Combined deletion of SCD1 from adipose tissue and liver does not protect mice from obesity

Matthew T. Flowers,* Lacombouh Ade,* Maggie S. Strable,† and James M. Ntambi1,*†
Departments of Biochemistry* and Nutritional Sciences,† University of Wisconsin-Madison, Madison, WI 53706

Abstract  Stearoyl-CoA desaturase 1 (SCD1) catalyzes the synthesis of monounsaturated fatty acids (MUFA) from saturated FA. Mice with whole-body or skin-specific deletion of SCD1 are resistant to obesity. Here, we show that mice lacking SCD1 in adipose and/or liver are not protected from either genetic- (agouti; A/y/a) or diet-induced obesity (DIO) despite a robust reduction in SCD1 MUFA products in both subcutaneous and epididymal white adipose tissue. Adipose SCD1 deletion had no effect on glucose or insulin tolerance or on hepatic triglyceride (TG) accumulation. Interestingly, lack of SCD1 from liver lowered the MUFA levels of adipose tissue and vice versa, as reflected by the changes in FA composition. Simultaneous deletion of SCD1 from liver and adipose resulted in a synergistic lowering of tissue MUFA composition. Adipose SCD1 deletion had no effect on glucose or insulin tolerance or on hepatic triglyceride (TG) accumulation. Interestingly, lack of SCD1 from liver lowered the MUFA levels of adipose tissue and vice versa, as reflected by the changes in FA composition. Simultaneous deletion of SCD1 from liver and adipose resulted in a synergistic lowering of tissue MUFA composition. Adipose SCD1 deletion had no effect on glucose or insulin tolerance or on hepatic triglyceride (TG) accumulation.

The current study suggests that SCD1 deletion from adipose and/or liver is insufficient to elicit protection from obesity, but it supports the existence of extensive lipid cross-talk between liver and adipose tissue. — Flowers, M. T., L. Ade, M. S. Strable, and J. M. Ntambi. Combined deletion of SCD1 from adipose tissue and liver does not protect mice from obesity. J. Lipid Res. 2012. 53: 1646–1653.

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Stearoyl-CoA desaturase 1 (SCD1) catalyzes the desaturation of saturated fatty acids (FA), primarily palmitate (16:0) and stearate (18:0), into the cis-monounsaturated fatty acid (MUFA) products palmitoleate (16:1n7) and oleate (18:1n9), respectively. The metabolic role for SCD1 has been extensively explored in mice with naturally occurring Scd1 mutations and those with global deletion of Scd1 (GKO mice) (1–3). These mice display a remarkable hypermetabolic phenotype that protects them from obesity, insulin resistance, and hepatic steatosis. To determine which tissue or tissues are primarily responsible for these metabolic changes, we have employed the Cre-lox system to explore the tissue-specific contributions of SCD1.

We previously found that mice with a liver-specific deletion of Scd1 (LKO mice) are protected from high-carbohydrate, but not high-fat, diet-induced obesity (DIO), unlike GKO mice that are resistant to both high-fat and high-carbohydrate DIO (4). This indicates that inhibition of liver SCD1 alone is insufficient to elicit the hypermetabolism and increased energy expenditure necessary to compensate for the increased energy intake associated with high-fat feeding. The reduced high-carbohydrate diet-induced adiposity in LKO and GKO mice was associated with a block in carbohydrate-induced increases in hepatic sterol regulatory element binding protein-1c (SREBP-1c) proteolytic processing, expression of FA synthesis genes, and hepatic triglyceride (TG) accumulation. We recently reported that mice with a skin-specific deletion of Scd1 (SKO mice) recapitulated the hypermetabolic phenotype observed in GKO mice, indicating that the skin is a major contributor to the altered energy metabolism observed in GKO mice (5). In contrast, SKO mice had normal carbohydrate-induced increase in SREBP-1c maturation and FA synthesis genes. These hepatic observations highlight that not all of the phenotypes of the SCD1 GKO mice can be attributed to SCD1 deletion in the skin.

Interestingly, mice intraperitoneally injected with Scd1-targeted antisense oligonucleotides (ASO) are also protected from the development of high-fat DIO and insulin resistance. This work was supported by National Institutes of Health Grants R01-DK-062388 (to J.M.N.), T32-DK-007665 (to M.T.F.), and T32-DK-007665 (to M.S.S.); and by an American Heart Association postdoctoral fellowship (to J.M.N.). The online version of this article (available at http://www.jlr.org) contains supplementary data in the form of four figures.

Abbreviations: a/a, non-agouti; A/y/a, agouti; AKO, adipose SCD1 knockout; ASO, antisense oligonucleotide; DIO, diet-induced obesity; GKO, global Scd1 knockout; LAKO, liver/adipose SCD1 knockout; LKO, liver Scd1 knockout; Lox, Scd1lox/lox; SCD1, stearoyl-CoA desaturase-1; SKO, skin Scd1 knockout; SREBP1c, sterol regulatory element binding protein-1c; TG, triglyceride.

1To whom correspondence should be addressed.
2email: ntambi@biochem.wisc.edu
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resistance (6, 7). This ASO therapy potently reduced SCD1 expression and activity in the liver and adipose tissue without affecting SCD1 expression in the muscle or skin. This suggests that inhibition of SCD1 in the liver and adipose tissue elicits obesity resistance via a mechanism that is physiologically distinct from that caused by skin SCD1 deletion. Because LKO mice are not protected from high-fat DIO (4), we hypothesized that deletion of adipose SCD1 (AKO mice) or combined deletion of liver and adipose SCD1 (LAKO) mice would elicit obesity resistance and other improved metabolic parameters previously observed in SCD1 GKO mice and those treated with ASO.

Adipose SCD1 was deleted from both white and brown adipose tissue by introducing the aP2-Cre allele into our SCD1flox/flox mice. Surprisingly, AKO and LAKO mice were not protected from obesity despite robust reductions in adipose MUFA content. These results indicate that deletion or inhibition of SCD1 in the white and brown adipose tissue, in the presence or absence of liver SCD1 activity, has minimal effect on whole-body energy expenditure. Our study also reveals that adipose-derived FA affect the FA composition of liver and vice versa. Lastly, we report that the MUFA composition of plasma TG is strikingly similar to that of liver TG and can potentially be used as a predictor of hepatic SCD activity.

MATERIALS AND METHODS

Animals, genotyping, and diets

FABP4(aP2)-Cre mice were obtained from Barbara Kahn at Beth Israel Deaconess Medical Center (8) and backcrossed to C57BL/6J male AKO mice were bred with either female Lox mice to produce Lox, AKO, LKO, or LAKO mice. Alternatively, male LAKO mice were weaned at 3 weeks of age and maintained on Purina 5008 until 26 weeks of age. Animals were fasted for 4 h and euthanized by an overdose of isoflurane anesthesia. The genotyping protocol for distinguishing the Scd1flox and wild-type Scd1 alleles has been described previously (4, 9).

For breeding strategies involving only one Cre transgene, we used a generic Cre-recombinase genotyping strategy available at the Jackson Laboratories website (jaxmice.jax.org). For LAKO breeding schemes, we designed genotyping primers specific to either albumin-Cre or aP2-Cre. Albumin-Cre was amplified using primer Alb-F (5′ GCC TGC AGG CAT TCA TCA 3′) and Cre-R (5′ GTG AAA CAG CAT TGC TGT CAC TT 3′) with a 54°C annealing temperature. AP2-Cre was amplified using aP2-F (5′ ATG ATC TGG CCC CCA TTG G 3′) and Cre-R with a 51°C annealing temperature. All in vivo experimental procedures were performed in accordance with the National Institutes of Health Guidelines for the Care and Use of Animals and were approved by the Institutional Animal Care and Use Committee at the University of Wisconsin-Madison.

Immunoblot analysis of SCD1

Liver and white adipose microsomes were prepared by sequential centrifugation. Tissues were first homogenized at 100 mg tissue/ml buffer in 0.1 M potassium phosphate buffer (pH 7.2) supplemented with 10 μg/ml leupeptin and 1 mM PMSF. The supernatant was subsequently centrifuged at 100,000 g for 1 h at 4°C. The pellet was rinsed once and resuspended in protease inhibitor free 0.1 M phosphate buffer. Brown adipose tissue was homogenized at 100 mg tissue/ml in lysis buffer containing 1 mM PMSF, 10 μg/ml leupeptin, 5 μg/ml pepstatin A, and 2 μg/ml aprotinin. SCD1 immunoblotting was performed on 10 μg of protein using a polyclonal SCD1 antibody (Santa Cruz Biotechnology; sc-14719).

Glucose and insulin tolerance tests

For glucose tolerance tests, mice were fasted for 4 h and subsequently injected intraperitoneally with 10% dextrose at a dose of 1 g/kg body weight. Blood was sampled by retroorbital puncture at 0, 20, 40, 90, and 180 min postinjection. For insulin tolerance tests, nonfasted mice were injected intraperitoneally with 0.75 U/kg body weight of human insulin (Novo Nordisk). Blood was collected by retroorbital puncture at 0, 15, 30, 45, and 60 min postinjection. Plasma glucose was analyzed using a colorimetric glucose oxidase method.

Tissue and plasma lipid analysis

Lipids were extracted from approximately 30 mg of tissue or 100 μl of plasma lipids using a modification of the Folch procedure (10). Samples were homogenized in 3 ml of CHCl3:MeOH (2:1) containing 10 mg/100 ml butylated hydroxytoluene. Hepatocanedic acid was added as an internal standard to correct for sample loss. Next, 1 ml of acidified saline (0.01 N HCl, 0.9% NaCl) was added. After vigorous vortexing and centrifugation, the organic layer was isolated, dried under nitrogen gas, and subjected to TLC on silica gel-60 plates (EMD Chemicals) in heptane/isopropyl ether/glacial acetic acid (60/40/5, v/v/v). The bands corresponding to standards were scraped and transferred to screw cap glass tubes containing pentadecanoic acid as an internal standard to control for transmethylation efficiency. FA were transmethylated in the presence of 14% boron trifluoride in methanol. The resulting methyl esters were extracted with hexane and analyzed by gas liquid chromatography as previously described (11). Total lipid contents were calculated from individual FA content in each fraction.

Real-time quantitative PCR

Total RNA was extracted with TRI reagent (Molecular Research), treated with Turbo DNase (Ambion), and reverse transcribed
using Multiscribe reverse transcriptase (Applied Biosystems). Real-time quantitative PCR was performed on an ABI StepOne Plus instrument using gene-specific primers and Power SYBR Green master mix (Applied Biosystems). Quantification of given genes is expressed as mRNA level normalized to a housekeeping gene (Arbp) using the ∆∆Ct method. Primer sequences are available upon request.

Data and statistical analysis
Data are expressed as mean ± SEM and were compared by one- or two-way ANOVA followed by Tukey’s posthoc test in GraphPad Prism. Comparisons with a P < 0.05 were considered significant.

RESULTS

To generate mice deficient in adipose Scd1 (AKO), we introduced the aP2-Cre transgene into our Scd1<sup>lox/lox</sup> (Lox) colony (4, 5). We also generated mice with liver-specific deletion of Scd1 (LKO) that expressed the albumin-Cre transgene (4), and crossed these with the AKO mice to obtain mice with simultaneous deletion of Scd1 from both the liver and adipose (LAKO). AKO and LAKO mice were born at the expected frequency and were indistinguishable from Lox mice. Importantly, AKO and LAKO mice do not display the closed eye fissures, dry skin, and alopecia previously observed in global (GKO) and skin-specific (SKO) Scd1-deficient mice (5). Immunoblot analysis confirmed the deletion of Scd1 from white and brown adipose tissue of AKO and LAKO mice, and from liver of LKO and LAKO mice (Fig. 1A). Furthermore, FA composition analysis of TG from both subcutaneous adipose tissue (Fig. 1B–E) and epididymal adipose tissue (supplementary Fig. I) reflects a marked decrease in the abundance of the SCD products palmitoleate (16:1n7) and oleate (18:1n9) and an increase in the SCD substrates palmitate (16:0) and stearate (18:0) in AKO and LAKO mice.

Effect of adipose Scd1 deletion on obesity and glucose tolerance
To test whether adipose and/or liver deletion of Scd1 influences the development of obesity, we used both dietary (high-fat DIO) and genetically induced (A<sup>y/a</sup> allele) obesity models compared with a/a mice fed a standard diet. The high-fat DIO model utilizes a hypercaloric, semipurified diet, which is highly abundant in the SCD product oleate. Despite the ample MUFA content of this high-fat diet, we have previously shown that both GKO and SKO mice, but not LKO mice, are remarkably resistant to obesity on this diet due to a hypermetabolic phenotype (4, 5). A<sup>y/a</sup> mice have ubiquitous expression of the agouti protein, causing leptin resistance, increased food intake, and decreased energy expenditure (2, 12). Thus, the A<sup>y/a</sup> model allows for obesity induction by feeding a standard diet. We found no significant difference in body weight or in epididymal or subcutaneous white adipose weights due to adipose and/or liver Scd1 deletion in either the DIO or A<sup>y/a</sup> models (Fig. 2A, C, D). Additionally, food intake was unaffected (Fig. 2E, F). Liver weight was not significantly affected in a/a mice fed a standard or high-fat diet; however, liver weight was reduced by loss of liver Scd1 in A<sup>y/a</sup> LKO and A<sup>y/a</sup> LAKO compared with Lox counterparts (Fig. 2B). Fasting glucose as well as glucose and insulin tolerance was not significantly affected by adipose Scd1 deletion in a/a mice fed a standard or high-fat diet (Fig. 3A–C). However, liver Scd1 deletion in both A<sup>y/a</sup> LAKO and A<sup>y/a</sup> LKO improved glucose tolerance (Fig. 3D).

Hepatic Scd1 activity influences adipose FA composition
Liver and adipose tissue TG levels and their FA composition can be influenced directly by several sources. First, dietary FA, primarily in the form of TG-rich chylomicrons, can be taken up into tissues via lipoprotein lipase and lipoprotein receptor-mediated mechanisms. Second, cells can de novo synthesize FA from acetyl-CoA, and the rate of de novo synthesis in liver is especially responsive to dietary nutrients and hormones, such as glucose and insulin, respectively (13). And finally, there is significant lipid crosstalk between the adipose and liver via the reciprocal exchange of adipose-derived free FA and hepatic-derived TG-rich VLDL. Importantly, intracellular saturated FA (palmitate and stearate), regardless of their origin, can be converted to MUFA via SCD1.

Liver Scd1 influenced adipose stores of oleate and the 18:1n9/18:0 ratio, but not stores of palmitoleate or 16:1n7/16:0 ratio, in A<sup>y/a</sup> mice but not in a/a mice (Fig. 1 and supplementary Fig. I). This suggests that under conditions of high hepatic lipogenesis, liver-derived TG contribute a greater amount to adipose TG stores than during conditions of low hepatic lipogenesis. Additionally, the percentage composition of palmitate, palmitoleate, stearate, and oleate in adipose TG was significantly affected by loss of adipose Scd1 in the subcutaneous fat, but not epididymal fat, of a/a mice. Upon introgression of the A<sup>y/a</sup> allele, both fat depots were significantly influenced by loss of Scd1 from liver or adipose. These fat depot-specific changes suggest that the FA composition of fat depots spatially oriented closer to the liver, such as visceral or epididymal fat, is influenced to a greater extent by liver-derived FA, especially under basal lipogenic conditions.

Aside from 16- and 18-carbon saturated FA and MUFA, the other predominant FA found in adipose is linoleic acid (18:2n6), which comprises approximately 25% of adipose FA. In a/a mice, loss of adipose and/or liver Scd1 had no significant effect on the percentage composition of 18:2n6 in epididymal or subcutaneous fat (data not shown). Loss of adipose Scd1 in A<sup>y/a</sup> AKO mice also had no effect on the abundance of 18:2n6 (supplementary Fig. II). Interestingly, loss of liver Scd1 in both A<sup>y/a</sup> LKO and A<sup>y/a</sup> LAKO mice caused elevated levels of 18:2n6 in the epididymal and subcutaneous adipose stores (supplementary Fig. II). To further explore the relationship between liver Scd1 and adipose 18:2n6 levels, we looked at 18:2n6 levels in liver TG and plasma TG. In a/a mice, 18:2n6 levels in liver were nonsignificantly elevated by approximately 12% in both LKO and LAKO mice compared with Lox and AKO (∼28.7% in LKO and LAKO compared with 25.5% in Lox and AKO), but plasma 18:2n6 levels were unchanged. However, both liver and plasma 18:2n6
Deletion of adipose and liver SCD1 does not prevent obesity significantly affected by loss of hepatic SCD1 in both the A/y/a LKO and A/y/a LAKO mice. (Fig. 4 and Fig. 5). Combined loss of liver and adipose SCD1 lowered hepatic TG, the 16:1n7/16:0 ratio, and the 18:1n9/18:0 ratio in high-fat fed LAKO mice as well (supplementary Fig. III). Deletion of liver and/or adipose SCD1 did not influence hepatic TG levels in the a/a mice and had a more modest effect on the FA composition. Thus, the hepatic rate of FA synthesis is an important determinant for the accumulation of SCD substrates in the absence of SCD1.

In A/y/a mice, the hepatic 16:1n7/16:0 and 18:1n9/18:0 ratios were predominantly determined by liver SCD1. Analysis of the desaturation indices of hepatic lipids also revealed a partial role of adipose SCD1 in determining the FA composition of the liver. In both A/y/a and a/a mice, the liver TG 16:1n7/16:0 ratio was independently and additively influenced by loss of liver and adipose SCD1 (Fig. 4). Adipose deletion of SCD1 in A/y/a AKO mice also reduced the hepatic 18:1n9/18:0 ratio. However, adipose-specific deletion of SCD1 did not protect mice from high-fat levels were increased in A/y/a LKO and A/y/a LAKO compared with A/y/a Lox and A/y/a AKO mice (supplementary Fig. II). Thus, lack of hepatic SCD1, when combined with the A/y allele, leads to a dramatic decrease in 18:1n9 availability and a concomitant increase in 18:2n6 enrichment of these liver and plasma TG, which subsequently influence the FA composition of the adipose stores.

**Effect of liver and adipose SCD1 on hepatic and TG composition**

Although A/y/a LKO and A/y/a LAKO mice were not protected from obesity, their liver weights were significantly lower than the A/y/a Lox and A/y/a AKO mice (Fig. 2). We measured hepatic TG levels in both A/y/a and a/a mice. Compared with their a/a counterparts, A/y/a mice had significantly elevated hepatic TG levels (Fig. 4). However, this A/y/a effect was severely blunted by loss of liver SCD1 in both A/y/a LKO and A/y/a LAKO mice. Consistent with this observation, the percentage composition of palmitate, palmitoleate, stearate, and oleate in hepatic TG were all significantly affected by loss of hepatic SCD1 in both the A/y/a LKO and A/y/a LAKO mice. (Fig. 4 and Fig. 5).

Fig. 1. Validation of adipose SCD1 deletion by immunoblot and FA composition. (A) SCD1 protein expression in liver, epididymal white adipose, subcutaneous white adipose, and brown adipose. Lox (LX), LKO (L), AKO (A), and LAKO (LA). (B, C) FA composition of subcutaneous fat in a/a (B) and A/y/a (C) mice fed a standard diet were analyzed by one-way ANOVA. (D, E) 16:1n7/16:0 (D) and 18:1n9/18:0 (E) desaturation indices for subcutaneous fat were analyzed by two-way ANOVA. Different letters indicate significant differences (*P < 0.05) between SCD1 genotypes within the a/a or A/y/a group. *P < 0.05 for A/y/a versus a/a, n = 5–7 per group.
of these particles in hepatocytes. Therefore, we compared the liver and plasma TG masses, desaturation indices, and FA compositions in both A\textsuperscript{y}/a and a/a mice with liver and/or adipose deletion of SCD1 (Figs. 4 and 5). Similar to the pattern observed for liver TG mass, plasma TG mass in a/a mice was not affected by loss of adipose and/or liver SCD1, but loss of liver SCD1 in A\textsuperscript{y}/a LKO and A\textsuperscript{y}/a LAKO led to a reduction of plasma TG levels. Strikingly, the

**Plasma TG FA composition parallels hepatic TG FA composition**

In the fasted state, plasma TG are predominantly transported by liver-derived VLDL, which obtain their neutral lipid core during the presecretory intracellular assembly of these particles in hepatocytes. Therefore, we compared the liver and plasma TG masses, desaturation indices, and FA compositions in both A\textsuperscript{y}/a and a/a mice with liver and/or adipose deletion of SCD1 (Figs. 4 and 5). Similar to the pattern observed for liver TG mass, plasma TG mass in a/a mice was not affected by loss of adipose and/or liver SCD1, but loss of liver SCD1 in A\textsuperscript{y}/a LKO and A\textsuperscript{y}/a LAKO led to a reduction of plasma TG levels. Strikingly, the

![Graph A](image1.png)

![Graph B](image2.png)

![Graph C](image3.png)

![Graph D](image4.png)

**Fig. 2.** Deletion of adipose and/or liver SCD1 does not protect mice from obesity. (A) Body weight, (B) liver weight, (C) epididymal fat pad weight, and (D) subcutaneous fat pad weight were measured at euthanasia in male mice at 6 months of age after being fed either a standard diet (SD) for both a/a and A\textsuperscript{y}/a, or high-fat diet (DIO) for a/a. Food intake for (E) A\textsuperscript{y}/a and (F) DIO mice were measured over the course of 5–7 days. A\textsuperscript{y}/a SD, a/a SD, and a/a DIO groups were each independently analyzed by one-way ANOVA. *P < 0.05 versus Lox, n = 5–11 per group.

![Graph E](image5.png)

![Graph F](image6.png)

**Fig. 3.** Effect of SCD1 deletion on glucose and insulin tolerance. Glucose tolerance tests were performed in (A) a/a mice fed a standard diet, (C) a/a mice fed a high-fat diet (DIO), and (D) A\textsuperscript{y}/a mice fed a standard diet. Insulin tolerance tests (B) were done in a/a mice fed the standard diet. Tests were done at 5–6 months of age. *P < 0.05 Lox versus LKO, *P < 0.05 Lox versus LAKO, *P < 0.05 AKO versus LAKO, n = 5–11 per group.
Deletion of adipose and liver SCD1 does not prevent obesity

expression and concomitant deletion of Scd1 in our studies are likely not restricted to adipocytes. The aP2-Cre transgene has also been detected during embryonic development (20) in macrophages and macrophage-rich tissues (21, 22), bone marrow (21), cardiac and skeletal muscle, intestine, stomach, pancreas, and in the central and peripheral nervous system (23). However, the endogenous aP2 gene is predominantly expressed in adipocytes, and the aP2-Cre-mediated recombination in these other tissues is likely incomplete due to the lower expression level of Cre. Nonetheless, aP2-Cre-mediated deletion of Scd1 from adipocytes and potentially other cell types failed to recapitulate the obesity resistance observed in GKO mice.

We recently reported that skin-specific deletion of SCD1 elicited a hypermetabolic phenotype comparable to that observed in GKO mice (5). These mice resisted adipocyte hypertrophy and maintained insulin sensitivity when challenged with high-fat feeding, which was associated with increased uncoupling protein expression in a variety of peripheral tissues, such as white and brown adipose tissue, muscle, and liver. Thus, altered skin lipid metabolism can indirectly influence metabolic flux and cell signaling in peripheral tissues. However, these data do not necessarily exclude a role for SCD1 in other tissues, such as brown or white adipose tissue, because the severity of the cutaneous phenotypes in SKO mice may mask the contributions of SCD1 in these tissues to global energy homeostasis. Therefore, it is possible that an extracutaneous mechanism for obesity resistance also exists in the GKO mouse. This is supported by studies in mice treated with Scd1-targeted ASO, which are reported to not affect the skin

desaturation indices (Fig. 4) and FA compositions (Fig. 5) of liver and plasma TG showed the same genotype-dependent changes, suggesting that the FA composition of plasma TG is predictive of both liver TG FA composition and SCD1 activity.

**DISCUSSION**

Although mice with a global deletion of Scd1 (GKO) display remarkable obesity resistance (2–4) and insulin sensitivity (14), the importance of adipose SCD1 for whole-body energy metabolism is unclear. SCD1 is highly expressed in both white and brown adipose tissue. Previous studies in mice with a whole-body deletion of SCD1 have documented increased insulin signaling and uncoupling protein expression in the white and brown adipose tissue (15–17). Due to the simultaneous deletion of Scd1 from all tissues in GKO mice, it is unclear whether metabolic changes in a particular tissue are a direct effect of local loss of SCD1 function or an indirect effect stemming from loss of SCD1 in distal tissues.

Tissue-specific deletion using the Cre-lox system relies upon the use of a tissue-specific promoter to drive the expression of the Cre-recombinase. The FABP4(aP2)-Cre transgene is highly expressed in both white and brown adipose tissue (8, 18). The differentiation of preadipocytes into mature adipocytes leads to the activation of several adipose-specific genes, including Fabp4 and Scd1 (19). Therefore, it is likely that the FABP4-Cre-mediated deletion of Scd1 occurs sometime during or immediately after adipocyte differentiation. It is noteworthy to acknowledge that aP2-Cre

Fig. 4. Effect of SCD1 deletion on liver and plasma TG mass and desaturation indices. (A, B) Liver TG mass (A) and plasma TG mass (B) in a/a and A/y/a mice fed a standard diet. (C, D) 16:1n7/16:0 TG desaturation index for liver (C) and plasma (D). (E, F) 18:1n9/18:0 desaturation index for liver (E) and plasma (F). Data were analyzed by two-way ANOVA. Different letters indicate significant differences (P < 0.05) between SCD1 genotypes within the a/a or A/y/a group. *P < 0.05 for A/y/a versus a/a, n = 5–7 per group.
However, the failure of adipose and/or liver Scd1 deletion to prevent obesity in either the A'/a or DIO model strongly suggests that loss of SCD1 function in these tissues is not the mechanism for obesity resistance in the GKO mice or ASO-treated mice. Furthermore, we did not observe increased expression of thermogenic genes (Ucp1, Ucp2, Adrb2, Dio2, Pparg1a) in the brown adipose tissue of AKO or LAKO mice (supplementary Fig. IV).

Intraperitoneally injected ASO resulted in the accumulation of ASO in several tissues. In addition to the reported effects of SCD1 ASOs on liver and white adipose tissue, we speculate that the ASO-mediated obesity resistance is due to additional inhibition of Scd1 in cells or tissues not targeted by the albumin-Cre and aP2-Cre. In the case of the adipose tissue, the ASO treatment may be eliciting effects on both preadipocytes and mature adipocytes, unlike the aP2-Cre-mediated recombination that requires activation of the aP2 promoter during adipocyte differentiation. Inhibition of SCD1 during the preadipocyte stage may cause distinct metabolic changes compared with deletion of Scd1 in the late stages of or after differentiation. Alternatively, the ASO may be eliciting off-target effects on other genes contributing to obesity resistance, unlike the Cre-lox system, which is specific to Scd1. Unlike the adult mouse liver, which expresses primarily Scd1, both Scd1 and Scd2 are highly expressed in the mouse adipose tissue (24). We found that the expression of Scd2 in white and brown adipose tissue was unaffected by deletion of Scd1 via aP2-Cre (supplementary Fig. IV). Therefore, it is also possible that the anti-obesity effects of published Scd1-targeted ASO (6, 7) occur due to the simultaneous inhibition of both Scd1 and Scd2. This would predict that there are two SCD-mediated mechanisms to obesity resistance in mice: i) deletion of Scd1 in skin and ii) inhibition of Scd1 and Scd2 from the liver and adipose.

The failure of Scd1 deletion from the liver to protect against the A'/a-induced obesity is somewhat surprising in light of our previous observations of reduced adiposity in LKO mice fed a semipurified, high-carbohydrate diet (4). Since the A'/a mice in our study were fed a cereal grain-based, high-carbohydrate, low-fat diet, we hypothesized that lack of liver SCD1 would reduce hepatic conversion of these dietary carbohydrates into TG, reduce liver VLDL TG secretion, and reduce overall body adiposity. Although we did observe reduced hepatic MUFA and TG content, this is presumably insufficient to affect overall energy homeostasis. The leptin-resistant phenotype of the A'/a mice results in both increased food intake and decreased energy expenditure. Thus, a hypermetabolic response that increases whole-body energy expenditure, such as that observed in GKO and SKO mice (5), may be necessary to combat the positive energy balance that exists in the A'/a and DIO obesity challenges.

Palmitoleate (16:1n7) released into circulation from the adipose tissue has been suggested to act as a beneficial lipokine that improves metabolic derangements, including insulin resistance and hepatic steatosis (25). The combined adipose deficiency of the aP2 (FABP4) and mal1 (FABP5) FA binding proteins caused elevated levels of adipose 16:1n7
concomitant with improved metabolic phenotype (25); this predicts that reducing normal levels of adipose 16:1n7 by deleting SCD1 would worsen metabolism. However, deletion of adipose SCD1 in our study had no effect on body weight, glucose, or insulin tolerance in standard diet-fed mice or on two models of obesity (DIO and Δ/Δ), despite a significant lowering of adipose levels of 16:1n7. Furthermore, simultaneous deletion of liver and adipose SCD1 actually had a beneficial effect of lowering liver TG levels and improving glucose tolerance compared with control mice. The FA ratios 16:1n7/16:0 and 18:1n9/18:0 in liver TG have been previously shown in human studies to correlate very well with the FA ratios found in plasma TG (26). In our current study, we found a remarkable similarity between the liver and plasma TG FA compositions that reflected both Δ/Δ and SCD1-genotype effects. Although the liver SCD1 genotype comprised a majority of the effect on the liver and plasma 18:1n9/18:0 ratio, we found the 16:1n7/16:0 to be influenced by both the liver and adipose SCD1 genotype. Additionally, we found the adipose 18:1n9/18:0 ratio to be influenced by hepatic SCD1 in Δ/Δ mice. Therefore, liver and adipose FA stores can reciprocally influence the composition of one another, presumably due to the exchange of liver-derived VLDL TG and adipose-derived free FA. We also observed that the magnitude of the hepatic SCD1-genotype effect on these hepatic FA ratios was dramatically influenced by the presence or absence of the Δ/Δ allele. Thus, diet and genetic factors that predispose an individual to insulin resistance and increased hepatic lipogenesis can affect the apparent correlation of hepatic FA ratios with hepatic SCD1 levels.

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