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 hyperlipemic 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 2 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 apoa-II and apoa-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;29:00-00.)

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

Stearoyl-CoA desaturase (SCD) is the rate-limiting enzyme in the synthesis of monounsaturated fatty acids. It creates a cis-double bond in the Δ9 position of palmitic (16:0) and stearic acid (18:0), thereby converting them to palmitoleic (16:1n7) and oleic acid (18:1n9). Oleic acid is the major fatty acid found in triglycerides (TG) and cholesterol esters (CE); likely because of its status as the preferred fatty acid substrate of acyl-CoA:cholesterol acyltransferase (ACAT), and the close proximity of SCD to diacylglycerol acyltransferase-2 in the endoplasmic reticulum (ER). 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.

In the absence of the Scd1 gene product LDLR-deficient mice have reduced hepatic steatosis (by ≈80%) and plasma TG (by ≈50%). Absence of SCD1 also provides striking protection from diet-induced weight gain and insulin resistance. A major unanswered question is whether the amelioration of these features of the metabolic syndrome 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 Scd1ab-2J or Scd1ab-J null alleles were back-crossed to C57BL/6 for 5 generations to produce N5 incipient congenic mice and then crossed to the B6.129S7-Ldlr−/−Her mutant strain.11 The Scd1+/− Ldlr−/− control groups consisted of both littermates of Scd1−/− Ldlr−/− mice and additional age- and sex-matched Scd1+/− Ldlr−/− mice that were not littermates (~63% of all animals studied). Mice deficient in SCD1 with the Scd1ab-J 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 Scd1ab-2J allele) were also studied. Sections of the aortic root were stained as described in Singaraga et al.12

Results

SCD1 Deficiency Increases Atherosclerosis in Ldlr−/− Mice

Mice with a spontaneous deletion in Scd1 (B6.ABJ/L-Scd1ab-J) were crossed with an existing dyslipidemic mouse model (B6.129S7-Ldlr−/−Her)11 to generate mice with combined deficiencies of both LDLR (Ldlr−/−) and SCD1 (Scd1−/−). After 12 weeks of an atherogenic Western diet,13 weights for male and female Scd1+/+ Ldlr−/− mice were 44% and 54% higher than initial values, respectively, whereas neither male nor female Scd1−/− Ldlr−/− mice showed a significant increase in body weight, as described elsewhere.7 Total plasma TG was reduced by 44% and 51%, and non-HDL cholesterol was reduced by 8% and 27% in male and female Scd1+/− Ldlr−/− mice, respectively, relative to Scd1+/− Ldlr−/− 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.7

Atherosclerotic lesion size was evaluated in multiple sections of the aortic root in this same cohort of Scd1−/− Ldlr−/− mice (males, n=6; females, n=10) and Scd1+/− Ldlr−/− control mice (males, n=11; females, n=11; Figure 1a and 1c). 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 Scd1ab-J 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-Scd1ab-2J).10 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 1c), 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+/+ Ldlr−/− mice (Figure 2a, 2b, and 2c). These findings were increased in the more advanced lesions of the Scd1−/− Ldlr−/− mice, with many extracellular cholesterol clefts 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+/+ Ldlr−/− and Scd1−/− Ldlr−/− mice (Figure 2d). The increased lesion size in Scd1−/− Ldlr−/− mice fed the Western diet for 12 weeks was characterized by greater absolute areas of macrophage infiltration in these animals versus Scd1+/+ Ldlr−/− controls. This macrophage infiltration was evident in both the large complex atheromatous lesions in the left coronary sinuses, as well as the smaller lesions of the right coronary and non-coronary sinuses. The majority of cells in early plaques were positive for monocyte/macrophage staining in both Scd1+/+ Ldlr−/− and Scd1−/− Ldlr−/− mice (Figure 2e).

Semiquantitative 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 clefts, 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−/− Ldlr−/− mice earned significantly higher lesion severity scores than Scd1+/+ Ldlr−/− controls (P=0.001; Figure 2f).

SCD1 Deficiency Promotes Inflammation in Ldlr−/− Mice

Our observation of increased atherosclerosis with SCD1 deficiency, despite favorable metabolic characteristics expected to reduce lipid accumulation, led us to investigate the cause of advanced atherosclerosis in this model. Prior derma-
ICAM-1 protein was increased more than 2-fold in mice fed a Western diet for 12 weeks were stained with Movat pentachrome to visualize extracellular matrix deposition and cellular components within the lesions. Representative sections include those from the aortic root at low (4x; a) and high-magnification (20x; b). Proteoglycan-rich, extracellular matrix thickening of the intima (sea-green color), necrotic cores (absence of purple/black nuclei, NC), and extracellular cholesterol clefts (needle-shaped lucencies; C) were observed. The absence of yellow stain indicates the lack of significant collagen deposition. Serial sections were also stained with hematoxylin and eosin (H&E; c). Smooth muscle cell content (d) in mice fed a Western diet for 12 weeks and macrophage content (e) in mice fed a Western diet for 5 weeks was determined by immunohistochromatic staining for α-actin and MOMA-2, respectively. f, Semiquantitative assessment of lesion severity (P = 0.001). n = 16 to 22 mice per group.

Figure 3. Evaluation of skin phenotype in Ldlr<sup>−/−</sup> mice lacking Scd1. a, Relative amount of mRNA encoding ICAM-1 was determined by quantitative RT-PCR in dorsal skin from mice fed a Western diet. Each value represents the amount of mRNA relative to that in Scd1<sup>+/+</sup>Ldlr<sup>/−</sup> mice (arbitrarily set at 1). n = 5 to 6 mice per group. b, ICAM-1 protein levels in whole skin homogenates were determined by immunoassay, n = 6 mice per group. c through e, Representative sections from the dorsal skin of Scd1<sup>−/−</sup>Ldlr<sup>/−</sup> mice (c) or Scd1<sup>−/−</sup>Ldlr<sup>/−</sup> mice with severe dermatitis (d and e) were stained with H&E (c and d) or toluidine blue (e) to visualize mast cells (arrows). Stratum corneum (SC), epidermis (E), dermis (D), fat tissue (F), and ulceration (U) are indicated. f, Severe dermatitis necessitated euthanasia during the course of the study in Scd1<sup>−/−</sup>Ldlr<sup>/−</sup> mice carrying the Scd1ab-J and Scd1ab-2J alleles but not Scd1<sup>−/−</sup>Ldlr<sup>/−</sup> mice (Scd1ab-J, P = 0.016; Scd1ab-2J, P = 0.023; 2-sided log rank test). n = 28 to 35 mice per group.
SCD1 Deficiency Alters HDL-Associated Proteins in Ldlr−/− Mice

We hypothesized that the chronic inflammation in SCD1-deficient mice may be associated with alterations in HDL-associated proteins, despite normal levels of HDL-cholesterol. 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, while enriched in serum amyloid A (SAA). 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 4b), whereas plasma apoA-I (females, \( \approx 37\% \) reduction, \( P = 0.0017 \); males, \( \approx 16\% \) reduction, \( P = 0.44 \); Figure 4c) and apoA-II are decreased (\( \approx 40\% \) reduction; females, \( P = 0.0007 \); males, \( P = 0.0003 \); Figure 4d). 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, are decreased nearly 75% (\( P = 0.001 \); Figure 5a), whereas mRNA levels of Clu, the gene that encodes apolipoprotein J, a protein that mediates the interaction of HDL with other proteins, 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 result from increased systemic inflammation or 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 from Scdl−/− Ldlr−/− and Scdl+/+ Ldlr−/− mice (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 bone marrow–derived cells is to evaluate the effect of SCD1 deficiency in bone marrow–derived cells on atherosclerosis of macrophages. Another way to examine whether the atherogenic effect of SCD1 deficiency results from a direct effect on SCD1 deficiency in bone marrow–derived cells is to evaluate the effect of SCD1 deficiency in bone marrow–derived cells on atherosclerosis.

We therefore evaluated the ability of bone marrow–derived macrophages from Scd1<sup>−/−</sup> mice to efflux cholesterol to apoA-1 and HDL acceptors. SCD1 deficiency had no effect on cholesterol efflux to 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. A 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, phenotypic components of the metabolic syndrome that have been linked to increased susceptibility to atherosclerosis. These surprising data suggested that SCD1-deficient mice must have some proatherogenic 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 and increased infiltration of macrophages and mast cells but only rare lymphocytes or neutrophils in the dermis. In addition, subcutaneous cyclosporin A can inhibit ICAM-1 expression and reduce mast cell numbers in the skin, restoring the wild-type skin phenotype. Histopathologic studies in these mice have demonstrated that the chronic inflammatory reaction is a foreign body response, with extreme sebaceous gland hypoplasia 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.

Inflammation is recognized to play a major role in all stages of atherogenesis, and plasma markers of systemic inflammation are predictive of cardiovascular events in humans. 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.

Moreover, chronic inflammatory diseases such as psoriasis, rheumatoid arthritis, periodontal disease, and systemic lupus erythematosus (SLE) are associated with in-
increased cardiovascular risk. The association between chronic inflammatory disease and cardiovascular disease is particularly pronounced for SLE, as women with SLE have an estimated 50-fold increased risk of myocardial infarction compared with age- and sex-matched controls. In the LDLR-deficient mouse model, used by several groups to study the link between chronic inflammation and atherosclerosis, 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.

Our observations of increased plasma IL-6, a marker of systemic inflammation that is associated with atherosclerosis, sICAM-1, an adhesion molecule that is elevated in serum of patients with inflammatory skin disorders, and IL-1β and IL-12p70, proinflammatory cytokines known to be elevated in psoriatic lesional skin, suggest that chronic inflammation of the skin may be contributing to the proatherogenic profile of SCD-1 deficient mice by creating an environment of chronic systemic inflammation. 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.

Our results show that absence of SCD1 has a proatherogenic effect on HDL composition. However, further studies are needed to fully explore the effect of SCD1 deficiency on HDL particle size, protein and lipid composition, as well as the effect on the function of these HDL particles. While we have not ruled out the possibility that the increased systemic inflammation with SCD1 deficiency is a consequence of increased atherosclerosis rather than its cause, alterations in multiple indicators of inflammation are consistent with a proatherogenic role for inflammation in the absence of murine SCD1.

Chen et al. have recently reported that SCD1 downregulation exacerbates the inflammatory response to acute proinflammatory stimuli. Although these authors evaluated dextran-sulfate sodium-induced colitis in SCD1-null mice, they attribute the accelerated response to reduced SCD1-mediated hepatic oleic acid biogenesis. We consider it unlikely that a reduction in hepatic SCD1 activity explains all the atherogenic effects on inflammatory markers in the SCD1-null model, however, because the phenotype of this model includes pronounced chronic dermal inflammation, a well-established contributor to systemic inflammation and cardiovascular disease in humans.

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 macrophages of atherosclerotic lesions.

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. 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, inflammatory markers, and potentially coronary heart disease, 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 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, as SCD1 deficiency is accompanied by a 50% reduction of HDL-cholesterol levels in the dyslipidemic Ldlr−/−Apob100/100 model, a change in HDL expected to be associated with increased atherosclerosis. However, ASO-mediated SCD1 deficiency in the CIH model is accompanied by an atheroprotective 20% increase in HDL-cholesterol levels. Our results indicate that in the absence of changes in HDL cholesterol levels, complete and chronic SCD1 deficiency is likely to exacerbate atherosclerosis.

These studies now provide strong support for the role of chronic inflammation in promoting atherosclerosis, even in the presence of antiatherogenic biochemical and metabolic characteristics.

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Disclosures

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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<sup>−/−</sup>Ldlr<sup>−/−</sup> and Scd1<sup>+/+</sup>Ldlr<sup>−/−</sup> 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 α-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 Scd1ab−/− allele) following a four-hour fast. Blood was allowed to clot for 1h 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 \( Ldlr^-/- \) 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 \( Scd1^-/-Ldlr^-/- \) and \( Scd1^+/-Ldlr^-/- \) 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. 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 \( \mu \)Ci/mL \([^3H]\)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 \( \mu \)g/mL apoA-I or 50 \( \mu \)g/mL human HDL. \([^3H]\)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 \([^{3}H]\)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>
<th>p</th>
<th>Chow</th>
<th>Western Diet</th>
<th>p</th>
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<tbody>
<tr>
<td></td>
<td>Scd1(^{+/-})→Ldlr(^{-/-})</td>
<td>Scd1(^{-/-})→Ldlr(^{-/-})</td>
<td></td>
<td>Scd1(^{+/-})→Ldlr(^{-/-})</td>
<td>Scd1(^{-/-})→Ldlr(^{-/-})</td>
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<tr>
<td><strong>TC, mg/dL</strong></td>
<td>285 ± 6 (12)</td>
<td>283 ± 12 (12)</td>
<td>0.92</td>
<td>1025 ± 55 (12)</td>
<td>997 ± 68 (12)</td>
<td>0.75</td>
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<tr>
<td><strong>CE, mg/dL</strong></td>
<td>339 ± 8 (12)</td>
<td>335 ± 14 (12)</td>
<td>0.81</td>
<td>1333 ± 73 (12)</td>
<td>1301 ± 90 (12)</td>
<td>0.79</td>
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<tr>
<td><strong>FC, mg/dL</strong></td>
<td>84.3 ± 2.1 (12)</td>
<td>85.2 ± 3.9 (12)</td>
<td>0.83</td>
<td>237 ± 13 (12)</td>
<td>227 ± 14 (12)</td>
<td>0.61</td>
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<tr>
<td><strong>PL, mg/dL</strong></td>
<td>490 ± 33 (12)</td>
<td>531 ± 29 (12)</td>
<td>0.37</td>
<td>668 ± 31 (12)</td>
<td>696 ± 23 (12)</td>
<td>0.47</td>
</tr>
</tbody>
</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 Ldlr<sup>−/−</sup> mice lacking SCD1. For the assessment of lesion complexity, lesions in aortic roots of Scd1<sup>+/+</sup>Ldlr<sup>−/−</sup> (left) and Scd1<sup>−/−</sup>Ldlr<sup>−/−</sup> (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× (<i>a</i>). Smooth muscle cell content (<i>b</i>) in mice fed a western diet for 12 weeks and macrophage content (<i>c</i>) 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<sup>−/−</sup> 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<sup>+/+</sup>Ldlr<sup>−/−</sup> mice. n = 8 mice per group.