SCD1 activity in muscle increases triglyceride PUFA content, exercise capacity, and PPARδ expression in mice

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Abstract Stearoyl-CoA desaturase (SCD)1 converts saturated fatty acids into monounsaturated fatty acids. Using muscle overexpression, we sought to determine the role of SCD1 expression in glucose and lipid metabolism and its effects on exercise capacity in mice. Wild-type C57Bl/6 (WT) and SCD1 muscle transgenic (SCD1-Tg) mice were generated, and expression of the SCD1 transgene was restricted to skeletal muscle. SCD1 overexpression was associated with increased triglyceride (TG) content. The fatty acid composition of the muscle revealed a significant increase in polyunsaturated fatty acid (PUFA) content of TG, including linoleate (18:2n6). Untrained SCD1-Tg mice also displayed significantly increased treadmill exercise capacity (WT = 6.6 ± 3 min, Tg = 71.9 ± 9.5 min; P = 0.0009). SCD1-Tg mice had decreased fasting plasma glucose, glucose transporter (GLUT)1 mRNA, fatty acid oxidation, mitochondrial content, and increased peroxisome proliferator-activated receptor (PPAR)δ and Pgc-1 protein expression in skeletal muscle. In vitro studies in C2C12 myocytes revealed that linoleate (18:2n6) and not oleate (18:1n9) caused a 3-fold increase in PPARδ and a 9-fold increase in CPT-1b with a subsequent increase in fat oxidation. The present model suggests that increasing delta-9 desaturase activity of muscle increases metabolic function, exercise capacity, and lipid oxidation likely through increased PUFA content, which increases PPARδ expression and activity. However, the mechanism of action that results in increased PUFA content of SCD1-Tg mice remains to be elucidated.—Rogowski, M. P., M. T. Flowers, A. D. Stamatikos, J. M. Ntambi, and C. M. Paton. SCD1 activity in muscle increases triglyceride PUFA content, exercise capacity, and PPARδ expression in mice. J. Lipid Res. 2013. 54: 2636–2646.

Supplementary key words peroxisome proliferator-activated receptor • stearoyl-CoA desaturase • oleate • linoleate • polyunsaturated fatty acid sparing

To combat the rise in obesity and metabolic diseases, a large number of studies have attempted to define the etiology of impaired glucose metabolism, dyslipidemia, and lipodystrophy (1–5). The results of these studies have generally shown that development of type 2 diabetes mellitus (T2DM) is dependent on a multitude of factors, including obesity due to overnutrition combined with physical inactivity; consumption of a high-fat/high-carbohydrate diet; and genetic predisposition (6–9). There have also been a number of studies designed to establish effective treatments and/or define drug targets to prolong the pre-diabetic state or to reduce the severity of established T2DM in which a wide range of potential targets have been identified. Despite the heterogeneity of causative factors leading to T2DM, there has been nearly unanimous agreement that impaired insulin signaling, increased intramuscular lipid deposition, and a loss of metabolic flexibility in skeletal muscle is critical for development of the disease (10, 11).

The current study was intended to define the role of a key lipogenic enzyme, stearoyl-CoA desaturase-1 (SCD1), in regulating skeletal muscle lipid metabolism and how its overexpression can actually promote fat oxidation, exercise capacity, and glucose tolerance by increasing triglyceride synthesis. SCD1 is an integral membrane protein of the endoplasmic reticulum that catalyzes the desaturation of long-chain saturated fatty acids (SFA) into monounsaturated fatty acids (MUFA). The enzyme is expressed in nearly all tissues of humans and mice where it converts primarily stearate (18:0) into oleate (18:1) and, to a lesser extent, palmitoleate (16:1). This conversion of saturated fatty acids to MUFA through SCD1 is a key step in the synthesis of unsaturated acylglycerols and is a primary mechanism for the production of triacylglycerol (TAG) for storage. The current study was intended to define the role of a key lipogenic enzyme, stearoyl-CoA desaturase-1 (SCD1), in regulating skeletal muscle lipid metabolism and how its overexpression can actually promote fat oxidation, exercise capacity, and glucose tolerance by increasing triglyceride synthesis. SCD1 is an integral membrane protein of the endoplasmic reticulum that catalyzes the desaturation of long-chain saturated fatty acids (SFA) into monounsaturated fatty acids (MUFA). The enzyme is expressed in nearly all tissues of humans and mice where it converts primarily stearate (18:0) into oleate (18:1) and, to a lesser extent, palmitoleate (16:1). This conversion of saturated fatty acids to MUFA through SCD1 is a key step in the synthesis of unsaturated acylglycerols and is a primary mechanism for the production of triacylglycerol (TAG) for storage.

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Abbreviations: Cpt-1b, carnitine palmitoyl transferase-1b; DAG, diacylglycerol; DI, desaturation index; gastrocnemius; GLUT, glucose transporter; IMTG, intramuscular triglyceride; IP GTT, intraperitoneal glucose tolerance; KO, knockout; PPAR, peroxisome proliferator-activated receptor; RER, respiratory exchange ratio; SCD, stearoyl-CoA desaturase; SKO, SCD1 knockout; TG, triglyceride; Tg, transgenic; T2DM, type 2 diabetes mellitus; WT, wild-type.

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extent, palmitate (16:0) into palmitoleate (16:1). Over the last 10+ years, a wealth of information has been collected to demonstrate that SCD1-knockout (KO) animals are completely resistant to the detrimental effects of high-fat diets (12–17). Those animals retain insulin sensitivity, glucose tolerance, and lower body weight relative to wild-type (WT) counterparts due in large part to increased energy expenditure (18). However, it is important to note that it is counterintuitive and unexpected that loss of SCD1 activity would actually protect from obesity and the related metabolic detriments. The increase in SFA content would be expected to increase inflammation and lipotoxicity and to promote insulin resistance, all leading to cardiovascular disease (CVD) (19, 20). However, we have recently shown that the antiobesity phenotype in SCD1-null animals is due to loss of SCD1 in the skin (18), which may be preventing the accumulation of SFA due to increased energy expenditure. In fact, recent studies examining the effect of tissue-specific deletion of SCD1 in liver and adipose have shown no protection from obesity and may even increase inflammation when skin expression is normal (13, 21, 22).

In light of the results described above, we began to speculate that increased SCD1 activity, especially in skeletal muscle, may actually provide more protection from obesity and T2DM than SCD1 knockout or inhibition. This is because increased oleate synthesis or availability is known to increase DGAT-mediated triglyceride (TG) synthesis at the expense of diacylglycerol (DAG) and free fatty acids (FFA) (23). Sequestering FFA and DAG into TG has been hypothesized to increase muscle TG synthesis, glucose uptake, and peroxisome proliferator-activated receptor (PPAR) expression. Furthermore, SCD1-overexpressing mice increase exercise capacity by more than 240%.

METHODS

Animals, diets, and tissue collection

We generated the muscle-specific SCD1 overexpressing mouse using the human skeletal muscle α-actin promoter (25) coupled with mouse SCD1 (actin-SCD1-Tg). Animals on a mixed FVB/C57Bl/6J background were backcrossed 10 generations to C57Bl/6J animals. Genotyping was performed by routine PCR of genomic tail DNA. Male mice were weaned at three weeks of age and then individually caged at eight weeks of age and fed a standard chow diet (Purina Formula 5008). All aspects of the study were approved by the Animal Care and Use Committees of the University of Wisconsin-Madison and Texas Tech University. Microsomes and mouse SCD1 Western blots were prepared as previously described (18).

Quantification of gene expression

mRNA was extracted from tissues and cells using Tri Reagent (Molecular Research Center, Cincinnati, OH) and reverse transcribed using AB high capacity cDNA synthesis kits (Applied Biosystems). Quantification of changes in target gene expression was performed using quantitative real-time PCR with an Eppendorf Realplex® and SYBR green master mix (Invitrogen) using β-actin as the reference gene. PCR primers (Integrated DNA Technologies, Coralville, IA) were designed to span exon-exon boundaries to prevent amplification of genomic DNA. All PCR products were confirmed using dissociation curve analysis and visualized by running on an EtBr-stained 2% agarose gel.

Glucose tolerance tests

Mice were fasted for 4 h in the early light-cycle prior to collecting a basal blood sample by retro-orbital puncture. Mice were then given an intraperitoneal injection of glucose solution (1 g glucose/kg body weight), and blood samples were collected 10, 20, 40, 80, and 120 min postinjection. Blood was collected into EDTA and glucose measured using a glucose oxidase colorimetric assay.

Fatty acid composition

Total lipids were extracted from tissues according to the method of Bligh and Dyer (26) and separated by silica gel TLC using petroleum ether/diethyl ether/acetone (80:30:1) as the developing solvent. Spots were visualized by spraying with fluorescein dissolved in ethanol and illuminated with UV light. The TG spots were scraped, methylated, and analyzed by gas-liquid chromatography to determine fatty acid composition as previously described (18). For exercised mice, total lipids were extracted and directly methylated prior to GC analysis. Total muscle TG was determined using an enzymatic assay (Wako Chemicals).

Tissue mitochondrial isolation

Mitochondria (mt) and mtDNA were isolated essentially as described (27). Briefly, approximately 30 mg of tissue was homogenized with 100 µl of the homogenate retained for analysis of total protein using the Bradford assay. The remaining homogenate as mitochondrial protein isolate. From the remaining isolated mitochondrial protein, mitochondria DNA was isolated and measured using a spectrophotometer prior to assay use or storage at −20°C.

Exercise testing

Grip time was assessed by placing animals on the top of the wire cage lids and inverting them. The time to fatigue was determined at the point in which the animals could no longer maintain their grip. Swim time to exhaustion was assessed by placing animals in 2,000 ml glass beakers filled with 30°C water and monitoring the length of time until the animals were no longer able to maintain their head (nose) above the water. Voluntary free-wheel running activity was measured over the course of five days in the dark cycle. Free-wheel revolutions were counted using the home cage wheel counter software (Columbus Instruments, Columbus, OH). The treadmill exercise protocol was assessed by measuring time to fatigue at 18 m/min and 0° incline on an automated mouse treadmill (AccuScan Instruments, Columbus, OH), with shock grid (1.00 mA). Exhaustive fatigue was defined as 10 continuous seconds of no longer attempting to leave the shock pad.

Metabolic cage studies

Mice were placed in metabolic chambers at the start of the dark cycle to collect respiratory gas exchange measurements. Oxygen consumption (VO₂), carbon dioxide production (VCO₂), respiratory exchange ratio (RER, VCO₂/VO₂), and total energy expenditure (TEE) were collected at 12 s time intervals for 48 h using the AccuScan metabolic cage system (AccuScan Instruments). Food intake was measured at the end of the light cycle after days 1 and 2.
Ex vivo glucose uptake and fatty acid oxidation

Red gastrocnemius (gastroc) muscle samples (~200 mg) were minced with a razor blade and cultured in 24-well plates with Hank’s Balanced Salt Solution (HBSS) for 12 h. For glucose uptake, HBSS was supplemented with 1 mM 2-deoxy-D-glucose (2-DG) containing 1 μCi 14C-labeled 2-DG with or without 200 nM insulin in the presence or absence of 100 μM palmitate (16:0). For fatty acid oxidation, muscle samples were cultured with 50 μM stearate (18:0) containing 1 μCi 14C-labeled 18:0 in HBSS. After 12 h, a piece of Whatman filter paper was saturated with 3M NaOH and placed over the tissue culture well; under the paper, 100 μl 70% perchloric acid was added to the HBSS and left for 90 min at 37°C. The 14CO2 content of the filter paper was measured using a liquid scintillation counter. To determine 14C-labeled 18:0 uptake, muscle samples were removed from the HBSS after 30 min and washed extensively in lipid free HBSS. Total lipids were extracted as described above, and 14C was measured using liquid scintillation counting.

In vitro fatty acid treatment

Mouse C2C12 myoblasts were subcultured (>60% confluence) in DMEM + 10% FBS and 1% penicillin/streptomycin until they were induced to differentiate into mature myocytes. To induce differentiation, cells were grown to confluence, and then media was changed to DMEM + 2% horse serum for 10 days. To examine the effect of individual fatty acids on the expression of PPARγ and its target gene carnitine palmitoyl transferase-1b (Cpt-1b), 100 μM palmitate (16:0), oleate (18:1), or linoleate (18:2n6) were added to serum-free DMEM after day 10 for 48 h. Fatty acid oxidation rate was determined by culturing the cells in the presence of 50 μM 3H-labeled 18:1 (0.5 μCi) for 4 h as previously described (28). To examine the effect of an individual fatty acid on PPARγ and Cpt-1b during the course of C2C12 differentiation, 100 μM 18:2n6 was added on days 4–10 or on days 8–10 in differentiation medium. Cells treated with normal medium throughout the 10-day differentiation period were used as control.

Statistical analyses

SAS Version 9.2 was used to conduct the statistical analyses in which values are expressed as mean ± SE. Differences between groups were evaluated using either the unpaired Student t-test or one-way ANOVA. For all analyses, experiments were performed at least three times.

RESULTS

We generated mice that overexpress mouse SCD1 in skeletal and cardiac muscle under the control of the human α-actin promoter (25). Four founders were identified that carried the SCD1 transgene (Fig. 1A); line 282 displayed the highest SCD activity and was selected for backcrossing. These mice display ~10-fold higher muscle SCD1 mRNA in soleus and gastrocnemius (gastroc) muscle (Fig. 1B), while brain, small intestine, liver, and lung remain unchanged. Diaphragm muscle SCD1 mRNA expression was increased over 3,000-fold in the Tg mice (Fig. 1C). We are uncertain as to why this muscle tissue was increased to such an extent; however, it is possible that its uniform composition (e.g., nearly 100% type I oxidative fibers) or the fact that it is a highly active muscle tissue even in sedentary mice could cause SCD1 mRNA overexpression to persist at greater levels compared with other muscle tissues. Microsomes obtained from mixed gastroc muscle displayed ~10-fold higher SCD1 protein content (Fig. 1D).

Actin-SCD1-Tg animals breed normally with expected litter sizes (data not shown) and, aside from a slightly reduced body weight (Fig. 1E), are indistinguishable from their WT counterparts. Despite no significant difference in SCD1 expression in the heart, gross assessment of the cardiac muscle displayed a slight but nonsignificant increase in heart weight that became significant after four weeks of exercise training (WT = 5.4 ± 0.5 g versus SCD1-Tg = 6.3 ± 0.4 g; P = 0.008) (Fig. 1F). A number of factors could account for the difference in heart weight after exercise training (free-wheel running), including increased TG and/or glycogen content, left ventricular hypertrophy, or a more generalized pathological or nonpathological enlargement of the heart. However, as the actin-SCD1-Tg animals ran farther and longer than their WT counterparts (described in detail below), it is likely that exercise dosage was higher in the Tg group and likely eliminates pathological hypertrophy as the culprit.

To gain a better perspective of the physiological relevance of the degree to which SCD1 was increased in skeletal muscle, we provided WT C57Bl/6 mice with access to free wheels for eight weeks. Since exercise training has been shown to increase muscle SCD1 expression (29, 30), we wanted to compare the effect of Tg overexpression with the physiological stimulus of exercise training. After eight weeks of free-wheel running, body weights were significantly lower in exercised mice versus sedentary mice (28.6 ± 0.7 versus 33.8 ± 1.4 g; P = 0.01), indicating that the animals engaged in voluntary exercise. Muscle SCD1 mRNA expression from WT mice increased 9.7 ± 1.3-fold versus sedentary mice (P = 0.01) (Fig. 1G), which was nearly identical to our Tg model (soleus = 9.3 ± 2-fold and gastroc = 5.8 ± 3-fold), suggesting that Tg overexpression levels fall within physiologically relevant levels.

Next, we determined the effect of SCD1 overexpression on muscle fatty acid (FA) composition. Red gastroc muscle was homogenized, and total lipids were extracted as described then separated using silica gel TLC, stained with fluorescein dissolved in ethanol, and illuminated with UV light for visual inspection. To quantify differences, total muscle triglycerides were measured using a colorimetric assay. Actin-SCD1-Tg mice had 112% more intramuscular triglyceride (IMTG) than WT mice (Tg = 111.4 ± 24 μg/g; WT = 52.3 ± 15 μg/g; P = 0.03) (Fig. 2A). Several factors may account for the increase in TG concentration, the most likely explanation being that oleic acid is a better substrate for DGAT-mediated TG esterification and increased TG synthesis. Previous reports have demonstrated that MUFA incorporation into TG increases the rate of esterification more than SFA does (31, 32). In light of these results, it is likely that increased MUFA synthesis in the skeletal muscle of SCD1-Tg animals enables DGAT-mediated DAG + fatty acyl-CoA → TG conversion more than it does in skeletal muscle of WT animals.

In addition to total TG content, we measured IMTG desaturation indices (Fig. 2B) and FA composition (Fig. 2C–E).
To determine whether higher 18:2n6 levels in SCD1-Tg mice might lead to increased metabolism and/or food intake, we performed macronutrient and energy expenditure tests using metabolic cages. Additionally, we collected measures of food intake over two consecutive days in WT and SCD1-Tg counterparts. There were no differences in total energy expenditure as assessed by oxygen consumption, but there were significant differences in RER and food intake (supplementary Fig. II). Uncoupling is not measured via gas exchange and may be higher in the SCD1-Tg mice since food intake was elevated (WT days 1 and 2: 3.9 ± 0.3 and 3.6 ± 0.1 g/day versus SCD1-Tg days 1 and day 2: 4.4 ± 0.8 and 4.5 ± 0.8 g/day; \( P = 0.1 \) and 0.01, respectively), but body weight was lower (Fig. 1E). Therefore, the increased TG 18:2n6 content may be due to hyperphagia; further detailed analyses of thermogenesis and uncoupling would be needed to conclusively determine whether it is occurring. RER, which is an indicator of fat versus carbohydrate utilization, was higher in the fed state (night cycle, WT versus SCD1-Tg day 1 and 2, \( P < 0.0001 \)) and lower in the fasted state (day cycle, WT versus SCD1-Tg day 1 and 2, \( P < 0.0001 \)). These data suggest that when glucose is present (fed state), the SCD1-Tg mice use more glucose, and when animals rely more on FFA in the fasted state, they preferentially use more fat for fuel.

In addition to the fact that oleic acid has been shown to increase DGAT activity, it is believed to increase SREBP-1c activation (33). SREBP-1c is a transcription factor that regulates the expression of several genes involved in lipogenic activation.
expressed the data as a percentage change relative to fasting levels (i.e., normalized to the differences in fasting values), we saw that the pattern of change during the GTT was identical between WT and SCD1-Tg mice (Fig. 4D).

These data suggest that increased SCD1 activity and the resulting increased IMTG content do not negatively affect glucose uptake. In fact, it suggests that increased IMTG may actually enhance basal glucose disposal. Saturated fat is thought to impair glucose uptake, but with increased SCD1 activity, the SFA effect should be mitigated by the conversion to MUFA. We assessed the ability of ex vivo prepared muscle samples to transport 1 mM 3H-labeled 2-deoxy-D-glucose (2-DG) in the presence or absence of SCD1 overexpression. To help explain the increase in TG content of actin-SCD1-Tg muscles samples, we measured SREBP-1c gene expression along with the fatty acid elongase enzyme Elovl6 and the esterification enzyme DGAT2. In white gastroc, there was a significant increase in SREBP-1c but no difference in Elovl6 or DGAT2 between WT and actin-SCD1-Tg mice (Fig. 3A). In red gastroc, SREBP-1c, Elovl6, and DGAT2 were all significantly elevated in the Tg animals (Fig. 3B).

Increased muscle lipid content is thought to decrease insulin-stimulated glucose uptake. Therefore, to determine the effect of SCD1 overexpression on whole-body glucose tolerance, we assessed glucose transporter (GLUT)1 and GLUT4 mRNA expression, fasting blood glucose, and intraperitoneal glucose tolerance (IP GTT). In both white (Fig. 4A) and red (4B) gastroc, there was a significant increase in GLUT1 expression, and in white gastroc, there was a significant decrease in GLUT4. GLUT1 is primarily responsible for noninsulin-stimulated glucose uptake (basal), whereas GLUT4 is the insulin-responsive glucose transported in skeletal muscle. It is unclear how SCD1 overexpression impacts GLUT1 and GLUT4 expressions; however, it has been reported that long-chain n-3 PUFAs increase GLUT1 expression (34) and oleate reduces GLUT4 expression (35).

The GLUT expression patterns suggest altered glucose uptake. To test this, we measured 4 h fasting blood glucose levels and IP GTT. There was a reduction in fasting glucose in actin-SCD1-Tg mice (Fig. 4C) versus WT. During the 2 h IP GTT, all postinjection values were significantly lower in the actin-SCD1-Tg mice. However, when we expressed the data as a percentage change relative to fasting levels (i.e., normalized to the differences in fasting values), we saw that the pattern of change during the GTT was identical between WT and SCD1-Tg mice (Fig. 4D).

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SCD1 overexpression, fat oxidation, and PPARα in muscle

exercise capacity by testing grip strength (Fig. 5A), swim time to exhaustion (Fig. 5B), and daily free-wheel running activity (Fig. 5C). We noted that SCD1-Tg animals were able to support their body weight longer (WT = 92.2 ± 3 s versus SCD1-Tg = 201.0 ± 20 s; \( P = 0.003 \)), swim longer (WT = 98.5 ± 2 min versus SCD1-Tg = 135 ± 0 min; \( P < 0.0001 \)), and ran farther (WT = 2.0 ± 0.8 km/night versus SCD1-Tg = 5.4 ± 1.0 km/night; \( P = 0.01 \)) than their WT counterparts. During the swim test, WT mice failed to keep their heads above water after ~90 min, whereas the SCD1-Tg mice were removed from the water because all animals were still swimming after 2 h and 15 min with no signs of distress. Free-wheel running activity was not different between WT and Tg animals after four weeks of access (e.g., in the trained state); however, in the first seven days (e.g., untrained state), Tg mice preferentially ran farther than WT mice.

To conclusively define the exercise capacity of untrained WT and actin-SCD1-Tg mice, we subjected the animals to a maximal treadmill-running protocol. Each mouse was acclimated to the treadmill for four consecutive days with an active shock pad and increasing speed from 0 to 18 m/min for a total of 3 min. Three minutes per day is insufficient to induce training while still enabling acclimation, thereby keeping the animals in an untrained state. On the day 5, animals were left to run at 18 m/min until volitional exhaustion, defined as 10 continuous seconds of no longer attempting to get off the shock pad. Animals continuing to run after 90 min were removed from the treadmill by the experimenter. WT mice \( (n = 4) \) ran for an average of 6.6 ± 3 min, whereas the actin-SCD1-Tg mice \( (n = 5) \) ran for...
different between genotypes (Fig. 6C). However, 14C incorporation into CO2 was significantly higher in SCD1-Tg samples, confirming the effect of increased fatty acid oxidation with SCD1 overexpression (Fig. 6D).

Mitochondrial biogenesis is driven, at least in part, by increased PPARγ activity, and constitutively active PPARγ has been shown to significantly increase exercise performance and running time to exhaustion in mice (37, 38). In light of these facts, we examined the expression of PPARγ and two of its target genes, carnitine palmitoyl transferase-1b (Cpt-1b) and citrate synthase (Cit Syn). PPARγ is a transcriptional regulator that is highly expressed in skeletal muscle and controls the expression of several genes involved in fatty acid oxidation. Cpt-1b is the rate-limiting enzyme for fatty acid entry into the mitochondria and Cit Syn is the rate-limiting enzyme that controls oxaloacetate + pyruvate condensation and subsequent citrate entry into the TCA cycle. In actin-SCD1-Tg red gastroc fibers, PPARγ expression was increased 23.1 ± 7-fold (P = 0.02), Cpt-1b was increased 7.0 ± 2-fold (P = 0.01), and Cit Syn was increased 8.8 ± 2-fold (P = 0.01) versus WT tissue (Fig. 7A).

The PPARγ target Adfp was increased 2.9 ± 0.6-fold (P = 0.008) with no differences in UCP3 or ERRα between genotypes. Recently, Gan et al. (39) demonstrated the effect of PPARα versus PPARδ overexpression in muscle on exercise performance and glucose metabolism. A major difference between the two models was that PPARδ specifically induced LDHb expression and not LDHa, which increased lactate conversion into pyruvate. We tested the expression levels of LDHa and LDHb, and we found that LDHb was significantly increased in the SCD1-Tg animals (2.4 ± 0.4-fold; P = 0.007), with no change in LDHa (Fig. 7B).

Protein expression of Pgc-1 (pan) and PPARγ displayed

71.9 ± 9.5 min (P = 0.0009) (Fig. 5D). WT exercise times ranged from 2.3 to 16.9 min, and actin-SCD1-Tg times ranged from 38.2 to 90 min (Fig. 5E). The combined data from the four separate exercise tests clearly indicate that overexpression of SCD1 in the skeletal (and cardiac) muscle causes a significant increase in exercise tolerance in untrained animals.

At least part of the increased exercise capacity in actin-SCD1-Tg mice may be mediated by increased mitochondrial content of the skeletal muscle. Mitochondria were purified from red gastroc samples, and the total DNA content was determined spectrophotometrically and expressed relative to tissue weight. WT muscle contained significantly less mitochondrial DNA than Tg muscle (0.18 ± 0.09 ng/mg versus 0.44 ± 0.2 ng/mg; P = 0.04) (Fig. 6A). The purity of the mitochondrial fractions was confirmed in Fig. 6B using the mitochondrial DNA specific primer sets for cytochrome c oxidase subunit-1 (mtCo1), cytochrome b (mtCytB), and the genomic DNA primer set for β-actin. The PCR successfully amplified mtCo1 and mtCytB DNA with no amplification of β-actin, indicating that we had no contamination of genomic DNA in our mtDNA samples (with a β-actin positive control to demonstrate that the primer set works in the presence of genomic DNA).

The combined increase in mitochondrial content suggests that SCD1 overexpression in skeletal muscle may be sufficient to increase muscle fatty acid β-oxidation. To confirm this, we cultured skeletal muscle samples in HBSS and measured 14C stearate uptake ex vivo and the corresponding increase in 14CO2 formation produced as a result of labeled fatty acid β-oxidation. There was no difference in fatty acid uptake between WT and SCD1-Tg muscle samples, indicating that entry into the cells was not different between genotypes (Fig. 6C). However, 14C incorporation into CO2 was significantly higher in SCD1-Tg samples, confirming the effect of increased fatty acid oxidation with SCD1 overexpression (Fig. 6D).

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similar increases in SCD1-Tg animals (Fig. 7C). No differences between SCD1-Tg expression of PPARα (1.3 ± 0.4-fold; \( P = 0.4 \)) or PPARγ (1.4 ± 0.3-fold; \( P = 0.2 \)) versus WT were observed in muscle. We measured liver PPARδ, PPARα, and PPARγ expression, and we found a slight but nonsignificant reduction in expression for PPARδ SCD1-Tg = 0.8 ± 0.4, \( P = 0.3 \); PPARα SCD1-Tg = 0.7 ± 0.3, \( P = 0.3 \); PPARγ SCD1-Tg = 0.5 ± 0.2, \( P = 0.1 \) (fold change versus WT). While these results do not prove that PPARδ is solely responsible for the noted phenotypes, they do suggest that PPARα and PPARγ are not likely to be involved.

Finally, we wanted to determine the mechanism by which SCD1 overexpression in skeletal muscle produced enhanced fatty acid oxidation. The most likely explanation for the metabolic phenotype is that a particular fatty acid species is acting as a ligand for PPARδ. This is based on the understanding that i) PPARδ is the most abundant PPAR isoform in skeletal muscle (40); ii) it is known to be activated by several fatty acids (41, 42); and iii) overexpression of PPARδ in muscle was shown to increase metabolic function and exercise capacity in mice (37–39). Therefore, we examined the effect of the three major fatty acid species identified in Fig. 5C (i.e., 16:0, 18:1, and 18:2n6) on PPARδ and Cpt-1b expression in C2C12 myocytes. Fully differentiated cells were treated with 100 μM BSA conjugated fatty acid for 48 h. We saw that neither 16:0 nor 18:1 increased PPARδ expression, although 18:2n6 treatment resulted in a 2.3 ± 0.3-fold increase (\( P < 0.003 \)) (Fig. 8A). There was a small yet significant effect of 16:0 on Cpt-1b with no observed increase after 18:1 treatment, whereas 18:2n6 increased its expression 10.3 ± 2.4-fold (\( P < 0.0001 \)) (Fig. 8B). We tested the effects of the three fatty acids on fat oxidation in vitro to determine whether the changes in mRNA related to changes in the rate of oxidation. Cells were pretreated with BSA (control), 16:0, 18:1, or 18:2n6 for 48 h, then media was changed and all cells were treated with 50 μM (0.5 μCi) \(^3\)H-labeled 18:1 for 4 h. The rate of fatty acid β-oxidation was determined by measuring the amount of \(^3\)H2O formation. Neither 16:0 nor 18:1 increased β-oxidation significantly (\( P = 0.2 \) and 0.1, respectively); however, 18:2n6 increased \(^3\)H2O content nearly 3-fold (\( P = 0.004 \) versus BSA) (Fig. 8C).

Once we determined which fatty acid was responsible for increasing PPARδ and Cpt-1b, we then determined the effect of 18:2n6 on target gene expression during differentiation. We saw that cells treated with 18:2n6 on days 4–10 or on days 8–10 displayed an equal increase in PPARδ over standard treatment (~3-fold increase) (Fig. 8D). Cpt-1b, on the other hand, increased from 5.9 ± 1.4-fold on days 4–10 to 12.0 ± 4.0-fold on days 8–10 treatment (Fig. 8E). The changes in mRNA were matched by changes in PPARδ and Pgc-1 protein levels from cells that were treated with 18:2n6 on days 4–10 or on days 8–10 (Fig. 8F). Further studies will need to examine the effect of 18:2n6 on skeletal muscle differentiation; however, these data suggest that if the effect occurs in mature myocytes, then supplementation with linoleic acid may provide a means by which dietary lipid could increase metabolic and aerobic function in skeletal muscle.

Fig. 6. Mitochondrial content and fat oxidation are increased in SCD1-Tg mice. Tg mice have significantly more muscle mitochondria than WT (\( P = 0.04 \)) (A). To confirm the purity of our mitochondrial DNA (mtDNA) prep, representative samples were used to amplify mtDNA specific genes (Co1 and CytB) with no amplification of genomic DNA (B). There was no difference in fatty acid uptake into muscle samples (C); however, \(^1^4\)C incorporation into \(^1^4\)CO₂ (produced as a result of \(^1^4\)C-labeled fatty acid β-oxidation) was significantly higher in SCD1-Tg samples (D). (WT n = 4, Tg n = 5; \(* P < 0.05 \) between WT and SCD1-Tg).

Fig. 7. PPARδ expression is increased in skeletal muscle in vivo. PPARδ was 23.1 ± 7-fold higher (\( P = 0.02 \)), Cpt-1b was 7.0 ± 2-fold higher (\( P = 0.01 \)), and Citrate synthase was 8.8 ± 2-fold higher in SCD1-Tg than WT (\( P = 0.01 \)) (A). Adfp and LDHβ were increased in the muscle of SCD1-Tg mice (B). Western blot analysis of Pgc-1 and PPARδ in three representative WT and SCD1-Tg mice, with β-actin as loading control (C). (n = 5 animals per group; \(* P < 0.05 \) between WT and SCD1-Tg).
increasing the rate and efficiency of the reaction (23). The results of this model suggest that increased SCD1 activity in skeletal muscle increases glucose uptake, fatty acid oxidation, and exercise capacity. The mechanisms by which these increases are achieved are unlike those of the SKO models in which loss of SCD1 in the skin creates a hypermetabolic phenotype. Given the dramatic increases of PUFA content in the skeletal muscle of SCD1-Tg mice, we suspect the primary mechanism for the observed improved metabolic function in these animals is the result of increased availability of potential ligands for the transcription factor PPARγ and its subsequent activation.

Rather than simply increasing total energy expenditure, we suspect that the increased rate of MUFA production enables the sparing of n3 and n6 PUFAs. Several long-chain PUFAs, including linoleic acid (18:2n6), are known ligands of the PPARγ and have been shown to increase its transcriptional activity (48, 49). In our C2C12 cell culture model, we were able to demonstrate the effect of 18:2n6 treatment on PPARγ and Cpt-1b expression. While there are a number of n3, n6, and even n9 PUFAs that are elevated in our model, we felt that 18:2n6 was the most likely candidate based on the absolute content compared with the other PUFAs in the TG fraction (18:2n6/1160120% versus 18:3n3/116010.2%, etc.). However, we also recognize that despite their 100-fold lower concentration, some of the minor PUFA species may have much more potent bioactive effects than 18:2n6. Thus, it would be necessary to examine the effect of individual fatty acid species on regulating metabolic function through PPARγ. To achieve this, studies will need to be conducted in which specific PUFAs are removed from the diet to determine which species is responsible for the phenotype. Additionally, actin-SCD1-Tg mice on a muscle-specific PPARγ-null background should be used to determine whether it mediates the increased metabolic

**DISCUSSION**

Over the last 10–15 years, the majority of research regarding SCD1 has focused either on in vivo KO models or in vitro models with suppression of desaturase activity. In KO models, there is a clear and consistent prevention of diet-induced obesity (12, 13, 17, 43–45), yet investigators could not explain these results in light of the fact that insulin sensitivity was increased with a concomitant impairment in TG and/or MUFA synthesis. These paradoxical results have been largely ignored due to the overwhelming success of the SCD1-KO models in preventing obesity. However, recent work has shown clearly that despite its ability to prevent obesity, loss of SCD1 is associated with an increased risk for atherogenesis in SCD1−/− × LDLR−/− double-knockout animals (46, 47). Furthermore, our previous work looking at skin-specific deletion of SCD1 (SKO) (18) indicates that increased energy expenditure accounts for the anti-obesity effect and that increased β-oxidation likely prevents accumulation of toxic DAG and SFA. In other words, the detrimental effect of increased DAG and SFA is masked by increased energy expenditure from the skin defect in SKO or global KO animals. Therefore, only when SCD1 is removed from tissues other than skin can we see its detrimental effects on glucose metabolism or fatty acid uptake (13, 22).

However, we have developed a model to increase MUFA synthesis specifically in skeletal muscle. It is quite likely that increasing the activity of SCD1 increases the rate of IMTG synthesis by increasing MUFA content of muscle, which increases the rate of DGAT activity. Increasing SCD1 activity promotes MUFA synthesis, which is invariably incorporated into TG and appears to have a sparing effect on PUFA (18). Increasing SCD1 activity also provides a better substrate for DGAT-mediated TG synthesis, thereby increasing the rate and efficiency of the reaction (23). The results of this model suggest that increased SCD1 activity in skeletal muscle increases glucose uptake, fatty acid oxidation, and exercise capacity. The mechanisms by which these increases are achieved are unlike those of the SKO models in which loss of SCD1 in the skin creates a hypermetabolic phenotype. Given the dramatic increases of PUFA content in the skeletal muscle of SCD1-Tg mice, we suspect the primary mechanism for the observed improved metabolic function in these animals is the result of increased availability of potential ligands for the transcription factor PPARγ and its subsequent activation.

Rather than simply increasing total energy expenditure, we suspect that the increased rate of MUFA production enables the sparing of n3 and n6 PUFAs. Several long-chain PUFAs, including linoleic acid (18:2n6), are known ligands of the PPARγ and have been shown to increase its transcriptional activity (48, 49). In our C2C12 cell culture model, we were able to demonstrate the effect of 18:2n6 treatment on PPARγ and Cpt-1b expression. While there are a number of n3, n6, and even n9 PUFAs that are elevated in our model, we felt that 18:2n6 was the most likely candidate based on the absolute content compared with the other PUFAs in the TG fraction (18:2n6 ~20% versus 18:3n3 ~0.2%, etc.). However, we also recognize that despite their ~100-fold lower concentration, some of the minor PUFA species may have much more potent bioactive effects than 18:2n6. Thus, it would be necessary to examine the effect of individual fatty acid species on regulating metabolic function through PPARγ. To achieve this, studies will need to be conducted in which specific PUFAs are removed from the diet to determine which species is responsible for the phenotype. Additionally, actin-SCD1-Tg mice on a muscle-specific PPARγ-null background should be used to determine whether it mediates the increased metabolic
effects. Lastly, while we suspect that the increased MUFA production observed in actin-SCD1-Tg mice results in increased PUFA sparing, the specific mechanism by which PUFAs accumulate in the skeletal muscle of these mice remains unknown and is a subject of further research.

Previous work has shown that overexpression of SCD1 in primary myocytes derived from lean humans suppressed fat oxidation (50). This is in direct contrast to our results, however, one major difference is that we saw an increase in PUFA content of muscle. It is somewhat surprising that muscle TG would contain significantly more PUFA when MUFA synthesis is increased. Although we only assessed TG fatty acid composition, it is expected that all neutral lipids in these samples would demonstrate a similar increase in PUFA content. Lastly, the WT and SCD1-Tg animals consumed the same diet, leading us to conclude that the increase in TG PUFAs is due to “sparring” of dietary sources.

We examined the role of SFA, MUFA, and PUFA on PPARα and Cpt-1b expression in vitro and found that PUFA causes a significant increase in their expression. In the mouse model, we expect this effect to be exponentially more pronounced due to the fact that PUFA sparing is likely occurring early in the development and differentiation of skeletal muscle cells in utero. It would be interesting to see what effect increased MUFA synthesis has on skeletal muscle differentiation and fiber type composition and whether these effects can be achieved simply by supplementing with PUFA in the diet. More importantly, our in vitro results suggest that supplementing cells with 18:2n6 may be sufficient to increase PPARα expression and activity. If this can be achieved in humans, it may provide a means by which dietary supplementation could increase cardiovascular and metabolic health. Subsequent investigations should be performed that examine the effects of dietary PUFA supplementation on skeletal muscle differentiation, metabolic function, and exercise capacity both in animal and human models.

Another aspect of increased skeletal muscle SCD1 activity is an increased exercise tolerance and capacity. Assessments of grip strength, swim time to exhaustion, voluntary free-wheel running, and forced treadmill running all indicate that overexpression of SCD1 dramatically increases cardiovascular endurance. It was reported that DGAT1-Tg animals had decreased 24 h activity and lower energy expenditure with no change in PPARα expression (51), whereas our model indicates that PPARα expression is increased >20-fold. We suspect that increased oleate synthesis is causing PUFA sparing, which leads to enhanced PPARα activity, while DGAT1 activity would simply be passive in its role toward fatty acid composition. Narkar and colleagues developed a constitutively active PPARα transgenic model, and they found that as a result, type I oxidative fiber content of muscle was increased (38). This enabled the animals to withstand a greater workload of activity both before training and after treadmill exercise training. The authors also noted that SCD1 expression (which is a PPARα target gene) was elevated in the VP16-PPARα model, and we suspect that a similar phenomenon exists with actin-SCD1 Tg animals wherein elevated PUFA levels are constitutively increasing PPARα activity. The fact that SCD1-Tg animals have a higher capacity to perform exercise poses the question of what their basal, nonexercise activity is. While it was not assessed in the current investigation, if these animals exhibited higher nonexercise activity, it could account for the observed reductions in body weight and fasting glucose.

The present data suggest that overexpression of SCD1, at least in muscle, provides a far greater benefit on metabolic function than does suppressing its activity. In fact, we hope these data will provide a paradigm shift that will re-examine the notion that suppressing either SCD1 or DGAT activity by pharmacological means may not provide the most ideal phenotype. Instead, nutritional and/or lifestyle interventions aimed at increasing muscle SCD1 activity and PUFA content should be strongly considered. If enhanced SCD1 activity in skeletal muscle is sufficient to decrease fasting glucose, while increasing fat oxidation and exercise capacity, then means to suppress its activity should be avoided or at least used with caution. Instead, it may prove to be more efficacious to use dietary supplementation of PUFA either alone or in combination with aerobic exercise training to increase overall metabolic health.

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