Endothelial lipase modulates HDL but has no effect on atherosclerosis development in apoE$^{-/-}$ and LDLR$^{-/-}$ mice[S]

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Abstract **Endothelial lipase (EL) is a determinant of high density lipoprotein-cholesterol (HDL-C) level, which is negatively correlated with atherosclerosis susceptibility. We found no difference in aortic atherosclerotic lesion areas between 26-week-old EL$^{+/+}$ apolipoprotein E-deficient (apoE$^{-/-}$) and EL$^{-/-}$ apoE$^{-/-}$ mice. To more firmly establish the role of EL in atherosclerosis, we extended our study to EL$^{-/-}$ and EL$^{+/+}$ low density lipoprotein receptor-deficient (LDLR$^{-/-}$) mice that were fed a Western diet. Morphometric analysis again revealed no difference in atherosclerosis lesion area between the two groups. Compared with EL$^{+/+}$ mice, we found increased HDL-C in EL$^{-/-}$ mice with apoE$^{-/-}$ or LDLR$^{-/-}$ background but no difference in macrophage content between lesions of EL$^{-/-}$ and EL$^{+/+}$ mice in apoE$^{-/-}$ or LDLR$^{-/-}$ background. EL inactivation had no effect on hepatic mRNAs of proteins involved in reverse cholesterol transport. A survey of lipid homeostasis in EL$^{+/+}$ and EL$^{-/-}$ macrophages revealed that oxidized LDL-induced ABCA1 was attenuated in EL$^{-/-}$ macrophages. This potentially proatherogenic change may have nullified any minor protective increase of HDL in EL$^{-/-}$ mice. Thus, although EL modulated lipoprotein profile in mice, there was no effect of EL inactivation on atherosclerosis development in two hyperlipidemic atherosclerosis-prone mouse models.**—Ko, K. W. S., A. Paul, K. Ma, L. Li, and L. Chan. **Endothelial lipase modulates HDL but has no effect on atherosclerosis development in apoE$^{-/-}$ and LDLR$^{-/-}$ mice.** ***J. Lipid Res.*** **2005.** 46: **2586–2594.**

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Endothelial lipase (EL; gene name *LIPG*) is a recently described member of the triglyceride lipase gene family that shares considerable sequence homology with LPL and HL (1, 2). EL is synthesized by endothelial cells, where its expression is regulated by cytokines and physical forces (3, 4) as well as by cholesterol feeding (5). EL expression has also been demonstrated in macrophage cell lines (1, 2). Taken together and based on our knowledge of the functions of LPL and HL, these findings suggest potential regulatory roles for EL in lipoprotein metabolism, vascular biology, and atherosclerosis.

LPL and HL have different degrees of both triglyceride lipase and phospholipase activities (6). The enzymatic activity of EL is distinct, it being mainly a phospholipase active toward all lipoprotein classes, although preferring HDL as a substrate (7). Accordingly, marked adenovirus-mediated overexpression of EL in low density lipoprotein receptor (LDLR) knockout mice reduced plasma VLDL and LDL-cholesterol by ~50% while almost reducing high density lipoprotein-cholesterol (HDL-C) to zero (2). Inactivation of EL through gene targeting (8, 9) or functional inhibition by antibody infusion (10) demonstrated that EL can indeed function as a key physiological regulator of HDL level, although details of its role in the catabolism of apolipoprotein B-containing lipoproteins have also expanded (11).

A difference in the tissue specificity of EL (secreted by vascular endothelial cells as well as macrophages) compared with that of LPL (secreted by muscle, heart, and adipose tissue as well as macrophages) and HL (secreted by hepatocytes as well as macrophages) could imply nonoverlapping vascular biologic functions, for example, in the provision of peroxisome proliferator-activated receptor ligands to endothelium (12) or macrophages (13). Aside from enzymatic activity, LPL (14) and HL (15) provide noncatalytic bridging function to uptake receptors, and such a role has also been demonstrated for EL (11, 16). The conspicuous expression of EL in placenta (2) and during en-

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Abbreviations: apoE, apolipoprotein E; EL, endothelial lipase; FPLC, fast-protein liquid chromatography; IDL, intermediate density lipoprotein; HDL-C, high density lipoprotein-cholesterol; LDLR, low density lipoprotein receptor; OxLDL, oxidized low density lipoprotein; SR-A, scavenger receptor A; SR-BI, scavenger receptor class B type I.

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[S] The online version of this article (available at http://www.jlr.org) contains an additional table.

dothelial cell tube formation (1) also suggest possible specialized roles during fetal development and angiogenesis.

New regulators of HDL level have drawn strong interest as possible modulators of atherosclerosis. Longstanding epidemiological data demonstrate an inverse relationship between plasma HDL-C level and coronary heart disease incidence (17), with at least 50% of variation in HDL-C being genetically determined (18). Both LPL and HL have been shown to affect atherosclerosis, and interestingly, both gene products possess both potentially proatherogenic and antiatherogenic functions (6, 19). There is mounting evidence for a role of EL in the regulation of HDL levels, and because inactivation of EL increases HDL, its action may be viewed as proatherogenic. The expression of EL in macrophages and endothelium, where it might serve noncatalytic, bridging functions to possibly promote lipid accumulation and lesion development, could also be seen as potentially proatherogenic. However, the actual contribution of EL to atherogenesis remains unproven, because there are other major lipases, LPL and HL, also present in the circulation and expressed by macrophages. In this study, we explore the role of EL in atherosclerosis by analyzing the outcome of placing targeted disruption of EL into the apolipoprotein E (apoE) and LDLR knockout mouse models of increased atherosclerosis susceptibility. Both models are characterized by hypercholesterolemia with decreased HDL compared with C57BL/6 mice, and although we found increased plasma phospholipids and HDL-C levels in these animals with genetic deletion of EL, we found no difference in atherosclerosis development compared with their EL wild-type counterparts.

MATERIALS AND METHODS

Animals

EL heterozygous knockout mice (bearing a deletion of exon 2) generated in our laboratory (8) were backcrossed to the C57BL/6J background for at least five generations and then crossed two more generations with homozygous apoE$^{-/-}$ C57BL/6J mice to obtain EL$^{+/-}$ apoE$^{-/-}$ mice. These EL heterozygotes were intercrossed, and EL homozygous wild-type and knockout siblings were used in all studies. A similar approach was taken to generate EL knockout and wild-type siblings in an LDLR$^{-/-}$ C57BL/6J background. Mice were weaned at 4 weeks of age onto normal chow, and in the case of LDL$^{-/-}$ background mice, at 8 weeks age animals were placed on a Western diet (88137; 0.15% cholesterol, 21% milk fat, and 19.5% casein) from Harlan Teklad (Madison, WI). PCR was performed for genotyping EL (20), apoE (wild-type allele-specific, 5′-GCCTAGCCGAGGGAGAGCCG-3′; common, 5′-TGTGACTTGGGAGCTCTGCAGC-3′; targeted allele-specific, 5′-GCCGCCCCGACTGCATCT-3′; 58°C annealing temperature, three-primer PCR gives 155 bp wild-type and 245 bp knockout bands), and LDLR (wild-type allele-specific, 5′-AGGTGAGATGACAGGAGATC-3′; common, 5′-ACCCCAAGACGTGCTCCCAGGATGA-3′; targeted allele-specific, 5′-CGCAGTGCTCCTCATCTGACT-3′; 58°C annealing temperature, separate two-primer PCR gives 400 bp wild-type and 800 bp knockout bands) loci using the given primers and conditions. C57BL/6J wild-type, apoE$^{-/-}$, and LDLR$^{-/-}$ mice were obtained from Jackson Laboratory (Bar Harbor, ME). All animal experiments were conducted according to protocols for the handling and treatment of animals approved by the Institutional Animal Care and Use Committee at Baylor College of Medicine.

Plasma lipid analysis

Blood was collected after overnight fasting by retro-orbital puncture, and EDTA-plasma was isolated by centrifugation at 5,000 *g* for 5 min at 4°C. Total plasma cholesterol, triglyceride (both from Sigma-Aldrich, St. Louis, MO), and phospholipid (Wako, Richmond, VA) were measured using enzymatic kits. Fractionation of plasma lipoproteins by fast-protein liquid chromatography (FPLC) was performed using two Superose 6 columns (Amersham-Pharmacia, Piscataway, NJ) as described (8). Plasma HDL-C was measured using a commercial kit (Wako).

Atherosclerotic lesion quantitation and histologic analysis

Homozygous EL wild-type and knockout mice were euthanized at 26 weeks of age, and atherosclerotic lesions were analyzed as described previously (21). Briefly, the aortic tissue was embedded in OCT (Electron Microscopy Sciences, Washington, PA) and frozen before cryostat sections (7 μm thickness) were prepared. After Oil Red O lipid staining and hematoxylin counterstaining, sections spanning 150 μm proceeding distally from the aortic origin from each mouse were used for quantitation of lesion area. Digitizing morphometric measurement of stained lesional areas was performed using a Zeiss microscope and AxioVision software as described previously (21) in a blinded manner.

Immunohistological analysis of lesion morphology was also performed using primary antibodies for Mac-3 (Santa Cruz Biotechnology, Santa Cruz, CA), human EL (Cayman Chemicals, Ann Arbor, MI), and human LPL (22). For quantification by image analysis, we set a threshold to automatically compute the areas positive for the antibody and then computed the ratio of positively stained area to the total lesion area as described (21).

Real-time quantitative RT-PCR analysis

Total RNA from liver and 3 day thioglycollate-elicited peritoneal macrophages was prepared using the RNeasy Mini Kit (Qiagen, Valencia, CA) with on-column DNase treatment. Reverse transcription of 0.5–5 μg of total RNA was performed using the SuperScript First-Strand System for RT-PCR (Invitrogen, Carlsbad, CA). Primers were designed using Primer3 software (23), and sequences are available in the supplementary data. Quantitative real-time RT-PCR was performed with SYBR Green QPCR Master Mix from Stratagene (Cedar Creek, TX) using 40 amplification cycles (95°C for 30 s, 55°C for 1 min, 72°C for 1 min) and detection of specific products with the Mx3000P system (Stratagene). Relative gene expression levels were determined from threshold cycle values normalized to actin.

Thioglycollate-elicited macrophages were plated on DMEM containing 10% FBS and cultured for 24 h (37°C, 5% CO_2) after withdrawal from the peritoneal cavity, and in some cases in the presence of oxidized low density lipoprotein (OxLDL; 100 μg/ml) or the phorbol ester tetradecanoyl phorbol acetate (100 ng/ml; Sigma-Aldrich) before harvesting for RNA isolation.

Statistical analysis

Data are presented as means ± SD, and experimental groups were compared with controls using paired or unpaired Student's *t*-tests. $P \leq 0.05$ was considered significant.

RESULTS AND DISCUSSION

Absence of EL does not affect atherosclerosis development in apoE$^{-/-}$ mice

The role of EL in atherosclerosis development was first tested by placing EL gene inactivation into mice with the apoE$^{-/-}$ background. The apoE$^{-/-}$ EL$^{-/-}$ and apoE$^{-/-}$

$EL^{+/+}$ mice in the C57BL/6 background were maintained on chow diet and were euthanized at age 26 weeks for quantitative morphometric cross-sectional analysis of aortic atherosclerosis lesion involvement. We detected no difference in cross-sectional lesion area between $apoE^{-/-}$ $EL^{-/-}$ and $apoE^{-/-}$ $EL^{+/+}$ mice (n = 19 for $EL^{-/-}$ and n = 18 for $EL^{+/+}$; **Fig. 1**). At 26 weeks, lesions are in the early stages of development (between stages II and III), characterized by lipoprotein accumulation, leukocyte (mainly T-cell and macrophage) gathering, and foam cell formation (24). A gender difference in overall lesion formation between females and males (less in males than in females) was obtained, but this has been observed previously by us (21) and others (25, 26). However, when we examined the male and female groups separately, we detected no difference in atherosclerosis lesion size between $apoE^{-/-}$ $EL^{-/-}$ and $apoE^{-/-}$ $EL^{+/+}$ mice among groups of the same gender ($P = 0.70$ for females, $P = 0.52$ for males).

Absence of EL does not affect atherosclerosis development in $LDLR^{-/-}$ mice

As we were completing these studies, Ishida et al. (27) reported that the absence of EL was associated with reduced atherosclerosis in $apoE^{-/-}$ mice. Therefore, we extended our experiments to examine whether the lack of EL expression has any effect on the accelerated atherosclerosis of another mouse model, the $LDLR^{-/-}$ mouse. As the mouse counterpart for familial hypercholesterolemia in humans, the $LDLR^{-/-}$ mouse is a model for a much more common lipid disorder than the $apoE^{-/-}$ mouse. $LDLR^{-/-}$ $EL^{-/-}$ and $LDLR^{-/-}$ $EL^{+/+}$ mice were fed regular chow until 8 weeks of age, when they were switched to a Western diet. The mice were euthanized at 26 weeks, and aortic atherosclerosis lesion sizes were quantified in these animals. In this model, the overall extent of atherosclerosis was 2- to 3-fold greater than that in $apoE^{-/-}$ mice of corresponding age; it represents mainly stage IV early advanced lesions, as defined by the appearance of an extracellular lipid core, proceeding to necrotic core formation with capping by foam cells (24). In mice with the $LDLR^{-/-}$ background, we detected a nonsignificant difference in lesion size between male and female animals, there being a tendency toward slightly more extensive lesions in female mice. When we compared the lesion size between $LDLR^{-/-}$ $EL^{-/-}$ and $LDLR^{-/-}$ $EL^{+/+}$ groups, we detected no difference. Similarly, when we divided the groups into male and female subgroups, we again failed to detect any difference in lesion involvement in either group ($P = 0.58$ for females, $P = 0.53$ for males; **Fig. 2**).

Inactivation of EL affects lipid levels and lipoprotein profiles in $apoE^{-/-}$ and $LDLR^{-/-}$ mice

It was previously shown by gene targeting that in the C57BL/6 background, EL is a major determinant of HDL level (8, 9). However, C57BL/6 mice are mainly "HDL" animals (28) and unsuitable for atherosclerosis studies, because they only develop fatty streak lesions (24, 28). In this study, we used $apoE^{-/-}$ (29, 30) and $LDLR^{-/-}$ (31) mice as our models, because they both exhibit enhanced atherosclerosis susceptibility, leading to complex lesions resembling human disease, and they both have been used extensively to analyze the atherogenic potential of different mouse genes in various cross-breeding experiments.

As we found no difference in atherosclerosis development between $EL^{-/-}$ and $EL^{+/+}$ mice whether they were in the $apoE^{-/-}$ or $LDLR^{-/-}$ background, we analyzed the lipoprotein profiles of the different groups of mice. We found that, similar to previous observations, compared with

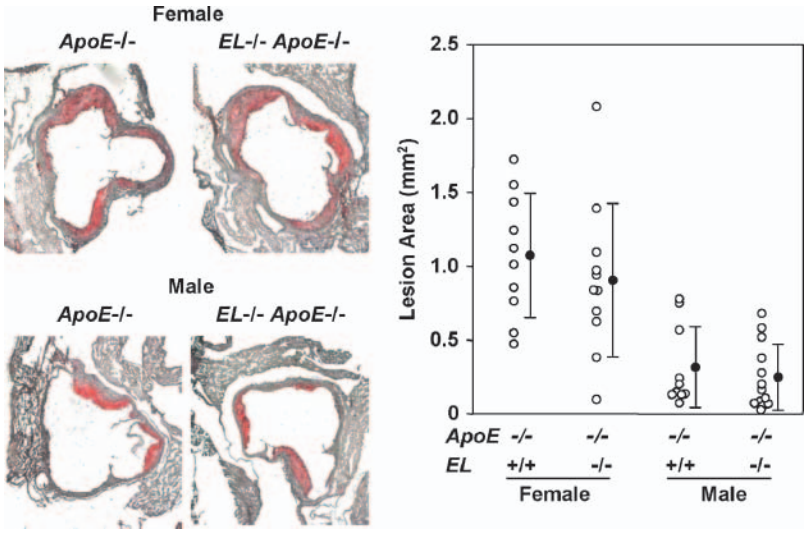

Fig. 1. Quantitation of atherosclerotic lesions in the aortic origin of apolipoprotein E-deficient ($apoE^{-/-}$) mice showing no change in the absence of endothelial lipase (EL). Left, representative photographs of Oil Red O-stained aortic origin sections from $apoE^{-/-}$ single knockout and $apoE^{-/-}$ $EL^{-/-}$ double knockout mice of both genders (magnification ×50). Right, quantitative analysis of aortic lesion size at 26 weeks of age under chow diet, with stained areas measured by morphometry. Values shown are means ± SD of groups of the indicated genders and genotypes.

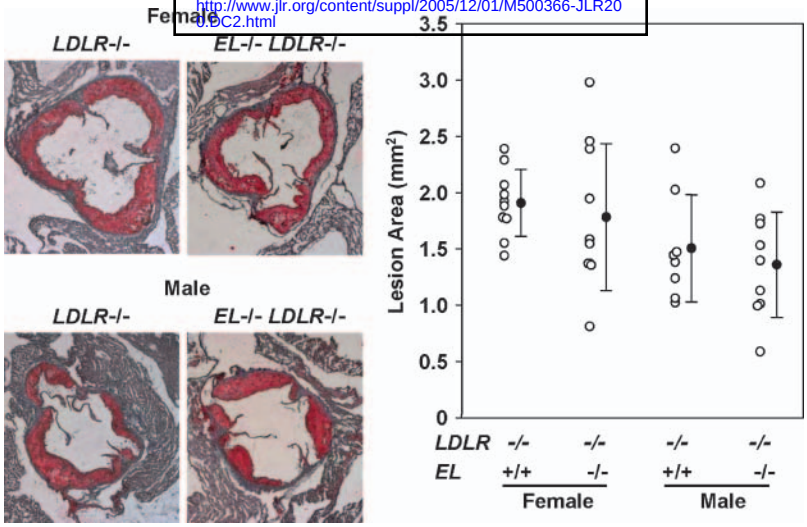

Fig. 2. Quantitation of atherosclerotic lesions in the aortic origin of low density lipoprotein receptor-deficient (LDLR$^{-/-}$) mice showing no change in the absence of EL. Left, representative photographs of Oil Red O-stained aortic origin sections from LDLR$^{-/-}$ single knockout and LDLR$^{-/-}$ EL$^{-/-}$ double knockout mice of both genders (magnification ×50). Right, quantitative analysis of aortic lesion size at 26 weeks of age under Western diet for 18 weeks, with stained areas measured by morphometry. Values shown are means ± SD of groups of the indicated genders and genotypes.

wild-type mice, apoE$^{-/-}$ mice with normal EL function were severely hyperlipidemic, with reduced HDL (45% of normal) and massively increased VLDL and intermediate density lipoprotein (IDL; 18-fold increase) (32, 33). Genetic disruption of EL in the apoE$^{-/-}$ background tended to further increase the level of all plasma lipids (**Table 1**), with the changes in cholesterol (22% increase) and triglyceride (42% increase) reaching significance only in female and male groups, respectively, whereas significantly increased phospholipid levels (38% increase) were found in both genders (Table 1). These results support previous reports that EL has mainly phospholipase activity (7); they also corroborate results showing EL to have moderate activity toward neutral lipids (modulation of apoB-containing lipoproteins) in some mouse backgrounds or circumstances (2, 11). FPLC analysis revealed that absence of EL had only modest effects on the lipoprotein cholesterol profile, and the increases in total plasma lipid levels were reflected mainly in increases in VLDL and HDL peaks of the lipid profiles (**Fig. 3**). In male mice, in which the change in total plasma cholesterol did not reach statistical significance, the absence of EL led to an increase in HDL-C in the FPLC analysis. Direct measurement of HDL-C levels (Table 1) confirmed a physiological role for EL in the regulation of HDL level in the apoE$^{-/-}$ model, in which HDL constitutes a minor lipoprotein fraction. Therefore, EL expression had no effect on atherosclerosis development in apoE$^{-/-}$ mice, despite the fact that it significantly modulates all of the major plasma lipoprotein fractions in this model.

TABLE 1. Fasting plasma lipid and HDL-C levels of EL wild-type and knockout mice in atherosclerosis backgrounds

Lipid	ApoE$^{-/-}$	EL$^{-/-}$ ApoE$^{-/-}$	LDLR$^{-/-}$	EL$^{-/-}$ LDLR$^{-/-}$
Cholesterol				
Female	373 ± 64	460 ± 69[a]	1,176 ± 240	1,267 ± 261
Male	449 ± 105	481 ± 94	1,245 ± 199	1,532 ± 255[b]
Triglyceride				
Female	57 ± 19	61 ± 21	202 ± 97	210 ± 164
Male	76 ± 26	108 ± 37[a]	238 ± 101	352 ± 127
Phospholipid				
Female	117 ± 30	162 ± 31[a]	446 ± 92	478 ± 152
Male	148 ± 45	203 ± 59[a]	500 ± 73	657 ± 92[a]
HDL-C	13 ± 3	20 ± 4[b]	26 ± 5	42 ± 4[a]

ApoE, apolipoprotein E; EL, endothelial lipase; HDL-C, high density lipoprotein-cholesterol; LDLR, low density lipoprotein receptor. Lipid values for single and double knockout mouse plasma are means ± SD in mg/dl. The number of samples was 12–16 for all measurements.
[a] $P < 0.01$ with respect to single knockout mice.
[b] $P < 0.05$ with respect to single knockout mice.

We asked whether the lack of an effect of EL on aortic atherosclerosis in LDLR$^{-/-}$ mice was also associated with differences in lipoprotein profiles between LDLR$^{-/-}$ mice that expressed EL and those that did not. As reported previously (34), LDLR$^{-/-}$ mice on a Western diet had severe hyperlipidemia. The absence of EL expression in LDLR$^{-/-}$ mice increased the plasma levels of all lipid classes, although the increase reached statistical significance for plasma cholesterol (23% increase) and phospholipids (31% increase) in male animals only (Table 1); also evident was an increase in plasma triglyceride (48% increase), which was of borderline significance ($P = 0.07$). FPLC analysis revealed that EL disruption also affected the plasma lipoprotein profile in LDLR$^{-/-}$ mice (Fig. 3). There was an increase in apoB-containing lipoprotein fractions (VLDL and LDL) and a modest increase in the HDL fraction, which was also confirmed by direct measurement of HDL-C levels in total plasma (Table 1). Thus, lipoprotein analysis in EL$^{+/+}$ and EL$^{-/-}$ mice with apoE$^{-/-}$ or LDLR$^{-/-}$ background showed that the inactivation of EL produced significant effects on the plasma

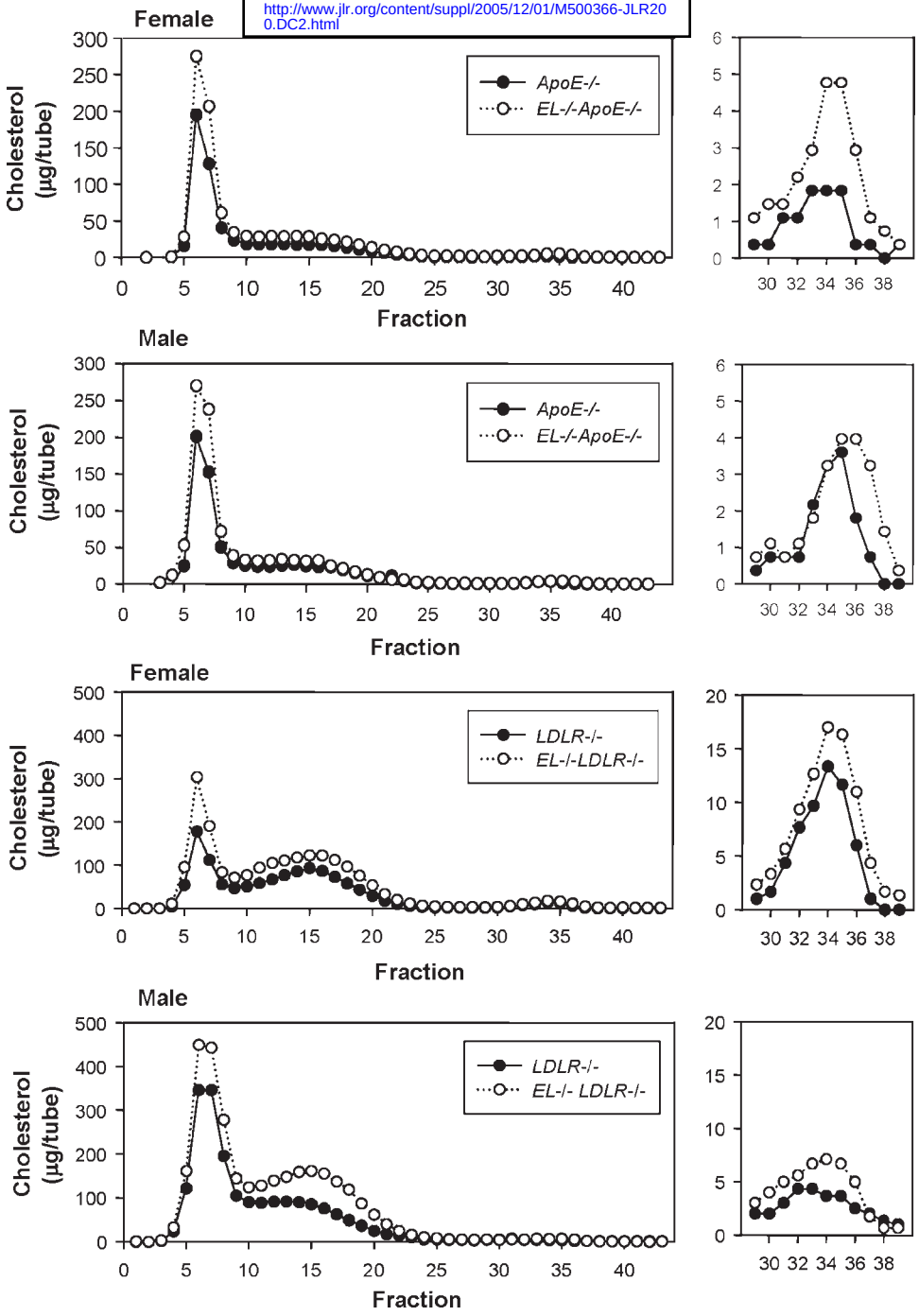

Fig. 3. Effect of the absence of EL on fast-protein liquid chromatography (FPLC) lipoprotein profiles of apoE$^{-/-}$ and LDLR$^{-/-}$ mice. Left, pooled plasma from mice of the indicated genotypes and genders were fractionated by FPLC and assayed for cholesterol. Right, the regions of the profiles where HDL eluted (fractions 29–39) were replotted with a magnified y axis scale to show differences attributable to the absence of EL.

lipoprotein level and distribution in both dyslipidemic mouse models.

Inactivation of EL does not affect lesion morphology

Ishida et al. (27) reported a difference in atherosclerotic lesion morphology between apoE$^{-/-}$ EL$^{+/+}$ and apoE$^{-/-}$ EL$^{-/-}$ mice. Therefore, we tested the hypothesis that inactivation of EL may have a discernible effect on the lesion morphology of apoE$^{-/-}$ and LDLR$^{-/-}$ mice, especially because the two models differ in stage of lesion development and pathogenesis (24, 28). Immunohistochemical analysis revealed no difference in lesional macrophage content between animals with or without EL in either the apoE$^{-/-}$ or LDLR$^{-/-}$ background, even with lesions being more advanced in the LDLR$^{-/-}$ animals (**Fig. 4**). EL immunoreactivity was weak compared with the data reported by Ishida et al. (27) (**Fig. 5**). Because both Ishida et al. (27) and we used apoE$^{-/-}$ mice in the C57BL/6J

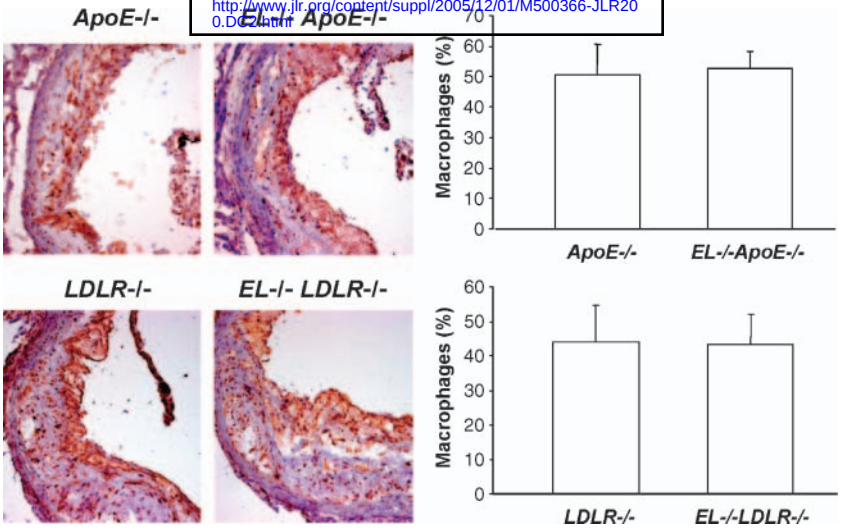

Fig. 4. Macrophage content of atherosclerotic lesions in the aortic origin showing no change in the absence of EL. Left, immunohistochemical staining for Mac-3 was used to quantify relative area occupied by macrophages in lesions from mice of the indicated genotypes (magnification ×200). Samples were from same mice used for Figs. 1, 2. Right, quantitative analysis of macrophage content of individual lesions (n = 8) in the aortic origin, with percentage of stained area to total lesion area measured by morphometry. Error bars represent means and SD of groups of the indicated genotypes.

background but different antibodies for the immunohistochemical analysis of the lesions, the difference in immunostaining intensity is likely related to differences in the affinity and/or specificity of the antibodies or to the method of staining rather than a true difference in EL expression. In contrast to EL, LPL staining was much more prominent throughout the lesion (Fig. 5), suggesting more widespread and higher levels of LPL expression compared with EL by cells in the lesion. Although the results shown were from LDLR$^{-/-}$ mouse samples, similar results were obtained with apoE$^{-/-}$ samples (data not shown). Thus, in addition to there being no significant difference in atherosclerosis development in terms of lesional area as a result of EL inactivation in either the apoE$^{-/-}$ or LDLR$^{-/-}$ background, there was also no difference in terms of lesion morphology indicative of altered macrophage migration or growth. We note that this is at odds with the observation of Ishida et al. (27), who found a decrease in macrophage content in lesions lacking EL.

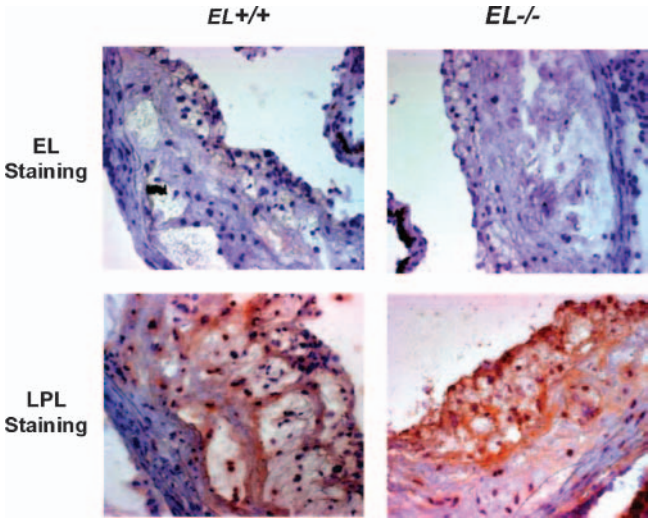

Fig. 5. Expression of EL and LPL within atherosclerotic lesions showing much higher protein levels of LPL than EL. Immunohistochemical staining for EL (top) and LPL (bottom) was performed on aortic sections from the same LDLR$^{-/-}$ mice used for Fig. 2 (magnification ×400).

Effects of EL inactivation on gene expression in liver and macrophages

Gene expression profiling was performed to determine whether the loss of EL function in the vascular system was associated with any change in the expression of genes that could potentially influence HDL levels or atherogenesis. Quantitative RT-PCR analysis of transcripts of major cholesterol transport genes in livers of apoE$^{-/-}$ mice revealed no significant difference affecting major liver genes involved in the synthesis (apoA-I, ABCA1), remodeling (LCAT, phospholipid transfer protein, HL), or catabolism [LDLR, scavenger receptor class B type I (SR-BI), CD36, scavenger receptor A (SR-A)] of lipoproteins, particularly HDL, in the presence or absence of EL expression (data not shown).

Macrophage expression of EL and other genes involved in lipid homeostasis was also examined in these mice to determine whether changes of expression of potentially proatherogenic or antiatherogenic genes might be operative in lesion development. Quantitative RT-PCR analysis of peritoneal macrophages in EL$^{+/+}$ mice revealed much lower EL expression relative to actin than that found in liver. At this level, EL was undetectable by Northern blotting (data not shown) (1). Treatment by OxLDL to mimic conditions in the lesion and lipid-load the cells failed to

induce macrophage expression of EL, as did phorbol ester treatment (data not shown). Although muscle, heart, and fat are thought to be the major sites of LPL expression, LPL transcript level in macrophages was significant and actually ~3 orders of magnitude greater than that of EL. There was no compensation in macrophages lacking in EL by upregulation of LPL or HL expression (**Fig. 6A**), and there were no differences in the expression of SR-A, CD36, and SR-BI in mice with or without EL (Fig. 6B); notably, however, the induction of ABCA1 by OxLDL was significantly attenuated (34%) in the absence of EL (Fig. 6B). Thus, EL deletion may result in macrophages with decreased cholesterol efflux capacity under conditions possible during atherosclerosis.

Conclusions and perspective

EL has been shown to be a major determinant of HDL level in mice of the C57BL/6 background (8–10). ApoE$^{-/-}$ and Western diet-fed LDLR$^{-/-}$ mice present two different levels of hyperlipidemia (~450 and 1,350 mg/dl cholesterol, respectively) that are associated with a preponderance of cholesterol in apoB-containing fractions (remnants and IDL/LDL, respectively). EL deficiency was able to increase HDL levels in apoE$^{-/-}$ mice, but there were also moderate increases in VLDL and IDL fractions of the FPLC profile. EL deficiency had a similar effect on HDL levels in the LDLR$^{-/-}$ model, whereas somewhat more pronounced overall effects were seen in the VLDL and IDL/LDL fractions. Thus, based on the observed changes in

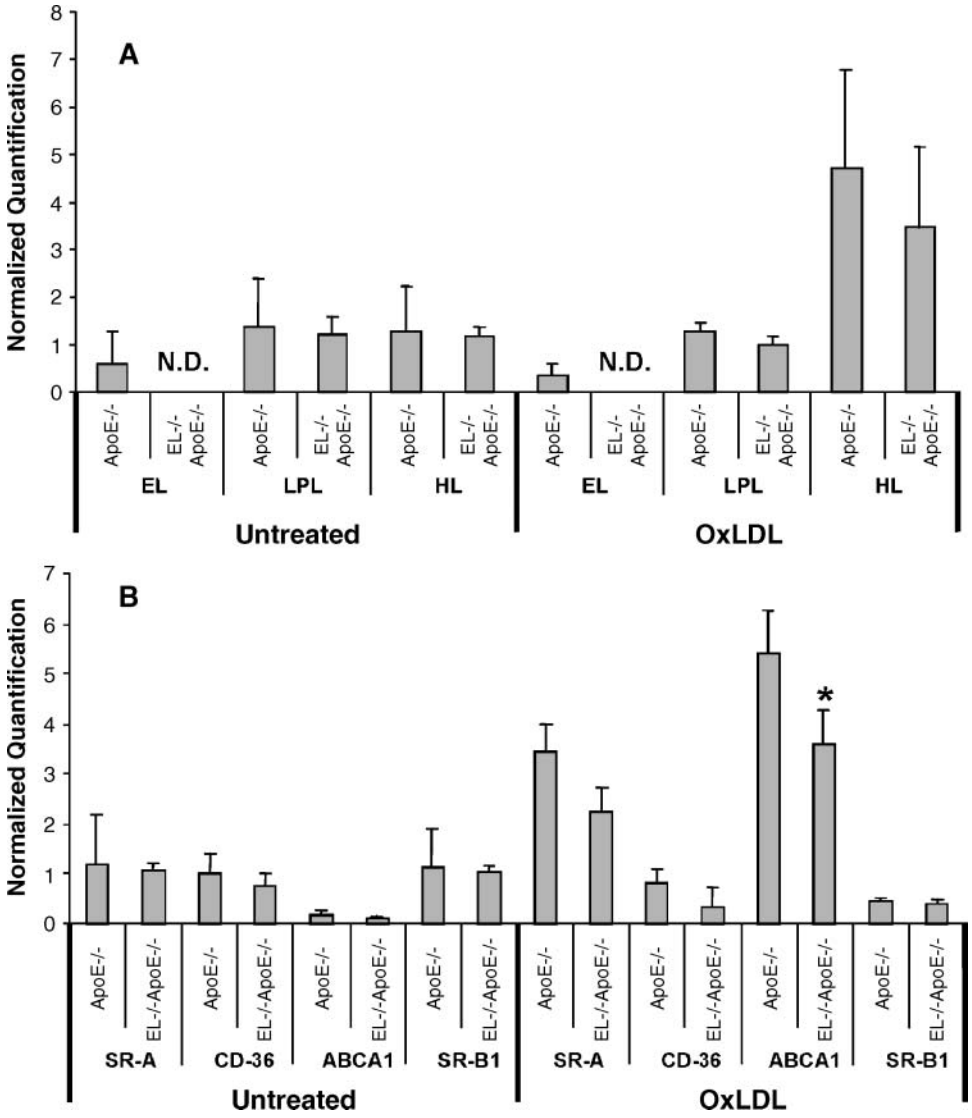

Fig. 6. Relative expression of lipases and lipid homeostasis genes in macrophages. Expression levels are relative to β-actin of vascular lipases (A) and lipid transporters (B) in peritoneal macrophages isolated from EL$^{+/+}$ and EL$^{-/-}$ mice that were untreated or treated with 100 μg/ml oxidized low density lipoprotein (OxLDL) for 16 h as determined by quantitative RT-PCR. Values shown are means ± SD (n = 4). N.D., nondetectable; SR-A, scavenger receptor A; SR-BI, scavenger receptor class B type I. * $P = 0.015$ with respect to wild-type mice. This figure shows the relative amount of each transcript in the presence and absence of EL; it was not meant to compare the level of any gene transcript relative to that of any other, which may differ by several orders of magnitude (e.g., between EL and LPL).

lipid level and lipoprotein profile, EL inactivation may be expected to be slightly antiatherogenic or neutral in the apoE$^{-/-}$ background and possibly proatherogenic in the LDLR$^{-/-}$ background. Overall, the lipid and lipoprotein changes attributable to EL inactivation appear to be mild, however, and based solely on these changes, it would be difficult to predict what effect, if any, EL inactivation might have on atherosclerosis.

The two experimental models (apoE$^{-/-}$ and LDLR$^{-/-}$), exposed to different diets and presenting with different lipid/lipoprotein levels, presented with varying degrees of atherosclerosis at the time of lesion analysis and, additionally, with a gender effect in the apoE$^{-/-}$ model. This allowed us to examine the role of EL at different stages of lesion development. Our data showed that inactivation of EL had no effect on lesion development or complexity in any of the models. Interestingly, previous studies on the other members of the vascular lipase family revealed a proatherogenic role of macrophage LPL, which was independent of any changes in plasma lipid levels; promotion of foam cell formation by LPL was proposed as a mechanism (22, 35). Furthermore, whole body (36) and macrophage-specific (37) HL knockout demonstrated a proatherogenic role of HL, which was associated with increased cholesterol efflux capacity of HDL in HL-deficient animals as well as increased OxLDL uptake in HL-expressing macrophages.

In contrast with our observations, Ishida et al. (27) reported that inactivation of EL retarded atherosclerosis in mice in the apoE$^{-/-}$ background. Their findings on the effect of EL inactivation on blood lipid levels and lipoprotein profiles were very similar to ours, and they concluded that the serum lipid profiles did not explain the attenuated atherosclerosis that they observed in apoE$^{-/-}$ mice. We agree with this assessment; indeed, we observed no significant proatherogenic or antiatherogenic effect mediated by the lipoprotein perturbations observed in EL$^{-/-}$ mice in the apoE$^{-/-}$ background. We further corroborated the lack of effect of EL on aortic atherosclerosis development in LDLR$^{-/-}$ mice. Ishida et al. (27) ascribed the protective effect of the absence of EL mainly to proatherogenic EL functions in the vascular wall in monocyte recruitment and cholesterol uptake, as they found a major reduction in the macrophage content in the lesion in apoE$^{-/-}$ mice. In contrast, we found no difference in macrophage content by quantitative morphometry in either apoE$^{-/-}$ or LDLR$^{-/-}$ mice in the presence or absence of EL.

In addition to similar macrophage contents in the lesions of EL$^{-/-}$ and EL$^{+/+}$ mice, we also found no difference in the transcript levels of HL, LPL, SR-A, CD36, and SR-BI in isolated macrophages under basal conditions or after OxLDL stimulation. There was, however, an attenuation of OxLDL-stimulated ABCA1 transcript levels in isolated EL$^{-/-}$ macrophages, which suggests a reduced capacity for cholesterol efflux. In our current understanding, macrophage-mediated cholesterol efflux is part of reverse cholesterol transport, and its attenuation would have a proatherogenic effect (38, 39), which could have nullified any small atheroprotective effect of the small HDL increase that occurs in EL$^{-/-}$ mice. Ishida et al. (27) attached much significance to EL expression by macrophages, whereas we found EL transcript levels in isolated macrophages to be barely detectable in wild-type mice. We further found a much higher level of expression of LPL transcripts (by 3 orders of magnitude compared with EL in EL$^{+/+}$ macrophages), which did not differ between EL$^{+/+}$ and EL$^{-/-}$ macrophages, and, by immunostaining, LPL protein levels greatly exceeded EL levels within the lesions of LDLR$^{-/-}$ mice (Fig. 5). Our experiments suggest that the extremely low level of EL expression does not play a significant role in modulating macrophage function or atherosclerosis development.

The precise reasons for the difference in findings between the study by Ishida et al. (27) and ours are not known. Both groups used apoE$^{-/-}$ mice in the C57BL/6 background in their experiments, demonstrating similar effects of EL inactivation on lipoprotein profiles and HDL-C levels in this model. In both groups, the targeting strategy led to the absence of functional EL expression. In our case, we documented the absence of any alternatively spliced message in the liver of our knockout mice. Any protein expressed from the targeted allele would have included a short peptide sequence encoded by exon 1 (14 residues in the N terminus, excluding signal peptide) plus 5 aberrant residues encoded by a short stretch of frameshifted exon 3 before a premature stop codon. Such a truncated protein would not be expected to have EL catalytic activity or noncatalytic bridging function. In fact, no transcript for such a peptide was detected. Therefore, the reason for the difference between our study and that of Ishida et al. (27) is unclear but may be related to environmental factors or the stage of development of the atherosclerotic lesions at the time of measurement. In summary, although both we and Ishida et al. (27) demonstrated a role for EL in the modulation of lipoprotein profile and HDL-C in vivo, under the experimental conditions in our laboratory, the absence of EL expression had no effect on atherosclerosis development in two different hyperlipoproteinemic mouse models.

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