Absence of stearoyl-CoA desaturase-1 ameliorates features of the metabolic syndrome in LDLR-deficient mice

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Running Title: Metabolic features of SCD1-deficient mice

Abbreviations: SCD, stearoyl-CoA desaturase; LDLR, LDL receptor; FAS, fatty acid synthase; ACC-1, acetyl-CoA carboxylase-1; ACS, acetyl-CoA synthetase; GPAT, glycerol-3-phosphate acyltransferase; MUFA, monounsaturated fatty acids; TG, triglycerides; CE, cholesteryl esters; HTG, hypertriglyceridemia; FH, familial hypercholesterolemia.
A combination of the interrelated metabolic risk factors obesity, insulin resistance, dyslipidemia and hypertension, often described as “metabolic syndrome”, is known to increase the risk of developing cardiovascular disease and diabetes. Stearoyl-CoA desaturase (SCD) activity has been implicated in the metabolic syndrome, but detailed studies on the beneficial metabolic effects of SCD deficiency have been limited. Here we show that absence of the Scd1 gene product reduces plasma triglycerides and reduces weight gain in severely hyperlipidemic low density lipoprotein receptor (LDLR)-deficient mice challenged with a western diet. Absence of SCD1 also increases insulin sensitivity as measured by intraperitoneal glucose and insulin tolerance testing. SCD1 deficiency dramatically reduces hepatic lipid accumulation while causing more modest reductions in plasma apolipoproteins, suggesting that in conditions of sustained hyperlipidemia, SCD1 functions primarily to mediate lipid stores. In addition, absence of SCD1 partially ameliorates the undesirable hypertriglyceridemic effect of antiatherogenic LXR agonists. Our results demonstrate that constitutive reduction of SCD activity improves the metabolic phenotype of the LDLR-deficient mice on a western diet.

Supplementary keywords: monounsaturated fatty acids, very low-density lipoprotein, high-density lipoprotein, apolipoprotein B, mouse model, liver, atherosclerosis, ATP binding cassette transporter A1, hyperlipidemia, western diet
Susceptibility to cardiovascular disease and diabetes is associated with the metabolic abnormalities of obesity, insulin resistance, dyslipidemia and hypertension (1-3). A key enzyme that has been implicated in these metabolic abnormalities is stearoyl-CoA desaturase (SCD), which introduces a \textit{cis}-double bond in the Δ-9 position in its substrates, thereby converting palmitic (16:0) and stearic acid (18:0), to palmitoleic (16:1n9) and oleic acid (18:1n9), respectively (4, 5). These nutritionally and physiologically important monounsaturated fatty acids (MUFA) are the major fatty acids found in triglycerides (TG) and cholesteryl esters (CE) (6).

A number of mammalian SCD genes have been reported, including a human \textit{SCD} on 10q24 (7) and a cluster of four genes on mouse chromosome 19 (\textit{Scd1} (8), \textit{Scd2} (9), \textit{Scd3} (10), and \textit{Scd4} (11)). These appear to have arisen from local duplications after divergence of the primate and rodent genomes. An additional SCD gene, \textit{SCD5}, which pre-dates separation of the primate and rodent lineages, has been identified recently and is expressed in the brain and pancreas (12-14). The most prominent site of expression for human \textit{SCD} is in adipose tissue (14), with lower expression in liver and brain (7). Like other lipogenic genes, mammalian SCD genes are highly regulated. Human \textit{SCD} is repressed in cultured cells by polyunsaturated fatty acids and cholesterol via sterol regulatory element-binding protein-1 (SREBP-1) (15). Furthermore, \textit{SCD} mRNA is elevated in skeletal muscle of obese humans (16), and several but not all (17) observational studies in humans have shown an association between increased indices of SCD activity and components of the metabolic syndrome, including insulin resistance (18-21), obesity (16, 22-24), and hypertension (25). The expression of mouse \textit{Scd1} in adipose tissue and liver (8, 10) and its regulation by the lipogenic transcription factors SREBP-1 and LXR (26) defines it as the most relevant murine ortholog for studying the metabolic function of SCD activity in humans.
Seven Scd1 mutant alleles have now been described in mice, including four spontaneous mutations, BALB/c-Scd1\textsuperscript{ab} (27), ABJ/Le-Scd1\textsuperscript{ab-J} (28), DBA/1LacJ-Scd1\textsuperscript{ab-2J/J} (29), and Kunming-Scd1\textsuperscript{Xyk} (30), one chemically-induced mutation, C57BL6/J-Scd1\textsuperscript{flk} (31), and two targeted mutations, 129S-Scd1\textsuperscript{tm1Ntam} (32) and B6129S1F2-Scd1\textsuperscript{tm1Wst} (33). The asebia series of alleles (ab, ab-J, and ab-2J) has been studied the most extensively. Homozygosity for each is associated with atrophic sebaceous glands, alopecia, and scaly skin, phenotypes which are also observed in mice carrying the targeted disruption of the gene (32).

SCD1-deficient mice have been observed to exhibit reduced plasma TG, increased insulin sensitivity, an increased metabolic rate, and resistance to diet-induced obesity (34, 35). However, the impact of SCD1 deficient on hyperlipidemic mice fed a western diet is unknown.

A recent study using homozygous 129S-Scd1\textsuperscript{tm1Ntam} mice fed a chow diet has shown that SCD1 can influence the plasma lipid response to a synthetic LXR agonist (26), T0901317, which increases cholesterol efflux in hyperlipidemic mice (36, 37). However, LXR activation exacerbates hypertriglyceridemia (HTG) in hyperlipidemic mice (36, 37), and the role of SCD1 in regulating the severe LXR-induced HTG observed in hyperlipidemic mice has not yet been determined.

Familial hypercholesterolemia (FH; OMIM 143890), characterized by markedly elevated LDL-cholesterol levels and premature atherosclerosis, was the first genetic disease of lipid metabolism to be clinically and molecularly defined (38). The risk to FH patients of developing coronary disease is further increased by the presence of metabolic syndrome
(39) or its individual components, including low HDL-cholesterol (40-44), high triglycerides (40), and insulin resistance (45-47).

The LDLR-deficient hyperlipidemic mouse mimics human FH and has now been used in numerous studies (48) as a model for the disrupted lipoprotein regulation and metabolic function that leads to diabetes and atherosclerosis. Unlike the most commonly used hyperlipidemic model, apoE-deficient mice (49), LDLR-deficient mice develop diet-induced diabetes and obesity when fed a western diet and also develop a lipoprotein phenotype similar to that seen in FH (48, 50-53).

Earlier studies on the beneficial metabolic effects of SCD1 deficiency have been confined to normolipidemic mice (6, 26, 34, 54-58), and the influence on metabolic parameters in hyperlipidemic mice is unknown. Our results reveal that SCD1 deficiency reduces hepatic and plasma TG, inhibits diet-induced weight gain and insulin resistance, and reduces the hypertriglyceridemic effect of an LXR agonist in hyperlipidemic LDLR-deficient mice.

**Results**

**SCD1 deficiency reduces weight gain and adiposity in Ldlr−/− mice.** An existing mouse strain with a spontaneous deletion in Scd1 (B6.Cg-Scd1<sup>ab−/−</sup>) and an existing dyslipidemic mouse model (B6.129S7-Ldlr<sup>tm1Her</sup>) (59) were crossed to generate mice with deficiencies of both LDLR (Ldlr<sup>/−</sup>) and SCD1 (Scd1<sup>/−</sup>). Mice at the age of 11-13 weeks were fed an atherogenic "western" diet (60) for 12 weeks. Male Scd1<sup>+/−</sup>Ldlr<sup>−/−</sup> mice had a similar weight to the control Scd1<sup>+/+</sup>Ldlr<sup>−/−</sup> mice at the beginning of the diet study (28.4 g vs. 29.1 g, p = 0.51), but they gained less weight after feeding a western diet, (Fig. 1a), despite tending to consume more food than controls (1.5g/day vs. 1.1g/day; p = 0.11, n = 4).
Female $Scd1^{-/-}Ldlr^{/-}$ mice also gained less weight than controls after feeding a western diet (Fig. 1b). After 12 weeks of a western diet, weights for male and female $Scd1^{+/-}Ldlr^{/-}$ mice were 44% and 54% higher than initial values, respectively, while neither male nor female $Scd1^{-/-}Ldlr^{/-}$ mice showed a significant increase in body weight. Both male and female $Scd1^{-/-}Ldlr^{/-}$ mice had smaller peri-epididymal or peri-uterine fat pads than control $Ldlr^{/-}$ mice (Fig. 1c).

To evaluate weight gain in terms of adiposity, fat mass and lean mass were determined using magnetic resonance (MR) relaxometry, a recently validated noninvasive method for the precise assessment of body composition (61) (Fig. 2a-c). Lean body mass was not different between SCD1-deficient mice and controls (Fig. 2b). However, SCD1-deficient mice had a significant 50% reduction in total fat mass compared to controls (males, $p = 0.0006$; females, $p = 0.0043$). Representative images in Fig. 2c show a decrease in both visceral and subcutaneous lipid in SCD1-deficient mice.

**SCD1 deficiency reduces hepatic steatosis in $Ldlr^{/-}$ mice.** Nonadipose tissue also exhibited a marked decrease in lipid accumulation. Histological examination of the livers revealed protection from hepatic steatosis (Fig. 3a, b), and hepatic TG levels were 5-fold higher in control $Scd1^{+/-}Ldlr^{/-}$ mice than in $Scd1^{-/-}Ldlr^{/-}$ mice (Fig. 3c).

**SCD1 deficiency reduces plasma lipids and improves lipoprotein profiles in $Ldlr^{-/-}$ mice.** To determine whether the reduced levels of tissue lipids in hyperlipidemic SCD1-deficient mice are reflected in plasma lipoprotein levels, fasting plasma lipid concentrations and lipoprotein profiles for $Scd1^{-/-}Ldlr^{/-}$ mice and control $Scd1^{+/-}Ldlr^{/-}$ mice were evaluated (Fig. 4 and Table 1). Total plasma TG is significantly reduced by approximately 51% in female SCD1-deficient mice ($p = 0.021$; Fig. 4a). Plasma TG also
tended to be reduced in male SCD1-deficient mice but this difference was not statistically significant (p = 0.23). Plasma total cholesterol (TC) was significantly reduced by approximately 26% in SCD1-deficient male mice (p = 0.023; Fig. 4b), but this trend was not observed in females. HDL cholesterol was not significantly altered in SCD1-deficient mice, while non-HDL cholesterol paralleled the reduction observed in plasma TC (Fig. 4c,d). FPLC analysis confirmed the decrease in VLDL-TG in SCD1-deficient mice with a small increase noted in VLDL cholesterol (Fig. 4e-f).

**SCD1 deficiency reduces plasma apolipoproteins in Ldlr⁻/⁻ mice.** Reductions of plasma apoB (p = 0.0022), apoE (p = 0.0022), and apoC-III (p = 0.0005) in females (Fig. 5a-c), consistent with a reduction in VLDL-TG, were evident. Similar trends were observed in males, but these reductions in plasma apolipoproteins were not significant (Table 1).

**SCD1 deficiency reduces fatty acid synthesis in Ldlr⁻/⁻ mice.** Decreases in hepatic and plasma lipid levels could result from decreased lipogenesis. Therefore, to explore the mechanism by which SCD1 deficiency decreases hepatic and plasma lipids in hyperlipidemic SCD1-deficient mice, we evaluated the incorporation of [³H]acetate into saponifiable lipids in primary hepatocytes isolated from Scd1⁻/⁻Ldlr⁻/⁻ mice and control Scd1⁺/⁺Ldlr⁻/⁻ mice (Fig. 6a). The incorporation of [³H]acetate into fatty acids in the saponifiable lipid fraction over time was reduced in Scd1⁻/⁻Ldlr⁻/⁻ hepatocytes relative to control Scd1⁺/⁺Ldlr⁻/⁻ hepatocytes (p < 0.0001), and incorporation was significantly reduced by approximately 48 to 60% at all time points (p < 0.001).

Acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS) are the two enzymes required for fatty acid synthesis (62, 63). Thus, we hypothesized that the reduced fatty
acid synthesis in hepatocytes may result from reduced levels of transcripts encoding these enzymes, as well as reduced levels of the transcription factor SREBP-1c (64), an important regulator of these transcripts. We examined the hepatic expression of various lipid-sensitive mRNAs and observed significant reductions in the genes that encode ACC-1 (p = 0.0059) and FAS (p = 0.0020) in Scd1<sup>−/−</sup>Ldlr<sup>−/−</sup> mice relative to Scd1<sup>+/−</sup>Ldlr<sup>−/−</sup> mice (Fig. 6b). However, SCD1 deficiency does not significantly alter the hepatic mRNA levels of genes that encode the transcription factors PPARα, LXRα, and SREBP-1c in LDLR-deficient mice challenged with a western diet, although trends toward reduction are seen, particularly for PPARα (p = 0.060) and SREBP-1c (p = 0.082).

**SCD1 deficiency reduces insulin resistance in Ldlr<sup>−/−</sup> mice.** The association between obesity, HTG, and diabetes is well documented (65, 66). To further investigate the effects of SCD1 on these parameters, the response to glucose challenge was assessed.

Male gender is a predisposing diabetes susceptibility factor in most mouse strains (67), so we were not surprised to observe a clear sexual dimorphism in diabetes susceptibility of Scd1<sup>+/−</sup>Ldlr<sup>−/−</sup> mice. At 7 and 11 weeks on a western diet, male Scd1<sup>−/−</sup>Ldlr<sup>−/−</sup> mice were protected from the impaired glucose tolerance evident in male Scd1<sup>+/−</sup>Ldlr<sup>−/−</sup> mice (Fig. 7a,b). Female LDLR-deficient mice were more successful than males at controlling their blood glucose when fed a western diet, but by 11 weeks female Scd1<sup>−/−</sup>Ldlr<sup>−/−</sup> mice showed improved glucose tolerance relative to female Scd1<sup>+/−</sup>Ldlr<sup>−/−</sup> mice (p = 0.03; Fig. 7c).

Insulin sensitivity assays were performed on male mice mice fed a western diet for 9 weeks and fasted overnight before intraperitoneal insulin injection (0.75 U/kg) and glucose was monitored over 90 min. Consistent with their lean phenotype, male Scd1<sup>−/−</sup>Ldlr<sup>−/−</sup> mice
showed both an improved response 15min after insulin injection and reduced blood glucose (Fig. 7d). Measurement of fasting glucose levels indicated that male \( Scd1^{+/+}Ldlr^{-/-} \) mice were protected from the hyperglycemia that had developed in \( Scd1^{+/+}Ldlr^{-/-} \) controls by 9 weeks on a western diet ((Fig. 7e). Male \( Scd1^{-/-}Ldlr^{-/-} \) mice were also protected from the markedly elevated fasting plasma insulin that had developed in \( Scd1^{+/+}Ldlr^{-/-} \) controls by 11 weeks on a western diet ((Fig. 7f). These data indicate that the improved glucose tolerance that was observed in mice lacking SCD1 is attributable in part to increased insulin sensitivity.

**SCD1 mediates the plasma lipid response to LXR agonist treatment in \( Ldlr^{-/-} \) mice.** Treatment of mice with a synthetic LXR agonist, T0901317, has been shown to be antiatherogenic in hyperlipidemic LDLR-deficient mice (36, 37), but its therapeutic utility has been limited by the accompanying severe HTG (36, 37) and hepatic steatosis (68, 69). Recent studies have shown that the lipogenic effect of LXR agonists is mediated through SCD1 (26), but the role of SCD1 in regulating the severe LXR-induced HTG observed in hyperlipidemic LDLR-deficient mice has not yet been determined. To determine whether SCD1 deficiency moderates the undesirable effects of LXR activation in hyperlipidemia, we fed female \( Scd1^{-/-}Ldlr^{-/-} \) mice and \( Scd1^{+/+}Ldlr^{-/-} \) controls a western diet for 12 days and treated with 10mg/kg of T0901317 by oral gavage daily for the final 3 days.

T0901317 treatment resulted in a 4.3-fold increase in plasma TG in LDLR-deficient mice (Fig. 8a). However, plasma TG was reduced by 48% in T0901317-treated \( Scd1^{-/-}Ldlr^{-/-} \) mice relative to \( Scd1^{+/+}Ldlr^{-/-} \) controls, a similar relative reduction to that induced by SCD1 deficiency in hyperlipidemic mice prior to treatment with an LXR agonist. Plasma TC was ~ 10-30% lower at all time-points in SCD1-deficient mice compared with controls, primarily due to a reduction in non-HDL cholesterol (Fig. 8b).
Interestingly, plasma HDL-cholesterol was increased by 73% in T0901317-treated Scd1<sup>-/-</sup>Ldlr<sup>-/-</sup> mice relative to Scd1<sup>+/+</sup>Ldlr<sup>-/-</sup> controls (Fig. 8c). To explore the molecular mechanism by which SCD1 influences plasma lipids in T0901317-treated hyperlipidemic mice, we assessed hepatic expression levels of various genes (Fig. 8d). We observed a 77% reduction in the level of FAS mRNA in SCD1-deficient mice (p = 0.0020). A reduction of FAS indicates that endogenous fatty acids are likely produced at a reduced rate and are less available for generation of triglycerides for secretion into the plasma. Trends toward decreases in transcripts of other lipogenic genes (acetyl-CoA synthetase, 78% reduction, p = 0.22; glycerol-3-phosphate acyltransferase, 51% reduction, p = 0.14) with SCD1 deficiency were also detected in mice fed the LXR agonist.

Hepatic ABCA1 protein expression was increased by 45% in T0901317-treated Scd1<sup>-/-</sup>Ldlr<sup>-/-</sup> mice relative to Scd1<sup>+/+</sup>Ldlr<sup>-/-</sup> controls (Fig. 8e, p = 0.002), which at least partly might explain the increased HDL levels (70, 71). However, we did not observe a significant alteration in the level of Abca1 mRNA (Fig. 8d), suggesting that the increased ABCA1 protein level is not due to increased synthesis of ABCA1, but rather due to alterations in post-transcriptional regulation.

**Discussion**

We have shown that an absence of SCD1 improves the metabolic phenotype of a mouse model of FH on a western diet. Absence of the Scd1 gene product reduces hepatic and plasma TG, and strongly inhibits diet-induced weight gain in LDLR-deficient mice. Absence of SCD1 also provides striking protection from diet-induced insulin resistance as measured by intraperitoneal glucose and insulin tolerance testing. Finally, we have
demonstrated that absence of SCD1 partially reduces the undesirable hypertriglyceridemic effect of antiatherogenic LXR agonists in hyperlipidemic mice.

Normolipidemic SCD1-deficient mice are known to be protected from insulin resistance and diet-induced obesity (34). The role of SCD1 in resistance to obesity has also been expanded to include the leptin-deficient model of obesity (35). We have now extended the findings and demonstrate that the absence of SCD1 provides significant protection from diet-induced obesity in the hyperlipidemic LDLR-deficient model.

Liver TG are reduced by 40-65% in SCD1-deficient mice (6, 54) and TG synthesis is also reduced (35, 54, 72). The most profound impact of absence of SCD1 on the metabolic features of LDLR-deficient mice in this study was a 5-fold reduction in hepatic steatosis, a greater relative reduction than the reductions of ~65% observed previously in chow-fed SCD1-deficient genetic models of obesity (35) and lipodystrophy (58).

Fatty liver is frequently observed in individuals with obesity, type 2 diabetes, and hyperlipidemia. Moreover, the degree of steatosis in non-alcoholic fatty liver disease is proportional to the degree of obesity (73), and insulin resistance is almost universally observed in non-alcoholic fatty liver disease (74, 75). Short term high-fat feeding in rodents in the absence of increases in peripheral fat accumulation has previously demonstrated a causal role for intracellular hepatic fat accumulation in the pathogenesis of hepatic insulin resistance (76). Furthermore, hepatic fat accumulation is often accompanied by a chronic, subacute state of inflammation, which can increase insulin resistance (77, 78). Thus the dramatic reduction in hepatic triglycerides that we observe in the absence of SCD1 may contribute in part to increasing insulin sensitivity. However, increased levels of saturated fatty acids (79, 80) and overexpression of SCD1 (81) decrease insulin signaling in
muscle cells, suggesting that absence of SCD1 may also contribute to increased insulin sensitivity in skeletal muscle in addition to the liver.

The role of SCD1 in regulating plasma TG has been evaluated in several studies and a reduction in plasma TG has not been consistently observed. Some studies have shown plasma TG reduced by over 50% (6, 55, 82), but two studies have shown no significant differences (56, 57). It is not known whether the phenotypic differences could be attributed to the variations in age, sex, diet, fasting protocol, or genetic background of mice in the different studies. SCD1 deficient mice have a markedly reduced rate of VLDL-TG production (35), and the effect on plasma TG levels may be more apparent in hyperlipidemic mice. Our results show that absence of SCD1 reduces plasma lipids (TC, TG) and improves lipoprotein profiles in LDLR-deficient mice.

A potential mechanism for the reduction in hepatic and plasma lipids in SCD1-deficient mice is reduced lipogenesis. We found that SCD1 deficiency markedly reduces fatty acid synthesis in hepatocytes and reduces hepatic mRNA levels of the two SREBP-1c regulated genes that encode enzymes required for long-chain fatty acid synthesis, ACC-1 and FAS. These data are consistent with previous studies that demonstrated reduced ACC-1 and FAS hepatic expression levels (34, 58, 83, 84) and reduced ACC activity (84) in SCD1-deficient chow-fed mice, and indicate that a reduction in hepatic de novo fatty acid synthesis is likely to be a major contributor to the decreased hepatic and plasma lipids we observe in hyperlipidemic SCD1-deficient mice.

T0901317 is a synthetic LXR agonist which has been shown to be atheroprotective in hyperlipidemic mice (36, 37), an effect that is postulated to be mediated by stimulating cholesterol efflux in macrophages (36, 37, 69). However, LXR activation has been
observed to lead to undesirable side effects, specifically HTG (36, 37) and hepatic steatosis. Our data demonstrate that absence of SCD1 significantly influences the plasma lipid response to antiatherogenic LXR agonist treatment, reducing non-HDL cholesterol and increasing beneficial HDL cholesterol. In addition, SCD1 deficiency is also able to partially improve LXR-induced HTG in the hyperlipidemic LDLR-deficient model. These data are consistent with data in a recent study of chow-fed mice that have shown that SCD1 can regulate the metabolic response to a synthetic LXR agonist (26).

Increased levels of SCD1 and its MUFA products inhibit cholesterol efflux mediated by ABCA1 (85-87) by a mechanism that may involve increased turnover rather than altered transcript levels (88). In addition, SCD1 deficiency can increase hepatic ABCA1 in SCD1-deficient mice fed a very-low fat diet (89). Our data now provide evidence that the increased plasma HDL cholesterol observed in hyperlipidemic LXR-treated mice is accompanied by increased levels of the ABCA1 protein but no significant alteration in the level of Abca1 mRNA, consistent with a mechanism involving alterations in post-transcriptional regulation.

In summary, our results establish the robust impact of SCD1 deficiency on the metabolic phenotype of the hyperlipidemic LDLR-deficient mouse model, including reduced hepatic and plasma TG, reduced diet-induced weight gain and insulin resistance, and a partially reduced hypertriglyceridemic response to an LXR agonist.

Methods

Animals and diet. Mice carrying the Scd1<sup>ab-J</sup> (28) or Scd1ab-2J (29) null alleles were back-crossed to C57BL/6 for five generations then crossed to the B6.129S7-Ldlr<sup>amHet</sup> mutant strain (59). Mice carrying the Scd1<sup>ab-J</sup> allele were used in all experiments except
those involving hepatocyte isolation in which mice carrying the Scd1<sup>ab-2J</sup> allele were used. Animals received a standard laboratory rodent chow diet (LabDiet 5010 Autoclavable Rodent Diet, PMI Nutrition International, Richmond, IN), or western diet (TD.88137, Harlan Teklad, Madison, WI). For LXR agonist treatment, animals received T0901317 (68) (10 mg/kg body weight) daily by oral gavage for 3 days. The weight of the food contained in the food bin and any that had been spilled or buried in each cage (to the nearest 0.1g) was recorded every 2 to 3 days for 8 consecutive days and food intake for each mouse was averaged over the 8 days. All studies were approved by the University of British Columbia Animal Care Committee.

**Adiposity measurements using MR imaging and relaxometry.** For imaging, mice were anesthetized with isofluorane and imaged in a Bruker Biospec 70/30 7 Tesla MRI Scanner (Bruker Biospin, Ettlingen, Germany) with and without fat suppression. Images were acquired in the abdominal region in 1.5 mm transverse slices with a MSME T1-weighted pulse sequence, acquiring a field of view of 4 cm and a matrix size of 128 x128. The echo time was 12 ms and the repetition time was 300 ms. MR signal from the body of nonanesthetized mice was acquired with a quadrature volume RF coil tuned to 300 MHz. Absolute fat and lean mass was calculated from the NMR data as described by Kunnecke et al. (61).

**Fat pad measurements and histology.** Peri-uterine and peri-epididymal white adipose depots were dissected and weighed. For routine histology, similar areas from liver tissue from mice after 16h starvation were formalin-fixed, embedded, sectioned, and stained with oil red O (counterstained with hematoxylin).

**Lipid and lipoprotein analysis.** Fast protein liquid chromatography (FPLC) was preformed to separate the 3 major lipoprotein classes, VLDL, LDL, and HDL, in pooled
plasma. For hepatic lipid analysis, liver tissue was homogenized in PBS and total lipids extracted using Folch solution (chloroform/methanol 2:1), dried under \( N_2 \), and resuspended in 2% Triton X-100. Unfractionated plasma, FPLC fractions, and tissue lipid extracts were assayed for cholesterol and TG concentrations by enzymatic assays with the use of commercially available reagents. Plasma HDL cholesterol levels were determined after precipitation of apoB-containing lipoproteins with phosphotungstic acid/Mg (Wako Diagnostics, Richmond, VA). Lipoproteins in the density < 1.21 g/mL fraction obtained by preparative ultracentrifugation were analyzed by SDS-PAGE on gradient gels (4-16%) for determination of apoB and apoE as described (90). Briefly, 10 \( \mu \)g of protein was added to each lane of the gel. Gels were stained with Coomassie blue and bands corresponding to apoB and apoE were quantified by scanning using a densitometer. Unfractionated plasma levels apoC-III were determined by immunonephelometry with the use of mouse-specific antibodies developed in rabbits. The distribution of lipids in plasma lipoproteins was assessed as described (91).

**Hepatocyte isolation and radiolabeling with \( ^3 \text{H} \)acetate.** Primary hepatocytes were isolated as described (92). Briefly, mice fed a western diet for four weeks were anesthetized by intraperitoneal injection of Somnotol (22 \( \mu \)l/50 g body weight) and the livers were perfused with Hanks' EGTA solution containing 1 mg/ml insulin followed by Hanks' collagenase solution (100 units/ml) containing 1 mg/ml insulin. The hepatocytes were dispersed in Hanks' collagenase solution and washed three times in Dulbecco's modified Eagle's medium (DMEM), then suspended in medium containing 10% fetal bovine serum and plated on 60 mm collagen-coated dishes (1 x 10^6 cells/ml). Hepatocytes were incubated for up to 3 h with DMEM containing 25 \( \mu \)Ci/ml \( ^3 \text{H} \)acetate and then washed twice with DMEM. Lipids were extracted with chloroform: methanol (2:1, v/v) and then saponified by heating to 80°C in methanolic KOH. Non-saponifiable lipids were...
removed by extraction with diethyl ether. The aqueous phase containing released fatty acids was acidified and the fatty acids were extracted with hexane. Incorporation of \([{}^3\text{H}]\)acetate into fatty acids was determined as \([{}^3\text{H}]\)fatty acids per mg of total cell protein.

**Physiological analysis.** Intraperitoneal glucose tolerance tests were performed on 12-hour fasted mice injected with 1.5 g/kg glucose. Blood samples were taken at 0, 15, 30, 60, and 90 minutes and blood glucose was measured with a glucometer (Lifescan, Milpitas, CA). Insulin tolerance tests were performed on 12-hour fasted mice injected with 0.75 U/kg human recombinant insulin (Novo Nordisk, Princeton, NJ). We measured plasma insulin by ELISA (Crystal Chem, Downers Grove, IL).

**Real-time PCR and immunoblotting.** We extracted total RNA from liver using the TRIzol reagent according to manufacturer’s instructions (Invitrogen Canada, Burlington, ON, CA). 1 microgram of DNase-treated RNA was reverse-transcribed using Superscript II (Invitrogen Canada, Burlington, ON, Canada) to generate RNAse H-treated cDNA for real-time PCR using SYBR green PCR Master Mix (Applied Biosystems, Foster City, CA) in an ABI Prism 7700 Sequence Detection System. We used *Gapdh* as the invariant control. mRNA levels in control mice were arbitrarily set at 1.

Western blotting was performed as previously described (93). Briefly, tissues were homogenized in low salt lysis buffer containing complete protease inhibitor (Roche Diagnostics, Laval, QC), and protein concentration was determined by the Lowry assay (94). Equivalent amounts of total protein were separated by SDS-PAGE, transferred to polyvinylidene difluoride (PVDF) membranes, and probed with anti-ABCA1 (93) or anti-GAPDH antibodies.
**Statistical analysis.** Data are presented as means plus or minus standard error. Initial analyses were performed by the unpaired two-tailed Student's *t* test. If the data did not fit the constraints of this parametric test, data were analyzed with the Mann-Whitney test. Data from body weight, [³H]acetate incorporation and tolerance tests were analyzed by two-way analysis of variance (ANOVA; time, within subjects; genotype, between subjects), using repeated measures for body weight and tolerance tests, all followed by Bonferroni post-tests. Areas under the glucose curves (AUC\text{glucose}) were calculated by the trapezoid rule. Statistical analysis was performed GraphPad Prism software and with the open-source R-package (GraphPad, San Diego, CA; R Development Core Team, 2006 (95)). *P* < 0.05 was considered significant.
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Fig. 1. Total body and fat pad weights of Ldlr<sup>−/−</sup> mice lacking stearoyl-CoA desaturase (SCD1). Body weight was measured in (a) male (b) and female Scd1<sup>+/−</sup> Ldlr<sup>−/−</sup> and Scd1<sup>−/−</sup>Ldlr<sup>−/−</sup> mice fed western diet starting at 12 weeks of age (males, p < 0.0001; females, p = 0.01; repeated-measures analysis of variance (ANOVA)). n = 6-12 mice per group. (c) Fat pad weights are shown. *p < 0.05. ***p < 0.001.

Fig. 2. Adiposity in Ldlr<sup>−/−</sup> mice lacking SCD1. (a) total body fat mass, and (b) total body lean mass from mice fed a western diet. n = 3-10 mice per group. (c) Transverse abdominal cross-sections of an SCD1-deficient mouse and a control mouse obtained by magnetic resonance imaging. Slices (1.5 mm thickness) at the kidneys were identified in sagittal images from each mouse. Fatty tissues are shown as bright areas. Spinal muscle, kidneys and subcutaneous (SC) and visceral (V) fat are indicated.

Fig. 3. Hepatic lipids in Ldlr<sup>−/−</sup> mice lacking SCD1. (a and b) Livers of female Scd1<sup>+/−</sup> Ldlr<sup>−/−</sup> and Scd1<sup>−/−</sup>Ldlr<sup>−/−</sup> mice fed a western diet stained with oil red O. (c) Liver triglyceride (TG), free cholesterol (FC), and cholesteryl ester (CE) content of Scd1<sup>+/−</sup>Ldlr<sup>−/−</sup> and Scd1<sup>−/−</sup>Ldlr<sup>−/−</sup> mice fed a western diet. n = 8 mice per group.

Fig. 4. Plasma lipids and lipoprotein profiles in Ldlr<sup>−/−</sup> mice lacking SCD1. (a) Plasma TG, (b) total cholesterol (TC) content, (c) HDL-cholesterol, and (d) non-HDL-cholesterol content of Scd1<sup>+/−</sup>Ldlr<sup>−/−</sup> and Scd1<sup>−/−</sup>Ldlr<sup>−/−</sup> mice fed western diet. n = 8-12 mice per group. (e-h), Fast protein liquid chromatography lipoprotein profiles of pooled plasma samples from Scd1<sup>+/−</sup>Ldlr<sup>−/−</sup> and Scd1<sup>−/−</sup>Ldlr<sup>−/−</sup> mice fed western diet. TG (e and f) levels were determined for each fraction from male (e) and female (f) mice. The lipoprotein peaks for VLDL, LDL, and HDL are indicated.
Fig. 5. Plasma apolipoproteins in \( Ldlr^- \) mice lacking SCD1. ApoB (a), apoE (b), and apoC-III (c) were measured in male and female \( Scd1^{+/+}Ldlr^- \) and \( Scd1^{-/-}Ldlr^- \) mice fed western diet. Apolipoprotein levels are expressed in relative units (RU) compared to the levels of male \( Scd1^{+/+}Ldlr^- \) mice. \( n = 6-12 \) mice per group.

Fig. 6. Fatty acid synthesis and hepatic gene expression in \( Ldlr^- \) mice lacking SCD1. (a) Assessment of fatty acid synthesis in hepatocytes from \( Scd1^{+/+}Ldlr^- \) and \( Scd1^{-/-}Ldlr^- \) mice fed a western diet. Primary hepatocytes were prepared from one mouse of each genotype and incubated for up to 3 hours with \( [3^3H] \)acetate, after which lipids were saponified and \( [3^3H] \)fatty acid content was measured (\( p < 0.0001; \) two-way ANOVA). Each value is the average of four independent dishes of hepatocytes and error bars are included unless obscured by symbols. ***\( p < 0.001 \). (b) Relative amount of various mRNAs in livers of \( Scd1^{+/+}Ldlr^- \) and \( Scd1^{-/-}Ldlr^- \) mice fed a western diet. Each value represents the amount of mRNA relative to that in \( Scd1^{+/+}Ldlr^- \) mice (arbitrarily set at 1 for each transcript in these mice). \( n = 6-17 \) mice per group.

Fig. 7. Glucose tolerance and insulin resistance in \( Ldlr^- \) mice lacking SCD1. (a-c) Intraperitoneal (IP) glucose tolerance tests (1.5 g/kg) were performed on mice fasted overnight after being fed a western diet for 7 weeks (a, males; \( p < 0.0001 \), repeated measures ANOVA) or 11 weeks (b, males, \( p = 0.014 \); c, females, \( p = 0.03 \), repeated measures ANOVA). Insets show areas under the glucose curves (AUC\text{glucose}; mg/dL x 90 min). (d) IP insulin tolerance tests (0.75 U/mL) were performed on male mice fasted overnight after being fed a western diet for 9 weeks (\( p = 0.0085 \), repeated measures ANOVA). (e) Glucose was measured in blood and (f) insulin was measured in plasma obtained from the saphenous vein of mice.
fasted overnight after being fed a western diet for 9 weeks and 11 weeks, respectively. \( n = 3-10 \) mice per group. \( \text{Scd1}^{+/-}\text{Ldlr}^{-/-} \) mice are indicated with a solid line, and \( \text{Scd1}^{-/-}\text{Ldlr}^{-/-} \) mice are indicated with a broken line. \(* p < 0.05. ** p < 0.01. *** p < 0.001.\)

Fig. 8. Plasma lipid response to LXR agonist treatment in \( \text{Ldlr}^{-/-} \) mice lacking SCD1. (a) Plasma TG and (b) TC content of \( \text{Scd1}^{+/-}\text{Ldlr}^{-/-} \) and \( \text{Scd1}^{-/-}\text{Ldlr}^{-/-} \) mice at the age of 10-12 months at baseline, and after being fed a western diet (WTD) for 9 days, and a western diet plus 10mg/kg T0901317 for 3 additional days. (c) Plasma HDL cholesterol at baseline and after T0901317 feeding. \( n = 7-8 \) mice per group. (d) Relative amount of mRNA encoding acetyl-CoA synthetase (ACS), fatty acid synthase (FAS), glycerol-3-phosphate acyltransferase (GPAT) and ABCA1 in liver after T0901317 feeding. Each value represents the amount of mRNA relative to that in \( \text{Scd1}^{+/-}\text{Ldlr}^{-/-} \) mice (arbitrarily set at 1 for each transcript in these mice). \( n = 4-5 \) mice per group. (e) Quantification and representative immunoblot of liver lysates from \( \text{Scd1}^{+/-}\text{Ldlr}^{-/-} \) and \( \text{Scd1}^{-/-}\text{Ldlr}^{-/-} \) mice with antibodies against ABCA1, and GAPDH as loading control. \( n = 5-8 \) mice per group.
### Table 1. Plasma Lipid and Apolipoprotein Levels in Ldlr\(^{-/-}\) mice lacking SCD1.

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Females</th>
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<tbody>
<tr>
<td></td>
<td>Scd1(^{+/+})Ldlr(^{-/-})</td>
<td>Scd1(^{-/-})Ldlr(^{-/-})</td>
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<tr>
<td>TG, mg/dL</td>
<td>885 ± 673 (11)</td>
<td>497 ± 294 (8)</td>
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<tr>
<td>Cholesterol, mg/dL</td>
<td>2192 ± 635 (11)</td>
<td>1614 ± 307 (8)</td>
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<tr>
<td>HDL-C, mg/dL</td>
<td>58.5 ± 14.5 (11)</td>
<td>64.4 ± 8.3 (7)</td>
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<tr>
<td>Non-HDL-C, mg/dL</td>
<td>2133 ± 627 (11)</td>
<td>1555 ± 306 (8)</td>
</tr>
<tr>
<td>ApoB</td>
<td>1.00 ± 0.60 (6)</td>
<td>0.66 ± 0.15 (6)</td>
</tr>
<tr>
<td>ApoE</td>
<td>1.00 ± 0.50 (6)</td>
<td>0.75 ± 0.18 (6)</td>
</tr>
<tr>
<td>ApoC-III</td>
<td>1.00 ± 0.37 (11)</td>
<td>0.77 ± 0.26 (7)</td>
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Apolipoprotein levels are expressed in relative units compared to the levels of male Scd1\(^{+/+}\)Ldlr\(^{-/-}\) mice. Data represent mean ± SD. The number of animals in each subgroup is indicated in parentheses.
Figure 1
Figure 2
Figure 3
Figure 4
Figure 5
Figure 6
Figure 7
Figure 8