Loss of stearoyl–CoA desaturase-1 function protects mice against adiposity

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Stearoyl–CoA desaturase (SCD) is a central lipogenic enzyme catalyzing the synthesis of monounsaturated fatty acids, mainly oleate (C18:1) and palmitoleate (C16:1), which are components of membrane phospholipids, triglycerides, wax esters, and cholesterol esters. Several SCD isoforms (*SCD1-3*) exist in the mouse. Here we show that mice with a targeted disruption of the *SCD1* isoform have reduced body adiposity, increased insulin sensitivity, and are resistant to diet-induced weight gain. The protection from obesity involves increased energy expenditure and increased oxygen consumption. Compared with the wild-type mice the *SCD1*−/− mice have increased levels of plasma ketone bodies but reduced levels of plasma insulin and leptin. In the *SCD1*−/− mice, the expression of several genes of lipid oxidation are up-regulated, whereas lipid synthesis genes are down-regulated. These observations suggest that a consequence of *SCD1* deficiency is an activation of lipid oxidation in addition to reduced triglyceride synthesis and storage.

Stearoyl–CoA desaturase (SCD) is the rate-limiting enzyme in the biosynthesis of monounsaturated fatty acids. It catalyzes the introduction of the *cis* double bond in the Δ9 position of fatty acyl–CoA substrates. The preferred desaturation substrates are palmitoyl–CoA and stearoyl–CoA, which are converted to palmitoleoyl–CoA (16:1) and oleoyl–CoA (18:1), respectively (1–4). These fatty acids are requisite components of membrane phospholipids, triglycerides, cholesterol esters, and wax esters (5–7). Effects on composition of phospholipids ultimately determine membrane fluidity, and the effects on the composition of cholesterol esters and triglycerides can affect lipoprotein metabolism and adiposity. SCD expression is sensitive to dietary factors including polyunsaturated fatty acids, cholesterol and vitamin A, hormonal changes (i.e., insulin and glucagon), developmental processes, temperature changes, thiazolinediones, metals, alcohol, peroxisomal proliferators, and phenolic compounds (3). High SCD activity has been implicated in a wide range of disorders including diabetes, atherosclerosis, cancer, obesity, and viral infection (3, 8–13).

The existence of multiple SCD isoforms in mice (6, 14–18) and rats makes it difficult to determine the role of each isoform in lipid metabolism. New insights into the physiological role of the *SCD1* gene and its endogenous products came from recent studies of the asebia mouse strains (ab^{j} and ab^{2j}) that have naturally occurring mutations in *SCD1* (17–19) as well as a laboratory mouse model with a targeted disruption (*SCD1*−/−) (6). We used these animal models to show that *SCD1*−/− mice are deficient in hepatic triglycerides and cholesterol esters (7, 20). The levels of palmitoleate (16:1) and oleate (18:1) are reduced, whereas palmitate and stearate are increased in the lipid fractions of *SCD1*−/− mice. On a high carbohydrate diet supplemented with triolein, the cholesterol ester levels are corrected but the triglyceride levels are not reversed to the levels found in the wild-type mouse (7).

Apart from the dramatic alterations in triglyceride and cholesterol metabolism, the *SCD1*−/− mice are considerably leaner than their wild-type counterparts. Here, we show changes in metabolic rate and in the expression of genes encoding enzymes involved in lipid metabolism.

Methods

Animals and Diets. *SCD1*−/− mice in SV129 background were generated and genotyped as described (5). The wild-type (*SCD1*+/+), heterozygous (*SCD1*+/−) and homozygous (*SCD1*−/−) mice are housed and bred in a pathogen-free barrier facility of the Department of Biochemistry (Univ. of Wisconsin, Madison) operating at room temperature in a 12-h light/12-h dark cycle. The breeding of these animals was in accordance with the protocols approved by the animal care research committee of the Univ. of Wisconsin. At 3 weeks of age, the mice were fed *ad libitum* a standard laboratory chow diet or a high-fat diet for 23 weeks. The high-fat diet contains 195 g/kg casein, 3 g/kg DL-methionine, 377 g/kg sucrose, 150 g/kg corn starch, 153 g/kg anhydrous milkfat, 10 g/kg corn oil, 1.5 g/kg cholesterol, 60.067 g/kg cellulose, 35 g/kg mineral mix AIN-76 (170915), 4 g/kg calcium carbonate, 10 g/kg vitamin mix Teklad (40060), 1.2 g/kg choline bitartrate, and 0.033 g/kg ethoxyquin (antioxidant).The weight of each mouse within each group was measured weekly; the data are presented as means ± SD ($n = 8$, $P < 0.001$). The glucose tolerance and insulin tolerance were determined as described (21).

Measurement of Oxygen Consumption. Gender matched *SCD1*−/− and wild-type littermates were investigated in indirect calorimeters as described (22). Oxygen consumption rate (VO_2) and CO_2 production rate (VCO_2) were continuously assayed over 4 consecutive 23-h periods, including 12 h dark (1800–0600) and 11 h light (0600–1700).

Gene Expression Analysis. RNA was isolated from livers of 10 individual 6-week-old female mice by using a standard method (23). Mouse genome U74A arrays were used to monitor the expression level of approximately 12,000 genes and expressed sequence tags (Affymetrix). Genes differentially expressed were identified by comparing expression levels in *SCD1*−/− and wild-type mice (24, 25). For Northern blot analysis, 20 μg of total liver RNA was separated on an 0.8% agarose/formaldehyde gel, transferred onto nylon membrane, and hybridized with cDNA probes for the corresponding genes.

Results

Reduced Body Weight in *SCD1*−/− Mice Fed a High-Fat Diet. Although the growth curves of male *SCD1*−/− mice were similar to those of wild-type siblings on chow diet, a high-fat diet revealed large differences in weight gain in both males (34.2 g vs. 39.5 g, $P < 0.01$, Fig. 1) and females (27.7 g vs. 31.9 g, $P < 0.05$).

Abbreviation: SCD, stearoyl–CoA desaturase.

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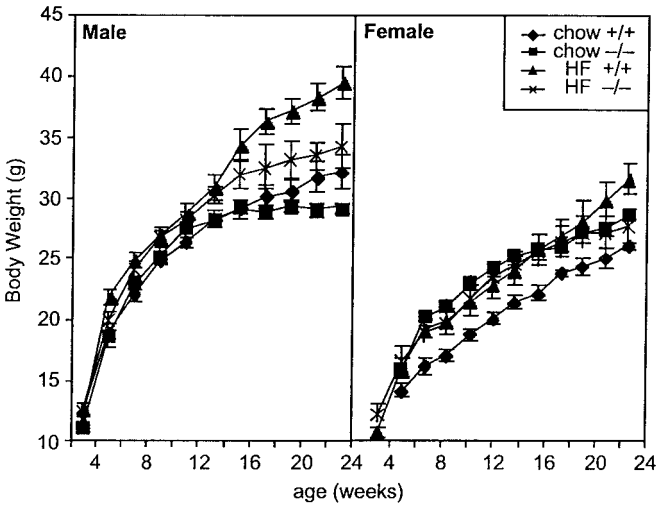

Fig. 1. Body weight of male and female wild-type and *SCD1*−/− mice fed a chow or high-fat diet.

Reduced Body Fat Mass in *SCD1*−/− Mice. On average, the *SCD1*−/− mice consumed 25% more food than wild-type mice (4.1 g/day vs. 5.6 g/day; $n = 9, P < 0.05$). Nonetheless, they were leaner and accumulated less fat in their adipose tissue (Fig. 2*A*). The epididymal fat pad mass was markedly reduced in male *SCD1*−/− relative to wild-type mice fed a chow diet (0.4 ± 0.1 mg vs. 0.8 ± 0.2; $n = 9, P < 0.05$; Fig. 2*B*) and a high-fat diet (1.0 ± 0.2 mg vs. 1.6 ± 0.2, $n = 12, P < 0.05$; Fig. 2*C*). The livers of the wild-type and *SCD1*−/− mice were grossly normal and of similar mass. In contrast, on a high-fat diet, the livers of the wild-type mice were lighter in color than those of the mutant mice (Fig. 2*C*), suggestive of hepatic steatosis. The masses of white adipose depots in *SCD1*−/− mice were globally reduced in mice on either the chow or the high-fat diet (Fig. 2*D*). The masses of other tissues, including brown adipose tissue, were not significantly altered. Thus, *SCD1*−/− mice were resistant to diet-induced weight gain and fat accumulation, despite increased food intake.

Increased Oxygen Consumption in *SCD1*−/− Mice. We carried out indirect calorimetry to investigate whether the resistance to weight gain is caused by increased energy expenditure. The *SCD1*−/− mice exhibited consistently higher rates of oxygen consumption (had higher metabolic rates) than their wild-type littermates throughout the day and night (Fig. 3*A*). After adjusting for allometric scaling and gender, the effect of the knockout allele was highly significant ($P = 0.00019$, multiple ANOVA, Fig. 3*B*).

Because the increase in O_2 consumption occurred during the fasting phase (daytime) as well as during the feeding phase, the animals are more active in oxidizing fat. Although ketone bodies were undetectable in plasma from either strain during postprandial conditions, β-hydroxybutyrate levels were much higher in

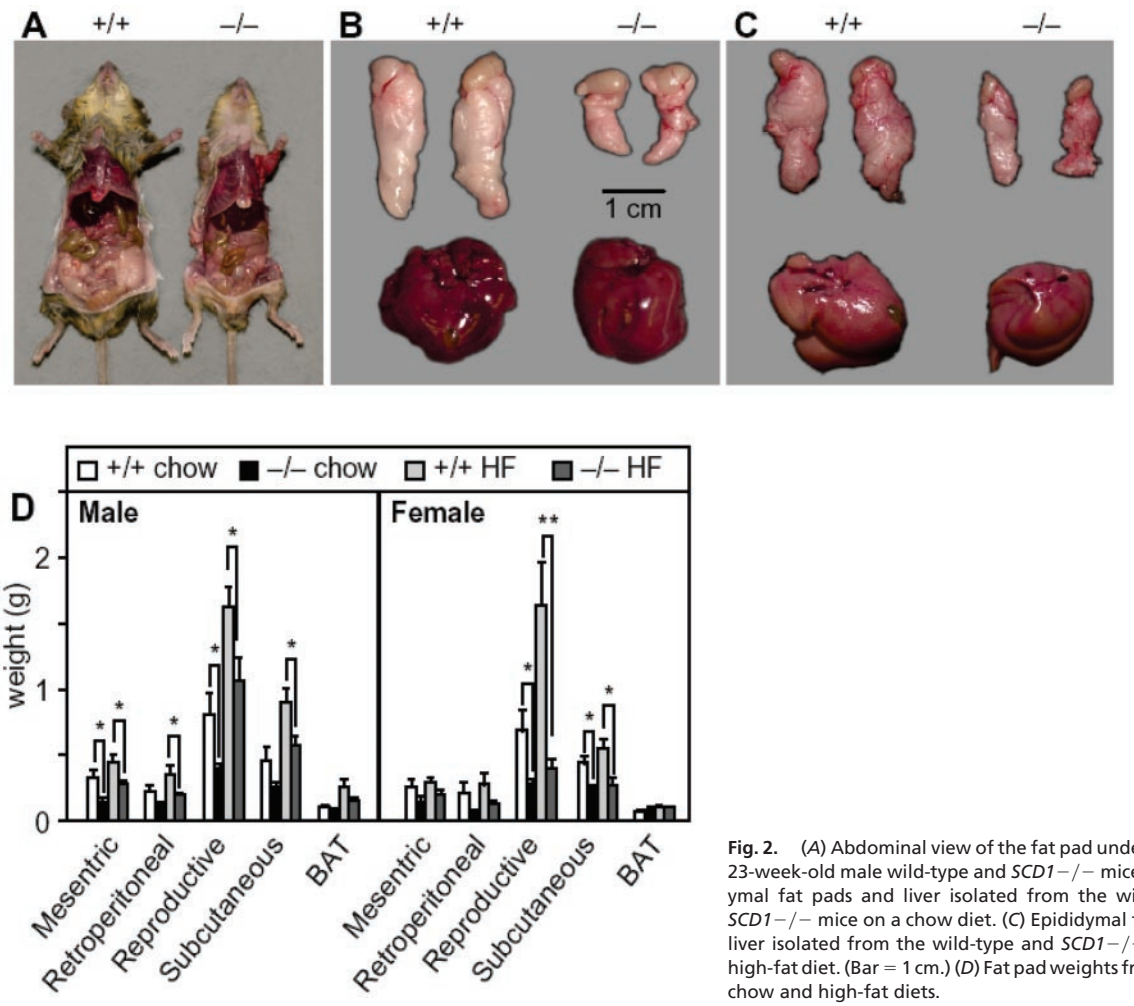

Fig. 2. (*A*) Abdominal view of the fat pad under the skin in 23-week-old male wild-type and *SCD1*−/− mice. (*B*) Epididymal fat pads and liver isolated from the wild-type and *SCD1*−/− mice on a chow diet. (*C*) Epididymal fat pads and liver isolated from the wild-type and *SCD1*−/− mice on a high-fat diet. (Bar = 1 cm.) (*D*) Fat pad weights from mice fed chow and high-fat diets.

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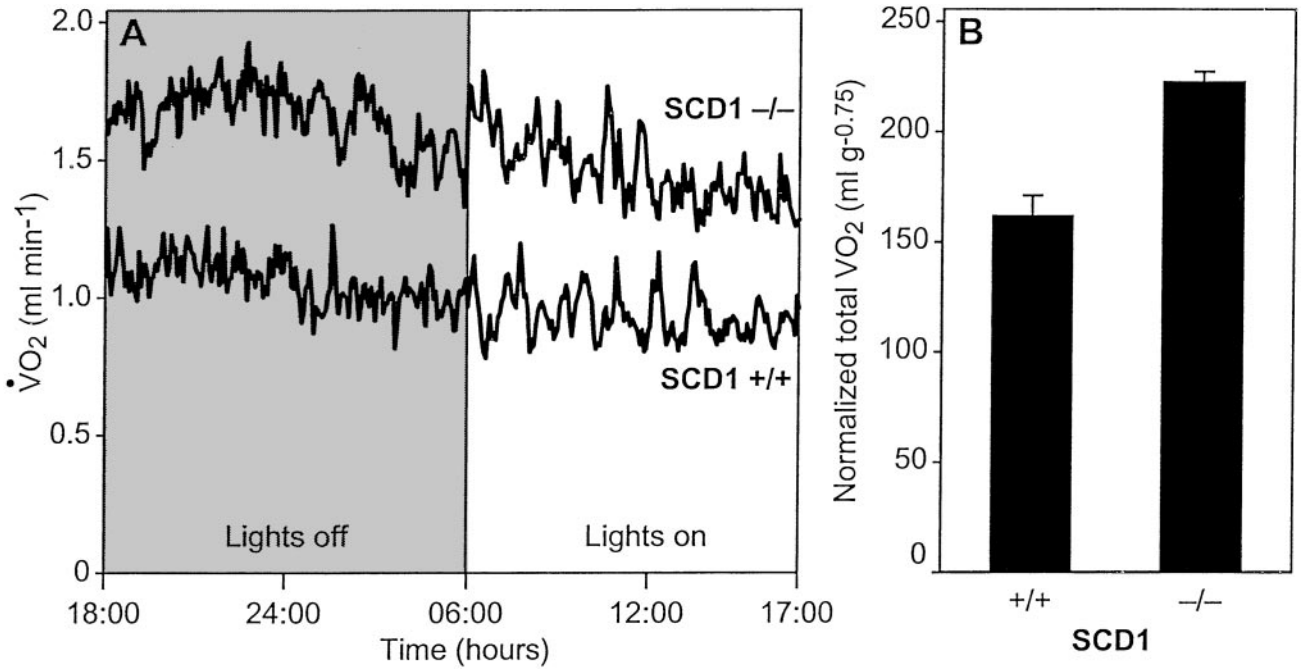

Fig. 3. (*A*) Metabolic rate and oxygen consumption of male mice on a chow diet. (*B*) Gender-adjusted, normalized total oxygen consumption over a 23-h period. Error bars denote SE.

the *SCD1*−/− mice after a 4-h fast (4.4 ± 0.6 mg/dl vs. 1.1 ± 0.7 mg/dl; $P < 0.001$), indicating a higher rate of β-oxidation in knockout mice. A similar but less dramatic difference was seen in females. These differences were also observed in mice on high-fat diet.

Increased Expression of Genes Involved in Fatty Acid Oxidation in *SCD1*−/− Mice. We used DNA microarrays to identify genes whose expression was altered in the livers of *SCD1*−/− mice. We identified 200 mRNAs that were significantly different between the livers of *SCD1*−/− and wild-type mice. The most striking pattern was seen in genes involved in lipogenesis and fatty acid β-oxidation. Lipid oxidation genes were up-regulated, whereas lipid synthesis genes were down-regulated in the *SCD1*−/− mice (Fig. 4*A*). Using the same RNA samples, the microarray data were verified with quantitative reverse-transcription–PCR using DNA primers that were designed for selected genes that showed differential expression (26). The results showed that the *PPARα*-target gene Fasting-Induced Adipocyte Factor (*FIAF*) was up-regulated in *SCD1*−/− mice ($P < 0.05$; Fig. 4*B*), whereas fatty acid synthase (*FAS*) was down-regulated ($P < 0.01$).

Northern blot analysis also supports changes in fatty acid oxidation and lipid biosynthesis. Probes for acyl–CoA oxidase (*ACO*), very long chain acyl–CoA dehydrogenase (*VLCAD*), and carnitine palmitoyltransferase-1 (*CPT-1*) indicate increases in β-oxidation (27, 28), whereas probes for *SREBP-1*, *FAS*, and glycerol phosphate acyl–CoA transferase (*GPAT*) point to a decrease in triglyceride biosynthesis (Fig. 4*C*).

Increased Insulin Sensitivity in *SCD1*−/− Mice. Reduced adipose tissue mass could either elicit insulin resistance or insulin sensitivity as demonstrated in several animal models (28). Fasting insulin levels were lower in the male *SCD1*−/− on chow diet (1.3 ± 0.3 ng/dl; $n = 7$) compared with wild-type mice (2.5 ± 0.9 ng/ml; $n = 7$). On a high-fat diet, insulin levels were similar between the two groups. Fasting glucose levels were similar between the *SCD1*−/− and wild-type mice. However, male and female *SCD1*−/− mice showed improved glucose tolerance compared with wild type (Fig. 5, $P < 0.05$). Thirty minutes after a glucose load, both male and female *SCD1*−/− mice tended to have lower fasting glucose levels (males: wild type, 345 ± 44 mg/dl; *SCD1*−/− mice, 202 ± 20, $n = 8$; females: wild type, 209 ± 20; *SCD1*−/− mice, 141 ± 9, $n = 5$). In addition, the glucose lowering effect of insulin was greater in the *SCD1*−/− mice than wild-type mice (data not shown). These data indicate that *SCD1*−/− mice have increased insulin sensitivity along with their loss of adiposity.

Discussion

These studies establish a critical role for SCD in the generation of body fat. The deletion of the SCD1 gene resulted in global changes in gene expression and altered metabolic activity that can account for the loss of body fat.

Genes encoding enzymes that participate in fatty acid oxidation were up-regulated in the *SCD1*−/−mice. *CPT*-1, *ACO*, *VLCAD*, and *FIAF* are known targets of *PPARα* (27, 28) and contain *PPARα* response regions in their promoters (28). Because *PPARα* mRNA is unchanged (data not shown), the up-regulation of enzymes of fatty acid β-oxidation in the *SCD1*−/− mice must be downstream of *PPARα* transcription. Thus, it is possible that loss of SCD1 function results in an increase in the concentration of a *PPARα* activator, perhaps a lipid ligand. The contents of saturated fatty acids (C16:0 and C18:0) are increased, whereas the contents of the polyunsaturated fatty acids of the *n*-6 and *n*-3 are not changed in the liver of the *SCD1*−/− mice (8, 10). One possible mechanism for our observations is that the saturated fatty acids induce the signal that activates the *PPARα* in the *SCD1*−/− mice, but this has yet to be determined. Alternatively the increased levels of C18:0- or C16:0-CoAs could inhibit acetyl–CoA carboxylase (*ACC*) through a well-known feedback mechanism; the resulting drop in malonyl–CoA can derepress *CPT-1*, resulting in increased transport of fatty acids into the mitochondria. Thus, the mechanism of increased lipid oxidation in the *SCD1*-deficient mouse could

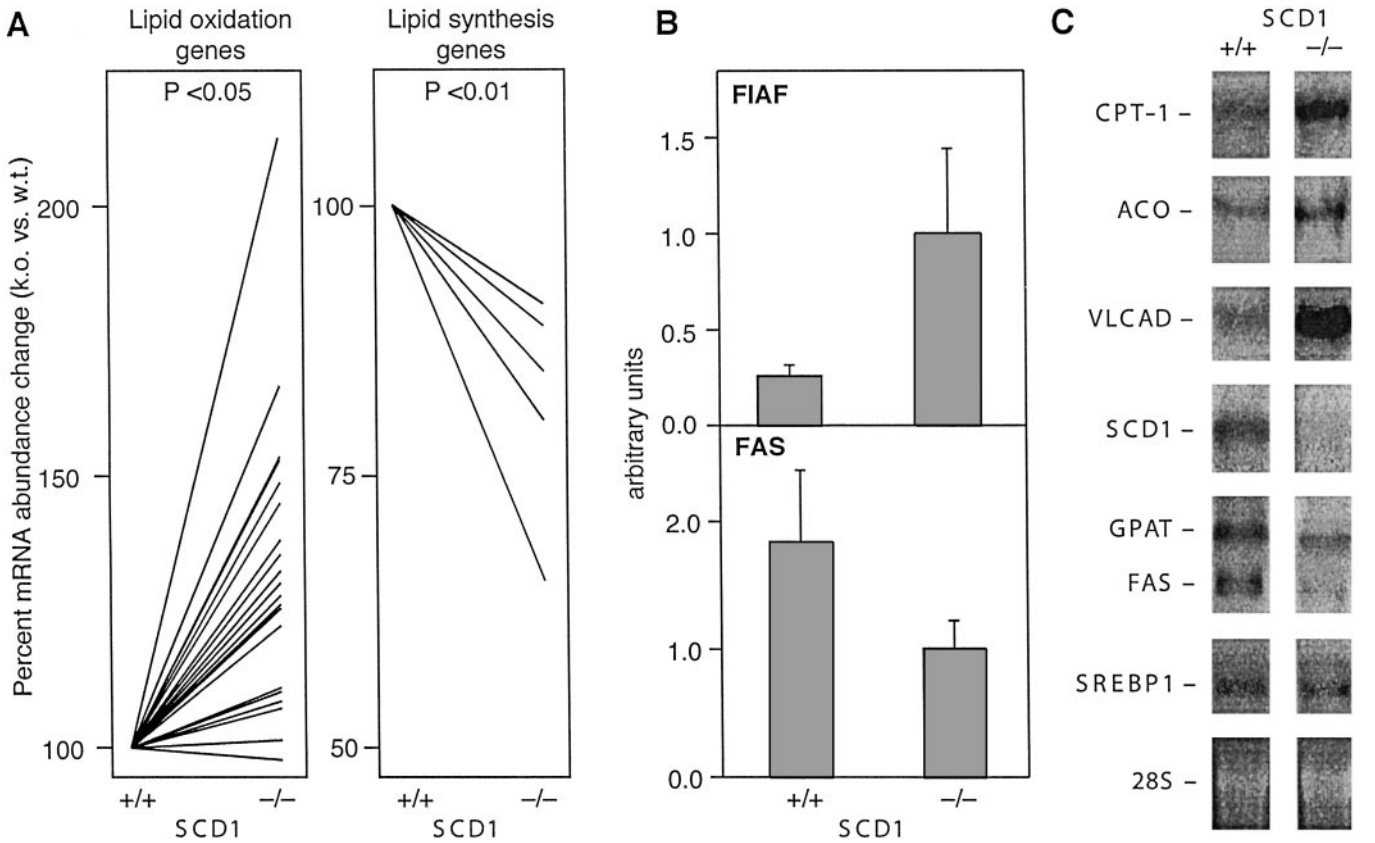

Fig. 4. (*A*) Expression levels of lipid oxidation (*Left*) and lipid synthesis (*Right*) genes between wild-type and *SCD1*−/− mice. (*B*) Quantitative reverse-transcription–PCR of *FIAF* and *FAS* gene expression, relative to wild-type mice. 18S RNA was used as a normalization control. (*C*) Northern blot analysis of lipid oxidation genes and lipid synthesis genes (*SREBP-1*, *FAS*, and *GPAT*) in the wild-type and *SCD1*−/− mice.

be caused by induction of *PPARα*-target genes as well enhanced availability of fatty acids for mitochondrial β-oxidation.

The *SCD1*−/− mice showed decreased expression in the liver of lipogenic genes *SREBP-1*, *FAS*, and *GPAT* (Fig. 4*C*). *SREBP-1c* is the main *SREBP-1* isoform expressed in the liver and regulates the expression of lipogenic genes (29). Insulin, dietary carbohydrate, fatty acids, and cholesterol regulate *SREBP-1* gene expression and protein maturation (29, 30). Thus, the down-regulation of *SREBP-1* gene expression in the *SCD1*−/− mice could have numerous effects on various metabolic pathways regulated by *SREBP-1*. For instance the induction of *SREBP-1* by insulin and cholesterol greatly enhances the synthesis and secretion of triglycerides by the liver (31). However, in the *SCD1* knockout mice, carbohydrate feeding fails to induce triglyceride synthesis and secretion by the liver (7, 20). In addition, the *SCD1* deficiency attenuates triglyceride synthesis and very low density lipoprotein secretion in the *ob*/*ob* mouse (32), implying that *SCD1* represents a crucial bottleneck in triglyceride synthesis in the mouse.

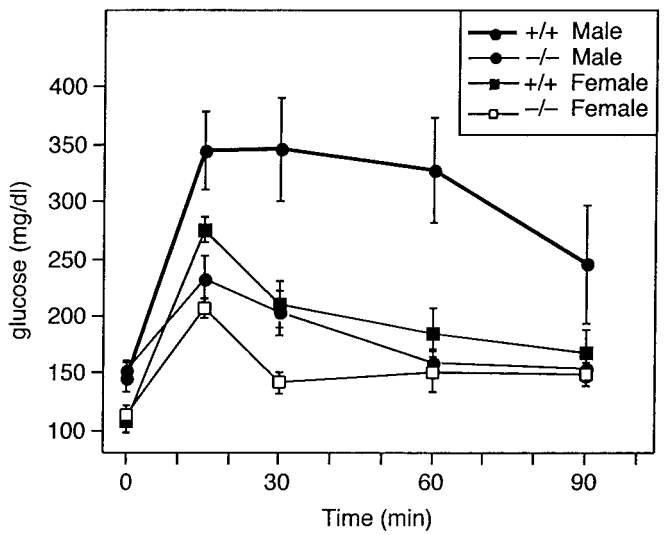

Fig. 5. Plasma glucose levels during the glucose tolerance test of male and female wild-type and *SCD1*−/− mice.

In contrast to human subjects and several mouse models of lipodystrophy (33–36), the loss of adiposity in the *SCD1*−/− mice led to increased rather than decreased insulin sensitivity. In lipodystrophy, there is a redistribution in the lipogenic burden away from adipose tissue, leading to triglyceride accumulation in the liver and in skeletal muscle. Skeletal muscle triglyceride levels have recently been shown to strongly correlate with impaired insulin-stimulated glucose disposal. The reduction in muscle triglyceride content (M. Rahman, M.M., and J.M.N., unpublished data) in the *SCD1*−/− mice may contribute to increased insulin sensitivity observed in these mice.

Lipodystrophic *Crebbp* heterozygous null mice (37) have increased energy expenditure and unlike other lipodystrophic mouse models, increased insulin sensitivity. This has been attributed to increased plasma leptin levels. We measured plasma leptin to determine whether changes in levels of plasma leptin could account for the protection from weight gain, increased

energy expenditure and insulin sensitivity in the *SCD1*−/− mice. Plasma leptin was significantly reduced in the *SCD1*−/− mice relative to the wild-type controls (on chow diet: males, 5.0 ± 0.5 vs. 25.3 ± 5.5 ng/ml, $P < 0.01$; females, 5.1 ± 0.9 ng/ml vs. 11.1 ± 1.2 ng/ml, $P < 0.001$). A similar large difference was observed in mice on high-fat diet. Plasma leptin remained lower in *SCD1*−/− mice even after correcting for reduced fat mass. Thus, the *SCD1*−/− mouse does not resemble the *Crebbp*+/− mouse, because the protection from adiposity is present despite lower leptin levels. These data suggest that *SCD1* acts downstream of leptin, and predict that loss of SCD function would ameliorate the severe obesity observed in leptin-deficient *ob*/*ob* mice. Indeed, double mutant *asebia ob*/*ob* mice weighed significantly less than C57BL/6–*ob*/*ob* mice (32).

In conclusion, our studies have revealed that *SCD1* gene deficiency leads to resistance to diet-induced obesity, increased insulin sensitivity, and increased metabolic rate. Because leptin represses the expression of the *SCD1* gene and the *SCD1* deficiency normalizes the hypometabolic phenotype of the *ob*/*ob* mice (32), our results are consistent with *SCD1* being a target of leptin signaling, as suggested by the gene array studies of Soukas *et al.* (38) and confirmed by Cohen *et al.* (32). In addition, the expression of *PPARα* target genes of lipid oxidation were up-regulated in mouse liver of *SCD1*−/− mice, whereas those of *SREBP-1* target genes of lipid synthesis were down-regulated. The studies suggest that *SCD1* deficiency either directly or indirectly induces a signal that activates the *PPARα* pathway to partition fat toward oxidation and down-regulates *SREBP-1* expression thereby reducing lipid synthesis and storage. These metabolic changes recommend SCD as a promising therapeutic target for the many disorders associated with the metabolic syndrome.

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