Identification and Characterization of Murine SCD4, a Novel Heart-specific Stearoyl-CoA Desaturase Isoform Regulated by Leptin and Dietary Factors*

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Stearyl-CoA desaturase (SCD) is the rate-limiting enzyme in the biosynthesis of monounsaturated fatty acids. Thus far, three isoforms of SCD (SCD1, SCD2, and SCD3) have been identified and characterized. Regulation of the SCD1 isoform has been shown to be an important component of the metabolic actions of leptin in liver, but the effects of leptin on SCD isoforms in other tissues have not been investigated. We found that although the mRNA levels of SCD1 and SCD2 were not affected by leptin deficiency in the hearts of ob/ob mice, the SCD activity and levels of monounsaturated fatty acids were increased, implying the existence of another SCD isoform. This observation has led to the cDNA cloning and characterization of a fourth SCD isoform (SCD4) that is expressed exclusively in the heart. SCD4 encodes a 352-amino acid protein that shares 79% sequence identity with the SCD1, SCD2, and SCD3 isoforms. Liver X receptor α (LXRα) agonists and a high carbohydrate-fat-free diet induced SCD4 expression, but unlike SCD1, SCD4 expression was not repressed by dietary polyunsaturated fatty acids. SCD4 mRNA levels were elevated 5-fold in the hearts of leptin-deficient ob/ob mice relative to wild type controls. Treatment of ob/ob mice with leptin decreased mRNA levels of SCD4, whereas levels of SCD1 and SCD2 were not affected. Furthermore, in the hearts of SCD1-deficient mice, SCD4 mRNA levels were induced 3-fold, whereas the levels of SCD2 were not altered. The current studies identify a novel heart-specific SCD isoform that demonstrates tissue-specific regulation by leptin and dietary factors.

Stearyl-CoA desaturase (SCD) catalyzes the committed step in the biosynthesis of monounsaturated fatty acids from saturated fatty acids. This reaction involves the introduction of a cis-double bond in the Δ-9 position (between carbons 9 and 10) in a spectrum of methylene-interrupted fatty acyl-CoAs. The preferred substrates are palmitoyl- and stearoyl-CoAs, which are desaturated into palmitoleoyl- and oleoyl-CoA, respectively (1, 2). The roles of monounsaturated fatty acids are diverse and crucial in living organisms. Palmitoleic and oleic acids are the major monounsaturated fatty acids in membrane phospholipids, triglycerides, and cholesterol esters (3). A proper ratio of saturated to monounsaturated fatty acids contributes to membrane fluidity, whereas changes in cholesterol esters and triglyceride levels affect lipoprotein and lipid metabolism (3–5). Apart from being components of lipids, monounsaturated fatty acids have also been implicated as mediators in signal transduction and differentiation of neurons and other cells (6). Monounsaturated fatty acids have also been shown to regulate food intake in the brain (7). Given the multiple roles of monounsaturated fatty acids, alterations in stearyl-CoA desaturase activity in mammals would be expected to have potent effects on lipid metabolism and to play a role in the propensity to develop obesity, atherosclerosis, and metabolic diseases (1, 2).

A number of mammalian SCD genes have been cloned and studied. Two SCD genes have been cloned in rats and three well-characterized SCD genes (SCD1, SCD2, and SCD3) have been cloned in mice (8–10). In addition, a single human SCD gene that is highly homologous to the mouse and rat SCD genes has been cloned and characterized (11). The expression of the mouse isoforms varies among tissues. SCD1 is the main isofrom expressed in mouse liver, whereas SCD2 is constitutively expressed in the brain (8, 9). In tissues such as the adipose and eyelids, both SCD1 and SCD2 are expressed. On the other hand, all three isoforms are expressed in skin, Harderian, and preputial glands (10, 13). The reason for having two or more SCD isoforms in the same tissue is not known but seems to be related to substrate specificity of the isoforms and their regulation through tissue-specific transcription factors (12).

We recently found that the regulation of SCD1 by leptin plays a crucial role in signaling the body to either store fat or burn it. Obese mice, which lack leptin, lost weight because of increased energy expenditure when genetically crossed with a strain of mice carrying a mutation in SCD1 (5). The missing SCD1 enzyme also corrected a major clinical problem called fatty liver, which is found in obese mice and humans. Leptin has also been found to reduce fat deposition in other tissues such as muscle and heart (28–31), but the role of SCD in mediating the effects of leptin in these tissues has not been established.

We report here the isolation of a fourth SCD isoform and have studied its tissue distribution and nutritional regula-
Regulation of SCD4 by Leptin and Dietary Factors

**EXPERIMENTAL PROCEDURES**

**Animals and Diets—**Male C57BL6J and ob/ob mice were obtained from the Jackson Laboratory (Bar Harbor, ME). 129sv/SvEv mice were purchased from Taconic (Germantown, NY). The mice (14–16 weeks old) were maintained on a 12-h dark/light cycle and were fed a normal nonpurified diet (5008 test diet; PMI Nutrition International Inc., Richmond, IN), a high carbohydrate fat-free diet (TD99252, Harlan Teklad, Madison, WI), or high carbohydrate fat-free diet supplemented with fish oil. In some experiments the chow diet was supplemented with 0.025% T9091317, an LXR agonist (Cayman, Ann Arbor, MI), which was fed to the mice for 2 days. All animal breeding was in accordance with protocols approved by the animal care research committee of the University of Wisconsin-Madison.

**Materials—**Radioactive [3H]labeled deoxyguanosine triphosphate (3000 Ci/mmol) was obtained from DuPont. Thin layer chromatography plates (TLC Silica Gel G60) were from Merck. [1-14C]Stearoyl-CoA and [1-14C]Palmitoyl-CoA were obtained from American Radiolabeled Chemicals Inc. (St. Louis, MO). The cDNA probes for SCD1 and SCD2 have been previously described (15). All other chemicals were purchased from Sigma.

**Subcutaneous Leptin Treatment—** ob/ob mice were individually housed and allowed to acclimatize for a number of days prior to the start of the experiment. The mice were treated with 200 ng/h of recombinant mouse leptin (Ampgen, Thousand Oaks, CA) and PBS for 12 days using subcutaneously placed Alzet miniosmotic pumps model 1002 (Alza Co., Palo Alto, CA) as previously described (27). As an additional control, a group of mice referred to as pair-fed was treated with PBS and given the same amount of food to eat as leptin-treated mice voluntarily consumed. All of the data presented were generated from these or a similar time course where mice were either treated with PBS or leptin for 12 days. Body mass and food intake were measured daily.

**Isolation and Analysis of RNA—** Total RNA was extracted from the hearts of 5 ob/ob male C57BL6 and SCD1−/− mice as well as the appropriate wild type controls using Trizol reagent (Invitrogen) (14). For Northern blot analysis, 15 μg of total RNA were separated by 1.0% agarose, 2.2 M formaldehyde gel electrophoresis and transferred onto a nylon membrane. The membrane was hybridized with 32P-labeled cDNA probes.

**Cloning and Expression of the Mouse SCD4 cDNA—** A BLAST search of the mouse genomic data base identified a gene with exon sequences highly homologous to the amino acid sequence of mouse SCD1, 2, and 3 (GenBank accession numbers AH 002092, M56270, and AP572057, respectively). Using the genomic sequence, a candidate open reading frame of the putative SCD4 was cloned by synthesizing a forward and a reverse primer. The primers were utilized to screen total RNA isolated from the hearts of SCD1−/− mice by PCR amplification. The amplification conditions consisted of an initial denaturation step at 94 °C for 3 min followed by 40 cycles at 94 °C for 30 s, 58 °C for 1 min, 72 °C for 1 min and finally 1 cycle at 72 °C for 10 min. The resulting PCR product was cloned into the T-easy vector (Promega, Madison, WI) and sequenced. The construct was named pTA-mSCD4. The 5′ upstream and 3′ downstream regions of the SCD4 cDNA that were missing from pTA-mSCD4 were obtained by a SMART cDNA library (Clontech, Palo Alto, CA) using the primers 5′-CTCTCTGCCTCTCCTCGGGAGTGCGGCTTCCA-3′ and 5′-TTGCGCAACCTCCTCGGAGCTGTCACAGC-3′, respectively. The 5′-RACE and 3′-RACE products were cloned in the T-easy vector and then sequenced. The complete SCD4 cDNA was then obtained using the forward primer (5′-ACACGAGACCTGACTGTCGAACCTGCAATGAGTAG-3′) and reverse primer (5′-CAGGAGACCTGTCCTGTTAGC-3′) and then sequenced. A Multiple-Tissue Northern blot (Clontech, catalog number 7762-1) was used to detect SCD4 expression in mouse heart, brain, spleen, lung, skeletal muscle, and kidney. The EcoRI-Sty1 fragment (186 bp) of the 5′-RACE product of SCD4 was used as the probe. It was tested against SCD1, 2, and 3 there was no cross-hybridization. The specific probes for SCD1, SCD2, and SCD3 derived from the 5′-untranslated region of the specific probes have been previously described (15). For additional tissues, RT-PCR was performed using the SCD4 primers 5′-AGATGGAGAGAAGATGFIRAC-3′ (forward) and 5′-AGGTGTGTGTATGGAAAGACAGACCC-3′ (reverse). SCD4 primers for quantitative RT-PCR are 5′-GCTCTCTGCTCTCCTCAGAAGG-CC-3′ (forward) and 5′-AGGGTTTATGGAACGGACACCC-3′ (reverse). Real time quantitative PCR using SCD1, SCD2, SCD3, SCD4, cyclophilin-A, glyceraldehyde-3-phosphate dehydrogenase-specific primers was performed as previously described (14).

To introduce an N-terminal hemagglutinin (HA) epitope tag into the cytomegalovirus promoter of the pcDNA3 expression vector (Invitrogen), PCR was performed with pTA-mSCD4 as the template, using a forward primer (TTGAATTCACCATGCTATGATTGCCTCGGACAGACAGACAGACAGAGCTGTCCTGTTAGC-3′) and reverse primer (5′-GCTCTCTGCTCTCCTCAGAAGG-CC-3′). The PCR product was ligated into pcDNA3, and the construct was named pcDNA3-HAmSCD4. HEK-293 (ATCC) cells were transfected with 60 μg of Superfect transfection reagent (Qiagen). 36 h after transfection, the cells were harvested, and the microsomes were isolated. Expression of HA-tagged SCD4 protein in microsomes was determined by immunoblot analysis. Microsomal protein (10 μg) was electrophoresed on an 8% SDS-PAGE and transferred to a nitrocellulose membrane (Millipore, Bedford, MA).
The membrane was blocked at room temperature for 1 h in Tris-buffered saline containing 1% bovine serum albumin and then incubated with 100 ng/ml anti-HA monoclonal antibody (clone 3F10; Roche Applied Science) in Tris-buffered saline containing 1% bovine serum albumin for 1 h at room temperature. After washing with Tris-buffered saline containing 0.1% Tween 20, the membrane was incubated with horseradish peroxidase-conjugated anti-rat IgG at a 1:20000 dilution (Sigma) for 30 min at room temperature. Visualization was performed with an ECL Western blot detection kit (Amersham Biosciences).

**Enzyme Assays**—Microsomes were isolated from hearts of ob/ob mice as well as from HEK-293 cells by differential centrifugation and suspended in a 0.1M potassium phosphate buffer (pH 7.2). Stearoyl-CoA desaturase activity was assayed at 23 °C with 3 μM [14C]stearoyl-CoA or [14C]palmitoyl-CoA, 2 mM NADH, and 100 μg of microsomal protein. After 5 min of incubation, 200 μl of 2.5 M KOH in 75% ethanol was added, and the reaction mixture was saponified at 85 °C for 1 h. The samples were cooled and acidified with 280 μl of formic acid. Free fatty acids were extracted with 700 μl of hexane and separated on a 10% AgNO3-impregnated TLC plate using chloroform:methanol:acetic acid:H2O (90:8:1:0.8). The TLC plates were analyzed with Instant Imager (Packard, Meriden, CT) overnight.

**Fatty Acid Analysis**—Total lipids were extracted from the hearts or HEK cells according to the method of Bligh and Dyer as previously described (14). The fatty acids were quantitated by GLC as described.
(13). Pentadecanoic acid (Sigma) was added as an internal standard for the quantitation of fatty acids.

RESULTS

Fig. 1 shows a Northern blot of total RNA isolated from hearts of ob/ob mice and wild type controls measuring the levels of SCD1 and SCD2. SCD1 and SCD2 mRNA levels were similar between wild type and ob/ob mice (Fig. 1, A and B). However, the SCD activity (Fig. 1C) and levels of C16:1 and C18:1 monounsaturated fatty acids, the major SCD products, were significantly elevated in ob/ob hearts (Fig. 1D), which first suggested the presence of another SCD isoform that was induced in the heart by leptin deficiency.

We searched the mouse genomic DNA data base (www.genomic.ucsc.edu) for other mouse SCD homologues, using the amino acid sequences of other members of the mouse SCD family. A predicted cDNA sequence with a 78–86% similarity to the other SCD isoforms was identified. We designed specific primers for the predicted SCD cDNA and used them in a PCR analysis to screen cDNAs prepared from total RNA isolated from hearts of mice with a targeted disruption in the SCD1 isoform (SCD1−/−). SCD1−/− mice were used to minimize cross-reactivity of the primers with SCD1 sequences expressed in other tissues. PCR product was sequenced, and the missing 5′- and 3′-untranslated region sequences were obtained by 5′- and 3′-RACE. The resulting cDNA had an in-frame termination codon 248 nucleotides upstream of the putative methionine initiator codon, suggesting that the entire open reading frame had been obtained, and it was designated SCD4.

The gene structure, predicted amino acid sequence and hydrophobicity plots of the mouse SCD4 cDNA and protein are shown in Fig. 2. The sequence of the gene obtained by BLAST analysis of the mouse genome resources of UCSC as well as sequence from two isolated BAC clones revealed that the mouse SCD4 spans 14 kb and has 6 exons and 5 introns (Fig. 2A). The open reading frame that terminates in exon 6 predicts a 353-amino acid protein with a theoretical molecular mass of 41 kDa sharing ~83% nucleotide homology with the other three SCD isoforms. Amino acid sequence alignment of SCD4 with SCD1, SCD2, and SCD3 indicates sequence identity of greater than 77% and includes 100% conservation of the three histidine motifs HRLWSH, HRAHH, and HNYHH that exist in other desaturases (Fig. 2B). The hydropathy plot shows that the SCD4 protein possesses four transmembrane domains (Fig. 2C), similar to other SCD isoforms.

To determine whether SCD4 functions as a Δ9-desaturase, HEK-293 cells were transiently transfected with a pcDNA3-HA-tagged SCD4 vector under the control of the cytomegalovirus promoter (pcDNA3-HA-mSCD4). Immunoblot analysis with an HA antibody showed that SCD4 protein was expressed in the microsomal fractions (Fig. 3A), migrating at a molecular mass of ~41 kDa similar to other SCD isoforms. SCD activity was measured in isolated membranes and compared with HEK-293 cells transfected with empty vector alone (pcDNA3). Fig. 3B shows that the densitometric quantitation (Fig. 3C) shows that SCD activity using 18:0 as a substrate was increased 8-fold in microsomes from pcDNA3-HA-mSCD4 transfected cells compared with cells transfected with empty vector alone (pcDNA3). SCD activity was increased 3-fold when 16:0 was used as substrate (data not shown).

To determine the tissue expression pattern of SCD4, a commercially prepared filter (Clontech) containing 2 μg of poly(A)+ RNA from several mouse tissues was hybridized with the SCD4-specific probe. Additional RNA isolated from other tissues (brown adipose, white adipose, and Harderian gland) was analyzed by RT-PCR using SCD4-specific primers. The Northern blot (Fig. 4A) and RT-PCR analysis of additional tissues (Fig. 4B) show that the heart is the only tissue expressing SCD4. SCD1 is most abundantly expressed in liver, lung, and kidney, whereas SCD2 is mainly expressed in brain, lung, and testis. SCD3 was expressed only in the Harderian gland (data not shown). The 3.1-kb mRNA transcript was the predominant species of SCD4, with two minor transcripts (2.8 and 1.5) that probably arise from the use of multiple GAAA repeats within exon 6 as cleavage sites for the addition of polyadenylate. The SCD4 mRNA is thus smaller than the 4.9 kb of the other SCD isoforms, because of a shorter 3′-untranslated region.

Numerous dietary studies indicate that hepatic SCD activity increases when animals are fed with high carbohydrate fat-free
diets, whereas it decreases when polyunsaturated fatty acids are ingested (16, 17). These effects are thought to be mediated, respectively, through SREBP-1c induction by insulin and inhibition of SREBP-1c expression and protein maturation by polyunsaturated fatty acids (18). In addition, LXR agonists or without the involvement of SREBP-1c can induce hepatic SCD1 gene expression by cholesterol (18, 19). To determine whether SCD4, like SCD1, is regulated by a high carbohydrate fat-free diet and polyunsaturated fatty acids and by the LXR agonist T0901317, 129SV mice were fed either a high carbohydrate fat-free diet or a chow diet containing 0.025% T0901317. As shown in Fig. 5A, the high carbohydrate fat-free diet induced the expression of SCD4 mRNA from eight mouse tissues were hybridized to 32P-labeled SCD1-, SCD2-, and SCD4-specific probes as described under “Experimental Procedures.” The blots were stripped and rehybridized with a β-actin probe to indicate the presence of mRNA in each lane. A 3.1-kb major transcript and a 1.5-kb minor transcript were detected only in the heart. B, RT-PCR of additional mouse tissues. WAT, white adipose tissue; BAT, brown adipose tissue; HG, Haderian gland.

ob/ob mice have a mutation in the leptin gene and are a model of obesity, insulin resistance, and diabetes. Previous studies showed that hepatic SCD1 and SCD2 expression were induced in the liver of ob/ob mice but were decreased when ob/ob mice were treated with leptin (5). We analyzed the mRNA level of the SCD4 isoform in the hearts of leptin-deficient ob/ob mice and ob/ob mice treated with either PBS or leptin. As an additional control, RNA isolated from saline-treated ob/ob mice that were pair-fed to the leptin treated group for 12 days was also analyzed. Pair-fed mice are given the same amount of food that leptin-treated mice voluntarily consume, allowing one to dissociate the effects of reduced food intake from those of leptin per se. After 12 days of treatment, food intake in the untreated wild type and ob/ob controls was 4.4 ± 0.2 and 6.4 ± 0.2 g, respectively (p < 0.04), whereas the leptin-treated and pair-fed mice ate 1.2 ± 0.1 and 1.1 ± 0.1 g, respectively (p < 0.001 for both leptin and pair-fed). By the end of the treatment period, leptin-treated ob/ob mice had lost a 34 ± 0.8% of their initial weight as compared with 23 ± 1.2% for pair-fed mice (p < 0.002), confirming that leptin has specific metabolic actions distinct from its anorectic effects. SCD activity was elevated 3-fold in ob/ob hearts relative to the wild type controls and was completely normalized to wild type levels in leptin-treated ob/ob
mice (Fig. 6A). SCD activity was reduced but to a lesser extent in the pair-fed ob/ob mice (Fig. 6A). To assess the effects of elevated SCD activity in the heart and its specific down-regulation by leptin, a separate and identical time course was performed. As expected from the SCD activity, ob/ob hearts were found to have a high 18:1 content that was reduced to levels comparable with those in wild type mice upon leptin treatment (Fig. 6B). To determine which SCD isoform(s) accounted for these effects, we measured RNA levels of SCD1, SCD2, and SCD4. Unlike SCD1 and SCD2, which were not induced in the hearts of ob/ob mice, SCD4 mRNA levels were increased 5-fold (Fig. 6C). The SCD4 mRNA levels were decreased by greater than 75% in leptin-treated ob/ob mice, whereas the expression of SCD1 and SCD2 isoforms were unchanged (Fig. 6C). These observations suggest that SCD4 is a target of leptin regulation in the heart.

We previously showed that administering leptin to ob/ob mice reduced SCD1 expression in liver (5). To determine whether SCD4 could compensate for SCD1 deficiency in the heart, we analyzed the mRNA levels of SCD4 in SCD1−/−/mice. Northern blot analysis (Fig. 7A) and quantitative RT-PCR (Fig. 7B) using an SCD4-specific DNA probe and primers showed that SCD4 mRNA levels were induced 3-fold in hearts of SCD1−/− relative to the SCD1+/+ mice (Fig. 7A). SCD2 expression was not altered between SCD1−/− and SCD1+/+ mice. The amounts of C18:1 and C16:1 monounsaturated fatty acids were reduced by only 30% (Fig. 7B) in hearts of SCD1−/− mice compared with a greater than 70% reduction in livers of SCD1−/− mice (5), where SCD1 is the major isoform expressed. These results suggest that SCD1 deficiency leads to an up-regulation of SCD4 expression and imply a distinct and major role for SCD4 in the synthesis of monounsaturated fatty acids in the heart.

**DISCUSSION**

SCD is a microsomal enzyme that catalyzes the Δ9-cis-desaturation of saturated fatty acyl-CoAs. The purification of this enzyme has been difficult mainly because it is a short-lived membrane protein (20, 21). However, we and others have previously isolated three SCD genes (SCD1, SCD2, and SCD3) from mouse 3T3-L1 preadipocytes and a mouse skin cDNA and genomic library that encode stearoyl-CoA desaturases. In this study, we have identified a new SCD isoform designated SCD4. The SCD4 cDNA encodes a 353-amino acid residue protein with four transmembrane regions that is more than 80% identical to the other three mouse SCD genes. SCD4 also contains three conserved histidine-rich motifs that are present in the other SCD isoforms and essential for Δ9-desaturase function (8–10). This enzyme desaturates both 18:0 and 16:0 to the corresponding monounsaturated fatty acids.

SCD4 maps to mouse chromosome 19 D2, where three other SCD genes are located within a 200-kb region. SCD4 lies between the SCD1 and SCD2 genes, indicating that this region is composed of a cluster of Δ9-desaturases. The tissue distribution of SCD4 was completely distinct from the other three isoforms. Although SCD1 and SCD2 are the predominant isoforms in liver and brain, respectively, SCD4 is expressed exclusively in the heart. The 3.1-kb transcript is the predominant species, and thus the SCD4 mRNA is smaller than the 4.9 kb of the other SCD isoforms, because of a shorter 3′-untranslated region. The function of the long 3′-untranslated mRNA regions in SCDs has been a longstanding subject of interest. Now with the identification of SCD4, which has a truncation in this region, it will be possible to determine whether this region is involved in the regulation of SCD mRNA stability. The two minor transcripts (2.8 and 1.5 kb) of SCD4 probably arise from the use of multiple GAAA repeats present within exon 6 as cleavage sites for the addition of polyadenylate. The mouse SCD1, SCD2, and SCD3 have no additional polyadenylation sites, but a second polyadenylation site has been found in the human SCD (11) and postulated to be involved in regulating its expression. Whether polyadenylation is a mode of regulation of SCD4 in heart will require further studies.

Many studies have shown that SCD1 gene expression is highly regulated by dietary and hormonal factors in liver (1). Both cholesterol and a high carbohydrate fat-free diet induce SCD1 expression in liver through an LXRα-mediated pathway and insulin-mediated activation of SREBP-1c, respectively. We used these dietary manipulations to study the regulation of SCD4 in the heart. As shown in Fig. 5, both SCD1 and SCD4 are induced by high carbohydrate diet, suggesting that both of...
these SCD isoforms are targets of SREBP-1c in the heart. However, analysis of the SCD4 promoter sequence did not reveal an SREBP binding site that is well conserved in the SCD1 and SCD2 promoters (22). It has been postulated that polyunsaturated fatty acids repress the transcription of the hepatic SCD1 gene and other lipogenic genes by suppressing SREBP-1c gene transcription or by reducing the maturation of SREBP-1 protein (23–26). Although SCD1, and to a smaller extent SCD2, were repressed by polyunsaturated fatty acids in the heart, SCD4 was not sensitive to polyunsaturated fatty acid repression (Fig. 5B), and its promoter sequence lacks the polyunsaturated fatty acid response element that is present in the SCD1 and SCD2 promoters. SCD1, SCD2, and SCD4 were induced by LXRA agonist, suggesting that the three isoforms can be regulated by cholesterol in heart. These experiments illustrate the tissue-specific regulation of the SCD isoforms in response to particular dietary factors.

We previously demonstrated that SCD1 is a target of leptin signaling in liver (5). In these studies we found that SCD1 mRNA expression is induced in leptin-deficient ob/ob mice and that leptin administration to ob/ob mice results in a dramatic reduction SCD1 mRNA levels and SCD enzymatic activity. ob/ob mice have massively enlarged livers that are engorged with lipid. When an SCD1 mutation is introduced into ob/ob mice (ab/ab; ob/ob), both the hepatomegaly and steatosis of ob/ob mice are normalized (5). In the present study, we found that SCD activity was up-regulated in ob/ob hearts and reduced to wild type levels upon leptin treatment. Furthermore, the levels of monounsaturated fatty acids were reduced to the levels comparable with those in wild type mice. Pair-fed mice showed a smaller decrease in SCD activity, suggesting that SCD activity in heart is specifically modulated by leptin. Triglyceride levels in the hearts of leptin-treated ob/ob mice were much lower than in wild type controls. These findings are the result of leptin-dependent regulation of a novel, heart-specific SCD isoform, because SCD1 and SCD2 mRNA levels were not induced in hearts of ob/ob mice, whereas SCD4 mRNA was induced 5-fold compared with wild type controls. SCD4 mRNA levels were decreased in hearts of leptin-treated ob/ob mice, whereas SCD1 and SCD2 were not altered. These observations suggest that SCD4 synthesizes the bulk of monounsaturated fatty acids in the heart and that elevated expression of SCD4 may be responsible for the development of the “fatty heart” observed in ob/ob mice. Previous studies have shown that accumulation of lipid in the heart can have deleterious consequences, analogous to those of fatty liver (28–31). Our observations indicate that SCD4 is the SCD isoform that is the target of leptin signaling in the heart. Therefore down-regulation of SCD4 expression by leptin may be one of the mechanisms by which leptin depletes lipid from the heart and exerts its anti-lipotoxic effects. Our results also suggest that leptin signaling in the heart is different from that in the liver and that tissue-specific factors regulate the tissue-specific expression of the SCD isoforms. Further studies will be required to determine whether the effects of leptin on SCD4 are direct or indirect through the central nervous system. In the hearts of SCD1−/− mice, the amounts of C18:1 and C16:1 monounsaturated fatty acids were reduced by only 25% com-
pared with a greater than 70% reduction in the livers of SCD1−/− mice (5). These observations suggest that SCD4 expression may be induced to compensate for the deficiency of SCD1 and suggest that SCD4 expression plays a distinct and critical role in the synthesis of monounsaturated fatty acids in the heart. This finding is also interesting because it indicates that there is molecular mechanism whereby the levels of monounsaturated fatty acids are sensed in tissues and, if found to be deficient, can lead to up-regulation of other SCD isoforms.

**Fig. 7.** A, SCD4 mRNA levels in SCD1−/− mice. Total RNA prepared from hearts of SCD1−/− or SCD1+/+ mice was used to measure the SCD2 and SCD4 mRNA levels. The fold change relative to that of wild type was calculated after correction for loading differences with rRNA. B, quantitative RT-PCR analysis. The fold change relative to that of wild type was calculated after correction for the levels of cyclophilin isofoms. C, found to be deficient, can lead to up-regulation of other SCD isoforms.

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