Macrophage Elovl6 Deficiency Ameliorates Foam Cell Formation and Reduces Atherosclerosis in Low-Density Lipoprotein Receptor-Deficient Mice

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**Objective**—Elovl6, a long-chain fatty acid elongase, is a rate-limiting enzyme that elongates saturated and monounsaturated fatty acids and has been shown to be related to obesity-induced insulin resistance via modification of fatty acid composition. In this study, we investigated the roles of Elovl6 in foam cell formation in macrophages and atherosclerosis in mice.

**Methods and Results**—To investigate the roles of Elovl6 in macrophages in the progression of atherosclerosis, we transplanted bone marrow cells of wild-type or Elovl6−/− mice into irradiated LDL-R−/− mice that were fed a western diet. Aortic atherosclerotic lesion areas and infiltration of macrophages were significantly smaller in Elovl6−/− bone marrow cells-transplanted LDL-R−/− mice than in wild-type. Accumulation of esterified cholesterol on exposure to acetylated-LDL was less severe in peritoneal macrophages from Elovl6−/− mice than those from wild-type. Cholesterol efflux and expression of cholesterol efflux transporters were increased in Elovl6−/− macrophages, although no difference in uptake of acetylated-LDL was found between the two groups. On analysis of fatty acid composition of the esterified cholesterol fraction in macrophages, n-6 polyunsaturated fatty acids were decreased by absence of Elovl6.

**Conclusion**—These findings suggest that Elovl6 in macrophages may contribute to foam cell formation and progression of atherosclerosis. (Arterioscler Thromb Vasc Biol. 2011;31:1973-1979.)

**Key Words:** Elovl6 | atherosclerosis | macrophage | fatty acid composition

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Elongation of very long-chain fatty acids family member 6 (Elovl6, also known as LCE, FACE, and MASR) belongs to a highly conserved family of endoplasmic reticulum enzymes that consists of at least 7 fatty acid elongases in mice and humans. Elovl6 specifically catalyzes the elongation of saturated fatty acids and monounsaturated fatty acids (MUFA) with 12, 14, and 16 carbons, thereby converting palmitate (C16:0) and palmitoleate (C16:1n-7) to stearate (C18:0) and vaccenate (C18:1n-7). These fatty acids are important constituents of triglycerides, esterified cholesterol, and phospholipids.

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Elovl6 is expressed in essentially all tissues, though high levels of it are found in the adrenal gland, liver, white adipose tissue, brain, testis, and skin, where lipogenesis and steroidogenesis are active.1-3 Expression of Elovl6 is directly regulated by sterol regulatory element-binding protein-1a, -1c, and -2, as well as other lipogenic enzymes such as fatty acid synthase (FAS) and stearoyl coenzyme A desaturase (SCD)-1,2,4 Dietary n-3 polyunsaturated fatty acids (PUFA), such as eicosapentaenoate and docosahexaenoate acid, cause profound suppression of Elovl6 expression.3,7 Absence of Elovl6 increases levels of palmitate and palmitoleate but reduces levels of stearate and oleate (C18:1n-9), and it provides protection from obesity-induced hyperinsulinemia, hyperglycemia, and hyperleptinemia despite the development of obesity and hepatosteatosis.8 Inhibition of Elovl6 is thus a potential therapeutic target for ameliorating insulin resistance, diabetes, and cardiovascular risk. A major unanswered question is whether inhibition of this elongase will lead to reduced susceptibility to atherosclerosis.

The differentiation of macrophages into lipid-laden foam cells is a critical event in the early stages of atherosclerosis. Traditionally, it has been assumed that the uptake of oxidized LDL by macrophages and the subsequent accumulation of esterified cholesterol formed via catalysis by acyl-coenzyme A:cholesterol acyltransferase (ACAT) is largely responsible for this process. Although free cholesterol, a substrate for ACAT, is supplied from the intracellular cholesterol pool,
little is known concerning the source and pathways involved in the supply of fatty acids as precursors for fatty acyl-CoA, another substrate of ACAT. Oleate, linoleate (C18:2n-6), palmitate, and palmitoleate were identified as the major fatty acids found in triglycerides and esterified cholesterol fractions of human fatty streak lesions. In particular, because oleate is the preferred fatty acid substrate of ACAT, we considered the possibility whether inactivation of Elovl6 in macrophages affects foam cell formation and the development of atherosclerosis.

To test whether Elovl6 in macrophages modulates susceptibility to atherosclerosis, we performed bone marrow (BM) transplantation from Elovl6 deficient mice into LDL-R−/− deficient mice. Our findings demonstrated that the absence of Elovl6 in macrophages increased cholesterol efflux, and that this was associated with significant suppression of atherosclerosis in LDL-R−/− deficient mice.

### Materials and Methods

#### Animal Procedures

Elovl6 knockout mice (Elovl6−/− mice, C57BL/6 background) and low-density lipoprotein receptor knockout mice (LDL-R−/− mice, C57BL/6 background) prepared as described previously were used in this study. The Elovl6−/− mice were crossed with the LDL-R−/− mice to produce LDL-R−/−/Elovl6−/− mice. Animals received a standard laboratory rodent chow diet or a western diet containing 21% (w/w) fat and 0.15% (w/w) cholesterol (D12079B; Research Diets Inc., NJ). All animal husbandry and animal experiments were consistent with the University of Tsukuba’s Regulations of Animal Experiments and were approved by the Animal Experiment Committee of the University of Tsukuba.

#### BM Transplantation

Donor BM cells were extracted from the tibias and femurs of male wild-type or Elovl6−/− mice with RPMI1640 medium containing 10 U/mL heparin and 2% fetal bovine serum. Male LDL-R−/− mice were lethally irradiated with a single dose of 900 rad using a MBR-1520R (Hitachi Medical Corporation, Tokyo, Japan). After irradiation, LDL-R−/− recipient mice were injected with 5×10^5 BM cells via the tail vein. Recipient mice were provided with neomycin- and polymyxin- (Wako Pure Chemicals, Osaka, Japan) supplemented water for 1 week before and 4 weeks after transplantation. Mice were fed a regular chow diet for the first 4 weeks after BM transplantation and then switched to a western diet for 16 weeks.

The hematologic chimerism of LDL-R−/− mice was determined in genomic DNA from blood by polymerase chain reaction (PCR) at 16 weeks post-transplantation. The primers used to detect the Elovl6 gene were described previously.

#### Plasma Analysis

Plasma samples were collected via orbital bleeding in irradiated LDL-R−/− recipient mice transplanted with BM cells isolated from wild-type or Elovl6−/− mice. Plasma lipids (total cholesterol, triglycerides, free fatty acids, and phospholipids) and glucose levels were measured using commercially available kits (Wako Pure Chemicals). In addition, plasma hematologic parameters (hemoglobin, red blood cells, and white blood cells) were measured in other irradiated LDL-R−/− recipient mice.

#### Measurement of Atherosclerotic Lesions

Atherosclerotic lesions were analyzed as previously described. In brief, mice were euthanized and their hearts and aortas were isolated. The degree of atherosclerosis was assessed by determining lesion sizes on both pinned-open aortas and serial cross-sections through the aortic root. The aorta was opened longitudinally along the ventral midline from the iliac arteries to the aortic root. After branching vessels were removed, the aorta was pinned out flat on a black rubber board. The lesions were stained with Sudan IV for 15 minutes, destained with 70% ethanol, and then fixed in 4% phosphate-buffered formalin. Aortic images were analyzed with Adobe Photoshop 7 software (Adobe Systems Incorporated, CA). Values are the percentage of the aortic surface covered by lesions. The hearts were perfused with 10% formalin and were fixed. The basal half of the hearts was embedded in Tissue-Tek OCT compound (Sakura Finetek Japan Co., Tokyo, Japan), and serial sections were captured using a Cryostat microtome. The sections were stained with Oil-Red O (0.3% in 60% isopropl alcohol) and counterstained with hematoxylin.

#### Immunohistochemical Analyses of Atherosclerotic Lesions

To detect macrophage infiltration in atherosclerotic lesions, we first incubated sections with primary antibody to mouse macrophage marker F4/80 (1:20; Serotec, Raleigh, NC) for 2 hours. After washes, the sections were incubated with biotinylated anti-rat antibody for 30 minutes at room temperature and then with avidin-biotin peroxidase complex (Vector Laboratories Inc., CA) for 30 minutes. Finally, the sections were developed with DAB (Wako Pure Chemicals) and counterstained with methylgreen.

#### Foam Cell Formation Assays

Mouse peritoneal macrophages (MPM) were incubated with 100 µg/mL acetylated-LDL (aLDL) (Biomedical Technologies Inc., Stoughton, USA) in DMEM medium containing 0.2% fatty acid-free bovine serum albumin (Sigma-Aldrich Japan, Tokyo, Japan) for 48 hours. Cells were extensively washed, fixed with 10% formalin, and stained with Oil-Red O. Cellular lipids were extracted with hexane/isopropyl alcohol on exposure to aLDL for 24 or 48 hours, and cellular cholesterol contents (total cholesterol, esterified cholesterol, and free cholesterol) were measured as previously described. For pretreatment of long chain unsaturated fatty acids, MPM were treated with medium alone, 50 µmol/L oleate (C18:1 n-9), or linoleate (C18:2 n-6) (Sigma-Aldrich Japan) in DMEM medium containing 0.2% bovine serum albumin for 24 hours before incubation with aLDL.

#### Uptake of 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine (Dil)-aLDL

Fluorescent Dil-labeled aLDL was prepared according to a method described elsewhere. MPM were incubated with 100 µg/mL aLDL for 3 hours at 37°C. After the cells were washed, Dil was extracted with isopropyl alcohol, and relative fluorescence intensity was determined at 524 nm (excitation) and at 567 nm (emission). Experiments were performed 3 times, and representative values are shown as means ± SEM.

#### Cholesterol Efflux Measurements

MPM were loaded with 1.2 µCi/mL (1,2,3,4-3H)-cholesterol and 100 µg/mL aLDL in DMEM medium containing 0.2% bovine serum albumin for 24 hours at 37°C. Cells were then incubated with DMEM medium containing 0.2% bovine serum albumin supplemented or not with 50 µg/mL of high-density lipoprotein (HDLL, Biomedical Technologies Inc.). Medium and cell lysates were collected after 48 hours incubation and radioactivity was determined by liquid scintillation counting. Cholesterol efflux was expressed as...
the percentage of radioactivity released into the medium relative to total radioactivity.

Quantitative Real-Time PCR

Total RNA was isolated using Sepasol Reagent (Nacalai Tesque, Kyoto, Japan), and was reverse-transcribed using the ThermoScript RT-PCR System (Applied Biosystems Japan, Tokyo, Japan). Quantitative real-time PCR was performed using SYBR Green Dye (Applied Biosystems Japan) in an ABI Prism 7300 PCR instrument (Applied Biosystems Japan) as previously described. Expression levels were normalized to GAPDH expression. The primers in this study were shown in Supplemental Table I, available online at http://atvb.ahajournals.org.

Fatty Acid Composition of Esterified Cholesterol Fraction in MPM

Total lipids in acLDL-treated MPM were extracted according to Bligh-Dyer’s procedure and esterified cholesterol was separated on 500 mg silica columns (Discovery DSC-NH2, Sigma-Aldrich Japan) exactly as described. After saponification, the fatty acids in each sample were methyl-esterified and the relative abundance of each fatty acid was quantified by gas chromatography.

Statistics

All values are expressed as the mean ± SEM. The significance of differences between means was determined using Student t test with SAS software (SAS Institute Inc, NC).

Results

Deficiency of Elovl6 in Macrophages Improves Development of Atherosclerosis in LDL-R−/− Mice

To assess the biological roles of Elovl6 in macrophages in the development of atherosclerotic lesions in vivo, irradiated LDL-R−/− mice, which represented an established animal model for the development of atherosclerosis, were transplanted with BM cells isolated from wild-type or Elovl6−/− mice. Successful reconstitution of recipients with cells of donor origin after BM transplantation was confirmed by PCR-assisted amplification of the Elovl6 gene (Supplemental Figure I). To induce atherosclerotic lesion development, we fed these transplanted mice a western diet for 16 weeks, and the degree of atherosclerosis was determined either by quantifying Sudanophilic surface lesions in pinned-out whole aortas or by quantifying Oil-Red O-stained lesions in cross-sections from the aortic root. Deficiency of Elovl6 in hematologic cells did not alter body weight, serum lipid levels, glucose levels, or hematologic parameters (Supplemental Tables II and III). Atherosclerotic lesions emerged in both groups with the western diet regimen for 16 weeks. En face analysis demonstrated that aortic surface lesion areas in LDL-R−/− mice transplanted with BM cells isolated from Elovl6−/− mice were significantly smaller than in those transplanted with BM cells isolated from wild-type mice (42% decrease, P<0.05) (Figure 1A and 1B). Consistent with aortic surface lesion area, the decrease in cross-sectional lesion area in LDL-R−/− mice transplanted with BM cells isolated from Elovl6−/− mice was also significant on quantification of Oil Red O-stained aortic root sections (32% decrease, P<0.05) (Figure 1C and 1D). In addition, we quantified macrophage contents in cross-sections from the aortic root by immunohistochemical analysis using F4/80 as a macrophage marker. Similarly, macrophage-infiltrated areas in aortic roots were decreased in LDL-R−/− mice transplanted with BM cells isolated from Elovl6−/− mice compared with those transplanted with BM cells isolated from wild-type mice (27% decrease, P<0.05) (Figure 1E and 1F). Taken together, these findings indicate that BM transplantation from Elovl6−/− mice attenuated the development of atherosclerotic lesions.
Elov6 Deficiency in MPM Suppresses Lipid Accumulation on Exposure to AcLDL

To examine whether macrophage Elov6 deficiency had an impact on foam cell formation, MPM isolated from wild-type or Elov6−/− mice were incubated with acLDL. We also detected lipid accumulation in these cells by Oil-Red O staining or by extraction of cholesterol. Deficiency of Elov6 was confirmed by expression of Elov6 mRNA (Supplemental Figure II). As shown by Oil-Red O staining, cellular lipid accumulation was obviously lower in Elov6−/− MPM than wild-type MPM (Figure 2A). With acLDL loading, cellular total cholesterol contents in MPM increased in time-dependent fashion. The increase in cellular total cholesterol content was significantly lower in Elov6−/− MPM than in wild-type (Figure 2B). Cellular esterified cholesterol accumulation in Elov6−/− MPM was suppressed by 45% at 48 hours after acLDL addition, whereas cellular-free cholesterol content was not affected by genotype (Figure 2C and 2D). Thus, the differences in cellular total cholesterol content between both wild-type and Elov6−/− macrophages mainly resulted from changes in esterified cholesterol content.

In addition, to examine whether responses of wild-type and Elov6−/− MPM were same as those of LDL-R−/− and LDL-R−/−/Elov6−/− MPM, we detected lipid accumulation in MPM incubated with acLDL. In LDL-R−/− background MPM, deficiency of Elov6 similarly suppressed accumulation of cellular total and esterified cholesterol (Supplemental Figure IIIA and IIIB). These findings suggested that the reduction of lipid accumulation by Elov6 deficiency in MPM was not affected by LDL-R deficiency.

Cholesterol Influx into MPM

To study the impact of Elov6 on cholesterol influx into MPM, we examined the expression profiles of lipid transporters involved in cholesterol influx. The expression of genes encoding CD36, scavenger receptor-A, or LDL-R was not affected by Elov6 deficiency in acLDL-un-treated condition (Figure 3A). In agreement with this observation, the uptakes of Dil-labeled acLDL by MPM were similar for the 2 genotypes (Figure 3B).

Cholesterol Efflux from MPM

Cholesterol efflux from foam cells preloaded with radiolabeled cholesterol and acLDL was evaluated. In contrast to cholesterol influx, cholesterol efflux from foam cells was significantly increased in Elov6−/− MPM compared with wild-type MPM, and these effects were more pronounced in the presence of HDL in medium (Figure 4A). Furthermore, we investigated the expression profiles of lipid transporters involved in cholesterol efflux after incubation with or without acLDL. The expressions of ATP-binding cassette, subfamily A, member 1 (ABCA1), ATP-binding cassette, subfamily G, member 1 (ABCG1), and scavenger receptor-B1, which are involved in cholesterol efflux from macrophages as components of reverse cholesterol transport, were markedly increased in Elov6−/− MPM compared with wild-type MPM after incubation with acLDL (Figure 4B). Taken together, these results suggested that macrophage Elov6 deficiency suppressed foam cell formation due to increase in cholesterol efflux.

Fatty Acid Composition of Esterified Cholesterol Fraction in MPM

To estimate the impact of Elov6 deficiency on fatty acid composition in MPM, the esterified cholesterol fraction was separated and the various fatty acid contents were measured.
To determine whether exogenous long-chain unsaturated fatty acids influence cholesterol accumulation in MPM, oleate (C18:1 n-9) and linoleate (C18:2 n-6) were administered before incubation with acLDL. Pretreatment with oleate and linoleate normalized the reduction of cellular cholesterol accumulation in Elovl6−/− MPM (Figure 5A). These findings suggested that the reduction of long-chain unsaturated fatty acids by Elovl6 deficiency played an unknown role in the decrease in foam cell formation.

Elovl6 Deficiency in BM Cells and MPM Did Not Alter Inflammatory Cytokines, Chemokines, and Adhesion Molecules

We investigated the expression of genes encoding inflammatory cytokines, chemokines, and adhesion molecules in the aorta of LDL-R−/− mice transplanted with BM cells isolated from wild-type or Elovl6−/−, which were fed a western diet. The expressions of genes encoding inflammatory cytokines (IL-1β, TNF-α, IL-6), chemokines (MCP1, CCR2), and
adhesion molecules (VCAM, ICAM) were almost not affected by Elovl6 deficiency in BM cells (Supplemental Figure VA). In agreement with these results, the expression of genes encoding inflammatory cytokines and chemokines was similar in Elovl6−/− MPM and wild-type MPM with incubation with aCDL (Supplemental Figure VB). Taken together, these findings suggested that the reduction of atherosclerosis by Elovl6 deficiency was due to some other mechanism and was not involved in inflammatory responses, chemotaxis, or adhesion molecule function as modulated by cholesterol loading.

Discussion

Accumulation of esterified cholesterol is a characteristic of macrophage foam cells, which are central to the development of atherosclerotic plaque.16,17 In the process of foam cell formation, cholesterol incorporated from modified LDL and taken up was re-esterified mainly by endogenous fatty acyl-CoA rather than exogenous fatty acids from modified LDL.9,18–20 It can be speculated that the fatty acids themselves and their regulation of fatty acid homeostasis play important roles in foam cell formation in addition to regulation of cholesterol content. One of the means of regulation of intracellular fatty acid levels and compositions is fatty acid biosynthesis by lipogenic enzymes, such as FAS, Elovl6, and SCD-1. FAS generates palmitate (C16:0),21 Elovl6 elongates saturated fatty acids and MUFA with C12–16, and SCD-1 synthesizes MUFA, mainly palmiloleate (C16:1 n-7) and oleate (C18:1 n-9).22 We found that expression of these enzymes tended to be increased in the aorta of atherosclerotic mice, though there were no significant differences (Supplemental Figure VI). The lipogenic enzymes in vessels and macrophages could be involved in the development of atherosclerosis. The roles of macrophage SCD-1 and FAS in atherosclerosis have been already reported. Macrophage SCD-1 deficiency did not alter atherosclerotic lesion sizes in LDL-R−/− mice and had no effect on cholesterol efflux from macrophages.23 In contrast, macrophage FAS deficiency decreased atherosclerotic lesions in apoE-deficient mice.24 In the present study, Elovl6-deficient hematopoietic cells derived from BM in LDL-R−/− mice ameliorated lipid accumulation on the aorta and the infiltration of macrophages, without differences in serum lipid parameters, aortic inflammatory cytokines, chemokines, or adhesion molecules from wild-type. These findings imply that the suppression of de novo fatty acid biosynthesis or change in long-chain fatty acid composition affects atherosclerosis.

The findings of the present study of macrophages from Elovl6−/− mice imply roles of Elovl6 in foam cell formation. It was reported that foam cell formation in FAS-deficient macrophages was diminished due to both increased cholesterol efflux and decreased uptake of oxidized LDL.24 In contrast, Elovl6 deficiency in MPM also suppressed the cellular accumulation of esterified cholesterol after incubation of aCDL, although uptake of aCDL as well as expression of related transporters did not change and expression of FAS was enhanced, suggesting that amelioration of foam cell formation by Elovl6 deficiency might proceed via mechanisms different from those in the case of FAS deficiency.

Therefore, suppression could be at the level of esterification. Because oleate, the major esterified cholesterol in foam cells, is a better substrate for ACAT than palmitate and miristate, inhibition of conversion from palmitate to stearate resulting in decreased endogenous MUFA and increased endogenous saturated fatty acids could lead to reduction in esterified cholesterol. The enhancement of expression of lipogenic enzymes in Elovl6−/− MPM might be compensated for the unbalanced fatty acid composition. Furthermore, in Elovl6−/− MPM, n-6 PUFA levels were decreased in the esterified cholesterol fraction, although in FAS-deficient MPM, levels of palmitate and stearate, which are products of the FAS reaction, and oleate were decreased and major PUFA levels were not changed.24 In foam cells, cholesteryl linoleate and cholesteryl arachidonate are along with cholesteryl oleate also major components of esterified cholesterol.9 One possible reason for amelioration of foam cell formation by Elovl6 deficiency is the suppression of esterification of cholesterol by decrease in n-6 PUFA levels. Although the causes of the decreases in n-6 PUFA in the esterified cholesterol fraction in Elovl6-deficient MPM are currently unclear, pretreatment with MUFA and n-6 PUFA compensated for the reduction of cellular cholesterol content in Elovl6−/− macrophages. The present findings suggest that the intracellular fatty acid composition that Elovl6 regulates is a novel determinant of the amount of esterified cholesterol.

Another new finding of the present study is activation of cholesterol efflux from Elovl6-deficient foam cells. Reduction of esterified cholesterol accompanied increased free cholesterol, which could lead to enhanced cholesterol efflux from foam cells. Increased free cholesterol was associated with activated gene expression of LXRα target genes such as ABCA1, ABCG1, and scavenger receptor-B1.25,26 The enhancement of expression of these lipid transporters in Elovl6−/− MPM might be related to LXRα activation via increases in cellular cholesterol content after aCDL addition.25,26 Though we did not detect increase in free cholesterol in Elovl6−/− MPM, cellular cholesterol levels might be increased with shorter incubation times. In addition, it is known that unsaturated fatty acids inhibit cholesterol efflux from macrophages by increasing degradation of ABCA1.27,28 Decrease in MUFA and PUFA may increase cholesterol efflux via increases in ABCA1. In contrast, cholesterol uptake in Elovl6−/− MPM was not altered, unlike the suppression of cholesterol uptake in FAS-deficient MPM. The main transporter involved in cholesterol influx, CD36, is a peroxisome proliferator-activated receptor-α target gene,29 which is activated by various fatty acids such as palmitate. In FAS-deficient MPM, decrease in palmitate levels in addition to those of oleate was observed, suggesting greater suppression in peroxisome proliferator-activated receptor-α regulation. It may be that Elovl6 deficiency is directly linked to the cholesterol efflux genes by an unknown mechanism.

In conclusion, we showed that Elovl6 plays a crucial role in the development of both foam cells and atherosclerotic lesions. These findings increase understanding of the pathophysiology of atherosclerosis and provide further evidence of the importance of fatty acid quality in the development of atherosclerosis.
Acknowledgments

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Disclosures

None.

References

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Supplemental Material

Quantitative Real-Time PCR

Quantitative Real-Time PCR was performed as same as Materials and Methods. Expression levels in the aorta were normalized to peptidylprolyl isomerase B (Cyclophilin) expression. The primers used were as follows: FAS forward 5'- ATCCTGGAACGAGAACGCATCT -3', reverse 5'- AGAGACGTGCTACTCTGGAGCT -3'; SCD-1 forward 5'- AGATCTCCAGTCTTACGACCAC -3', reverse 5'- CTTTCATTCCAGGACGGATGCTCT -3'; acetyl-Coenzyme A carboxylase (ACC-1) forward 5'- GGGCACAGACCGTGAGTT -3', reverse 5'- CAGGATCAGCTGGGATACGTG -3'; interleukin 1 beta (IL-1β) forward 5'- AGTTGACGAGCCCCAAAGAT -3', reverse 5'- GGAGACGCAAGCCAGAAGG -3'; tumor necrosis factor α (TNF-α) forward 5'- TCGTAGCAAAACCACCAAGT -3', reverse 5'- AGATGCAAATCGGCTGACG -3'; IL-6 forward 5'- TAGTCCCTCTACTCCCCCAAATTCC -3', reverse 5'- TTGGTCTCTAGCCACTCTCTC -3'; monocyte chemotactic protein-1 (MCP1) forward 5'- CCCACTCAGCTGTCTAGCTACT -3', reverse 5'- ATTTGTTCCCCAGATCCGGT -3'; chemokine receptor 2 (CCR2) forward 5'- TGGCTGTGGTTCCTGCTTCTCA -3', reverse 5'- CCTACAGCGAACAGGGTGT -3'; vascular cell adhesion molecule (VCAM) forward 5'- GTGAAGATGGTGGGAGTTGT -3', reverse 5'- GGGCATGGAGTCACCGATT -3'; intercellular adhesion molecule (ICAM) forward 5'- CCGCAGGTCCAATTCACACT -3', reverse 5'- TCCAGCGAGGACCACATACAG -3'; Cyclophilin forward 5'- TGGGTCACAGTTCTTCCATAACCA-3', reverse 5'- ATGACATCCTTCCAGTGTTGTC -3'. 
**Supplemental Table I.** Primer sequence used for Real-Time PCR

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<th>Gene Name</th>
<th>Forward Primers</th>
<th>Reverse Primers</th>
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<td>Elovl6</td>
<td>ACAATGGACCTGTCAGCAA</td>
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<td>CD36</td>
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<td>GAPDH</td>
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**Supplemental Table II.** Body weight, plasma lipids, and plasma glucose levels of LDL-R⁻/⁻ mice transplanted with bone marrow cells isolated from wild-type or Elovl6⁻/⁻ mice after 16 weeks of western diet. Values are means ± SEM (n=11-12 per group). TG, triglycerides; FFA, free fatty acids; PL, phospholipids

<table>
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<tr>
<th>Recipient</th>
<th>Donor</th>
<th>Body Weight (g)</th>
<th>Total Cholesterol (mg/dL)</th>
<th>TG (mg/dL)</th>
<th>FFA (mEq/L)</th>
<th>PL (mg/dL)</th>
<th>Glucose (mg/dL)</th>
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<tr>
<td>Wild-type</td>
<td>LDL-R⁻/⁻</td>
<td>27.5 ± 1.0</td>
<td>1262 ± 85</td>
<td>857 ± 66</td>
<td>3.35 ± 0.29</td>
<td>869 ± 47</td>
<td>267 ± 14</td>
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<tr>
<td>LDL-R⁻/⁻</td>
<td>Elovl6⁻/⁻</td>
<td>27.0 ± 0.7</td>
<td>1228 ± 104</td>
<td>766 ± 61</td>
<td>2.80 ± 0.19</td>
<td>819 ± 58</td>
<td>262 ± 10</td>
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**Supplemental Table III.** Hematological parameters (Hb, RBC, and WBC) of LDL-R<sup>−/−</sup> mice transplanted with bone marrow cells isolated from wild-type or Elovl6<sup>−/−</sup> mice after 8 weeks of western diet. Values are means ± SEM (n=3 per group). Hb, hemoglobin; RBC, red blood cell; WBC, white blood cell

<table>
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<th>Recipient</th>
<th>Donor</th>
<th>Hb (g/dL)</th>
<th>RBC (×10000 cells/μL)</th>
<th>WBC (cells/μL)</th>
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<td>LDL-R&lt;sup&gt;−/−&lt;/sup&gt;</td>
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<td>13.5 ± 0.3</td>
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<td>Elovl6&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>12.0 ± 0.8</td>
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</tbody>
</table>
**Supplemental Figure I.** PCR genotyping of blood-derived DNA or tail-derived DNA from wild-type mouse (lane 1), Elovl6<sup>−/−</sup> mouse (lane 2), and LDL-R<sup>−/−</sup> mice transplanted with bone marrow cells isolated from wild-type (lanes 3-5) or Elovl6<sup>−/−</sup> (lanes 6,7).
Supplemental Figure II. Expression of Elovl6 mRNA in peritoneal macrophages before incubation with acLDL. Values are means ± SEM (n=3 dishes per group). ** p < 0.01 versus the wild-type.
Supplemental Figure III. Foam cell formation in peritoneal macrophages isolated from wild-type, Elovl6−/−, LDL-R−/−, or LDL-R−/− / Elovl6−/− mice. Cellular levels of total cholesterol (A) and esterified cholesterol (B) in macrophages incubated with or without acLDL (100 μg/mL) for 48 hr. Values are means ± SEM (n=3-4 per group). * p < 0.05, ** p < 0.01 versus the respective wild-type or LDL-R−/−.
Supplemental Figure IV. Expression of lipogenic enzymes mRNA in peritoneal macrophages isolated from wild-type or Elovl6$^{-/-}$ mice. Macrophages were incubated with acLDL (100 μg/mL) for 48 hr. Values are means ± SEM (n=6 per group). * $p < 0.05$ versus the respective wild-type.
Supplemental Figure V. (A) Expression of inflammatory cytokines, chemokines, and adhesion molecules mRNA in aorta of LDL-R⁻/⁻ mice transplanted with bone marrow cells isolated from wild-type or Elovl6⁻/⁻ mice, which were fed a western diet for 16 weeks. Values are means ± SEM (n=7-10 per group). * p < 0.05 versus the respective wild-type. (B) Expression of inflammatory cytokines and chemokines mRNA in peritoneal macrophages isolated from wild-type or Elovl6⁻/⁻ mice incubated with acLDL (100 μg/mL) for 48 hr. Values are means ± SEM (n=3 per group).
**Supplemental Figure VI.** Expression of Elovl6, SCD-1, and FAS mRNA in aorta of LDL-R⁻/⁻ mice, which were fed a normal diet or a western diet. Values are means ± SEM (n=5-6 per group).

![Graph showing relative mRNA expression of Elovl6, SCD-1, and FAS in LDL-R⁻/⁻ mice fed normal or western diet.](image-url)