Increased Hepatic Expression of Endothelial Lipase Inhibits Cholesterol Diet–Induced Hypercholesterolemia and Atherosclerosis in Transgenic Rabbits

Chuan Wang,* Kazutoshi Nishijima,* Shuji Kitajima, Manabu Niimi, Haizhao Yan, Yajie Chen, Bo Ning, Fumikazu Matsuhisa, Enqi Liu, Jifeng Zhang, Y. Eugene Chen, Jianglin Fan

Objective—Endothelial lipase (EL) is a key determinant in plasma high-density lipoprotein-cholesterol. However, functional roles of EL on the development of atherosclerosis have not been clarified. We investigated whether hepatic expression of EL affects plasma lipoprotein metabolism and cholesterol diet–induced atherosclerosis.

Approach and Results—We generated transgenic (Tg) rabbits expressing the human EL gene in the liver and then examined the effects of EL expression on plasma lipids and lipoproteins and compared the susceptibility of Tg rabbits with cholesterol diet–induced atherosclerosis with non-Tg littermates. On a chow diet, hepatic expression of human EL in Tg rabbits led to remarkable reductions in plasma levels of total cholesterol, phospholipids, and high-density lipoprotein-cholesterol compared with non-Tg controls. On a cholesterol-rich diet for 16 weeks, Tg rabbits exhibited significantly lower hypercholesterolemia and less atherosclerosis than non-Tg littermates. In Tg rabbits, gross lesion area of aortic atherosclerosis was reduced by 52%, and the lesions were characterized by fewer macrophages and smooth muscle cells compared with non-Tg littermates.

Conclusions—Increased hepatic expression of EL attenuates cholesterol diet–induced hypercholesterolemia and protects against atherosclerosis.

Visual Overview—An online visual overview is available for this article. (Arterioscler Thromb Vasc Biol. 2017;37:1282-1289. DOI: 10.1161/ATVBHA.117.309139.)

Key Words: atherosclerosis ■ hypercholesterolemia ■ lipoprotein ■ rabbits ■ transgenes
remains unanswered whether targeting EL will be beneficial to patients with cardiovascular disease. EL has multiple functions in lipoprotein metabolism in humans.23,24 Given the known differences in lipoprotein metabolism between mice and humans, it is necessary to study EL pathophysiologic functions using animals that have lipoprotein metabolism features similar to humans. To this end, we generated Tg rabbits expressing human EL in the liver. The rationale of using rabbits for this undertaking was that rabbits have been widely used for the study of human lipid metabolism and atherosclerosis.25 Like humans, but unlike mice, rabbits have abundant plasma cholesteryl ester transfer protein activity, an important regulator of cholesterol metabolism, and they are sensitive to a cholesterol diet and develop atherosclerosis rapidly because of hepatic apoB100 and intestinal apoB48 synthesis.25 Our studies revealed that hepatic expression of human EL protects against cholesterol diet–induced atherosclerosis in Tg rabbits.

Materials and Methods
Materials and Methods are available in the online-only Data Supplement.

Results
Generation of Human EL Tg Rabbits
In a total, we implanted 770 microinjected embryos into 29 surrogates and obtained 18 pups. Among them, 2 pups were found to have the human EL (hEL) transgene by Southern blotting. Tg founder (E18) showed detectable hEL expression (see below) and bred to generate F1 progeny for the current study. Tg rabbits showed no apparent abnormalities in terms of body weight and other organs. Tg hEL mRNA was expressed in the liver of Tg rabbits confirmed by real-time reverse transcription polymerase chain reaction (data not shown). Western blotting analysis revealed that hEL proteins were detected in both pre- and post-heparin plasma along with the liver. EL proteins in pre- and post-heparin plasma were ≈40 kDa in size, similar to the N-terminal fragment of EL,26 whereas in the liver, there were 2 bands of EL proteins presumably representing full-length (≈68 kDa) EL and N-terminal EL (≈40 kDa; Figure 1A). The latter may be those of cleaved dimeric forms of EL.26 Using hEL-specific ELISA kits, we further measured plasma levels of hEL and found that in pre-heparin plasma, hEL levels in Tg rabbits were 987.7±355.7 pg/mL in male Tg and 1670.3±469.4 pg/mL in female Tg rabbits (Figure 1B), which is similar to normal human plasma levels of EL mass.27

In human pre-heparin plasma, EL mass ranged up to 1387.7 pg/mL; therefore, Tg hEL levels in Tg rabbits are equivalent to the upper portion of human EL levels. After heparin injection, plasma levels of hEL were increased by 1.5-fold in male and 1.4-fold in female Tg rabbits, suggesting that ≈60% of EL proteins are in circulation, whereas the rest of them (≈40%) are assumedly associated with heparan sulfate proteoglycans on the cellular surface,29 as shown in the human plasma.27

Effects of hEL Expression on Plasma Lipids and Lipoproteins
Analysis of plasma lipids revealed that expression of hEL led to a marked reduction of plasma lipids (Figure 2). Plasma levels of total cholesterol (TC) were reduced by 67% in males and 47% in females, phospholipid levels were reduced by 61% in males and 52% in females, HDL-C levels were reduced by 88% in males and 70% in females, and HDL-phospholipids levels were reduced by 66% in males and 57% in females. Plasma triglyceride levels were also reduced but were only statistically significant in males (36% decrease, P<0.01) compared with non-Tg rabbits.

Analysis of plasma lipoproteins by agarose gel electrophoresis revealed that both α-migrating (HDLs) and β-migrating lipoproteins (very low-density lipoproteins [VLDLs] and LDLS) in Tg rabbits were decreased compared with non-Tg rabbits (Figure 3A). This change was more prominent in male Tg than in female Tg rabbits. Western blotting analysis of the whole plasma showed that plasma apoE and apoAI contents were concomitantly reduced in Tg rabbits, but apoB contents were unchanged compared with non-Tg rabbits (Figure 3B). As shown in the high-performance liquid chromatography assay, HDL peaks surpassed VLDL/LDL peaks in non-Tg rabbits; however, in Tg rabbits, the heights of these 2 peaks were reversed, even though both became shorter (Figure 3C).

Analysis of lipoprotein fractions isolated by sequential gradient ultracentrifugation revealed that there was a marked reduction of HDL₂ (d=1.06–1.10 g/mL) and HDL₃ (d=1.10–1.21 g/mL) accompanied by decreased contents of apoA1 and apoE in these fractions in Tg rabbits (Figure 3D). ApoB-containing particles (VLDL and LDL) were not prominently changed in Tg rabbits. Reduction of apoAI and apoE contents in HDLs was also shown by SDS-PAGE using the same density fractions (Figure 3E). Furthermore, HDL₂ fractions of Tg rabbits showed a marked reduction of both TC and triglyceride contents in both male and female Tg rabbits compared with each counterpart non-Tg rabbit (Figure II in the online-only Data Supplement).

Cholesterol-Rich Diet Experiments
To investigate the response of Tg rabbits to a cholesterol-rich diet, Tg and non-Tg littermates were fed a cholesterol-rich diet for 16 weeks. Compared with those of non-Tg rabbits, Tg rabbits showed constantly and significantly lower hypercholesterolemia: lower TC and lower HDL-C levels than non-Tg rabbits throughout the experiment period (Figure 4). Triglyceride levels of Tg rabbits were slightly (but not statistically significant) lower during the experiment period (Figure 4). Analysis of lipoprotein profiles by agarose gel electrophoresis revealed that β-migrating lipoproteins (β-VLDLs

**Nonstandard Abbreviations and Acronyms**

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Definition</th>
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<tr>
<td>EL</td>
<td>endothelial lipase</td>
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<tr>
<td>hEL</td>
<td>human EL</td>
</tr>
<tr>
<td>HDL</td>
<td>high-density lipoprotein</td>
</tr>
<tr>
<td>LDL</td>
<td>low-density lipoprotein</td>
</tr>
<tr>
<td>Tg</td>
<td>transgenic</td>
</tr>
<tr>
<td>TC</td>
<td>total cholesterol</td>
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<tr>
<td>TG</td>
<td>triglycerides</td>
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<td>VLDL</td>
<td>very low-density lipoprotein</td>
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and remnant lipoproteins) were remarkably reduced in Tg rabbits (Figure 4, bottom).

We further analyzed lipoprotein fractions isolated from cholesterol-fed rabbits. There were 2 striking changes in the lipoproteins of cholesterol-fed Tg rabbits compared with non-Tg rabbits. First, there was a remarkable reduction of apoB-containing particles, including VLDLs, intermediate density lipoproteins, and LDLs associated with reduced contents of apoB and apoE in Tg rabbits. Second, similar to the Tg rabbits on the chow diet, there was a prominent reduction of HDL2-3 in which apoAI and apoE were also decreased (Figure 5A and 5B). Quantitation of TC and triglycerides in these fractions showed that all lipoproteins were reduced in Tg rabbits compared with non-Tg rabbits (Figure III in the online-only Data Supplement).

Quantification of Aortic and Coronary Atherosclerosis

Analysis of en face aortic lesion areas revealed that the whole aortic atherosclerotic lesions of Tg rabbits were significantly

![Figure 1. Analysis of human endothelial lipase (hEL) in pre- and post-heparin plasma using Western blotting (A) and ELISA (B). Plasma fractions and liver proteins were fractionated by 10% SDS-PAGE under reducing conditions, followed by immunoblotting with hEL monoclonal antibody. Plasma concentrations of hEL protein in pre-heparin and post-heparin plasma were measured using ELISA kits. Tg indicates transgenic.]

![Figure 2. Plasma lipids of human endothelial lipase transgenic (Tg) and non-Tg rabbits. Plasma lipids were analyzed from fasting EDTA plasma of both male (top) and female rabbits (bottom). Data are expressed as mean±SD. **P<0.01, ***P<0.001 vs non-Tg rabbits. HDL-C indicates high-density lipoprotein-cholesterol; PL, phospholipid; TC, total cholesterol; and TG, triglycerides.]

![Downloaded from http://atvb.ahajournals.org by guest on January 17, 2018]
Figure 3. Plasma lipoproteins and apolipoproteins. Plasma (4 μL) was electrophoresed on a 1% agarose gel and stained for neutral lipids with Fat Red 7B (A). Plasma (0.5 μL) was resolved by 4% to 20% SDS-PAGE, followed by immunoblotting with apoB, apoE, and apoAI antibodies (Abs; B). Plasma lipoprotein profiles were analyzed by high-performance liquid chromatography (C). Plasma lipoproteins were separated by sequential gradient ultracentrifugation. An equal volume (8 μL) of each fraction was resolved by electrophoresis in a 1% agarose gel. Lipoproteins were visualized using Fat Red 7B staining, and apolipoproteins were identified by immunoblotting with apoB, apoE, and apoAI Abs (D). An equal volume of each fraction (5 μL) was resolved by electrophoresis by 4% to 20% SDS-PAGE. Apolipoproteins were visualized using either CBB (Coomassie Brilliant Blue) staining or immunoblotting with apoB, apoE, and apoAI Abs (E). HDL indicates high-density lipoprotein; LDL, low-density lipoprotein; Tg, transgenic; and VLDL, very low-density lipoprotein.
reduced by 52%, with a 42% reduction in the aortic arch, a 62% reduction in the thoracic aorta, and a 54% reduction in the abdominal aorta compared with non-Tg rabbits (Figure 6). Histological examinations showed that the aortic lesions of both Tg and non-Tg rabbits were mainly composed of infiltrating macrophages and smooth muscle cells intermingled with extracellular matrix. The microscopic lesion size of the aortic arch was markedly decreased in Tg rabbits because of reduced numbers of both macrophages (53% decrease) and smooth muscle cells (63% decrease) compared with non-Tg rabbits (Figure 7). Analysis of coronary atherosclerosis revealed that Tg rabbits had smaller lesions in both left and right coronary arteries (33% decrease in left and 42% decrease in right coronary arteries, \( P > 0.05 \)) than non-Tg rabbits (Figure IV in the online-only Data Supplement).

**Discussion**

In the current study, we generated Tg rabbits expressing human EL in the liver and characterized the effects of overexpression of EL on plasma lipoproteins and cholesterol diet–induced atherosclerosis. Consistent with the previous studies, hepatic expression of EL in Tg rabbits on a chow diet led to a remarkable reduction of plasma TC, phospholipids, HDL-C, and HDL-phospholipids, suggesting that EL indeed plays an important role in maintaining the HDL homeostasis. It should be noted that in Tg rabbits, \( \approx 60\% \) of the EL proteins were present in pre-heparin plasma associated with lipoproteins, with rest of them bound to the luminal surface of endothelial surface heparan sulfate proteoglycans because they are releasable to the circulation by heparin injection. The presence of free EL immunoreactive proteins in the circulation has also been reported in WHHL rabbits and humans, and measurement of the pre-heparin plasma EL activity showed that high EL activity is associated with high risk of coronary heart disease. Besides its phospholipase activity, EL possesses a noncatalytic function as lipoprotein lipase and hepatic lipase, which may facilitate binding of plasma lipoproteins to the heparan sulfate proteoglycans, leading to enhancement of lipoprotein uptake and degradation in the arterial intima. Taken together and based on our observations above, we initially postulated that hEL Tg rabbits should be extremely susceptible to cholesterol diet–induced atherosclerosis.

To our surprise, cholesterol-fed hEL Tg rabbits developed lower hypercholesterolemia and less aortic and coronary atherosclerosis than did non-Tg rabbits, suggesting that increased expression of EL is not proatherogenic but rather antiatherogenic. Several mechanisms may be operative for EL antiatherogenic effects. First, Tg rabbits had lower plasma TC levels with a remarkable reduction in apoB-containing lipoproteins in addition to low HDLs. When rabbits were fed with a cholesterol-rich diet, they develop hypercholesterolemia because of the elevation of hepatically and intestinally derived cholesteryl ester–rich remnant lipoproteins, called \( \beta \)-VLDLs. It is these \( \beta \)-VLDLs that are atherogenic in cholesterol-fed rabbits. Even though plasma HDL levels (antiatherogenic lipoproteins) were concomitantly lower in Tg rabbits, the net effects of EL overexpression were atheroprotective owing to lowering plasma \( \beta \)-VLDLs. Therefore, EL antiatherogenic effects are basically dependent on plasma \( \beta \)-VLDL or apoB levels.

![Figure 4. Plasma total cholesterol (TC), triglycerides (TG), and high-density lipoprotein-cholesterol (HDL-C) levels of transgenic (Tg) and non-Tg rabbits fed a cholesterol-rich diet. All rabbits were male at the age of 4–5 months. Data are expressed as mean±SD. *P<0.05, **P<0.01, ***P<0.001 vs non-Tg rabbits. Plasma (2 μL) was electrophoresed on a 1% agarose gel and stained for neutral lipids with Fat Red 7B (bottom).](http://atvb.ahajournals.org/).
It has been reported that deficiency of EL led to increased small LDL levels in hepatic lipase knockout mice, whereas expression of EL in mouse models with elevated apoB-containing particles markedly reduces VLDL and LDL levels and accelerates the turnover rates of LDLs. However, whether increased hepatic EL expression in Tg rabbits enhances the clearance of apoB containing particles awaits for the vigorous lipoprotein catabolism study in future. In addition, it is necessary to elucidate whether EL exerts such a function through either EL catalytic or noncatalytic mechanism. To

**Figure 5.** Analysis of plasma lipoproteins isolated from rabbits at 15 wk after cholesterol diet feeding. An equal volume (2 μL) of each fraction was resolved by electrophoresis in a 1% agarose gel. Lipoproteins were visualized using Fat Red 7B staining, and apolipoproteins were identified by immunoblotting with apoB, apoE, and apoAl antibodies (A). An equal volume of each fraction (5 μL) was resolved by electrophoresis by 4% to 20% SDS-PAGE. Apolipoproteins were visualized using CBB (Coomassie Brilliant Blue) staining (B). Size of apoCl-III (between 10 and 15 kDa) shown on SDS-PAGE is larger than those predicted possibly because of glycosylation.

**Figure 6.** Analysis of atherosclerotic lesions of aorta. Male transgenic (Tg) and non-Tg rabbits were fed a cholesterol diet for 16 wk and then the aortic lesions were quantified. Representative pictures of aortas stained with Sudan IV are shown on the left. The lesion area (defined by the sudanophilic area) was quantified using an image analysis system. Each dot represents the lesion area of an individual animal. *P<0.05, **P<0.01 vs non-Tg rabbits.
examine this issue, we attempted to compare plasma triglyceride lipase and phospholipase activity of Tg rabbits with non-Tg rabbits. We found that although the majority (≈ 60%) of hEL proteins exists in the pre-heparin plasma of Tg rabbits, their triglyceride lipase and phospholipase A1 activity were not significantly increased compared with non-Tg rabbits (Figure V in the online-only Data Supplement). Because the current method for measuring phospholipase A1 activity was not specific for EL as other lipases such as hepatic lipase also exhibit phospholipase activity, it is still immature to conclude which EL (catalytic versus noncatalytic) plays a major role in mediating lipoprotein metabolism in Tg rabbits including enhancement of hepatic uptake and clearance of apoB-containing particles. It remains unclear whether EL expressed by extrahepatic organs exhibits the same antiatherogenic effects as hepatically expressed EL shown in this study.

A noteworthy finding in this study was that increased EL in Tg rabbits did not affect the particular cell types in the lesions because both macrophages and smooth muscle cells were similarly reduced in number compared with non-Tg rabbits. This strengthened the above notion that EL antiatherogenic functions are virtually through lowering plasma atherogenic lipoproteins rather than mediating arterial wall macrophage infiltration or smooth muscle cell proliferation. Nevertheless, the current study using Tg rabbits along with our previous EL knockdown study strongly suggests that therapeutic inhibition of EL expression may not be an appropriate strategy for the treatment of atherosclerosis. In our previous study, we investigated the effect of EL antisense oligonucleotides on HDL metabolism and atherosclerosis in both wild-type rabbits and WHHL (Watanabe heritable hyperlipidemic) rabbits. Injection with rabbit EL antisense oligonucleotides (40 mg/kg) for 6 weeks resulted in 50% reduction of hepatic expression of EL but did not lead to a significant change in plasma TC and HDL-C levels. Although there was an increase of large-sized (>12 nm) phospholipid-rich HDL particles compared with mismatched oligonucleotide control, such a mild change in HDL particle components failed to affect the aortic lesion size in WHHL rabbits.

In conclusion, our results support the contention that EL functions in the metabolism of both HDL and apoB-containing lipoproteins, thereby playing a key role in plasma cholesterol homeostasis. Overexpression of EL in the liver protected against cholesterol-induced hypercholesterolemia and atherosclerosis. It remains to be verified whether inhibition of EL serves as a therapeutic target for the treatment of atherosclerosis.

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Disclosures
None.

References
Human Endothelial Lipase Inhibits Atherosclerosis

Highlights

- Increased hepatic expression of endothelial lipase in transgenic rabbits decreases plasma total cholesterol triglyceride and high-density lipoprotein-cholesterol levels.
- Endothelial lipase inhibits cholesterol diet–induced hypercholesterolemia in transgenic rabbits.
- Endothelial lipase protects against cholesterol-induced aortic and coronary atherosclerosis in transgenic rabbits.

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Materials and Methods

Production of transgenic (Tg) rabbits
Tg rabbits were generated by the methods established in our laboratory as reported previously1, 2. The DNA construct used for microinjection was composed of human endothelial lipase (hEL) cDNA under the control of a liver-specific apoE promoter along with four copies of the chicken β globin insulator (S-Fig. I), which prevents the position effect of transgene insulators3, 4. Tg founders were identified by Southern blotting and mated with non-Tg rabbits to produce F1 progeny. Rabbits were fed with a chow diet (RM-4, Funabashi Farmer) containing 16.5% protein, 4.2% fat, and 13% crude fiber. In this study, rabbits at the age of 3~6 months were used. All rabbits were fed either with a chow diet or cholesterol-rich diet containing 0.3% cholesterol and 3% soybean oil for 16 weeks5. The rabbits were allowed access to diet and water ad libitum. All animal experiments were performed with the approval of the Animal Care Committee of the Universities of Yamanashi and Saga and conformed to the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health. Plasma lipids and apolipoproteins along with enzymes were analyzed using the methods described in the supplemental materials.

Analysis of EL protein
Post-heparin plasma was prepared from a blood sample taken 10 min after injection of heparin at a dose of 30 units/kg of body weight6. Pre-heparin and post-heparin plasma hEL proteins were measured using an enzyme-linked immunosorbent assay system (Immuno-Biological Laboratories Com., Ltd., Gunma, Japan). In addition, EL proteins were analyzed by Western blotting7. The plasma fractions (d<1.24 g/mL) were isolated by ultracentrifugation and fractionated by 10% SDS–polyacrylamide gel electrophoresis (SDS-PAGE), followed by immunoblotting with mouse monoclonal antibody (Ab) against the whole-length hEL protein (working dilution: 1:500) (Abnova Corporation, Taiwan), and immune-complexed proteins were identified by reaction with a horseradish peroxidase-conjugated goat Ab against mouse IgG, followed by enhanced chemiluminescence detection8.

Analysis of plasma phospholipase A1 activity and triglyceride lipase activity
Pre- and post-heparin plasma phospholipase A1 activity was measured using an EnzChek® phospholipase A1 assay kit (Thermo Fisher Scientific, Waltham, MD). 50 µl sample containing 0.5 µl plasma and 49.5 µl phospholipase A1 reaction buffer was
added to 50 µl liposomes containing fluorescent labeled substrate (PED-A1), dioleoylphosphatidylcholine, and dioleoylphosphatidylglycerol in a 96-well microplate. Fluorescence intensity was constantly measured at 25oC for 60 min using SpectraMax Gemini EM fluorescence microplate reader (Molecular Devices, Sunnyvale, CA). The maximum excitation/emission wavelengths for phospholipase A1 selective PED-A1 substrate were determined at 485 nm (excitation) and 538 nm (emission). Plasma total triglyceride lipase activity (including LPL, HL and EL) was measured using a commercial lipase fluorometric assay kit (Cell Biolabs, San Diego, CA). 100 µl diluted plasma (100x) were incubated with 100 µl fluorometric substrate at 37oC for 30 min, and then with 20 µl stop solution. The fluorescence was measured using SpectraMax Gemini EM fluorescent microplate reader at 485 nm and fluorescence emission at 525 nm with a 495 nm filter cutoff.

Analysis of plasma lipids and lipoproteins
Plasma levels of total cholesterol (TC), triglycerides (TG), phospholipids (PL), HDL cholesterol (HDL-C), and HDL phospholipid (HDL-PL) were measured using enzymatic assay kits (Wako Pure Chemical Industries Ltd., Osaka). Plasma lipoproteins were analyzed by a 1% agarose gel electrophoresis (Helena Laboratories, Saitama, Japan) and stained with Fat Red 7B. Furthermore, the plasma lipoproteins were analyzed by high performance liquid chromatography (HPLC) according to the procedure described before. Apolipoprotein (apo)A, apoB, and apoE contents in the whole plasma were determined by Western blotting. Two µl of plasma was subjected to 4~20% SDS-PAGE, followed by immunoblotting with goat polyclonal Abs against apoB, apoE (Rockland Inc., Limerick, PA, USA), and sheep anti–apoAI (Bio-Rad AbD Serotec, Kidlington, UK). Plasma lipoproteins were further isolated by sequential gradient ultracentrifugation and then subjected to a 1% agarose gel electrophoresis and stained with Fat Red 7B or transferred to a nitrocellulose membrane for immunoblotting with anti-apoB, apoE, and apoAI polyclonal Abs (1:1000). Lipoprotein fractions were fractionated by 4~20% SDS-PAGE. Apolipoproteins were visualized by Coomassie Brilliant Blue (CBB) staining or immunoblotted with anti-apoB, apoE, and apoAI Abs. TC and TG contents in each density fraction were measured using the Wako assay kits described above.

Quantification of aortic and coronary atherosclerosis
At 16 weeks after cholesterol diet feeding, all rabbits were euthanized by injection of an overdose of sodium pentobarbital solution. The whole aortas were stained with Sudan IV for evaluation of the gross lesion size as described previously. For microscopic
evaluation of the lesion area, the aortic arch was cut into 10 sections and embedded in paraffin. Serial sections (3 μm thick) were stained with hematoxylin–eosin (H&E) and elastica van Gieson (EVG) or immunohistochemically stained with mAbs against macrophages, RAM11, (1:400) and smooth muscle α-actin, HHF35 (1:200). The microscopic lesion area and the macrophage and smooth muscle cell (SMC) contents in the lesions were quantified using a WinROOF image analysis system3. To assess coronary atherosclerosis, rabbit hearts were sectioned into 5 blocks, and the coronary lesions (expressed as stenosis %) of the left and right coronary arteries were quantified by the method described previously10.

Statistical Analysis
All data are expressed as mean ± SD except Figure 7. Student’s t-test (for parametric data) and Mann-Whitney U-test (for non-parametric data) were used for statistical analyses using SPSS 22.0 software. P values < 0.05 were considered significant.

References:


S-Figure I. Transgenic DNA construct for microinjection. The construct consisted of the whole length human EL cDNA (1.5 kb), liver-specific apoE promoter, 4 copies of insulators, and human apoE poly A signal and liver regulatory element. Sal I linearized fragments (11.5 kb) at 2.5 ng/ml in TE buffer were used for microinjection.
S-Figure II. The quantitation of cholesterol and TG contents in lipoprotein fractions isolated from rabbits on a normal chow diet. Cholesterol and TG contents in each density fraction were quantified. Data are expressed as mean ± SD. *p<0.05, **p<0.01 vs. Non-Tg rabbits.
S-Figure III. The quantitation of cholesterol and TG contents in lipoprotein fractions of rabbits fed a cholesterol-rich diet for 15 weeks. Total cholesterol (TC) and triglycerides (TG) contents in each density fraction were quantified. Data are expressed as mean ± SD. *p<0.05 vs. Non-Tg rabbits.
S-Figure IV. Histological analysis of coronary arterial atherosclerosis in lesions (left) and quantitatively measured lesion size expressed as stenosis (%) of the lumen area. LCA indicates left coronary artery trunks; RCA, right coronary artery trunks. Data are expressed as mean ± SD.
S-Figure V. Analysis of plasma triglyceride lipase activity (A) and phospholipase A1 activity (B)

Pre-heparin and post-heparin plasma was isolated from non-Tg and Tg rabbits fed a cholesterol diet for 15 weeks and stored at -80°C until. Triglyceride lipase and phospholipase A1 activity were measured as described in the Materials and Methods. Data are expressed as mean ± SD (n=7 for non-Tg and n=5 for Tg).