Despite Antiatherogenic Metabolic Characteristics, SCD1-Deficient Mice Have Increased Inflammation and Atherosclerosis

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Objective—Absence of stearoyl-CoA desaturase-1 (SCD1) in mice reduces plasma triglycerides and provides protection from obesity and insulin resistance, which would be predicted to be associated with reduced susceptibility to atherosclerosis. The aim of this study was to determine the effect of SCD1 deficiency on atherosclerosis.

Methods and Results—Despite an antiatherogenic metabolic profile, SCD1 deficiency increases atherosclerosis in hyperlipidemic low-density lipoprotein receptor (LDLR)-deficient mice challenged with a Western diet. Lesion area at the aortic root is significantly increased in males and females in two models of SCD1 deficiency. Inflammatory changes are evident in the skin of these mice, including increased intercellular adhesion molecule (ICAM)-1 and ulcerative dermatitis. Increases in ICAM-1 and interleukin-6 are also evident in plasma of SCD1-deficient mice. HDL particles demonstrate changes associated with inflammation, including decreased plasma apolipoprotein A-II and apolipoprotein A-I and paraoxonase-1 and increased plasma serum amyloid A. Lipopolysaccharide-induced inflammatory response and cholesterol efflux are not altered in SCD1-deficient macrophages. In addition, when SCD1 deficiency is limited to bone marrow–derived cells, lesion size is not altered in LDLR-deficient mice.

Conclusions—These studies reinforce the crucial role of chronic inflammation in promoting atherosclerosis, even in the presence of antiatherogenic biochemical and metabolic characteristics. (Arterioscler Thromb Vasc Biol. 2009;29:341-347.)

Key Words: atherosclerosis ■ inflammation ■ apolipoproteins ■ lipoproteins ■ hyperlipoproteinemia

SCD1-deficient mice are protected from insulin resistance and diet-induced obesity and have a markedly reduced rate of VLDL-TG production. We have recently shown that SCD1 deficiency improves the metabolic phenotype of a hyperlipidemic LDLR-deficient mouse model of familial hypercholesterolemia (FH). On a Western diet, LDLR-deficient mice develop diet-induced diabetes and obesity and develop atherosclerosis over 2 to 3 months.

Absence of SCD1 reduces hepatic steatosis and plasma TG (by ≈50%) and provides striking protection from diet-induced weight gain and insulin resistance in LDLR-deficient mice. A major unanswered question is whether the amelioration of these features in SCD1-deficient mice will lead to reduced susceptibility to atherosclerosis.

In this study, we show that despite these antiatherogenic metabolic characteristics, SCD1 deficiency surprisingly increases lesion size in hyperlipidemic LDLR-deficient mice and that this acceleration in atherosclerosis is likely to result from chronic inflammation primarily of the skin, which then leads to changes in markers of inflammation in plasma and proinflammatory changes in HDL.
Methods
An extended Methods section is available in the online supplemental materials (please see http://atvb.ahajournals.org). Mice carrying the Scd1<sup>ab-5</sup> or Scd1<sup>ab-23</sup> null alleles were back-crossed to C57BL/6 for five generations to produce N<sub>x</sub> incipient congenic mice and then crossed to the B6.129S7-Ldlr<sup>m1Her</sup>mutant strain. The Scd1<sup>+/−</sup>Ldlr<sup>−/−</sup> control groups consisted of both littermates of Scd1<sup>+/−</sup>Ldlr<sup>−/−</sup> mice and additional age- and sex-matched Scd1<sup>+/−</sup>Ldlr<sup>−/−</sup> mice that were not littermates (≈63% of all animals studied). Mice deficient in SCD1 with the Scd1<sup>ab-5</sup> allele were used in all experiments except those involving analysis of atherosclerotic lesions and paraoxonase-1 (PON1) activity, in which mice carrying a separately derived SCD1 deletion (the Scd1<sup>ab-23</sup> allele) were also studied. Sections of the aortic root were stained as described in Singaraga et al.<sup>12</sup>

Results
SCD1 Deficiency Increases Atherosclerosis in Ldlr<sup>−/−</sup> Mice
Mice with a spontaneous deletion in Scd1 (B6.ABJ/Le-Scd1<sup>ab-5</sup>) were crossed with an existing dyslipidemic mouse model (B6.129S7-Ldlr<sup>m1Her</sup>)<sup>11</sup> to generate mice with combined deficiencies of both LDLR (Ldlr<sup>−/−</sup>) and SCD1 (Scd1<sup>−/−</sup>). After 12 weeks of an atherogenic “Western” diet,<sup>13</sup> weights for male and female Scd1<sup>−/−</sup>Ldlr<sup>−/−</sup> mice were 44% and 54% higher than initial values, respectively, whereas neither male nor female Scd1<sup>−/−</sup>Ldlr<sup>−/−</sup> mice showed a significant increase in body weight, as described elsewhere. Total plasma TG was reduced by 44% and 51%, and non-HDL cholesterol was reduced by 8% and 27% in male and female Scd1<sup>−/−</sup>Ldlr<sup>−/−</sup> mice, respectively, relative to Scd1<sup>+/−</sup>Ldlr<sup>−/−</sup> controls. HDL cholesterol levels were unchanged by SCD1 deficiency. Absence of SCD1 also increased insulin sensitivity as measured by intraperitoneal glucose and insulin tolerance testing.<sup>7</sup>

Atherosclerotic lesion size was evaluated in multiple sections of the aortic root in this same cohort of Scd1<sup>−/−</sup>Ldlr<sup>−/−</sup> mice (males, n=6; females, n=10) and Scd1<sup>+/−</sup>Ldlr<sup>−/−</sup>-control mice (males, n=11; females, n=11; Figure 1a and 1b). Unexpectedly, both male and female SCD1-deficient mice have significantly increased lesion size relative to controls. Lesion area was increased by 74% in males (P=0.0002) and by 41% in females (P=0.0004).

In view of these observations in mice with the Scd1<sup>ab-5</sup> allele, we wished to examine whether these findings could be replicated in another cohort of mice carrying a different spontaneous null allele of Scd1 (B6.D1-Scd1<sup>ab-23</sup>).<sup>10</sup> These mice were crossed with the same LDLR-deficient model and housed at a different specific pathogen-free barrier animal facility. Again, lesion area at the aortic root was increased in SCD1-deficient mice (129% increase in males; P=0.0001; 70% increase in females; P<0.0001; Figure 1b and supplemental Figure 1), thus supporting our initial findings. The effect remained significant when all mice that were not littermates were excluded from the analysis (data not shown).

Aortic root sections from the first cohort of mice were stained with Movat pentachrome and hematoxylin and eosin (H&E) for histological examination. Extracellular matrix thickening and acellular areas containing cholesterol crystals were apparent in the deeper portion of the lesions from Scd1<sup>+/−</sup>Ldlr<sup>−/−</sup> mice (Figure 2a and 2b and supplemental Figure IIc). These findings were increased in the more advanced lesions of the Scd1<sup>−/−</sup>Ldlr<sup>−/−</sup> mice, with many extracellular cholesterol clefs in the large necrotic core underlying foam cell–rich regions. Staining for smooth muscle actin was evident in the media and fibrous caps of advanced lesions of both Scd1<sup>+/−</sup>Ldlr<sup>−/−</sup> and Scd1<sup>−/−</sup>Ldlr<sup>−/−</sup> mice (supplemental Figure 1b). The increased lesion size in Scd1<sup>−/−</sup>Ldlr<sup>−/−</sup> mice fed the Western diet for 12 weeks was characterized by greater absolute areas of macrophage infiltration in these animals versus Scd1<sup>+/−</sup>Ldlr<sup>−/−</sup>-controls. This macrophage infiltration was evident in both the large complex atheromatous lesions in the left coronary sinususes, as well as the smaller lesions of the right coronary and noncoronary sinususes. The majority of cells in early plaques were positive for monocyte/macrophage staining in both Scd1<sup>+/−</sup>Ldlr<sup>−/−</sup> and Scd1<sup>−/−</sup>Ldlr<sup>−/−</sup> mice (supplemental Figure IIc).

Semi-quantitative morphological examination of sections stained with Movat pentachrome and H&E was used to assign lesion severity scores on a 0 to 5+ scale based on the following parameters: foam cell characteristics, cholesterol clefs, presence of necrotic core, degree and composition of fibrous cap, infiltration into the media, extracellular matrix deposition, calcification, and plaque cellular characteristics. When examined in a blinded fashion, the aortic roots of Scd1<sup>−/−</sup>Ldlr<sup>−/−</sup>-mice earned significantly higher lesion severity scores than Scd1<sup>+/−</sup>Ldlr<sup>−/−</sup>-controls (P=0.001; Figure 2c).

SCD1 Deficiency Promotes Inflammation in Ldlr<sup>−/−</sup> Mice
Prior dermatologic and immunologic studies have indicated that SCD1-deficient mice have skin that is rich in macrophages and mast cells,<sup>14,15</sup> indicative of chronic dermal
inflammation. \textit{Icam1} mRNA was increased more than 2-fold in the skin of Scd1\(^{-/-}\)/Ldlr\(^{-/-}\) mice relative to control Scd1\(^{+/+}\)/Ldlr\(^{+/+}\) mice (males, \(P=0.017\); females, \(P=0.093\); Figure 3a), and skin ICAM-1 protein was increased more than 2-fold in Scd1\(^{-/-}\)/Ldlr\(^{-/-}\) mice (males, \(P=0.0091\); females, \(P=0.0022\); Figure 3b).

Scd1\(^{-/-}\)/Ldlr\(^{-/-}\) mice have obvious skin abnormalities, including a hyperplastic epidermis and stratum corneum (supplemental Figure IIIa). Severe spontaneous ulcerative dermatitis (supplemental Figure IIIb and IIIc) necessitated euthanasia of 14\% to 16\% of SCD1-deficient mice (Figure 3c). Diffuse inflammatory infiltration that included mast cells (supplemental Figure IIIc), exudation of inflammatory cells and fibrin onto the surface of the skin, and proliferation of fibrous tissue and granulation in the dermis was evident (supplemental Figure IIIb and IIIc). We also observed mild to moderate lymphadenopathy, particularly protruding cervical and brachial lymph nodes, in mice with severe dermatitis. No Scd1\(^{+/+}\)/Ldlr\(^{-/-}\) mice developed any skin lesions by the end of the study.

These findings prompted us to examine whether SCD1-deficient mice also have markers of systemic inflammation that may be contributing to atherosclerosis. Interleukin (IL)-6 is increased by 67\% (\(P=0.043\); Figure 4a), and soluble ICAM-1, an adhesion molecule that is elevated in serum of patients with the inflammatory skin disorders psoriasis\(^{16}\) and atopic eczema,\(^{17}\) was also increased in Scd1\(^{-/-}\)/Ldlr\(^{-/-}\) mice (\(P=0.0035\); Figure 4b). Two additional proinflammatory cytokines known to be elevated in psoriatic skin lesions, IL-1\(^{18}\) and IL-12p70,\(^{19}\) were detected in plasma from Scd1\(^{-/-}\)/Ldlr\(^{-/-}\) mice but not Scd1\(^{+/+}\)/Ldlr\(^{-/-}\) controls (Figure 4c and 4d).

Circulating levels of MCP-1 were decreased 2-fold in Scd1\(^{-/-}\)/Ldlr\(^{-/-}\) mice (\(P=0.017\); Figure 4e). White adipose
tissue is the major source of MCP-1 in obese mice,20 and the decreased levels of MCP-1 may be attributed to the significant decrease in white adipose tissue seen in Scd1L−/−Ldlr−/− mice.7 The levels of RANTES (CCL5) were not significantly different (Figure 4f).

In the absence of a proinflammatory dietary stimulus, several inflammatory indicators in plasma were below the limit of detection in both Scd1L−/−Ldlr−/− and Scd1+/+Ldlr−/− mice. Weak trends toward increased inflammatory indicators were seen in Scd1L−/−Ldlr−/− mice relative to Scd1+/+Ldlr−/− controls, but these increases were not significant (supplemental Figure IV).

SCD1 Deficiency Alters HDL-Associated Proteins in Ldlr−/− Mice

Inflammation has been shown to have a proatherogenic effect on the composition of HDL particles, such that they become depleted in specific proteins, such as apoA-I, apoA-II, and PON1, whereas they are enriched in serum amyloid A (SAA).21 Indeed, in SCD1-deficient mice, plasma SAA is dramatically increased (females, 52-fold increase, P=0.0087; males, 2.9-fold increase, P=0.0043; Figure 5b), whereas plasma apoA-I (females, ~37% reduction, P=0.0017; males, ~16% reduction, P=0.44; Figure 5c) and apoA-II are decreased (~40% reduction; females, P=0.0007; males, P=0.0003; Figure 5d). The changes in plasma SAA and apoA-II were paralleled by significant changes in mRNA encoding these genes (Saa1, P=0.017; Saa2, P=0.017; Apoa2, P=0.039; Figure 5a). Furthermore, hepatic mRNA levels of Pon1, the gene that encodes PON1, an enzyme that contributes to the antioxidant properties of HDL,22 are decreased by nearly 75% (P<0.001; Figure 5a), whereas mRNA levels of Clu, the gene that encodes apoJ/clusterin, an acute phase HDL-associated protein,23 are increased by more than 2-fold (P=0.0001; Figure 5a). No changes were observed in mRNA levels of Lcat (Figure 5a). SCD1-deficient mice also had a significantly lower serum PON1 activity (P=0.0028; Figure 5e). These data indicate that SCD1 has a proatherogenic effect on HDL protein composition that may be attributed to chronic inflammation.

SCD1 Deficiency Does Not Alter Macrophage Function

The atherogenic effect of SCD1 deficiency could also result from a direct effect of SCD1 deficiency on macrophage function. If the increased macrophage infiltration is attributable to a direct effect of SCD1 deficiency in macrophages, we would expect an increased inflammatory response in SCD1-deficient macrophages.

We therefore evaluated the effect of SCD1 deficiency on the inflammatory response of thioglycollate-elicited peritoneal exudate cells (Figure 6a). Inflammatory gene expression was induced by lipopolysaccharide (LPS), an agonist of toll-like receptor 4 signaling, and mRNA levels of several LPS-induced inflammatory proteins were assessed. No significant differences were observed in genes encoding IL-6, TNF-α, IL-1β, IL-12p35, iNOS, IP-10, GARG-16, or COX2, suggesting that the increased atherosclerosis observed with
SCD1 deficiency is not attributable to an altered macrophage inflammatory response.

**Macrophage SCD1 Deficiency Does Not Alter Atherosclerosis in Ldlr<sup>−/−</sup> Mice**

Another way to examine whether the atherogenic effect of SCD1 deficiency results from a direct effect on SCD1 deficiency in macrophages is to evaluate the effect of SCD1 deficiency in bone marrow–derived cells on atherosclerosis in vivo. Bone marrow from Scd1<sup>−/−</sup> and Scd1<sup>+/+</sup> mice was transplanted into LDLR-deficient mice. At 6 weeks after bone marrow transplantation, the diet was switched from regular chow diet to Western diet. After 6 weeks on the Western diet, no skin lesions were observed in the transplanted mice, nor did SCD1 deficiency in bone marrow–derived cells have an effect on serum lipids (supplemental Table 1). Most importantly, no effect was observed between the two transplanted groups on atherosclerotic lesion size (0.207±0.018 mm<sup>2</sup> in Scd1<sup>+/+</sup>→Ldlr<sup>−/−</sup> mice versus 0.189±0.029 mm<sup>2</sup> in Scd1<sup>−/−</sup>→Ldlr<sup>−/−</sup> mice; Figure 6b and 6c), indicating that loss of macrophage SCD1 does not directly play a significant role in atherogenesis in these mice.

Overexpression of SCD1 has been reported to result in decreased cholesterol efflux in HEK293 and CHO cells.24 SCD1 deficiency had no effect on cholesterol efflux to apoA-I or HDL under our experimental conditions (Figure 6d), further supporting the fact that SCD1 deficiency is not associated with altered macrophage function.

**Discussion**

Despite an antiatherogenic lipid and metabolic profile, absence of SCD1 promotes inflammation and atherosclerosis in a mouse model of FH on a Western diet. Absence of SCD1 also increases plasma IL-6, IL-1β, IL-12p70, and sICAM-1 levels and has a proinflammatory effect on the components of HDL particles, increasing SAA and apoJ/clusterin and reducing apoA-I, apoA-II, and PON1. Specific deficiency of SCD1 in bone marrow–derived cells does not influence atherosclerotic lesion size.

We have recently shown that SCD1-deficient mice have relatively reduced plasma triglycerides and are protected from obesity and insulin resistance,7 phenotypic components of the metabolic syndrome that have been linked to increased susceptibility to atherosclerosis.25,26 These surprising data suggested that SCD1-deficient mice must have proatherothogenic stimulus that overcomes the antiatherogenic metabolic characteristics expected to reduce lipid accumulation in the aorta. Chronic inflammation has been reported in the skin of chow-fed SCD1-deficient mice, indicated by increased mRNA encoding ICAM-1.15 and increased infiltration of macrophages and mast cells but only rare lymphocytes or neutrophils in the dermis.14 In addition, subcutaneous cyclosporin A can inhibit ICAM-1 expression and reduce mast cell numbers in the skin, restoring the wild-type skin phenotype.15 Histopathologic studies in these mice have demonstrated that the chronic inflammatory reaction is a foreign body response, with extreme sebaceous gland hyperplasia in SCD1-deficient animals resulting in hair fiber perforation of the follicle base and a foreign body response to fragments of hair fiber in the dermis.10

Inflammation is recognized to play a major role in all stages of atherogenesis,27 and plasma markers of systemic inflammation are predictive for cardiovascular events in humans.28,29 Indeed, standard preventive drug therapies such as aspirin and statins are known to have antiinflammatory properties and have been shown to be most beneficial in individuals with elevated inflammatory markers at baseline, even in those with relatively low serum cholesterol levels.30

In the LDLR-deficient mouse model, used by several groups to study the link between chronic inflammation and atherosclerosis,31,32 plasma markers of systemic inflammation increase in response to dietary cholesterol, and these markers are associated with increased lesion area independent of plasma lipoprotein levels.33 Our observations of increased plasma IL-6, a marker of systemic inflammation that is associated with atherosclerosis,28 sICAM-1, an adhesion molecule that is elevated in serum of patients with inflammatory skin disorders,16,17 and IL-1β and IL-12p70, proinflammatory cytokines known to be elevated in psoriatic lesional skin,18,19 suggest that chronic inflammation of the skin may be contributing to the proatherogenic profile of SCD1-deficient mice. During an inflammatory response, HDL particles are known to become depleted in apoA-I, apoA-II, and PON1 and enriched in SAA, a liver-derived protein increased by Western diets and correlated with lesion area in LDLR-deficient mice.21,33 Our results show that absence of SCD1 has a proatherogenic effect on HDL composition.

Atherosclerotic lesion size and macrophage cholesterol efflux are not altered in LDLR-deficient mice transplanted with bone marrow from SCD1-deficient mice. These observations, in addition to the lack of altered LPS-induced inflammatory response in SCD1-deficient peritoneal macrophages, suggest that macrophage SCD1 does not play a significant role in atherogenesis in this model. However, it should be noted that these results are from early lesions only, and a longer term study is needed of SCD1 in lesion macrophages.

The SCD1-deficient mouse model affords a unique opportunity to compare and contrast directly the effects of an antiatherogenic metabolic profile with proinflammatory pathways. In this instance, proinflammatory pathways overcome the favorable metabolic profile. However, significant unanswered questions remain regarding possible atherogenic effects of circulating lipoproteins or tissue lipids with increased saturated fatty acids (SFA) or decreased monounsaturated fatty acids (MUFA), as shown in SCD1-deficient mice.2,24

The relevance of these findings to the development of SCD inhibitors for treatment of the metabolic syndrome in humans is unclear. Observational studies in humans have shown an association between increased indices of SCD activity and components of the metabolic syndrome,35–37 inflammatory markers,38 and potentially coronary heart disease,39 suggesting that the atherogenic inflammation observed in this mouse model of SCD1 deficiency may not extend to humans with reduced SCD1 activity. The findings in this study represent the effects of long-term complete SCD1 deficiency in all
tissues in mice. Antisense oligonucleotides (ASOs) may also be expected to result in near complete deficiency of SCD1 expression in some extrahepatic tissues, which could lead to atherogenic inflammation in rodent models similar to that observed here. By contrast, pharmaceutical compounds are generally not used at levels that would cause complete inhibition of the target enzyme through a 24-hour cycle, and would not be distributed throughout all tissues in the body. 

While this manuscript was in review, two different studies reported on the relationship between atherosclerosis and SCD1 deficiency mediated by ASOs. Both groups treated mice with identical SCD1-targeted ASOs, but the experiments yielded different results: increased atherosclerosis in the Ldlr−/− Apob100/100 model and reduced atherosclerosis in the chronic intermittent hypoxia (CIH) model. One clue to the discrepancy could be model-specific effects on HDL-cholesterol levels. Our results indicate that in the absence of antiatherogenic biochemical and metabolic characteristics, the presence of antiatherogenic biochemical and metabolic characteristics.

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Disclosures
M.R.H is a founder and serves on the board of directors of Xenon Pharmaceuticals.

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Supplemental Material

Supplemental Methods

Animals and diet. Animals received a standard laboratory rodent chow diet (LabDiet 5010 Autoclavable Rodent Diet, PMI Nutrition International, Richmond, IN), or western diet (TD.88137, Harlan Teklad, Madison, WI). All studies except those involving bone marrow transplantation were approved by the University of British Columbia Animal Care Committee.

Histological analysis. At 11-13 weeks of age, Scd1⁻/⁻Ldlr⁻/⁻ and Scd1⁺/⁺Ldlr⁻/⁻ mice were placed on the western diet. After a period of 12 weeks, the mice were fasted overnight, then anaesthetized by intraperitoneal injection of 250 mg/kg 2,2,2-tribromoethanol (Sigma-Aldrich, Oakville, ON, Canada). After exsanguination, mice were perfused transcardially with phosphate-buffered saline (PBS). Hearts with attached aortas were then removed and fixed in a 4% solution of paraformaldehyde in phosphate-buffered saline prior to embedding and freezing in Tissue-Tek OCT (Sakura Finetek USA Inc., Torrance, CA). Sixteen consecutive 10-μm sections were obtained working from the apex of the heart towards the aortic origin (1), beginning from the point where all three aortic valve cusps became clearly visible. Four slides were made from each animal. From the 16 sections, every fourth section (40 μm apart) was stained for oil red O (ORO) and hematoxylin to visualize neutral lipid. Adjacent sections were stained with hematoxylin and eosin or Movat pentachrome as described in Singaraga et al (2). Movat pentachrome staining was used for visualization of proteoglycan-rich, extracellular matrix thickening of the intima (sea-green colour) and necrotic cores (absence of purple/black nuclei).

Image analysis was performed with Image Pro Plus (Media Cybernetics, Silver Springs, MD) or ImageJ (version 1.41b; National Institutes of Health, Baltimore, MD). Measurements were made at a magnification of 4× after calibration of the image analysis
software using a micrometer image scanned at a magnification identical to that used for the aortic root tissue.

It was critical to manually evaluate lesion area from the luminal edge to the intima-media border rather than using threshold-based quantification of oil red O staining of neutral lipids, as we observed inconsistent staining between two studies and within lesions that contain regions of extracellular matrix and unesterified cholesterol. Values reported represent the mean lesion area from 4 sections for each animal.

Semi-quantitative assessment of lesion severity and inflammatory cell infiltration was performed in a randomized and blinded fashion by a registered cardiovascular pathologist (BMM) using a 0 to 5+ scale. To assess reproducibility of this analysis, randomly selected slides were used to assess intra-observer variability on two separate occasions. Qualitative morphologic assessment of lesion complexity was performed with light microscopy for the following parameters: foam cell characteristics, cholesterol clefts, presence of necrotic core, degree and composition of fibrous cap, infiltration into the media, extracellular matrix deposition, calcification and plaque cellular characteristics. Sections were graded (0-5+) based on the following scale: 0, few or no apparent foam cells and no apparent intimal lesion; 1+, small, foam-cell predominant plaque; 2+, intermediate plaque with multilayered or diffuse foam cells, occasional cholesterol clefts, and few or no apparent acellular degenerative areas; 3+ mixed plaque with fibrous cap that may have cholesterol clefts and an atheromatous core superficially covered by smooth muscle cells; 4+, mixed plaque composed of smooth muscle cells, collagen, and elastic fragments, with a consistent acellular core, fibrous cap, possible calcification and common cholesterol clefts; 5+, advanced complex lesion with multilayered and diffuse foam cells, many deep cholesterol clefts, consistently, large acellular cores, a large amount of extracellular matrix, a consistent fibrous cap and consistent calcification.
Dorsal skin tissue was embedded and frozen in Tissue-Tek OCT and 10-μm sections were prepared and stained with hematoxylin and eosin. Skin samples from severe dermatitis lesions were fixed, embedded in paraffin, and then sectioned for staining with hematoxylin and eosin or toluidine blue.

**Immunohistochemical Studies.** For the assessment of smooth muscle cells, cryosections were immunolabeled with a primary mouse monoclonal antibody against smooth muscle \( \alpha \)-actin, clone 1A4 (Thermo Fisher Scientific, Fremont, CA). For the assessment of macrophage infiltration in early lesions, mice were euthanized after 5 weeks on the western diet, hearts with attached aortas were removed and embedded in OCT, and cryosections were immunolabeled with a primary rat monoclonal antibody against mouse monocytes/macrophages, clone MOMA-2 (AbD Serotec, Raleigh, NC).

**Quantitative RT-PCR.** We extracted total RNA from dorsal skin, liver tissue, and cells from mice fed a western diet using the TRIzol reagent according to manufacturer’s instructions (Invitrogen Canada, Burlington, ON, CA). 1 microgram of DNase-treated RNA was reverse-transcribed using Superscript II (Invitrogen Canada, Burlington, ON, Canada) to generate RNAse H-treated cDNA for real-time PCR using Power SYBR green PCR Master Mix (Applied Biosystems, Foster City, CA) in an ABI 7500 Fast Real-Time PCR System. We used Gapdh as the invariant control. mRNA levels in control mice were arbitrarily set at 1.

**Measurements of inflammatory molecules.** For whole skin ICAM-1 protein analysis, dorsal skin tissue was homogenized in PBS containing complete protease inhibitor (Roche Diagnostics, Laval, Quebec, CA) and stored at -20°C overnight. Supernatants were collected by centrifugation (2000g, 5min) and protein concentration was determined by the assay of Lowry et al. (3). Levels of murine inflammatory protein molecules in plasma and whole skin homogenates from mice fed a western diet were measured using commercial immunoassay kits (R&D Systems, Minneapolis, MN). The threshold of detection for these assays was 12, 29, 2, and 2pg/mL for interleukin-6 (IL-6), intercellular adhesion molecule (ICAM-1), monocyte chemoattractant protein 1 (MCP-1) and regulated upon activation, normal T
expressed and presumably secreted protein (RANTES), respectively. Levels of interleukin (IL)-1β and IL-12p70 in all mice, and levels of MCP-1 and RANTES in mice before commencement of western diet were measured using Milliplex multi-analyte profiling assays (Millipore, Billerica, MA) and quantified using a Luminex instrument.

**Apolipoprotein analysis.** Unfractionated plasma levels apoA-I and apoA-II in male and female mice fed western diet were determined by immunonephelometry with the use of mouse-specific antibodies developed in rabbits. Levels of murine SAA in plasma were measured using a commercial immunoassay kit (BioSource, Camarillo, CA), which had a threshold of detection of 270 ng/mL.

**Paraoxonase (PON1) activity.** After a period of 12 weeks on a western diet, blood was collected from female Scd1+/+Ldlr−/− mice and Scd1−/−Ldlr−/− mice (homozygous for the Scd1abJ allele) following a four-hour fast. Blood was allowed to clot for 1 h on ice and then serum was separated by centrifugation at 4°C. Serum was mixed with sucrose (final concentration 0.6%) as a cryoprotectant and frozen at -80°C under nitrogen for less than 3 months. Serum PON1 activity toward phenyl acetate (arylesterase activity) was determined photometrically in the presence of CaCl2 (1mM)(4), where one unit = 1 µmol phenylacetate hydrolyzed per min.

**Bone Marrow Transplantation.** Bone marrow transplantation (BMT) experiments were performed at the Gorlaeus laboratories of the Leiden/Amsterdam Center for Drug Research in Leiden, The Netherlands in accordance with the national laws. BMT protocols were approved by the Ethics Committee for Animal Experiments of Leiden University. To induce bone marrow aplasia, female Ldlr−/− recipient mice were exposed to a single dose of 9 Gy (0.19 Gy/min, 200 kV, 4 mA) total body irradiation using an Andrex Smart 225 Röntgen source (YXLON International) with a 6-mm aluminum filter 1 day before the transplantation. Bone marrow was isolated by flushing the femurs and tibias from female Scd1−/− and Scd1+/+ mice. Irradiated recipients received 0.5x10⁷ bone marrow cells by tail vein injection. Animals received a standard laboratory rodent chow diet or western diet (Diet W, Special
Diet Services, Witham, UK). The hematologic chimerism of the \( \text{Ldlr}^{-/-} \) mice was determined using genomic DNA from bone marrow by polymerase chain reaction (PCR) at 12 weeks after transplant.

**Macrophage functional studies.** After 5 days on a western diet, thioglycollate-elicited peritoneal macrophages obtained from \( \text{Scd1}^{-/-}\text{Ldlr}^{-/-} \) and \( \text{Scd1}^{+/+}\text{Ldlr}^{-/-} \) mice were counted and plated in 24-well plates at a density of 400,000 cells per well and the media was changed after 2h. After an additional 24h, the media was changed to fresh DMEM/10% FBS or DMEM/10% FBS containing 100ng/mL lipopolysaccharide (\( E. \text{coli} \) O113:H10; Associates of Cape Cod). After 6 h, RNA was isolated from at least two independent wells from each animal for each condition.

For cholesterol efflux studies, bone marrow-derived cells were labeled with 0.5 \( \mu \text{Ci/mL} [^{3}\text{H}]\)cholesterol in DMEM/0.2% bovine serum albumin for 24 hours. Cholesterol efflux was studied by incubation of the cells with DMEM/0.2% BSA alone or supplemented with 10 \( \mu \text{g/mL} \) apoA-I or 50 \( \mu \text{g/mL} \) human HDL. \( [^{3}\text{H}]\)Cholesterol released to HDL after 24h incubation was measured by liquid scintillation counting. Cholesterol efflux is expressed as the radiolabel released as a percentage of \( [^{3}\text{H}]\)cholesterol within cells before addition of acceptor.

**Statistical analysis.** Data are presented as means plus or minus standard error. Initial analyses were performed by the unpaired two-tailed Student's \( t \) test. Data that did not follow a normal distribution as judged by Kolmogorov-Smirnov tests were analyzed with the Mann-Whitney test for unpaired data. For cytokine data the minimum detectable limit was assigned to those values below the limit of detection and the Wilcoxon signed-rank test was used. Analyses of the cumulative frequency of dermatitis were performed by a two-sided log rank test. Statistical analysis was performed with GraphPad Prism software and with the open-source R-package (GraphPad, San Diego, CA; R Development Core Team, 2006 (5)). \( P < 0.05 \) was considered significant.
References
Supplemental Table I. Serum lipid levels in \( Ldlr^{-/-} \) mice transplanted with bone-marrow derived cells lacking SCD1.

<table>
<thead>
<tr>
<th></th>
<th>Chow</th>
<th>Western Diet</th>
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<tbody>
<tr>
<td></td>
<td>( Scd1^{+/+} \rightarrow Ldlr^{-/-} )</td>
<td>( Scd1^{+/+} \rightarrow Ldlr^{-/-} )</td>
</tr>
<tr>
<td>TC, mg/dL</td>
<td>285 ± 6 (12)</td>
<td>283 ± 12 (12)</td>
</tr>
<tr>
<td>CE, mg/dL</td>
<td>339 ± 8 (12)</td>
<td>335 ± 14 (12)</td>
</tr>
<tr>
<td>FC, mg/dL</td>
<td>84.3 ± 2.1 (12)</td>
<td>85.2 ± 3.9 (12)</td>
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<tr>
<td>PL, mg/dL</td>
<td>490 ± 33 (12)</td>
<td>531 ± 29 (12)</td>
</tr>
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</table>

TC, total cholesterol; CE, cholesterol esters; FC, free cholesterol; PL, phospholipids. Data represent mean ± SEM. The number of animals in each subgroup is indicated in parentheses.
Supplemental Fig I. Lesion area in Ldlr<sup>−/−</sup> mice lacking SCD1. Lesions in aortic roots of Scd1<sup>+/−</sup>Ldlr<sup>−/−</sup> (left) and Scd1<sup>−/−</sup>Ldlr<sup>−/−</sup> (right) mice carrying the Scd1<sup>ab-2j</sup> alleles were stained with oil red O to detect accumulation of lipids and photographed. Scale bar, 0.5 mm.
Supplemental Fig II. Lesion morphology in \( \text{Ldlr}^{-/-} \) mice lacking SCD1. For the assessment of lesion complexity, lesions in aortic roots of \( \text{Scd1}^{+/+}\text{Ldlr}^{-/-} \) (left) and \( \text{Scd1}^{-/-}\text{Ldlr}^{-/-} \) (right) mice fed a western diet for 12 weeks were stained with hematoxylin and eosin (H&E). Images of representative sections from the aortic root were captured at a magnification of 20× (a). Smooth muscle cell content (b) in mice fed a western diet for 12 weeks and macrophage content (c) in mice fed a western diet for 5 weeks was determined by immunohistochemical staining for α-actin and MOMA-2, respectively.
Supplemental Fig III. Skin of Ldlr<sup>−/−</sup> mice lacking SCD1. Skin sections of Scd1<sup>−/−</sup>Ldlr<sup>−/−</sup> mice (a) or Scd1<sup>−/−</sup>Ldlr<sup>−/−</sup> mice with dermatitis (b,c) were stained with hematoxylin and eosin (H&E) (a,b) or toluidine blue (c) to visualize mast cells (arrows). Stratum corneum (SC), epidermis (E), dermis (D), fat tissue(F), and ulceration (U).
Supplemental Fig IV. Inflammation in \( Ldlr^{-/-} \) mice lacking SCD1. Plasma cytokine concentrations were determined before commencement of western diet. Data are represented as proportion of the mean plasma cytokine concentration relative to that in \( Scd1^{+/+}Ldlr^{-/-} \) mice. \( n = 8 \) mice per group.