SKIN-SPECIFIC DELETION OF STEAROYL-CoA DESATURASE-1 ALTERS SKIN LIPID COMPOSITION AND PROTECTS MICE FROM HIGH-FAT DIET-INDUCED OBESITY

Harini Sampath1,4, Matthew T. Flowers2, Xueqing Liu1, Chad M. Paton2, Ruth Sullivan3, Kiki Chu2, Minghui Zhao2, and James M. Ntambi1,2

From the Departments of 1Nutritional Sciences and 2Biochemistry, and 3UW-Madison Research Animal Resources Center, University of Wisconsin-Madison, Madison, WI, 53706.

Running Title: Skin SCD1 deletion increases energy expenditure

Address Correspondence to: James M. Ntambi, Ph.D., University of Wisconsin-Madison, 433, Babcock Drive, Madison, WI, 53706, Tel. 608-265-3700; Fax.608-265-3272; E-mail: ntambi@biochem.wisc.edu

Stearoyl Co-A desaturase-1 (SCD1) catalyzes the synthesis of monounsaturated fatty acids and is an important regulator of whole body energy homeostasis. Severe cutaneous changes in mice globally deficient in SCD1 also indicate a role for SCD1 in maintaining skin lipids. We have generated mice with a skin-specific deletion of SCD1 (SKO) and report here that SKO mice display marked sebaceous gland hypoplasia and depletion of sebaceous lipids. In addition, SKO mice have significantly increased energy expenditure and are protected from high-fat diet induced obesity, thereby recapitulating the hypermetabolic phenotype of global SCD1 deficiency. Genes of fat oxidation, lipolysis and thermogenesis, including uncoupling proteins and PPAR-gamma co-activator -1 alpha are upregulated in peripheral tissues of SKO mice. However, unlike mice globally deficient in SCD1, SKO mice have an intact hepatic lipogenic response to acute high-carbohydrate feeding. Despite increased basal thermogenesis, SKO mice display severe cold intolerance due to rapid depletion of fuel substrates, including hepatic glycogen, in order to maintain core body temperature. These data collectively indicate that SKO mice have increased cold perception due to loss of insulating factors in the skin. This results in upregulation of thermogenic processes for temperature maintenance at the expense of fuel economy, illustrating crosstalk between the skin and peripheral tissues in maintaining energy homeostasis.

Obesity is a multi-factorial disease stemming from a combination of genetic, dietary, and lifestyle factors and the interaction between these components (1-3). The microsomal enzyme, stearoyl-CoA desaturase-1 (SCD1), is a critical control point in the development of metabolic diseases, including obesity and insulin resistance. SCD1 catalyzes the conversion of saturated fatty acids, such as palmitate (16:0) and stearate (18:0), into their delta-9 monounsaturated products, palmitoleate (16:1 n-7) and oleate (18:1 n-9), respectively. Mice lacking the SCD1 enzyme due to a global deletion of the Scd1 gene (GKO) are lean and protected from diet- and leptin-deficiency induced obesity. These mice have a marked increase in energy expenditure and almost complete protection from high-fat diet induced weight gain and glucose intolerance (4-10).

Since SCD1 is expressed in multiple tissues including liver, brown and white adipose tissue, skeletal muscle, and skin, it has been difficult to determine the relative contributions of these tissues to the dramatically altered metabolic phenotypes of GKO mice. Studies using antisense oligonucleotide (ASO)-mediated approaches to knock down Scd1 expression have reported protection from diet-induced weight gain and hepatic insulin resistance upon hepatic SCD1 inhibition (11-13). However, while the liver is a major target of these ASOs, they have also been reported to affect expression of target genes in adipose tissue (13,14), and possibly other organs (15). Using Cre-recombinase mediated inhibition of hepatic Scd1, we recently reported that chronic deletion of SCD1 specifically in liver does not protect mice from high-fat diet induced obesity (16), suggesting that extra-hepatic tissues may play a more prominent role in the increased energy expenditure phenotype of global SCD1 deficiency (16).

In addition to their hypermetabolic phenotype, global SCD1 deficiency also elicits marked cutaneous phenotypes including dry skin,
alopecia and sebocyte hypoplasia (7,17,18). Given the severity of this skin phenotype in GKO mice, we sought to establish a specific role for SCD1 in the skin. In our current study, we used the Cre-lox system to generate mice with a skin-specific deletion of SCD1 (SKO). We report here that SKO mice have a severe paucity of lipid-enriched sebocytes in the skin, resulting in dry skin, alopecia, and marked alterations in levels of key skin lipids. Unlike mice with global or liver-specific deletion of SCD1 (7,16), SKO have an intact hepatic lipogenic response to dietary stimuli. However, deletion of skin SCD1 completely recapitulates the increased energy expenditure phenotype of GKO mice (7) and protects SKO mice from high-fat diet induced obesity, hepatic steatosis and glucose intolerance. However, deletion of skin SCD1 completely recapitulates the increased energy expenditure phenotype of GKO mice (7) and protects SKO mice from high-fat diet induced obesity, hepatic steatosis and glucose intolerance. Elevation of genes encoding for cold-inducible factors, including peroxisome proliferator-activated receptor gamma co-activator-1 alpha (Pgc-1α) and uncoupling proteins (Ucops) in brown and white adipose tissue and skeletal muscle of SKO mice, suggest upregulation of thermogenic processes for maintenance of core body temperature in SKO mice. Furthermore, the hypermetabolic phenotype of SKO mice, coupled with the loss of insulating factors in the skin, result in severe cold intolerance in SKO mice that is ameliorated by prior feeding with a high-fat diet. To the best of our knowledge, this study represents the first example of skin-specific deletion of a lipogenic enzyme resulting in profound changes in systemic energy metabolism. These data elucidate an as yet under-appreciated role for skin SCD1 in triggering the altered metabolic phenotypes caused by global SCD1 deletion.

MATERIALS AND METHODS

Animals and diets
The generation of mice having the third exon of the Scd1 gene flanked by loxP sites (Scd1<sup>floxflo</sup>) has been previously described (16). To generate skin-specific Scd1-knockout mice, female Scd1<sup>floxflo</sup> mice were crossed with Keratin-14 Cre mice (K14-Cre; mixed Swiss Webster; C57Bl/6J; CBA/J; Jackson Laboratory, Bar Harbor, ME) (19) that were fully backcrossed onto the C57Bl/6J background to generate compound heterozygous (Scd1<sup>floxflo,Cre+</sup>) mice. Male Scd1<sup>floxflo,Cre+</sup> mice were subsequently mated with female Scd1<sup>floxflo,Lox</sup> (Lox) mice, generating Scd1<sup>floxflo,Lox,Cre+</sup> mice (SKO). For litter expansion, male SKO mice were bred with female Lox mice. All mice were on a fully backcrossed C57Bl/6j background, and Lox and SKO mice were born in an expected ratio of 1:1. Genotyping was performed by PCR using genomic DNA isolated from a tail clip, as previously described (16). Mice were maintained on a 12 hr light/dark cycle with free access to water and either a standard chow diet (Purina 5008) or special formulations as indicated. Detailed diet formulations and feeding conditions are presented in Supplementary Methods. All animals were sacrificed by isoflurane overdose without fasting, unless otherwise indicated, and tissues and plasma were rapidly removed, snap-frozen in liquid nitrogen, and stored at -80° C. For preparation of nuclear protein, fresh liver was homogenized immediately upon excision. All in vivo experimental procedures were approved by the Animal Care Research Committee of the University of Wisconsin-Madison.

Cold tolerance tests
Cold tolerance tests were performed as previously described (20) in 12-week old male and female Lox and SKO mice that were individually caged and allowed ad libitum access to food and water.

Histology
Fresh liver, white adipose and skin samples from similar sites in age and gender-matched Lox and SKO mice were fixed in 10% buffered formalin and paraffin-embedded for sectioning and staining with H&E. For Oil Red O staining of skin samples, frozen skin samples were embedded in cryosectioning medium prior to sectioning and staining.

Energy expenditure
Energy balance was assessed using the PhysioScan Oxygen Consumption/Carbon Dioxide Production System (AccuScan Instruments Inc.) at The University of Cincinnati Mouse Metabolic Phenotyping Center. 12-week old male mice were placed in the PhysioScan chamber with food 3 hours before the dark cycle and energy expenditure was measured for the next 48 hours, as previously described (21). VO<sub>2</sub> and VCO<sub>2</sub> values were normalized to total body weights, as
there were no significant differences in body weights between the genotypes. Heat was calculated from recorded VO₂ values.

Analytical Procedures
Glucose tolerance tests, basal plasma glucose and plasma insulin measurements were performed in mice fasted for 4 hours as previously described (16). Hepatic glycogen was measured by an enzyme coupled spectrophotometric assay as previously described (16). Plasma insulin was measured using an insulin RIA, and plasma leptin was measured with a mouse leptin ELISA (both from Millipore). Plasma cholesterol and TG were analyzed by colorimetric enzyme assay using the Infinity TG and cholesterol reagents (Thermo Electron). Skin free cholesterol (FC) was measured using a free cholesterol colorimetric enzyme assay (Wako). Plasma T₃ and T₄ were measured at Anilytics, Inc. (Gaithersburg, MD).

Lipid analyses
Skin surface lipids were extracted by dipping carcasses into 100 mL of chloroform:methanol (2:1, v/v) followed by 100 mL of acetone. Lipid extracts were pooled, dried down under a stream of N₂, and resuspended in equal volumes of chloroform:methanol (2:1, v/v) for loading onto TLC plates. Hepatic lipids were extracted from 30 mg of frozen liver by the Folch method (22). Hepatic and skin lipids were separated and analyzed by TLC and gas chromatography as previously described, with penta- and heptadecanoic acids added as internal standards (17,23,24). Details on developing solvents are presented in Supplemental Methods.

Real-Time Quantitative PCR
Quantitative PCR was performed as previously described (16). Results are expressed as mean ± SEM after normalizing to expression of a housekeeping gene (Arbp or Cyclophilin) using the ΔΔCt method. Primer sequences are available upon request.

Western Blot analysis
Nuclear extracts (25) and microsomal fractions (26) were prepared as described for immunoblotting of SREBP and SCD1, respectively. Polyclonal anti-SREBP-1 (BD Pharmingen), SREBP-2 (kindly provided by Dr. Jay Horton), Histone H₃ (Santa Cruz), or SCD1 (Santa Cruz) were used as primary antibodies, followed by the appropriate IgG-HRP conjugated secondary antibody. Proteins were visualized by ECL.

Data and statistical analyses
Data are expressed as mean±SEM with comparisons carried out using a Student’s t-test or analysis of variance (ANOVA) where appropriate, using the program GraphPad Prism. When a significant F-ratio was obtained (significance p < 0.05), post-hoc analysis was conducted between groups using a multiple comparison procedure with Bonferroni/Dunn post-hoc comparison. p-values < 0.05 were considered significant.

RESULTS
Skin-specific SCD1 deletion causes sebocyte hypoplasia
We previously generated mice with a floxed allele of Scd1 to allow for tissue-specific deletion of the gene (16). Scd1lox/lox mice are phenotypically indistinguishable from wild-type (WT) mice and are used as the control mice throughout this study. The keratin-14 gene is expressed ubiquitously in the skin, including in the undifferentiated cells of the sebaceous gland (27,28), which is the only region of skin known to express SCD1 (29). In order to generate skin-specific SCD1 knockout mice (SKO), we crossed female Scd1lox/lox mice to male mice expressing Cre-recombinase under a keratin-14 promoter (19). The resulting male Scd1lox/lox;Cre/+ mice were indistinguishable from WT mice in terms of appearance, viability, body weights and food intake, indicating that the presence of Cre recombinase itself did not cause any apparent toxicity. We subsequently crossed these mice to female Scd1lox/lox mice to generate Scd1lox/lox (Lox) and Scd1lox/lox;Cre/+ (SKO) mice. SKO mice could be easily distinguished by their closed eye fissures, dry skin, and alopecia (Supplementary Figure 1), which were apparent at weaning. RT-PCR analysis of skin RNA from SKO mice revealed a 95% reduction in skin Scd1 expression, relative to Lox controls (Figure 1A), without changes in expression in liver, white (WAT) or brown adipose tissue (BAT), skeletal muscle (SM) or other tissues of SKO mice (Figure 1A). SCD1
protein levels were also significantly decreased only in skin of SKO mice (Figure 1B). A basal level of SCD1 protein was still detectable in skin of SKO mice, likely due to contamination from residual subcutaneous adipose tissue in skin samples. Given the closed eye fissure phenotype of the SKO mice, we also measured expression of Scd1 in the Harderian gland, an eye-associated sebaceous gland (30,31), and found that Scd1 gene expression was decreased by over 50% in Harderian glands of SKO mice, accompanied by significant atrophy of the Harderian glands in these mice (data not shown).

In order to identify any structural changes in the skin, paraffin-embedded skin samples were sectioned and stained with hematoxylin and eosin (H&E). Skin sections from SKO mice revealed a marked paucity of lipid-filled sebaceous glands with foamy sebocytes that would be expected around the hair follicle (Figure 1C). In the region where the sebaceous gland would be expected to be located (black arrows), some follicles had nodular clusters of cells, vaguely reminiscent of a sebaceous gland, but these cells never achieved the foamy vacuolation expected in mature sebocytes. Oil Red O staining of frozen skin sections confirmed the virtual absence of lipid-rich sebaceous glands in SKO mice (Figure 1C). In the region where the sebaceous gland would be expected to be located (black arrows), some follicles had nodular clusters of cells, vaguely reminiscent of a sebaceous gland, but these cells never achieved the foamy vacuolation expected in mature sebocytes. Oil Red O staining of frozen skin sections confirmed the virtual absence of lipid-rich sebaceous glands in SKO mice (Figure 1C). In the region where the sebaceous gland would be expected to be located (black arrows), some follicles had nodular clusters of cells, vaguely reminiscent of a sebaceous gland, but these cells never achieved the foamy vacuolation expected in mature sebocytes.

**Skin SCD1 deficiency selectively decreases sebaceous lipids on skin surface**

Given the marked sebocyte hypoplasia in the skin (Figure 1C), we hypothesized that SKO mice may have significant changes in skin lipid composition. We extracted and separated skin surface lipids by thin-layer chromatography (TLC). Sebaceous lipids in mice consist mainly of cholesterol esters (CE), wax mono-esters (WE), triglycerides (TGs), and wax di-esters (WDEs). We did not detect any significant changes in levels of either CE or WE in skin of SKO mice (Figure 2B). However, levels of the two largest lipid fractions, TGs (Figure 2A and 2B) and WDEs (Figure 2B), were reduced by 72% and 84%, respectively, in skin lipids from SKO mice (Table 1). The fatty acid composition of TGs and WDEs was analyzed by gas chromatography (Table 1). Given the large changes in total TG and WDE levels, almost all fatty acids were significantly decreased in these fractions in SKO mice (Table 1). Notably, however, levels of palmitate (16:0) and stearate (18:0) were not significantly decreased in the WDEs (Table 1) of SKO mice. Products of delta-9 desaturation including 16:1, 18:1, 20:1, and 22:1 were all dramatically reduced in both TGs and WDEs (Table 1) from SKO mice, relative to Lox controls. Consequently, the delta-9 desaturation index of skin surface lipids was significantly lowered in both the TG (Figure 2D) and the WDE fractions (Figure 2E) from SKO mice, relative to Lox controls, indicating that the decreased sebaceous lipids in the skin of SKO mice results from a specific loss of delta-9 desaturase activity in the skin of these mice.

**SKO mice have increased energy expenditure and are resistant to high-fat diet-induced obesity, adiposity and glucose intolerance**

In order to determine if skin-specific deletion of SCD1 has any effects on energy metabolism, we measured energy expenditure in individually-caged 12-week old male Lox and
SKO mice via indirect calorimetry. O2 consumption and CO2 production were measured for two consecutive 24-hour periods with alternating 12-hour dark and light cycles. SKO mice had significantly higher O2 consumption (Figure 3A) and CO2 production (Figure 3B) than Lox mice during both day and night cycles, indicating increased resting metabolic rate and increased energy expenditure in SKO mice. In fact, O2 consumption (Supplementary Figure 2A) and CO2 production (Supplementary Figure 2B) were increased to a similar extent in SKO mice as in mice with a global deletion of SCD1 (GKO). Similarly, heat output (kcal/hour) was significantly increased in SKO mice (Supplementary Figure 2C) during both day and night cycles, consistent with increased energy expenditure. Respiratory quotients were similar in both Lox and SKO mice (Supplementary Figure 2D). Taken together, these data indicate that skin-specific deletion of SCD1 completely recapitulates the increased metabolic rate observed due to global SCD1 deletion.

Given that SKO mice have increased energy expenditure relative to Lox mice, we hypothesized that they would be protected from high-fat diet (HFD)-induced obesity. 8-week-old male Lox and SKO mice were individually caged and fed a rodent chow diet or a hypercaloric HFD (Research Diets 12492), containing 35% fat by weight (60% fat kcals). SKO mice were hyperphagic on both diets and ate 2-fold and 1.5-fold more than Lox mice on the chow and HFD, respectively (Table 2). Body weights were similar in both Lox and SKO animals on a chow diet (Figure 3C). However, despite similar starting body weights, Lox mice gained 80% more body weight on the HFD than SKO mice (Figure 3D). Glucose tolerance was assessed in SKO mice by performing oral glucose tolerance tests after 7-weeks of chow- or HFD- feeding. SKO mice showed significantly improved glucose tolerance curves after both chow- (Figure 3E) and HFD-feeding (Figure 3F), compared to Lox mice. Relative to Lox mice, peak plasma glucose levels were 46% and 38% lower after chow- and HFD-feeding, respectively, in SKO mice. While 180-minute plasma glucose levels remained elevated 1.4-fold and 1.9-fold over basal levels in chow- or HFD-fed Lox mice, respectively, they returned to baseline values in SKO mice, indicating improved glucose clearance in SKO mice. After HFD-feeding, plasma insulin levels were significantly elevated in Lox mice, relative to chow-fed counterparts (Table 2). SKO mice, on the other hand, were protected from this diet-induced hyperinsulinemia (Table 2). Together with their improved glucose clearance (Figures 3E, 3F), these data suggest significantly improved insulin sensitivity in SKO mice.

After 8-weeks of feeding, animals were sacrificed and epididymal (E-WAT) and subcutaneous (SC-WAT) white adipose tissue depots were weighed. Corresponding with their lower body weights, SKO mice had 53% and 76% lower E-WAT and 37% and 69% lower SC-WAT accumulation than Lox mice on chow and HFD, respectively (Table 2). While SKO mice did accumulate more adipose tissue after HFD-feeding relative to chow-fed counterparts, they were largely resistant to the increased adiposity observed in Lox controls upon HFD-feeding (Table 1, Figure 3D). Commensurate with their decreased adiposity, both chow- and HFD-fed SKO mice had lower levels of plasma leptin relative to Lox controls (Table 2). H&E staining of E-WAT sections also revealed significantly smaller adipocytes in SKO mice under both chow- and HFD-fed conditions (Figure 3G).

Since increased adiposity is a risk factor for hepatic lipid accumulation, livers from chow- and HFD-fed Lox and SKO mice were stained with H&E. Chow-fed livers from both genotypes did not appear different from each other (Figure 3H). However, after HFD-feeding, Lox mice had significant accumulation of hepatic lipids, which was not apparent in liver sections from SKO mice (Figure 3H). Hepatic lipids were extracted and separated by TLC (Supplementary Figure 2E), and bands corresponding to liver free fatty acids (FFAs), TGs and CEs were scraped and quantified by gas chromatography. While HFD-feeding increased TGs and CEs in both Lox and SKO mice, SKO mice consistently had reduced hepatic TGs and CEs under both dietary conditions (Table 2). FFA levels were not different between SKO and Lox mice under chow-fed conditions (Table 2) but were decreased in SKO livers after HFD-feeding, potentially due to increased esterification of fatty acids into TGs and CEs (Table 2). Analysis of fatty acid composition of these hepatic lipids revealed a generalized decrease in most major fatty acids rather than a selective decrease in any
particular fatty acids (Supplementary Table 1A-C). Plasma TG was not significantly affected by genotype (Table 2). Plasma cholesterol levels increased significantly in Lox mice upon HFD-feeding, but SKO mice did not show a similar increase (Table 2). Hepatic glycogen levels, a measure of the intermediate energy stores of the animal, were consistently decreased in SKO mice under both chow and HFD-fed conditions (Table 2). Together with decreased adipose mass (Table 2), the reduced hepatic glycogen indicates reduced availability of energy reserves in SKO mice, consistent with their increased energy expenditure (Figure 3A, 3B).

**Hepatic induction of genes of de novo lipogenesis is not impaired in SKO mice**

In order to determine if the lower hepatic lipid accumulation and protection from diet-induced obesity are a consequence of decreases in the de novo lipogenesis pathway, we measured hepatic expression of key lipogenic genes (Figure 4A). On a chow diet, SKO mice had significantly lower expression of lipogenic genes including acetyl-CoA carboxylase (Acc), fatty acid synthase (Fas), and Scd1 (Figure 4A). Also, expression of the lipogenic transcription factor, sterol-regulatory element binding protein-1c (Srebp-1c) and its requisite co-activator, PPAR-gamma co-activator-1c (Pgc-1c) were significantly lower in SKO mice, relative to Lox counterparts. After HFD-feeding, levels of all lipogenic genes, except Srebp-1c, were normalized in SKO mice, relative to Lox controls (Figure 4A). Although the magnitude of change from chow to HFD were similar in both SKO and Lox mice, Srebp-1c gene expression remained significantly lower in SKO mice (Figure 4A), suggesting either that this level of induction of Srebp-1c and Pgc-1c is sufficient to restore lipogenic gene expression, or that an alternative Srebp-1c-independent mode of regulation normalizes lipogenic genes in HFD-fed SKO mice.

SREBP-1c is also regulated post-translationally by proteolytic processing of the precursor protein and transit of the mature form to the nucleus (33). Under chow-fed conditions, SKO mice had significantly lower nuclear levels of the mature SREBP-1 protein (Figure 4B), corresponding with the decreased expression of hepatic lipogenic genes (Figure 4A). After HFD-feeding, nuclear levels of SREBP-1 were still lower in SKO mice, relative to Lox controls, but the difference in nuclear SREBP-1 protein levels between Lox and SKO mice was attenuated. This was consistent with the rescue of lipogenic gene expression in HFD-fed SKO mice (Figure 4A), suggesting that impaired lipogenesis is not likely to be the cause of decreased hepatic lipid accumulation in these mice. In contrast, GKO mice had lower nuclear SREBP-1 protein (Supplementary Figure 3A) and reduced hepatic lipogenic gene expression (Supplementary Figure 3B) relative to control mice on a chow-diet, which was not rescued even after HFD-feeding.

To further confirm that SKO mice are able to induce de novo lipogenic genes in response to a dietary stimulus, we subjected Lox and SKO mice to a fasting-refeeding protocol known to robustly induce de novo lipogenesis in the liver. Consistent with their increased energy expenditure, SKO mice lost more body weight in response to fasting than Lox controls (2.7±0.5 g vs. 1.4±0.5 g; p=0.0002). However, upon being given access to food (high-carbohydrate diet), SKO mice consumed significantly more food than Lox counterparts (3.02±0.56 g vs. 2.07±0.24 g; p=0.0009), and regained all body weight lost. Thus, this acute fasting-refeeding protocol did not cause any net changes in body weight in either Lox or SKO mice. As anticipated, Lox mice responded to refeeding a lipogenic diet by dramatically increasing both nuclear levels of mature SREBP-1 (Figure 4C) as well as hepatic lipogenic genes, including Acc, Fas, Scd1, Srebp-1c, and Pgc-1c (Figure 4D). Similarly, SKO mice also responded to refeeding with a robust increase in SREBP-1 maturation (Figure 4C) and hepatic expression of key lipogenic genes, to the same extent as Lox mice (Figure 4D). In contrast, GKO mice subjected to the same fasting-refeeding challenge had significantly lower levels of both nuclear SREBP-1 (Supplementary Figure 3C) as well as hepatic lipogenic gene expression (Supplementary Figure 3D). These results clearly demonstrate that unlike global Scd1 deletion, skin-specific deletion of Scd1 does not impair the hepatic de novo lipogenic pathway and that impaired lipogenesis is not likely to be the basis of protection from diet-induced obesity in SKO mice. Thus, the reduction of hepatic lipogenesis due to global Scd1 deletion (Supplemental Figure 3) are likely to be mediated by Scd1 in the liver (16).
Expression of genes of fatty acid oxidation, lipolysis and uncoupling are induced in SKO mice

Given that hepatic lipogenesis was unaffected in SKO mice after HFD-feeding, we hypothesized that net increases in catabolic processes in the liver and other peripheral tissues may underlie the protection from diet-induced obesity and adiposity observed in SKO mice. We therefore measured markers of fatty acid oxidation, lipolysis and thermogenesis in peripheral tissues including liver, skeletal muscle, brown and white adipose tissues of chow or HFD-fed SKO mice.

Expression of the key genes of fatty acid oxidation, carnitine palmitoyl transferase-1 (Cpt-1) and acyl-CoA oxidase (Aox), were modestly elevated in livers of SKO mice, relative to Lox counterparts, under both dietary conditions (Figure 5A). Expression of the transcriptional co-activator Pgc-1α, which mediates aspects of the fasted response in liver, was increased by 1.5-fold in livers of chow-fed SKO mice; after HFD-feeding, Pgc-1α levels were increased in Lox controls and hence were not significantly different between Lox and SKO mice (Figure 5A). Expression of uncoupling proteins (Ucp)-2 and -5 was significantly induced in SKO mice after both chow- as well as HFD-feeding. While the exact contribution of hepatic uncoupling to energy expenditure is still under debate, it is possible that the increased levels of Ucups in liver contribute to increased proton dissipation, thereby increasing resting metabolic rate and energy expenditure (34).

In rodents and humans, skeletal muscle is a major site of energy metabolism and contributes significantly to whole body thermogenesis. Even slight increases in levels of UCP-2 and UCP-3 in skeletal muscle have been shown to be protective against obesity (35-40). We observed a 1.7- to 2-fold increase in expression of both Ucp-2 and Ucp-3 in skeletal muscle of SKO mice (Figure 5B), which could contribute to their observed protection from diet-induced obesity. PPAR-gamma co-activator-1α (Pgc-1α), a known co-activator of genes encoding for uncoupling proteins (41), was also induced in muscle of SKO mice (Figure 5B). In addition to these thermogenic genes, expression of lipoprotein lipase (Lpl) was modestly induced in muscle of SKO mice (Figure 5B), suggesting increased lipid uptake in skeletal muscle. Increased lipid uptake through LPL in skeletal muscle has been suggested to channel lipids towards oxidation rather than storage (42). Increased white adipose tissue (WAT) uncoupling via UCP-1 has been shown to confer protection against diet-induced obesity by increasing the thermogenic capacity of WAT (43-45). In WAT of chow-fed SKO mice, we detected a 2.5-fold (p=0.05) increase in Ucp-1 expression, relative to Lox controls (Figure 5C). After HFD-feeding, Ucp-1 levels tended to remain higher in SKO mice by up to 4-fold on average (Figure 5C); however, inter-animal variability kept this difference from being statistically significant. We also detected a 1.9- to 2.8-fold increase in levels of Pgc-1α, an important co-activator of Ucp-1 (46,47), in WAT of chow- and HFD-fed SKO mice, respectively (Figure 5C). Expression of Ucp-3, which is also generally restricted to BAT and muscle in rodents (48,49), was modestly increased by 1.4-fold (p=0.06) and 1.7-fold (p=0.007) in WAT of chow- and HFD-fed SKO mice, respectively (Figure 5C). Ucp-2 expression was not increased in WAT of SKO mice (not shown). Under chow-fed conditions, oxidative genes including Cpt-1 and long-chain acyl-CoA dehydrogenase (Lcad) were increased by 2.3- and 3.4-fold, respectively, in SKO mice (Figure 5C); this difference was not observed in HFD-fed animals (Figure 5C). Hormone-sensitive lipase (Hsl) expression was modestly increased by 1.3- to 1.6-fold in chow- and HFD-fed SKO mice, respectively (Figure 5C), suggesting a more catabolic state in WAT of SKO mice, consistent with their decreased adipose mass (Table 2). We did not observe any significant differences in levels of Ppar-gamma, Fas, or Scd1 in WAT of SKO mice (data not shown), suggesting that there was no defect in adipocyte differentiation that could account for decreased adipose mass in SKO mice.

Brown adipose tissue (BAT) is a major site of thermogenesis in rodents and expresses high levels of UCP-1. UCP-1 is induced by cold-exposure and contributes to heat generation at the expense of ATP production, thereby increasing energy expenditure in the whole animal (46,50,51). Ucp-1 expression was modestly elevated in BAT of both chow- and HFD-fed SKO mice, relative to Lox counterparts (Figure 5D). Furthermore, expression of BAT Pgc-1α, which is known to be
robustly induced in response to cold exposure (51), was increased by 2- and 2.5-fold in chow- and HFD-fed SKO mice, respectively (Figure 5D). Expression of genes of fatty acid oxidation including Cpt-1 and Lcad were induced by 1.2- and 1.6-fold, respectively, in SKO mice under both dietary conditions (Figure 5D). Furthermore, expression of Hsl and beta-3 adrenergic receptor (β3-Ar) expression was elevated in chow-fed SKO mice (Figure 5D). Taken together, these results indicate a catabolic state of increased lipolysis and lipid oxidation in BAT of SKO mice, along with increased uncoupling via Ucp-1 and Pgc-1α.

Thyroid hormone signaling is also an activator of BAT thermogenesis (52,53), and in addition to being derived from the circulation, T3 can be generated in situ, by Type II iodothyronine deiodinase (DIO2), which is chiefly expressed in BAT of rodents (53,54). Increased BAT Dio2 expression has been shown to increase energy expenditure, potentially through increased uncoupling via Ucp-1 and Pgc-1α, and confer resistance to diet-induced obesity in mice (55-57). While activation of plasma membrane receptors such as β3-AR and consequent generation of cAMP has been shown to activate Dio2 expression, the Dio2 gene can also be regulated independently of cAMP, especially under conditions such as HFD-feeding (56,57). We found that in BAT of chow-fed SKO mice, Dio2 expression was modestly increased by 1.5-fold, relative to Lox counterparts (Figure 5D). However, upon HFD-feeding, SKO mice had 3.3-fold higher Dio2 expression than Lox counterparts (Figure 5D), corresponding with their protection from HFD-induced obesity.

SKO mice have severe cold sensitivity due to rapid hepatic glycogen depletion and hypoglycemia

Environmental temperatures are a strong determinant of adaptive thermogenesis in homeotherms. Therefore, in order to test if response to cold-exposure is altered in the hypermetabolic SKO mice, we subjected Lox and SKO animals to a 4°C cold challenge and monitored changes in core body temperature. Starting body temperatures were not different between Lox and SKO mice (Figure 6A). However, upon cold exposure, SKO animals displayed a severe inability to maintain body temperature (Figure 6A), and all SKO mice went into a state of extreme lethargy progressing to death within three hours. For subsequent experiments, we adopted a modified cold exposure protocol of subjecting all animals to 4°C for 90-minutes, in order to be able to study animals prior to the onset of torpor. Despite starting at similar body temperatures, within 90-minutes of cold exposure, SKO mice displayed an 8.4°C drop in body temperature, while Lox mice only had a 2.4°C change (Figure 6B). While basal plasma glucose levels were similar in SKO and Lox mice maintained on a chow diet (Table 2), SKO mice had a significant reduction in plasma glucose levels within 90- minutes of cold exposure (Figure 6C), suggesting hypoglycemia to be a potential cause of death in cold-exposed SKO mice. Hepatic glycogen levels were approximately 50% lower in chow-fed SKO mice under basal conditions (Table 2), indicating decreased energy reserves in these mice, consistent with their increased energy expenditure. After cold exposure for 90-minutes, both Lox and SKO mice had reduced hepatic glycogen stores (Figure 6D), as expected, since hepatic glycogen is an important energy source during cold exposure (20,58). However, consistent with their hypoglycemia (Figure 6C), cold-exposed SKO mice had a near complete depletion of hepatic glycogen to about 11% of the values seen in Lox controls (Figure 6D).

Given the increased energy expenditure observed in SKO mice coupled with alopecia and loss of skin sebaceous lipids, we hypothesized that SKO mice may lose more heat through their skin and therefore utilize more substrates for heat production. If this were the case, we hypothesized that prior feeding of SKO mice with a hypercaloric diet may help bolster their fuel reserves and attenuate the cold intolerance observed in these mice. We therefore placed SKO mice on a 20%-by weight high-fat diet for 3-weeks. We formulated the diets with either saturated fat from fully-hydrogenated coconut oil or monounsaturated fat (MUFA) from high-oleic safflower oil as the predominant fat source. Since both diets elicited the same response in Lox and SKO mice, only the data from the MUFA-feeding are shown. Over the 3-weeks of HFD-feeding, SKO mice gained 0.82±0.69 g, while Lox mice
gained 5.85±0.70 g of body weight. After HFD-feeding for 3-weeks, Lox and SKO mice were subjected to another cold tolerance test. HFD-fed SKO were now able to regulate their body temperatures similar to Lox counterparts (Figure 6E) and were able to tolerate prolonged cold exposure, similar to Lox controls (over 24 hours; data not shown). Unlike chow-fed SKO mice (Figure 6C), HFD-fed SKO mice were able to maintain plasma glucose at steady state levels even after cold exposure (Figure 6D). While chow-fed SKO mice showed a drastic depletion of hepatic glycogen upon cold exposure (Figure 6D), prior high-fat feeding in SKO mice did not result in a similar ablation of hepatic glycogen during cold exposure (Figure 6D). This sparing of hepatic glycogen corresponded well with the prevention of hypoglycemia (Figure 6C) and improved cold tolerance (Figure 6E) observed in SKO mice fed a HFD prior to cold exposure.

DISCUSSION

Apart from acting as the organism’s first line of defense, the skin is also a site of active lipid synthesis. The lipid profile of the skin affects one of its most important functions of serving as a barrier against heat and water loss to the environment (59,60). Mice with a global SCD1 deficiency have severe impairments in the structure of the skin, characterized by sebaceous gland hypoplasia, dry skin, and alopecia (17,18,61-63). SCD1 deficient mice also have a hypermetabolic phenotype and have been shown to be resistant to weight gain, despite persistent hyperphagia (4,6-8). However, the contributions of SCD1 in various peripheral tissues to this global metabolic phenotype were as yet unclear. The results of our present study indicate that the cutaneous phenotypes resulting from global SCD1 deletion are a direct result of the loss of SCD1 and local delta-9 MUFA synthesis in the skin. Furthermore, these data suggest that these cutaneous alterations are intricately linked to changes in systemic energy metabolism. To the best of our knowledge, these mice represent the first model of skin-specific deletion of a lipogenic enzyme resulting in global changes in energy homeostasis.

The reduction of sebaceous lipids upon deletion of skin SCD1 (Figure 2B) may be of value in the treatment of human diseases such as acne vulgaris, which are associated with increased sebaceous activity. While the mechanisms for sebocyte hypoplasia due to SCD1 deficiency are yet to be determined, it is tempting to speculate that the concurrent accumulation of FC in the skin of SKO mice (Figures 2A and 2F) plays a role in this process. Indeed, other mouse models, including mice overexpressing human apolipoprotein C1 (64), a constituent of very-low density lipoproteins, and mice deficient in acyl-CoA:cholesterol acyltransferase-1 (65), the major enzyme of cholesterol esterification, all have increased levels of skin FC and similar cutaneous phenotypes as SKO mice (64,65). Alternatively, skin SCD1 deficiency could result in altered levels of important metabolites that regulate sebocyte differentiation and proliferation. For instance, loss of SCD1 activity in the skin may result in decreased synthesis of ligands for activation of peroxisome proliferator-activated receptor (PPAR) isoforms in the skin that are thought to be required for sebocyte development (66,67). Alternatively, decreased retinol esterification in the skin has been recently suggested as a potential cause for sebocyte atrophy (68). Since one of the storage forms of retinol is as retinyl oleate, decreased skin oleate pools in SKO mice (Table 1) may result in decreased retinol esterification, thereby contributing to the sebocyte hypoplasia in SKO mice (Figure 1C). Given the heterogeneity of cells in the skin, future studies specifically focused on sebaceous cells are necessary to resolve the mechanistic contribution of SCD1 to sebocyte differentiation and proliferation.

It has been suggested that global loss of SCD1 may cause an alteration in skin barrier permeability and increase water loss through the skin (18,63). While we did not measure trans-epidermal water loss in our current study, our findings of increased skin FC and ceramides (Figure 2) were in contrast to reported decreases in skin sterols (18) and ceramides (63) in mice with global SCD1 deficiency, suggesting important differences between these mouse models and our current model of skin SCD1 deletion. Similar to the SKO mice, a separate mouse model of global SCD1 deficiency (asebia J1 mice) has been shown to have intact barrier function despite sebocyte atrophy (61), suggesting that loss of sebaceous lipids per se does not alter barrier function in mice.
(18,61). As observed in SKO mice (Figure 2A and 2C), asebia J1 mice also do not have decreased epidermal lipids including ceramides and cholesterol (61). Since increases in skin surface FC and ceramides have been reported to be essential for recovery following acute skin barrier disruption (59,60,69), the increase in these lipids in skin of SKO mice (Figure 2) may represent a compensatory mechanism to preserve barrier homeostasis in the context of sebocyte hypoplasia.

At 25°C, room temperature is 5 to 7 °C below thermoneutrality in a mouse (70), and metabolic efficiency is compromised under these conditions in order to maintain core body temperature (71,72). Organisms have developed a variety of ways to sacrifice fuel efficiency in order to maintain temperature homeostasis, one of which is the uncoupling of respiration from ADP phosphorylation via mitochondrial uncoupling proteins (UCPs) (73). UCP-1 in BAT of rodents is robustly induced in response to cold, and has been established as an important regulator of temperature and energy homeostasis (50,51,71,74). Additionally, uncoupling in other tissues such as skeletal muscle via UCP-2 and -3 also contributes to the thermogenic response to cold exposure (50,51,74). The induction of BAT Ucp-1 (Figure 5D) along with Pgc-1α in BAT and skeletal muscle (Figures 5D and 5B) of SKO mice maintained at room temperature suggests increased need for heat generation in SKO mice, as both Ucp-1 and Pgc-1α are known to be strongly induced by cold exposure (50,51,75).

The importance of ambient temperature to the development of obesity in rodents has been elegantly demonstrated in mice lacking UCP1. UCP1−/− mice are resistant to diet-induced obesity at temperatures below 20°C, but prone to obesity when maintained at thermoneutrality (58,71,76). Since SKO mice lack insulating factors including fur and sebaceous lipids, it is likely that cold perception is heightened in these animals, thereby necessitating an increase in basal thermogenesis for temperature maintenance, at the expense of fuel economy. This increased thermogenic demand in SKO mice is met partially through increased food consumption (Table 2) as well as increased lipid and glucose oxidation, as evidenced by higher O2 consumption (Figure 3A) and decreased fat and glycogen storage (Table 2). Additionally, SKO mice are acutely cold intolerant, rapidly depleting energy substrates upon cold exposure (Figure 6). These observations lend further support to the hypothesis that increased cold perception in SKO mice underlies their protection from diet-induced obesity. Interestingly, similar phenotypes of resistance to diet-induced obesity have been described in other mouse models that exhibit cutaneous changes, including mice globally deficient in DGAT1 (68,77,78), mice overexpressing human apolipoprotein C1 (64,79), and mice lacking the Vitamin D receptor (VDRKO) (44,80). While a link between the skin phenotypes and energy expenditure has not been inferred in these mouse models, the data from our current study suggest that in addition to ambient temperatures, cutaneous changes are an important consideration in metabolic studies utilizing transgenic mice.

Induction of UCP-1 in WAT has been shown to be protective against diet-induced obesity in multiple mouse models including liver X receptor knockout and VDRKO mice (43,44,50,75). In SKO mice, the increases in WAT Ucp-1 levels were relatively subtle, as compared to other obesity-resistant models that have reported as much as a 25-fold increase in WAT Ucp-1 expression (44). While some SKO mice in our current study did indeed exhibit changes as large as 30-fold or even higher in WAT Ucp-1 expression, most animals had a more modest elevation of WAT Ucp-1. Also, while adipocytes from WAT of SKO mice were significantly smaller (Figure 3G), we did not observe a perceptible change in the color of fat pads or a significant increase in cells with multiple lipid droplets, as is characteristic of brown adipocytes. Nonetheless, the modest but consistent increases in Ucp-1 in WAT of SKO mice (Figure 5C), in conjunction with the increased expression of Ucps in BAT (Figure 5D) and skeletal muscle (Figure 5B), could plausibly contribute to significantly increased uncoupling in peripheral tissues of these animals. Such an elevation in uncoupling would be expected to place a tremendous demand on the energy reserves of the animal, thereby dissipating excess calories as heat and conferring protection from diet-induced obesity, as observed in SKO mice.

Uncoupling in liver, skeletal muscle and adipose tissue can be activated through PPARα activation, increased thyroid hormone signaling,
increased free fatty acid levels, or increased β-adrenergic signaling (50,75,81). Increased fatty acid uptake through Lpl in skeletal muscle (Figure 5B) or increased lipolysis through Hsl in WAT (Figure 5C) could both transiently increase the local levels of free fatty acids, thereby contributing to induction of Ucpe in SKO mice. Alternatively, it is possible that increased signaling through either the β3-AR or through serum or tissue T3 is responsible for increased uncoupling in SKO mice. Consistent with this hypothesis, expression of BAT Dio2 was significantly elevated in SKO mice, especially upon HFD-feeding (Figure 5D). Dio2 expression in BAT leads to local increases in T3 signaling without changes in circulating T3 levels, and increased BAT Dio2 is strongly associated with increased energy expenditure and obesity resistance in mice (52-56,58). Taken together with the increased markers of lipid oxidation and uncoupling in BAT (Figure 5D), the large increase in Dio2 gene levels (Figure 5D) could contribute to the protection from diet-induced obesity observed in SKO mice.

In summary, the results of our current study elucidate a role for skin SCD1 and local synthesis of delta-9 MUFAs not only in maintaining the structure and lipid composition of the skin, but also in altering global energy balance. Unlike in GKO mice (Supplementary Figure 3) and mice with liver-specific ablation of SCD1 (16), hepatic lipogenesis is not severely impaired in SKO animals (Figure 4), further confirming our previous finding that de novo lipogenesis is mainly regulated by hepatic SCD1 (16). While studies targeting hepatic and adipose SCD1 using antisense mediated approaches have described protection from diet-induced obesity and insulin resistance (11-13), we have previously reported that chronic deletion of hepatic SCD1 does not protect mice against the detrimental effects of high-fat diets (16). In fact, the results of our current study suggest that a major part of the hypermetabolic phenotype of global SCD1 deletion in mice is mediated by loss of SCD1 in the skin, and not the liver. These data do not necessarily exclude a role for SCD1 in other tissues such as brown or white adipose tissue, since the severity of the cutaneous phenotypes in SKO mice may mask the contributions of SCD1 in these tissues to global energy homeostasis. Nevertheless, these data reveal an as yet underappreciated role for local synthesis of MUFAs in the skin to maintaining skin lipid composition and illustrate an example of crosstalk between the skin and peripheral organs in the regulation of whole body energy metabolism.
REFERENCES

1. Mensah, G. A., Mokdad, A. H., Ford, E., Narayan, K. M., Giles, W. H., Vinicor, F., and Deedwania, P. C. (2004) *Cardiol Clin* **22**(4), 485-504

2. Grundy, S. M. (2007) *J Clin Endocrinol Metab* **92**(2), 399-404

3. Muoio, D. M., and Newgard, C. B. (2006) *Annu Rev Biochem* **75**, 367-401

4. Miyazaki, M., Dobrzyn, A., Man, W. C., Chu, K., Sampath, H., Kim, H. J., and Ntambi, J. M. (2004) *J Biol Chem* **279**(24), 25164-25171

5. Miyazaki, M., Kim, Y. C., Gray-Keller, M. P., Attie, A. D., and Ntambi, J. M. (2000) *J Biol Chem* **275**(39), 30132-30138

6. Miyazaki, M., Kim, Y. C., and Ntambi, J. M. (2001) *J Lipid Res* **42**(7), 1018-1024

7. Ntambi, J. M., Miyazaki, M., Stoehr, J. P., Lan, H., Kendziorski, C. M., Yandell, B. S., Song, Y., Cohen, P., Friedman, J. M., and Attie, A. D. (2002) *Proc Natl Acad Sci U S A* **99**(17), 11482-11486

8. Cohen, P., Miyazaki, M., Socci, N. D., Hagge-Greenberg, A., Liedtke, W., Soukas, A. A., Sharma, R., Hudgins, L. C., Ntambi, J. M., and Friedman, J. M. (2002) *Science* **297**(5579), 240-243

9. Rahman, S. M., Dobrzyn, A., Dobrzyn, P., Lee, S. H., Miyazaki, M., and Ntambi, J. M. (2003) *Proc Natl Acad Sci U S A* **100**(19), 11110-11115

10. Rahman, S. M., Dobrzyn, A., Lee, S. H., Dobrzyn, P., Miyazaki, M., and Ntambi, J. M. (2005) *Am J Physiol Endocrinol Metab* **288**(2), E381-387

11. Jiang, G., Li, Z., Liu, F., Ellsworth, K., Dallas-Yang, Q., Wu, M., Ronan, J., Esau, C., Murphy, C., Szalkowski, D., Bergeron, R., Doebber, T., and Zhang, B. B. (2005) *J Clin Invest* **115**(4), 1030-1038

12. Gutierrez-Juarez, R., Pocai, A., Mulas, C., Ono, H., Bhanot, S., Monia, B. P., and Rossetti, L. (2006) *J Clin Invest* **116**(6), 1686-1695

13. Brown, J. M., Chung, S., Sawyer, J. K., Degirolamo, C., Alger, H. M., Nguyen, T., Zhu, X., Duong, M. N., Wibley, A. L., Shah, R., Davis, M. A., Kelley, K., Wilson, M. D., Kent, C., Parks, J. S., and Rudel, L. L. (2008) *Circulation* **118**(14), 1467-1475

14. Choi, C. S., Savage, D. B., Kulkarni, A., Yu, X. X., Liu, Z. X., Morino, K., Kim, S., Distefano, A., Samuel, V. T., Neschen, S., Zhang, D., Wang, A., Zhang, X. M., Kahn, M., Cline, G. W., Pandey, S. K., Geisler, J. G., Bhanot, S., Monia, B. P., and Shulman, G. I. (2007) *J Biol Chem* **282**(31), 22678-22688
15. Levin AA, Y. R., Geary RS. (2008) Basic principles of the pharmacokinetics of antisense oligonucleotide drugs. In. Antisense Drug Technology, 2 Ed., CRC Press, Boca Raton, FL

16. Miyazaki, M., Flowers, M. T., Sampath, H., Chu, K., Otzelberger, C., Liu, X., and Ntambi, J. M. (2007) Cell Metab 6(6), 484-496

17. Miyazaki, M., Man, W. C., and Ntambi, J. M. (2001) J Nutr 131(9), 2260-2268

18. Sundberg, J. P., Boggess, D., Sundberg, B. A., Eilertsen, K., Parimoo, S., Filippi, M., and Stenn, K. (2000) Am J Pathol 156(6), 2067-2075

19. Dassule, H. R., Lewis, P., Bei, M., Maas, R., and McMahon, A. P. (2000) Development 127(22), 4775-4785

20. Lee, S. H., Dobrzyn, A., Dobrzyn, P., Rahman, S. M., Miyazaki, M., and Ntambi, J. M. (2004) J Lipid Res 45(9), 1674-1682

21. Althage, M. C., Ford, E. L., Wang, S., Tso, P., Polonsky, K. S., and WicE, B. M. (2008) J Biol Chem 283(26), 18365-18376

22. Folch, J., Lees, M., and Sloane Stanley, G. H. (1957) J Biol Chem 226(1), 497-509

23. Sampath, H., Miyazaki, M., Dobrzyn, A., and Ntambi, J. M. (2007) J Biol Chem 282(4), 2483-2493

24. Miyazaki, M., Dobrzyn, A., Elias, P. M., and Ntambi, J. M. (2005) Proc Natl Acad Sci USA 102(35), 12501-12506

25. Shimomura, I., Bashmakov, Y., and Horton, J. D. (1999) J Biol Chem 274(42), 30028-30032

26. Miyazaki, M., Jacobson, M. J., Man, W. C., Cohen, P., Asilmaz, E., Friedman, J. M., and Ntambi, J. M. (2003) J Biol Chem 278(36), 33904-33911

27. Braun, K. M., Niemann, C., Jensen, U. B., Sundberg, J. P., Silva-Vargas, V., and Watt, F. M. (2003) Development 130(21), 5241-5255

28. Lo Celso, C., Berta, M. A., Braun, K. M., Frye, M., Lyle, S., Zouboulis, C. C., and Watt, F. M. (2008) Stem Cells 26(5), 1241-1252

29. Zheng, Y., Prouty, S. M., Harmon, A., Sundberg, J. P., Stenn, K. S., and Parimoo, S. (2001) Genomics 71(2), 182-191

30. Payne, A. P. (1994) J Anat 185 (Pt 1)(Pt 1), 1-49

31. Hillenius, W. J., Phillips, D. A., and Rehorek, S. J. (2007) Ann Anat 189(5), 423-433

32. Miyazaki, M., Kim, H. J., Man, W. C., and Ntambi, J. M. (2001) J Biol Chem 276(42), 39455-39461
33. Horton, J. D., Goldstein, J. L., and Brown, M. S. (2002) *J Clin Invest* **109**(9), 1125-1131

34. Fleury, C., Neverova, M., Collins, S., Raimbault, S., Champigny, O., Levi-Meyrueis, C., Bouillaud, F., Seldin, M. F., Surwit, R. S., Ricquier, D., and Warden, C. H. (1997) *Nat Genet* **15**(3), 269-272

35. Li, B., Nolte, L. A., Ju, J. S., Han, D. H., Coleman, T., Holloszy, J. O., and Semenkovich, C. F. (2000) *Nat Med* **6**(10), 1115-1120

36. Han, D. H., Nolte, L. A., Ju, J. S., Coleman, T., Holloszy, J. O., and Semenkovich, C. F. (2004) *Am J Physiol Endocrinol Metab* **286**(3), E347-353

37. Neschen, S., Katterle, Y., Richter, J., Augustin, R., Scherneck, S., Mirhashemi, F., Schurmann, A., Joost, H. G., and Klaus, S. (2008) *Physiol Genomics* **33**(3), 333-340

38. Clapham, J. C., Arch, J. R., Chapman, H., Haynes, A., Lister, C., Moore, G. B., Piercy, V., Carter, S. A., Lehner, I., Smith, S. A., Beeley, L. J., Godden, R. J., Herrity, N., Skehel, M., Changani, K. K., Hockings, P. D., Reid, D. G., Squires, S. M., Hatcher, J., Trail, B., Latcham, J., Rastan, S., Harper, A. J., Cadenas, S., Buckingham, J. A., Brand, M. D., and Abuin, A. (2000) *Nature* **406**(6794), 415-418

39. Harper, M. E., Green, K., and Brand, M. D. (2008) *Annu Rev Nutr* **28**, 13-33

40. Son, C., Hosoda, K., Ishihara, K., Bevilacqua, L., Masuzaki, H., Fushiki, T., Harper, M. E., and Nakao, K. (2004) *Diabetologia* **47**(1), 47-54

41. Wu, Z., Puigserver, P., Andersson, U., Zhang, C., Adelmant, G., Mootha, V., Troy, A., Cinti, S., Lowell, B., Scarpulla, R. C., and Spiegelman, B. M. (1999) *Cell* **98**(1), 115-124

42. Mead, J. R., Irvine, S. A., and Ramji, D. P. (2002) *J Mol Med* **80**(12), 753-769

43. Kalaany, N. Y., Gauthier, K. C., Zavacki, A. M., Mammen, P. P., Kitazume, T., Peterson, J. A., Horton, J. D., Garry, D. J., Bianco, A. C., and Mangelsdorf, D. J. (2005) *Cell Metab* **3**(5), 333-341

44. Narvaez, C. J., Matthews, D., Broun, E., Chan, M., and Welsh, J. (2008) *Endocrinology* **9**, 9

45. Strom, K., Hansson, O., Lucas, S., Nevsten, P., Fernandez, C., Klint, C., Moverare-Skrtic, S., Sundler, F., Ohlsson, C., and Holm, C. (2008) *PLoS ONE* **3**(3), e1793

46. Uldry, M., Yang, W., St-Pierre, J., Lin, J., Seale, P., and Spiegelman, B. M. (2006) *Cell Metab* **3**(5), 333-341

47. Seale, P., Kajimura, S., Yang, W., Chin, S., Rohas, L. M., Uldry, M., Tavernier, G., Langin, D., and Spiegelman, B. M. (2007) *Cell Metab* **6**(1), 38-54
48. Vidal-Puig, A., Solanes, G., Grujic, D., Flier, J. S., and Lowell, B. B. (1997) *Biochem Biophys Res Commun* **235**(1), 79-82

49. Vidal-Puig, A. J., Grujic, D., Zhang, C. Y., Hagen, T., Boss, O., Ido, Y., Szczepanik, A., Wade, J., Mootha, V., Cortright, R., Muoio, D. M., and Lowell, B. B. (2000) *J Biol Chem* **275**(21), 16258-16266

50. Lowell, B. B., and Spiegelman, B. M. (2000) *Nature* **404**(6778), 652-660

51. Puigserver, P., Wu, Z., Park, C. W., Graves, R., Wright, M., and Spiegelman, B. M. (1998) *Cell* **92**(6), 829-839

52. de Jesus, L. A., Carvalho, S. D., Ribeiro, M. O., Schneider, M., Kim, S. W., Harney, J. W., Larsen, P. R., and Bianco, A. C. (2001) *J Clin Invest* **108**(9), 1379-1385

53. Kim, B. (2008) *Thyroid* **18**(2), 141-144

54. Gereben, B., Zeold, A., Dentice, M., Salvatore, D., and Bianco, A. C. (2008) *Cell Mol Life Sci* **65**(4), 570-590

55. Pelletier, P., Gauthier, K., Sideleva, O., Samarut, J., and Silva, J. E. (2008) *Endocrinology* **149**(12), 6471-6486

56. Watanabe, M., Houten, S. M., Mataki, C., Christoffolete, M. A., Kim, B. W., Sato, H., Messaddeq, N., Harney, J. W., Ezaki, O., Kodama, T., Schoonjans, K., Bianco, A. C., and Auwerx, J. (2006) *Nature* **439**(7075), 484-489

57. Wondisford, F. E. (2006) *Cell Metab* **3**(2), 81-82

58. Ukropec, J., Anunciado, R. P., Ravussin, Y., Hulver, M. W., and Kozak, L. P. (2006) *J Biol Chem* **281**(42), 31894-31908

59. Elias, P. M., and Feingold, K. R. (1992) *Semin Dermatol* **11**(2), 176-182

60. Smith, K. R., and Thiboutot, D. M. (2008) *J Lipid Res* **49**(2), 271-281

61. Fluhr, J. W., Mao-Qiang, M., Brown, B. E., Wertz, P. W., Crumrine, D., Sundberg, J. P., Feingold, K. R., and Elias, P. M. (2003) *J Invest Dermatol* **120**(5), 728-737

62. Lu, Y., Bu, L., Zhou, S., Jin, M., Sundberg, J. P., Jiang, H., Qian, M., Shi, Y., Zhao, G., Kong, X., and Hu, L. (2004) *Mol Genet Genomics* **272**(2), 129-137

63. Biczsek, E., Jenke, B., Holz, B., Gunter, R. H., Thevis, M., and Stoffel, W. (2007) *Biol Chem* **388**(4), 405-418

64. Jong, M. C., Gijbels, M. J., Dahlmans, V. E., Gorp, P. J., Koopman, S. J., Ponec, M., Hofker, M. H., and Havekes, L. M. (1998) *J Clin Invest* **101**(1), 145-152
65. Yagyu, H., Kitamine, T., Osuga, J., Tozawa, R., Chen, Z., Kaji, Y., Oka, T., Perrey, S., Tamura, Y., Ohashi, K., Okazaki, H., Yahagi, N., Shionoiri, F., Iizuka, Y., Harada, K., Shimano, H., Yamashita, H., Gotoda, T., Yamada, N., and Ishibashi, S. (2000) *J Biol Chem* **275**(28), 21324-21330

66. Karnik, P., Tekeste, Z., McCormick, T. S., Gilliam, A. C., Price, V. H., Cooper, K. D., and Mirmirani, P. (2008) *J Invest Dermatol* **4**, 4

67. Michalik, L., and Wahli, W. (2007) *Biochim Biophys Acta* **1771**(8), 991-998

68. Shih, M. Y., Kane, M. A., Zhou, P., Streeper, R. S., Yen, C. L., Napoli, J. L., and Farese, R. V., Jr. (2008) *J Biol Chem* **20**, 20

69. Mirza, R., Hayasaka, S., Takagishi, Y., Kambe, F., Ohmori, S., Maki, K., Yamamoto, M., Murakami, K., Kaji, T., Zadworny, D., Murata, Y., and Seo, H. (2006) *J Invest Dermatol* **126**(3), 638-647

70. Overton, J. M., and Williams, T. D. (2004) *Physiol Behav* **81**(5), 749-754

71. Feldmann, H. M., Golozoubova, V., Cannon, B., and Nedergaard, J. (2009) *Cell Metab* **9**(2), 203-209

72. Lodhi, I. J., and Semenkovich, C. F. (2009) *Cell Metab* **9**(2), 111-112

73. Silva, J. E. (2006) *Physiol Rev* **86**(2), 435-464

74. Jensen, D. R., Knaub, L. A., Konhilas, J. P., Leinwand, L. A., MacLean, P. S., and Eckel, R. H. (2008) *J Lipid Res* **49**(4), 870-879

75. Rousset, S., Alves-Guerra, M. C., Mozo, J., Miroux, B., Cassard-Doulcier, A. M., Bouillaud, F., and Ricquier, D. (2004) *Diabetes* **53** Suppl 1(1), S130-S135

76. Liu, X., Rossmeisl, M., McClaine, J., Riachi, M., Harper, M. E., and Kozak, L. P. (2003) *J Clin Invest* **111**(3), 399-407

77. Chen, H. C., Smith, S. J., Tow, B., Elias, P. M., and Farese, R. V., Jr. (2002) *J Clin Invest* **109**(2), 175-181

78. Smith, S. J., Cases, S., Jensen, D. R., Chen, H. C., Sande, E., Tow, B., Sanan, D. A., Raber, J., Eckel, R. H., and Farese, R. V., Jr. (2000) *Nat Genet* **25**(1), 87-90

79. Jong, M. C., Voshol, P. J., Muurling, M., Dahlmans, V. E., Romijn, J. A., Pijl, H., and Havekes, L. M. (2001) *Diabetes* **50**(12), 2779-2785

80. Xie, Z., Komuves, L., Yu, Q. C., Elalieh, H., Ng, D. C., Leary, C., Chang, S., Crumrine, D., Yoshizawa, T., Kato, S., and Bikle, D. D. (2002) *J Invest Dermatol* **118**(1), 11-16

81. Rodgers, J. T., Lerin, C., Gerhart-Hines, Z., and Puigserver, P. (2008) *FEBS Lett* **582**(1), 46-53
ACKNOWLEDGMENTS

Indirect calorimetry measurements were performed at the University of Cincinnatti Genome Research Institute. We thank Dr. Makoto Miyazaki for help with generation of skin-SCD1 knockout mice and Dr. Jay Horton for providing the SREBP-2 antibody.

This work was supported by a Wisconsin Distinguished Graduate Fellowship (to H.S.) and NIH grant RO1DK-62388 (to J.M.N).

Current Address: Center for Study of Weight Regulation and Associated Disorders, Oregon Health and Science University, Portland, OR 97239

The abbreviations used are: AOX, acyl CoA oxidase; BAT, brown adipose tissue; CE, cholesterol esters; CPT, carnitine palmitoyl transferase; FAS, fatty acid synthase; FFA, free fatty acids; GKO, global SCD1-knockout; LCAD, long-chain acyl-CoA dehydrogenase; LOX, Scd1^{flox/flox}; PGC1, PPAR-gamma coactivator-1; PPAR, peroxisome proliferator-activated receptor; SCD, stearoyl CoA desaturase; SREBP, sterol regulatory element-binding protein; SKO, skin-specific SCD1-knockout; T_3, triiodothyronine; T_4, thyroxine; UCP, uncoupling protein; WAT, white adipose tissue; WDE, wax di-esters; WE, wax mono-esters
Table 1. Fatty acid composition of skin surface lipids
Skin surface triglycerides and wax diesters were scraped after separation by thin-layer chromatography. Fatty acids were methylated and quantified by gas chromatography with penta- and hepta-decanoic acids added as internal standards. Five 12-week old male mice of each genotype were individually analyzed. Results are presented as mean ± SEM and represent total freely extractable lipids on the surface of the skin of the animal in mmol. *, p<0.05 vs. Lox counterparts.

Table 2. Food intake, body weight, serum and liver metabolites
16-week old male mice were sacrificed by isofluorane overdose after being fed chow or HFD for 8-weeks. Plasma glucose and insulin were measured in the same mice after 7-weeks of dietary treatment with a prior 4-hour fast. All other measurements were conducted in non-fasted animals. BW, body weight; CE, cholesterol esters; FFA, free fatty acids; T₃, triiodothyronine; T₄, thyroxine; TG, triglycerides; Data are presented as mean ± SEM. *, p<0.05 vs. Lox counterparts; #, p<0.05 vs. chow-fed counterparts.

Figure 1. SCD1 is deleted specifically from skin of SKO mice and causes marked sebocyte hypoplasia and reductions in sebaceous lipids
(A) Real-time PCR analysis: Analysis of Scd1 mRNA levels in various tissues revealed that Scd1 expression was specifically decreased only in skin of SKO mice. Results are expressed as mean ± SEM. BAT, brown adipose tissue, SM, skeletal muscle, WAT, white adipose tissue. (B) Western blot analysis of SCD1 protein: Protein from liver, white and brown adipose tissue, and skin revealed no changes in SCD1 levels in any tissues except skin, where it was significantly decreased in SKO mice. (C) Skin histology: Skin sections of 12-week old male mice were stained with H&E (upper panels) or Oil Red O (lower panels). Both staining methods revealed a marked absence of lipid-engorged sebaceous glands (black arrows) along the hair follicles in SKO mice. Figures are representative of several animals of each genotype. Scale bars in all panels represent 500 µm. (D) Skin Scd3 expression: Expression of Scd3, a marker of differentiated sebocytes in the skin, was measured by RT-PCR. Scd3 expression was significantly reduced in skin of SKO mice. Results are expressed as mean ± SEM.

Figure 2. Skin surface lipids are altered in SKO mice
(A-C) Skin surface lipids: Skin surface lipids were extracted from Lox and SKO mice as described in the Methods section and separated by thin-layer chromatography. (D,E) Desaturation index: Delta-9 desaturation ratios of skin surface TGs (D) and WDEs (E) were calculated from fatty acid compositions of these lipid species analyzed by gas chromatography. (F) Levels of skin free cholesterol were measured using a colorimetric enzyme assay (Wako). *, p<0.05 vs. Lox counterparts.

Figure 3. SKO mice have increased energy expenditure and are protected from HFD-induced adiposity and glucose intolerance
(A,B) O₂ consumption (A) and CO₂ production (B) were measured in individually caged 12-week old male mice in indirect calorimetry chambers. There were no significant differences in body weights of animals (Lox, 26.55 ± 0.45 and SKO, 27.05 ± 0.61 g), and data is expressed as mL/kg body weight/minute of A) O₂ consumed or B) CO₂ produced. (C,D) 8-week old male mice were fed chow (C) or high-fat diets (D) for 8-weeks, and body weights were measured weekly. (E,F) Oral glucose tolerance was assessed after 7-weeks of feeding chow (E) or HFD (F). Mice
were fasted for 4 hours and administered 2 g glucose/ kg BW by oral gavage. Data are presented as mean \pm SEM.

(G,H) White adipose tissue (G) and liver (H) pieces were fixed in buffered formalin, and sections were stained with H&E for histological examination. Bar represents 250 \mu m in all figures. *, \( p<0.05 \) vs. Lox counterparts.

Figure 4. Hepatic lipogenic gene expression is not decreased in HFD-fed or fasted-refed SKO mice

(A, B) Hepatic lipogenic gene expression (A) and nuclear levels of SREBP-1, SREBP-2 and histone H3 (B) were measured in Lox and SKO mice after 8-weeks of Chow- or HFD-feeding. *, \( p<0.05 \) vs. Lox counterparts

(C, D) Induction of nuclear levels of SREBP-1 (C) and lipogenic genes (D) were measured in livers of mice fasted for 12-hours or fasted and refed a high-carbohydrate lipogenic diet for 12-hours. Data are presented as mean \pm SEM. *, \( p<0.05 \) vs. Lox counterparts

Acc, acetyl-CoA carboxylase; F, fasted; Fas, fatty acid synthase; Pgc-1\( \beta \), PPAR-gamma co-activator-1 beta; RF, fasted-refed; Scd1, stearoyl-CoA desaturase 1; Srebp-1c, sterol regulatory element binding protein-1c

Figure 5. Expression of oxidative, lipolytic and thermogenic genes is altered in peripheral tissues of SKO mice

(A-D) Expression of genes involved in uncoupling, lipid uptake, lipolysis and fatty acid oxidation was measured in liver (A), skeletal muscle (B), WAT (C), and BAT (D) of chow- or HFD-fed SKO and Lox mice. Data are presented as mean \pm SEM. *, \( p<0.05 \) vs. Lox counterparts

Aox, acyl-CoA oxidase, \( \beta 3\)-Ar, beta-3 adrenergic receptor; Cpt-1, carnitine palmitoyl transferase-1; Dio2, Type II iodothyronine deiodinase; Hsl, hormone sensitive lipase; Lcad, long chain acyl-CoA dehydrogenase; Lpl, lipoprotein lipase; Pgc-1\( \alpha \), PPAR-gamma co-activator-1 alpha; Ucp, uncoupling protein

Figure 6. Cold tolerance is impaired in SKO mice due to rapid depletion of hepatic glycogen

(A,B) 12-week old Chow-fed Lox and SKO mice were exposed to 4°C for 120 minutes (A) or for a shortened duration of 90 minutes (B) with ad libitum access to chow diet and water. Rectal temperatures were measured at the indicated time points with a YSI Series Precision thermometer.

(C) Blood was collected by cardiac puncture following euthanasia, and blood glucose was measured by the glucose oxidase method.

(D) Hepatic glycogen was measured by an enzyme-coupled spectrophotometric assay.

(E) 9-week old Lox and SKO mice were fed a high-fat diet for 3-weeks and subjected to a cold tolerance test. Detailed diet composition is available in Supplementary Methods. *, \( p<0.05 \) vs. Lox counterparts
### Table 1. Fatty acid composition of skin surface lipids

| Skin surface lipids | Triglycerides (mmol/animal) | Wax diesters (mmol/animal) |
|---------------------|-----------------------------|--------------------------|
|                     | Lox                         | SKO                      | Lox                         | SKO |
| 16:0                | 2.58 ± 7.91                 | 1.58 ± 0.18              | 0.99 ± 0.62                  | 0.54 ± 0.02 |
| 16:1 (n-7)          | 2.33 ± 3.26                 | 0.81 ± 0.20*             | 2.48 ± 1.63                  | 0.13 ± 0.06* |
| 18:0                | 1.01 ± 3.61                 | 0.64 ± 0.05*             | 0.63 ± 0.12                  | 0.74 ± 0.10 |
| 18:1 (n-9)          | 9.65 ± 4.56                 | 2.20 ± 0.33*             | 1.67 ± 0.54                  | 0.14 ± 0.00* |
| 18:2 (n-6)          | 4.88 ± 2.71                 | 1.20 ± 0.19*             | 0.38 ± 0.26                  | 0.02 ± 0.00* |
| 18:3 (n-3)          | 1.91 ± 1.01                 | 0.07 ± 0.02*             | 0.92 ± 0.06                  | 0.07 ± 0.02* |
| 20:0                | 0.024 ± 0.00                | 0.05 ± 0.00*             | 1.21 ± 0.36                  | 0.25 ± 0.02* |
| 20:1                | 0.11 ± 0.05                 | 0.04 ± 0.00*             | 0.98 ± 0.51                  | 0.04 ± 0.03* |
| 20:3 (n-6)          | ND                          | ND                       | 0.24 ± 0.11                  | ND* |
| 20:3 (n-9)          | ND                          | ND                       | 0.29 ± 0.35                  | ND* |
| 20:4                | ND                          | ND                       | 1.66 ± 0.14                  | 0.12 ± 0.03* |
| 20:5                | ND                          | ND                       | 0.26 ± 0.10                  | ND* |
| 22:0                | 0.013 ± 0.00                | 0.02 ± 0.00*             | 0.53 ± 0.10                  | 0.05 ± 0.03* |
| 22:1                | ND                          | ND                       | 0.46 ± 0.32                  | 0.03 ± 0.01* |
| 24:00               | ND                          | ND                       | 0.19 ± 0.03                  | 0.01 ± 0.00* |
| Total               | 25.52 ± 12.34               | 7.11 ± 0.89*             | 13.79 ± 3.70                 | 2.24 ± 0.10* |
|                          | Lox                  | SKO                  |
|--------------------------|----------------------|----------------------|
|                          | Chow | HFD | Chow | HFD |
| **Body weight (g)**      | 26.38 ± 0.42 | 41.28 ± 0.98# | 25.84 ± 0.28 | 26.96 ± 1.13* |
| **Food intake (g/d)**    | 3.71 ± 0.20 | 2.71 ± 0.22# | 7.45 ± 0.48* | 4.15 ± 0.18*# |
| **Epididymal fat mass (g)** | 0.45 ± 0.06 | 3.07 ± 0.60# | 0.21 ± 0.02* | 0.74 ± 0.21*# |
| **Subcutaneous fat mass (g)** | 0.28 ± 0.03 | 1.49 ± 0.35# | 0.18 ± 0.01* | 0.47 ± 0.09*# |
| **Plasma TG (mg/dL)**    | 62.5 ± 4.7 | 69.0 ± 8.0 | 53.6 ± 3.5 | 72.4 ± 5.0# |
| **Plasma Cholesterol (mg/dL)** | 110.2 ± 3.5 | 204.8 ± 27.9# | 95.2 ± 4.5 | 119.3 ± 11.5* |
| **Plasma glucose (mg/dL)** | 159.1 ± 14.3 | 191.3 ± 21.6# | 140.4 ± 9.6 | 168.4 ± 11.1# |
| **Plasma insulin (ng/mL)** | 0.77 ± 0.11 | 2.45 ± 0.93# | 0.80 ± 0.36 | 0.83 ± 0.16* |
| **Plasma leptin (ng/mL)** | 3.32 ± 0.67 | 38.49 ± 7.18# | 0.42 ± 0.13* | 4.36 ± 1.78*# |
| **Plasma T₃ (ng/dL)**    | 87.54 ± 1.58 | 88.00 ± 2.40 | 77.09 ± 4.87 | 87.05 ± 13.85 |
| **Plasma T₄ (ug/dL)**    | 2.80 ± 0.25 | 2.18 ± 0.42 | 1.25 ± 0.23 | 1.12 ± 0.25 |
| **Plasma T₃/T₄ ratio**   | 0.03 ± 0.01 | 0.04 ± 0.01 | 0.07 ± 0.03 | 0.08 ± 0.01 |
| **Liver glycogen** (mg/g liver) | 64.59 ± 3.65 | 62.15 ± 3.31 | 29.82 ± 1.47* | 25.71 ± 0.96* |
| **Liver TG** (µmol/g)    | 20.8 ± 5.2 | 98.7 ± 14.2# | 12.7 ± 0.7* | 33.9 ± 11.3*# |
| **Liver FFAs** (µmol/g)  | 1.88 ± 0.18 | 1.79 ± 0.17 | 1.96 ± 0.16 | 1.28 ± 0.17*# |
| **Liver CE** (µmol/g)    | 4.26 ± 0.74 | 10.52 ± 1.07# | 3.19 ± 0.48* | 8.69 ± 0.26*# |
A. Scd1 gene expression

B. Scd1 GKO Lox SKO Lox SKO

C. Lox SKO

D. Scd3 gene expression

E. Sampath_ Figure 1

F. Liver WAT BAT Kidneys SM Skin

G. 95.5%

H. Sampath_ Figure 1

I. mRNA levels (Normalized for Lox mice)

J. Sampath_ Figure 1

K. Sampath_ Figure 1

L. Sampath_ Figure 1

M. Sampath_ Figure 1

N. Sampath_ Figure 1

O. Sampath_ Figure 1

P. Sampath_ Figure 1

Q. Sampath_ Figure 1

R. Sampath_ Figure 1

S. Sampath_ Figure 1

T. Sampath_ Figure 1

U. Sampath_ Figure 1

V. Sampath_ Figure 1

W. Sampath_ Figure 1

X. Sampath_ Figure 1

Y. Sampath_ Figure 1

Z. Sampath_ Figure 1
Cholesterol esters,
wax mono- and
di-esters
Triglycerides
Free fatty acids
Free cholesterol

Desaturation ratio (Arb. units)

Desaturation index: TG

Desaturation index: WDE

Free Cholesterol

* p<0.05 vs. Lox

Downloaded from http://www.jbc.org/ by guest on March 24, 2020
A) Hepatic lipogenic genes

B) Western blot analysis of Lox and SKO mice fed Chow or HFD.

C) Western blot analysis of Lox and SKO mice under fasting and refeeding conditions.

D) Hepatic lipogenic genes under fasting-refeeding conditions.

Sampath_Figure 4

Downloaded from http://www.jbc.org/ on March 24, 2020 by guest.
A. Hepatic gene expression

B. Skeletal muscle gene expression

C. WAT gene expression

D. BAT gene expression

* p<0.05 vs. Lox
Supplementary Methods

Animals and diets

The high-fat diet (HFD) used in the long-term diet-induced obesity studies was from Research Diets (RD12492) and the high-carbohydrate (HC) diet used in the fasting-refeeding study was from Harlan Teklad, Inc. (TD03045). The caloric contributions (% fat: % carbohydrate: % protein; kcal/g) for the chow, HF, and HC diets as indicated by the manufacturer are (chow, 16.7: 56.4: 26.8; 3.50 kcal/g), (HF, 59.9: 20.1: 20.0; 5.24 kcal/g), and (HC, 2.6: 76.7: 20.7: 3.53 kcal/g). For all diet studies, mice were individually caged 1-week prior to the start of the study. For the diet-induced obesity studies, 8-week old male mice were fed chow or HFD for 8-weeks. Oral glucose tolerance tests were performed after a 4-hour fast after 7-weeks of feeding. Mice were sacrificed at the end of 8-weeks of feeding without fasting. For the fasting-refeeding studies, 10-12 week old male mice were either fasted for 12 hours (fasted group) or fasted for 12 hours followed by refeeding the HC diet for 12 hours (refed group). For cold-tolerance tests, 10- to 12-week old male and female mice were individually caged 1-week prior to cold exposure and allowed ad libitum access to food through the course of the cold challenge. The high-fat diets used in the cold tolerance experiments were specially formulated in order to be able to control the composition of the fat source. These diets were designed to contain 20% fat by weight from either fully-hydrogenated coconut oil (saturated fat -- data not shown) or high-oleic safflower oil (monounsaturated fat) as the predominant fat source, supplemented to a 20% fat-free base (TD88232 from Harlan Teklad).

Lipid analyses

Skin surface neutral lipids were separated along with authentic standards by thin-layer chromatography using heptane:isopropyl ether:acetic acid (60:40:3, v/v/v) as a solvent system (1). For better resolution of wax esters, wax diesters and cholesterol esters, a benzene:hexane (65:35, v/v) solvent system was utilized (2). For separation of ceramides, plates were washed with chloroform: methanol (9:1 v/v) and heat activated for 1 hour at 110 deg C; ceramides were resolved by sequential development in chloroform/methanol/acetic acid (190:9:1, v/v/v), twice (3). Plates were sprayed uniformly with 10% cupric sulfate in 8% aqueous phosphoric acid, allowed to dry at room temperature, and then charred at 110 deg C for 30 minutes for visualization of lipid species (4). For fatty acid analysis by gas chromatography, lipids were visualized by spraying with 5 mM 2′,7′-dichlorofluorescein in ethanol. Sebaceous lipids including triglycerides, wax esters, wax diesters and cholesterol esters were scraped and methylated as previously described (2). Hepatic lipids were extracted by the Folch method (5) and separated by TLC as previously described (1). Fatty acid composition of skin and hepatic lipids was quantified by gas chromatography as previously described, with penta- and heptadecanoic acids added as internal standards (1).

REFERENCES

1. Sampath, H., Miyazaki, M., Dobrzyn, A., and Ntambi, J. M. (2007) J Biol Chem 282(4), 2483-2493
2. Miyazaki, M., Man, W. C., and Ntambi, J. M. (2001) J Nutr 131(9), 2260-2268
3. Miyazaki, M., Dobrzyn, A., Elias, P. M., and Ntambi, J. M. (2005) Proc Natl Acad Sci U S A 102(35), 12501-12506
4. Entezami, A. A., Venables, B. J., and Daugherty, K. E. (1987) Journal of Chromatography A 387, 323-331
5. Folch, J., Lees, M., and Sloane Stanley, G. H. (1957) J Biol Chem 226(1), 497-509
Legends to Supplementary tables and figures

Supplementary Table 1. Fatty acid composition of hepatic lipids.

(A-C) Hepatic lipids were extracted from 30 mg of frozen liver from 16-week old male mice fed chow or HFD for 8 weeks. Lipids were separated by thin-layer chromatography, and bands corresponding to hepatic TG (A), CE (B) and FFA (C) were scraped, methylated, and quantified by gas chromatography with penta- and hepta-decanoic acids as internal standards. Results are presented as mean ± SEM and represent at least five animals in each group. *, p<0.05 vs. Lox counterparts.

Supplementary Figure 1. Cutaneous phenotype of SKO mice.

Alopecia and closed eye fissures of SKO mice: SKO mice developed dry scaly skin with hair loss and closed eye fissures, as has been reported in GKO mice. 12-week old male littermates are shown here.

Supplementary Figure 2. Energy expenditure in SKO mice is increased to the same extent as in GKO mice and hepatic lipid accumulation is significantly decreased in SKO mice

(A,B) O$_2$ consumption (A) and CO$_2$ production (B) were measured in individually caged 12-week old male in indirect calorimetry chambers. There were no significant differences in body weights of animals (Lox, 26.55 ± 0.45; GKO, 26.72 ± 0.28 and SKO, 27.05 ± 0.61 g), and data is expressed as ml/kg body weight/minute of (A) O$_2$ consumed or (B) CO$_2$ produced.

(C) Heat and (D) respiratory quotient were calculated from VO$_2$ and VCO$_2$ measurements. Six mice of each genotype were measured over two consecutive 24-hour periods consisting of alternating 12-hour dark and light cycles.

(E) Hepatic lipids were extracted from frozen liver samples of chow- and HFD-fed Lox and SKO mice and separated by thin-layer chromatography with heptane:isopropyl ether:acetic acid (60:40:3, v/v/v) as the developing solvent. *, p<0.05 vs. Lox counterparts.

Supplementary Figure 3. Hepatic SREBP-1 and lipogenic gene expression are differentially regulated in SKO and GKO mice

(A, B) Nuclear levels of SREBP-1, SREBP-2 and histone H$_3$ (A) and hepatic lipogenic gene expression (B) were measured in Lox and GKO mice after 8-weeks of Chow- or HFD-feeding.

(C, D) Induction of nuclear levels of SREBP-1 (C) and lipogenic genes (D) were measured in livers of mice fasted for 12-hours or fasted and refed a high-carbohydrate lipogenic diet for 12-hours. Data are presented as mean ± SEM. *, p<0.05 vs. Lox counterparts Acc, acetyl-CoA carboxylase; F, fasted; Fas, fatty acid synthase; Pgc-1β, PPAR-gamma co-activator-1 beta; RF, fasted-refed; Scd1, stearoyl-CoA desaturase 1; Srebp-1c, sterol regulatory element binding protein-1c.
## SUPPLEMENTARY TABLES

### Supplementary Table 1A. Fatty acid composition of hepatic triglycerides.

| TG (µmol/g) | Lox Chow | SKO Chow | Lox HFD | SKO HFD  |
|-------------|----------|----------|--------|---------|
| 12:0        | 0.07 ± 0.04 | 0.08 ± 0.01 | 0.13 ± 0.02 | 0.08 ± 0.03* |
| 12:1        | ND       | ND       | 0.20 ± 0.19 | 0.06 ± 0.02 |
| 14:0        | 0.25 ± 0.12 | 0.16 ± 0.02 | 0.75 ± 0.11 | 0.32 ± 0.16* |
| 14:1        | ND       | ND       | 0.45 ± 0.07 | 0.35 ± 0.08* |
| 16:0        | 5.28 ± 2.00 | 3.65 ± 0.18 | 30.34 ± 7.87 | 9.21 ± 3.14* |
| 16:1        | 0.59 ± 0.22 | 0.27 ± 0.01 | 1.70 ± 0.33 | 0.47 ± 0.36* |
| 18:0        | 2.33 ± 0.62 | 2.11 ± 0.24 | 5.34 ± 1.28 | 4.25 ± 2.64 |
| 18:1(n-9)   | 5.23 ± 1.50 | 3.52 ± 0.18 | 30.63 ± 5.36 | 10.24 ± 4.23* |
| 18:1 (n-7)  | 0.44 ± 0.18 | 0.24 ± 0.02 | 1.70 ± 0.43 | 0.57 ± 0.21* |
| 18:2 (n-6)  | 2.44 ± 0.75 | 1.75 ± 0.15 | 17.88 ± 4.12 | 5.32 ± 2.36* |
| 18:3 (n-6)  | 0.06 ± 0.02 | 0.04 ± 0.00 | 0.05 ± 0.01 | 0.03 ± 0.01* |
| 18:3 (n-3)  | 0.11 ± 0.03 | 0.07 ± 0.01 | 0.69 ± 0.12 | 0.19 ± 0.09* |
| 20:0        | 0.10 ± 0.03 | 0.07 ± 0.01 | 0.64 ± 0.13 | 0.28 ± 0.10* |
| 20:1 (n-9)  | 0.04 ± 0.01 | 0.01 ± 0.00 | 0.08 ± 0.02 | 0.04 ± 0.03 |
| 20:3 (n-9)  | 0.08 ± 0.02 | 0.06 ± 0.00 | 0.48 ± 0.09 | 0.19 ± 0.07* |
| 20:4        | 0.07 ± 0.03 | 0.06 ± 0.00 | 0.19 ± 0.04 | 0.12 ± 0.02* |
| 20:5 (n-3)  | 0.04 ± 0.01 | 0.04 ± 0.01 | 0.14 ± 0.04 | 0.02 ± 0.01* |
| 22:0        | 0.03 ± 0.02 | 0.02 ± 0.00 | 0.05 ± 0.02 | 0.02 ± 0.00* |
| 22:1 (n-9)  | 0.01 ± 0.00 | 0.01 ± 0.00 | 0.09 ± 0.10 | 0.03 ± 0.03 |
| 22:6        | 0.21 ± 0.05 | 0.17 ± 0.06 | 0.61 ± 0.16 | 0.17 ± 0.09* |
| **Total**   | **18.35 ± 1.92** | **12.70 ± 0.67** | **98.68 ± 14.23** | **33.92 ± 11.27*** |

*, p<0.05 vs. Lox counterparts
Supplementary Table 1B. Fatty acid composition of hepatic cholesterol esters.

| CE (µmol/g) | Lox chow | SKO chow | Lox HFD | SKO HFD |
|-------------|----------|----------|---------|---------|
| 12:0        | 0.07 ± 0.03 | 0.04 ± 0.00 | 0.06 ± 0.03 | 0.07 ± 0.03 |
| 14:0        | 0.08 ± 0.02 | 0.05 ± 0.01 | 0.14 ± 0.05 | 0.13 ± 0.05 |
| 14:1        | 0.23 ± 0.07 | 0.15 ± 0.02 | 0.37 ± 0.08 | 0.37 ± 0.05 |
| 16:0        | 1.09 ± 0.21 | 0.79 ± 0.06 | 2.10 ± 0.73 | 1.80 ± 0.73 |
| 16:1 (n-9)  | 0.18 ± 0.08 | 0.10 ± 0.01 | 0.31 ± 0.05 | 0.19 ± 0.04* |
| 18:0        | 1.85 ± 0.25 | 1.39 ± 0.15 | 3.55 ± 2.02 | 3.30 ± 1.74 |
| 18:1 (n-9)  | 0.52 ± 0.17 | 0.22 ± 0.02 | 1.66 ± 0.24 | 1.03 ± 0.29 |
| 18:1 (n-7)  | 0.02 ± 0.01 | 0.01 ± 0.00 | 0.05 ± 0.01 | 0.03 ± 0.02 |
| 18:2 (n-6)  | 0.09 ± 0.03 | 0.05 ± 0.00 | 0.43 ± 0.14 | 0.21 ± 0.10* |
| 18:3 (n-6)  | 0.02 ± 0.00 | 0.01 ± 0.00 | 0.03 ± 0.01 | 0.04 ± 0.01 |
| 18:3 (n-3)  | 0.02 ± 0.01 | 0.02 ± 0.01 | 0.07 ± 0.04 | 0.06 ± 0.03 |
| 20:0        | 0.05 ± 0.01 | 0.04 ± 0.00 | 0.10 ± 0.04 | 0.09 ± 0.04 |
| 20:1 (n-9)  | 0.01 ± 0.00 | 0.14 ± 0.27 | 0.02 ± 0.01 | 0.11 ± 0.22 |
| 20:4        | 0.05 ± 0.02 | 0.04 ± 0.00 | 0.10 ± 0.02 | 0.10 ± 0.01 |
| 22:0        | 0.01 ± 0.00 | 0.02 ± 0.02 | 0.02 ± 0.00 | 0.02 ± 0.02 |
| **Total**   | **4.26 ± 0.74** | **3.19 ± 0.48** | **10.52 ± 1.07** | **8.69 ± 0.26*** |

*, p<0.05 vs. Lox counterparts
Supplementary Table 1C. Fatty acid composition of hepatic free fatty acids.

| FFA (nmol/g) | Lox Chow     | SKO Chow     | Lox HFD      | SKO HFD      |
|--------------|--------------|--------------|--------------|--------------|
| 12:0         | 13.48 ± 4.27 | 18.31 ± 6.00 | 19.64 ± 5.87 | 21.26 ± 3.14 |
| 12:1         | 0.71 ± 1.58  | 3.05 ± 2.93  | ND           | 2.12 ± 2.92  |
| 14:0         | 18.72 ± 4.00 | 17.28 ± 1.63 | 18.99 ± 3.48 | 18.16 ± 2.48 |
| 14:1         | 40.07 ± 8.78 | 40.36 ± 3.17 | 44.05 ± 4.83 | 41.32 ± 5.03 |
| 16:0         | 1169.79 ± 63.18 | 1233.32 ± 158.87 | 649.58 ± 142.02 | 565.28 ± 137.99 |
| 16:1         | 109.25 ± 17.58 | 82.97 ± 5.54* | 119.17 ± 20.75 | 98.54 ± 10.88* |
| 18:0         | 183.64 ± 43.30 | 159.92 ± 5.91 | 166.30 ± 25.58 | 168.77 ± 15.70 |
| 18:1 (n-9)   | 215.32 ± 68.57 | 144.08 ± 17.20* | 404.76 ± 96.39 | 236.37 ± 84.39* |
| 18:1 (n-7)   | 16.49 ± 3.65  | 10.50 ± 1.65* | 22.78 ± 5.75  | 15.56 ± 3.95* |
| 18:2 (n-6)   | 49.55 ± 11.50 | 31.05 ± 4.03* | 219.40 ± 72.48 | 107.75 ± 58.48* |
| 18:3 (n-3)   | 2.92 ± 2.20   | 0.60 ± 0.83*  | 6.06 ± 8.45   | 1.32 ± 0.94*  |
| 20:0         | 4.14 ± 1.12   | 2.35 ± 0.48   | 7.40 ± 1.78   | 7.88 ± 0.45   |
| 20:1 (n-9)   | 6.69 ± 0.70   | 6.85 ± 2.14   | 11.09 ± 3.03  | 10.99 ± 3.70  |
| 20:3 (n-9)   | 2.51 ± 1.62   | 2.07 ± 0.55   | 9.09 ± 3.35   | 6.09 ± 2.03   |
| 20:3 (n-6)   | 5.53 ± 1.48   | 6.19 ± 2.72   | 31.02 ± 11.53 | 19.50 ± 6.96  |
| 20:4         | 19.59 ± 4.73  | 17.23 ± 1.82  | 21.23 ± 3.12  | 20.86 ± 0.96  |
| 20:5         | 2.68 ± 2.07   | 0.00 ± 0.00*  | 1.35 ± 1.17   | 1.98 ± 1.95   |
| 22:0         | 2.49 ± 0.46   | 1.56 ± 0.32*  | 4.12 ± 1.47   | 4.89 ± 1.66   |
| 22:1 (n-9)   | ND            | ND            | ND            | 0.64 ± 1.44   |
| 22:5 (n-3)   | ND            | ND            | 3.38 ± 0.73   | 2.89 ± 0.57   |
| 22:6         | ND            | ND            | 11.19 ± 5.41  | 5.91 ± 3.55   |
| Totals       | 1875.67 ± 180.24 | 1788.52 ± 166.57 | 1962.64 ± 163.73 | 1282.85 ± 165.16* |

* p<0.05 vs. Lox counterparts
**A**

Lox  GKO  Lox  GKO

SREBP-1  SREBP-2  Histone H3

Chow  HFD

**B**

Lipogenic genes

| Gene   | Chow | HFD |
|--------|------|-----|
| Acc    | Bar  | Bar |
| Fas    | Bar  | Bar |
| Scd1   | Bar  | Bar |
| Srebp-1c | Bar | Bar |
| Pgc    | Bar  | Bar |
| Pgc-1β | Bar  | Bar |

* p<0.05 vs. Lox

**C**

Lox  GKO

SREBP-1  SREBP-2  Histone H3

**D**

| Gene   | Fasted | Refed |
|--------|--------|-------|
| Acc    | Bar    | Bar   |
| Fas    | Bar    | Bar   |
| Scd1   | Bar    | Bar   |
| Srebp-1c | Bar | Bar |
| Pgc    | Bar    | Bar   |
| Pgc-1β | Bar    | Bar   |

* p<0.05 vs. Lox

Chow  HFD  Fasted  Refed

mRNA levels (Rel. to Chow-fed Lox mice)

mRNA levels (Rel. to Chow-fed Lox mice)
Skin-specific deletion of stearoyl-CoA desaturase-1 alters skin lipid composition and protects mice from high-fat diet-induced obesity
Harini Sampath, Dr., Matthew T. Flowers, Dr., Xueqing Liu, Dr., Chad M. Paton, Dr., Ruth Sullivan, Dr., Kiki Chu, Dr., Minghui Zhao and James M. Ntambi, Dr.

J. Biol. Chem. published online May 8, 2009

Access the most updated version of this article at doi: 10.1074/jbc.M109.014225

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

Supplemental material:
http://www.jbc.org/content/suppl/2009/07/28/M109.014225.DC1