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

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Abstract A combination of the interrelated metabolic risk factors obesity, insulin resistance, dyslipidemia, and hypertension, often described as the "metabolic syndrome," is known to increase the risk of developing cardiovascular disease and diabetes. Stearoyl-coenzyme A desaturase (SCD) activity has been implicated in the metabolic syndrome, but detailed studies of 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 liver X receptor agonists. Our results demonstrate that constitutive reduction of SCD activity improves the metabolic phenotype of LDLR-deficient mice on a Western diet.—MacDonald, M. L. E., R. R. Singaraja, N. Bissada, P. Ruddle, R. Watts, J. M. Karasinska, W. T. Gibson, C. Fievet, J. E. Vance, B. Staels, and M. R. Hayden. **Absence of stearoyl-CoA desaturase-1 ameliorates features of the metabolic syndrome in LDLR-deficient mice. *J. Lipid Res.* 2008.** 49: **217–229.**

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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-coenzyme A desaturase (SCD), which introduces a *cis* double bond in the Δ-9 position in its substrates, thereby converting palmitic acid (16:0) and stearic acid (18:0) to palmitoleic acid (16:1n7) and oleic acid (18:1n7), respectively (4, 5). These nutritionally and physiologically important MUFAs are the major fatty acids found in triglycerides (TGs) and cholesteryl esters (6).

A number of mammalian SCD genes have been reported, including a human *SCD* on 10q24 (7) and a cluster of four genes on mouse chromosome 19 [*Scd1* (8), *Scd2* (9), *Scd3* (10), and *Scd4* (11)]. These appear to have arisen from local duplications after the divergence of the primate and rodent genomes. An additional SCD gene, *SCD5*, which predates the separation of the primate and rodent lineages, was identified recently and is expressed in the brain and pancreas (12–14). The most prominent site of expression for human *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 *SCD* is repressed in cultured cells by polyunsaturated fatty acids and cholesterol via sterol-regulatory element binding protein-1 (SREBP-1) (15). Furthermore, *SCD* mRNA is increased in skeletal muscle of obese

Abbreviations: ACC-1, acetyl-coenzyme A carboxylase-1; apoE, apolipoprotein E; FH, familial hypercholesterolemia; FPLC, fast-protein liquid chromatography; HTG, hypertriglyceridemia; LDLR, low density lipoprotein receptor; LXR, liver X receptor; SCD, stearoyl-coenzyme A desaturase; SREBP-1, sterol-regulatory element binding protein-1; TC, total cholesterol; TG, triglyceride.

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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 *Scd1* in adipose tissue and liver (8, 10) and its regulation by the lipogenic transcription factors SREBP-1 and liver X receptor (LXR) (26) define 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: four spontaneous mutations, BALB/c-*Scd1*^{ab} (27), ABJ/Le-*Scd1*$^{ab\text{-}J}$ (28), DBA/1LacJ-*Scd1*$^{ab\text{-}2J}$/J (29), and Kunming-*Scd1*^{Xyk} (30); one chemically induced mutation, C57BL6/J-*Scd1*^{flk} (31); and two targeted mutations, 129S-*Scd1*^{tm1Ntam} (32) and B6129S1F2-*Scd1*^{tm1Wst} (33). The *asebia* series of alleles (*ab*, *ab-J*, and *ab-2J*) has been studied most extensively. Homozygosity for each is associated with atrophic sebaceous glands, alopecia, and scaly skin, phenotypes that 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 deficiency on hyperlipidemic mice fed a Western diet is unknown.

A recent study using homozygous 129S-*Scd1*^{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; Online Mendelian Inheritance in Man accession 143890), characterized by markedly increased 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 the metabolic syndrome (39) or its individual components, including low HDL-cholesterol (40–44), high TGs (40), and insulin resistance (45–47).

The low density lipoprotein receptor (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, apolipoprotein E (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.

METHODS

Animals and diet

Mice carrying the *Scd1*$^{ab\text{-}J}$ (28) or *Scd1*$^{ab\text{-}2J}$ (29) null allele were backcrossed to C57BL/6 for five generations and then crossed to the B6.129S7-*Ldlr*^{tm1Her} mutant strain (59). Mice carrying the *Scd1*$^{ab\text{-}J}$ allele were used in all experiments except those involving hepatocyte isolation, in which mice carrying the *Scd1*$^{ab\text{-}2J}$ allele were used. Animals received a standard laboratory rodent chow diet (LabDiet 5010 Autoclavable Rodent Diet; PMI Nutrition International, Richmond, IN) or a Western diet (TD.88137; Harlan Teklad, Madison, WI). For LXR agonist treatment, animals received T0901317 (60) (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 0.1 g) was recorded every 2–3 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 MRI and relaxometry

For imaging, mice were anesthetized with isoflurane 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 × 128. The echo time was 12 ms, and the repetition time was 300 ms. MR signals from the bodies of nonanesthetized mice were acquired with a quadrature volume RF coil tuned to 300 MHz. Absolute fat and lean mass were calculated from the NMR data as described by Kunnecke et al. (61).

Fat pad measurements and histology

Periuterine and periepididymal white adipose depots were dissected and weighed. For routine histology, similar areas from liver tissue from mice after 16 h of 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 performed to separate the three major lipoprotein classes, VLDL, LDL, and HDL, in pooled plasma. For hepatic lipid analysis, liver tissue was homogenized in PBS and total lipids were 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 the determination of apoB and apoE as described (62). Briefly, 10 μg 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 of 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 (63).

Hepatocyte isolation and radiolabeling with [^{3}H]acetate

Primary hepatocytes were isolated as described (64). Briefly, mice fed a Western diet for 4 weeks were anesthetized by intraperitoneal injection of Somnotol (22 μl/50 g body weight) and the livers were perfused with Hanks' EGTA solution containing 1 mg/ml insulin followed by Hanks' collagenase solution (100 U/ml) containing 1 mg/ml insulin. The hepatocytes were dispersed in Hanks' collagenase solution and washed three times in DMEM, then suspended in medium containing 10% fetal bovine serum and plated on 60 mm collagen-coated dishes (1×10^6 cells/ml). Hepatocytes were incubated for up to 3 h with DMEM containing 25 μCi/ml [^{3}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. Nonsaponifiable 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}H]acetate into fatty acids was determined as ^{3}H-labeled fatty acids per milligram of total cell protein.

Physiological analysis

Intraperitoneal glucose tolerance tests were performed on 12 h fasted mice injected with 1.5 g/kg glucose. Blood samples were taken at 0, 15, 30, 60, and 90 min, and blood glucose was measured with a glucometer (Lifescan, Milpitas, CA). Insulin tolerance tests were performed on 12 h 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 the manufacturer's instructions (Invitrogen Canada, Burlington, Ontario, Canada). One microgram of DNase-treated RNA was reverse-transcribed using SuperScript II (Invitrogen 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 described previously (65). Briefly, tissues were homogenized in low-salt lysis buffer containing complete protease inhibitor (Roche Diagnostics, Laval, Quebec, Canada), and protein concentration was determined by the assay of Lowry et al. (66). Equivalent amounts of total protein were separated by SDS-PAGE, transferred to polyvinylidene difluoride membranes, and probed with anti-ABCA1 (65) or anti-GAPDH antibody.

Statistical analysis

Data are presented as means ± SEM. 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, [^{3}H]acetate incorporation, and tolerance tests were analyzed by two-way ANOVA (time, within subjects; genotype, between subjects) using repeated measures for body weight and tolerance tests, all followed by Bonferroni posttests. Areas under the glucose curves were calculated by the trapezoid rule. Statistical analysis was performed with GraphPad Prism software (GraphPad, San Diego, CA) and with the open-source R package (67). $P < 0.05$ was considered significant.

RESULTS

SCD1 deficiency reduces weight gain and adiposity in *Ldlr*$^{-/-}$ mice

An existing mouse strain with a spontaneous deletion in Scd1 (B6.Cg-*Scd1*$^{ab\text{-}J}$) and an existing dyslipidemic mouse model (B6.129S7-*Ldlr*^{tm1Her}) (59) were crossed to generate mice with deficiencies of both LDLR (*Ldlr*$^{-/-}$) and SCD1 (*Scd1*$^{-/-}$). Mice at the age of 11–13 weeks were fed an atherogenic Western diet (68) for 12 weeks. Male *Scd1*$^{-/-}$ *Ldlr*$^{-/-}$ mice had similar weight to the control *Scd1*$^{+/+}$ *Ldlr*$^{-/-}$ mice at the beginning of the diet study (28.4 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 (1.5 vs. 1.1 g/day; $P = 0.11$, $n = 4$). Female *Scd1*$^{-/-}$*Ldlr*$^{-/-}$ mice also gained less weight than

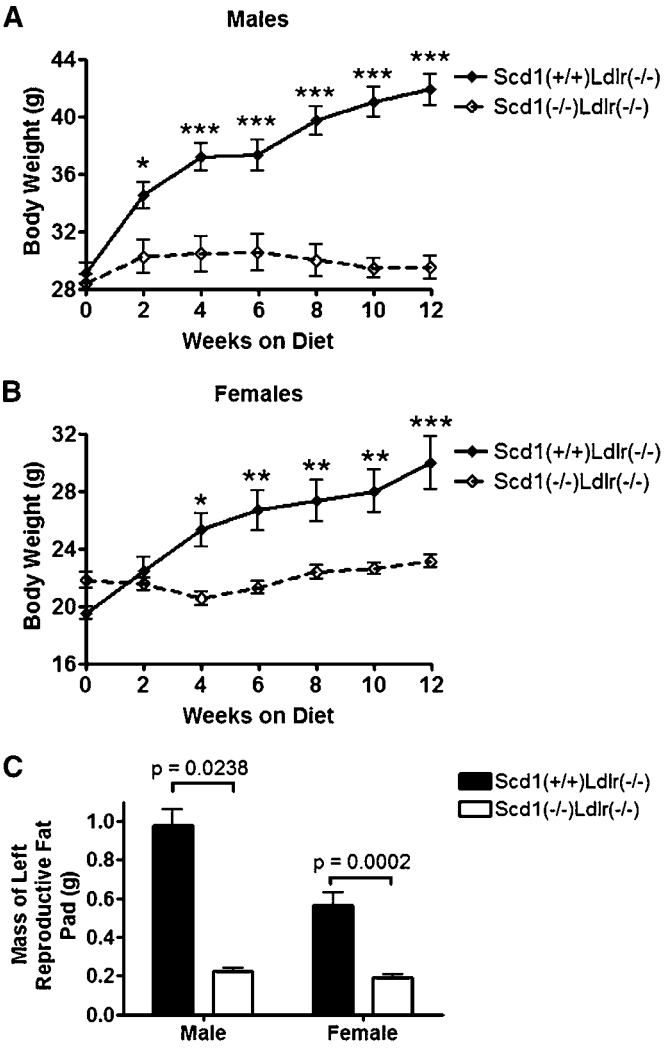

Fig. 1. Total body and fat pad weights of low density lipoprotein receptor-deficient (*Ldlr*$^{-/-}$) mice lacking stearoyl-coenzyme A desaturase (SCD1). A, B: Body weight was measured in male (A) and female (B) *Scd1*$^{+/+}$*Ldlr*$^{-/-}$ and *Scd1*$^{-/-}$*Ldlr*$^{-/-}$ mice fed a Western diet starting at 12 weeks of age (males, $P < 0.0001$; females, $P = 0.01$; repeated-measures ANOVA). n = 6–12 mice per group. C: Fat pad weights are shown. Data shown are means ± SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

controls after feeding a Western diet (Fig. 1B). After 12 weeks on a Western diet, weights for male and female *Scd1*$^{+/+}$ *Ldlr*$^{-/-}$ mice were 44% and 54% higher than initial values, respectively, whereas neither male nor female *Scd1*$^{-/-}$ *Ldlr*$^{-/-}$ mice showed a significant increase in body weight. Both male and female *Scd1*$^{-/-}$*Ldlr*$^{-/-}$ mice had smaller periepididymal or periuterine fat pads than control *Ldlr*$^{-/-}$ mice (Fig. 1C).

To evaluate weight gain in terms of adiposity, fat mass and lean mass were determined using 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 with 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**, **Table 1**). Total plasma TG was significantly reduced by ~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 ~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, whereas 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 [^{3}H]acetate into saponifiable lipids in primary hepatocytes isolated from *Scd1*$^{-/-}$*Ldlr*$^{-/-}$ mice and control *Scd1*$^{+/+}$*Ldlr*$^{-/-}$ mice

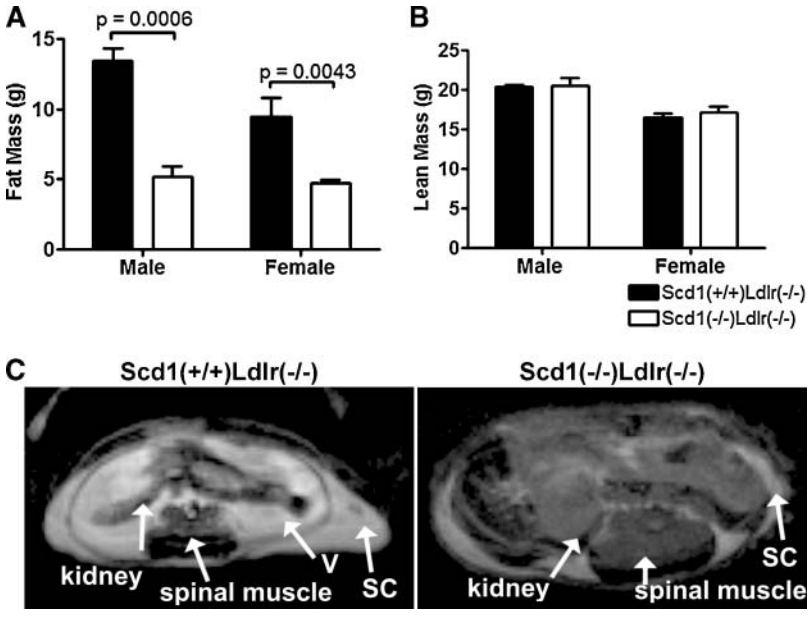

Fig. 2. Adiposity in *Ldlr*$^{-/-}$ mice lacking SCD1. A, B: Total body fat mass (A) and total body lean mass (B) from mice fed a Western diet. n = 3–10 mice per group. Data shown are means ± SEM. C: Transverse abdominal cross-sections of an SCD1-deficient mouse and a control mouse obtained by magnetic resonance imaging. Slices (1.5 mm thick) at the kidneys were identified in sagittal images from each mouse. Fatty tissues are show as bright areas. Spinal muscle, kidneys, and subcutaneous (SC) and visceral (V) fat are indicated.

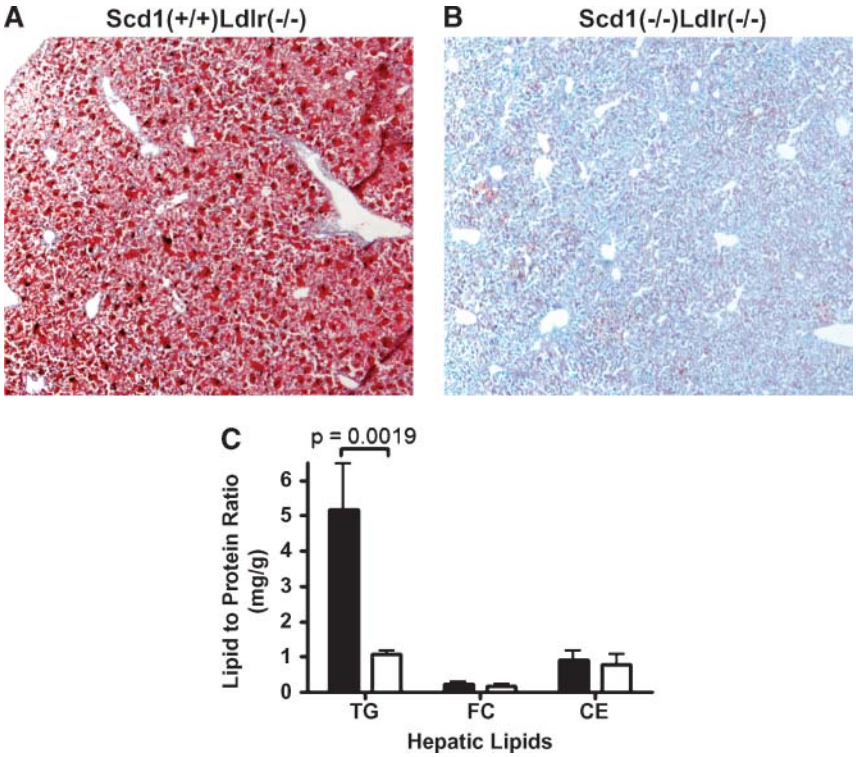

Fig. 3. Hepatic lipids in $Ldlr^{-/-}$ mice lacking SCD1. A, B: Livers of female $Scd1^{+/+}Ldlr^{-/-}$ (A) and $Scd1^{-/-}Ldlr^{-/-}$ (B) mice fed a Western diet stained with Oil Red O. C: Liver triglyceride (TG), free cholesterol (FC), and cholesteryl ester (CE) contents of $Scd1^{+/+}Ldlr^{-/-}$ and $Scd1^{-/-}Ldlr^{-/-}$ mice fed a Western diet. Data shown are means ± SEM. n = 8 mice per group.

(**Fig. 6A**). The incorporation of [^{3}H]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 ~48–60% at all time points ($P < 0.001$).

Acetyl-coenzyme A carboxylase (ACC) and FAS are the two enzymes required for fatty acid synthesis (69, 70). Thus, we hypothesized that the reduced fatty acid synthesis in hepatocytes may result from reduced levels of transcripts encoding these enzymes as well as from reduced levels of the transcription factor SREBP-1c (71), 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 $Scd1^{-/-}Ldlr^{-/-}$ mice relative to $Scd1^{+/+}Ldlr^{-/-}$ mice (Fig. 6B). However, SCD1 deficiency did not significantly alter the hepatic mRNA levels of genes that encode the transcription factors peroxisome proliferator-activated receptor α, LXRα, and SREBP-1c in LDLR-deficient mice challenged with a Western diet, although trends toward reduction were seen, particularly for peroxisome proliferator-activated receptor α ($P = 0.060$) and SREBP-1c ($P = 0.082$).

SCD1 deficiency reduces insulin resistance in $Ldlr^{-/-}$ mice

The association between obesity, HTG, and diabetes is well documented (72, 73). 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 (74), so we were not surprised to observe a clear sexual dimorphism in diabetes susceptibility of $Scd1^{+/+}Ldlr^{-/-}$ mice. At 7 and 11 weeks on a Western diet, male $Scd1^{-/-}Ldlr^{-/-}$ mice were protected from the impaired glucose tolerance evident in male $Scd1^{+/+}Ldlr^{-/-}$ 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 $Scd1^{-/-}Ldlr^{-/-}$ mice showed improved glucose tolerance relative to female $Scd1^{+/+}Ldlr^{-/-}$ mice ($P = 0.03$) (Fig. 7C).

Insulin sensitivity assays were performed on male 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 $Scd1^{-/-}Ldlr^{-/-}$ mice showed both an improved response at 15 min 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 increased 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

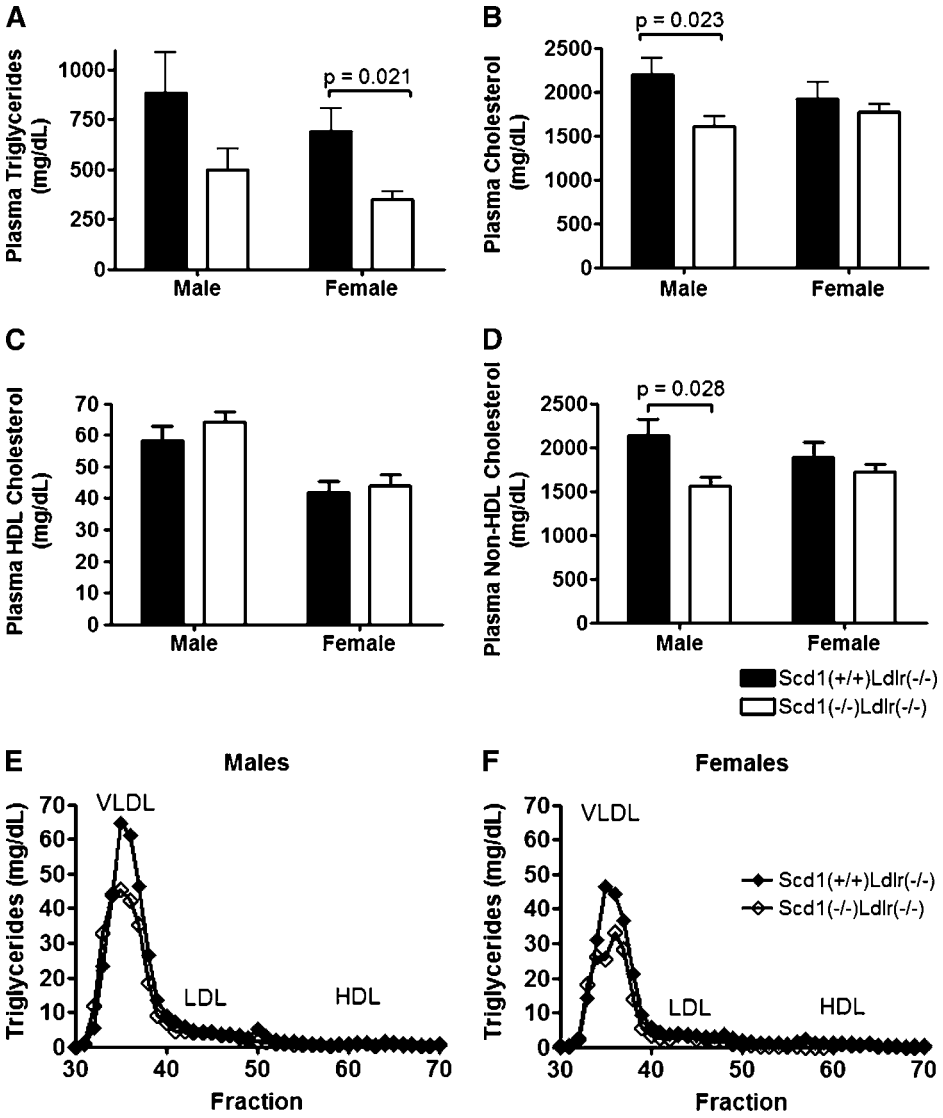

Fig. 4. Plasma lipids and lipoprotein profiles in $Ldlr^{-/-}$ mice lacking SCD1. A–D: Plasma TG (A), TC (B), HDL-cholesterol (C), and non-HDL-cholesterol (D) contents of $Scd1^{+/+}Ldlr^{-/-}$ and $Scd1^{-/-}Ldlr^{-/-}$ mice fed a Western diet. n = 8–12 mice per group. Data shown are means ± SEM. E, F: Fast-protein liquid chromatography lipoprotein profiles of pooled plasma samples from $Scd1^{+/+}Ldlr^{-/-}$ and $Scd1^{-/-}Ldlr^{-/-}$ mice fed a Western diet. TG levels were determined for each fraction from male (E) and female (F) mice. The lipoprotein peaks for VLDL, LDL, and HDL are indicated.

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 (60, 75). 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 them with 10 mg/kg 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 before treatment with an LXR agonist. Plasma TC was ~10–30% lower at all time points in SCD1-deficient mice compared with controls, primarily as a result of a reduction in non-HDL-cholesterol (Fig. 8B).

TABLE 1. Plasma lipid and apolipoprotein levels in LDL receptor-deficient mice lacking stearoyl-coenzyme A desaturase 1

Lipid	Males			Females		
	$Scd1^{+/+}Ldlr^{-/-}$	$Scd1^{-/-}Ldlr^{-/-}$	*P*	$Scd1^{+/+}Ldlr^{-/-}$	$Scd1^{-/-}Ldlr^{-/-}$	*P*
Triglyceride, mg/dl	885 ± 673 (11)	497 ± 294 (8)	0.23	689 ± 414 (12)	350 ± 114 (9)	0.021
Cholesterol, mg/dl	2,192 ± 635 (11)	1,614 ± 307 (8)	0.023	1,927 ± 611 (12)	1,769 ± 269 (9)	0.86
HDL-cholesterol, mg/dl	58.5 ± 14.5 (11)	64.4 ± 8.3 (7)	0.34	41.7 ± 12.2 (12)	44.0 ± 10.9 (9)	0.66
Non-HDL-cholesterol, mg/dl	2,133 ± 627 (11)	1,555 ± 306 (8)	0.028	1,885 ± 612 (12)	1,725 ± 268 (9)	0.86
ApoB	1.00 ± 0.60 (6)	0.66 ± 0.15 (6)	0.18	1.35 ± 0.42 (6)	0.41 ± 0.15 (6)	0.0022
ApoE	1.00 ± 0.50 (6)	0.75 ± 0.18 (6)	0.24	1.29 ± 0.41 (6)	0.45 ± 0.14 (6)	0.0022
ApoC-III	1.00 ± 0.37 (11)	0.77 ± 0.26 (7)	0.17	0.97 ± 0.31 (12)	0.53 ± 0.15 (10)	0.0005

ApoB, apolipoprotein B. Apolipoprotein levels are expressed in relative units compared with the levels of male $Scd1^{+/+}Ldlr^{-/-}$ mice. Data represent means ± SD. The number of animals in each subgroup is indicated in parentheses.

Interestingly, plasma HDL-cholesterol was increased by 73% in T0901317-treated $Scd1^{-/-}Ldlr^{-/-}$ mice relative to $Scd1^{+/+}Ldlr^{-/-}$ 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 the generation of TGs 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 $Scd1^{-/-}Ldlr^{-/-}$ mice relative to $Scd1^{+/+}Ldlr^{-/-}$ controls (Fig. 8E) ($P = 0.002$), which might at least partly explain the increased HDL levels (76, 77). 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 attributable to increased synthesis of ABCA1 but rather to alterations in posttranscriptional 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 these findings and demonstrate that the absence of SCD1 provides significant protection from diet-induced obesity in the hyperlipidemic LDLR-deficient model.

Liver TGs are reduced by 40–65% in SCD1-deficient mice (6, 54), and TG synthesis is also reduced (35, 54, 78). 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 nonalcoholic fatty liver disease is proportional to the degree of obesity (79), and insulin resistance is almost universally observed in nonalcoholic fatty liver disease (80, 81). Short term high-fat feeding in rodents in the absence of increases in peripheral fat accumulation previously demonstrated a causal role for intracellular hepatic fat accumulation in the pathogenesis of hepatic insulin resistance (82). Furthermore, hepatic fat accumulation is often accompanied by a chronic, subacute state of inflammation, which can increase insulin resistance (83, 84). Thus, the dramatic reduction in hepatic TGs that we observed in the absence of SCD1 may contribute in part to increasing insulin sensitivity. However, increased levels of saturated fatty acids (85, 86) and overexpression of SCD1 (87) 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 >50% (6, 55, 88), 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 and TG) and improves lipoprotein profiles in LDLR-deficient mice.

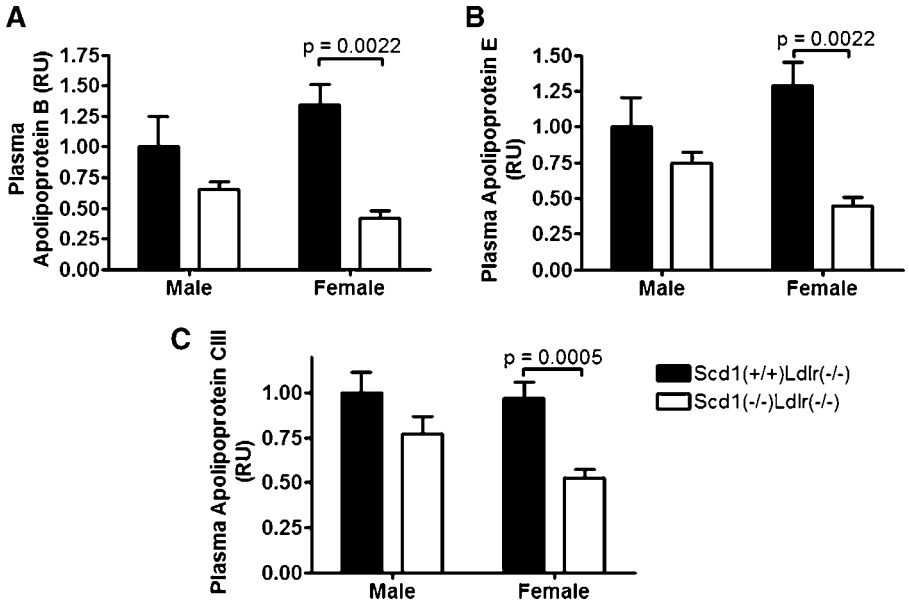

Fig. 5. Plasma apolipoproteins in $Ldlr^{-/-}$ mice lacking SCD1. Apolipoprotein B (apoB; A), apoE (B), and apoC-III (C) were measured in male and female $Scd1^{+/+}Ldlr^{-/-}$ and $Scd1^{-/-}Ldlr^{-/-}$ mice fed a Western diet. Apolipoprotein levels are expressed in relative units (RU) compared with the levels of male $Scd1^{+/+}Ldlr^{-/-}$ mice. Data shown are means ± SEM. n = 6–12 mice per group.

A potential mechanism for the reduction of 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, 89, 90) and reduced ACC activity (90) 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 observed in hyperlipidemic SCD1-deficient mice.

T0901317 is a synthetic LXR agonist that 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, 75). However, LXR activation has been observed to lead to undesirable side effects, specifically HTG (36, 37) and

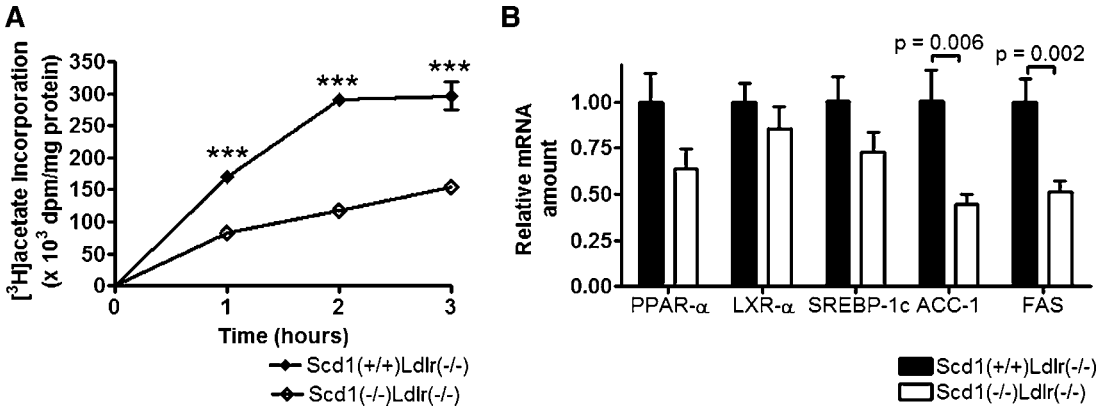

Fig. 6. Fatty acid synthesis and hepatic gene expression in $Ldlr^{-/-}$ mice lacking SCD1. A: Assessment of fatty acid synthesis in hepatocytes from $Scd1^{+/+}Ldlr^{-/-}$ and $Scd1^{-/-}Ldlr^{-/-}$ mice fed a Western diet. Primary hepatocytes were prepared from one mouse of each genotype and incubated for up to 3 h with [^{3}H]acetate, after which lipids were saponified and ^{3}H-labeled 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. Data shown are means ± SEM. *** $P < 0.001$. 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). ACC-1, acetyl-coenzyme A carboxylase-1; LXR-α, liver X receptor-α; PPAR-α, peroxisome proliferator-activated receptor α; SREBP-1c, sterol-regulatory element binding protein-1c. Data shown are means ± SEM. n = 6–17 mice per group.

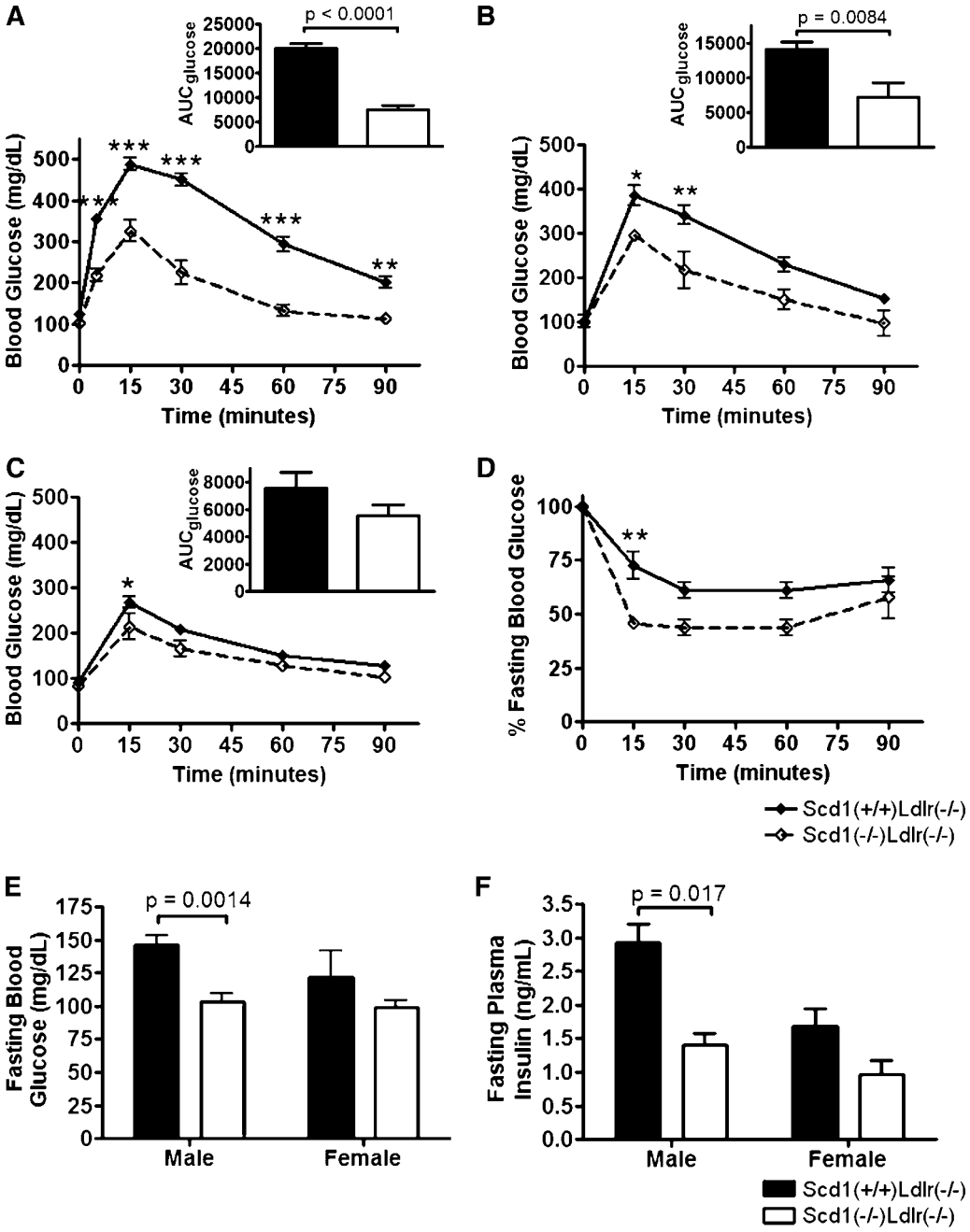

Fig. 7. Glucose tolerance and insulin resistance in $Ldlr^{-/-}$ mice lacking SCD1. A–C: Intraperitoneal glucose tolerance tests (1.5 g/kg) were performed on mice fasted overnight after being fed a Western diet for 7 weeks (A, males; $P < 0.0001$; repeated-measures ANOVA) or 11 weeks (B, males, $P = 0.014$; C, females, $P = 0.03$; repeated-measures ANOVA). Insets show areas under the glucose curves ($AUC_{glucose}$; mg/dl/90 min). D: Intraperitoneal 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). $Scd1^{+/+}Ldlr^{-/-}$ mice are indicated with solid lines, and $Scd1^{-/-}Ldlr^{-/-}$ mice are indicated with dashed lines. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. E, F: Glucose was measured in blood (E) and insulin was measured in plasma (F) 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. Data shown are means ± SEM.

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 showing that SCD1 can regulate the metabolic response to a synthetic LXR agonist (26).

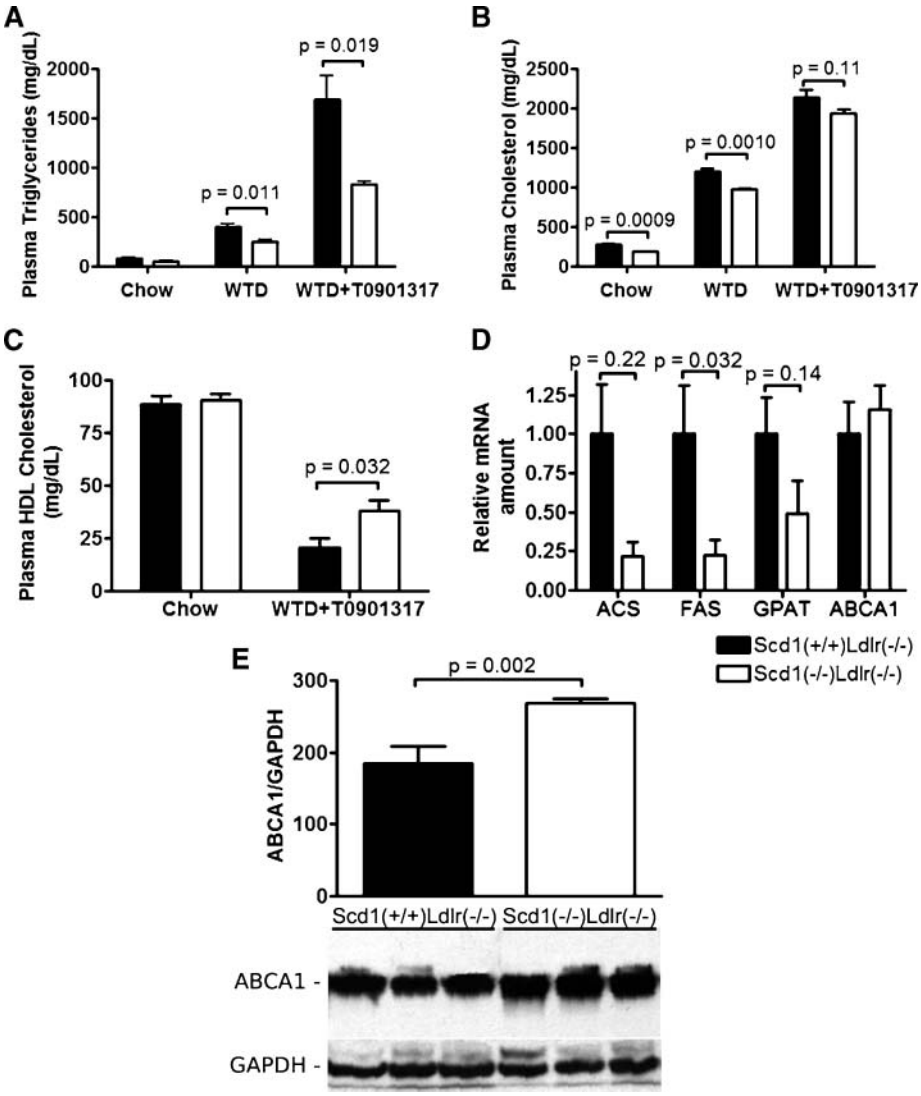

Fig. 8. Plasma lipid response to LXR agonist treatment in $Ldlr^{-/-}$ mice lacking SCD1. A, B: Plasma TG (A) and TC (B) contents of $Scd1^{+/+}Ldlr^{-/-}$ and $Scd1^{-/-}Ldlr^{-/-}$ mice 10–12 months of age at baseline and after being fed a Western diet (WTD) for 9 days and a Western diet plus 10 mg/kg 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), FAS, glycerol-3-phosphate acyltransferase (GPAT), and ABCA1 in liver after T0901317 feeding. 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 = 4–5 mice per group. E: Quantification and representative immunoblot of liver lysates from $Scd1^{+/+}Ldlr^{-/-}$ and $Scd1^{-/-}Ldlr^{-/-}$ mice with antibodies against ABCA1, with GAPDH as a loading control. n = 5–8 mice per group. Data shown are means ± SEM.

Increased levels of SCD1 and its MUFA products inhibit cholesterol efflux mediated by ABCA1 (91–93) by a mechanism that may involve increased turnover rather than altered transcript levels (94). In addition, SCD1 deficiency can increase hepatic ABCA1 in SCD1-deficient mice fed a very-low-fat diet (95). 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 posttranscriptional 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.

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