Loss of stearoyl-CoA desaturase 1 inhibits fatty acid oxidation and increases glucose utilization in the heart

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Dobrzyn P, Sampath H, Dobrzyn A, Miyazaki M, Ntambi JM. Loss of stearoyl-CoA desaturase 1 inhibits fatty acid oxidation and increases glucose utilization in the heart. Am J Physiol Endocrinol Metab 294: E357–E364, 2008. First published November 27, 2007; doi:10.1152/ajpendo.00471.2007.—Stearoyl-CoA desaturase (SCD) is a lipogenic enzyme that catalyzes the synthesis of monounsaturated fatty acids (FA). SCD1 deficiency activates metabolic pathways that promote FA β-oxidation and decrease lipogenesis in liver. In the present study, we show that FA transport and oxidation are decreased, whereas glucose uptake and oxidation are increased in the heart of SCD1−/− mice. Protein levels of FA transport proteins such as FA translocase/CD36 and FA transport protein as well as activity of carnitine palmitoyltransferase 1, the rate-limiting enzyme for mitochondrial fat oxidation, were significantly lower in the heart of SCD1−/− mice compared with SCD1+/+ mice. Consequently, the rate of palmitoyl-CoA oxidation was decreased significantly in the heart of SCD1−/− mice. mRNA levels of peroxisome proliferator-activated receptor-α, a key transcription factor controlling genes of FA oxidation, were significantly reduced in SCD1−/− mice. Phosphorylation of insulin receptor substrate-1 (IRS-1) and the association of ep85 subunit of phosphatidylinositol 3-kinase with IRS-1 were significantly higher under both basal and insulin-stimulated conditions in SCD1−/− hearts. This increased insulin sensitivity translated to a 1.8-fold greater 2-deoxyglucose uptake and 2-fold higher rate of glucose oxidation in the myocardium compared with SCD1+/+ counterparts. The results suggest that SCD1 deficiency causes a shift in cardiac substrate utilization from FA to glucose by upregulating insulin signaling, decreasing FA availability, and reducing expression of FA oxidation genes in the heart. This increase in cardiac insulin sensitivity and glucose utilization due to SCD1 deficiency could prove therapeutic in pathological conditions such as obesity that are characterized by skewed cardiac substrate utilization.

The heart has a major energy requirement based on its large work output. Although the cardiomyocytes are capable of using carbohydrates and ketone bodies as energy sources, fatty acids (FA) are considered the preferred fuel. Under aerobic conditions, the heart derives 60–90% of the energy necessary for contractile function from FA oxidation while the majority of the rest is obtained from carbohydrates (glucose and lactate) (34, 38). There is evidence to suggest that impaired substrate metabolism contributes to contractile dysfunction and to the progressive left ventricular remodeling that are characteristic of heart failure. In disease states like ischemia-reperfusion, diabetes, or obesity, cardiac substrate utilization is shifted into an excessive use of FA in place of glucose (5, 34, 37, 38). It has been suggested that this shift in metabolism plays a role in the development of cardiomyopathy, which leads to both impaired contractile function and ischemic injury (37). Thus the regulation of substrate metabolism in the heart plays a fundamental role in the control of normal cardiac function.

Stearoyl-CoA desaturase (SCD), the rate-limiting enzyme in the biosynthesis of monounsaturated FAs, has recently been shown to be a critical control point in regulation of liver and skeletal muscle metabolism (12, 13, 27). Studies of a mouse model with a targeted disruption of the SCD1 gene have provided evidence that lack of SCD1 function increases energy expenditure and basal thermogenesis (27) and significantly reduces body adiposity (23, 29). SCD1 deficiency leads to a decrease in the content of hepatic triglycerides (TG) and cholesterol esters and downregulates de novo FA synthesis in the liver (29). SCD1−/− mice also have reduced levels of TG in the very low-density lipoprotein and low-density lipoprotein fractions relative to SCD1+/+ animals and are resistant to high-carbohydrate and high-fat diet-induced liver steatosis (6). In liver (14), skeletal muscle (10), and brown adipose tissue (BAT) (23), SCD1 deficiency was also shown to increase the rate of FA β-oxidation. The molecular mechanism that accounts for the increased FA oxidation due to SCD1 deficiency is not completely understood. However, our recent studies have established that, in skeletal muscle and liver, SCD1 deficiency upregulates the AMP-activated protein kinase (AMPK) pathway (10, 14) and expression of genes of FA oxidation (29).

SCD is also involved in the regulation of carbohydrate metabolism. SCD1−/− mice have increased whole body glucose tolerance and significantly elevated insulin sensitivity in skeletal muscle (31) and BAT (32). Our studies provided evidence for significant and specific alternations in the levels of insulin signaling components in the skeletal muscle and BAT of SCD1−/− mice, as demonstrated by an increase in basal tyrosine phosphorylation of the insulin receptor (IR) and IR substrates (IRS1, IRS2), increased phosphorylation of protein kinase B, and enhanced GLUT4 membrane translocation (31, 32). Our studies showed that, in skeletal muscles of SCD1−/− mice, activation of insulin signaling was associated with downregulation of de novo ceramide synthesis (10) and decreasing protein-tyrosine phosphatase-1B gene expression (31), suggesting these two mechanisms as viable for increased insulin signaling due to SCD1 deficiency.

In the heart, three isoforms of SCD (SCD1, SCD2, and SCD4) are expressed and regulated in a hormone-dependent manner. Stimulation of the insulin signaling pathway upregulates the SCD1 subunit of phosphatidylinositol 3-kinase with IRS-1 were significantly reduced in SCD1−/− hearts. This increased insulin sensitivity translated to a 1.8-fold greater 2-deoxyglucose uptake and 2-fold higher rate of glucose oxidation in the myocardium compared with SCD1+/+ counterparts. The results suggest that SCD1 deficiency causes a shift in cardiac substrate utilization from FA to glucose by upregulating insulin signaling, decreasing FA availability, and reducing expression of FA oxidation genes in the heart. This increase in cardiac insulin sensitivity and glucose utilization due to SCD1 deficiency could prove therapeutic in pathological conditions such as obesity that are characterized by skewed cardiac substrate utilization. Additionally, the heart is a highly insulin-sensitive organ and its metabolism is finely tuned to be able to rely on both FA and glucose as energy sources. In disease states like ischemia-reperfusion, diabetes, or obesity, cardiac substrate utilization is shifted into an excessive use of FA in place of glucose (5, 34, 37, 38). It has been suggested that this shift in metabolism plays a role in the development of cardiomyopathy, which leads to both impaired contractile function and ischemic injury (37). Thus the regulation of substrate metabolism in the heart plays a fundamental role in the control of normal cardiac function.

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fashion (26). The molecular and metabolic implications of overlapping expression of these various SCD isoforms are as yet unclear. Because SCD1\(^{+/−}\) mice have increased FA oxidation in liver, skeletal muscle, and BAT, we hypothesized that fat oxidation in the heart would also be affected by SCD1 deficiency. We show here that the lack of SCD1 decreases FA uptake and oxidation while increasing glucose transport and oxidation in the heart. The results of our current study support the notion that this decrease in FA β-oxidation occurs as a result of downregulation of oxidative gene expression and decreased plasma FA availability. We also show that SCD1 deficiency causes a shift in cardiac substrate utilization from FA to glucose in hearts of SCD1\(^{+/−}\) mice. Interestingly, this shift in substrate metabolism does not affect the cardiac function in SCD1\(^{+/−}\) mice.

**MATERIALS AND METHODS**

**Animals.** The generation of SCD1\(^{+/−}\) mice has been described previously (27). Pure-bred homozygous (SCD1\(^{+/−}\)) and wild-type (SCD1\(^{+/+}\)) male mice (12 wk old) on a 12:9SV background were used. Mice were maintained on a 12:12-h dark-light cycle and fed a normal nonpurified diet (5008 test diet; PMI Nutrition International, Richmond, IN). The breeding of these animals was in accordance with the protocols reviewed and approved by the Animal Care Research Committees of the University of Wisconsin-Madison.

**Materials.** [\(^{1}H\)]carnitine, \([^{14}C]\)palmitic acid, \([^{14}C]\)stearoyl-CoA, 2-deoxy-[\(^{14}C\)]glucose, \([^{14}C]\)mannitol, and \([^{14}C,^{15}N]\)glucose were purchased from American Radiolabeled Chemicals (St. Louis, MO). IR, IRS-1, 6-phosphatidyl-dinositol 3-kinase (PI 3-kinase), fatty acid translocase/Cd36 (FAT/Cd36), and fatty acid transport protein (FATP) antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Carnitine palmitoyltransferase (CPT) 1 antibody were from Alpha Diagnostic (San Antonio, TX). Other chemicals were purchased from Sigma (St. Louis, MO) or Fisher Scientific (Pittsburgh, PA).

**Echocardiography.** Transthoracic echocardiography was performed using a Sonos 5500 ultrasonograph with a 12-MHz transducer (Philips). Noninvasive acquisition of two-dimensional guided M-mode images at the tip of papillary muscles and Doppler studies were recorded on anesthetized mice (50 mg/kg ketamine). The thickness of the posterior and anterior walls of the left ventricle (LV) chamber and the opening of the aortic valve were calculated. From these images, heart rate and the time between closure of the mitral and aortic valves. From the posterior and anterior walls of the left ventricle (LV) chamber and Doppler studies were recorded on anesthetized mice (50 mg/kg ketamine). The thickness of the posterior and anterior walls of the left ventricle (LV) chamber and the LV diameter during systole and diastole were measured using the leading edge-to-leading edge convention. All parameters were measured over at least three consecutive cardiac cycles. These parameters were used to calculate LV mass and fractional shortening as previously described (18). Pulse-wave Doppler was used to measure the velocity of blood through the mitral and aortic valves. From these images, heart rate and the time between closure of the mitral valve and the opening of the aortic valve were calculated.

**Blood sampling.** Mice were killed by cervical dislocation. Blood was collected aseptically by direct cardiac puncture and centrifuged (13,000 g, 5 min, 4°C) to collect plasma. Plasma cholesterol, TG, insulin, and glucose levels were measured by using commercial kits (Roche Applied Science, Indianapolis, IN; Linco Research, St. Charles, MI; and Sigma). Plasma free fatty acids (FFA) were measured by gas-liquid chromatography as previously described (27).

**Isolation and analysis of RNA.** Total RNA was isolated from hearts of SCD1\(^{+/−}\) and SCD1\(^{+/−}\) mice using TRIzol reagent (Invitrogen, Carlsbad, CA). DNase-treated RNA was reverse transcribed with Superscript III (Invitrogen), and real-time quantitative PCR was performed on an ABI Prism 7500 Fast Instrument. SYBR green was used for detection and quantification of genes, which are expressed as mRNA level normalized to cyclophilin using the ΔΔC\(_T\) method. Primer sequences were as follows: CPT1: 5′-TCTATGAGGGGTCGCAG-3′ (forward), 5′-CTGCTAGGGTGTAGCA-3′ (reverse); peroxisome proliferator-activated receptor (PPAR)-α: 5′-TCAAGGGACTACGATGGTCA-3′ (forward), 5′-CCGAATAGTGTCGGGAAAG-3′ (reverse); acetyl-CoA oxidase (ACO): 5′-TCTCTTTGAGACAGGGCCCAG-3′ (forward), 5′-GTTCCGACTGCCCCAGTG-3′ (reverse); PPARy coactivator (PGC)-1α: 5′-TCTAGTTGTCGCTTCTGTG-3′ (forward), 5′-ACAGAGCGGATCTTGG-3′ (reverse).

**Measurement of lipids.** Heart lipids were extracted by the method of Bligh and Dyer (2) and measured as described (10). Briefly, the lipids were separated by thin-layer chromatography (TLC) on silica gel-60 plates (Merck) in heptane-isopropyl ether-glacial acetic acid (60:40:4, vol/vol/vol) with authentic standards. The bands corresponding to TG and free fatty acid (FFA) standards were scraped off the plate and transferred to screw cap glass tubes containing methylenedecanoic acid as an internal standard. FA were then transmethylated in the presence of 14% boron trifluoride in methanol. The resulting methyl esters were extracted with hexane and analyzed by gas-liquid chromatography. Total contents were calculated from individual FA content in each fraction.
pellet was then resuspended in 800 μl of double-distilled H2O and extracted with 600 μl of butanol. Three hundred microliters of the butanol phase were counted by liquid scintillation.

**Western blot analysis.** The phosphorylation assays were carried out as described (31). Animals were injected with 0.075 U insulin/g body wt or an equal volume of PBS. Mice were killed 15 min later, and LVs were used for protein isolation. The samples were homogenized and centrifuged at 100,000 g for 1 h in ice-cold 50 mM HEPES buffer (pH 7.4) containing 150 mM NaCl, 10 mM sodium pyrophosphate, 2 mM Na2VO4, 10 mM NaF, 2 mM EDTA, 2 mM phenylmethylsulfonyl fluoride, 5 μg/ml leupeptin, 1% Nonidet P-40, and 10% glycerol. Cytosolic proteins were immunoprecipitated with anti-IR β-antibody or anti-IRS-1 antibody. Immunoprecipitates were subjected to SDS-PAGE on a 9% acrylamide gel. Proteins were transferred and immobilized on nitrocellulose membrane. The membranes were then immunoblotted with anti-phosphotyrosine or anti-PI 3-kinase p85 antibodies.

Protein levels of α1- and α2-AMPK and phosphorylation of AMPK at Thr172 were determined in 50 μg of clarified homogenate protein using specific antibodies that were obtained from Dr. Grahame Hardie (University of Dundee, Dundee, UK). To measure FAT/CD36 and FATP protein levels, 100 μg of clarified homogenate protein were loaded on 9% SDS-PAGE, whereas CPT1 protein content was measured in 50 μg of purified mitochondrial protein. The separated proteins were transferred to nitrocellulose membranes that were blocked using antibodies. The proteins were visualized using enhanced chemiluminescence as described by the manufacturer and quantified by densitometry.

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

**Statistical analysis.** Results were analyzed using the Student’s t-test. A difference of P < 0.05 was considered significant. Values are presented as means ± SD (n = 6 mice/group).

### RESULTS

**Decreased level of FA transport proteins and lipid content in the heart of SCD1−/− mice.** The concentrations of FFA (1,164 μmol/dl in SCD1+/+ mice vs. 768 μmol/dl in SCD1−/− mice), TG (256 mg/dl in SCD1+/+ mice vs. 167 mg/dl in SCD1−/− mice), and cholesterol (174 mg/dl in SCD1−/− mice vs. 101 mg/dl in SCD1−/− mice) in plasma were significantly (P < 0.05) decreased in SCD1−/− mice relative to SCD1+/+ mice, whereas fasting glucose levels are similar between both groups of mice (29).

FA uptake by cardiac myocytes occurs by two main transport processes: protein-mediated transport, which accounts for ~80% of total FA uptake (4), and by simple diffusion. FAT/CD36 and FATP are the major proteins responsible for membrane FA transport (7). We measured both FAT/CD36 and FATP protein levels in the hearts of SCD1−/− and SCD1+/+ mice by Western blotting. The content of both proteins was significantly lower in the hearts of SCD1−/− mice (Fig. 1A), suggesting lower rates of FA transport. In addition, to shed
light on the rate of cardiac FA uptake from the plasma, we analyzed the incorporation of intravenously injected \(^{14}C\)palmitate in the cardiac lipids. The rate of incorporation of \(^{14}C\)palmitate in total lipids was 54% lower in the heart of SCD1 \(^{-/-}\) mice relative to SCD1 \(^{+/+}\) mice (Fig. 1B). This decreased rate of palmitate incorporation into lipids in the heart of SCD1 \(^{-/-}\) mice was accompanied by reductions in intracellular FFA (Fig. 1C) and TG (Fig. 1D) in the myocardium.

We showed previously that SCD4 expression is induced in the heart of SCD1 \(^{-/-}\) mice, possibly to compensate for the SCD1 deficiency (26). To determine if SCD1 deficiency results in any functional increase in SCD enzyme activity in the hearts of SCD1 \(^{-/-}\) animals, we assayed SCD activity by measuring the conversion of a \(^{14}C\)stearyl-CoA to \(^{14}C\)oleoyl-CoA. Although SCD activity was slightly decreased in the myocardium of SCD1 \(^{-/-}\) mice compared with SCD1 \(^{+/+}\) mice (Fig. 1E), it was not significantly lower than in SCD1 \(^{+/+}\) mice (P = 0.12).

**Decreased FA β-oxidation in the heart of SCD1 \(^{-/-}\) mice.** The rate of FA β-oxidation is controlled by their rate of transfer into the mitochondria through CPT1 (1, 33). To address this process, we measured CPT1 mRNA and protein levels as well as enzyme activity in the hearts of SCD1 \(^{-/-}\) mice. CPT1 mRNA level was decreased by 65% in the myocardium of SCD1 \(^{-/-}\) mice relative to SCD1 \(^{+/+}\) mice (Fig. 2A). Both CPT1 protein level and activity were also decreased significantly in the heart of SCD1 \(^{-/-}\) mice relative to SCD1 \(^{+/+}\) controls (Fig. 2, B and C). To determine if this correlates with a decrease in mitochondrial fat oxidation in SCD1 \(^{-/-}\) mice, we measured oxidation of \(^{14}C\)palmitoyl-CoA in heart mitochondria. Palmitoyl-CoA β-oxidation was 37.1% lower in SCD1 \(^{-/-}\) mice relative to SCD1 \(^{+/+}\) controls (Fig. 2D), correlating with decreased CPT1 levels.

FA oxidation has been shown to be modulated by the activity of AMPK, which is activated by phosphorylation at a threonine residue (21). We therefore measured AMPK phosphorylation and AMPK α-subunit protein levels in heart homogenates of SCD1 \(^{-/-}\) and SCD1 \(^{+/+}\) mice. Neither AMPK phosphorylation nor protein levels of the α-subunit were affected by SCD1 deficiency (Fig. 2E), indicating that AMPK is not involved in decreasing FA oxidation that we observed in the heart of SCD1 \(^{-/-}\) mice.

**PPARα pathway is downregulated in the heart of SCD1 \(^{-/-}\) mice.** Another important factor responsible for transcriptionally regulating genes of FA catabolism, including CPT1, is PPARα, a transcription factor predominantly expressed in liver and heart (15). When activated, it promotes expression of FA oxidation genes (15). In the heart, PPARα activation has also been shown to upregulate the FAT/CD36 level (42). Because we observed decreased levels of FAT/CD36 protein and CPT1 mRNA, we were interested in determining if these changes may be mediated by decreased PPARα activity. Therefore, we measured expression of two genes that are regulated by PPARα, ACO and PPARα itself. As expected, the mRNA levels of ACO were decreased significantly in the heart of SCD1 \(^{-/-}\) mice (Fig. 3A). mRNA levels of PPARα were also...
decreased by 33% in the heart of SCD1−/− mice compared with SCD1+/+ animals (Fig. 3B), suggesting decreased PPARα activity as a viable mechanism for decreased FA oxidation in the myocardium of SCD1−/− mice. To shed light on the molecular mechanism of PPARα downregulation in SCD1−/− heart, we analyzed the gene expression of PGC1α, an inducible coregulator of PPARα (16). mRNA level of PGC1α was not affected by SCD1 deficiency (Fig. 3C), indicating that this factor is not involved in downregulation of PPARα observed in the heart of SCD1−/− mice. Polyunsaturated fatty acids (PUFA) are other very potent endogenous regulators of PPARα activity (35). The intracellular content of nonesterified PUFA was decreased by 30% in the heart of SCD1−/− compared with SCD1+/+ mice (Fig. 3D). It is thus possible that reduced PPARα activity in SCD1−/− hearts is associated with decreased intracellular PUFA content.

Increased insulin signaling and glucose utilization in the heart of SCD1−/− mice. The intracellular signaling pathway by which insulin mediates glucose transport involves signal transduction through the IR and increasing tyrosine kinase activity toward IRSs (40), leading to activation of PI 3-kinase, a key signaling intermediate in insulin-mediated glucose utilization (20). To measure insulin-stimulated signaling in the heart, mice were injected with insulin, and hearts were collected 15 min after the injection. As reported previously (31), basal plasma insulin levels were significantly lower in SCD1−/− mice relative to SCD1+/+ mice (Fig. 4A). After insulin injection, plasma insulin levels were increased in both SCD1+/+ and SCD1−/− mice to equal levels (Fig. 4A). Tyrosine phosphorylation of IR in response to insulin was 50% greater in the heart of SCD1−/− animals (Fig. 4B). Consequently, IRS-1 tyrosine phosphorylation after insulin injection was also, 2-fold higher in the heart of SCD1−/− mice compared with SCD1+/+ mice (Fig. 4B). There were no significant differences in total protein levels of IR and IRS-1 between the two groups of mice (data not shown). When IRSs are phosphorylated, they associate with the α85 subunit of PI 3-kinase, resulting in its activation (40) and leading to increased glucose uptake. The association of α85 subunit of PI 3-kinase with IRS-1 in basal conditions as well as after insulin administration were significantly higher in the SCD1−/− mice compared with SCD1+/+ mice (Fig. 4B).

We also measured in vivo glucose uptake and oxidation in the heart of SCD1−/− and SCD1+/+ mice. 2-Deoxyglucose uptake was a 1.8-fold higher (Fig. 4C) and glucose oxidation was 2-fold higher in the heart of SCD1−/− mice compared with the SCD1+/+ mice (Fig. 4D). Taken together, these data indicate increased insulin sensitivity, glucose uptake, and oxidation in the hearts of SCD1−/− mice.

Heart function is not significantly different between SCD1−/− and SCD1+/+ mice. To check whether metabolic changes associated with SCD1 deficiency lead to an alteration in heart function, cardiac structural and functional parameters were investigated using transthoracic echocardiography and Doppler. LV weights normalized to body weight, measured by both wet weights obtained at necropsy (data not shown) and echocardiography (Table 1), were ~15% higher in SCD1−/− compared with SCD1+/+ mice. LV diameter in the heart of SCD1−/− mice was significantly larger; however, the LV posterior and anterior wall thicknesses were not different between groups (Table 1). The structural differences were not accompanied by functional abnormalities of the heart of SCD1−/− mice. Heart rate (beats/min) measured during echocardiography was similar in SCD1−/− and SCD1+/+ mice (Table 1). LV fractional shortening, used as a measure of systolic function, and the isovolumetric relaxation time, used as a measure of diastolic function, were not significantly different between groups (Table 1). Also, the myocardial performance index, a Doppler-based measure of left ventricular function, and the velocity of blood flow across the mitral
with SCD1 to the heart. Indeed, in the present paper, we show that metabolism by changing the proportion of substrate available is tempting to speculate that SCD1 deficiency could alter cardiac metabolism by changing the proportion of substrate available to the heart. Indeed, in the present paper, we show that metabolism by changing the proportion of substrate available to the heart.

Table 1. Echocardiographic analysis of heart function and structure of SCD1+/+ and SCD1−/− mice

<table>
<thead>
<tr>
<th></th>
<th>SCD1+/+ (n = 10)</th>
<th>SCD1−/− (n = 8)</th>
</tr>
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<tbody>
<tr>
<td>HR, beats/min</td>
<td>445.00±31.1</td>
<td>476.13±26.0</td>
</tr>
<tr>
<td>AWD, mm</td>
<td>0.80±0.2</td>
<td>0.84±0.1</td>
</tr>
<tr>
<td>PWd, mm</td>
<td>0.81±0.1</td>
<td>0.86±0.1</td>
</tr>
<tr>
<td>LVd, mm</td>
<td>3.30±0.4</td>
<td>3.79±0.3*</td>
</tr>
<tr>
<td>LV mass/body wt, mg/g</td>
<td>3.63±0.5</td>
<td>4.18±0.3*</td>
</tr>
<tr>
<td>Fractional shortening, %</td>
<td>51.33±6.3</td>
<td>54.05±3.7</td>
</tr>
<tr>
<td>IVRT, s</td>
<td>0.012±0.004</td>
<td>0.017±0.002</td>
</tr>
<tr>
<td>MPI</td>
<td>0.44±0.1</td>
<td>0.48±0.2</td>
</tr>
<tr>
<td>Ea/Aa</td>
<td>1.87±0.6</td>
<td>1.63±0.2</td>
</tr>
<tr>
<td>E/Ea</td>
<td>30.83±3.0</td>
<td>27.62±4.1</td>
</tr>
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Values are means ± SE; n, no. of mice. SCD, stearoyl-CoA desaturase; HR, heart rate; AWD, anterior wall in diastole; PWd, posterior wall in diastole; LVd, left ventricular diameter in diastole; LV mass/body wt, left ventricular mass/body wt; IVRT, isovolumic relaxation time (the time between the closure of the aortic valve and the opening of the mitral valve); MPI, myocardial performance index = the ratio of isovolumic contraction and relaxation to ejection time [MPI = (a − b)/b where a is the time of mitral valve closure and b is aortic ejection time]; Ea, early diastolic maximal velocity from tissue Doppler; Aa, late diastolic maximal velocity from tissue Doppler. Fractional shortening = (LVd − LVds)/LVd. *P < 0.05 vs. SCD1+/+ mice.

Fig. 4. Increased glucose uptake and glucose oxidation in the heart of SCD1−/− mice. A: insulin levels were measured in basal condition and 15 min after insulin injection. B: insulin receptor (IR) and IR substrate (IRS)-1 phosphorylation and association of IRS-1 with the op85 subunit of phosphatidylinositol 3-kinase (PI 3-kinase) were investigated by immunoblotting in basal condition and after insulin administration. C: 2-deoxyglucose uptake measured in vivo in the heart of SCD1+/+ and SCD1−/− mice. D: glucose oxidation was determined using [U-14C]glucose as substrate. Data are representative of 6 animals in each group. *P < 0.05 vs. SCD1+/+ mice.

Discussion

We previously showed that SCD1 deficiency increases FA β-oxidation in liver, skeletal muscle, and BAT (10, 14, 23). The higher rate of FA oxidation in these tissues makes it tempting to speculate that SCD1 deficiency could alter cardiac FA oxidation by changing the proportion of substrate available to the heart. Indeed, in the present paper, we show that metabolism by changing the proportion of substrate available to the heart.

Although lipids are required for the energy needs and the structural integrity of the heart, they also have toxic effects at higher doses. High levels of FA and their fatty acyl-CoA esters are detrimental to myocardial structure and function (25). In the present study, we found decreased levels of FFA, TG, and cholesterol in plasma of SCD1−/− mice compared with SCD1+/+ controls. Lowering the plasma and intracellular levels of lipid intermediates has been proposed as beneficial for cardiac function (9, 42). Decreased plasma FFA, TG, and cholesterol was also observed in FAT/CD36-deficient mice that show decreased FA utilization and increased glucose utilization in the heart (7, 42). FAT/CD36 and FATP have been identified as the major membrane FA transporters in the heart (7). We found that FAT/CD36 and FATP levels as well as [14C]palmitate uptake from plasma are decreased in the myocardium of SCD1−/− compared with SCD1+/+ mice. These results suggest that lower FA uptake is responsible for decreased FFA and TG content are decreased in the myocardium of SCD1−/− mice. The activity and protein content of CPT1 and the rate of FA oxidation are decreased, whereas glucose uptake and glucose oxidation are increased in SCD1−/− mice. Because FFA and TG contents in both blood plasma and cardiomyocytes are reduced significantly in SCD1−/− mice, it is likely that increased whole body energy expenditure and reduced de novo hepatic lipogenesis due to SCD1 deficiency decreases FA availability for utilization by the heart. On the other hand, however, the reduced rate of FA oxidation in SCD1−/− heart compared with SCD1+/+ was also shown when the measurements of mitochondrial oxidation were performed in vitro and under similar conditions. This suggests that intracellular regulatory mechanisms are also involved in the development of the SCD1−/− phenotype in the heart.
creased FFA and TG accumulation in the heart of SCD1−/− mice. Because obesity-related cardiomyopathy is associated with the accumulation of myocardial TG, possibly stemming from elevation of myocardial long-chain FA uptake (34, 38), a decrease in FA uptake caused by SCD1 deficiency could be beneficial in the treatment or prevention of this lipotoxic cardiomyopathy.

The oxidation of FA is controlled by their rate of transfer into the mitochondria through CPT1 (1, 33). CPT1 activity and the rate of β-oxidation are increased in liver, skeletal muscle, and BAT of SCD1−/− compared with SCD1+/+ mice (10, 14, 23). Interestingly, however, both CPT1 mRNA and protein levels and activity were decreased in the heart of SCD1−/− mice. As a result of lower FA availability and uptake and decreased rate of FA transport into mitochondria by CPT1, the rate of mitochondrial FA oxidation was decreased in the heart of SCD1−/− animals. The utilization of FA and glucose is tightly coupled in the myocardium (34, 38). When FAs are unavailable as a source of ATP, the heart extends its use of carbohydrates as an energy supply. Furthermore, we have previously observed that insulin sensitivity and glucose uptake are enhanced by SCD1 deficiency (31, 32). Therefore, we expected that glucose oxidation would be increased in the heart of SCD−/− mice to compensate for ATP supply. Indeed, the rate of glucose oxidation was enhanced in the heart of SCD1−/− mice. We also established that loss of SCD1 function in mice leads to increased tyrosine phosphorylation of IR and IRS-1 and greater IRS association with the α-85 subunit of PI 3-kinase in the heart despite lower levels of plasma insulin. This increased insulin signaling could be responsible for enhanced glucose uptake in the heart of SCD1−/− mice. This is consistent with previous results obtained during hyperinsulinemic-euglycemic clamp studies displaying markedly increased insulin-stimulated glucose flux in the heart of SCD1−/− mice (17). The decrease in fat oxidation coupled with increased insulin sensitivity then leads to a shift in substrate utilization from FA to glucose in the SCD1-deficient heart.

AMPK is an important factor that regulates metabolic pathways such as FA oxidation, glucose transporter translocation, glucose uptake, and glycolysis (34, 39). Thus activation of AMPK in the heart can potentially increase both fat and glucose metabolism (8, 38, 41). SCD1 deficiency is known to activate AMPK in liver and skeletal muscle (10, 14). However, in the heart, AMPK phosphorylation and protein levels were not affected by SCD1 deficiency, indicating that AMPK is unlikely to play a role in the shift in substrate oxidation in the myocardium of SCD1−/− mice.

The transcription factor PPARα is highly expressed in tissues with a high capacity for FA oxidation, including hepatocytes, cardiomyocytes, the renal cortex, and skeletal muscles. Activation of PPARα promotes FA oxidation, ketone body synthesis, and glucose sparing (15). Because the expression of PPARα is decreased significantly in the myocardium of SCD1−/− mice, it raises the possibility that the decreased expression of genes of FA oxidation such as CPT1 is mediated by decreased PPARα activity in the heart of SCD1−/− mice. Decreased ACO gene expression additionally confirms reduced PPARα activity in the SCΔ1−/− heart. Cardiac-specific overexpression of PPARα has been shown to cause insulin resistance and increased FA oxidation in the heart (30), whereas ablation of FAT/CD36 in the context of PPARα cardiac overexpression largely reverses these effects and promotes glucose uptake and oxidation in the heart (42). These studies underscore the role of PPARα in regulating substrate utilization in the heart. PGC1α and nonesterified PUFA are two main regulators of PPARα activity (16, 35). Although cardiac PGC1α expression is not affected by SCD1 deficiency, the intracellular PUFA contents are reduced by 30% in the heart of SCD1−/− mice, suggesting a viable mechanism for the decreased PPARα activity observed in SCD1-deficient hearts.

Three different forms of SCD, namely, SCD1, SCD2, and SCD4, are present in the mouse heart (12, 28). SCD4, which is expressed exclusively in the heart, demonstrates tissue-specific regulation by leptin (26). To determine if decreased FA oxidation and increased glucose oxidation in the myocardium of SCD1−/− mice might be directly dependent on inhibition of SCD activity in the heart, we measured the enzyme activity in microsomes isolated from the heart of SCD1−/− and SCD1+/+ mice. There was no significant difference in SCΔ1 enzyme activity either group of mice. Furthermore, there were no significant differences in levels of palmitoleic acid (data not shown), a product of de novo desaturation by SCD, in cardiac lipids between SCD1+/+ and SCD1−/− mice. These results indicate that the heart-specific SCD4 isoform likely compensates for the lack of SCD1 in the heart, as previously suggested (26). This relative lack of a change in Δ9-desaturase activity in the heart could account for the tissue-specific differences in fat oxidation observed in the heart, as opposed to other tissues, such as skeletal muscle, liver, or BAT. Ongoing studies examining the specific role of SCD4 in cardiac metabolism should further clarify the functions of this resident Δ9-desaturase in heart metabolism.

In summary, we show here for the first time that SCD1 deficiency affects cardiac metabolism by shifting substrate utilization toward glucose oxidation and away from FA uptake and oxidation. We postulate that increased insulin signaling and downregulation of the PPARα pathway are the major reasons for this shift, since SCD activity and AMPK phosphorylation are unchanged in the myocardium of SCD1−/− mice. Echocardiographic analysis showed that changes in substrate utilization caused by SCD1 deficiency do not disturb heart function. Thus SCD1 repression represents a viable approach for decreasing FA uptake and oxidation in the heart and thereby aid in the prevention and treatment of lipotoxic cardiomyopathy observed in the diabetic and obese states.

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