Dietary saturated fats have often been implicated in the promotion of obesity and related disorders. It has been shown recently that saturated fats act through the transcription factor SREBP-1c (sterol regulatory element-binding protein-1c) and its requisite coactivator, peroxisome proliferator-activated receptor-γ coactivator-1β (PGC-1β), to exert their pro-lipogenic effects. We show here that a diet high in the saturated fat stearate induces lipogenic genes in wild-type mice, with the induction of the Scd1 (stearoyl-CoA desaturase-1) gene preceding that of other lipogenic genes. However, in Scd1−/− mice, stearate does not induce lipogenesis, and Srebp-1c and Pgc-1β levels are markedly reduced. Instead, genes of fatty acid oxidation such as Cpt-1 (carnitine palmitoyltransferase-1) as well as Pgc-1α are induced. Mitochondrial fatty acid oxidation is increased, and white adipose tissue and hepatic glycogen stores are depleted in stearate-fed Scd1−/− mice. Furthermore, AMP-activated protein kinase is also induced by stearate feeding in Scd1−/− mice. These results indicate that the desaturation of saturated fats such as stearate by SCD is an essential step mediating their induction of lipogenesis. In the absence of SCD1, stearate promotes oxidation, leading to protection from saturated fat-induced obesity. SCD1 thus serves as a molecular switch in the promotion or prevention of lipid-induced disorders brought on by consumption of excess saturated fat.

Along with the alarming rise in obesity to epidemic proportions, related complications such as diabetes mellitus, insulin resistance, and cardiovascular disease are also growing and represent some of the leading health-care concerns in the world (1–3). It has become increasingly clear that although dietary fat intake is correlated with the onset of obesity and related conditions, the type of dietary fat has a significant impact on its health effects. Although saturated fats have been associated with increased lipogenesis and risk for lipid-induced disorders (4, 5), the mechanisms by which they accelerate disease progression are poorly understood. Furthermore, growing evidence suggests that dietary fat may function differently from stored lipids or fatty acids synthesized de novo (6, 7), further complicating the understanding of the roles of particular fatty acids in cellular metabolism.

The 18-carbon saturated fatty acid stearate (18:0) represents one of the most abundant dietary saturated fatty acids (8) and has been implicated in the induction of hepatic lipogenesis (4). Apart from dietary intake, stearate can also be derived through de novo synthesis by elongation of palmitate, the end production of fatty acid synthesis. Once in the cell, stearate has multiple fates, including elongation, oxidation, or esterification into complex lipids (8). Furthermore, stearate also serves as a major substrate for the enzyme stearoyl-CoA desaturase (SCD),3 which rapidly converts it to the monounsaturated fatty acid oleate (9). Oleic acid is one of the most abundant dietary and tissue fatty acids and has been shown to be involved in the development of obesity and lipid-induced disorders (10, 11). First, unlike stearate, oleate is a preferred substrate for the synthesis of triglycerides and cholesteryl esters (9). Non-human primates placed on a long-term high monounsaturated fat diet have been shown to accumulate higher amounts of hepatic lipids corresponding with higher rates of coronary artery atherosclerosis relative to animals fed a high saturated fat or polyunsaturated fat diet (12). Also, SCD activity and consequent accumulation of triglycerides and oleate have been shown to be increased in skeletal muscle samples from obese human subjects (13).

Further insight into the potential role of oleate in lipid-induced disorders comes from studies in mice with a targeted mutation in the Scd1 gene (Scd1−/−). SCD is the key enzyme involved in the synthesis of monounsaturated fatty acids and catalyzes the insertion of a double bond between carbons 9 and 10 of long chain saturated fatty acids (9). This enzyme displays specificity for palmitoyl- and stearoyl-CoA as substrates, converting them to palmitoleoyl- and oleoyl-CoA, respectively. Scd1−/− mice are lean and protected from diet-induced obesity and insulin resistance (7, 14–18). They accumulate very little hepatic and whole body lipids compared with wild-type (WT) animals. This is accompanied by a great reduction in 16:1 and 18:1 fatty acids, decreased rates of lipogenesis, as well as increased metabolic rate and lipid oxidation (16–18), suggesting a possible correlation between the monounsaturated products of SCD1 and hepatic lipid metabolism.

3 The abbreviations used are: SCD, stearoyl-CoA desaturase; AMPK, AMP-activated protein kinase; FAS, fatty-acid synthase; GPAT, glycerol-3-phosphate acyltransferase; PGC-1, PPAR-γ coactivator-1; PPAR, peroxisome proliferator-activated receptor; SREBP, sterol regulatory element-binding protein; TO, triolein; TS, tristearin; WAT, white adipose tissue; ACC, acetyl-CoA carboxylase; TG, triglycerides; WT, wild type.

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* Effects of Dietary Saturated Fat*

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A recent study indicated that the lipogenic effects of dietary saturated fat are mediated by two transactivating factors, SREBP-1c (sterol regulatory element-binding protein-1c) and PGC-1α (PPAR-γ coactivator-1α) (4). SREBP-1c is a transcription factor belonging to the helix-loop-helix-leucine zipper family of transcription factors (19–21). It is activated by insulin and glucose as well as by fructose (16, 20, 22) and is a key transcriptional activator of hepatic lipogenic genes such as acetyl-CoA carboxylase (Acc), fatty-acid synthase (Fas), glycerol-3-phosphate acyltransferase (Gpat), and Scd1 (21). Overexpression of SREBP-1 in livers of transgenic mice leads to marked increases in de novo lipogenesis and development of fatty livers (23). Also, SREBP-1c expression is significantly increased in the fatty liver of leptin-deficient ob/ob mice, underscoring the role of this transcription factor in the development of obesity and related disorders (23, 24). Several studies have shown that SREBP on its own is a very weak transcriptional activator and requires the presence of coactivators to exert maximal effects (25). PGC-1α is one such requisite coactivator of SREBP-1c that is induced in response to a short term high saturated fat diet, whereupon it coactivates SREBP-1c to up-regulate de novo lipogenesis (4).

Somewhat in contrast with this, studies from our laboratory have suggested that mice that cannot desaturate dietary saturated fat because of Scd1 deficiency have lower levels of SREBP-1c and decreased expression of lipogenic genes (16). Furthermore, given the fact that oleate, the product of SCD, is a preferred substrate for complex lipid synthesis, we hypothesized that intracellular oleate generated by Scd1 is directly involved in the development of obesity and in the induction of lipogenesis previously attributed to saturated fat. In this study, we demonstrate that dietary stearate-mediated induction of lipogenesis requires its conversion into oleate by SCD. In the Scd1−/− mouse, which is unable to carry out this desaturation process, dietary stearate does not promote lipogenesis or weight gain but instead promotes oxidation, leaving the animal protected from saturated fat-induced obesity.

**MATERIALS AND METHODS**

**Animals and Diets**—The generation of Scd1−/− mice has been described previously (16). The breeding and care of animals are in accordance with the protocols approved by the Animal Care Research Committee of the University of Wisconsin, Madison. Pre-bred homozygous (Scd1+/−) and wild-type (Scd1+/+) mice on a pure 129 SvEv background were used. Six mice (n = 6) at 8 weeks of age were placed on 20% high fat diets for 8 weeks, 7 days, or 2 days. Diets were made by supplementing fat-free basal mix (TD88232; Harlan Teklad) with 20% by weight of tristearin (69498; Sigma) or triolein (99% purity, T7140; Sigma) (36% fat calories) and 1% by weight corn oil. Dietary fat absorption was assessed as described previously (16). Food intake was measured daily, and body weights were measured every 2 days during the feeding period. 1 week before the end of the 8-week study, mice were fasted overnight and 10% dextrose was delivered by oral gavage at a dose of 1 g/kg body weight. Plasma glucose was measured at 0, 30, and 90 min post-gavage to assess oral glucose tolerance. Animals were given ad libitum access to food and water after gavage. At the end of each of the feeding periods, mice were anesthetized with isoflurane and sacrificed by cervical dislocation. Tissues were rapidly excised and frozen in liquid nitrogen or placed in 10% buffered formalin for hematoxylin and eosin staining. Portions of fresh liver were also aliquoted for isolation of nuclear protein.

**Materials**—Radioactive [14C]stearoyl-CoA and [3H]oleoyl-CoA were purchased from American Radiolabeled Chemicals (St. Louis) and PerkinElmer Life Sciences. TLC plates (TLC Silica Gel G60) were from Merck. Scd1 and SREBP-1 antibodies were purchased from Santa Cruz Biotechnology and Pharmingen, respectively. Phospho-AMPK and AMPK antibodies were from Cell Signaling Technology, Inc. (Beverly, MA), and Upstate Biotechnology, Inc., respectively. SAMS peptide was a gift from Dr. K. W. Saepe (University of Wisconsin, Madison). All other materials were from Sigma unless indicated otherwise.

**Glucose and Glycogen Determinations**—Plasma glucose was analyzed using a colorimetric glucose oxidase method (Sigma), and glycogen content was measured according to the methods of Roehrig and Allred (26).

**Plasma Insulin**—Plasma insulin was analyzed using a radioimmunoassay kit (Linco Research SRI-13K).

**Lipid Analysis**—Total lipids were extracted from liver according to the methods of Bligh and Dyer (27) and analyzed by TLC followed by gas chromatography, as described previously (28). Total plasma triglyceride was measured using a commercially available enzymatic kit (Roche Diagnostics). Plasma fatty acid compositions were also quantitated by methylation and separation by gas chromatography, as described above.

**Isolation and Analysis of RNA**—Total RNA was isolated from livers of WT and Scd1−/− mice using TRIzol reagent (Invitrogen) and then treated with DNase. cDNA was prepared by reverse transcription with random hexamer primers and amplified by PCR using gene-specific primers in the presence of SYBR Green on an ABI 7500 fast machine (Applied Biosystems). Relative abundance of mRNA was calculated by normalizing to cyclophilin. Primer sequences are available upon request.

**Western Blot Analysis**—SREBP protein levels were detected in nuclear extracts by Western blotting as described previously (14). SCD1 protein levels were detected by Western blotting using 30 μg of microsomal protein and immunoblotting with polyclonal anti-SCD1. AMPKα1 was immunoprecipitated using previously described methods (17) from 400 μg of total cellular protein using anti-AMPKα1 antibody. Immunoprecipitates were resolved by SDS-PAGE as above and blotted with anti-phospho-AMPKα1/α2 (Ser-485) antibody. The proteins were visualized with a chemiluminescence detection system (Pierce) and quantified by densitometry.

**SCD Activity Assay**—Liver microsomes were isolated, and SCD activity was assayed as described previously (29). Briefly, SCD activity was assayed at 23 °C with 3 μM [14C]stearoyl-CoA, 2 mM NADH, and 100 μg of microsomal protein. Reactions were incubated for 15 min and terminated by addition of 200 μl of 2.5 M KOH in 75% ethanol. The reaction mixture was saponified at 85 °C for 1 h, and samples were cooled and acidified with 280 μl of formic acid. Free fatty acids were extracted with 700 μl of hexane and separated on a 10% AgNO₃-impreg-
TABLE 1
Food intake, body weight, and white adipose tissue mass after TS or TO feeding
8-Week-old WT (n = 6) and Scd1−/− mice (n = 6) were placed on 20-g weight % TS or TO diets for 2 months (WT only), 7 days, or 2 days. Food intake was measured daily, and body weights (BW) were measured every 2 days during the feeding period. Epididymal fat pads were weighed after sacrifice and normalized to body weight. *p < 0.05 for the following: *, compared with TO-fed counterparts; #, compared with WT counterparts.

| Duration diet | 2 months | 7 days | 2 days |
|---------------|----------|--------|--------|
| Food intake (g/day/g BW) | | | |
| WT | 0.18 ± 0.02 | 0.12 ± 0.006 | 0.24 ± 0.07 | 0.16 ± 0.03 | 0.31 ± 0.02 | 0.22 ± 0.01 |
| Scd1−/− | 0.36 ± 0.04 | 0.28 ± 0.004 | 0.42 ± 0.27 | 0.28 ± 0.01 |
| BW (g) | | | |
| WT | 27.40 ± 1.60* | 30.24 ± 1.35 | 20.00 ± 1.93* | 23.31 ± 1.56 | 21.16 ± 1.61 | 22.79 ± 2.00 |
| Scd1−/− | 17.6 ± 1.37* | 20.00 ± 3.76 | 17.6 ± 1.37* | 20.00 ± 3.76 | 23.10 ± 1.89 | 24.06 ± 1.43 |
| Change in BW (g) | | | |
| WT | 4.55 ± 1.53* | 6.17 ± 1.36 | 0.33 ± 0.25* | 2.55 ± 0.91 | −0.96 ± 1.33 | −1.11 ± 2.78 |
| Scd1−/− | −3.76 ± 0.28* | 0.5 ± 0.65* | −1.56 ± 1.73 | −0.34 ± 1.16 |
| WAT mass (g) | | | |
| WT | 0.55 ± 0.13 | 0.97 ± 0.18 | 0.23 ± 0.07* | 0.42 ± 0.07 | 0.31 ± 0.07* | 0.43 ± 0.06 |
| Scd1−/− | 0.06 ± 0.01* | 0.42 ± 0.15 | 0.22 ± 0.01 | 0.31 ± 0.07* |
| WAT normalized to BW (mg/g BW) | | | |
| WT | 20.00 ± 3.74* | 32.41 ± 6.35 | 10.90 ± 2.80* | 17.77 ± 2.24 | 16.36 ± 2.28 | 18.98 ± 2.36 |
| Scd1−/− | 2.11 ± 1.89* | 20.61 ± 4.37 | 9.61 ± 1.66* | 12.61 ± 2.51* |

SCD1 Mediates the Lipogenic Effects of Dietary Saturated Fat

Stearate and oleate were chosen as the saturated and polyunsaturated fatty acids which most closely resemble dietary saturated and polyunsaturated fatty acids, respectively. The sum of palmitate and stearate is among the most abundant dietary fatty acids (8, 34). Although these diets are not strictly representative of human diet patterns, they were formulated to resemble as such in order to minimize confounding effects associated with the presence of fats of varying lengths and degrees of saturation. Dietary fat absorption was not different between WT and Scd1−/− mice (data not shown).

WT animals were fed TS or TO diets for 8 weeks. Chow-fed animals gained an average of 4.1 ± 0.3 g body weight during the 2-month period (data not shown). Thus, TS-fed animals did not gain significantly more weight than chow-fed WT animals (Table 1). However, despite consuming less food, TO-fed animals gained 1.4 times more body weight than TS-fed animals (Table 1). TO-fed animals also accumulated 1.6 times as much white adipose tissue (WAT) as TS-fed animals (Table 1) and 1.3 times as much WAT as chow-fed animals (data not shown) over the 8-week period. Fasting plasma insulin levels were not significantly different between TS- and TO-fed animals (0.50 ± 0.14 and 0.81 ± 0.32 ng/ml, respectively). Hematoxylin and eosin staining of liver sections revealed significantly higher hepatic lipid accumulation in TO-fed animals compared with TS-fed counterparts (Fig. 1A). Because increased adiposity is a known risk factor for insulin resistance (35, 36), we performed an oral glucose tolerance test at the end of the 8-week feeding period to assess whole body glucose tolerance. Although basal plasma glucose levels were no different between the animals (Fig. 1B), the ability to clear plasma glucose was markedly impaired after TO feeding as compared with TS feeding (Fig. 1B).
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Figure 1. Hepatic lipid accumulation and oral glucose tolerance in WT mice after 2 months of TS or TO feeding. A, fresh liver sections were stored in 10% buffered formalin and stained by hematoxylin and eosin to visualize lipid droplets (white arrows). Sections are representative of several WT animals in each group after 2 months of feeding. B, oral glucose tolerance (OGTT) was assessed in WT animals fed TS or TO diets for 8 weeks. Each point represents mean ± S.D. for 6 mice in each group. *, p < 0.05 compared with TO-fed counterparts.

The understanding of the molecular effects of saturated fats has often been complicated because of endogenous desaturation of these fatty acids into oleate. Therefore, to delineate the differential molecular mechanisms of stearate and oleate in the development of obesity and related conditions, we placed both WT and Scd1−/− mice on TS or TO diets for 7 days. A shorter 2-day feeding period was also included to look at acute changes in gene expression effected by dietary fat. Because these diets are hypercaloric and extremely purified compared with chow, we have chosen to limit our comparisons to the effects of the two high fat diets, rather than comparing them to the effects of a regular chow diet. During both feeding periods, food intake and body weights were monitored on a daily basis. Similar to the long term feeding experiment, TS-fed animals were hyperphagic relative to TO-fed counterparts during the 7-day feeding period (Table 1). Despite this, TO-fed WT and Scd1−/− animals gained 2.2 and 4.3 g more body weight and accumulated 1.6- and 10-fold larger epididymal fat pads, respectively, than TS-fed counterparts (Table 1). Notably, TS-fed Scd1−/− animals did not gain weight on this diet and displayed a virtual absence of white adipose tissue after 7 days of TS feeding. However, they did not appear sickly and remained hyperphagic and alert throughout the feeding period. Also, the depletion of white adipose tissue was not indicative of lipodystrophy as lipid accumulation in other tissues, including liver, was also decreased, as discussed below. Parameters such as food intake, body weight, and adiposity showed the same trends after 2 days of feeding (Table 1). TS-fed animals consumed more food but gained less weight and accumulated less white adipose tissue than TO-fed counterparts in both WT and Scd1−/− backgrounds. Plasma insulin levels were not different between TS- and TO-fed WT or Scd1−/− animals after 7 or 2 days of feeding. As reported previously (14), Scd1−/− mice had lower plasma insulin levels relative to WT counterparts (0.21 ± 0.09 versus 1.10 ± 0.43 ng/ml, respectively).

Dietary Oleate Is Readily Incorporated into Hepatic and Plasma Lipids—Dietary fat intake and increased adiposity are known risk factors for hepatic steatosis and hypertriglyceridemia (1–3, 35). To determine whether TS and TO feeding differentially affect hepatic and plasma lipid accumulation in WT and Scd1−/− mice, we measured triglycerides (TG) in liver and plasma after 7 days of TS or TO feeding. TO-fed WT and Scd1−/− animals accumulated 1.8 and 3.9 times as much hepatic TG, respectively, relative to TS-fed counterparts (Fig. 2A). Compared with TS feeding, TO feeding also increased plasma TG levels in WT and Scd1−/− mice by 1.6- and 2.4-fold, respectively (Fig. 2B). Interestingly, although TO feeding normalized hepatic TG in Scd1−/− mice (Fig. 2A), plasma TG remained 25% lower in TO-fed Scd1−/− mice relative to TO-fed WT mice (Fig. 2B). As shown before (7, 16), 2 days of oleate feeding did not rescue hepatic or plasma TG in Scd1−/− mice (data not shown).

The cellular ratio of saturated to monounsaturated fatty acids is important in regulating cellular signaling (37–39). To determine whether the degree of fatty acid desaturation of cellular lipids is differentially affected by TS or TO feeding, we measured fatty acid composition of hepatic TG and total plasma lipids. In WT mice, palmitoleate content of hepatic TG was 50% higher in TO-fed animals than in TS-fed animals (Table 2, part A), which was surprising given that they did not derive this fatty acid from the diet. Palmitoleate content, on the other hand, was increased by 3-fold in TS-fed WT mice compared with TO-fed counterparts (Table 2, part A). Interestingly, TS-fed WT mice did not accumulate significant amounts of stearate in liver, despite consuming it in the diet. TO-fed WT mice accumulated 3 times as much oleate in hepatic TG compared...
with TS-fed mice (Table 2, part A). This oleate reflected dietary fat composition and accounted for 53% of hepatic TG in TO-fed mice. Collectively, these differences in the degree of fatty acid desaturation suggest that SCD activity may be modulated differentially by stearate and oleate in WT mice.

In Scd1<sup>−/−</sup> mice, TO feeding caused 3-fold greater palmitate accumulation than TS feeding, suggesting a greater rate of de novo lipid synthesis. Coupled with SCD1 deficiency, this leads to accumulation of the saturated fatty acid palmitate. Similar to WT animals, oleate content of hepatic TG was 10-fold greater in TO-fed Scd1<sup>−/−</sup> mice relative to TS-fed counterparts and accounted for 49% of fatty acids in hepatic TG. Interestingly, similar to WT mice, stearate content of hepatic TG was not increased by TS feeding in Scd1<sup>−/−</sup> mice. Furthermore, stea rate was not found to accumulate in any of the hepatic lipid fractions after TS feeding in either WT or Scd1<sup>−/−</sup> mice (data not shown).

Fatty acid composition of plasma lipids was also differentially affected by the type of dietary fat. In WT mice, plasma fatty acids (Table 2, part B) reflected fatty acid composition of hepatic TG (Table 2, part A), with oleate accounting for almost 60% of plasma fatty acids. However, this was not the case in Scd1<sup>−/−</sup> mice. Despite significant enrichment of hepatic TG with oleate (Table 2, part A), plasma oleate accounted for less than 10% of plasma fatty acids in TO-fed Scd1<sup>−/−</sup> mice. Also of interest, there was no significant accumulation of steareate in plasma lipids of any of the TS-fed animals, despite high dietary intake of steareate or the inability to convert steareate to oleate because of SCD1 deficiency (Table 2, part B).

These data clearly demonstrate that the intake of excess dietary oleate leads to increased adiposity, correlating with increased hepatic and plasma lipid accumulation. Interestingly, the fatty acid compositions of hepatic and plasma lipids do not necessarily reflect dietary fat composition, suggesting significant differences in regulation of fat metabolism by stearate- and oleate-enriched high fat diets. Most importantly, stearate does not accumulate in hepatic or plasma lipids of WT or Scd1<sup>−/−</sup> mice despite high stearate feeding. Furthermore, high oleate feeding causes oleate enrichment of hepatic (Table 2, part A) but not plasma (Table 2, part B) lipids in Scd1<sup>−/−</sup> mice.

**Dietary Stearate Induces SCD Expression and Activity**—The Δ-9 desaturase, SCD1, prefers 16- and 18-carbon saturated fatty acids as substrates, rapidly desaturating them into their monounsaturated products (9, 14). We have shown previously that high fat feeding increases SCD1 expression in mice prone to obesity (15). However, the differential effects, if any, of different dietary fats are as yet unknown. Based on the differences in fatty acid composition observed after TS and TO feeding in WT mice (Table 2), we hypothesized that SCD activity may be induced by dietary stearate while being repressed by dietary oleate.

SCD1 expression in livers of WT mice was measured by real-time PCR. TS-fed animals had a more than 5-fold higher expression of hepatic SCD1 compared with TO-fed counterparts (Fig. 3A). This difference in gene expression translated to a comparable change in protein levels (Fig. 3B). TS feeding also

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**TABLE 2**

Fatty acid composition of hepatic and plasma lipids

A. fatty acid composition of hepatic triglycerides (μmol/g) was determined by gas chromatography after 7 days of TS or TO feeding in WT and Scd1<sup>−/−</sup> mice. B. fatty acid composition of total plasma lipids (nmol/100 μl) was determined by gas chromatography after 7 days of TS- or TO-feeding in WT and Scd1<sup>−/−</sup> mice. Data represent the mean ± S.D. for six animals in each group. p < 0.05 for the following: *, compared with TO-fed counterparts; #, compared with WT counterparts.

|            | WT | TS | Scd1<sup>−/−</sup> | Scd1<sup>−/−</sup> |
|------------|----|----|-------------------|-------------------|
| A. Hepatic TG fatty acid composition (μmol/g) |    |    |                   |                   |
| 16:0       |    |    |                   |                   |
| 16:1       |    |    |                   |                   |
| 18:0       |    |    |                   |                   |
| 18:1       |    |    |                   |                   |
| B. Plasma fatty acids (nmol/100 μl) |    |    |                   |                   |
| 16:0       |    |    |                   |                   |
| 16:1       |    |    |                   |                   |
| 18:0       |    |    |                   |                   |
| 18:1       |    |    |                   |                   |
**SCD1 Mediates the Lipogenic Effects of Dietary Saturated Fat**

Desaturation of Dietary Saturated Fat Is Required for Induction of de Novo Lipogenesis—Dietary saturated fats have been implicated in the induction of lipogenesis and in promoting the obesogenic pathway (4). However, the rapid intracellular desaturation of stearate in WT mice (Fig. 3) combined with the differential effects of stearate in WT and Scd1−/− animals in our study further reiterate the need to separate the molecular effects of stearate from those of endogenously synthesized oleate in the lipogenic pathway.

In this regard, we measured expression of several genes encoding lipogenic enzymes in livers of WT and Scd1−/− mice. Relative expression of genes was compared between dietary treatment groups as well as genotypes. In WT mice, TS feeding indeed caused greater induction of key lipogenic genes such as Acs1 (acyl-CoA synthetase 1), Acc, Fas, and Gpat, relative to TO-fed counterparts. TO-fed WT and Scd1−/− mice had 2–4-fold lower lipogenic gene expression compared with TO-fed counterparts. TO-fed WT and Scd1−/− mice had similar levels of lipogenic gene expression, which were significantly lower than TS-fed WT animals. Thus, the order of lipogenic gene expression was as follows: WT TS > WT TO = Scd1−/− TO > Scd1−/− TS. This differential induction of lipogenic genes after stearate feeding in WT and Scd1−/− mice provides further insight into the mechanism of action of saturated fat.

The induction of lipogenic genes by saturated fat in WT mice previously has been shown to be mediated by SREBP-1c and its requisite coactivator PGC-1β (4). Because stearate induces lipogenic genes in WT animals but not in Scd1−/− mice, we hypothesized that Scd1−/− mice may be protected from stearate-induced up-regulation of SREBP-1c. To test this hypothesis, we measured expression of Srebp-1c and Pgc-1β as well as nuclear levels of SREBP-1. TS-fed Scd1−/− mice had a 5-fold reduction of Srebp-1c and Pgc-1β expression relative to both TS-fed WT mice as well as TO-fed Scd1−/− mice (Fig. 4B). This translated to virtually undetectable levels of the mature form of SREBP-1 (Fig. 4C), corresponding with the extremely low lipogenic gene expression observed in TS-fed Scd1−/− animals (Fig. 4A). SREBP-2 gene expression and maturation were not changed in any group (data not shown). When high levels of oleate were added to the diets of Scd1−/− mice, SREBP-1 protein levels and gene expression, as well as Pgc-1β gene expression were restored to levels similar to WT animals (Fig. 4B). These data indicate that in the absence of SCD, dietary stearate does not promote Srebp-1c or Pgc-1β expression and consequent lipogenesis, and thus, oleate, whether dietary or endogenous, is required for maximal induction of SREBP-1 and Pgc-1β.

Although this repression of SREBP-1 and Pgc-1β was observed after 7 days of oleate deprivation in Scd1−/− mice, a very short 2-day feeding regimen did not cause any changes in Srebp-1c or Pgc-1β expression in any of the groups (data not shown). This further indicates that depletion of oleate in TS-fed
SCD1 Mediates the Lipogenic Effects of Dietary Saturated Fat

Scd1<sup>−/−</sup> mice may mediate the decrease in SREBP-1 and Pgc-1β levels in these animals after 7 days of stearate feeding.

SCD1 Is Induced by Stearate Prior to Other Lipogenic Genes—Because stearate induces lipogenic genes in WT but not in Scd1<sup>−/−</sup> mice, we hypothesized that endogenously synthesized oleate serves as an intracellular signal for induction of lipogenesis. If this is true, induction of Scd1 by stearate should precede the induction of other lipogenic genes. To test this hypothesis, we measured lipogenic gene expression after 2 days of TS or TO feeding to observe the acute effects of these two dietary fatty acids on Scd1 expression. Relative to oleate, stearate did not significantly induce lipogenic genes in WT or Scd1<sup>−/−</sup> mice after 2 days (Fig. 4D). In fact, Acc and Fas expression was significantly higher in TO-fed WT animals than in TS-fed counterparts after 2 days of feeding. Unlike other lipogenic genes, however, Scd1 gene expression was already induced 4-fold in TS-fed WT mice, relative to TO-fed mice by the 2-day time point (Fig. 4D). These results clearly indicate that induction of Scd1 by stearate occurs prior to induction of other lipogenic genes. In the absence of SCD, TS feeding does not induce lipogenesis either after a short 2-day feeding (Fig. 4D) or after a longer 7-day feeding period (Fig. 4A), underscoring the role of SCD in stearate-induced lipogenesis.

Stearate Increases Fatty Acid Oxidation in the Absence of SCD—TS-fed WT and Scd1<sup>−/−</sup> mice were significantly leaner than TO-fed counterparts, despite higher food intake (Table 1). Furthermore, although increased SCD activity (Fig. 3) could explain the lack of accumulation of stearate after TS feeding in WT mice (Table 2), the fate of dietary stearate in TS-fed Scd1<sup>−/−</sup> mice was puzzling. We have shown previously that Scd1<sup>−/−</sup> mice have higher rates of hepatic fatty acid oxidation (31) accompanied by increased oxygen consumption and energy expenditure (14). Because energy balance is affected both by energy consumption and expenditure, we hypothesized that the lower adiposity and lack of stearate accumulation in TS-fed animals could be a result of increased fatty acid oxidation.

Mitochondrial fatty acid oxidation was measured after 7 days of TS or TO feeding. TS-fed WT and Scd1<sup>−/−</sup> mice had 1.5- and 2.4-fold higher rates of fatty acid oxidation, respectively, than TO-fed counterparts (Fig. 5A). Notably, TS-fed Scd1<sup>−/−</sup> mice had the highest rates of fatty acid oxidation, corresponding with their protection from stearate-induced obesity. Expression of key genes of fatty acid oxidation such as Cpt-1, Fiaf (fasting induced adipocyte factor), and Lcad (long chain acyl-CoA dehydrogenase) were 1.8–7.7-fold higher after TS feeding than after TO feeding in both WT and Scd1<sup>−/−</sup> animals (Fig. 5B). Aox (acyl-CoA oxidase), a gene encoding an enzyme of peroxisomal oxidation, was not significantly different in TS- or TO-fed Scd1<sup>−/−</sup> mice.

Peroxisome proliferator-activated receptor-α (PPAR-α) is a nuclear receptor that is known to control expression of genes of lipid oxidation (40, 41). Expression of Ppar-α was increased by 2–4-fold in TS-fed WT and Scd1<sup>−/−</sup> animals (Fig. 5A). PPAR-γ coactivator-1α (PGC-1α) is a protein that is known to mediate the fasted response in liver and can coactivate members of the nuclear receptor family, including PPAR-α, to activate genes encoding enzymes of mitochondrial fatty acid oxidation (42). Expression of Pgc-1α was also increased by 2–4-fold in TS-fed WT and Scd1<sup>−/−</sup> animals (Fig. 5A).

Although TS feeding caused greater induction of genes of fatty acid oxidation in both WT and Scd1<sup>−/−</sup> mice, we were surprised by the relatively lower fold change of these genes in Scd1<sup>−/−</sup> mice compared with WT counterparts (Fig. 5A). We hypothesized that because TS-fed Scd1<sup>−/−</sup> mice are so lean by the end of the 7-day feeding period, their relatively lower expression of oxidative genes may be a compensatory effect of increased fatty acid oxidation earlier in the feeding period. Therefore, we measured expression of fatty acid oxidation genes after a 2-day short term feeding. As expected, after a short term feeding regimen, Scd1<sup>−/−</sup> mice had higher expression of oxidative genes relative to WT counterparts (Fig. 5C), with TS-fed Scd1<sup>−/−</sup> mice displaying the highest induction of fatty acid oxidation genes relative to all other groups. In WT mice, all...
oxidative genes except Aox continued to be higher in TS-fed animals compared with TO-fed animals (Fig. 5C).

These results demonstrate that dietary stearate has two clear fates within the cell. It is either rapidly desaturated by SCD1, as in the case of TS-fed WT animals (Fig. 3), or in the absence of SCD1, it promotes increased mitochondrial fatty acid oxidation and is oxidized (Fig. 5A). Thus, TS-fed Scd1−/− mice do not accumulate significant amounts of saturated or monounsaturated lipids in their tissues or plasma, causing them to be protected from weight gain and adiposity despite consuming a very high saturated fat diet.

**Glycogen Depletion and AMPK Activation in Stearate-fed Scd1−/− Mice**—Apart from white adipose tissue, liver glycogen is a sensitive indicator of the intermediate energy stores of the animal. We measured hepatic glycogen levels and found that TS-fed WT and Scd1−/− animals had 2.4- and 6.6-fold lower hepatic glycogen accumulation, respectively, compared with their TO-fed counterparts (Fig. 6A). Notably, the TS-fed Scd1−/− mice had severe depletion of hepatic glycogen.

Another potent sensor of cellular energy status is the AMP-activated protein kinase, which is not only activated by increasing cellular AMP levels but has also been shown to be regulated by glycogen stores (43). Because glycogen stores were depleted and fatty acid oxidation rates were increased significantly by stearate feeding, especially in Scd1−/− mice, we measured AMPK activity and phosphorylation in the liver after TS or TO feeding. TS-fed Scd1−/− mice had almost 2-fold higher AMPKα1 activity than TO-fed counterparts and over 40% higher activity than TS-fed WT animals (Fig. 6A). These changes in activity were accompanied by a corresponding increase in AMPKα1 serine phosphorylation (Fig. 6B). Total levels of AMPKα1 (Fig. 6B), as well as AMPKα2 activity and protein levels (data not shown), were unchanged. Also, AMPK activity was not increased by TS feeding in WT mice, indicating that unlike Scd1−/− mice, TS-fed WT animals were not in a state of energy deficit.
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Thus, mitochondrial fatty acid oxidation and AMPK activity were both highest in TS-fed Scd1−/− mice, in addition to depleted white adipose tissue and hepatic glycogen stores in these animals. These changes underscore the role of SCD as a molecular switch mediating the cellular fate of dietary saturated fat and help explain the protection from stearate-induced obesity in Scd1−/− mice.

DISCUSSION

Studies examining the molecular effects of dietary saturated fat have been complicated by the ability of cells and animal models to desaturate these lipids into monounsaturated fatty acids. Thus, the use of the Scd1−/− mouse model circumvents this pathway to specifically examine the molecular effects of saturated fats. Furthermore, the use of two different high fat diets that differ only in the degree of desaturation of the fat allows us to eliminate other potential confounding factors such as caloric content of diet, carbohydrate source, and differences in chain length of dietary fat, all of which have been shown to have effects on cellular metabolism (44–46). Although these single-fat purified diets are not completely representative of human dietary fat intake, they serve as viable models to study the molecular effects of particular fatty acids in an effort to better understand disease processes.

Based on our observations, we propose that cellular oleate generated by SCD is likely to mediate the lipogenic effects previously attributed to saturated fats (4, 5). To support the essential role of SCD in this lipogenic process, we present here three distinct lines of evidence. 1) Scd1−/− mice are protected from stearate-induced lipogenesis. 2) Stearate induces Scd1 gene expression prior to that of other lipogenic genes. 3) Oleate deprivation leads to loss of SREBP-1 processing and reduction of Srebp-1c and Pgc-1β expression in Scd1−/− mice, leading to a consequent reduction in de novo lipogenesis. In the absence of SCD, stearate promotes glycogen depletion and AMPK activation as well as fatty acid oxidation, resulting in depletion of adipose stores and protection from saturated fat-induced obesity. It is likely that palmitate, which can be elongated to stearate, would behave in a similar manner (16), as SCD1 does not show a preference for stearate over palmitate as a substrate (47).

**Scd1−/− Mice Are Protected from Stearate-induced Lipogenesis**—We have shown that SCD is induced in response to glucose, fructose, and high fat feeding (9, 15, 16), but differential regulation depending on type of dietary fat is as yet unestablished. The results of this study clearly demonstrate that SCD is induced in response to dietary stearate in WT animals. Although stearate also induces other lipogenic genes in WT mice, Scd1−/− mice are protected from this lipogenic effect, indicating that the endogenous conversion of stearate into oleate by SCD is essential for the lipogenic effects of dietary saturated fat. Other activators of SCD, including fructose (16), have been shown to cause a concomitant increase in de novo lipogenesis in WT animals, whereas Scd1−/− mice are protected from this effect. Based on these observations, we propose that the endogenous product of SCD, oleate, acts as a signal of energy influx and induces lipogenesis.

**Searate Induces SCD Prior to Other Lipogenic Genes**—Further evidence for the critical role of SCD in the lipogenic pathway comes from our observation that induction of Scd1 by stearate precedes the induction of other lipogenic genes. SCD1 is induced as early as 2 days after stearate feeding, although induction of genes such as Acc, Fas, and Gpat does not occur until later in the feeding period. These observations help clarify why Scd1−/− mice are protected from the lipogenic effects of dietary saturated fat. The early induction of Scd1 by stearate also suggests that stearate directly induces SCD1 and that this induction may be independent of changes in SREBP-1c.

**Oleate Deprivation Leads to Reduction of SREBP-1c and PGC-1β in Scd1−/− Mice**—A recent study found that the induction of lipogenic genes by dietary saturated fat involves the transcription factor SREBP-1c and its coactivator PGC-1β (4). Similar to this, we also found that relative to chow feeding (data not shown), both TS- and TO-enriched high fat diets increased Srebp-1c and Pgc-1β expression by 3-fold in WT mice. In Scd1−/− mice, however, dietary stearate caused a 5-fold reduction of Srebp-1c and Pgc-1β expression relative to TO feeding and almost undetectable levels of nuclear SREBP-1 (Fig. 4, B and C). Thus, it is likely that the extremely low expression of lipogenic genes in TS-fed Scd1−/− mice (Fig. 4, A and D) is mediated by decreased SREBP-1c and PGC-1β levels. These data indicate that saturated fats do not directly up-regulate these nuclear factors as previously thought (4) but must first be desaturated by SCD1 in order to elicit a lipogenic response.

Levels of SREBP can be modulated at the level of gene expression as well as protein maturation (20). In this study, by 7 days, but not 2 days (data not shown), of stearate feeding, both nuclear levels of SREBP-1 as well as Srebp-1c gene expression were reduced in Scd1−/− mice, suggesting that a decrease in maturation because of oleate deprivation may precede a decrease in Srebp-1c gene expression. This lack of changes in SREBP-1 levels due to acute dietary manipulation suggests that the requirement for cellular oleate in induction of SREBP-1c may be at the level of SREBP-1 maturation, which subsequently affects its own gene expression. The mechanisms by which oleate affects SREBP-1c maturation are not known, but it is plausible that stearate feeding in Scd1−/− mice causes changes in parameters such as fluidity of the endoplasmic reticulum membrane, thereby causing a reduction in the proteolytic maturation of SREBP. In support of this notion, it has been reported previously that membrane fluidizing compounds such as cetyltrimethylammonium bromide lead to induction of SREBP-regulated reporter constructs (48).

**Differential Roles for Dietary Versus Endogenously Synthesized Oleate**—A high monounsaturated fat diet has been shown to cause enrichment not only of hepatic lipids but also of plasma lipids with oleate, which correlates with higher rates of coronary artery atherosclerosis in a non-human primate model (12). In this study, unlike hepatic triglyceride composition, plasma oleate levels remained significantly lower in oleate-fed Scd1−/− mice relative to WT mice (Table 2, part B). This could be due to increased oleate uptake and oxidation in peripheral tissues such as brown adipose tissue or skeletal muscle. Alternatively, it is possible that dietary and endogenous oleate are functionally compartmentalized separately from each other within the liver. Previous studies from our laboratory and others have provided evidence for this notion of compartmentalization of intracellular...
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lar lipids depending on their source (6, 7). The existence of separate pools of hepatic oleate could explain the reduced levels of plasma oleate in Scd1−/− mice (Table 2, part B). Dietary oleate may be preferentially retained in hepatic TG in these mice, where it may serve critical roles in determining cell structure, membrane fluidity, or cellular signaling. In further support of the “functional compartmentalization” hypothesis, we find that in WT mice, dietary stearate, which is converted to oleate (Fig. 3), induces a more robust lipogenic response than dietary oleate (Fig. 4A). This differential induction of de novo lipogenesis indicates that endogenously synthesized oleate is more readily available to influence lipogenic gene expression in WT mice.

Effects of Dietary Stearate on Oxidation: Potential Roles for PGC-1α and AMPK—Because Scd1−/− mice cannot desaturate cellular stearate, they respond to dietary intake of stearate by up-regulating fatty acid oxidation (Fig. 6). Coupled with their inability to induce de novo lipogenesis, this increase in oxidation leads to weight loss and decreased adiposity (Table 1). Interestingly, whereas WT mice induce SCD activity and lipogenesis in response to stearate, they also up-regulate fatty acid oxidation (Fig. 6). Although seemingly paradoxical at first, this dual effect of stearate in WT mice is likely to be a protective mechanism to prevent cellular build-up of saturated fats, which are known to have cytotoxic effects (49, 50). Consumption of a diet extremely high in stearate, such as in our study, likely results in influx of stearate above and beyond the threshold for desaturation by SCD. Thus, oxidative pathways may be concurrently up-regulated in WT mice to clear cellular stearate as rapidly as possible.

In this study, we found that genes of fatty acid oxidation, including the transcriptional coactivator Pgc-1α, were induced by stearate feeding (Fig. 5B). It was recently shown that stearate, but no other fatty acid, can activate PGC-1α (4), although the physiological consequence of such an activation is unclear. Our current feeding study also points to PGC-1α as a possible candidate in mediating the induction of oxidative genes by dietary stearate. Ongoing studies should clarify the exact role, if any, of PGC-1α in mediating the induction of oxidative genes after stearate feeding.

Another regulator of fatty acid oxidation, AMPK, is a potent sensor of acute changes in energy status and is activated by rising levels of cellular AMP. There is also evidence that AMPK may sense glycogen stores as a measure of the intermediate energy status of the animal (43). We have shown previously that AMPK is activated by SCD1 deficiency, but the mechanisms leading to this activation are not fully understood (31). In this study, stearate feeding caused depletion of adipose and hepatic glycogen stores to extremely low levels (Fig. 6A) in Scd1−/− mice. In marked contrast from Scd1−/− mice, WT animals did not show as severe a depletion of adipose tissue or hepatic glycogen after stearate feeding, possibly due to their ability to induce de novo lipogenesis in response to dietary stearate (Fig. 4). Concomitant with depletion of hepatic glycogen, TS-fed Scd1−/− animals, but not WT animals, also had increased AMPK activity (Fig. 6B). These data suggest reciprocal regulation of AMPK and glycogen stores in Scd1−/− mice. Given that Scd1−/− mice have higher metabolic rates and increased rates of energy expenditure (14, 51), possibly leading to increased cellular AMP concentrations, it is possible that similar mechanisms may be involved in AMPK activation in the SCD1-deficient state.

In summary, the results of this study indicate that dietary stearate has two clear fates within the cell (Fig. 7). In WT animals, stearate acutely induces SCD1 and is rapidly desaturated to generate endogenous oleate. This endogenous oleate mediates the induction of de novo lipogenesis generally associated with intake of dietary saturated fat, further up-regulating SCD1 levels and ultimately leading to fat storage and obesity (Fig. 7). In the absence of SCD, cellular stearate promotes oxidation and is cleared from the cell. Continuous oxidation results in depletion of white adipose tissue, and depletion of hepatic glycogen stores may signal activation of AMPK, which is known to be activated by SCD1 deficiency. Thus, in the absence of SCD, dietary stearate does not accumulate in the body but is instead oxidized, leading to protection from saturated fat-induced obesity in Scd1−/− mice.

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