Endothelial lipase is synthesized by hepatic and aorta endothelial cells and its expression is altered in apoE-deficient mice

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Abstract **Both LPL and HL are synthesized in parenchymal cells, are secreted, and bind to endothelial cells. To learn where endothelial lipase (EL) is synthesized in adult animals, the localization of EL in mouse and rat liver was studied by immunohistochemical analysis. Furthermore, to test whether EL could play a role in atherogenesis, the expression of EL in the aorta and liver of apolipoprotein E knockout (EKO) mice was determined. EL in both mouse and rat liver was colocalized with vascular endothelial cells but not with hepatocytes. In contrast, HL was present in both hepatocytes and endothelial cells. By in situ hybridization, EL mRNA was present only in endothelial cells in liver sections. EL was also present at low levels in aorta of normal mice. We fed EKO mice and wild-type mice a variety of diets and determined EL expression in liver and aorta. EKO mice showed significant expression of EL in aorta. EL expression was lower in the liver of EKO mice than in normal mice. Cholesterol feeding decreased EL in liver of both types of mice. In the aorta, EL was higher in EKO than in wild-type mice, and cholesterol feeding had no effect. Together, these data suggest that EL may be upregulated at the site of atherosclerotic lesions and thus could supply lipids to the area.**—Yu, K. C-W., C. David, S. Kadambi, A. Stahl, K-I. Hirata, T. Ishida, T. Quertermous, A. D. Cooper, and S. Y. Choi. **Endothelial lipase is synthesized by hepatic and aorta endothelial cells and its expression is altered in apoE-deficient mice.** ***J. Lipid Res.*** **2004.** 45: **1614–1623.**

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Two laboratories independently cloned an enzyme that belongs to the pancreatic lipase gene family (1, 2). This enzyme is now referred to as endothelial lipase (EL) because it was first identified at a high level in embryonic endothelial cells. It was also discovered in cholesterol-loaded macrophages (1). The level seemed to decrease with maturation of endothelial cells, and in the adult, the liver and thyroid gland are the tissues with the highest levels. Furthermore, Northern blot analysis shows that it is present in HepG2 cells, a hepatoma cell line often used as a model of hepatocytes. Thus, its cell of origin in the adult is uncertain because the liver has both endothelial cells and macrophages as well as hepatocytes.

EL has high molecular homology with HL and LPL (1, 2). It contains ~500 amino acids and has a molecular mass of ~55 kDa, with conservation of the catalytic triad as well as potential heparin and lipoprotein binding sites. However, a potential lid region is not well conserved compared with the other lipases of its family. LPL is expressed in muscle, adipose, heart, mammary gland, brain, and macrophages (3, 4). It is secreted and binds to endothelial cells in those organs. HL is synthesized by hepatocytes and remains there, adherent to both hepatocytes and endothelial cells, or is transported to endothelial cells in the adrenal glands and ovaries (5, 6). Thus, both HL and LPL are synthesized by parenchymal cells, secreted, and bind to endothelial cells. Whether EL is processed like HL and LPL is unknown.

As a lipase, EL hydrolyzes water-soluble substrates (1) but does not have a high triglyceride lipase activity, at least in the presence of serum; rather, this enzyme has a readily detectable phospholipase A1 activity (1, 2). In mice, overexpression of EL using either a viral vector or transgenic animals results in a significantly lower plasma concentration of HDL cholesterol and apolipoprotein A-I (apoA-I)

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Abbreviations: apoA-I, apolipoprotein A-I; CA, cholic acid; DAPI, 4,6-diamidino-2-phenylindole; EKO, apolipoprotein E knockout; EL, endothelial lipase; HF, high-fat; IL-1β, interleukin-1β; NC, normal chow; NC+Chol, normal chow plus 2% cholesterol; TNF-α, tumor necrosis factor-α; vWF, von Willbrand factor.

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(1, 2, 7). Conversely, in animals with mutation of the EL gene (7) or infused with an anti-EL antibody, HDL cholesterol levels are significantly increased (8), suggesting that EL modulates plasma HDL levels in vivo. It has recently been reported that EL expression is increased in cultured human umbilical vein and coronary artery endothelial cells by inflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) (9, 10). This, along with its effect on HDL, suggests that this enzyme could be involved in the development of atherosclerosis.

The present study was designed to identify the cellular origin of EL and to ascertain whether it moves from parenchymal cells to endothelial cells. Because liver is the major organ in lipoprotein metabolism and EL expression was detected at a high level in the liver by Northern blot analysis (1), we studied the localization of EL using rat and mouse liver sections. In addition, to learn if the inflammatory response and its ability to modulate plasma levels of HDL contribute to atherogenesis, we studied the regulation of EL expression in liver and aorta in response to a high-fat (HF) diet in normal and apoE-deficient animals, an animal model for atherosclerosis.

MATERIALS AND METHODS

Animals and diets

Both wild-type C57BL/J6 and apoE knockout (EKO) mice (Jackson Laboratory, Bar Harbor, ME) were fed the following diets for 4 weeks: normal chow (NC), normal chow plus 2% cholesterol (NC+Chol), a HF diet (11), and a HF diet supplemented with 1% cholic acid (HF+CA). HF diets with or without CA were purchased from Harlan Teklad (Madison, WI). Cholesterol was purchased from Sigma (St. Louis, MO).

Western blot analysis

Eighty mircograms of total liver membranes were subjected to 7% SDS-PAGE followed by transfer to a nitrocellulose membrane (Bio-Rad, Hercules, CA). The membrane was then incubated with anti-EL antiserum for 2 h at room temperature. After washing and incubation with secondary antibodies conjugated with HRP, the membrane was developed with ECL (Amersham Pharmacia Biotech).

Specificity of anti-EL antibodies

The specificity of polyclonal anti-EL antibodies was tested using Western blots as described above. Briefly, media collected from COS cells and CHO cells producing human EL and HL, respectively, were subjected to electrophoresis followed by transfer to a nitrocellulose membrane. The membrane was then incubated with polyclonal anti-EL or anti-HL antibodies followed by secondary antibodies labeled with HRP. Anti-EL antibodies recognized only EL and not HL (data not shown). Conversely, anti-HL antibodies bound only to rat HL and not EL. Thus, these antibodies are specific for the corresponding antigens and do not cross-react with other lipases that are likely to be present on endothelial cells.

In situ hybridization

In situ hybridization was performed as described previously (1) using slides that were generated from paraformaldehyde-fixed, paraffin-embedded mouse liver sections according to established methods or were purchased from Novagen (Madison, WI). A 611 bp *Eco*RI mouse EL cDNA fragment encoding the carboxyl-terminal 52 amino acids and 3′ untranslated region was cloned into pBluescript KS(+). This fragment was used for in vitro RNA probe transcription. Both antisense and sense cRNA probes were labeled with [^{35}S]dUTP. Hybridization, washing, and probe detection were performed using SureSite II (Novagen) according to the manufacturer's instructions.

Immunostaining

The tissues were fixed with PBS containing 4% paraformaldehyde and then placed in OCT embedding medium (12) and frozen over dry ice. Sections were cut to 8 μm thickness and placed onto glass slides. The sections were incubated in PBS plus 0.1% Triton X-100 for 5 min, in PBS plus 3% BSA for 30 min, and then with antibodies. For staining endothelial cells, an anti-von Willebrand factor (vWF) antibody (Sigma) was used followed by FITC-labeled secondary antibodies (Molecular Probes, Inc., Eugene, OR). For staining HL and EL, polyclonal anti-HL or anti-EL antibodies were used, respectively, followed by rhodamine-labeled secondary antibodies. Digital images of the stained sections were obtained using a Molecular Dynamics Multiprobe confocal laser microscope (Sunnyvale, CA).

Human adult thyroid tissue sections were purchased from Novagen. Tissues were fixed in 4% paraformaldehyde, embedded in paraffin, and sectioned at 5 μm thickness. Before use, paraffin was removed with three 5-min washes in xylene and two 5-min washes in 100% ethanol as suggested by the manufacturer's instructions. The sections were washed in PBS containing 1% BSA, 10% fetal calf serum, and 1% normal donkey serum to block nonspecific binding. Primary and secondary antibodies were diluted in blocking solution and incubated for 1 h. The sections were mounted in 90% glycerol/PBS containing 1 mg/ml paraphenylenediamine. Monoclonal anti-CD31 antibodies were purchased from BD Pharmingen. Nuclei were counterstained with 4,6-diamidino-2-phenylindole (DAPI).

Real-time PCR

Total RNA was prepared from livers and aortas obtained from wild-type or apoE-deficient mice using a kit from Qiagen, and reverse transcriptase reaction was performed on 1 μg of RNA using random hexamer reverse transcriptase (Gibco BRL, Life Technologies, Vienna, Austria). The primers and probe for mouse EL were designed using Primer Express 1.5 (Applied Biosystems, Foster City, CA). The probe for EL was labeled with a reported dye (FAM) and quencher dye (TAMRA). The primers and TaqMan™ probe for GAPDH were purchased from ABI. Sequences of primers and probe are available upon request. The probe for GAPDH was labeled with a reporter dye (VIC) and TAMRA. RT-PCR was performed using 40 amplification cycles (95°C for 15 s, 55°C for 1 min, 72°C for 30 s). The level of GAPDH RNA was quantified and used to normalize the concentration of EL in each sample.

RESULTS

Immunohistochemical analysis of liver sections

The liver is one of the organs that expresses the highest levels of EL (1). It is also the most important organ in lipoprotein metabolism. To learn where EL is localized in the liver, we performed a series of immunohistochemical experiments using anti-EL antibodies. For a reference, mouse and rat liver sections were incubated with anti-

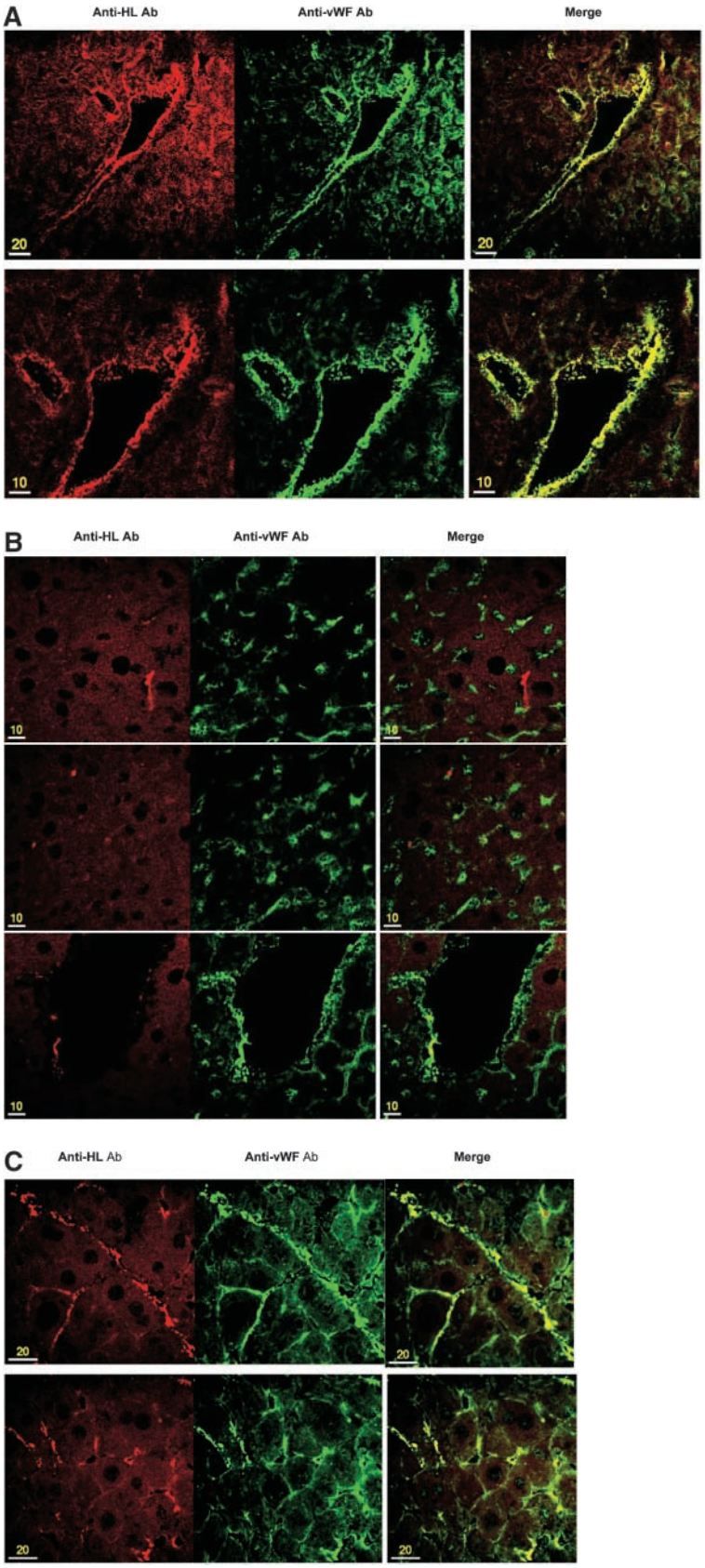

Fig. 1. Immunostaining of rat HL in rat and mouse livers. Livers were prepared for immunostaining with antibodies (Ab) against endothelial von Willbrand factor (vWF) and rat HL as described in Materials and Methods. A: A rat liver section showing the colocalization of the HL (red) with hepatocytes and with endothelial cells (green). B: A wild-type mouse liver section showing the colocalization of the HL (red) with hepatocytes but not with endothelial cells (green). C: A liver section from a mouse transgenic for rat HL showing the colocalization of the HL (red) with hepatocytes and with endothelial cells (green). μ, unit of the scale bars.

HL antibody followed by rhodamine-conjugated secondary antibody. Endothelial cells were localized with anti-vWF antibody and a FITC-conjugated secondary antibody. In rat liver, HL localizes to both hepatocytes and endothelial cells (**Fig. 1A**). In mouse liver, HL localizes with hepatocytes but not endothelial cells (Fig. 1B). Liver sections obtained from mice transgenic for rat HL have HL on endothelial cells as well as parenchymal cells (Fig. 1C).

Using the same technique, rat and mouse liver sections were stained with anti-EL antibodies and anti-vWF IgG. EL colocalizes with endothelial cells in the rat liver (**Fig. 2A**) and mouse liver (Fig. 2B). Unlike HL, EL is present only in endothelial cells and is virtually absent in hepatocytes. To further confirm these results, primary mouse hepatocytes and endothelial cells were isolated and stained for EL. Similar to the liver sections, EL was expressed only by the primary hepatic endothelial cells and not by the primary hepatocytes (data not shown).

Immunostaining of EL in other tissues

Northern blot analysis shows that EL is present in placenta, liver, lung, ovary, thyroid gland, and testis but not in adrenal gland (1). The tissue distribution of EL was further assessed by immunostaining. EL was not present in heart or muscle (data not shown). It was detected in the lung as previously reported (data not shown) and adrenal glands (**Fig. 3A**) but not in ovaries (Fig. 3B). We also performed immunohistochemical analysis of thyroid sections because EL expression was quite high in thyroid (1). In thyroid, as shown in Fig. 3C, EL is expressed in the endothelial cells of the large vessels (red). Nuclei were counterstained with DAPI (blue). Thus, EL protein colocalizes with organs in which its mRNA is present and is generally found on endothelial cells in the organ.

In situ hybridization of EL in mouse liver

Immunostaining data show that EL is present in endothelial cells of the liver. To determine whether, analogous

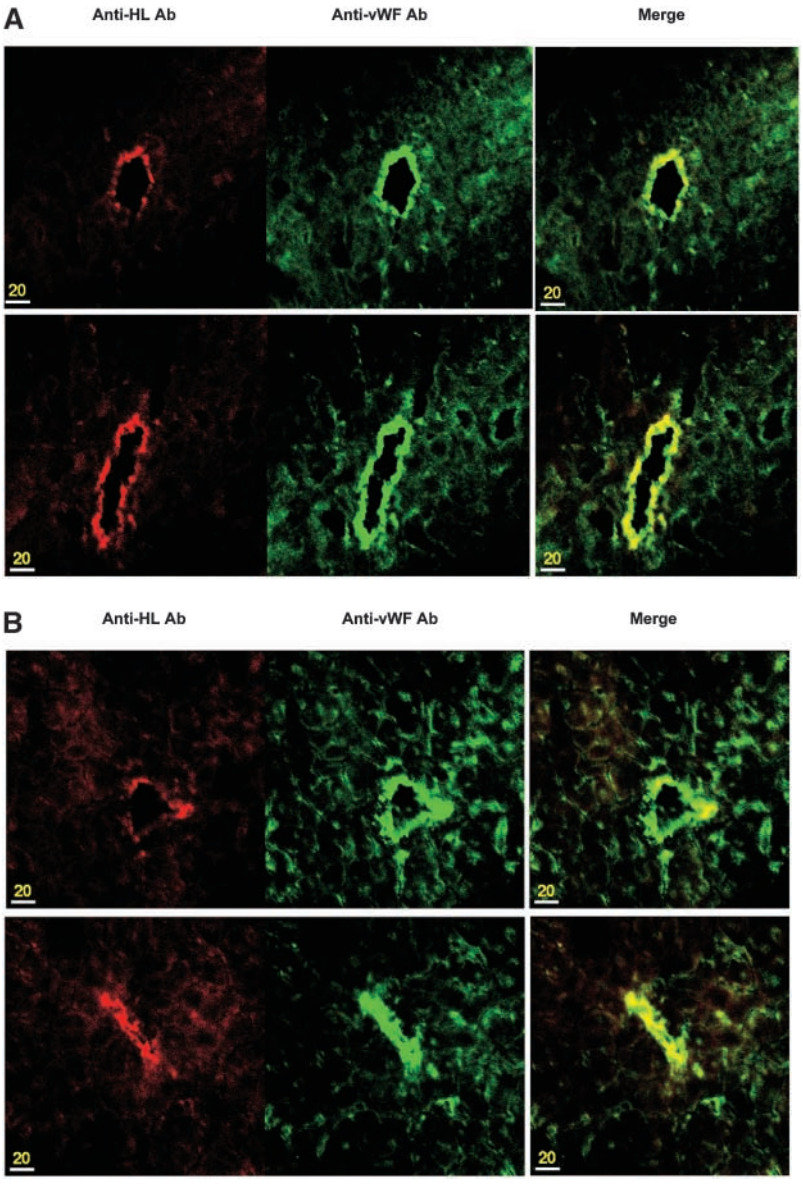

Fig. 2. Immunostaining of endothelial lipase (EL) in rat and mouse livers. Livers were prepared for immunostaining with antibodies against endothelial vWF and EL as described in Materials and Methods. A: A rat liver section showing the colocalization of the EL (red) with endothelial cells (green). B: A wild-type mouse liver section showing the colocalization of the EL (red) with endothelial cells (green).

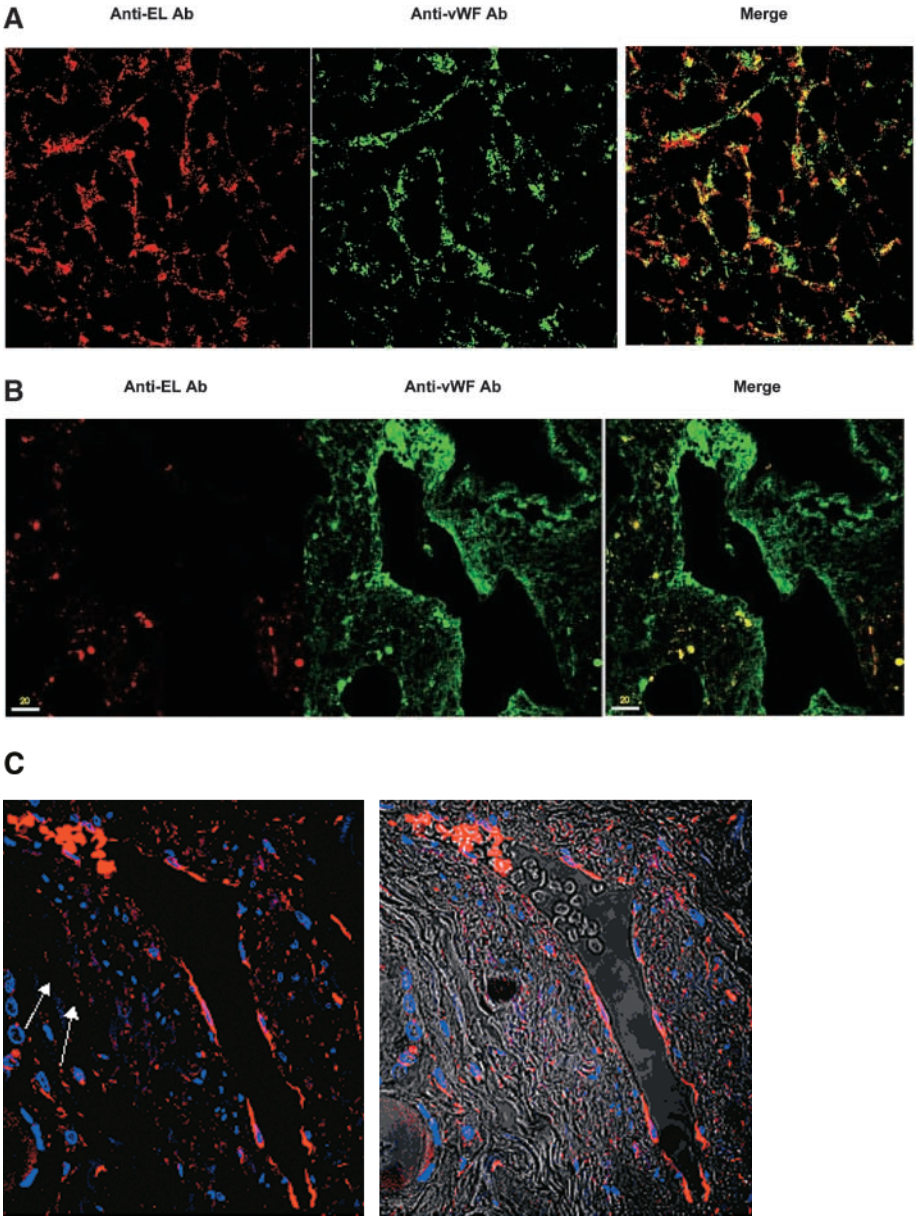

Fig. 3. Immunostaining of EL in adrenal glands, ovaries, and thyroids. Wild-type mouse livers were prepared for immunostaining with antibodies against endothelial vWF and EL as described in Materials and Methods. A: Presence of EL (red) colocalized with endothelial cells (green) in a section of the adrenal glands. B: Absence of EL in a section of the ovary despite the presence of endothelial cells (green). C: Presence of EL (red) in thyroid sections obtained from human adult. Nuclei were counterstained with 4,6-diamidino-2-phenylindole (blue). Arrows indicate the presence of erythrocytes in the sections.

to HL and LPL, EL is synthesized and secreted by hepatocytes and then translocated to endothelial cells or whether EL is synthesized by endothelial cells, in situ hybridization with an EL cRNA probe was performed on mouse and rat liver sections. If EL is synthesized in the hepatocyte and transferred to endothelial cells, then it would be analogous to HL and LPL. EL mRNA was readily identified in endothelial cells surrounding the large blood vessel in the mouse liver and could be seen in endothelial cells of the sinusoids. It was not detected in hepatocytes (**Fig. 4A**). Similar results were obtained with rat liver sections (Fig. 4B). Thus, unlike HL and LPL, EL is synthesized by the endothelial cells and remains localized there.

Expression of EL in aorta and liver in EKO mice

EL mRNA level is increased by inflammatory cytokines such as TNF-α and IL-1β in cultured endothelial cells (9, 10). EL modulates plasma HDL levels in transgenic and knockout animals (7). Together, these observations suggest that EL could play a role in the development of atherosclerosis, which is an inflammatory condition. Using EKO mice fed a HF diet, the expression of EL in aorta and

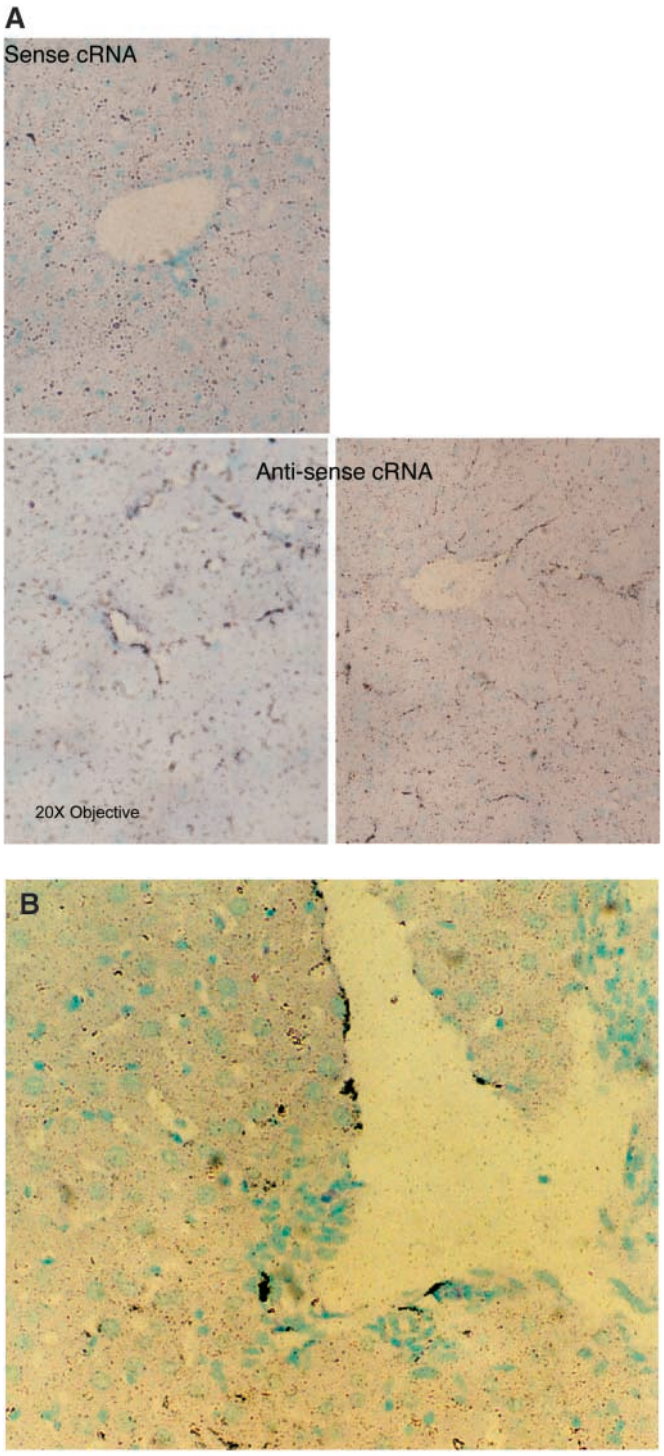

Fig. 4. In situ hybridization of EL expression. A: ^{35}S-labeled cRNA sense and antisense probes for EL were hybridized to sections of mouse liver (20× objective). B: Similarly, rat liver sections were hybridized with the same probe and only the antisense probes showed a signal above background and rat liver (20× objective).

liver was compared by immunohistochemistry. EL is expressed at a high level in the aorta of EKO animals (**Fig. 5A**, upper panels), whereas the expression of EL in the aorta of wild-type animals appears to be minimal (Fig. 5A, lower panels).

Using Western blotting, we then determined the effect of an atherogenic phenotype on the level of EL expression in the liver. EL expression was significantly reduced in the liver of EKO animals (Fig. 5B, lanes 6–9) compared with wild-type animals (Fig. 5B, lanes 1–5). These data together suggest that the regulation of EL expression is tissue specific and that the expression of the enzyme may be upregulated at the site of atherosclerotic lesions.

Effect of fat, cholesterol, and bile acid feeding on the expression of EL in EKO mice

To learn how the effect of the apoE$^{-/-}$ gene on the expression of EL in liver and aortic endothelial cells was determined, animals were fed diets of different cholesterol content and atherogenic potential. Animals were fed one of four diets: NC, NC plus 2% cholesterol (NC+Chol), a diet containing saturated fat and cholesterol (HF), and another diet of saturated fat containing cholesterol and 1% CA (HF+CA) for 4 weeks. Plasma triglyceride and cholesterol levels were measured (**Table 1**). Serum cholesterol levels increased significantly in EKO animals but not in wild-type mice fed 2% cholesterol (NC+Chol) compared with those fed the NC diet. The addition of saturated fat (HF) caused an increase in cholesterol in the wild-type mice and a further increase in EKO mice. Serum triglyceride levels of the mice were not affected by cholesterol feeding.

The level of EL mRNA in the liver and aorta of those animals was determined using RT-PCR. On all of the diets, EL expression in the liver of EKO mice was significantly lower than in livers of control mice ($P < 0.05$) (**Fig. 6A**). Cholesterol feeding, whether alone or with saturated fat, decreased EL in livers of both control and EKO mice. Interestingly, the addition of CA to a diet containing saturated fat (HF+CA) significantly increased EL expression in the liver compared with a diet containing saturated fat alone (HF) (Fig. 6A). This is consistent with the notion that bile salts play a role in the hepatic inflammation (13, 14) seen in this model of atherosclerosis. In contrast to the liver, none of the diets affected EL levels in the aorta of either control or EKO mice (Fig. 6B). On each diet, EL expression was higher in endothelial cells of EKO mice than in those of control mice. This was not statistically significant because of the variability and small number in each group. However, when the data were pooled to compare the two groups, EL expression was significantly higher in EKO mice compared with control mice ($P < 0.01$; data not shown). These data together demonstrate that there is a complex and tissue-specific regulation of EL expression.

DISCUSSION

In the present study, by use of in situ hybridization we demonstrated that EL is expressed in hepatic endothelial cells and not in hepatocytes. In addition, it was observed that the level of EL expression in hepatic endothelial cells

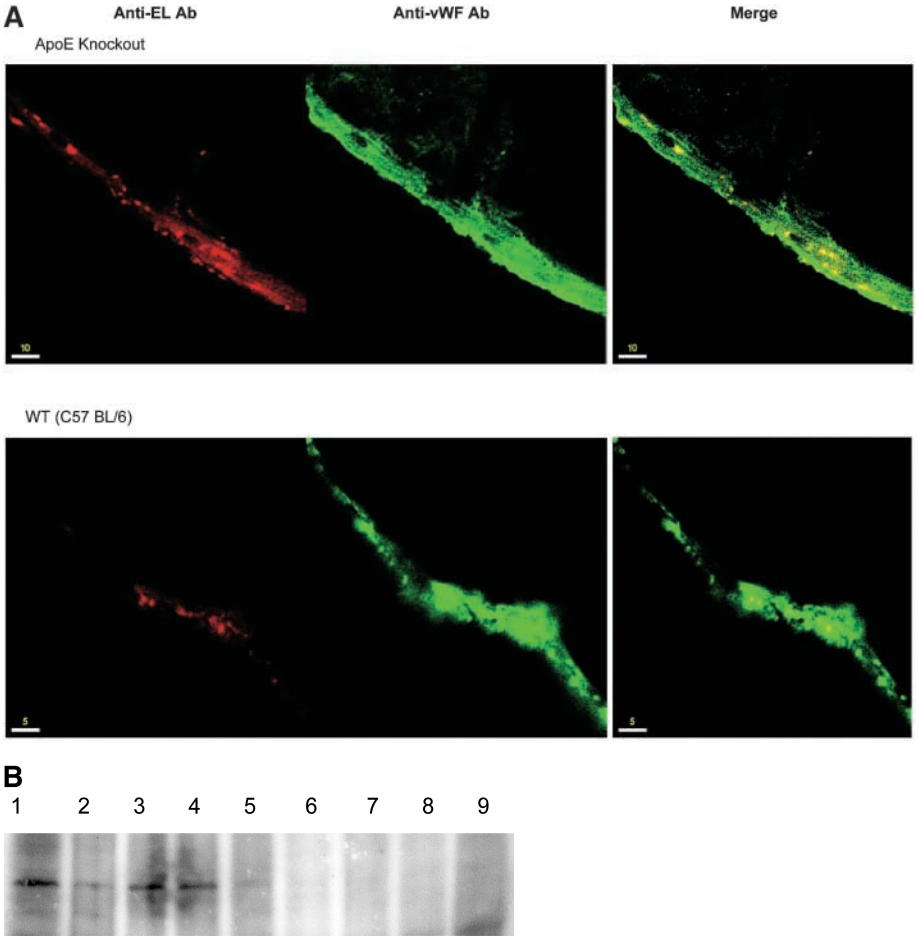

Fig. 5. Expression of EL in the aorta and liver of wild-type and apolipoprotein E knockout (EKO) mice. Immunostaining and Western blotting were used to determine the expression of EL in livers of wild-type and EKO mice as described in Materials and Methods. A: An EKO mouse aorta (upper panels) and a wild-type (WT) mouse aorta (lower panels) section showing the colocalization of the EL (red) with endothelial cells (green). B: Western blots showing the protein expression of EL in total liver membranes prepared from wild-type mice (lanes 1–5) and EKO mice (lanes 6–9).

is lower in EKO mice and higher in the aorta of EKO mice, suggesting a tissue-specific regulation of EL expression. This was confirmed by cholesterol feeding, which reduced EL mRNA levels in hepatic endothelial cells and did not affect mRNA levels in aortic endothelial cells.

The tissue-specific expression of EL in the adult is different from that of LPL and HL. In rats and humans, HL is synthesized by the hepatocytes and remains there, adherent to both hepatocytes and endothelial cells, or is transported to the endothelial cells in the adrenal glands and ovaries. In mice, HL circulates in the plasma, presumably because of a lack of heparin binding that results from variation in the heparin binding region in this species. LPL is synthesized in parenchymal cells of muscle, adipose, heart, mammary gland, and brain and functions while bound to the luminal surface of endothelial cells in these organs (15, 16). Thus, both HL and LPL are synthesized at one site and translocate to another site for function.

EL is found at a high level in embryonic endothelial cells, but its level decreases with maturation. In the adult, it is detected in a number of tissues, including placenta, lung, liver, testis, thyroid, and ovary. EL mRNA was detected at a high level on Northern blots of RNA from whole liver and HepG2 cells (1). Thus, it was possible that in the adult the enzyme is synthesized in parenchymal cells and secreted and bound to endothelial cells. Immu-

TABLE 1. Plasma cholesterol and triglyceride levels in WT and EKO mice

Animals and Diet	Cholesterol (n = 3)	Triglycerides (n = 3)
	mg/dl	
NC		
WT	77.7 ± 0.88	34.6 ± 2.3
EKO	684 ± 187	109.7 ± 16.7
NC + 2% cholesterol		
WT	59.0 ± 6.6	22.45 ± 2.6
EKO	1,097 ± 59	52.49 ± 4.09
HF		
WT	146 ± 28.58	27.3 ± 7.7
EKO	1,583 ± 18.9	74.7 ± 6.06
HF + 2% cholic acid		
WT	150 ± 22.7	43.7 ± 5.78
EKO	1,351 ± 131	65.1 ± 8.27

EKO, apolipoprotein E knockout; HL, high fat; NC, normal chow; WT, wild type.

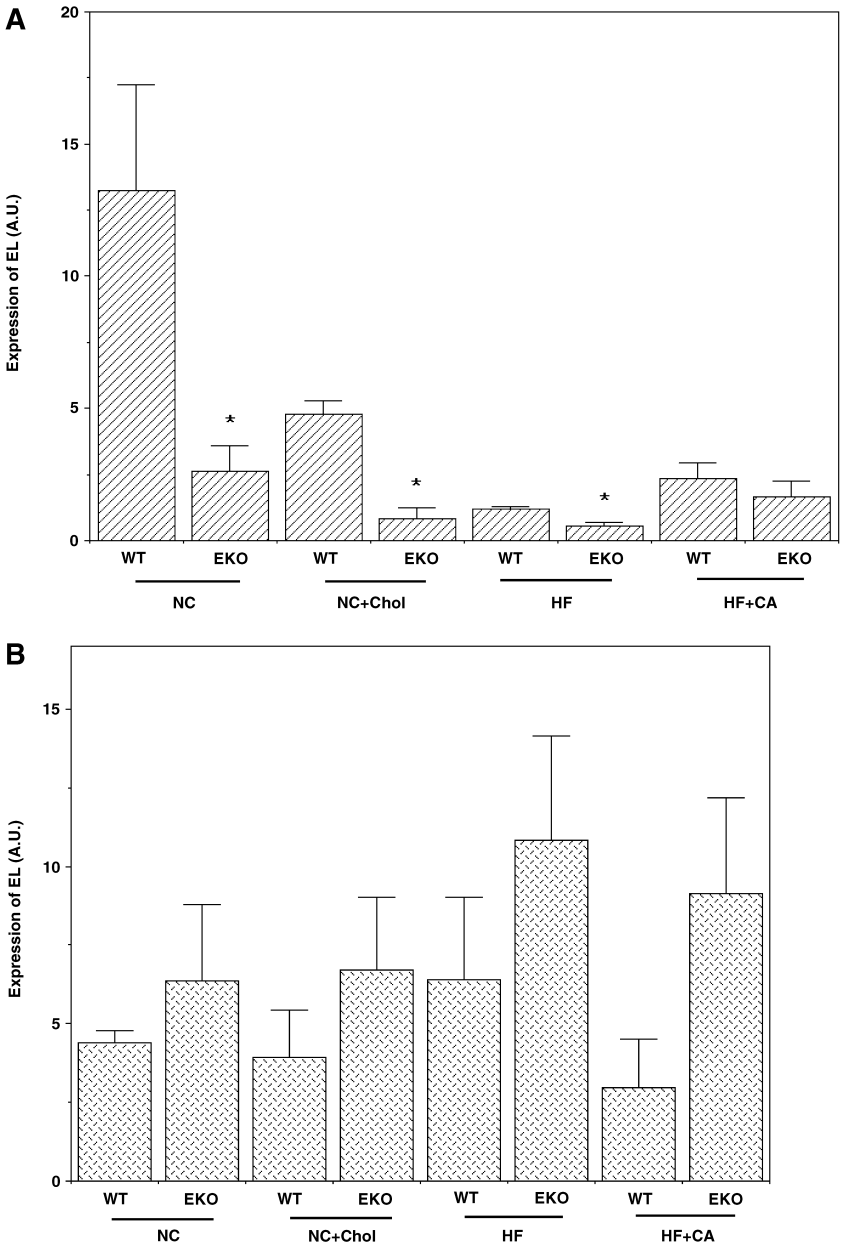

Fig. 6. Effect of fat, cholesterol, and bile acid feeding on the expression of EL in EKO mice. Wild-type (WT) and EKO mice were fed with normal chow (NC), normal chow plus 2% cholesterol (NC+Chol), a high-fat (HF) diet, and a HF diet plus 1% cholic acid (HF+CA) for 4 weeks. Real-time PCR was performed as described in Materials and Methods using total RNA prepared from aorta (A) and liver (B) of those animals. Data are expressed as mean arbitrary units (A.U.) ± SD (n = 4). * $P < 0.05$.

nostaining data show that EL is present in the endothelial cells lining the vessels in the mouse liver but is virtually undetectable in the hepatocytes. In situ hybridization revealed that, unlike HL, EL mRNA is present in endothelial cells but not in hepatocytes. Thus, EL is different from HL and LPL in that EL functions at the site where it is synthesized. The difference in distribution between cell types suggests nonoverlapping functions for HL and EL, despite their similar localization and molecular sequences. Interestingly, all of these lipases are expressed in macrophages. Indeed, in the other tissues where it is abundant, such as lung and thyroid, EL is localized to endothelial cells.

The role of LPL and HL in the development of atherosclerosis has been investigated by several laboratories. These enzymes appear to have both proatherogenic and antiatherogenic roles. LPL is antiathrogenic because the enzyme plays a role in clearing plasma chylomicron and VLDL remnant particles (17, 18) as well as LDL by bridging the lipoproteins to the cell surface receptors (19). However, in the vessel wall, LPL may have proatherogenic properties. For instance, macrophages secrete significant amounts of LPL (20–22), and the absence of macrophage-derived LPL reduced the formation of atherosclerotic lesions. However, LPL within the vessel wall increases lipo-

protein retention in the subendothelial matrix (23–25), and it was shown that LPL may act as a monocyte adhesion protein (26). Together, these properties could contribute to the formation of atherosclerotic lesions.

HL is involved in the selective uptake of HDL (27, 28) and thus decreases HDL levels. There is an inverse relationship between HDL levels and the incidence of atherosclerotic coronary artery disease (29, 30). This may be because HL decreases HDL levels by converting HDL_2 to HDL_3 and facilitates the selective uptake of HDL cholesteryl ester (27, 28). Transgenic animals as well as knockout animals were used to study whether HL plays a role in the development of atherosclerosis in vivo. Busch et al. (31) reported that aortic cholesterol levels were reduced in mice that overexpress human HL. In contrast, HL deficiency also reduced the susceptibility to atherosclerosis in EKO mice (32). Thus, it is not clear yet whether or not HL contributes directly to the development of atherosclerosis, although its level is related to susceptibility to atherosclerosis.

EL could potentially affect the development of atherogenesis through its effects on HDL levels and via local effects, because it is synthesized by the endothelial cells and its expression is upregulated by inflammatory cytokines such as TNF-α and IL-1β (9). Like LPL and HL, EL can bind apoB-containing lipoproteins and thus potentially facilitates their retention and cellular uptake, and it can increase the selective uptake of cholesteryl esters from HDL. Overexpression of EL reduced HDL levels (1, 2, 7); conversely, animals deficient in EL showed significantly increased levels of plasma HDL (7). Furthermore, it is established that atherosclerosis is an inflammatory disease and that EL expression can be induced by inflammatory cytokines. In the present studies using EKO mice, an animal model of atherosclerosis, it was found that EL expression is enhanced in the aorta and reduced in the liver of mice developing atherosclerosis. Thus, during atherogenesis, EL in endothelial cells might contribute to cholesteryl ester uptake and EL from macrophages could contribute to the retention of lipoproteins in the lesion. In animals lacking both EL and apoE, there was a significant decrease in atherosclerotic lesions compared with apoE-deficient mice (T. Ishida, S. Y. Choi, J. Spin, et al., unpublished data). Together, these data suggest that, similar to LPL and HL, EL also has proatherogenic effects.

The change in EL expression in apoE-deficient mice raises the question of what factors in addition to cytokines might alter EL expression and whether there may be organ-specific regulation of its levels. Indeed, cholesterol feeding decreased EL mRNA level in hepatic endothelial cells. The effect was augmented somewhat by the addition of saturated fat to the diet but not further augmented by the addition of CA. The latter not only further increases serum cholesterol levels but also causes hepatic inflammation. Quantification of EL in the aorta is more difficult because of the small amount of tissue available, but none of these regimes seemed to alter EL mRNA levels in aortic endothelial cells. The mechanism of induction in apoE-deficient mice is open to speculation, but this effect certainly could be attributable to the inflammation in the aorta of these animals that occurs as part of the atherosclerotic process.

In summary, the present studies demonstrated that EL is synthesized by hepatic endothelial cells and not by hepatocytes. It is present in the aorta, and its expression in the aorta is higher in animals developing atherosclerosis compared with normal animals. In contrast, its expression is downregulated in the liver of those animals. Additionally, in liver but not in aorta, its expression may be regulated by cholesterol flux. Thus, EL could play a role in the development of the atherosclerosis by altering plasma levels of lipoproteins as well as by directly affecting cholesterol and lipid flux at the site of lesions.

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