, despite the fact that the diets were relatively rich in oleic acid, we still saw evidence of liver damage. However, unlike the findings in $SCD^{-/-}$ mice fed low-fat diets (27), we found no evidence that the hypercholesterolemia was a result of the accumulation of lipoprotein X (characteristic of animals/humans suffering from cholestasis). The LDL fraction from chol + SO-fed animals had mobility similar to that of "normal" LDL on agarose gel electrophoresis and was essentially all precipitated by phosphotungstate, indicating association with apolipoprotein B/E (apoB/apoE) (data not shown). It is possible that the liver damage was a result of the accumulation of potentially toxic derivatives of cholesterol such as hydroxyl- or epoxy-cholesterol or their derivatives (27). Alternatively, the sequestration of de novo-synthesized oleic acid into the expanded cholesteryl ester pool may have diverted the fatty acid from other essential functions.

The changes in fatty acid composition of the tissues clearly indicate that feeding SO in the absence of added dietary cholesterol inhibited SCD activity. Despite this, there was essentially no impact on body weight or adipose tissue depot size. However, when fed in combination with dietary cholesterol, SO did reduce body weight. At least part of the effect is due to decreased food intake (the 450 kJ decrease in energy intake over the course of the experiment could theoretically account for a reduction in adipose tissue of approximately 11 g). Although a cholesterol-mediated reduction in SREBP expression, leading to reduced hepatic ACC and FAS expression, may also contribute to this effect, it is not presently known to what extent hepatic lipogenesis contributes to adipose tissue triacylglycerol in the hamster. Alternatively, impaired lipid absorption, possibly as a result of altered bile acid metabolism resulting from the observed liver damage, may also contribute to reduced feed efficiency.

We were also interested in investigating the impact of inhibiting SCD activity on lipoprotein metabolism. The previously published effects of SCD deficiency on hepatic lipid metabolism led us to speculate that reduced SCD activity may impact the ability to adapt to diets high in saturated fat and cholesterol. We chose to study the effect of SO (in the presence and absence of added dietary cholesterol) in the male Golden Syrian Hamster, because we have previously demonstrated specific interactions between dietary fat and cholesterol in this species that are analogous to those seen in humans and have shown that accumulation of hepatic cholesteryl ester is characteristic of hamsters fed such diets (10, 12).

On the low-cholesterol diet, there was no significant impact of SO on plasma cholesterol or its distribution between different lipoprotein fractions. It has previously been reported that $SCD^{-/-}$ mice fed a chow diet have significantly elevated plasma cholesterol compared with wild-type animals (7). However, a further report indicated that there was no significant difference in lipoprotein cholesterol profile between wild-type and SCD1-deficient mice (28) fed a chow diet. Feeding cholesterol to hamsters produced the expected increase in plasma cholesterol, particularly that associated with the potentially atherogenic VLDL and LDL fractions. As expected, hepatic LDLR

mRNA concentrations were reduced by cholesterol feeding (12). The LDLR is known to be regulated by SREBP2, which is posttranscriptionally regulated by dietary cholesterol, through inhibition of the release of the active nuclear form of the protein from the endoplasmic reticulum by increases in intracellular sterol concentrations (29). This then results in reduction in SREBP2 gene expression, inasmuch as it is autoregulated through a sterol response element in its promoter (30). When cholesterol was fed in combination with SO, hamsters exhibited an even more dramatic hypercholesterolemia that was associated with a specific accumulation of free cholesterol and was predominantly a result of an increase in LDL cholesterol. This could not be attributed to a further downregulation of LDLR gene expression, because hepatic LDLR mRNA was not affected by the addition of SO. It is possible that LDLR activity was regulated posttranslationally. It has recently been shown that overexpression of the protease PCSK9 induces degradation of LDLR (31) and that it is regulated by SREBP1c (32). In the present study, however, there was no significant effect of cholesterol and/or SO on hepatic PCSK9 mRNA concentrations.

It is of note that VLDL cholesterol and triacylglycerol were reduced in animals fed cholesterol and SO. Similar findings have been reported in SCD-deficient mice (7, 24). MTP is known to be essential for the assembly of VLDL and is involved in the lipidation of apoB (33). However, MTP mRNA levels were not significantly different between animals fed cholesterol with or without SO, suggesting that changes in expression of MTP could not explain these findings. LDL is normally viewed as a product of VLDL metabolism, and this suggests either enhanced conversion of VLDL to LDL or, perhaps more likely, the direct secretion of lipoproteins from the liver within the LDL density range.

Hepatic cholesteryl ester was reduced by inhibition of SCD activity, particularly when animals were fed additional cholesterol. Hepatic triacylglycerol concentration was not affected in animals on the low-cholesterol diet, but was reduced in those animals fed additional cholesterol. This supports previous findings that fatty acid incorporation into hepatic lipids is inhibited when SCD activity is reduced (7, 34) even, as in the present study, when there is a significant amount of oleic acid in the diet. We also demonstrated a reduction in the proportion of oleic acid associated with these hepatic lipid pools. Thus, our results support the suggestion that there is an absolute requirement for de novo synthesis of oleic acid to sustain cholesteryl ester and triacylglycerol synthesis. The reduced capacity to store cholesterol within the liver appears to be the most likely cause of the hypercholesterolemia seen in the hamsters fed cholesterol and SO. Without the ability to convert cholesterol to this relatively inert form, the liver may respond by incorporating free cholesterol into VLDL particles (perhaps of increased density) and secreting it back into the plasma.

We also investigated the effect of inhibiting SCD activity on the expression of a number of genes in the liver that code for proteins involved in lipid metabolism. Previous work indicates that knockout of the SDC1 gene in mice results in downregulation of a range of hepatic lipogenic genes and that this is associated with a decrease in hepatic SREBP1c expression (5). More recently Jiang et al. (18) treated mice with antisense oligonucleotide inhibitors of SCD1 and demonstrated a downregulation of lipogenic genes in both liver and adipose tissue. In the present study, SO alone had little effect on hepatic mRNA concentrations for any of the genes. However, when cholesterol was added to the diet, ACC and FAS mRNA were decreased. This was associated with a decrease in mRNA for both SREBP1a and -1c. These transcription factors are known regulators of lipogenic gene expression (35, 36). Hepatic concentrations of LPL and SCD mRNA were actually increased by dietary cholesterol, and this effect was further potentiated by inhibiting SCD activity. Both of these genes have been shown to contain sterol-regulatory elements within their promoters and therefore might have been expected to be reduced by the change in SREBP expression. However, dietary cholesterol has previously been shown to increase hepatic SCD gene expression in rats (37) and mice (38), and it has been suggested that this is dependent on expression of liver X receptor α (LXRα) (39). LXRα is a nuclear receptor that is activated by oxygenated derivatives of cholesterol and is known to regulate the expression of a range of genes for enzymes and other proteins involved in cholesterol and lipid metabolism (40). It is possible that increasing the intake of dietary cholesterol could lead to the generation of increased LXR ligand(s) and this may, in turn lead to the upregulation of SCD gene expression. This is supported by the recent discovery of an LXR response element within the promoter of the mouse SCD1 gene (41). The inhibition of SCD activity, and the accompanying reduction in the amount of cholesterol esterified, could lead to further generation of potential LXR ligands. A similar mechanism could also be postulated for the impact of cholesterol on LPL gene expression, which has also been reported to have an LXR response element within its promoter (42). The highly significant correlations between ABCA1 mRNA and SCD and LPL mRNA support this hypothesis. ABCA1 is well-established as a "target" gene for LXR (43). However, it is of note that ACC and FAS have both been reported to have LXR response elements within their promoters (44, 45), and we observed that expression of these genes was actually suppressed by the combination of dietary cholesterol and SO. Thus, mechanisms whereby cholesterol interacts with the inhibition of desaturase activity in the regulation of hepatic gene expression warrant further investigation. Overall, the changes in mRNA concentrations for ACC and FAS suggest that a reduction in hepatic lipogenesis may at least partly explain the reduction in adiposity in response to inhibition of SCD activity in cholesterol-fed animals. This in turn may be a result of reduced expression of SREBP (1a and 1c). Interestingly, these changes are not seen in adipose tissue itself, where FAS mRNA actually increases in animals fed cholesterol and SO (unpublished observations).

In conclusion, inhibition of SCD by SO in the hamster has little impact on body weight or lipoprotein metabolism despite clearly reducing tissue accumulation of oleic acid. However, when diets are enriched with cholesterol, there

are profound effects, with a reduction in overall body weight and adipose tissue deposition marked by hypercholesterolemia and evidence of liver damage. It remains unclear why the effects on body weight and adiposity are only seen in animals fed cholesterol. Although these data lend some support to a possible role of SCD in regulating adiposity, they also raise the possibility that, at least in some circumstances, reduced SCD activity can be associated with disordered lipoprotein metabolism and accumulation of potentially atherogenic lipoproteins in the plasma. This study also highlights the potential role of SCD in producing oleic acid that is specifically channelled into hepatic cholesteryl ester, thereby perhaps protecting the liver from potentially toxic effects of free cholesterol and/or its derivatives.

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