Lack of stearoyl-CoA desaturase 1 upregulates basal thermogenesis but causes hypothermia in a cold environment

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Abstract **Stearoyl-CoA desaturase (SCD) is a microsomal enzyme involved in the biosynthesis of oleate and palmitoleate. Mice with a targeted disruption of the SCD1 isoform (SCD1$^{-/-}$) exhibit reduced adiposity and increased energy expenditure. To address whether the energy expenditure is attributable to increased thermogenesis, we investigated the effect of SCD1 deficiency on basal and cold-induced thermogenesis. SCD1$^{-/-}$ mice have increased expression of uncoupling proteins in brown adipose tissue (BAT) relative to controls. The β3-adrenergic receptor (β3-AR) expression was increased and the phosphorylation of cAMP response element binding protein and the protein level of peroxisome proliferator-activated receptor-γ coactivator-1α were increased in the SCD1$^{-/-}$ mice. Both lipolysis and fatty acid oxidation were increased in the SCD1$^{-/-}$ mice. When exposed to 4°C, SCD1$^{-/-}$ mice showed hypothermia, hypoglycemia, and depleted liver glycogen. High levels of dietary oleate partially compensated for the hypothermia and rescued plasma glucose and liver glycogen. These results suggest that SCD1 deficiency stimulates basal thermogenesis through the upregulation of the β3-AR-mediated pathway and a subsequent increase in lipolysis and fatty acid oxidation in BAT. The hypothermia and hypoglycemia in cold-exposed SCD1$^{-/-}$ mice and the compensatory recovery by oleate indicate an important role of SCD1 gene expression in thermoregulation.**—Lee, S-H., A. Dobrzyn, P. Dobrzyn, S. M. Rahman, M. Miyazaki, and J. M. Ntambi. **Lack of stearoyl-CoA desaturase 1 upregulates basal thermogenesis but causes hypothermia in a cold environment. *J. Lipid Res.* 2004.** 45: **1674–1682.**

Supplementary key words brown adipose tissue • uncoupling protein • lipid metabolism

Stearoyl-CoA desaturase (SCD) is the rate-limiting enzyme in the biosynthesis of monounsaturated fatty acids. It catalyzes the introduction of the *cis* double bond in the Δ9 position of fatty acyl-CoA substrates. The major monounsaturated fatty acids of triglyceride (TG), cholesteryl esters, and membrane phospholipids are palmitoleic and oleic acids (1). The ratio of stearic acid to oleic acid is one of the factors influencing cell membrane fluidity, and an alteration in this ratio is implicated in obesity, aging, and various diseases such as diabetes, heart disease, and cancer (2).

Four SCD isoforms exist in the mouse genome. SCD1 is expressed in liver and brown and white adipose tissue, whereas SCD2 is expressed in brain and brown and white adipose tissue (3). SCD3 is expressed mainly in the skin and Harderian gland (4), whereas SCD4 is expressed in heart (5). Recent studies of the *asebia* mouse strains (*ab*^{j} and *ab*^{2j}) with a naturally occurring mutation in SCD1 and a laboratory mouse model with a targeted disruption (SCD1$^{-/-}$) provide new insights into the physiological role of the SCD1 gene and its endogenous products (6, 7). Mice with a targeted disruption of the SCD1 gene had reduced adiposity. TG synthesis in liver was decreased relative to that in the wild type, which suggests that SCD1 gene expression is highly correlated with fat accumulation (7). The reduction in fat accumulation of SCD1$^{-/-}$ mice was the result of downregulation in the expression of lipogenic genes (FAS, SREBP1, GPAT) and upregulation in the expression of genes involved in fatty acid oxidation (8). In addition, SCD1 deficiency decreased the obese phenotype of the *ob/ob* mouse (9), suggesting that obesity is highly correlated with SCD1 expression.

Energy expenditure is an important factor in the regulation of body weight. Heat production (thermogenesis) represents a major form of energy expenditure and plays a significant role in the maintenance of energy balance.

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Abbreviations: β3-AR, β3-adrenergic receptor; BAT, brown adipose tissue; CREB, cAMP response element binding protein; DAG, diacylglycerol; HSL, hormone-sensitive lipase; PCA, perchloric acid; PGC1α, peroxisome proliferator-activated receptor-γ coactivator-1α; PL, phospholipid; SCD, stearoyl-CoA desaturase; TG, triglyceride; UCP, uncoupling protein.

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Brown adipose tissue (BAT) is the primary site of thermogenesis and potentially functions to regulate body weight in rodents (10, 11). Therefore, enhanced function of BAT is a significant factor that increases energy expenditure and reduces obesity. Uncoupling protein 1 (UCP1) is predominantly expressed in BAT of rodents and functions to uncouple oxidative respiration from ATP synthesis, resulting in dissipation of energy as heat (12). The UCP1 gene in BAT is activated in response to cold exposure and plays a part in the maintenance of body temperature, as demonstrated in UCP1 knockout mice that showed cold sensitivity and lower oxygen consumption (13, 14). Two structurally homologous UCPs, UCP2 and UCP3, have been identified in BAT of rodents (15, 16); however, their role in nonshivering thermogenesis remains unclear (14).

Recently, there has been growing interest in the role of β3-adrenergic receptor (β3-AR) because of its predominant expression in adipose tissue and potential as a pharmacological target to control energy expenditure and lipid accretion (17). Pharmacological studies indicate that the β-AR subtype responsible for the stimulation of oxygen consumption and UCP expression is exclusively the β3 subtype (18). Stimulation of β3-ARs leads to nonshivering thermogenesis via the activation of UCPs in brown fat (19). Activated β3-AR is coupled to the adenylyl cyclase and stimulates subsequent catalytic responses through signal transduction via protein kinase A (19), which phosphorylates multiple targets, including cAMP response element binding protein (CREB) (20) and hormone-sensitive lipase (HSL) (21). On the other hand, it is believed that free fatty acids serve as substrates for mitochondrial oxidation and provide a signal to activate UCP1 (22). Fatty acids mimic the noradrenaline effects and stimulate UCP1 expression in cultured brown adipocytes, even though the physiological route is unknown (14, 23, 24).

In a previous study, we have shown that SCD1 knockout mice have enhanced oxygen consumption and metabolic rate (8). However, the physiological role and significance of SCD1 deficiency in thermoregulation has not been investigated. The objective of the current study was to examine whether deficiency of SCD1 induces the thermogenic activity of BAT. We found that SCD1 deficiency increased basal thermogenesis through the activation of a β3-AR-mediated pathway and subsequent increases in lipolysis and fatty acid oxidation in BAT of mice. However, upon cold exposure, SCD1$^{-/-}$ mice develop hypothermia, which is associated with hypoglycemia and depletion of liver glycogen. Dietary oleate partially compensates for hypothermia and hypoglycemia in cold-exposed SCD1$^{-/-}$ mice, suggesting that endogenously synthesized oleate plays a role in the control of thermoregulation associated with glycogen and lipid metabolism.

EXPERIMENTAL PROCEDURES

Animals and diets

The generation of targeted SCD1$^{-/-}$ mice has been previously described (25). Prebred homozygous (SCD1$^{-/-}$) and wild-type (SCD1$^{+/+}$) mice on a pure 129 SV background were used. The breeding and care of these animals is 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 a standard chow diet (No. 5008 test diet; PMI Nutrition International, Inc., Richmond, VA). At 12 weeks of age, SCD1$^{+/+}$ and SCD1$^{-/-}$ mice were fed control oil (soybean oil), tristearin, or triolein-supplemented diet (20% by weight) for 3 weeks and exposed to 4°C for 4 h. The high-fat diet was purchased from Harlan Teklad (Madison, WI) and contained 30% casein, 20% fat, 18.8% sucrose, 37% corn starch, 6.3% cellulose, 0.2% calcium carbonate, DL-methionine, salt mix (AIN-76), and vitamin mix (AIN-76).

Materials

Antibodies for UCP1 and phosphorylated CREB (pCREB) were purchased from Alpha Diagnostic (San Antonio, TX) and Cell Signaling (Beverly, MA), respectively. Antibodies for β3-AR and peroxisome proliferator-activated receptor-γ coactivator-1α (PGC1α) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). α-[^{32}P]dCTP and L-[^{3}H]carnitine were obtained from NEN Life Science (Boston, MA) and American Radiolabeled Chemicals (St. Louis, MO), respectively. TLC plates (TLC Silica Gel G60) were from Merck (Darmstadt, Germany). All chemicals were purchased from Sigma Chemical (St. Louis, MO) unless otherwise specified.

Isolation and analysis of RNA

Total RNA was isolated from BAT with Trizol Reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. Twenty micrograms of total RNA was fractionated on 1.0% agarose-2.2 M formaldehyde gels and transferred to Hybond N^{+} nylon membrane. After ultraviolet cross-linking, the membrane was hybridized with cDNA probes labeled with [^{32}P]dCTP by a random primer labeling kit (Promega, Madison, WI). After washing, the membranes were exposed to X-ray film at −80°C, and signals were quantified by densitometry on an ImageQuant densitometer (Molecular Dynamics, Sunnyvale, CA). The following previously described cDNAs were used for Northern analysis: UCP1 (26), UCP2 (26), and UCP3 (27). The probes for β3-AR and HSL were prepared by RT-PCR using the following primers: for β3-AR, forward (5′-AGGCAACCTGCTGGTAATCATAGC-3′) and reverse (5′-ACAACGAACACTCGAGCATAGACG-3′) (GenBank accession number NM_013462); for HSL, forward (5′-TTTTGACCTGGACACAGAGACACC-3′) and reverse (5′-CTGTCTCGTTGCGTTTGTAGTGCT-3′) (GenBank accession number NM_010719).

Western blotting

BAT was homogenized in ice-cold 50 mM HEPES buffer (pH 7.4) containing 150 mM NaCl, 10 mM sodium pyrophosphate, 2 mM Na_3VO_4, 10 mM NaF, 2 mM EDTA, 2 mM PMSF, 5 μg/ml leupeptin, 1% Nonidet P-40, and 10% glycerol and centrifuged at 12,000 rpm for 10 min at 4°C. The proteins were resolved by 12% SDS-PAGE, transferred to polyvinylidene difluoride membranes, and blotted by using antibodies for UCP1, β3-AR, pCREB, and PGC1α. The proteins were visualized using the enhanced chemiluminescence detection system (Amersham Biosciences) as described by the manufacturer and quantified by densitometry.

Preparation of mitochondria and CPT1 activity assay

Mitochondria were isolated as previously described by Vance (28). Carnitine palmitoyltransferase 1 (CPT1) activity was measured as described by Brener (29). A total of 200 μg of mitochondrial protein was added to assay medium containing 20 mM HEPES, pH 7.3, 75 mM KCl, 2 mM KCN, 1% fat-free BSA, 70 μM palmitoyl-CoA, and 0.25 mM L-[^{3}H]carnitine. After incubation at 37°C for 3 min, the reaction was stopped by adding 0.5 ml of 4 M ice-cold per-

chloric acid (PCA). After centrifugation at 13,000 *g* for 10 min, the pellet was washed with 500 μl of 2 mM PCA, resuspended in 800 μl of water, and extracted with 600 μl of butanol. Three hundred microliters of butanol phase was counted by liquid scintillation.

Fatty acid oxidation

Fatty acid oxidation was measured as described by Mannaerts et al. (30) with minor modifications. BAT was homogenized in Krebs-Henseleit bicarbonate buffer and centrifuged at 800 *g* for 10 min. The resultant supernatant was used for the assay. The reaction mixture contained 0.2 mM [^{14}C]palmitic acid, 4 mM ATP, 50 μmol of CoA, 0.5 mM L-carnitine, 2 mM DTT, and 7.2 mg/ml albumin with or without 2 mM KCN in 2 ml. The reaction was started by adding the substrate and incubating the preparation at 37°C for 10 min. The reaction was terminated by adding 1 ml of 6% PCA, followed by centrifugation. The supernatant was extracted three times with 1 ml of petroleum ether to remove residual radiolabeled palmitate. The radioactivity of the aqueous phase was measured.

Protein content

The protein concentration was determined with the Bio-Rad protein assay (Hercules, CA) using BSA as a standard.

Lipid analysis

Total lipids were extracted from BAT according to the method of Bligh and Dyer (31). For analysis of TG, 1,2-diacylglycerol (DAG), FFAs, and phospholipids (PLs), BAT was homogenized and the lipids were extracted with 3 ml of chloroform-methanol (2:1). After centrifugation, the organic phase was collected and dried under nitrogen and then dissolved in 100 μl of hexane. The extracts were separated by TLC using hexane-diethylether-acetic acid (80:20:1) as a solvent system. The bands were scraped from the plates, methylated, and analyzed by gas-liquid chromatography as previously described (7).

Primary culture of brown adipocytes and in vitro lipolysis

BAT precursor cells were isolated from interscapular brown fat depots from SCD1$^{+/+}$ and SCD1$^{-/-}$ mice, as described (32). Briefly, BAT was minced in HEPES-buffered Ringer solution containing 0.2% (w/v) collagenase type II (Sigma) and digested for 30 min at 37°C. The brown adipocytes were grown at 37°C in an atmosphere of 5% CO_2 in air, and the culture medium consisted of DMEM supplemented with 10% newborn calf serum, 4 nM insulin, 25 μg/ml sodium ascorbate, 10 mM HEPES, 4 mM glutamine, 50 U/ml penicillin, and 50 μg/ml streptomycin. After 10 days, brown adipocytes were incubated in Krebs buffer containing 10 μM T3 for 10 min, and medium was collected, deproteinated with 31% PCA, and neutralized with 5 M K_2CO_3. After centrifugation for 10 min at 3,000 *g*, the supernatant was used to determine glycerol using a commercially available enzymatic kit (Roche Molecular Biochemicals, Indianapolis, IN).

Analytical procedures

Plasma glucose was analyzed using a colorimetric method (Sigma), and glycogen content was measured as described (33).

Statistical analysis

Statistical analysis was performed with Student's unpaired *t*-test, with statistical significance set at $P < 0.05$.

RESULTS

SCD1 deficiency increases expression of UCPs in BAT

To determine whether SCD1 deficiency influences basal thermogenic activity, we measured UCP expression in BAT from SCD1$^{-/-}$ and SCD1$^{+/+}$ mice. Northern blot analysis shows that UCP1 mRNA levels were increased by 1.9-fold in SCD1$^{-/-}$ mice relative to SCD1$^{+/+}$ mice (**Fig. 1A**). UCP2 and UCP3 mRNA levels were also increased in SCD1$^{-/-}$ mice by 1.9- and 2.0-fold, respectively, relative to SCD1$^{+/+}$. pAL15 mRNA levels used here as a control were not changed. Consistent with the increased mRNA level, the UCP1 protein level was 1.7-fold higher in SCD1$^{-/-}$ mice than in SCD1$^{+/+}$ mice (Fig. 1B).

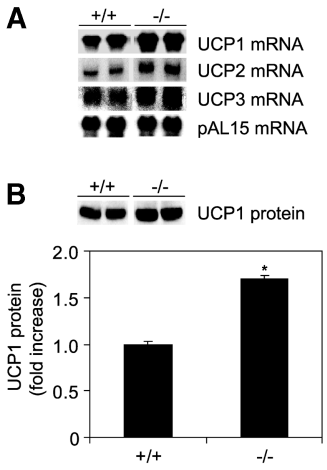

Fig. 1. Effect of stearoyl-CoA desaturase 1 (SCD1) deficiency on the expression of uncoupling proteins (UCPs) in brown adipose tissue (BAT). A: UCP mRNA levels. The interscapular BATs were taken from SCD1$^{+/+}$ and SCD1$^{-/-}$ mice. Northern blot analysis was performed to measure UCP mRNAs and normalized to the pAL15 mRNA level. B: UCP1 protein. Western blot analysis was performed to measure UCP1 protein. Data shown are means ± SE (n = 4). * $P < 0.01$ versus SCD1$^{+/+}$ mice.

SCD1 deficiency activates the β3-AR-mediated pathway in BAT

In BAT, β3-AR is responsible for the regulation of thermogenic pathways, including the adenylyl cyclase, the protein kinase A cascade, and the consequent activation of UCP1 (19). To determine whether the β3-AR participates in the upregulation of UCPs in BAT, the mRNA and protein levels of β3-AR were compared between SCD1$^{-/-}$ and SCD1$^{+/+}$ mice. The mRNA and protein levels of β3-AR were 1.6- and 2.0-fold higher (**Fig. 2A, B**), respectively, in SCD1$^{-/-}$ mice relative to SCD1$^{+/+}$ mice. The β1- and β2-AR mRNA levels measured by RT-PCR were not influenced by SCD1 deficiency (data not shown). The transcription factor CREB mediates adrenergically induced gene expression via β3-AR and protein kinase A to activate UCP1 expression (34). To determine whether CREB is activated by phosphorylation in SCD1$^{-/-}$ mice, we measured the levels of pCREB in BAT by Western blotting using an antibody against phosphoserine-133, which detects specifically the pCREB protein (35). As shown in Fig. 2B, the level of pCREB in BAT of SCD1$^{-/-}$ mice was increased by 1.6-fold relative to wild-type controls. The CREB mRNA was not influenced by SCD1 deficiency (data not shown).

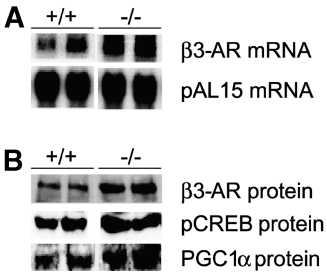

Fig. 2. Effect of SCD1 deficiency on the expression of β3-adrenergic receptor (β3-AR), the phosphorylation of cAMP response element binding protein (CREB), and peroxisome proliferator-activated receptor-γ coactivator-1α (PGC1α) protein level in BAT. A: Northern blot analysis of β3-AR mRNA normalized to the pAL15 mRNA control. B: Western blot analysis of β3-AR, phosphorylated CREB, and PGC1α protein.

Another activator of UCP1 transcription is PGC1α, a coactivator of nuclear receptors implicated in adaptive thermogenesis and mitochondrial biogenesis. The PGC1α effects are mediated through the β3-adrenergic pathway (36, 37). To determine whether PGC1α is responsible for increased UCP1 expression, we measured PGC1α mRNA and protein levels in BAT of SCD1$^{-/-}$ and SCD1$^{+/+}$ mice. The PGC1α protein level was increased by 1.9-fold in SCD1$^{-/-}$ mice relative to SCD1$^{+/+}$ mice (Fig. 2B). However, the PGC1α mRNA level was not affected by SCD1 deficiency (data not shown).

SCD1 deficiency increases lipolysis and fatty acid oxidation in BAT

Because activated β3-AR induces lipolysis in adipose tissue and lipolysis is believed to be closely connected to the activation of UCP1 in BAT, we measured HSL expression to determine whether upregulation of UCPs results from increased lipolysis in BAT of SCD1$^{-/-}$ mice. The HSL mRNA level was increased by 2.4-fold in BAT of SCD1$^{-/-}$ mice relative to wild-type mice (**Fig. 3A**). In vitro experiments showed that T3-induced glycerol release increased by 1.8-fold in cultured brown adipocytes isolated from

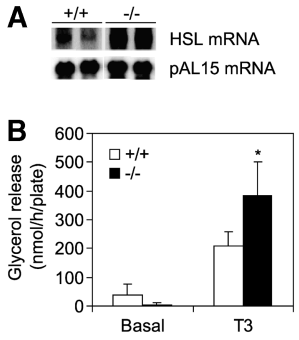

Fig. 3. Effect of SCD1 deficiency on the expression of hormone-sensitive lipase (HSL) and in vitro lipolysis. A: Northern blot analysis of HSL mRNA normalized to the pAL15 mRNA control. B: In vitro lipolysis. In vitro lipolysis was measured from primary cultures of brown adipocytes from SCD1$^{+/+}$ and SCD1$^{-/-}$ mice. Data shown are means ± SE (n = 4). * $P < 0.05$ versus SCD1$^{+/+}$ mice.

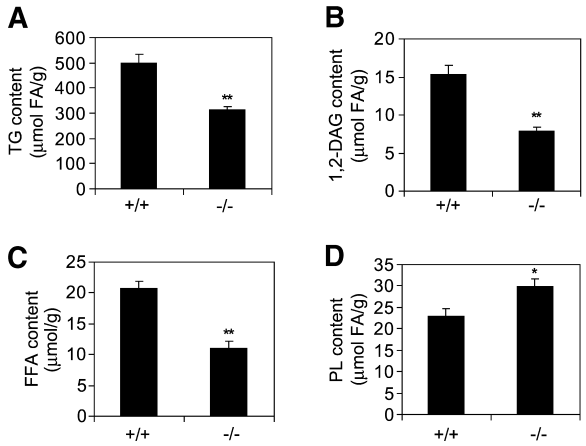

Fig. 4. Effect of SCD1 deficiency on the BAT content of triglyceride (TG; A), 1,2-diacylglyceride (DAG; B), FFAs (C), and phospholipids (PL; D). The lipids were extracted and separated by TLC. The individual lipid fractions were methyl esterified and quantitated by gas-liquid chromatography as described in Experimental Procedures. Data shown are means ± SE (n = 4). * $P < 0.01$ and ** $P < 0.001$ versus SCD1$^{+/+}$ mice.

SCD1$^{-/-}$ mice relative to those from SCD1$^{+/+}$ mice (Fig. 3B).

On the basis of the observed changes in HSL expression in BAT and the increased in vitro lipolysis in the brown adipocytes from SCD1$^{-/-}$ mice, we determined whether the contents of TG, FFA, 1,2-DAG, and PL fractions were affected by SCD1 deficiency in BAT. TG (**Fig. 4A**) and 1,2-DAG (Fig. 4B) contents were decreased by 37% and 49%, respectively, in BAT of SCD1$^{-/-}$ mice. The FFA fraction was decreased by 46% in SCD1$^{-/-}$ mice (Fig.4C), and plasma FFA content of SCD1$^{-/-}$ mice was lower than that of SCD1$^{+/+}$ mice (840.6 ± 94.8 vs. 1,104.7 ± 93.1 μmol/l) (**Table 1**). The PL fraction was increased by 1.3-fold in SCD1$^{-/-}$ mice. The fatty acid composition in each fraction was analyzed to determine whether SCD1 deficiency influences fatty acid composition in BAT. **Table 2** shows the relative percentages of the major fatty acids from each fraction. In the TG fraction, SCD1$^{-/-}$ mice had an 82% decrease in relative amounts of palmitoleate (16:1) and a 40% decrease in oleate (18:1). In the 1,2-DAG and FFA fractions, the relative amounts of 16:1 and 18:1 also decreased in SCD1$^{-/-}$ mice. The decreases of these monounsaturated fatty acids were accompanied by significant increases in the composition of the saturated fatty acids (16:0 and 18:0). The PL fraction of SCD1$^{-/-}$ mice showed 92% and 70% decreases in 16:1 and 18:1, respectively. However, the contents of 18:2 and 20:4 were increased in the PL fraction of SCD1$^{-/-}$ mice, and only minor changes were found in the composition of other fatty acids.

The simultaneous decrease of FFA in BAT and plasma suggests that fatty acid oxidation would be increased in BAT of SCD1$^{-/-}$ mice. To investigate whether fatty acid oxidation is increased in BAT of SCD1$^{-/-}$ mice, we measured BAT CPT1 mRNA level and CPT1 activity as well as the rate of fatty acid oxidation in mitochondria of BAT.

TABLE 1. TG and FFA content in plasma, and TG content in BAT and liver at 22°C and after cold exposure for 3 h

Variable	22°C$^{+/+}$	22°C$^{-/-}$	4°C$^{+/+}$	4°C$^{-/-}$
Plasma TG (μmol/l)	1,253.1 ± 91.8	893.3 ± 252.8	882.1 ± 251.5	495.9 ± 21.0
Plasma FFA (μmol/l)	1,104.7 ± 93.1	840.6 ± 94.8	959.6 ± 63.6	1247.6 ± 200.7
BAT TG (μmol/g)	276.0 ± 66.3	165.0 ± 27.5	421.7 ± 55.4	124.4 ± 46.9
Liver TG (μmol/g)	29.1 ± 6.9	13.9 ± 2.1	41.5 ± 5.9	17.1 ± 4.6

BAT, brown adipose tissue; TG, triglyceride; $^{+/+}$, stearoyl-CoA desaturase 1 (SCD1)$^{+/+}$ mice; $^{-/-}$, SCD1$^{-/-}$ mice.

CPT1 mRNA level and activity were increased in BAT of SCD1$^{-/-}$ mice by 1.6- and 1.4-fold, respectively, relative to SCD1$^{+/+}$ mice (**Fig. 5A, B**). The rate of fatty acid oxidation was 1.9-fold higher in BAT of SCD1$^{-/-}$ mice relative to that of SCD1$^{+/+}$ mice (Fig. 5C).

SCD1 is required to maintain body temperature in cold-exposed mice

To determine whether deficiency of the SCD1 gene affects thermoregulation in a cold environment, a temperature challenge study was performed. At room temperature, the core temperature was not different between SCD1$^{-/-}$ and SCD1$^{+/+}$ mice (37.46 ± 0.40 vs. 37.07 ± 0.82°C). After 3 h of cold exposure at 4°C, SCD1$^{-/-}$ mice showed severe cold sensitivity, and 25% of the SCD1$^{-/-}$ mice died when their body temperature decreased to 20°C, whereas SCD1$^{+/+}$ mice survived and maintained their body temperature within the normal range (**Fig. 6A**). These results suggest that SCD1 plays a significant role in cold-induced thermogenesis and that normal mechanisms to maintain body temperature in times of cold stress are not functioning correctly in SCD1$^{-/-}$ mice. Because it has been known that thermogenesis is primarily mediated by UCP1 and that its expression is induced by cold exposure, UCP1 mRNA level was measured after a 3 h exposure of SCD1$^{-/-}$ and SCD1$^{+/+}$ mice at 4°C. As shown in Fig. 6B, cold exposure increased UCP1 mRNA level in SCD1$^{+/+}$ as well as SCD1$^{-/-}$ mice. However, the difference was greater in SCD1$^{+/+}$ mice than in SCD1$^{-/-}$ mice, which suggests that UCP1 is probably expressed maximally in both SCD1$^{+/+}$ and SCD1$^{-/-}$ mice after cold exposure.

TABLE 2. Relative fatty acid composition of TG, 1,2-DAG, FFAs, and PL fractions in BAT from SCD1$^{+/+}$ and SCD1$^{-/-}$ mice

Variable	16:0	16:1	18:0	18:1n-9	18:2n-6	18:3n-3	20:4n-6	22:6n-3
					%			
TG								
$^{+/+}$	38.8	4.0	8.6	29.3	11.6	0.3	0.0	0.0
$^{-/-}$	41.5	0.7	20.6	17.5	12.4	0.4	0.0	0.0
1,2-DAG								
$^{+/+}$	30.0	8.4	7.4	34.5	15.3	0.0	0.0	0.0
$^{-/-}$	43.8	1.2	35.3	8.9	10.3	0.0	0.0	0.0
FFA								
$^{+/+}$	20.2	13.4	5.1	37.0	18.0	0.0	0.0	0.0
$^{-/-}$	29.4	2.1	24.2	9.5	15.6	0.0	0.0	0.0
PL								
$^{+/+}$	25.1	3.9	16.8	15.8	22.3	0.6	10.1	1.6
$^{-/-}$	20.1	0.3	26.4	4.7	31.0	0.6	12.5	1.3

DAG, diacylglycerol; PL, phospholipid.

SCD1 deficiency induces hypoglycemia in cold-exposed mice

During environmental cold exposure, a decrease in core temperature is prevented by increasing heat production via thermogenesis. Thermogenesis is mainly fueled by carbohydrate and lipids, whereas the contribution of protein oxidation remains minor. To determine why SCD1$^{-/-}$ mice experience hypothermia at 4°C, we measured plasma glucose in cold-exposed SCD1$^{-/-}$ and SCD1$^{+/+}$ mice. Plasma glucose level was not changed between SCD1$^{-/-}$ and SCD1$^{+/+}$ mice at 22°C; however, glucose level was decreased by 55% in SCD1$^{-/-}$ mice after 3 h of cold exposure (Fig. 6C). Because plasma glucose level is maintained by hepatic glycogenolysis, we measured glycogen content in liver of SCD1$^{-/-}$ and SCD1$^{+/+}$ mice after cold exposure. At room temperature, liver glycogen content was not changed between SCD1$^{-/-}$ and SCD1$^{+/+}$ mice. However, after cold exposure, liver glycogen of SCD1$^{-/-}$ mice was decreased by 90% and was almost de-

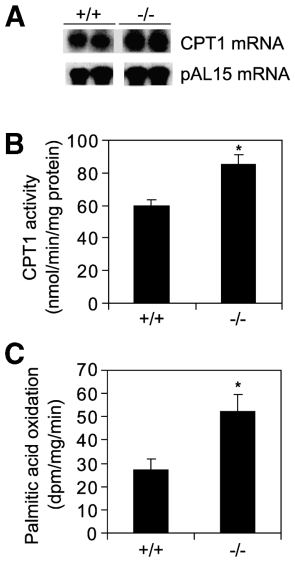

Fig. 5. Effect of SCD1 deficiency on BAT CPT1 mRNA level, carnitine palmitoyltransferase 1 (CPT1) enzyme activity, and β-oxidation rate. A: Northern blot analysis of CPT1 mRNA normalized to the pAL15 mRNA control. B: CPT1 enzyme activity. C: Rate of palmitic acid β-oxidation. Data shown are means ± SE (n = 4). * $P < 0.01$ versus SCD1$^{+/+}$ mice.

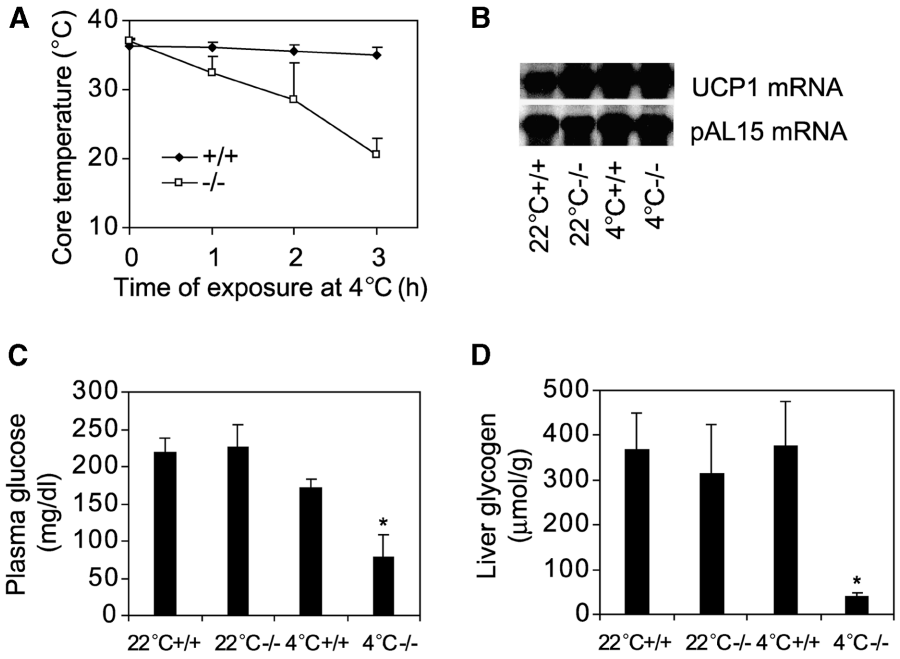

Fig. 6. Effect of SCD1 deficiency on core temperature (A), UCP1 mRNA levels (B), plasma glucose (C), and hepatic glycogen content (D) after cold exposure. SCD1$^{+/+}$ and SCD1$^{-/-}$ mice were exposed to 22°C or 4°C for 3 h (n = 5 mice/group). Core temperature was measured using a rectal probe. 22°C$^{+/+}$, SCD1$^{+/+}$ mice exposed to 22°C; 22°C$^{-/-}$, SCD1$^{-/-}$ mice exposed to 22°C; 4°C$^{+/+}$, SCD1$^{+/+}$ mice exposed to 4°C; 4°C$^{-/-}$, SCD1$^{-/-}$ mice exposed to 4°C. Data shown are means ± SE (n = 4). * $P < 0.001$ versus 4°C$^{+/+}$.

pleted relative to that of SCD1$^{+/+}$ mice (Fig. 6D). These results suggest that the depletion of glycogen and glucose may contribute to the hypothermia in SCD1$^{-/-}$ mice exposed to 4°C.

In a cold environment, de novo lipogenesis enhances cold acclimation by increasing lipid utilization and heat production (38, 39). To determine whether cold exposure changes fat stores in plasma, BAT, and liver, the amount of TG was analyzed. As shown in Table 1, plasma TG content is lower in SCD1$^{-/-}$ relative to SCD1$^{+/+}$ mice at 22°C. Cold exposure further decreased plasma TG content in SCD1$^{+/+}$ and SCD1$^{-/-}$ mice by 30% and 44%, respectively. Plasma FFA content was also decreased in SCD1$^{-/-}$ mice relative to SCD1$^{+/+}$ mice at 22°C. Cold exposure decreased plasma FFA content in SCD1$^{+/+}$ mice by 13%. However, plasma FFA content was increased by 1.5-fold in SCD1$^{-/-}$ mice. BAT and liver TG content was decreased in SCD1$^{-/-}$ mice relative to SCD1$^{+/+}$ mice at 22°C. Upon cold exposure, BAT and liver TG content was increased by 1.5- and 1.6-fold, respectively, in SCD1$^{+/+}$ mice. However, TG content was not changed in the BAT and liver of SCD1$^{-/-}$ mice.

Dietary oleate partially rescues the hypothermia induced by hypoglycemia in cold-exposed SCD1$^{-/-}$ mice

Because oleate is the primary product of SCD1 gene expression and is synthesized either de novo or by desaturation of exogenous stearate from the diet, we investigated whether dietary supplementation of triolein to the mice for 3 weeks would reverse the hypothermia observed in cold-exposed SCD1$^{-/-}$ mice. Tristearin was used as a control for the triolein effect. SCD1$^{-/-}$ mice fed the tristearin diet decreased body temperature quickly relative to SCD1$^{+/+}$ mice (**Fig. 7A**). However, SCD1$^{-/-}$ mice fed the triolein-supplemented diet showed higher body temperature at 3 and 4 h after cold exposure relative to SCD1$^{-/-}$ mice fed the tristearin diet, although the body temperature continued to decrease. Dietary triolein or tristearin did not influence body temperature in SCD1$^{+/+}$ mice during cold exposure. To determine whether triolein feeding influenced UCP1 expression, we analyzed UCP1 mRNA levels in BAT of SCD1$^{+/+}$ and SCD1$^{-/-}$ mice. UCP1 mRNA levels were not changed in either SCD1$^{+/+}$ or SCD1$^{-/-}$ mice upon triolein supplementation (Fig. 7B). To determine whether partial recovery of body temperature by triolein supplementation is associated with glycogen metabolism, plasma glucose and liver glycogen levels were measured. Dietary triolein increased plasma glucose (Fig. 7C) and liver glycogen (Fig. 7D) in SCD1$^{-/-}$ mice by 1.9- and 2.2-fold, respectively, but did not rescue plasma glucose and liver glycogen to the levels found in SCD1$^{+/+}$ mice. TG content was also measured to determine whether dietary triolein influences fat accumulation in BAT and liver. As shown in **Table 3**, long-term feeding of high levels of dietary triolein increased the TG content of BAT and liver of SCD1$^{+/+}$ and SCD1$^{-/-}$ mice. The TG levels in liver and BAT of SCD1$^{-/-}$ were not increased to the levels found in SCD1$^{+/+}$ mice.

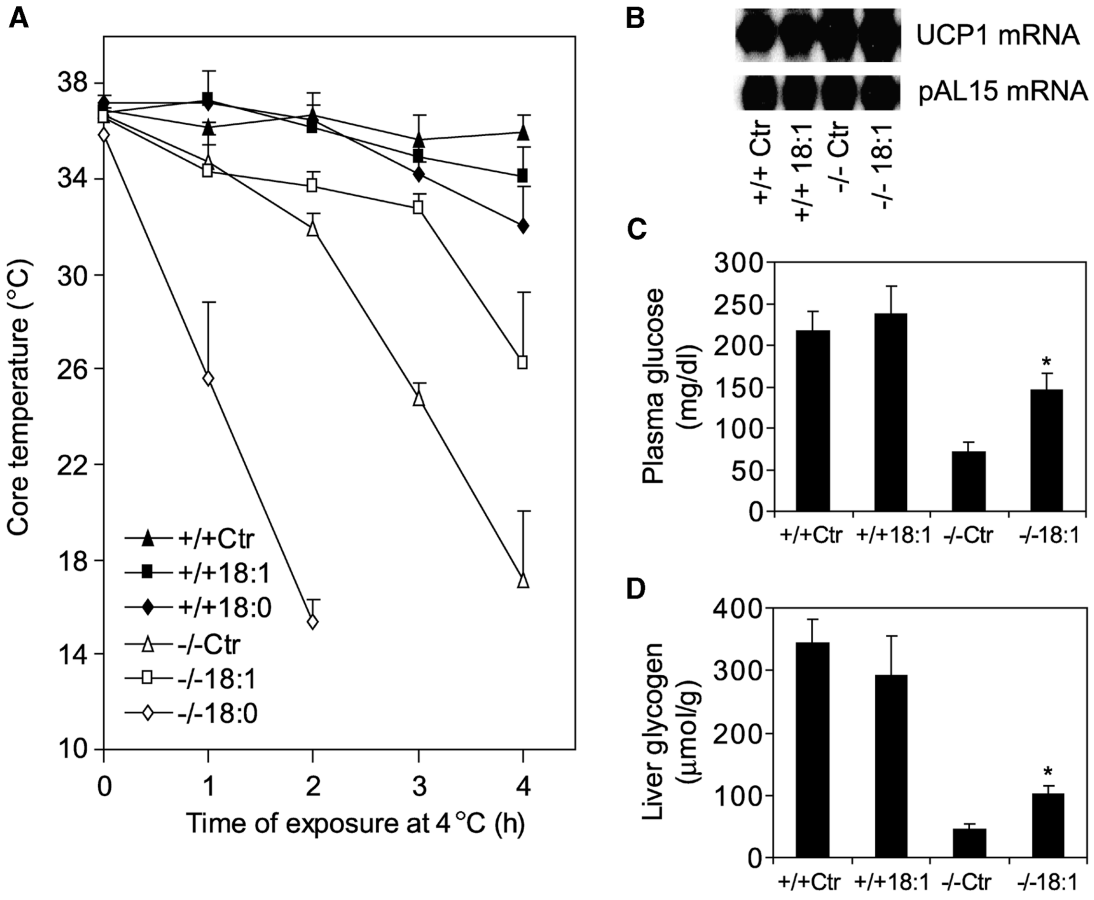

Fig. 7. Effect of triolein supplementation on core temperature (A), UCP1 mRNA level (B), plasma glucose (C), and hepatic glycogen content (D) after cold exposure. SCD1$^{+/+}$ and SCD1$^{-/-}$ mice were fed control, triolein, or tristearin-supplemented diets (20% by weight) for 3 weeks and exposed to 4°C for 4 h. $^{+/+}$Ctr, SCD1$^{+/+}$ mice fed a control diet; $^{+/+}$18:1, SCD1$^{+/+}$ mice fed a triolein diet; $^{+/+}$18:0, SCD1$^{+/+}$ mice fed a tristearin diet; $^{-/-}$Ctr, SCD1$^{-/-}$ mice fed a control diet; $^{-/-}$18:1, SCD1$^{-/-}$ mice fed a triolein diet; $^{-/-}$18:0, SCD1$^{-/-}$ mice fed a tristearin diet. Data shown are means ± SE (n = 3). * $P < 0.001$ versus $^{-/-}$Ctr.

DISCUSSION

Mice with a targeted disruption of the SCD1 isoform are protected against diet-induced adiposity, have increased energy expenditure, and upregulate expression of hepatic genes encoding enzymes of fatty acid β-oxidation. The SCD1$^{-/-}$ mice exhibit consistently higher rates of oxygen consumption (higher metabolic rates) than their wild-type littermates throughout the day and night. In this study, we extend these observations and show that lack of SCD1 stimulates basal thermogenesis and upregulates the β3-AR-mediated pathway in BAT. SCD1 deficiency also increased lipolysis and fatty acid oxidation in BAT. However, when the SCD1$^{-/-}$ mice were exposed to a cold environment, they failed to increase thermogenesis despite increased expression of UCP1. The mice instead developed hypothermia and hypoglycemia, demonstrating an important role of SCD1 gene expression in thermoregulation.

The mechanism of increased UCP1 expression in BAT of SCD1$^{-/-}$ mice is currently unknown. The results presented here suggest that it may involve the activation of the β3-AR-specific signaling pathway. Figure 2 shows that β3-AR mRNA and protein expression were increased in SCD1$^{-/-}$ mice. The increase in β3-AR expression was accompanied by increased activation of pCREB, which is the primary downstream factor of β3-AR-mediated pathways in BAT. Phosphorylation on the serine-133 residue of CREB in response to noradrenergic activation activates the transcription of the UCP1 gene (34, 35). Increased levels of PGC1α protein in BAT also support the activation of UCP1 expression in SCD1$^{-/-}$ mice because PGC1α strongly coactivates several nuclear receptors (peroxisome proliferator-activated receptor α, peroxisome proliferator-activated receptor γ, retinoic acid receptor, and thyroid hormone receptor) that bind to the UCP1 enhancer, and recent reports indicate that a major portion of the cAMP effect is mediated by PGC1α (36, 37). The CREB and PGC1α mRNA levels were not different between SCD1$^{+/+}$ and SCD1$^{-/-}$ mice, suggesting that SCD1 deficiency induces the activation of CREB by increasing its phosphorylation and regulates PGC1α expression at a posttranscriptional level.

The increased expression of HSL (Fig. 3A) and the reduction in levels of TG and 1,2-DAG content (Fig. 4A, B) suggest increased lipolysis in BAT of SCD1$^{-/-}$ mice. The plasma fatty acid levels would be expected to increase.

TABLE 3. TG content in BAT and liver after triolein supplementation in cold-exposed $SCD1^{+/+}$ and $SCD1^{-/-}$ mice

Variable	$^{+/+}$Control Diet	$^{+/+}$18:1 Diet	$^{-/-}$Control Diet	$^{-/-}$18:1 Diet
BAT TG (μmol/g)	411.2 ± 12.4	480.5 ± 28.7	165.5 ± 29.5	227.7 ± 19.3
Liver TG (μmol/g)	40.8 ± 3.0	52.6 ± 1.9	24.9 ± 3.4	33.9 ± 2.4

At 3 weeks of age, $SCD1^{+/+}$ and $SCD1^{-/-}$ mice were fed a control or a triolein-supplemented diet (20% of total fatty acids) for 3 weeks and then exposed to 4°C for 4 h (n = 3 mice/group). $^{+/+}$, $SCD1^{+/+}$ mice; $^{-/-}$, $SCD1^{-/-}$ mice.

However, the content of FFA fraction in BAT (Fig. 4C) and in the plasma (Table 1) was decreased in $SCD1^{-/-}$ mice. The increased CPT1 gene expression and enzyme activity as well as the increased oxidation of palmitic acid (Fig. 5) provide evidence of the more efficient FFA oxidation in BAT of $SCD1^{-/-}$ mice.

We hypothesized that SCD1 deficiency enhances cold-induced thermogenesis because SCD1 deficiency upregulates thermogenic activity in BAT at room temperature. Paradoxically, we found that $SCD1^{-/-}$ mice showed very severe hypothermia after only 3 h of cold exposure. Moreover, UCP1 was not responsible for cold-induced hypothermia in $SCD1^{-/-}$ mice. Survival in the cold depends on increased energy expenditure and thus requires sustained substrate mobilization from the main energy stores, liver glycogen and adipose tissue. We found that $SCD1^{-/-}$ mice had reduced levels of liver glycogen, which suggests that $SCD1^{-/-}$ mice use glycogen more efficiently than $SCD1^{+/+}$ mice for thermogenesis. The muscle and BAT glycogen content was not significantly decreased in $SCD1^{-/-}$ mice upon cold exposure (data not shown). This observation suggests that liver glycogen plays a role in whole-body metabolism and serves to maintain a constant level of glucose in the blood while glycogen of the peripheral tissues is used for local energy demand. On the other hand, increase in lipid utilization during cold exposure is a strategy to spare limited carbohydrate reserves. An increase in the use of lipids allows the maintenance of heat production for a longer period and therefore improves chances of survival in the cold (40). Actually, cold induces a large increase of de novo lipogenesis in thermogenic tissue such as BAT and liver after cold exposure, suggesting that lipogenesis is an essential step of cold acclimation (38, 39). We found a decrease in hepatic TG content and an increase in plasma FFA level in $SCD1^{-/-}$ mice after cold exposure.

The reduced lipogenesis and reduced lipid utilization in $SCD1^{-/-}$ mice could be responsible for the reduced heat production in the absence of carbohydrate sources. Therefore, we tested the possibility that dietary oleate would help in maintaining body temperature by increasing TG synthesis in $SCD1^{-/-}$ mice during cold exposure. Our results showed that high levels of dietary oleate partially rescued the deficiency of glycogen and TG in liver of $SCD1^{-/-}$ mice (Fig. 7). Dietary stearate or palmitate did not rescue the glycogen or TG deficiency in the livers of $SCD1^{-/-}$ mice (data not shown). These results support the hypothesis that glycogen and TG levels in liver of $SCD1^{-/-}$ mice might be affected by the presence of endogenously synthesized oleate, consistent with previous results (7). We do not exclude the possibility that cold injury may occur in the plasma membrane of $SCD1^{-/-}$ mice after cold exposure because $SCD1^{-/-}$ mice fed tristearin exhibited very severe cold sensitivity and died after 2 h of cold exposure. An altered lipid profile (low ratio of MUFA to saturated fatty acids) may change membrane fluidity and induce lipid-phase transition (41).

In conclusion, our study provides information on the role of SCD1 deficiency in stimulating the metabolic rate and the molecular events of basal thermogenesis. As depicted in **Fig. 8**, lack of the SCD1 gene coordinates the signal leading from β3-AR activation to phosphorylation of CREB and activation of PGC1α, which mediate the activation of UCP1. The resulting increase in lipolysis in $SCD1^{-/-}$ mice might provide the substrate for mitochondrial oxidation and induce the simultaneous stimulation of UCP1 expression and basal thermogenesis. These phenomena together result in increased energy expenditure and reduction in adiposity. However, SCD1 deficiency induced hypothermia, which is associated with hypoglycemia after cold exposure. The hypothermia and hypoglycemia in cold-exposed $SCD1^{-/-}$ mice and the compensatory recovery by dietary oleate suggest a significant role of SCD1 gene expression in thermoregulation, which is associated with glycogen and lipid metabolism.

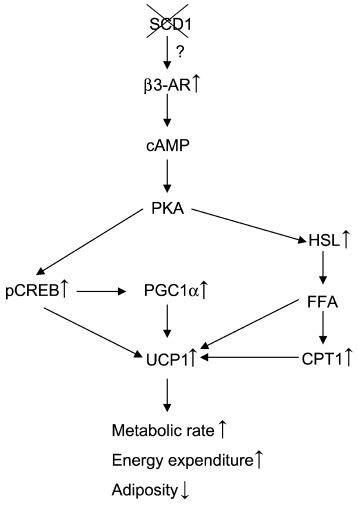

Fig. 8. Proposed scheme for the role of SCD1 in thermogenesis. SCD1 deficiency induces a signal that activates the β3-AR signaling pathway. The increased UCP1 expression results in increased metabolic rate and energy expenditure and a reduction in adiposity. PKA, protein kinase A.

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