Update on the Role of Endothelial Lipase in High-Density Lipoprotein Metabolism, Reverse Cholesterol Transport, and Atherosclerosis

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Endothelial lipase (EL) is a phospholipase that belongs to the lipoprotein lipase (LPL) family, which includes LPL and hepatic lipase (HL). Similar to LPL and HL, EL regulates lipoprotein metabolism, mainly high-density lipoprotein (HDL) metabolism and HDL cholesterol (HDL-C) levels in humans and mice. Existing data strongly suggest that inhibition of EL in humans would be expected to increase the HDL-C level. However, it has not been definitively established whether the effect of EL activity on HDL-C levels translates into effects on reverse cholesterol transport or atherosclerosis. The available data regarding the impact of EL expression and activity on HDL metabolism, reverse cholesterol transport, and atherosclerosis are reviewed. (Circ J 2010; 74: 2263–2270)

Key Words: Apolipoprotein; Atherosclerosis; High-density lipoprotein; Lipids; Lipoprotein

The benefit of low-density lipoprotein cholesterol (LDL-C) reduction therapy to avoid or decrease the likelihood of atherosclerotic disease has been widely established. However, accumulating clinical evidence implies limitations of LDL reduction therapies, as there remains substantial residual risk. Plasma levels of high-density lipoprotein-cholesterol (HDL-C) are a negative risk factor for cardiovascular disease (CVD). The antiatherogenic function of HDL is mediated at least in part by the promotion of reverse cholesterol transport (RCT). In addition, the HDL particle itself or its major associated protein, apolipoprotein A-I (apoA-I), has a variety of antioxidative, antiproliferative, and antiinflammatory activities. Thus, HDL-C raising therapy would be of great interest for reducing residual risk. Current approaches to raising plasma HDL-C levels have limitations; for example, niacin modestly increases plasma HDL-C levels but there are issues with tolerability, and fibrates have relatively small effects on HDL-C in most patients. Some experimental apoA-I-based infusion therapies have reportedly attenuated the progression or induced the regression of atherosclerosis. A large-scale clinical trial of the cholesteryl ester transfer protein (CETP) inhibitor, torcetrapib, in combination with atorvastatin failed to reduce the incidence of the major cardiovascular events, despite a marked increase in plasma HDL-C levels and a decrease in plasma LDL-C levels. Although the effect of CETP inhibition will be clarified by the results of ongoing clinical studies with the next generation of CETP inhibitors, it is considered that the effectiveness of HDL-raising therapies should be evaluated by the effect on RCT or clinical outcomes.

Endothelial lipase (EL) is a phospholipase that belongs to the lipoprotein lipase (LPL) gene family. EL is a regulator of plasma HDL-C levels, and here we review recent findings regarding the role EL in RCT and atherosclerosis.

EL Belongs to the LPL Family

Well-characterized members of the triglyceride lipase family include LPL and hepatic lipase (HL). LPL is mainly synthesized by adipocytes, skeletal muscle cells, and cardiac muscle cells, whereas HL is synthesized by the liver. LPL and HL are well known to regulate plasma cholesterol and triglyceride (TG) levels. LPL has dominantly TG lipase activity and hydrolyzes TG-rich lipoproteins, whereas HL has both TG lipase and phospholipase activity and hydrolyzes LDL and HDL, generating small LDL and small HDL.

Mature EL is a 68-kD glycoprotein, and it shares amino acid sequence similarity with lipases, having 44% amino acid identity to LPL and 41% amino acid identity to HL. However, the sequence homology of the lid lesion, which determines the specificity of lipases, largely differs between EL and LPL or HL (Table). EL is a unique lipase because it is synthesized mainly by vascular endothelial cells, and to a lesser extent by macrophages and smooth muscle cells. EL has a signal sequence in the amino-terminus, and therefore is secreted from these vascular cells, after which it binds to cell surface proteoglycans where it exerts its action.
EL and HDL Metabolism

In contrast to LPL and HL, EL is primarily an A1 phospholipase and hydrolyzes HDL-phospholipids at the sn-1 position. Strauss et al reported that EL efficiently cleaves nonesterified fatty acid (NEFA) from HDL-phospholipids, supplying NEFA for EL-expressing cells.13 Jahangiri et al showed that the reduction in HDL particle size mediated by EL was related to the amount of phospholipid hydrolysis.13 It has been reported that EL overexpression accelerates both renal apoA-I catabolism13 and hepatic cholesterol uptake through scavenger receptor class B type I (SR-B1).14 As a result, EL promotes the remodeling and elimination of HDL particles. In fact, plasma HDL-C levels are increased in EL knockout (−/−) mice and decreased in EL transgenic mice, indicating the inverse dosage effect of EL on plasma HDL-C levels in mice.15 The HDL particle size is increased in EL−/− mice, and turnover studies demonstrated that the clearance of HDL from the plasma was delayed in these mice.16 Similarly, studies have indicated that in humans the plasma EL mass inversely correlates with plasma HDL-C levels and positively correlates with features of atherosclerosis and metabolic syndrome.17 These findings indicate that EL is a major determinant of plasma HDL-C levels.

EL has conserved heparin-binding properties and lipid-biding domains,18 and therefore interacts directly with heparan sulfate proteoglycans (HSPG) and circulating lipids/lipoproteins. EL acts as bridge between lipoprotein and endothelial cells, promoting lipoprotein incorporation.19 As is the case with LPL and HL, EL has noncatalytic bridging functions in addition to its catalytic phospholipase activity, and both of these functions are considered to participate in modulating plasma HDL-C levels.20 Adenovirus-mediated hepatic EL overexpression accelerates plasma HDL clearance through both catalytic and noncatalytic bridging functions in vivo, and both the catalytic and noncatalytic bridging functions of hepatic EL promoted HDL-C selective uptake in in-vitro studies.21 The delayed HDL clearance in EL−/− mice is likely mediated by both the hydrolyzing and ligand-binding functions of EL.22 These findings suggest that EL inhibition would increase plasma HDL-C levels by inhibiting HDL and apoA-I catabolism.

Because all of these lipases bind to the endothelium through HSPG, they are released by heparin administration and the post-heparin plasma is high in lipase activity and mass compared with preheparin plasma. Previous studies have shown that heparin treatment increases plasma EL mass 3-fold.17 The EL mass in both the pre- and post-heparin plasma inversely correlates with the plasma HDL-C levels.

Regulation of EL Expression and Activity

It has been reported that EL expression is highly regulated by a variety of factors (Figure 1). For instance, tumor necrosis factor α, interleukin (IL)-1β and biomechanical forces have induced EL mRNA expression in human endothelial cells.23 Although phospholipase- and TG-lipase activities are barely detected in unstimulated endothelial cells, cytokine treatment stimulates phospholipase- and TG-lipase activities concomitant with an increase in EL expression.24 Moreover, lipopolysaccharide induces macrophage EL expression via activation of toll-like receptor 4.25,26 Shimokawa et al reported that angiotensin II and hypertension induce EL expression in vascular smooth muscle cells.27 Thus, the expression and activity of EL is induced by inflammatory stimuli in vascular cells existing in atherosclerotic lesions, whereas LPL or HL expression is reduced in response to these stimuli. Indeed, Paradis et al reported that in humans the plasma EL mass positively correlates with inflammatory markers C-reactive protein, IL-6, and the sPLA2-IIa concentration.28 Furthermore, Badellino et al showed that experimental low-dose endotoxemia in humans resulted in a 2.5-fold increase in the EL concentration, which was associated with decreases in both total and HDL-phospholipids;29 that study paper also demonstrated a strong correlation of EL mass with several markers of inflammation.28 Also, statins reduce EL expression and phospholipase activity in endothelial cells and macrophages, and reduced plasma EL mass accompanies increased plasma HDL-C levels in humans.30,31 According to those findings, upregulation of EL in inflammatory states, including atherosclerosis and metabolic syndrome,32 may contribute to the low HDL-C levels seen in these conditions. Intervention to reduce inflammation might be expected to raise plasma HDL-C levels by decreasing EL expression.

It has been postulated that EL undergoes tissue-specific regulation in vivo. Yu et al reported that hepatic EL expression decreased in apoE−/− mice while aortic EL expression increased.33 Feeding wild-type mice with a high-fat diet also decreased hepatic EL expression. A similar finding was obtained with LDL receptor (LDLR)−/− mice (Yasuda et al, unpublished data 2010). Therefore, it is considered that the cellular cholesterol content might regulate hepatic EL expression, although the precise mechanism is still unknown.

EL activity is also regulated through posttranscriptional mechanisms. Griffon et al recently reported that EL forms a dimer in human plasma, and that the homo-dimer formation is essential for the maintenance of EL activity,34 as is the case with LPL and HL. In addition, EL activity is regulated

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EL, endothelial lipase; LPL, lipoprotein lipase; HL, hepatic lipase; PL, phospholipid; TG, triglyceride; HDL, high-density lipoprotein; LDL, low-density lipoprotein; RCT, reverse cholesterol transport; KO, knockout; NA, not available.
by proprotein convertases (PCs). PCs induce site-specific proteolysis, which plays a crucial role in regulating many fundamental biological pathways, including the sequential initiation of activation of blood coagulation factors and activation of caspases and digestive enzymes. Jin et al have shown that EL is proteolytically processed into 40-kDa and 28-kDa fragments and inactivated by PCs.\(^{35}\) Overexpression of profurin, an inhibitor of PCs, results in a reduction of plasma HDL-C levels in wild-type mice, but this effect is not observed in \(EL^{-/-}\) mice. Administration of profurin to mice inhibits the cleavage of EL and increases plasma phospholipase activity, so PC inhibitors may reduce plasma HDL-C levels by increasing EL activity. Shimamura et al reported that angiopoietin-like 3 (ANGPTL3) is known to act as an endogenous EL inhibitor.\(^{36}\) ANGPTL3 knockout mice show low plasma HDL-C levels accompanied by increased phospholipase activity, indicating that EL activity may be elevated in these mice. Furthermore, plasma ANGPTL3 levels significantly correlate with plasma HDL-C levels in humans. Inhibition of PC cleavage reduces the activation of ANGPTL3, which may attenuate the inhibitory effect of ANGPTL3 on EL activity. Thus, PCs regulate EL activity directly through cleavage of EL protein and indirectly through modulating ANGPTL3 levels in the liver. Thus, the hepatic PC–ANGPTL3–EL–HDL pathway is a novel mechanism controlling HDL metabolism and cholesterol homeostasis.\(^{37}\)

McCoy et al reported that human heat-inactivated serum inhibited EL phospholipase activity,\(^{38}\) indicating that some endogenous EL inhibitor might exist in human serum. Apolipoprotein A-II (ApoA-II) constitutes ~20% of the total HDL protein mass, and has been reported to inhibit several protein activities related to HDL metabolism. Broedl et al reported that EL has less activity in human apoA-I/A-II double transgenic mice compared with human apoA-I single transgenic mice.\(^{39}\) These findings suggest that apoA-II may act as an inhibitor of EL activity in vivo.

EL has 5 potential N-linked glycosylation sites, and the effects of each site have been evaluated by site-directed mutagenesis of the asparagine. Miller et al reported that Asn-60 mutation markedly reduced its secretion, Asn-116 mutation increased phospholipase activity, and Asn-373 mutation reduced EL activity and its lipid-binding function.\(^{9}\) Brown et al reported that the specific hydrolytic activity toward LDL by EL-N116A was significantly greater than wild-type EL in vitro.\(^{40}\) In addition, adenovirus encoding wild-type EL reduced plasma HDL-C levels dominantly, whereas that encoding Asn-116 mutant EL reduced both HDL-C and LDL-C in \(LDLR^{-/-}\) mice. In contrast, Skropeta et al reported that EL N118A, N375A, and N473A reduced phospholipase activity, and N62A increased phospholipase activity by 6-fold compared with wild-type EL.\(^{41}\) There is a discrepancy between these data on the effects of glycosylation, partially because the 2 studies used different substrates, either glycerol-stabilized emulsion or reconstituted HDL. According to the data,
the glycosylation of the EL protein may regulate plasma HDL-C levels in vivo by affecting EL phospholipase activity.

**Association of EL Single-Nucleotide Polymorphisms With HDL-C Level or CVD**

Several EL gene polymorphisms have been reported to be associated with plasma HDL-C levels or cardiovascular events. The most common coding variant in *EL* is the T111I variant. Although early small reports suggested an association of the T111I variant with HDL-C levels, larger studies have definitively established a lack of association; furthermore, this variant has normal lipase activity compared with wild-type EL.42,43 Interestingly, Shimizu et al reported that the T111I polymorphism was associated with acute myocardial infarction independently of plasma HDL-C levels.44 Tang et al reported a similar finding in a Chinese population, which showed that the T111I variant was associated with protection from coronary artery disease (CAD).45 The most common functional EL variant in Caucasians is the N396S variant, which has been reported to decrease EL activity in vitro and is definitively associated with elevated plasma HDL-C levels.42 It will be important to determine whether the N396S variant is associated with CAD. Brown et al reported that a low-frequency G26S variant exists in persons of African descent and that this variant is associated with elevated plasma HDL-C levels because of reduced secretion.46 Genome wide association studies have revealed that a common variant at the *LIPG* (EL) locus is significantly associated with plasma HDL-C levels.47 Taking all the findings together, genetic variation in EL resulting in loss of function can elevate plasma HDL-C levels but of the relationship with CVD remains uncertain.

**Does EL Inhibition Promote RCT?**

HDL plays a central role in the process of RCT. The first step is cholesterol efflux from macrophages to HDL. A hypothesis can be made that EL inhibition would raise HDL-C levels and activate RCT in vivo. A number of studies regarding the EL effect on RCT have already been performed. Yancey et al reported that EL changes HDL composition and turns it into smaller particles.48 In their cell culture studies, EL-rich serum decreased cholesterol efflux via SR-BI by 90% and increased efflux via ATP binding-cassette transporter 1 by 63%, probably because of increased production of pre-beta HDL in the serum. Brown et al reported that plasma and HDL from *EL*-/- mice has greater efflux capacity than those from wild-type mice.49 Qui et al reported that macrophage EL promotes apoA-I-mediated cholesterol efflux through...
catalytic- and noncatalytic-dependent mechanisms through promotion of apoA-I binding to macrophages and lysophosphatidylcholine (lyso-PC) production, which can promote apoA-I-mediated cholesterol efflux. These findings indicate that EL works both ways (promoting and inhibiting) in macrophage cholesterol efflux.

The next step in RCT is hepatic cholesterol uptake. Strauss et al revealed that EL mediates the binding of HDL to the cell surface through its catalytic and noncatalytic functions in HepG2 cells, and promotes the uptake of HDL holoparticles, as well as selective uptake of its cholesterol ester (CE). Additionally, Nijstad et al reported that hepatic EL overexpression promotes HDL-CE selective uptake in wild-type mice but not in SR-BI−/− mice, indicating the SR-BI-mediated selective uptake of HDL-CE requires the remodeling of large alpha-migrating HDL particles by EL. These findings support the concept that EL is a key regulator of the hepatic uptake of HDL-CE.

The final step in RCT is cholesterol secretion from the liver to feces. Interestingly, EL overexpression has been reported to markedly reduce plasma HDL-C levels and increases hepatic cholesterol content without affecting biliary sterol secretion. Furthermore, Brown et al have recently reported that EL- and/or HL-deficient mice exhibited the same degree of in vivo RCT as wild-type mice, despite increased plasma HDL-C levels. These findings suggest that, when the complete RCT is defined as cholesterol flux from macrophages to feces, the net effect of EL on RCT remains speculative (Figure 2). However, if we look at the redistribution of cholesterol from the diseased vessel wall to the liver, which is a fundamental process in plaque regression, EL inhibition may increase plasma HDL-C levels without decreasing the EL ligand-binding function, which may promote cholesterol efflux from the diseased vessel wall to the liver.

**Does EL Inhibition Promote or Attenuate Atherosclerosis?**

The effect of altered expression of the TG-lipase members has been previously reported. LPL−/− mice developed spontaneous atherosclerosis; whereas LPL transgenic mice had decreased atherosclerosis because of the reduction in remnant lipoprotein. Moreover, LPL overexpression generates small dense LDL, resulting in an exacerbation of atherosclerosis in rabbits. HL deficiency reduces atherosclerosis in apoE−/− mice, but it has an antiatherogenic function in LDLR−/− mice. These results indicate that the effects of the TG-lipase family on atherosclerosis have not been fully elucidated, probably because both lipases have both pro- and antiatherogenic effects on atherosclerosis depending on where it is expressed.

Although EL inactivation was previously expected to inhibit atherosclerosis by raising plasma HDL-C levels, the effect of EL inactivation on atherosclerosis seems more complex than expected. Ishida et al have previously reported that targeted inactivation of EL increased the plasma HDL-C level and inhibited atherosclerosis in apoE−/− mice. On the other hand, Ko et al independently reported that EL inactivation in hyperlipidemic mice did not affect atherosclerosis even though the plasma HDL-C level was raised. Because...
apoE−/− or LDLR−/− mice are extremely high in athereogenic apoB-containing lipoproteins, it is considered to be difficult to determine the effect of relatively small changes in HDL-C levels on formation of atherosclerosis in these mice. In this context, it still remains unclear whether EL acts as a pro- or antiatherogenic molecule overall. Brown et al. showed that EL and HDL deficiency increases small dense LDL partly through LPL activation. In addition, EL overexpression reduces plasma apoB-containing lipoprotein levels in mice, while EL deficiency increases very-LDL-C (VLDL-C) levels against an apoE- or LDL-R-receptor deficient background. Therefore, EL can promote the catabolism of apoB lipoproteins as well as HDL. However, considering that plasma EL mass does not correlate with plasma LDL-C or TG levels, the action of EL on apoB lipoproteins is limited in humans. Ahmed et al. reported that EL activity is essential for HDL-induced adhesion molecule inhibition through PPARα activation in endothelial cells. These lines of evidence imply that EL has some antiatherogenic functions.

In contrast, Kojima et al. reported that monocyte adhesion was reduced on endothelial cells from EL−/− mice and increased on that from EL transgenic mice. It is also reported that macrophages from EL−/− mice or EL-depleted macrophages have less capacity to take up LDL and oxidize LDL independently of its lypolytic function. These findings suggest that EL acts as a bridge between monocytes and endothelial cells, lipoproteins and macrophages, which are the initial steps of atherosclerosis. Azumi et al. reported that EL is expressed in endothelial cells, smooth muscle cells, and macrophages within human atherosclerotic lesions. Hence, considering that EL is upregulated by inflammatory stimuli, EL may play a principal role in the vicious cycle of inflammation at the site of atherosclerotic lesions. EL inhibition by statins decreases the hydrolysis of phospholipids and subsequent lyso-PC productions, thus EL may increase proinflammatory lyso-PC production. The data from human and mouse studies indicate that EL has some proatherogenic effects, especially locally at atherosclerotic lesions (Figure 3).

All in all, EL seems to have diverse effects on the formation of atherosclerosis, depending on the cell type, the degree of inflammation, species etc. The net effects of these functions may explain the inconsistent results of EL inactivation on atherosclerosis that have been reported previously. Although there are conflicting results regarding EL’s pro- or antiatherogenic functions, speculation is raised that vascular EL, particularly at the site of atherosclerotic lesions, may accelerate the process of atherosclerosis in apoE−/− mice, whereas elevation of the VLDL fraction affected by hepatic EL deficiency might be crucial for developing atherosclerosis in LDLR−/− mice. These findings might explain the result that EL deficiency reduces atherosclerosis against the apoE knockout background, but not the LDLR knockout background.

**Conclusions**

EL is clearly a determinant of plasma HDL-C levels in humans, and inhibition of EL in humans would be expected to raise plasma HDL-C levels. However, the effect of EL inhibition on atherosclerotic CVD in humans is harder to predict. EL appears to have a variety of functions that may modulate atherosclerosis. Thus, the net effect of EL inhibition on atherosclerosis may be complicated and varied among the tissues or cells where EL is expressed, or by the presence of inflammation. The effect of EL inactivation has been proposed, mainly from findings obtained with genetically modified mice. However, it should be noted that the effects of EL deletion and pharmacological inactivation of EL, by EL inhibitors if available, are substantially different. Because EL−/− mice have undergone genetic deletion of LIPG, they do not express EL. In contrast, animals or humans administered with an EL inhibitor do express EL, but it is catalytically inactive. Thus, it is considered that the presence of EL after the administration of EL inhibitor, even if it is catalytically inactive, may promote the clearance of HDL from the plasma, and partly affect the plasma HDL level through its bridging function. From these findings, we speculate that vascular EL may have a relatively proatherogenic role by supplying lipids to the vessel wall or by reducing plasma HDL-C levels, whereas hepatic EL may have an antiatherogenic role by promoting hepatic cholesterol uptake. Therefore, selective inactivation of EL in blood vessels under inflammatory conditions may have beneficial effects on the prevention of atherosclerosis. It is expected that the pharmacological inactivation of EL may inactivate EL at the site of atherosclerosis to increase HDL-C, but would not interfere with its bridging function in the liver.

Finally, the cardiovascular effect of EL inhibition is hardly clarified in mice because there are so many differences in lipid metabolism between humans and mice. Therefore, to provide a useful insight as to whether EL inhibition is beneficial for atherosclerotic diseases in humans, the effects of EL inhibitors should be evaluated in animals that have similar lipid metabolism to humans, for example hamsters, rabbits or monkeys. Overall, EL remains an interesting potential target for therapeutic inhibition as a novel strategy to raise HDL-C and reduce the risk of atherosclerotic CVD.

**References**


