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Role of enterocyte stearoyl-Co-A desaturase-1 in LDLR-null mice

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Abstract After crossing floxed stearoyl-CoA desaturase-1 (*Scd1*^{fl/fl}) mice with LDL receptor-null (*ldlr*^{-/-}) mice, and then Villin Cre (*VilCre*) mice, enterocyte *Scd1* expression in *Scd1*^{fl/fl}/*ldlr*^{-/-}/*VilCre* mice was reduced 70%. On Western diet (WD), *Scd1*^{fl/fl}/*ldlr*^{-/-} mice gained more weight than *Scd1*^{fl/fl}/*ldlr*^{-/-}/*VilCre* mice ($P < 0.0023$). On WD, jejunum levels of lysophosphatidylcholine (LysoPC) 18:1 and lysophosphatidic acid (LPA) 18:1 were significantly less in *Scd1*^{fl/fl}/*ldlr*^{-/-}/*VilCre* compared with *Scd1*^{fl/fl}/*ldlr*^{-/-} mice ($P < 0.0004$ and $P < 0.026$, respectively). On WD, *Scd1*^{fl/fl}/*ldlr*^{-/-}/*VilCre* mice compared with *Scd1*^{fl/fl}/*ldlr*^{-/-} mice had lower protein levels of lipopolysaccharide-binding protein (LBP), cluster of differentiation 14 (CD14), toll-like receptor 4 (TLR4), and myeloid differentiation factor-88 (MyD88) in enterocytes and plasma, and less dyslipidemia and systemic inflammation. Adding a concentrate of tomatoes transgenic for the apoA-I mimetic peptide 6F (Tg6F) to WD resulted in reduced enterocyte protein levels of LBP, CD14, TLR4, and MyD88 in *Scd1*^{fl/fl}/*ldlr*^{-/-} mice similar to that seen in *Scd1*^{fl/fl}/*ldlr*^{-/-}/*VilCre* mice. Adding LysoPC 18:1 to WD did not reverse the effects of enterocyte *Scd1* knock-down. Adding LysoPC 18:1 (but not LysoPC 18:0) to chow induced jejunum *Scd1* expression and increased dyslipidemia and plasma serum amyloid A and interleukin 6 levels in *Scd1*^{fl/fl}/*ldlr*^{-/-} mice, but not in *Scd1*^{fl/fl}/*ldlr*^{-/-}/*VilCre* mice. **We conclude that enterocyte *Scd1* is partially responsible for LysoPC 18:1- and WD-induced dyslipidemia and inflammation in *ldlr*^{-/-} mice.**—Mukherjee, P., G. Hough, A. Chattopadhyay, V. Grijalva, E. I. O'Connor, D. Meriwether, A. Wagner, J. M. Ntambi, M. Navab, S. T. Reddy, and A. M. Fogelman. **Role of enterocyte stearoyl-Co-A desaturase-1 in LDLR-null mice.** *J. Lipid Res.* 2018. 59: 1818–1840.

Supplementary key words atherosclerosis • apolipoprotein A-I mimetic peptides • Tg6F • lipopolysaccharide-binding protein • cluster of differentiation 14 • toll-like receptor 4 • coenzyme A • low density lipoprotein receptor-null

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Microarray analysis of the jejunum of LDL receptor-null (*ldlr*^{-/-}) mice fed a Western diet (WD), a WD supplemented with transgenic tomatoes expressing the apoA-I mimetic peptide 6F (Tg6F), or a WD supplemented with control transgenic tomatoes revealed stearoyl-CoA desaturase-1 (*Scd1*) as the gene with the greatest differential expression (1). By quantitative real-time PCR, *Scd1* gene expression in the jejunum of *ldlr*^{-/-} mice fed WD increased ~25-fold compared with chow-fed *ldlr*^{-/-} mice, and the increase was almost completely inhibited by addition of Tg6F to WD (see supplemental Fig. S1C in Ref. 1).

Previously, we also reported (1, 2) that adding 1 mg of lysophosphatidylcholine (LysoPC) 18:1 per gram of chow or 1 μ g of lysophosphatidic acid (LPA) 18:1 per gram of chow to the chow diet of *ldlr*^{-/-} mice resulted in dyslipidemia and systemic inflammation similar (but quantitatively less) to that seen when the mice were fed WD. In contrast, adding the same dose of LysoPC 18:0 or LPA 18:0 to chow did not cause these changes (1, 2).

To our surprise, in a subsequent study, we found that standard mouse chow contains dramatically higher levels of oleic acid (C18:1)-containing phospholipids and higher levels of LysoPC 18:1 compared with WD (3). Moreover, WD contained almost no LysoPC 18:1, but WD dramatically increased the content of LysoPC 18:1 in the tissue of the jejunum (3). We hypothesized that fatty acids in enterocytes

Abbreviations: CD14, cluster of differentiation 14; CH25H, cholesterol 25-hydroxylase; 6F, the peptide D-W-L-K-A-F-Y-D-K-F-F-E-K-F-K-E-F-F without end blocking groups; fl/fl, floxed; IFN- β , interferon- β ; IL-6, interleukin 6; LBP, lipopolysaccharide-binding protein; *ldlr*^{-/-}, LDL receptor-null; LPA, lysophosphatidic acid; Lpcat3, lysophosphatidylcholine acyltransferase 3; LPS, lipopolysaccharide; LysoPC, lysophosphatidylcholine; MD-2, lymphocyte antigen 96; MyD88, myeloid differentiation factor-88; 25-OHC, 25-hydroxycholesterol; PC, phosphatidylcholine; SAA, serum amyloid A; *Scd1*, stearoyl-Co-A desaturase-1; Tg6F, transgenic tomatoes expressing the 6F peptide; TLR4, toll-like receptor 4; *VilCre*, Villin Cre; WD, Western diet.

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are acted upon by enzymes, which are induced by WD, resulting in the remodeling of LysoPC to oleic acid-containing species (3). The synthesis of oleic acid in mammals is largely due to the action of *Scd1*. Therefore, we set out to determine whether targeting the *Scd1* gene in enterocytes would reduce levels of LysoPC 18:1 and LPA 18:1, and would ameliorate dyslipidemia and systemic inflammation in *ldlr*^{-/-} mice.

To target the *Scd1* gene in enterocytes, we crossed *Scd1* floxed (*Scd1*^{fl/fl}) mice with *ldlr*^{-/-} mice, and then with Villin Cre (*VilCre*) mice. We report here that on WD, *Scd1*^{fl/fl}/*ldlr*^{-/-}/*VilCre* mice were protected from diet-induced obesity, as was the case for mice globally deficient in *Scd1* (4, 5). The *Scd1*^{fl/fl}/*ldlr*^{-/-}/*VilCre* mice had lower levels of LysoPC 18:1 and LPA 18:1 in the tissue of the jejunum compared with *Scd1*^{fl/fl}/*ldlr*^{-/-} mice. On WD, dyslipidemia and systemic inflammation were significantly improved in *Scd1*^{fl/fl}/*ldlr*^{-/-}/*VilCre* mice compared with *Scd1*^{fl/fl}/*ldlr*^{-/-} mice.

An important function of enterocytes is to provide a barrier between gut bacteria and the tissues of the host. The response of enterocytes to lipopolysaccharide (LPS) derived from gut bacteria is via a series of proteins that include LPS-binding protein (LBP), cluster of differentiation 14 (CD14), and toll-like receptor 4 (TLR4), which interacts with lymphocyte antigen 96 (MD-2) to initiate signaling through myeloid differentiation factor-88 (MyD88). Kim et al. (6) reported that a high-fat diet induced inflammation in mice by increasing bacterial LPS levels in the intestinal lumen as well as in the plasma by altering gut microbiota composition and increasing intestinal permeability through the induction of TLR4. The processing of LPS involves an initial interaction with LBP that helps CD14 to extract LPS from bacterial cell walls. CD14 then delivers LPS to TLR4-MD2, which prompts the dimerization and activation of TLR4 (7). TLR4 signals through multiple pathways, including through MyD88 (8), causing increased intestinal permeability that results in intestinal inflammation (9). Here, we report that enterocyte knockdown of *Scd1* in *ldlr*^{-/-} mice on WD resulted in lower protein levels of LBP, CD14, TLR4, and MyD88 in both enterocytes and plasma, and was similar in magnitude to the decrease in these proteins observed upon adding Tg6F to WD.

We also report that enterocyte knockdown of *Scd1* resulted in a marked reduction in the ability of LysoPC 18:1 to cause dyslipidemia and increase plasma levels of serum amyloid A (SAA) and interleukin 6 (IL-6) when added to mouse chow. Together these studies demonstrate that *Scd1* is partially responsible for LysoPC 18:1- and WD-induced dyslipidemia and inflammation in *ldlr*^{-/-} mice.

MATERIALS AND METHODS

Materials

Transgenic tomatoes expressing the 6F peptide (10) were grown and freeze-dried at the University of California, Davis, and shipped frozen to the University of California, Los Angeles, where

a concentrate of these tomatoes (Tg6F) was prepared as described (3). Analysis revealed that the freeze-dried tomato powder from Davis contained the same quantity of the 6F peptide as was found in the tomatoes previously grown at the Donald Danforth Plant Science Center in Saint Louis, MO (3) (data not shown). Studies in *ldlr*^{-/-} mice confirmed that concentrates prepared from tomatoes grown at the two sites were biologically similar in their potency (data not shown). In some experiments, as noted in the figure legends, instead of removing the ethyl acetate/acetic acid from the Tg6F mixture by sublimation with argon gas as described previously (3), a machine (Genevac Rocket 4D Synergy RK0644; SP Scientific Inc., UK) was used to rapidly remove the ethyl acetate/acetic acid. This machine allows the processing of much larger volumes (the machine allows processing of 5 liters versus the argon method, which is limited to 50 ml per tube). Moreover, the machine removes the ethyl acetate/acetic acid much more rapidly (30–60 min versus many hours). The biologic activity of Tg6F preparations made using either the argon method or the machine, when added to WD and fed to *ldlr*^{-/-} mice, was similar (data not shown). Unless otherwise stated, all other materials were from sources previously described (3).

Mice and diets

Scd1^{fl/fl} mice on a C57BL/6J background were obtained from Dr. James M. Ntambi at the University of Wisconsin, Madison (5). These mice were crossed with *ldlr*^{-/-} mice originally purchased from Jackson Laboratories on a C57BL/6J background that were from the breeding colony of the Department of Laboratory and Animal Medicine at the David Geffen School of Medicine at the University of California, Los Angeles, to yield *Scd1*^{fl/fl}/*ldlr*^{-/-} mice. *Scd1*^{fl/fl}/*ldlr*^{-/-} mice were crossed with Villin Cre (*VilCre*) mice on a C57BL/6J background that were purchased from Jackson Laboratories (stock #004586) to yield *Scd1*^{fl/fl}/*ldlr*^{-/-}/*VilCre* mice. The gender and age of the mice are stated in the legend to each figure. The mice were given unlimited access to standard mouse chow (Ralston Purina) prior to the start of experiments. To ensure that the mice ate all of their food during the experiments, the mice were not allowed unlimited access to food, as was the case prior to starting the experiment. During the experiments, they were given a fixed amount of diet each night, and any remaining food was removed each night and weighed to determine the percent not eaten. Unless otherwise stated, the mice were given precisely 4 g of diet per mouse each night (each cage contained four mice that received 16 g of diet each night). When the mice were switched to WD, it was from Teklad, Harlan (catalog #TD88137) or from Research Diets (catalog #D12079B). If the source of WD is not stated, it was from Teklad, which was the source for WD in our previous publications (1–3). When Tg6F was added to the diets, it was added at 0.06% by weight of diet as described (3).

Harvesting of the jejunum and preparation of enterocytes. After 2 weeks of treatment, the mice were fasted overnight in cages with clean bedding, and blood was removed under mild isoflurane anesthesia. The mice were euthanized with an overdose of isoflurane and perfused to remove blood; the small intestine was washed; the jejunum was harvested; and enterocytes were prepared as previously described (11).

Assays

Determination of mRNA levels for Scd1. Enterocyte mRNA levels were determined by quantitative real-time PCR for *Scd1*, as previously described (1, 11), and normalized to TATA-box binding protein mRNA (12).

Immunohistochemistry. Immunohistochemistry was performed as previously described (3) using an antibody to mouse *Scd1* purchased from Cell Signaling Technology, Danvers, MA (catalog #2794).

ELISA and immunoblots. ELISAs for LBP, CD14, TLR4, and MyD88 were performed on enterocytes using the materials and reagents described in the figure legends. Immunoblots for LBP, CD14, TLR4, and MyD88 were performed on enterocytes and plasma, as described (13), using the reagents described in the figure legends.

Other assays. Plasma lipids and SAA levels were determined as described (3, 14, 15), and LysoPC and LPA species were determined by LC-MS/MS as described previously (3). IL-6 levels were determined using a commercially available kit manufactured by Life Technologies, Grand Island, NY, purchased from Thermo Fisher Scientific (catalog #KMC0062), and used according to the manufacturer's instructions.

Statistical analysis

In comparing more than two groups, statistical analysis was initially performed by ANOVA. After determining that statistically significant differences were present by ANOVA, further comparisons were made by unpaired two-tailed *t*-test or, where appropriate, by paired *t*-test. All statistical analyses were performed using GraphPad Prism version 7.02 (GraphPad Software, San Diego, CA). Statistical significance was considered achieved if $P < 0.05$.

RESULTS

Relative expression of *Scd1* in enterocytes

As shown in **Fig. 1**, by quantitative real-time PCR, the relative expression of *Scd1* in enterocytes taken from the jejunum decreased from 1 ± 0.07 in *Scd1^{fl/fl}/ldlr^{-/-}* mice to 0.30 ± 0.05 in *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice. There was no decrease in the relative expression of hepatic *Scd1* in the *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice (data not shown). **Figure 2** demonstrates that on WD, the *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice had dramatically reduced levels of *Scd1* protein in enterocytes in the jejunum compared with *Scd1^{fl/fl}/ldlr^{-/-}* mice.

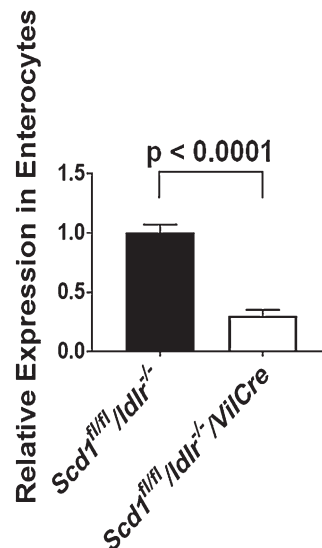
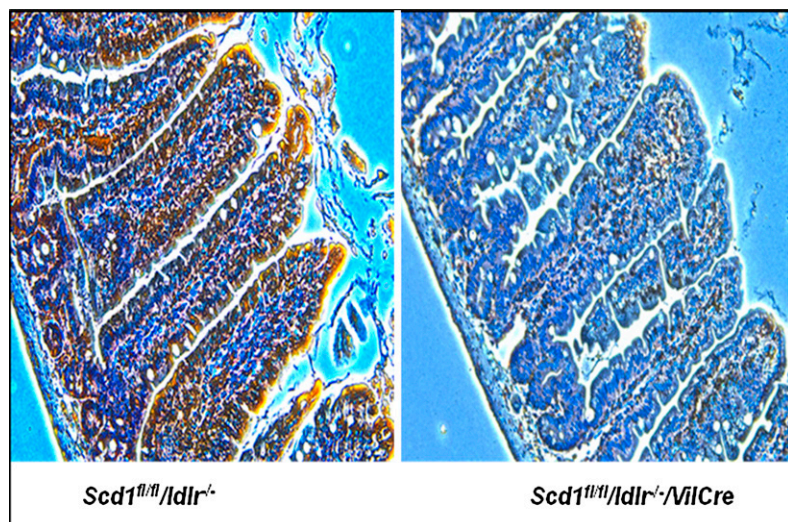


Fig. 1. Knockdown of enterocyte *Scd1*. Enterocytes were prepared from the jejunums of *Scd1^{fl/fl}/ldlr^{-/-}* and *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* female mice (age 4 months, $n = 6$ per group) on a chow diet. Enterocyte mRNA was isolated and quantitative real-time PCR was performed as described in the Materials and Methods. The results shown are the mean \pm SEM.

Knockdown of *Scd1* in enterocytes protected against weight gain on WD

In the first set of experiments, prior to starting WD, *Scd1^{fl/fl}/ldlr^{-/-}* mice weighed 22.16 ± 0.86 g (mean \pm SEM); after 2 weeks on WD, they weighed 23.87 ± 1.0 g ($P < 0.0001$). Prior to starting WD, *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice weighed 22.46 ± 0.76 g (mean \pm SEM); after 2 weeks on WD, they weighed 22.66 ± 0.84 g ($P = 0.5191$). **Figure 3A** shows the weight change in grams after 2 weeks on WD. **Figure 3B** shows the percent change in body weight determined by comparing the weight of each mouse just prior to starting WD with its weight after 2 weeks on WD. WD consumption was not different in *Scd1^{fl/fl}/ldlr^{-/-}* mice compared with *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice. Both the *Scd1^{fl/fl}/ldlr^{-/-}* mice and the *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice ate $\geq 95\%$

Fig. 2. *Scd1* protein in enterocytes was determined by immunohistochemistry. The jejunums of *Scd1^{fl/fl}/ldlr^{-/-}* and *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* female mice (age 4 months, $n = 6$ per group) on WD were stained for *Scd1* protein as described in the Materials and Methods. Representative sections from a *Scd1^{fl/fl}/ldlr^{-/-}* mouse and a *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mouse are shown in the figure.

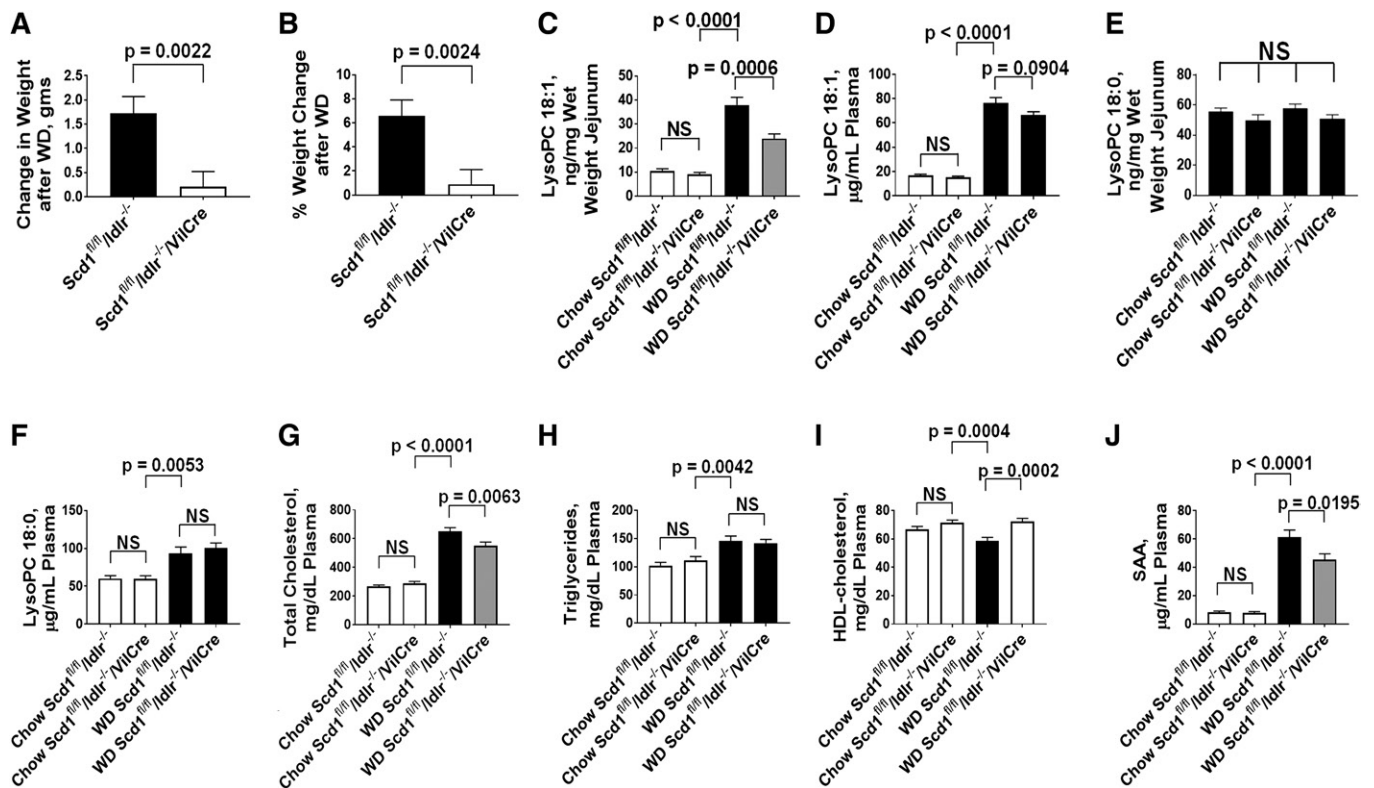


Fig. 3. A: Despite consuming the same amount of WD, *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice gained significantly less weight on WD compared with *Scd1^{fl/fl}/ldlr^{-/-}* mice. *Scd1^{fl/fl}/ldlr^{-/-}* and *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* female mice (age 4–6 months, n = 35–36 per group) were weighed and continued on chow or switched to WD. After 2 weeks, the mice were weighed again, and the difference in grams between the two weights for each mouse was determined. The data shown are the mean ± SEM. B: Despite consuming the same amount of WD, the percent body weight gained in *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice was significantly less on WD compared with *Scd1^{fl/fl}/ldlr^{-/-}* mice. The percent change in body weight for the mice described in A was determined by comparing the weight of each mouse just prior to starting WD with its weight after 2 weeks on WD. The data shown are the mean ± SEM. C: LysoPC 18:1 levels in the tissue of the jejunum increased significantly less in *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice on WD compared with *Scd1^{fl/fl}/ldlr^{-/-}* mice. After 2 weeks, LysoPC 18:1 was determined as described in the Materials and Methods in the jejunums of the mice described in A. The results shown are the mean ± SEM. D: LysoPC 18:1 levels in plasma increased on WD compared with chow, but were not significantly less after knockdown of *Scd1* in enterocytes. LysoPC 18:1 levels in the plasma of the mice described in A were determined as described in the Materials and Methods. The results shown are the mean ± SEM. E: LysoPC 18:0 levels in the tissue of the jejunum of mice fed chow or WD were not different, and LysoPC 18:0 levels were not different after knockdown of *Scd1* in enterocytes. LysoPC 18:0 levels were determined as described in the Materials and Methods in the jejunums of the mice described in A. The results shown are the mean ± SEM. F: LysoPC 18:0 levels in plasma increased on WD compared with chow, but were not less after knockdown of *Scd1* in enterocytes. LysoPC 18:0 levels in the plasma of the mice described in A were determined as described in the Materials and Methods. The results shown are the mean ± SEM. G: Plasma total cholesterol levels increased on WD compared with chow and increased significantly less following *Scd1* knockdown in enterocytes. Total cholesterol levels in plasma of the mice described in A were determined as described in the Materials and Methods. The results shown are the mean ± SEM. H: Plasma triglyceride levels increased on WD compared with chow, but did not change following *Scd1* knockdown in enterocytes. Triglyceride levels in the plasma of the mice described in A were determined as described in the Materials and Methods. The results shown are the mean ± SEM. I: Plasma HDL-cholesterol levels decreased on WD compared with chow in *Scd1^{fl/fl}/ldlr^{-/-}* mice, but not in *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice. HDL-cholesterol levels in the plasma of the mice described in A were determined as described in the Materials and Methods. The results shown are the mean ± SEM. J: Plasma SAA levels increased on WD compared with chow and increased significantly less following *Scd1* knockdown in enterocytes. SAA levels in the plasma of the mice described in A were determined as described in the Materials and Methods. The results shown are the mean ± SEM.

of the WD each day, and there was no difference between the two genotypes.

Knockdown of *Scd1* in enterocytes results in less LysoPC 18:1 in jejunum

As shown in Fig. 3C, LysoPC 18:1 levels in the tissue of the jejunum increased significantly less in *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice on WD compared with *Scd1^{fl/fl}/ldlr^{-/-}* mice on WD; the levels of LysoPC 18:1 in the jejunum were not different on chow. On chow, there was no difference in plasma LysoPC 18:1 levels (Fig. 3D). On WD, there was a

significant increase in LysoPC 18:1 in plasma compared with chow, but the difference in plasma levels of LysoPC 18:1 in *Scd1^{fl/fl}/ldlr^{-/-}* mice compared with *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice did not reach statistical significance (Fig. 3D). In this first set of experiments, the levels of LysoPC 18:0 in the jejunum were not significantly different in mice fed chow compared with WD, and were not significantly different in *Scd1^{fl/fl}/ldlr^{-/-}* mice compared with *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice (Fig. 3E). LysoPC 18:0 levels in plasma significantly increased in mice fed WD compared with chow, but there was no significant difference between *Scd1^{fl/fl}/ldlr^{-/-}*

mice compared with *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice on WD (Fig. 3F).

Knockdown of *Scd1* in enterocytes reduced the WD-mediated increase in plasma cholesterol and SAA levels, and prevented the WD-mediated decrease in plasma HDL-cholesterol levels

WD increased plasma total cholesterol levels compared with chow; *Scd1* knockdown in enterocytes modestly, but significantly, ameliorated the increase (Fig. 3G) without altering plasma triglyceride levels in these experiments (Fig. 3H). On WD, plasma HDL-cholesterol levels significantly decreased in *Scd1^{fl/fl}/ldlr^{-/-}* mice, but not in *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice (Fig. 3I). Plasma SAA levels significantly increased on WD compared with chow, but increased significantly less in *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice compared with *Scd1^{fl/fl}/ldlr^{-/-}* mice (Fig. 3J).

Knockdown of enterocyte *Scd1* decreased levels of LPA 18:1 (but not LPA 18:0) in the jejunum

We previously reported that a significant source of LPA 18:1 in the small intestine was LysoPC 18:1 that was converted to LPA 18:1 by lysophospholipase D (autotaxin) (2). To determine whether knockdown of *Scd1* in enterocytes would lead to changes in LPA 18:1 levels, we performed a second set of experiments. In the second set of experiments, prior to starting WD, *Scd1^{fl/fl}/ldlr^{-/-}* mice weighed 21.61 ± 0.25 g (mean \pm SEM); after 2 weeks on WD, they weighed 22.27 ± 0.33 g ($P = 0.1298$). Prior to starting WD, *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice weighed 21.58 ± 0.25 g (mean \pm SEM); after 2 weeks on WD, they weighed 20.8 ± 0.31 g ($P = 0.0669$). WD consumption was not different in *Scd1^{fl/fl}/ldlr^{-/-}* mice compared with *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice. Both the *Scd1^{fl/fl}/ldlr^{-/-}* mice and the *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice ate $\geq 95\%$ of the WD each day, and there was no difference between the two genotypes. **Figure 4A** shows the weight change in grams after 2 weeks on WD. **Figure 4B** shows the percent change in body weight determined by comparing the weight of each mouse just prior to starting WD with its weight after 2 weeks on WD. Despite the fact that WD consumption was not different in *Scd1^{fl/fl}/ldlr^{-/-}* mice compared with *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice, the *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice lost $3.7 \pm 0.79\%$ of their body weight while the *Scd1^{fl/fl}/ldlr^{-/-}* mice gained $3.0 \pm 0.77\%$ of their body weight on WD.

The experiments shown in Fig. 4C demonstrate that knockdown of enterocyte *Scd1* reduced levels of both LysoPC 18:1 and LPA 18:1 in the tissue of the jejunum. Levels of LysoPC 18:0 and LPA 18:0 were not decreased in the jejunum by enterocyte *Scd1* knockdown (Fig. 4D). In this experiment, as shown in Fig. 4E, the decrease in plasma levels of LysoPC 18:1 in *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice compared with *Scd1^{fl/fl}/ldlr^{-/-}* mice was highly significant. As shown in Fig. 4F, there was a small, but significant, decrease in plasma levels of LysoPC 18:0 in *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice compared with *Scd1^{fl/fl}/ldlr^{-/-}* mice.

In the mice described in Fig. 4A, plasma total cholesterol levels in the *Scd1^{fl/fl}/ldlr^{-/-}* mice were 735 ± 13 mg/dl compared with 647 ± 11 mg/dl in the *Scd1^{fl/fl}/ldlr^{-/-}/VilCre*

mice ($P < 0.0001$). Knockdown of *Scd1* in the enterocytes of the mice described in Fig. 4A resulted in a modest, but significant, decrease in plasma triglycerides (Fig. 4G). Plasma SAA levels in *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice on WD were significantly less than in *Scd1^{fl/fl}/ldlr^{-/-}* mice; adding Tg6F to WD at 0.06% of diet by weight significantly reduced plasma SAA levels in *Scd1^{fl/fl}/ldlr^{-/-}* mice, but not in *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice (Fig. 4H).

A comparison of the knockdown of enterocyte *Scd1* in male and female mice

To compare male and female mice with knockdown of *Scd1* in enterocytes, we performed a third set of experiments. In these experiments, as in the second set of experiments, all of the mice consumed WD for 2 weeks. In this third set of experiments, we compared males and females in four groups: *Scd1^{fl/fl}/ldlr^{-/-}* mice on WD alone; *Scd1^{fl/fl}/ldlr^{-/-}* mice on WD to which 0.06% by weight of Tg6F was added; *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice on WD alone; and *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice on WD to which 0.06% by weight of Tg6F was added.

Figure 5A shows the weights of the males and females just prior to receiving WD. The males in all groups were heavier; the male *Scd1^{fl/fl}/ldlr^{-/-}* mice that received WD without Tg6F were slightly, but significantly, heavier than the other groups of males, which did not differ in weight. There was no significant difference in the weights of the females prior to WD (Fig. 5A). Similar differences in weight between males and females were seen after 2 weeks on WD (Fig. 5B). **Figure 5C** demonstrates that male *Scd1^{fl/fl}/ldlr^{-/-}* mice not receiving Tg6F gained weight on WD, but when Tg6F was added to WD, the *Scd1^{fl/fl}/ldlr^{-/-}* mice did not gain weight. *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice did not gain weight on WD. Adding Tg6F to the WD that was fed to the *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice did not cause a significant change. **Figure 5D** demonstrates similar results in females. Despite the differences in body weight between males and females, the percent change in body weight after 2 weeks on WD was similar for males and females in each group (Fig. 5E). In this third set of experiments, as was the case in the first two sets of experiments, the mice ate $\geq 95\%$ of the WD each day, and there was no difference between groups. These results show that knockdown of enterocyte *Scd1* produced changes in both males and females that were similar to those achieved by adding Tg6F to WD in *Scd1^{fl/fl}/ldlr^{-/-}* mice.

Unlike body weight, LysoPC 18:1 content in the jejunum was similar in *Scd1^{fl/fl}/ldlr^{-/-}* males and females (Fig. 5F). Moreover, the reduction in LysoPC 18:1 content in the jejunum on adding Tg6F to WD or knockdown of enterocyte *Scd1* was also similar (Fig. 5F). *Scd1^{fl/fl}/ldlr^{-/-}* male and female mice receiving WD + Tg6F had significantly lower levels of LysoPC 18:1 compared with male or female *Scd1^{fl/fl}/ldlr^{-/-}* mice receiving WD alone. LysoPC 18:1 levels in *Scd1^{fl/fl}/ldlr^{-/-}* female mice receiving WD + Tg6F were not different from those of female *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice on WD without or with Tg6F. Male *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice on WD alone had modestly, but significantly, higher levels compared with male *Scd1^{fl/fl}/ldlr^{-/-}* mice receiving

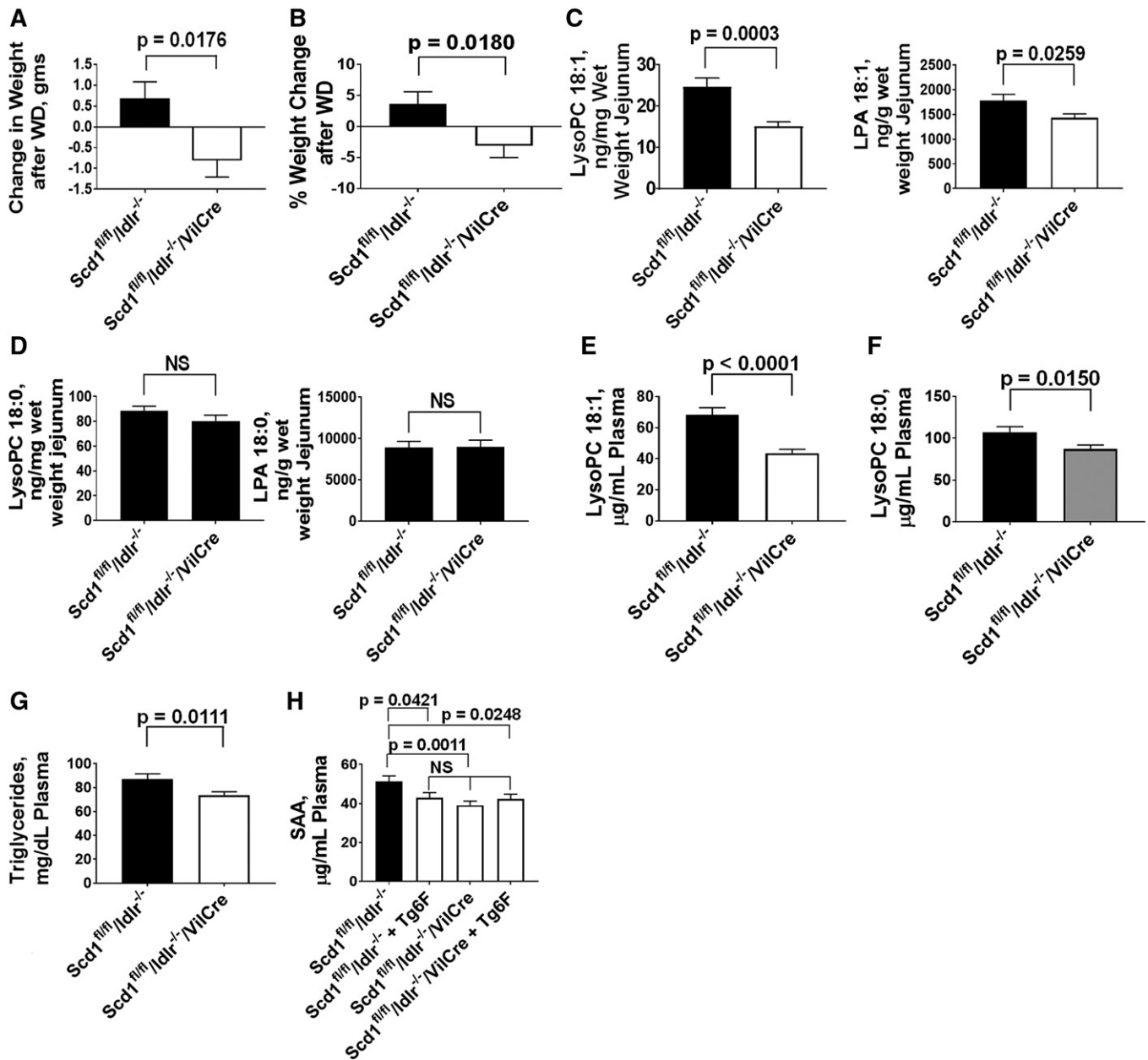


Fig. 4. A: Despite consuming the same amount of WD, the change in body weight in *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice was significantly different compared with *Scd1^{fl/fl}/ldlr^{-/-}* mice. Female mice (age 4–6 months, $n = 40$ per group) were weighed and switched from chow to WD. After 2 weeks, the mice were weighed again, and the difference in grams between the two weights for each mouse was determined. The data shown are the mean \pm SEM. B: Despite consuming the same amount of WD, the percent change in body weight in *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice was significantly different compared with *Scd1^{fl/fl}/ldlr^{-/-}* mice. The mice described in A were weighed and switched from chow to WD. After 2 weeks, the mice were weighed again. The percent change in body weight for the mice was determined by comparing the weight of each mouse just prior to starting WD with its weight after 2 weeks on WD. The data shown are the mean \pm SEM. C: Knockdown of enterocyte *Scd1* decreased LysoPC 18:1 and LPA 18:1 in the tissue of the jejunum. After 2 weeks, the jejunums from the mice described in A were harvested and LysoPC 18:1 (left panel) and LPA 18:1 (right panel) were determined as described in the Materials and Methods. The results shown are the mean \pm SEM. D: Knockdown of enterocyte *Scd1* did not alter levels of LysoPC 18:0 or LPA 18:0 in the tissue of the jejunum. LysoPC 18:0 (left panel) and LPA 18:0 (right panel) in the jejunums of the mice described in A were determined as described in the Materials and Methods. The results shown are the mean \pm SEM. E: On WD, plasma levels of LysoPC 18:1 were significantly lower following knockdown of enterocyte *Scd1*. LysoPC 18:1 plasma levels were determined as described in the Materials and Methods in the *Scd1^{fl/fl}/ldlr^{-/-}* and *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice described in A. The results shown are the mean \pm SEM. F: On WD, plasma levels of LysoPC 18:0 were slightly, but significantly, lower following knockdown of enterocyte *Scd1*. LysoPC 18:0 plasma levels were determined as described in the Materials and Methods in the *Scd1^{fl/fl}/ldlr^{-/-}* and *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice described in A. The results shown are the mean \pm SEM. G: Knockdown of enterocyte *Scd1* resulted in a modest, but significant, decrease in plasma triglyceride levels. Plasma triglycerides were determined as described in the Materials and Methods in the *Scd1^{fl/fl}/ldlr^{-/-}* and *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice described in A. The results shown are the mean \pm SEM. H: Knockdown of enterocyte *Scd1* resulted in a significant decrease in plasma SAA levels, and adding Tg6F to WD did not further decrease plasma SAA levels. Tg6F was added at 0.06% by weight to WD for some of the mice described in A, and plasma SAA levels were determined as described in the Materials and Methods. The results shown are mean \pm SEM.

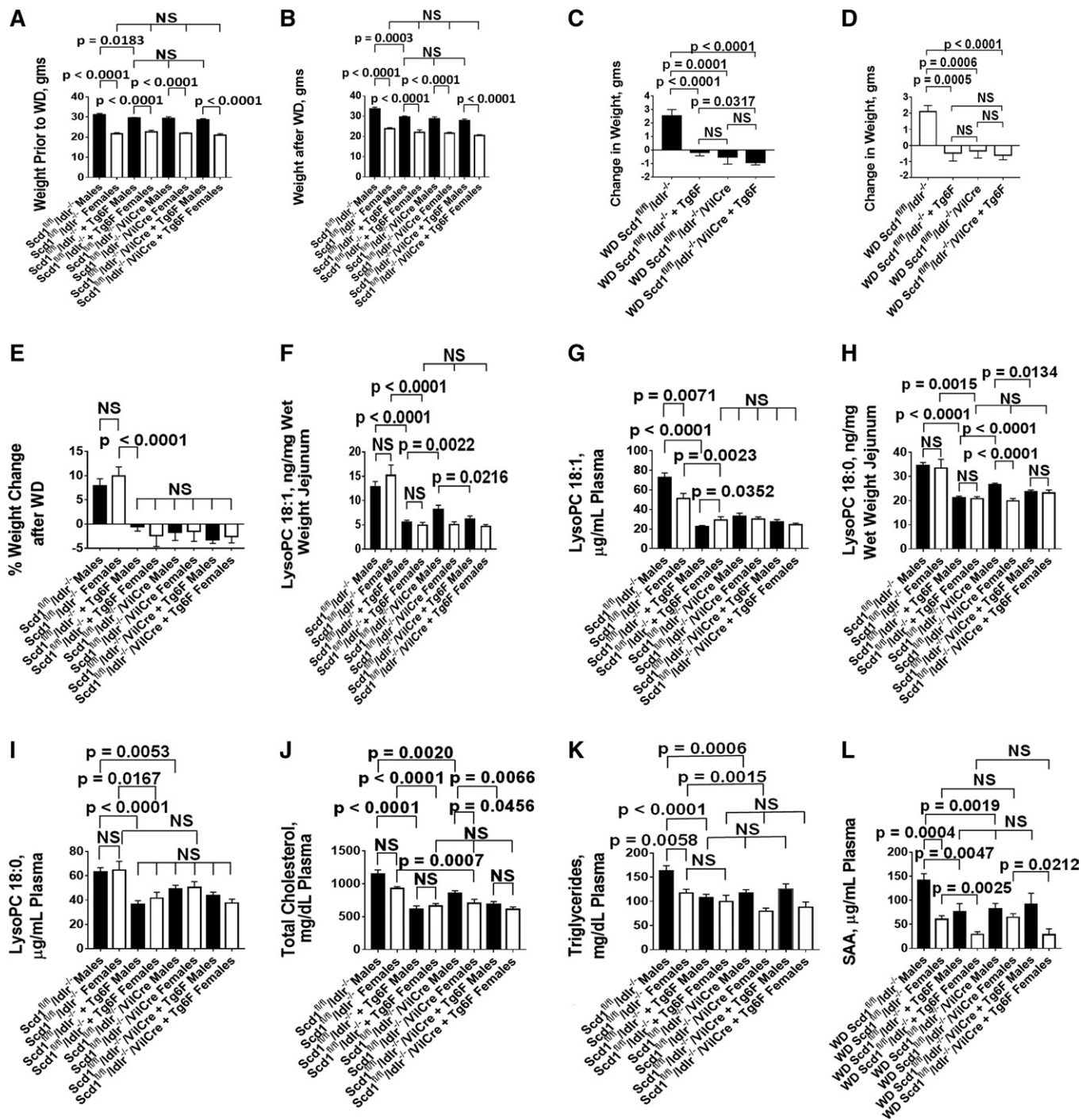


Fig. 5. A: Prior to the start of WD, male mice weighed more than female mice. Male *Scd1^{fl/fl}/ldlr^{-/-}* mice or *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice (age 6–8 months, n = 20 per group) or female *Scd1^{fl/fl}/ldlr^{-/-}* or *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice (age 6–8 months, n = 10 per group) were weighed prior to WD. The mice were then fed WD alone, or WD to which 0.06% by weight of Tg6F was added (+ Tg6F) as described in the Materials and Methods. The Tg6F used in these experiments was prepared using the machine that rapidly removes ethyl acetate/acetic acid from the Tg6F mixture as described in the Materials and Methods. The data shown are the weights prior to starting WD. The results are the mean \pm SEM. B: After 2 weeks on WD, male mice weighed more than female mice. The mice described in A were weighed after 2 weeks on WD. The results shown are the mean \pm SEM. C: Change in weight on WD for male mice. The weight of each male mouse shown in A prior to starting WD was subtracted from its weight after 2 weeks on WD. The data shown are mean \pm SEM. D: Change in weight on WD for female mice. The weight of each female mouse shown in A prior to starting WD was subtracted from its weight after 2 weeks on WD. The data shown are mean \pm SEM. E: Despite the difference in body weights between males and females, the percent change in weight on WD compared with the weight of each mouse prior to WD was similar in males and females. The percent change in body weight for the mice described in A was determined by comparing the weight of each mouse just prior to starting WD with its weight after 2 weeks on WD. The results shown are the mean \pm SEM. F: Knockdown of enterocyte *Scd1* lowered LysoPC 18:1 levels in the jejunum to the same or almost the same level as achieved by adding Tg6F to WD. LysoPC 18:1 levels were determined in the jejunum as described in the Materials and Methods for the mice described in A. The results shown are the mean \pm SEM. G: Knockdown of enterocyte *Scd1* lowered LysoPC 18:1 levels in plasma similar to Tg6F. Plasma

WD + Tg6F or male *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice on WD + Tg6F (Fig. 5F). These results show that knockdown of *Scd1* was either as effective or nearly as effective as adding Tg6F to WD in lowering LysoPC 18:1 levels in the jejunum.

On WD without Tg6F, plasma LysoPC 18:1 levels were modestly, but significantly, lower in female *Scd1^{fl/fl}/ldlr^{-/-}* mice (Fig. 5G). Adding Tg6F to WD significantly decreased plasma LysoPC 18:1 levels in both males and females, as was the case for enterocyte knockdown of *Scd1*. Adding Tg6F to WD in *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice did not further decrease plasma LysoPC 18:1 levels in either males or females (Fig. 5G).

In this experiment, adding Tg6F to WD or knockdown of *Scd1* in enterocytes also significantly reduced levels of LysoPC 18:0 in the jejunum of both males and females (Fig. 5H). Adding Tg6F to WD did not further lower LysoPC 18:0 levels in female *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice, but modestly did so in male mice (Fig. 5H). Plasma LysoPC 18:0 levels were also significantly decreased by addition of Tg6F to WD in both males and females. Knockdown of *Scd1* in enterocytes significantly decreased plasma levels of LysoPC 18:0 in males, but the decrease in females did not reach statistical significance (Fig. 5I).

In this third set of experiments, plasma total cholesterol levels were not significantly different in males or females, except in *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice where there was a modest difference. Adding Tg6F to WD significantly reduced plasma total cholesterol levels (Fig. 5J). Enterocyte knockdown of *Scd1* significantly decreased plasma total cholesterol levels in both males and females and adding Tg6F to WD modestly, but significantly, caused a further decrease in males (Fig. 5J). Plasma triglyceride levels were significantly less in female *Scd1^{fl/fl}/ldlr^{-/-}* mice compared with *Scd1^{fl/fl}/ldlr^{-/-}* male mice. Adding Tg6F to WD significantly decreased plasma triglyceride levels in *Scd1^{fl/fl}/ldlr^{-/-}* male mice, but the change in female mice did not reach statistical significance. Knockdown of *Scd1* in enterocytes significantly reduced plasma triglycerides in both male and female mice, and adding Tg6F to WD after enterocyte knockdown of *Scd1* did not further reduce plasma triglyceride levels (Fig. 5K).

Female *Scd1^{fl/fl}/ldlr^{-/-}* mice on WD had significantly lower levels of plasma SAA compared with male *Scd1^{fl/fl}/ldlr^{-/-}* mice on WD (Fig. 5L). Both males and females had significantly lower levels of plasma SAA when Tg6F was added to WD (Fig. 5L). Male *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice had significantly lower plasma SAA levels compared with

male *Scd1^{fl/fl}/ldlr^{-/-}* mice, and when Tg6F was added to WD, there was no further decrease in plasma SAA levels in the male *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice (Fig. 5L). In this experiment, female *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice did not have lower plasma SAA levels compared with female *Scd1^{fl/fl}/ldlr^{-/-}* mice. However, on addition of Tg6F to WD in the female *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice, plasma SAA levels significantly decreased and were not different from female *Scd1^{fl/fl}/ldlr^{-/-}* mice receiving Tg6F (Fig. 5L).

LBP, CD14, TLR4, and MyD88 protein levels in jejunum and plasma

Kim et al. (6) reported that a high-fat diet increases bacterial LPS in the intestinal lumen and plasma by altering the composition of the gut microbiome and increasing intestinal permeability through induction of TLR4. The pathway leading to TLR4 activation and signaling involves LPS first interacting with LBP and CD14, which deliver LPS to TLR4 and its partner, MD-2. Subsequently, TLR-4 signals through MyD88 (7–9). We hypothesized that Tg6F and/or enterocyte knockdown of *Scd1* might modulate this pathway resulting in lower levels of these key proteins. As shown in Fig. 6A, adding Tg6F to WD or knockdown of *Scd1* in enterocytes significantly decreased LBP protein levels in enterocytes of female *Scd1^{fl/fl}/ldlr^{-/-}* mice. Figure 6B shows that, in male mice, adding Tg6F to WD significantly decreased LBP protein levels in enterocytes, but the decrease in levels in male mice with enterocyte knockdown of *Scd1* did not reach statistical significance. After adding Tg6F to the male mice with enterocyte knockdown of *Scd1*, the decrease was highly significant (Fig. 6B). On comparing the values for female and male mice from Fig. 6A and B, there was only a significant difference for mice with enterocyte knockdown of *Scd1*; and after adding Tg6F, this difference was lost (Fig. 6C). As shown in Fig. 6D, combining the data from females with that of the males resulted in highly significant decreases in enterocyte LBP protein levels for both the addition of Tg6F to WD and for the knockdown of enterocyte *Scd1*, which was further reduced on addition of Tg6F. The ELISA results for LBP were confirmed with immunoblots for enterocytes (Fig. 6E) and plasma (Fig. 6F).

A similar trend was seen for CD14 in female mice, but the only value to reach statistical significance was for *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice on WD supplemented with Tg6F (Fig. 7A). In male mice, statistical significance was achieved in

LysoPC 18:1 levels were determined as described in the Materials and Methods for the mice described in A. The results shown are the mean \pm SEM. H: Knockdown of enterocyte *Scd1* decreased LysoPC 18:0 levels in the jejunum similar to Tg6F. LysoPC 18:0 levels in the jejunum were determined as described in the Materials and Methods for the mice described in A. The results shown are the mean \pm SEM. I: LysoPC 18:0 levels in plasma were decreased by adding Tg6F to WD or knockdown of *Scd1* in enterocytes. Plasma LysoPC 18:0 levels were determined as described in the Materials and Methods for the mice described in A. The results shown are the mean \pm SEM. J: Plasma total cholesterol levels were significantly less in mice receiving Tg6F or in mice with knockdown of enterocyte *Scd1*. Plasma total cholesterol levels were measured as described in the Materials and Methods for the mice described in A. The results shown are the mean \pm SEM. K: Plasma triglyceride levels were significantly less in mice with knockdown of enterocyte *Scd1* and in male mice receiving Tg6F. Plasma triglyceride levels were measured as described in the Materials and Methods for the mice described in A. The results shown are the mean \pm SEM. L: Female *Scd1^{fl/fl}/ldlr^{-/-}* mice had significantly lower levels of plasma SAA compared with male *Scd1^{fl/fl}/ldlr^{-/-}* mice. Plasma SAA levels were measured as described in the Materials and Methods for the mice described in A. The results shown are the mean \pm SEM.

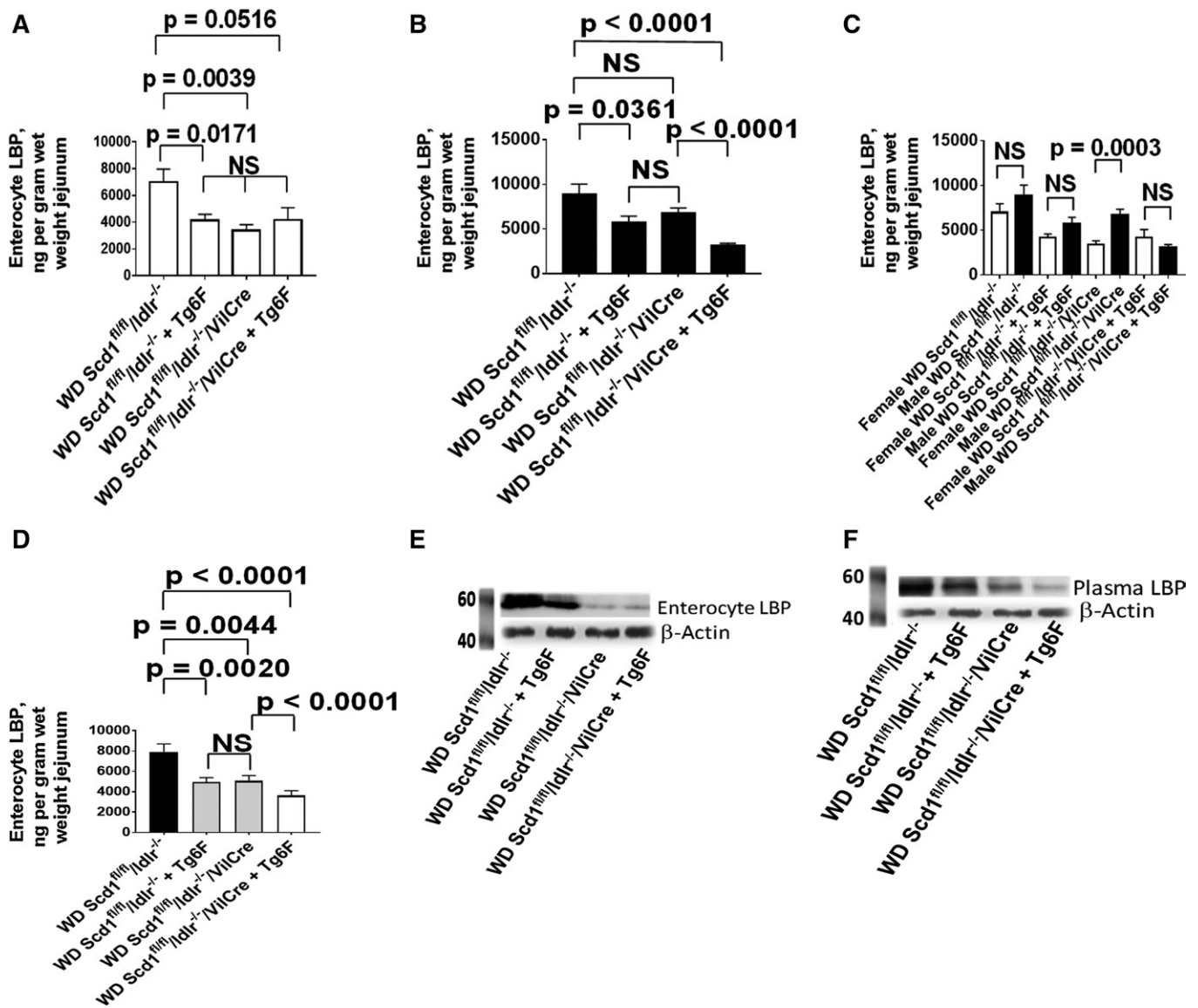


Fig. 6. A: Enterocyte LBP levels in female mice. Enterocytes were prepared from the 10 female mice described in Fig. 5A, and LBP levels were determined by ELISA as described in the Materials and Methods using the primary antibody from Enzo Life Sciences (catalog #ALX-804-502-C100) at a dilution of 1:500. The secondary antibody was rabbit anti-mouse antibody from Jackson ImmunoResearch (catalog #315-035-003) and was used at a dilution of 1:2,000. The data shown are mean \pm SEM. B: Enterocyte LBP levels in male mice. The enterocyte lysate from each pair of sequentially numbered male mice described in Fig. 5A were pooled to yield 10 pools that were analyzed for LBP by ELISA as described in A. The data shown are mean \pm SEM. C: The values from A and B were compared for each group. The data shown are mean \pm SEM. The white bars represent female mice; the black bars represent male mice. D: The values from A and B were combined for each group and the resulting four groups were compared. The data shown are mean \pm SEM. E: Immunoblot for enterocyte LBP confirms ELISA data. An immunoblot was performed as described in the Materials and Methods using pooled lysate from three female mice randomly selected from the mice described in Fig. 5A. The numbers on the left are molecular mass standards (in kilodaltons). F: Immunoblot for plasma LBP. An immunoblot was performed as described in the Materials and Methods on pooled plasma from three female mice randomly selected from the mice described in Fig. 5A. The numbers on the left are molecular mass standards (in kilodaltons).

the *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice, and was further decreased upon addition of Tg6F (Fig. 7B). As shown in Fig. 7C, there was no significant difference between female or male mice in any group. When the data from the female and male mice were combined, the decrease in CD14 protein levels was statistically significant for all groups compared with *Scd1^{fl/fl}/ldlr^{-/-}* mice (Fig. 7D). Enterocyte CD14 levels were not different in *Scd1^{fl/fl}/ldlr^{-/-}* mice on WD with Tg6F added compared with *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice on WD without Tg6F. In this experiment, adding Tg6F to WD

in *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice produced a further decrease in enterocyte CD14 protein levels (Fig. 7D). The ELISA results (Fig. 7A–D) were confirmed with immunoblots for enterocytes (Fig. 7E) and plasma (Fig. 7F).

Enterocyte TLR4 protein levels were decreased in female *Scd1^{fl/fl}/ldlr^{-/-}* mice with Tg6F added to WD compared with *Scd1^{fl/fl}/ldlr^{-/-}* mice on WD without Tg6F (Fig. 8A). Knockdown of *Scd1* decreased enterocyte TLR4 protein levels to those seen in *Scd1^{fl/fl}/ldlr^{-/-}* mice on WD with Tg6F. Addition of Tg6F to WD in *Scd1^{fl/fl}/ldlr^{-/-}/VilCre*

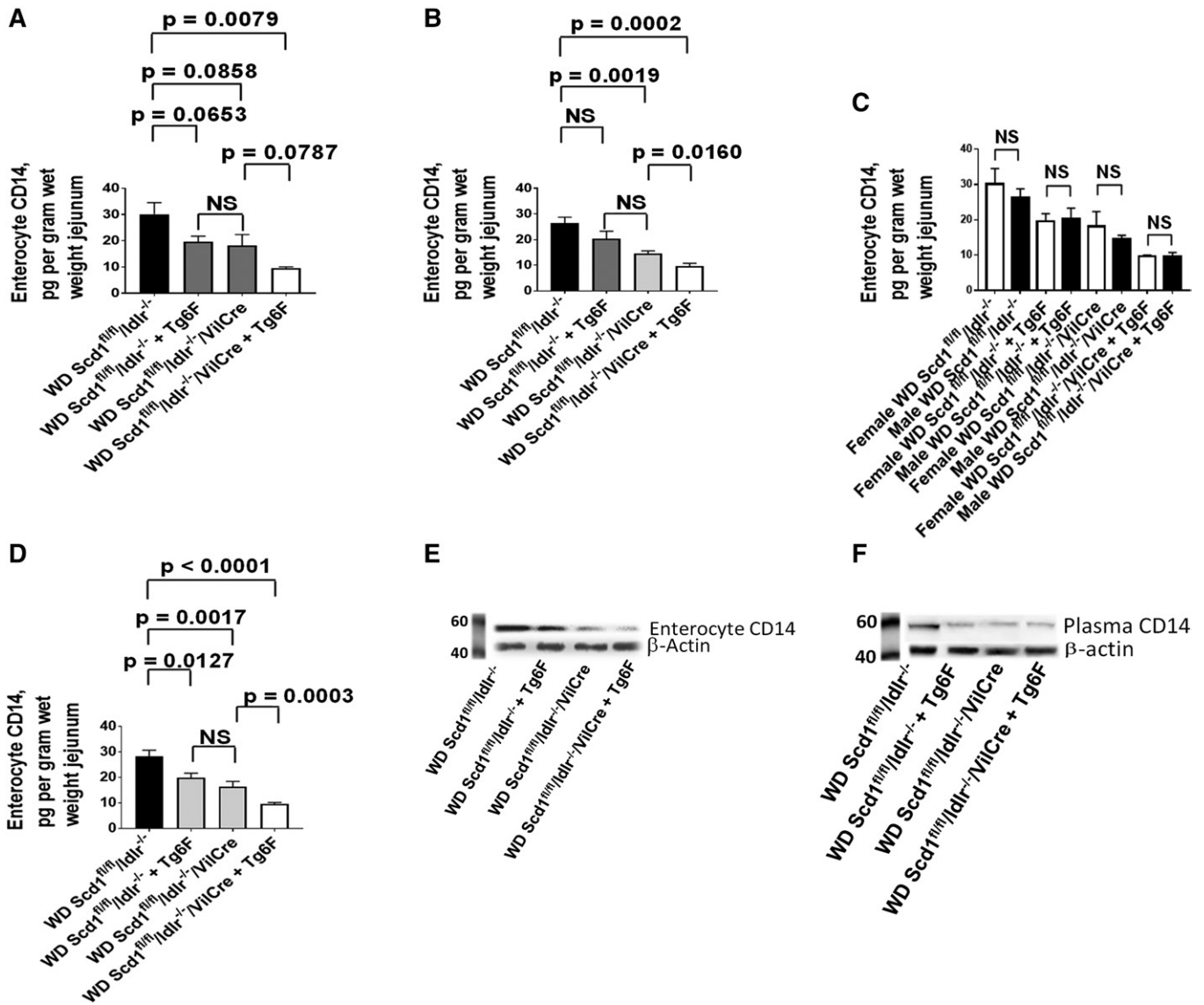


Fig. 7. A: Enterocyte CD14 levels in female mice. Enterocytes were prepared from the 10 female mice described in Fig. 5A, and the enterocyte lysates were combined into five pools that were each analyzed. CD14 levels were determined using an ELISA kit from LifeSpan Biosciences, Inc. (catalog #LS-F2629-1, lot number 110023). The primary antibody was used at a dilution of 1:1,000. The secondary antibody was rabbit anti-mouse antibody from Jackson ImmunoResearch (catalog #315-035-003) and was used at a dilution of 1:1,500. The data shown are mean \pm SEM. B: Enterocyte CD14 levels in male mice. The enterocyte lysate from the first 10 male mice in Fig. 5A were combined to yield five pools that were analyzed for CD14 by ELISA as described in A. The data shown are mean \pm SEM. C: The values from A and B were compared for each group. The data shown are mean \pm SEM. The white bars represent female mice; the black bars represent male mice. D: The values from A and B were combined for each group and the resulting four groups were compared. The data shown are mean \pm SEM. E: Immunoblot for enterocyte CD14 confirms ELISA data. An immunoblot was performed as described in the Materials and Methods using pooled lysate from three female mice randomly selected from the mice described in Fig. 5A. The numbers on the left are molecular mass standards (in kilodaltons). F: Immunoblot for plasma CD14. An immunoblot was performed as described in the Materials and Methods on pooled plasma from three female mice randomly selected from the mice described in Fig. 5A. The numbers on the left are molecular mass standards (in kilodaltons).

mice further decreased enterocyte TLR4 protein levels (Fig. 8A). Similar results were seen in male mice (Fig. 8B). The results obtained with ELISA were confirmed with immunoblots for enterocytes (Fig. 8C) and plasma (Fig. 8D).

Enterocyte MyD88 protein levels trended lower in female *Scd1^{fl/fl}/ldlr^{-/-}* mice on WD with Tg6F and in female *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice with or without Tg6F added to WD, but none of these values reached statistical significance

(Fig. 9A). Similar trends were seen in male mice (Fig. 9B), but only the addition of Tg6F to WD in *Scd1^{fl/fl}/ldlr^{-/-}* males reached statistical significance. Comparing females to males for the various groups revealed no significant differences (Fig. 9C); therefore, the data from females and males were combined (Fig. 9D). Upon combining the data from females and males, there was a significant reduction in MyD88 protein (as judged by optical density) in *Scd1^{fl/fl}/ldlr^{-/-}* mice on WD with Tg6F and in *Scd1^{fl/fl}/ldlr^{-/-}/VilCre*

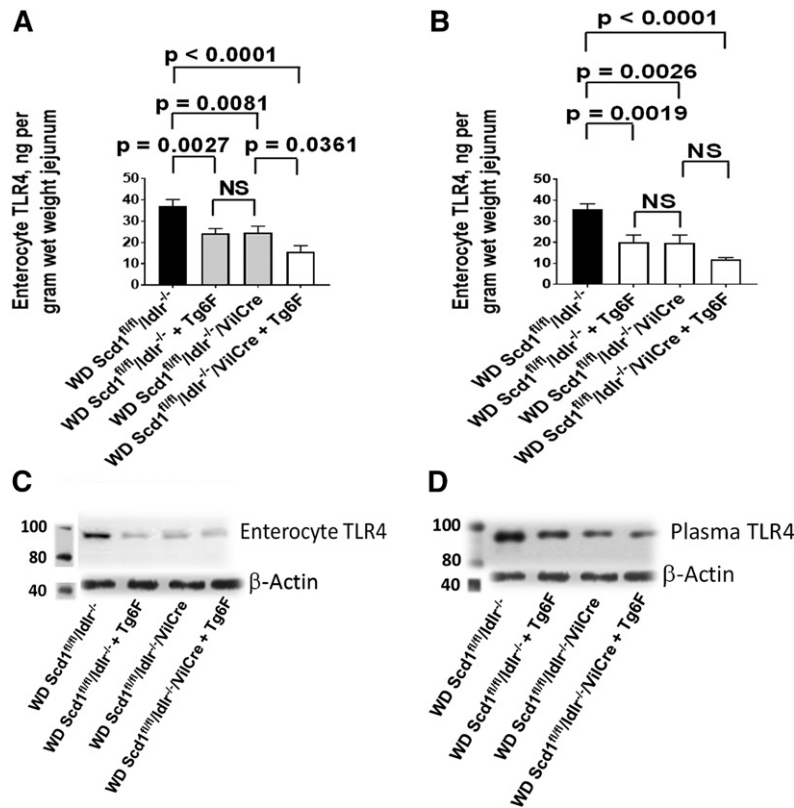


Fig. 8. A: Enterocyte TLR4 levels in female mice. Enterocytes were prepared from female mice (aged 4–6 months, $n = 20$ per group), some of which were used in the experiments described in Fig. 4A. The enterocyte lysates were diluted 1:10, and TLR4 levels were determined using sandwich ELISA kit material from LifeSpan BioSciences (catalog #LS-C180173-100) with primary antibody from Novus (catalog #76B357.1) used at a dilution of 1:500. The secondary antibody was goat anti-mouse IgG HRP conjugate from Invitrogen (catalog #31430) and was used at a dilution of 1:10,000. The data shown are mean \pm SEM. B: Enterocyte TLR4 levels in male mice. Enterocytes were prepared from male mice (aged 6–8 months, $n = 10$ per group), some of which were used in the experiments described in Fig. 5A. The enterocyte lysates were diluted 1:10, and TLR4 levels were determined using sandwich ELISA kit material from LifeSpan BioSciences (catalog #LS-C180173-100) with primary antibody from Novus (catalog #76B357.1) used at a dilution of 1:500. The secondary antibody was goat anti-mouse IgG HRP conjugate from Invitrogen (catalog #31430), and was used at a dilution of 1:10,000. The data shown are mean \pm SEM. C: Immunoblot for enterocyte TLR4 confirms ELISA data. An immunoblot was performed as described in the Materials and Methods using pooled lysate from three female mice randomly selected from the mice described in Fig. 5A. The numbers on the left are molecular mass standards (in kilodaltons). D: Immunoblot for plasma TLR4. An immunoblot was performed as described in the Materials and Methods on pooled plasma from three female mice randomly selected from the mice described in Fig. 5A. The numbers on the left are molecular mass standards (in kilodaltons).

mice; there was no further decrease on adding Tg6F to WD in *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice (Fig. 9D). The ELISA results were confirmed with immunoblots in enterocytes (Fig. 9E) and in plasma (Fig. 9F).

The ELISA results in Figs. 6, 7, and 9 frequently required the combination of data from females and males in order to achieve statistical significance. The ELISA experiments described in Figs. 6, 7, and 9 were conducted with limited volumes of enterocyte lysates, which in some cases required the pooling of samples. As a result, tests of statistical significance were limited by the relative small number of data points, which was overcome by combining the data from females and males. That was not the case for Fig. 8, where statistical significance was achieved without the need to pool samples or combine data from females and males. In order to confirm the ELISA results for Figs. 6, 7, and 9 (LBP, CD14, and MyD88, respectively), we repeated these

experiments using ample enterocyte lysates available from the mice described in Fig. 8A. As shown in **Fig. 10A**, there was a significant reduction in enterocyte LBP protein in *Scd1^{fl/fl}/ldlr^{-/-}* mice on WD with Tg6F and also in *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice with a further significant decrease on adding Tg6F to WD in *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice. As shown in Fig. 10B, there was a significant reduction in enterocyte CD14 protein in *Scd1^{fl/fl}/ldlr^{-/-}* mice on WD with Tg6F and also in *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice; there was no further decrease on adding Tg6F to WD in *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice. As shown in Fig. 10C, there was a significant reduction in enterocyte MyD88 protein in *Scd1^{fl/fl}/ldlr^{-/-}* mice on WD with Tg6F and also in *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice; there was no further decrease on adding Tg6F to WD in *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice. Thus, the data in Fig. 10 confirm the findings in Figs. 6, 7, and 9.

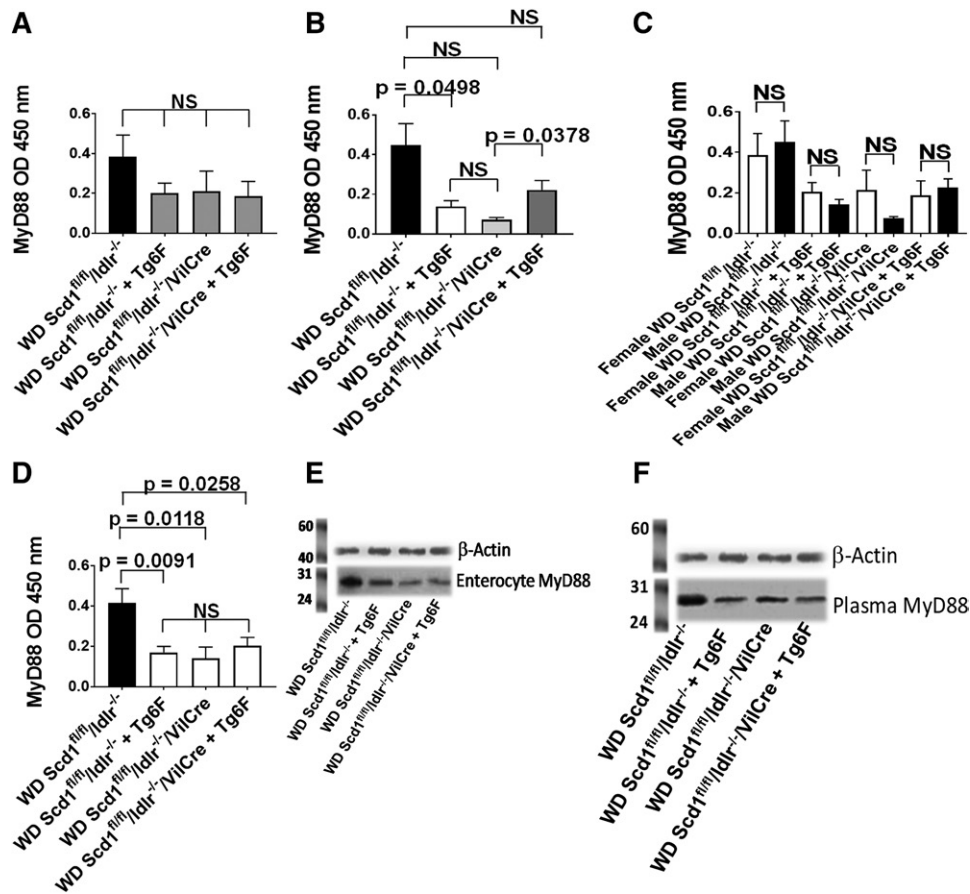


Fig. 9. A: Enterocyte MyD88 levels in female mice. Enterocytes were prepared from the 10 female mice described in Fig. 5A, and enterocyte lysates were pooled into three pools that were each analyzed. MyD88 levels were determined using an ELISA as described in the Materials and Methods. The primary antibody was from LifeSpan Biosciences, Inc. (catalog #LS-384717-100), which was used at a dilution of 1:1,000. The secondary antibody was donkey anti-rabbit from Jackson ImmunoResearch (catalog #711-035-152) and was used at a dilution of 1:4,000. The data shown are mean \pm SEM. B: Enterocyte MyD88 levels in male mice. The enterocyte lysate from the first 10 male mice in Fig. 5A were pooled to yield three pools that were analyzed for MyD88 by ELISA as described in A. The data shown are mean \pm SEM. C: The values from A and B were compared for each group. The data shown are mean \pm SEM. The white bars represent female mice; the black bars represent male mice. D: The values from A and B were combined for each group and the resulting four groups were compared. The data shown are mean \pm SEM. E: Immunoblot for enterocyte MyD88. An immunoblot was performed as described in the Materials and Methods using pooled lysate from three female mice randomly selected from the mice described in Fig. 5A. The numbers on the left are molecular mass standards (in kilodaltons). F: Immunoblot for plasma MyD88 gives similar results to enterocyte MyD88. An immunoblot was performed as described in the Materials and Methods on pooled plasma from three female mice randomly selected from the mice described in Fig. 5A. The numbers on the left are molecular mass standards (in kilodaltons).

Adding LysoPC 18:1 to WD fails to worsen dyslipidemia and inflammation in mice with enterocyte knockdown of *Scd1*

The data in Figs. 1–10 suggest that one explanation for the favorable effects of knockdown of enterocyte *Scd1* is a decrease in LysoPC 18:1 and LPA 18:1 levels that ameliorates dyslipidemia and systemic inflammation. If that were the case, one could expect that adding LysoPC 18:1 to WD in mice with knockdown of enterocyte *Scd1* might increase dyslipidemia and systemic inflammation above that seen in mice with enterocyte knockdown of *Scd1* on WD. The experiments in Fig. 11 tested this hypothesis with the WD used in Figs. 1–10 (Teklad WD; catalog #TD88137), a diet that is low in the essential fatty acid, linoleic acid (16).

Additionally, we also tested the hypothesis using a WD that is rich in linoleic acid (Research Diets WD; catalog #D12079B). The primary difference between these two diets is that 1% butter fat in the former diet is replaced by 1% corn oil in the latter diet. Figure 11A demonstrates that the mice in the different groups were of similar weight prior to starting the WD. Figure 11B demonstrates that there was no significant difference in the weights of the mice after 2 weeks on the diets. Figure 11C shows that the change in weight for individual mice in grams was not different between groups. The mice ate >95% of the diet each day and there was no significant difference between the two diets (Fig. 11D). Figure 11E demonstrates that total plasma cholesterol levels did not vary significantly between groups.

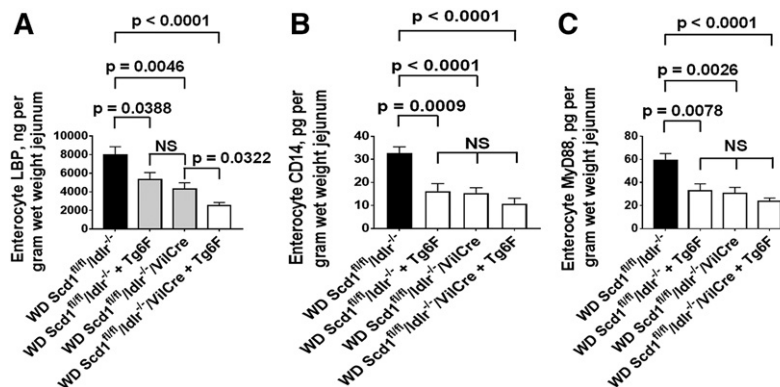


Fig. 10. A: Enterocyte LBP levels in female mice. Enterocytes were prepared from the female mice (aged 4–6 months, $n = 10$ per group) described in Fig. 8A. LBP levels were determined by ELISA as described in the Materials and Methods using the primary antibody from Enzo Life Sciences (catalog #ALX-804-502-C100) at a dilution of 1:1,000. The secondary antibody was rabbit anti-mouse antibody from Jackson ImmunoResearch (catalog #315-035-003), and was used at a dilution of 1:2,000. The data shown are mean \pm SEM. B: Enterocyte CD14 levels in female mice. Enterocytes were prepared from the 10 female mice described in A, and CD14 levels were determined by ELISA. The primary antibody was from Thermo Fisher Scientific (catalog #12-0149-82), and was used at a dilution of 1:1,000. The secondary antibody was from Jackson ImmunoResearch (catalog #315-035-003), and was used at a dilution of 1:2,500. The data shown are mean \pm SEM. C: Enterocyte MyD88 levels in female mice. Enterocytes were prepared from the 10 female mice described in A, and ELISA was performed as described in the Materials and Methods. The primary antibody was from LifeSpan Biosciences, Inc. (catalog #LS-384717-100), which was used at a dilution of 1:1,000. The secondary antibody was donkey anti-rabbit from Jackson ImmunoResearch (catalog #711-035-152), and was used at a dilution of 1:8,000. The data shown are mean \pm SEM.

Figure 11F shows that plasma triglyceride levels were not significantly different between groups. Figure 11G demonstrates that plasma HDL-cholesterol levels were higher on the linoleic acid-rich diet, but there was no significant difference in mice receiving LysoPC 18:1 and those that did not receive LysoPC 18:1. Figure 11H shows that there was no significant difference in plasma SAA levels between groups.

Adding LysoPC 18:1 to chow induced *Scd1*, dyslipidemia, and systemic inflammation in *Scd1^{fl/fl}/ldlr^{-/-}* mice, but not in *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice

The failure to increase dyslipidemia and inflammation after adding LysoPC 18:1 to WD in *ldlr^{-/-}* mice with knock-down of enterocyte *Scd1* might indicate that the dyslipidemia and systemic inflammation induced by LysoPC 18:1 requires induction of *Scd1*. To determine whether LysoPC 18:1 requires the induction of *Scd1* to cause dyslipidemia and systemic inflammation, we added LysoPC 18:1 or LysoPC 18:0 to the chow of mice with and without knock-down of enterocyte *Scd1*. As shown in Fig. 12A, addition of LysoPC 18:1 to chow induced *Scd1* expression in the jejunum from *Scd1^{fl/fl}/ldlr^{-/-}* mice. In contrast, addition of LysoPC 18:0 to chow did not induce *Scd1* expression in these mice. As expected, addition of either LysoPC 18:1 or LysoPC 18:0 did not induce *Scd1* expression in *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice (Fig. 12B). As demonstrated in Fig. 12C, addition of LysoPC 18:1 to chow modestly, but significantly, increased plasma total cholesterol levels in *Scd1^{fl/fl}/ldlr^{-/-}* mice, but not in *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice. SAA plasma levels were modestly, but significantly, increased on addition to chow of LysoPC 18:1 in *Scd1^{fl/fl}/ldlr^{-/-}* mice, but not in *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice (Fig. 12D). Addition

of LysoPC 18:1 to chow also significantly increased another marker of systemic inflammation, plasma IL-6 levels, in *Scd1^{fl/fl}/ldlr^{-/-}* mice, but not in *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice (Fig. 12E). Measurement of LysoPC 18:1 levels in the jejunum (Fig. 12F) and plasma (Fig. 12G) revealed that after addition of LysoPC 18:1 to chow, the content of LysoPC 18:1 significantly increased in the jejunum and plasma of *Scd1^{fl/fl}/ldlr^{-/-}* mice, but not in *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice. Addition of LysoPC 18:1 to chow did not alter levels of LysoPC 18:0 in the jejunum (Fig. 12H) or plasma (Fig. 12I) in either *Scd1^{fl/fl}/ldlr^{-/-}* mice or in *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice.

LysoPC 16:0 acts like LysoPC 18:0 and not like LysoPC 18:1

The experiments in Fig. 12 compared the addition of LysoPC 18:1 to that of LysoPC 18:0. LysoPC 16:0 is a preferred substrate for lysophosphatidylcholine acyltransferase 3 (Lpcat3) (17–19). Therefore, we compared adding LysoPC 18:1, LysoPC 18:0, or LysoPC 16:0 to chow. As shown in Fig. 13, there was no difference between adding LysoPC 18:0 or LysoPC 16:0 to chow; neither was different from no addition, and each was significantly different from adding LysoPC 18:1. Only adding LysoPC 18:1 to chow elevated plasma total cholesterol levels (Fig. 13A), plasma triglyceride levels (Fig. 13B), and plasma SAA levels (Fig. 13C).

LPA 18:1 induces *Scd1* expression

We previously reported that LPA 18:2 (but not LPA 18:0) induced *Scd1* expression (see supplementary Fig. S5C in Ref. 1), but we had not previously determined whether LPA 18:1 similarly induces *Scd1* expression. Because the

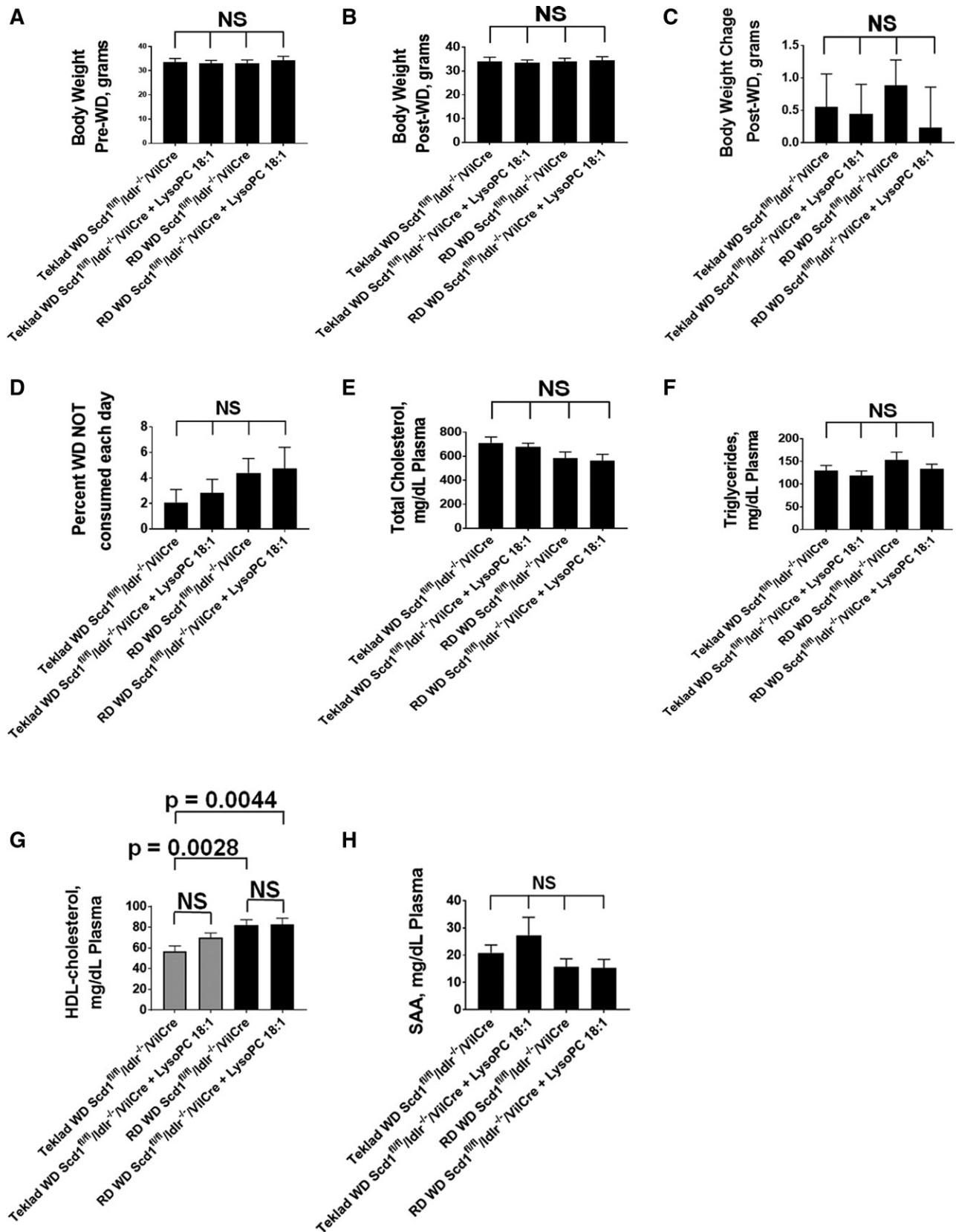


Fig. 11. A: Body weights prior to the start of the experiment to test whether adding LysoPC 18:1 to WD would worsen dyslipidemia and inflammation in *Scd1^{fl/fl}/Idlr^{-/-}/VilCre* mice. Male *Scd1^{fl/fl}/Idlr^{-/-}/VilCre* mice (age 10–12 months, n = 10 per group) that had been maintained on a chow diet were weighed just prior to being switched to Teklad WD (catalog #TD88137) or WD from Research Diets (RD) (catalog #D12079B) with or without LysoPC 18:1 added at 1 mg per gram of diet. The data shown are mean ± SEM. B: Body weights 2 weeks after the mice described in A were switched to 3 g per mouse per day of Teklad WD (catalog #TD88137) with or without LysoPC 18:1 added at 1 mg per gram of diet or 3 g per mouse per day of WD from Research Diets (RD) (catalog #D12079B) with or without LysoPC 18:1

focus of this work has been on *Scd1* and LysoPC 18:1, and because we hypothesize that the conversion of LysoPC 18:1 to LPA 18:1 is responsible for many of the observed effects of LysoPC 18:1, we thought it was important to directly determine whether LPA 18:1 induces *Scd1* expression. Prior to the start of this experiment, the mice destined to receive LPA 18:1 added to their chow at 1 μ g per gram of chow weighed 37.98 ± 1.6 g (mean \pm SEM), and the mice that were not to receive LPA 18:1 weighed 35.59 ± 0.69 g ($P = 0.1964$). Two weeks after starting the experiment, the mice receiving LPA 18:1 weighed 38.21 ± 1.4 g and the mice that did not receive LPA 18:1 weighed 34.16 ± 0.73 g ($P = 0.0169$). As shown in **Fig. 14A** and **B**, the mice receiving LPA 18:1 gained a small, but significant, amount of weight during the 2 week experiment, while the mice not receiving LPA 18:1 lost $\sim 4\%$ of their body weight. Why would the mice continued on the same chow that they had eaten prior to the start of the experiment lose weight? The most likely explanation is that the mice had unlimited access to chow prior to the start of the experiment, and the fixed amount administered during the experiment was less than they had been eating. As stated in the Materials and Methods, to ensure that the mice ate all of their food during the experiments, the mice were not allowed unlimited access to food during the experimental period. During the experiments, they were given a fixed amount of diet each night. During the experiments, any remaining food was removed each night and weighed to determine the percent not eaten. As noted in the Materials and Methods, unless otherwise stated, the mice were given precisely 4 g of diet per mouse each night. In this experiment, the diet was chow without or with added LPA 18:1. These mice ate 4–5 g of chow per mouse per day when they were given unlimited access to chow. The 4 g per mouse per day administered during the experiment for this group of mice likely was a little below what they were eating prior to the start of the experiment. Consequently, they lost some weight over the course of the 2 weeks. Remarkably, the mice receiving LPA 18:1 administered in the same amount of chow given to those not receiving LPA, actually gained a small amount of weight. There was no chow left each night in either group during the experiment described in **Fig. 14**, so the amount of diet eaten was the same in each group. As shown in **Fig. 14C**, the mice receiving chow supplemented with LPA 18:1 demonstrated a highly significant increase in *Scd1* expression in the jejunum compared with the mice not receiving LPA 18:1. Accompanying the increase in *Scd1* expression in the mice receiving LPA 18:1, there were also increases in plasma total cholesterol levels (**Fig. 14D**) and plasma triglyceride levels (**Fig. 14E**). Plasma HDL-cholesterol levels

were significantly decreased in the mice receiving LPA 18:1 (**Fig. 14F**), and plasma SAA levels were significantly increased (**Fig. 14G**). Thus, the mice receiving chow supplemented with LPA 18:1 acted similarly to mice that received WD, although the magnitudes of the changes were less, particularly for SAA.

DISCUSSION

To our knowledge, this is the first report of an enterocyte-specific knockdown of *Scd1*. Despite normal expression of *Scd1* in liver and elsewhere in the body, we show that enterocyte *Scd1* plays a significant role in a portion of the dyslipidemia and systemic inflammation that occurs when *ldlr*^{-/-} mice are fed WD. Our previous reports (1–3, 10, 11) suggested that tissue levels of LysoPC 18:1 and LPA 18:1 in the small intestine play a significant role in a portion of WD-mediated dyslipidemia and systemic inflammation in *ldlr*^{-/-} mice. The data in **Fig. 12** strongly suggest that the ability of LysoPC 18:1 to contribute to dyslipidemia and systemic inflammation requires the induction of *Scd1*. The data in **Fig. 14** are consistent with our previous work (1, 2), indicating that the conversion of LysoPC 18:1 to LPA 18:1 is responsible for many of the changes induced by LysoPC 18:1. Adding one-thousandth the dose of LPA 18:1 to chow (**Fig. 14**) compared with LysoPC 18:1 (**Figs. 12, 13**) produced similar biologic effects. Additionally, the data show that the ability of Tg6F to ameliorate dyslipidemia and systemic inflammation on WD is also dependent to a significant extent on enterocyte *Scd1*.

What are the similarities and differences in phenotype between mouse models with knockdown of *Scd1* by different methods and in different tissues?

Mice globally deficient in *Scd1* were found to be resistant to diet-induced weight gain (4). Mice globally deficient in *Scd1* because of a naturally occurring mutation were protected from obesity and insulin resistance when fed WD, but surprisingly had increased plasma SAA levels and increased aortic atherosclerosis, despite improvement in plasma total cholesterol and triglycerides (20).

Brown et al. (21) treated *ldlr*^{-/-} mice transgenic for human apoB100 with antisense oligonucleotides to *Scd1*. This virtually abolished *Scd1* protein in liver and adipose tissue, but not in skeletal muscle and skin (21). Similar to the findings of MacDonald et al. (20), the *Scd1*-deficient mice were protected from insulin resistance and obesity, but had increased aortic atherosclerosis (21).

Miyazaki et al. (5) reported that global knockout of *Scd1* in mice protected them from diet-induced obesity. Despite

added at 1 mg per gram of diet. The data shown are mean \pm SEM. C: The difference in body weights was determined by subtracting the weight of each mouse in A from its weight in B. The data shown are mean \pm SEM. D: The percent of WD not eaten each day for the mice described in B was determined by weighing the WD remaining the next morning and calculating this weight as a percent of the 3 g provided per mouse per day. The data shown are mean \pm SEM. E: Plasma total cholesterol levels were determined as described in the Materials and Methods for the mice described in B. The data shown are mean \pm SEM. F: Plasma triglyceride levels were determined as described in the Materials and Methods for the mice described in B. The data shown are mean \pm SEM. G: Plasma HDL-cholesterol levels were determined as described in the Materials and Methods for the mice described in B. The data shown are mean \pm SEM. H: Plasma SAA levels were determined as described in the Materials and Methods for the mice described in B. The data shown are mean \pm SEM.

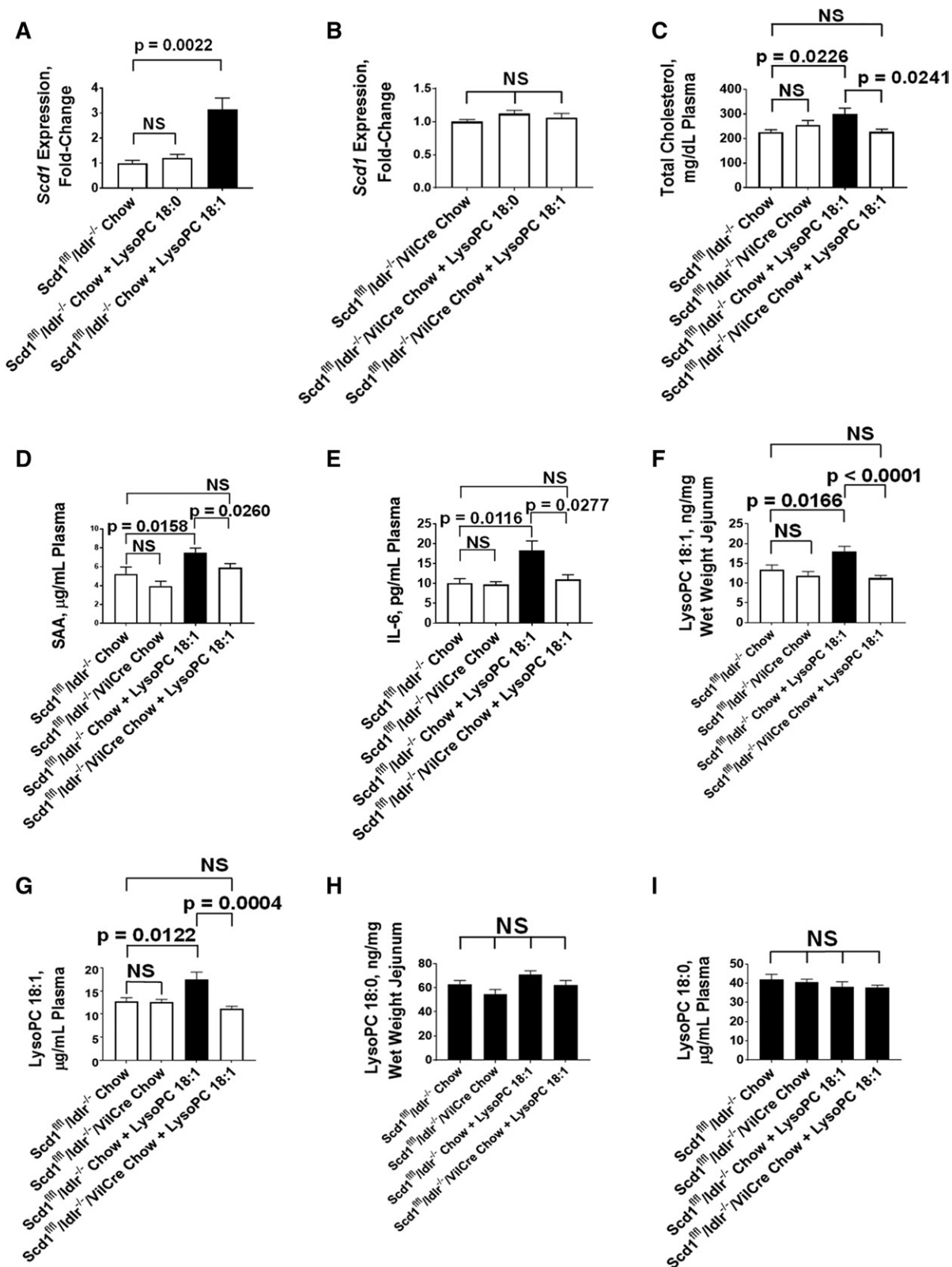


Fig. 12. A: Adding LysoPC 18:1 (but not LysoPC 18:0) to chow induces the expression of *Scd1* in *Scd1^{fl/fl}/ldlr^{-/-}* mice. Male *Scd1^{fl/fl}/ldlr^{-/-}* mice or male *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice (4–5 months of age, n = 20 per group) were fed chow without or with LysoPC 18:1 or LysoPC 18:0 added at 1 mg per gram of chow. After 2 weeks, the mice were euthanized, the jejunum was harvested, and the expression of *Scd1* was determined by quantitative real-time PCR as described in the Materials and Methods. The data shown are mean \pm SEM for *Scd1^{fl/fl}/ldlr^{-/-}* mice. B: The expression of *Scd1* in *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice was not altered by the addition of either LysoPC 18:1 or LysoPC 18:0 to chow. The expression of *Scd1* in the jejunum of the *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice in A was determined by quantitative real-time PCR as described in the Materials and Methods. The data shown are mean \pm SEM. C: Adding LysoPC 18:1 to chow increases plasma total cholesterol in *Scd1^{fl/fl}/ldlr^{-/-}* mice, but not in *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice. The mice described in A were fed chow without or with LysoPC 18:1 added at 1 mg per gram of

higher food intake, these mice showed enhanced glucose tolerance, lower fasting plasma glucose, and improved insulin sensitivity on both high-carbohydrate and high-fat diets (5). In contrast, mice with liver-specific *Scd1* knockout were protected from high-carbohydrate diet-induced weight gain and adiposity, but there was no difference in plasma glucose, insulin levels, glucose clearance, or insulin sensitivity (5). Liver-specific *Scd1* knockout mice were protected against liver steatosis and plasma triglyceride levels were significantly lower (5). However, on the high-fat diet, the *Scd1* liver-specific knockout mice were not protected against weight gain and adiposity (5). Plasma triglycerides modestly decreased without any improvement in glucose clearance and insulin sensitivity (5). Plasma cholesterol levels and fast-protein LC cholesterol profiles were not affected on either diet (5).

A skin-specific knockout of *Scd1* resulted in increased energy expenditure because of loss of insulating factors in the skin resulting in upregulation of thermogenic processes for temperature maintenance and protection from high-fat diet-induced obesity (22).

Hyun et al. (23) found that adipose-specific knockout of *Scd1* resulted in a significant decrease in adiponectin, increased expression of GLUT1 in adipose tissue, and a modest, but significant, increase in TNF- α expression without any significant change in insulin sensitivity.

Combined deletion of *Scd1* from adipose tissue and liver did not protect mice from obesity (24). Thus, tissue-specific deletion of *Scd1* in mice has given different results from the global knockout, and deletion of *Scd1* from different tissues has resulted in different phenotypes.

In the experiments reported here, enterocyte-specific knockdown largely prevented WD-induced weight gain. In some cases, enterocyte-specific knockdown of *Scd1* induced a modest, but significant, weight loss on WD associated with a modest, but significant, decrease in plasma total cholesterol levels and amelioration of the WD-induced decrease in HDL-cholesterol levels. Enterocyte knockdown of *Scd1* significantly decreased plasma triglyceride levels in some experiments, but not in others. Knockdown of enterocyte *Scd1* resulted in decreased SAA levels

on WD, except in one of the three experiments with female mice. In two of the three experiments in female mice and in the experiment with male mice, plasma SAA levels on WD in *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice were significantly lower than in *Scd1^{fl/fl}/ldlr^{-/-}* mice. These results are different from the global knockout in *ldlr^{-/-}* mice on WD in which diet-induced obesity was improved and plasma lipids were improved, but SAA levels and aortic atherosclerosis increased (20). Our results are also different from the studies achieving *Scd1* knockdown in liver and adipose tissue using antisense oligonucleotides (21). However, our studies are similar with respect to weight gain to those previously reported in global knockout mice in which food intake was carefully monitored. MacDonald et al. (25) fed the same WD as used in most of our studies (Teklad, Harlan, catalog #TD88137) to *ldlr^{-/-}* mice with a spontaneously occurring mutation resulting in global knockout of *Scd1*. Despite a trend in the global knockout mice to eat more WD each day, they gained significantly less weight (25), as was the case for the mice with enterocyte knockdown of *Scd1* in our studies. Also in our studies, *Scd1^{fl/fl}/ldlr^{-/-}* mice receiving LPA 18:1 added to chow gained a small, but significant, amount of weight, while mice not receiving LPA 18:1, but consuming the same amount of chow each day, lost weight (Fig. 14A, B). This may indicate that enterocyte LPA 18:1 levels are important in determining nutrient absorption from the same amount of food. The data in Fig. 4C show that, on WD, *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice had lower levels of LPA 18:1 in the jejunum and lost weight on WD (Fig. 4A, B). In contrast, on WD, *Scd1^{fl/fl}/ldlr^{-/-}* mice had higher enterocyte LPA 18:1 levels and gained weight. These observations are consistent with the hypothesis that enterocyte LPA 18:1 levels may influence nutrient absorption. Future research is required to determine whether this is the case.

What might be the mechanisms involved in explaining the phenotype of the enterocyte *Scd1* knockdown?

Enterocyte knockdown of *Scd1* partially protected against WD-mediated elevation of plasma SAA levels and was quantitatively similar to adding Tg6F to WD. The major source

chow. After 2 weeks, plasma total cholesterol levels were determined as described in the Materials and Methods. The data shown are mean \pm SEM. D: Adding LysoPC 18:1 to chow increases plasma SAA levels in *Scd1^{fl/fl}/ldlr^{-/-}* mice, but not in *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice. The mice described in A were fed chow without or with LysoPC 18:1 added at 1 mg per gram of chow. After 2 weeks, plasma SAA levels were determined as described in the Materials and Methods. The data shown are mean \pm SEM. E: Adding LysoPC 18:1 to chow increases plasma IL-6 levels in *Scd1^{fl/fl}/ldlr^{-/-}* mice, but not in *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice. The mice described in A were fed chow without or with LysoPC 18:1 added at 1 mg per gram of chow. After 2 weeks, plasma IL-6 levels were determined as described in the Materials and Methods. The data shown are mean \pm SEM. F: Adding LysoPC 18:1 to chow increases the levels of LysoPC 18:1 in the jejunum of *Scd1^{fl/fl}/ldlr^{-/-}* mice, but not in *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice. The mice described in A were fed chow without or with LysoPC 18:1 added at 1 mg per gram of chow. After 2 weeks, the jejunum was harvested from the mice and LysoPC 18:1 levels were determined as described in the Materials and Methods. The data shown are mean \pm SEM. G: Adding LysoPC 18:1 to chow increases the levels of LysoPC 18:1 in the plasma of *Scd1^{fl/fl}/ldlr^{-/-}* mice, but not in *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice. The mice described in A were fed chow without or with LysoPC 18:1 added at 1 mg per gram chow. After 2 weeks, plasma LysoPC 18:1 levels were determined as described in the Materials and Methods. The data shown are mean \pm SEM. H: Addition of LysoPC 18:1 to chow did not alter levels of LysoPC 18:0 in the jejunum of *Scd1^{fl/fl}/ldlr^{-/-}* mice or in the jejunum of *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice. The mice described in A were fed chow without or with LysoPC 18:1 added at 1 mg per gram of chow. After 2 weeks, the jejunum was harvested from the mice and LysoPC 18:0 levels were determined as described in the Materials and Methods. The data shown are mean \pm SEM. I: Addition of LysoPC 18:1 to chow did not alter levels of LysoPC 18:0 in the plasma of *Scd1^{fl/fl}/ldlr^{-/-}* mice or in the plasma of *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice. The mice described in A were fed chow without or with LysoPC 18:1 added at 1 mg per gram of chow. After 2 weeks, LysoPC 18:0 levels were determined in the plasma as described in the Materials and Methods. The data shown are mean \pm SEM.

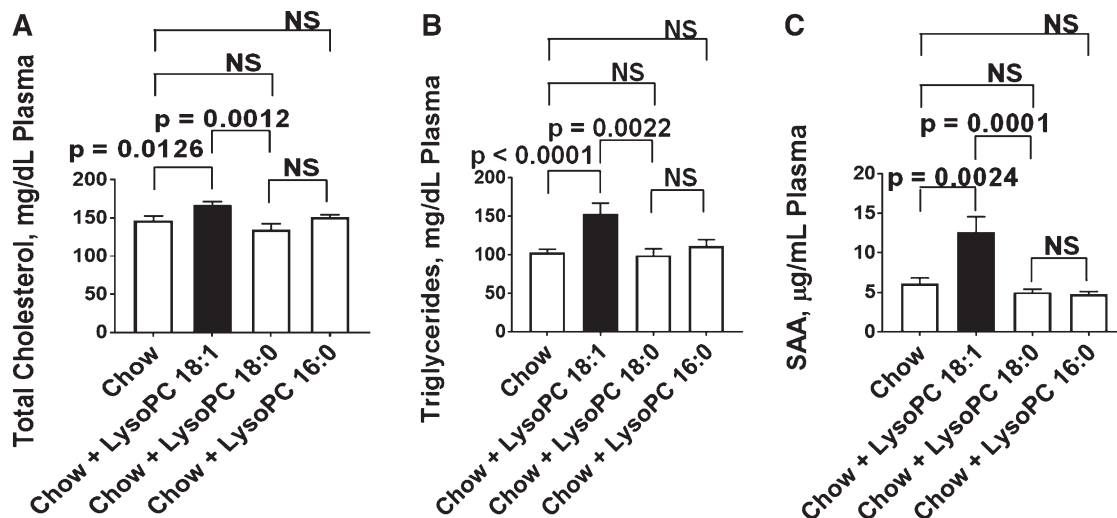


Fig. 13. A: LysoPC 18:1 (but not LysoPC 18:0 or LysoPC 16:0) increases plasma total cholesterol in *ldlr*^{-/-} mice. Female *ldlr*^{-/-} mice (age 7–8 months, n = 15 per group) were fed chow or chow to which LysoPC 18:1, LysoPC 18:0, or LysoPC 16:0 was added (at 1 mg per gram of chow). After 2 weeks, plasma total cholesterol was determined as described in the Materials and Methods. The data shown are mean ± SEM. B: LysoPC 18:1 (but not LysoPC 18:0 or LysoPC 16:0) increases plasma triglycerides in *ldlr*^{-/-} mice. Plasma triglycerides were determined as described in the Materials and Methods for the mice described in A. The data shown are mean ± SEM. C: LysoPC 18:1 (but not LysoPC 18:0 or LysoPC 16:0) increases plasma SAA levels in *ldlr*^{-/-} mice. Plasma SAA levels were determined as described in the Materials and Methods for the mice described in A. The data shown are mean ± SEM.

of plasma SAA is derived from the liver. How could knocking down enterocyte *Scd1* alter the production of SAA by the liver? An important function of enterocytes is to provide a barrier between gut bacteria and the tissues of the host. A high-fat diet induces inflammation in mice by increasing bacterial LPS levels in the intestinal lumen and plasma and increasing intestinal permeability through the induction of TLR4 (6). The processing of LPS involves an initial interaction with LBP that helps CD14 to extract LPS from bacteria. CD14 then delivers LPS to TLR4-MD-2 prompting dimerization and activation of TLR4 (7). TLR4 signals through multiple pathways, including through MyD88 (8), to cause increased intestinal permeability and intestinal inflammation (9). As shown in Figs. 6–10, enterocyte knockdown of *Scd1* in *ldlr*^{-/-} mice on WD resulted in lower protein levels of LBP, CD14, TLR4, and MyD88 in both enterocytes and plasma, and was similar in magnitude to the decrease in these proteins upon adding Tg6F to WD. Based on these experiments, we hypothesize that hepatic production of SAA is decreased in part by a reduction of LPS reaching the liver or by a reduction in cytokine production in the intestine leading to lower levels of cytokines reaching the liver through the portal circulation. Future research will be required to test this hypothesis.

According to the manufacturer's specifications, oleic acid accounts for 20.9% of the fatty acids in the WD used in most of our studies (Teklad, Harlan). With such an abundance of oleic acid in the diet, why would *Scd1* expression influence the formation of LysoPC 18:1 or LPA 18:1? We think that the findings of Miyazaki et al. (26) may provide the answer. They (26) reported that mice with a naturally occurring null mutation for *Scd1* were deficient in hepatic cholesterol esters and triglycerides despite the

presence of normal activities of acyl-CoA:cholesterol acyltransferase and glycerol phosphate acyltransferase. Feeding diets supplemented with triolein or tripalmitolein to the *Scd1*-deficient mice resulted in increased levels of oleic and palmitoleic fatty acids in liver, but failed to restore the levels of these fatty acids in cholesteryl esters or triglycerides (26). Using confocal microscopy and fluorescence resonance energy transfer, it was determined that acyl-CoA:diacylglycerol acyltransferase 2 (DGAT2) and SCD1 were colocalized in the endoplasmic reticulum. SCD1 was also present in the mitochondria-associated membrane (27). Based on these findings, they concluded that the subcellular localization of SCD1 relative to DGAT2 accounted for the preference for endogenous monounsaturated fatty acids in triglyceride synthesis and the use of endogenously generated monounsaturated fatty acids for incorporation into cholesterol esters in lipoproteins (27).

We believe that a similar explanation may apply as to why enterocyte *Scd1* determines the levels of LysoPC 18:1 and LPA 18:1 in the jejunum. We hypothesize that, in enterocytes, when the enzymes involved in the conversion of LysoPC to phosphatidylcholine (PC) incorporate oleic acid, they preferentially use endogenously synthesized oleic acid.

Hishikawa et al. (28) reported that *Lpcat3* preferentially utilizes linoleic acid. Wang et al. (18) reported that in the absence of *Lpcat3* in the intestine, there was a compensatory increase in phospholipids containing oleic acid. The abundance of individual phospholipid species in the livers of the high-fat-fed intestine-specific *Lpcat3*-deficient mice resembled their abundance in the enterocytes, indicating that the phospholipid composition of the liver is at least in part dependent on intestinal *Lpcat3* activity (18).

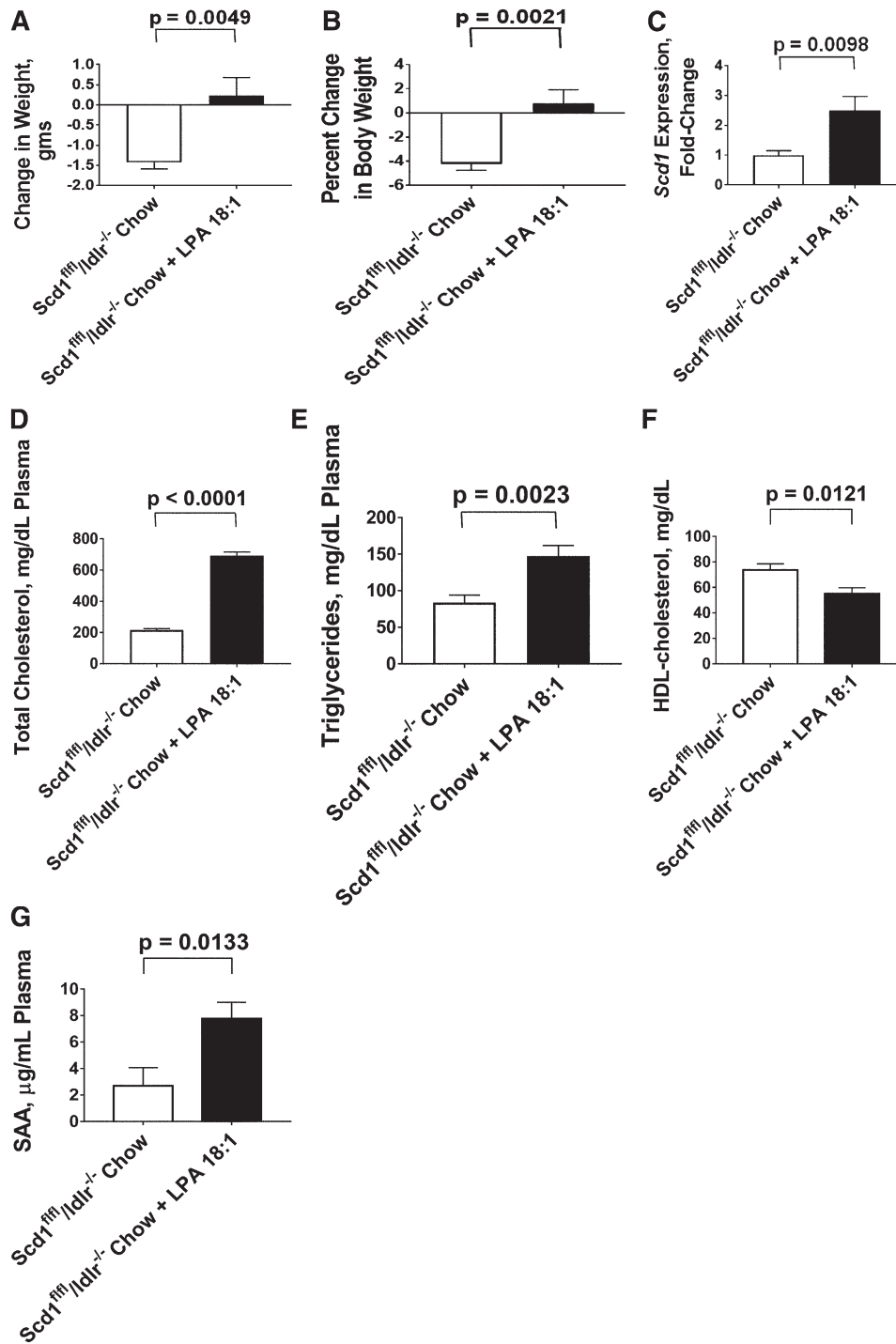


Fig. 14. A: Change in weight after adding LPA 18:1 to mouse chow. Male *Scd1^{fl/fl}/ldlr^{-/-}* mice (age 10–11 months, n = 10 per group) were fed chow or chow + LPA 18:1 added at 1 μ g per gram of chow as described in the Materials and Methods. After 2 weeks, the change in weight for the mice was determined by subtracting the weight for each mouse prior to the start of the experiment from its weight at the end of the experiment. The data shown are mean \pm SEM. B: Percent change in weight after adding LPA 18:1 to mouse chow. The percent change in weight for each mouse described in A was determined. The data shown are mean \pm SEM. C: LPA 18:1 induces *Scd1* expression in the jejunum. *Scd1* expression was determined as described in Materials and Methods in the mice described in A. The data shown are mean \pm SEM. D: LPA 18:1 induces an increase in plasma total cholesterol levels. Plasma total cholesterol was determined as described in the Materials and Methods in the mice described in A. The data shown are mean \pm SEM. E: LPA 18:1 induces an increase in plasma triglyceride levels. Plasma triglyceride was determined as described in the Materials and Methods in the mice described in A. The data shown are mean \pm SEM. F: LPA 18:1 induces a decrease in plasma HDL-cholesterol levels. Plasma HDL-cholesterol was determined as described in the Materials and Methods in the mice described in A. The data shown are mean \pm SEM. G: LPA 18:1 induces an increase in plasma SAA levels. Plasma SAA was determined as described in the Materials and Methods in the mice described in A. The data shown are mean \pm SEM.

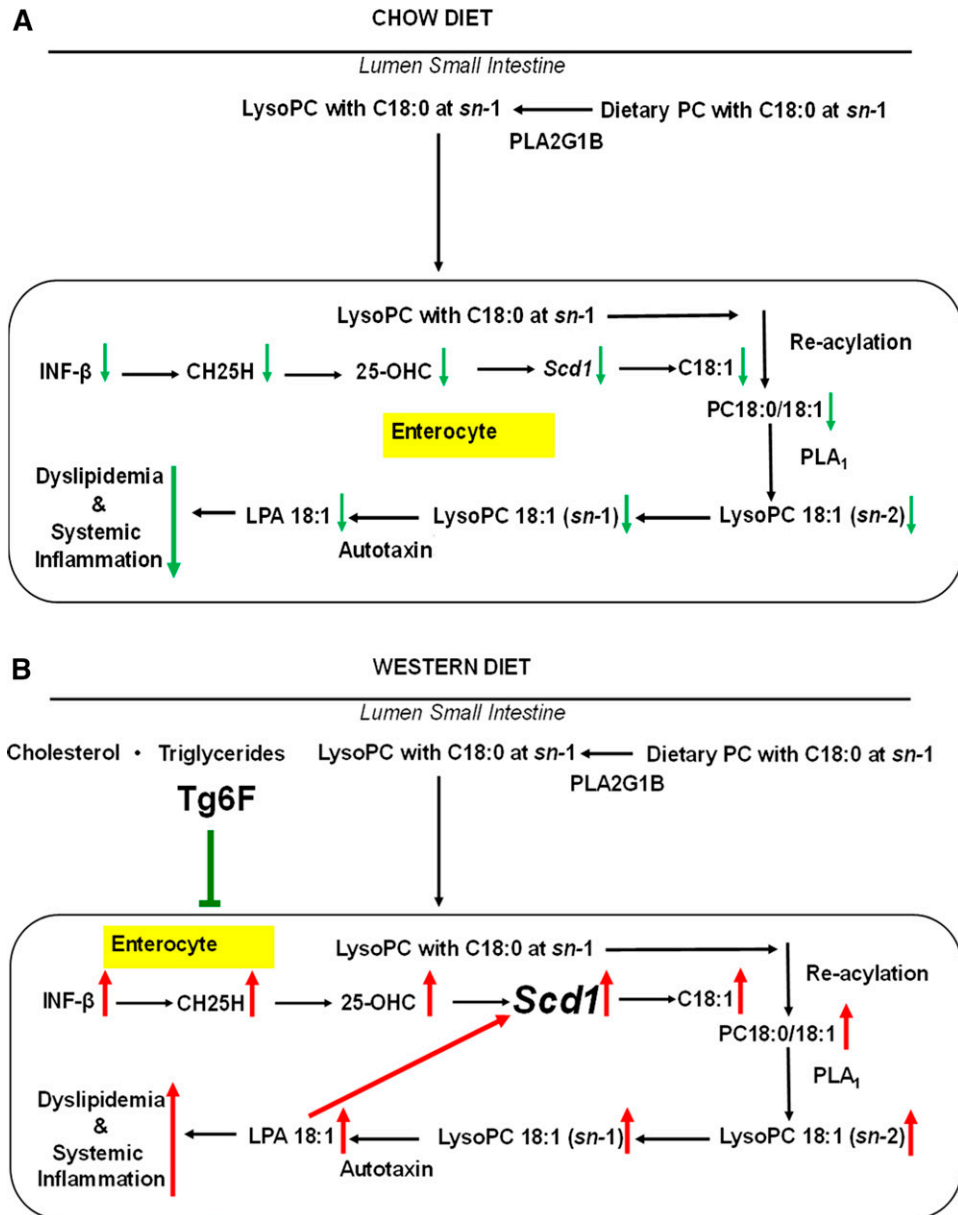


Fig. 15. A: A hypothesis to explain the role of enterocyte *Scd1* and Tg6F in WD-mediated dyslipidemia and systemic inflammation in *ldlr*^{-/-} mice. On a chow diet, the levels of IFN-β, CH25H, and 25-OHC are low, as is the level of *Scd1* expression in enterocytes. As a result, the levels of endogenously synthesized oleic acid (C18:1) are also low in the enterocytes. In the lumen of the duodenum and jejunum, dietary PC containing stearic acid (C18:0) at the *sn*-1 position with an unspecified fatty acid at the *sn*-2 position is acted upon by phospholipase A₂ group 1B (PLA2G1B) to yield LysoPC 18:0 with the acyl group at the *sn*-1 position. Subsequently, this LysoPC 18:0 enters the enterocytes and undergoes reacylation. On a chow diet, the levels of endogenously synthesized C18:1 are low, but they are not absent. Consequently, small amounts of PC with C18:0 at the *sn*-1 position and C18:1 at the *sn*-2 position (PC18:0/18:1) are formed. In the example shown, phospholipase A₁ (PLA₁) acts on PC18:0/18:1 to yield LysoPC 18:1 with C18:1 at the *sn*-2 position. The *sn*-1 position is thermodynamically more stable for accommodating an acyl group in lysophospholipids compared with the *sn*-2 position (34, 35). Consequently, the C18:1 isomerizes to the *sn*-1 position, and the resulting LysoPC 18:1 is then acted upon by autotaxin to yield LPA 18:1. On a chow diet, the amount of LPA 18:1 formed is too low to induce dyslipidemia and systemic inflammation. B: A hypothesis to explain the role of enterocyte *Scd1* and Tg6F in WD-mediated dyslipidemia and systemic inflammation in *ldlr*^{-/-} mice. On WD, the levels of IFN-β, CH25H, and 25-OHC are high, as is the level of *Scd1* expression in enterocytes. As LysoPC 18:1 and LPA 18:1 levels rise, there is a positive feed-back loop further inducing *Scd1* expression. Tg6F partially ameliorates the sequence that leads to dyslipidemia and systemic inflammation.

We previously reported that LPA 18:2 (but not LPA 18:0) was highly effective in causing dyslipidemia and systemic inflammation (1). If there is robust remodeling of phospholipids on WD, why would reducing levels of LysoPC 18:1 by knockdown of enterocyte *Scd1* result in improvement of dyslipidemia and systemic inflammation? Should we not expect linoleic acid-containing lysophospholipids to compensate for the absence of the oleic acid lysophospholipids? Why would we not expect LysoPC 18:2 and LPA 18:2 to be sufficient to induce WD-mediated dyslipidemia and systemic inflammation? Getz and Reardon (16) noted that the Teklad WD used in our studies is low in the essential fatty acid, linoleic acid. Indeed, according to the manufacturer's analysis, the levels of linoleic acid in the WD used in these studies, and in all of our previous studies (Teklad, Harlan, catalog #TD88137) are similar to the levels of linoleic acid used by others in models of essential fatty acid deficiency (29). Could it be that the low linoleic acid levels in our WD impaired the ability to synthesize LPA 18:2, thus exaggerating the importance of LysoPC 18:1 and LPA 18:1? We think this is unlikely on the Teklad WD, because in our previous studies, the levels of LPA 18:2 in the jejunum, liver, and plasma of *ldlr*^{-/-} mice on the Teklad WD were significantly higher than the levels on mouse chow, which is not deficient in essential fatty acids (see Figs. 8–10 in Ref. 2). Moreover, the experiments in Fig. 11 in this work yielded similar results whether we used a WD rich in linoleic acid (Research Diets WD) or one relatively deficient in linoleic acid (Teklad WD). Thus, we are left with the possibility that LysoPC 18:1 and LPA 18:1 may play a special role in the induction of dyslipidemia and systemic inflammation that is dependent on enterocyte *Scd1*.

The data in Fig. 12 show that, when added to mouse chow, the ability of LysoPC 18:1 to induce dyslipidemia and systemic inflammation was dependent on the induction of enterocyte *Scd1*. The data in Fig. 12F and G demonstrate that, upon addition to mouse chow, the ability of LysoPC 18:1 to induce elevated levels of LysoPC 18:1 in the tissue of the jejunum and plasma was also dependent on enterocyte *Scd1* (i.e., following enterocyte knockdown of *Scd1*, adding LysoPC 18:1 to chow did not increase LysoPC 18:1 levels in either the tissue of the jejunum or the plasma). It may be that with enterocyte knockdown of *Scd1*, absorption of LysoPC in general is impaired. LysoPC 18:0 levels in the jejunum were not decreased by knockdown of enterocyte *Scd1* in two experiments (Figs. 3E, 4D), but were in one (Fig. 5H), so we cannot exclude this possibility. Another possibility is that dietary LysoPC 18:1 rapidly enters a recycling pool, and with enterocyte knockdown of *Scd1*, LysoPC 18:1 is not reformed, but when enterocyte *Scd1* is intact, there is abundant synthesis of LysoPC 18:1. LysoPC 18:0 neither induces *Scd1* expression (Fig. 12A) nor causes dyslipidemia and systemic inflammation (2). Perhaps the failure of LysoPC 18:0 to cause dyslipidemia and systemic inflammation is due to its inability to induce *Scd1*. One might also question why, with knockdown of *Scd1*, levels of LysoPC 18:0 did not increase? In addition to the possibility of impaired absorption noted above, it is also possible that reacylation

of LysoPC to PC was enhanced using fatty acid moieties other than oleic acid. Future research will be needed to determine which (if any) of these explanations is correct. Because this work was limited to studies in *ldlr*^{-/-} mice, future studies will also be needed to determine whether these observations apply to wild-type mice.

Why does LysoPC 16:0 or LysoPC 18:0 fail to induce *Scd1*, dyslipidemia, and systemic inflammation?

Figure 13 demonstrates that LysoPC 16:0 acts like LysoPC 18:0 in its inability to cause dyslipidemia and systemic inflammation following addition to mouse chow. We previously reported that LysoPC 18:0 is not acted upon by autotaxin to form LPA 18:0; and yet upon feeding LysoPC 18:0, there was abundant formation of LPA 18:0 (2). Because others have shown that LysoPC 18:0 is a good substrate for autotaxin, we concluded (2) that this might indicate that LysoPC 18:0 is processed in a compartment different from where LysoPC 18:1 is processed. If LysoPC 18:0 and LysoPC 18:1 are converted to their respective LPA species in different cellular compartments, it might explain some of the different biologic activities of the two LysoPC species. Future research will be required to determine whether this is indeed the case.

What might be the mechanism by which Tg6F decreases protein levels of LBP, CD14, TLR4, and MyD88 in enterocytes and plasma?


Wurfel et al. (30) reported that passage of plasma over an anti-apoA-I column removed more than 99% of LBP detectable by ELISA. Additionally, anti-LBP monoclonal antibody coprecipitated apoA-I (30). These data conclusively established that LBP in plasma was associated with apoA-I. This group also reported that LBP was associated with a particle containing apoA-I, phospholipid, and factor H-related proteins (31). Tg6F is an apoA-I mimetic peptide in a concentrate of tomato polyphenols. It seems likely that in the intestinal lumen, the 6F peptide and the tomato polyphenols exist as a micelle. We hypothesize that enterocyte LBP is sequestered into the 6F-tomato polyphenol micelles in the lumen of the intestine. Because the 6F peptide is found in the lumen of the intestine after oral administration, but it is not detected in plasma (10), we presume that it is either not absorbed and/or it is degraded in the lumen of the intestine. If LBP was sequestered in the lumen of the intestine with the Tg6F, this would result in less bacterial LPS entering the enterocyte and could account for the observed decreased levels of enterocyte LBP, CD14, TLR4, and MyD88. Future research will be required to determine whether this hypothesis has merit.

What might be the mechanism by which Tg6F decreases *Scd1* expression?

Kim, Miyazaki, and Ntambi (32) reported that cholesterol is a potent inducer of *Scd1* expression. Indeed, they found that feeding mice cholesterol overcame the ability of polyunsaturated fatty acids to decrease the expression of *Scd1* and its protein and activity (32). We recently reported that WD induced the expression of interferon- β (IFN- β)

and cholesterol 25-hydroxylase (CH25H) with a resulting increase in levels of 25-hydroxycholesterol (25-OHC) in the jejunum that was ameliorated by Tg6F (11). Shibata et al. (33) studied the effect of 25-OHC on mouse macrophages using MS. They quantified 569 lipid species in three independent experiments and found that cholesteryl esters containing oleic acid were quantitatively the most abundant species to accumulate (33). These results are consistent with 25-OHC inducing *Scd1* and Tg6F preventing *Scd1* induction by lowering levels of 25-OHC.

In the current studies, adding Tg6F to WD consumed by *Scd1^{fl/fl}/ldlr^{-/-}/VilCre* mice either did not further reduce the measured parameters or only modestly did so. This suggests that the mechanism of action of Tg6F in ameliorating dyslipidemia and systemic inflammation may at least in part be due to its ability to decrease the expression of enterocyte *Scd1*. **Figure 15** presents a hypothesis to explain the role of *Scd1* and Tg6F in WD-mediated dyslipidemia and systemic inflammation in *ldlr^{-/-}* mice. Figure 15A depicts conditions on a chow diet where the levels of IFN- β , CH25H, and 25-OHC are low, as is the level of *Scd1* expression in enterocytes. As a result, the levels of endogenously synthesized oleic acid (C18:1) are also low in the enterocytes. In the schematic representation of our hypothesis shown in Fig. 15, dietary PC containing stearic acid (C18:0) at the *sn*-1 position with an unspecified fatty acid at the *sn*-2 position is acted upon by phospholipase A₂ group 1B (PLA2G1B) to yield LysoPC 18:0 with the acyl group at the *sn*-1 position. Subsequently, it enters the enterocytes and undergoes reacylation. On a chow diet, levels of endogenously synthesized C18:1 are low, but they are not absent. Consequently, small amounts of PC with C18:0 at the *sn*-1 position and C18:1 at the *sn*-2 position (PC18:0/18:1) are formed. In the example shown, phospholipase A₁ (PLA₁) acts on PC18:0/18:1 to yield LysoPC 18:1 with C18:1 at the *sn*-2 position. The *sn*-1 position is thermodynamically more stable for accommodating an acyl group in lysophospholipids compared with the *sn*-2 position (34, 35). Consequently, the C18:1 isomerizes to the *sn*-1 position, and the resulting LysoPC 18:1 is then acted upon by autotaxin to yield LPA 18:1. On a chow diet, the amount of LPA 18:1 formed is too low to induce dyslipidemia and systemic inflammation. In contrast, Fig. 15B depicts conditions on WD where the levels of IFN- β , CH25H, and 25-OHC are high, as is the level of *Scd1* expression in enterocytes. As LysoPC 18:1 and LPA 18:1 levels rise, we hypothesize that there is a positive feed-back loop further inducing *Scd1* expression. Figure 15B also indicates that Tg6F ameliorates this sequence that leads to dyslipidemia and systemic inflammation. This hypothesis provides a basis for future research. Such research will require the use of labeled precursors and determination of lipid species in enterocyte membranes and chylomicrons and studies of subcellular compartments.

The data reported here add to our previous studies (1–3, 10, 11) and the studies of Hui (36) showing that lysophospholipid metabolism in the small intestine can play an important role in dyslipidemia and systemic inflammation. 

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