Endothelial lipase: Its role in cardiovascular disease

Marie-Eve Paradis BSc, Benoit Lamarche PhD

Endothelial lipase (EL) has recently been identified as a new member of the triglyceride lipase gene family. EL shares a relatively high degree of homology with lipoprotein lipase and hepatic lipase, but it appears to be more specific at hydrolyzing phospholipids than lipoprotein lipase and hepatic lipase. EL is also the only identified lipase that is synthesized and expressed by endothelial cells. Data from in vitro and in vivo animal studies have suggested that EL may play a key role in modulating the metabolism of high density lipoproteins. Data are less consistent in clarifying how EL contributes to the metabolism of apolipoprotein B-containing lipoproteins. Investigations in humans are scarce. To date, increased plasma EL concentrations have been associated with a deteriorated lipoprotein-lipid profile along with elevated plasma triglyceride and apolipoprotein B concentrations, as well as with smaller low density lipoprotein particle size. Elevated proinflammatory cytokine concentrations and an increased prevalence of the metabolic syndrome have also been observed among individuals with elevated plasma EL concentrations. Taken together, data suggest that EL is one of several key regulatory enzymes of lipoprotein-lipid metabolism and that a proinflammatory state, such as the metabolic syndrome, may be implicated in the processes relating plasma EL concentrations and lipoprotein concentrations. EL should thus be considered to play an important role in the pathophysiology of cardiovascular disease.

Key Words: Inflammation; LDL size; LIPG; Lipoproteins

In 1999, two independent research groups (1,2) reported the discovery of endothelial lipase (EL), a new member of the triglyceride lipase (TG) lipase gene family, which before this landmark discovery included pancreatic lipase, lipoprotein lipase (LPL) and hepatic lipase (HL). Jaye et al (1) cloned the EL gene using a differential display method in human macrophage-like THP-1 cells after exposure to oxidized low density lipoprotein (LDL). Hirata et al (2) cloned the EL gene through subtractive hybridization in cultured human umbilical vein endothelial cells undergoing tube formation. EL has 40% homology with HL and 45% homology with LPL (1). Despite strong similarities and conserved features among members of the lipase family, EL has been identified as the only lipase to be synthesized and secreted by endothelial cells to date. EL is also different from LPL and HL because it is more specific in hydrolyzing phospholipids than TG (3). It has, therefore, been suggested that LPL, HL and EL define a spectrum of lipase activities, with LPL acting mainly as a TG lipase, EL acting almost exclusively as a phospholipase and HL exerting both TG and phospholipase activities. In vitro, EL has hydrolyzed phospholipids in chylomicrons, very low density lipoprotein (VLDL), intermediate density lipoprotein and LDL. However, the lipase activity of EL specific to high density lipoprotein (HDL) appears to be more important than for other lipoprotein subclasses (3). The affinity of HL and EL for HDL phospholipids also varies according to the nature of the phospholipids (4). The majority of studies in animals have suggested that EL may be an important determinant of HDL metabolism, thus supporting evidence from in vivo studies. Recent work in mice suggested that the impact of EL on HDL metabolism, similarly to HL, may be related to both its lipolytic activity and nonlipolytic functions (5). Finally, EL messenger (m) RNA and protein concentrations in endothelial cells have been shown to be upregulated in response to inflammatory cytokine stimulation (6,7). Accordingly, data from the Study of Inherited Risk of Coronary Atherosclerosis (SIRCA) indicated that there was a positive correlation between plasma EL concentrations and interleukin-6 and C-reactive protein concentrations.
concentrations (8). The present short review provides an overview of how EL may be implicated in the etiology of cardiovascular disease (CVD) by focusing on its theoretical and demonstrated impact on the metabolism of apolipoprotein (apo) B-containing lipoproteins and HDL as well as on the link between EL and inflammation.

EL AND apoB-CONTAINING LIPOPROTEINS
Evidence from in vitro studies
EL has distinct lipoprotein preferences in vitro compared with LPL and HL. LPL and HL preferentially hydrolyze TG-rich lipoproteins such as chylomicrons, VLDL and intermediate density lipoprotein, whereas EL is more active against HDL but is not HDL-specific. Indeed, data from in vitro studies have shown that EL catalyzes the hydrolysis of apoB-containing lipoproteins (3) and mediates the bridging of apoB-containing lipoprotein to cell surface heparan sulphate proteoglycans, two processes that upregulate the increase of cellular uptake and degradation of lipoproteins (9).

Evidence from in vivo studies using animal models
The modulation of apoB metabolism in vivo by EL has not been thoroughly investigated and conflicting results have been reported. Jaye et al (1) first observed a modest reduction in VLDL/LDL cholesterol (C) concentrations in mice overexpressing EL. More recently, data have suggested that overexpression of EL significantly reduced total C, non-HDL-C, TG and phospholipid concentrations in a mouse model of elevated apoB-containing lipoproteins (10). Mice over-expressing EL were also characterized by decreased LDL size and particle number, while LDL were being cleared more rapidly from the circulation compared with mice lacking EL (10). These are not consistent findings, because others have reported that knocking out EL gene in mice had no significant effect on VLDL or LDL particle size (11). Other studies in which EL has been inhibited through various approaches have also yielded inconsistent results. Ishida et al (12) reported that plasma LDL-C concentrations were increased by 90% in an EL knockout mouse model fed a chow diet while data from Ma et al (13) indicated that knocking out the EL gene had no effect on plasma LDL-C concentrations of mice even when they were fed a high-fat, high-C diet. Mice generally have very low plasma concentrations of LDL. The extent that data from these mouse models are relevant to human LDL metabolism can, therefore, be questioned. Ultimately, only data in humans will show how EL may contribute to the metabolism of apoB-containing lipoproteins.

Evidence from human studies
We have recently investigated (14,15) the impact of EL on the plasma lipid profile of 80 moderately obese but otherwise healthy men. Plasma EL mass was measured using an in-house ELISA developed by Daniel J Rader (unpublished). Our data suggested that men with high as opposed to low concentrations of plasma EL also had elevated plasma C, LDL-C, TG, LDL-TG, apoB, VLDL-apoB and LDL-apoB (14). Plasma EL concentrations were negatively correlated with LDL peak particle size (r=-0.58, P<0.0001) and positively correlated with the proportion of LDL with a diameter less than 255 Å (r=0.44, P<0.0001). A significant difference of 6 Å in LDL peak particle size was observed between men with arbitrarily high versus low concentrations of plasma EL, the smallest LDL particles being observed among the high EL mass subgroup of men (P<0.0001). Multivariate regression analyses indicated that plasma EL concentrations explained 28% (P<0.0001) of the variance in LDL peak particle diameter, and that this contribution was independent of concomitant variations in plasma TG concentrations and obesity indices, including levels of visceral adipose tissue measured by computed tomography (15). Our data and those from Badellino et al (16) also suggested that plasma EL concentrations in individuals with the metabolic syndrome as defined by the National Cholesterol Education Program were approximately 50% higher than concentrations measured in healthy patients (16).

EL AND HDL METABOLISM
Evidence from in vitro studies
The characterization of the intrinsic lipase activity of EL revealed its efficacy at hydrolyzing HDL more than other lipoprotein fractions (3). This led to the hypothesis that EL may be a key regulatory factor of in vivo HDL metabolism. Caiazza et al (17) have recently shown that apoA-I and apoA-II regulate the binding affinity of EL for HDL and that they have a major influence on the rate that EL hydrolyzes phospholipids in reconstituted HDL. Other evidence from in vitro studies have provided further support to the concept that EL is an important enzyme regulating HDL metabolism. Indeed, EL has been shown to mediate both the selective uptake of HDL-C esters (18) and HDL binding to cell-surface heparin sulphate proteoglycans (9,18). Based on these in vitro findings, it has been hypothesized that elevated concentrations of EL in the plasma may contribute to an overall lowering of HDL concentrations.

Evidence from in vivo animal studies
Some of the most convincing arguments that have related EL to HDL metabolism have been obtained from studies conducted using various animal models. Overexpression of EL in mice has been shown to reduce plasma HDL-C and apoA-I concentrations (11,12,19,20). The reduced plasma HDL-C concentrations associated with overexpression of EL has been explained, at least partly, by a dose-dependent increase in the fractional catabolic rate of HDL-apo (19). It has been hypothesized based on these data that the phospholipase activity of EL may contribute to a depletion of the HDL particle in phospholipids, resulting in a lipid-poor form of apoA-I that may be more rapidly catabolized. Changes in the phospholipid content of HDL have also been shown to modulate C efflux from Fu5AH cells (21,22). Along the same lines, the potential of serum from mice overexpressing EL to efflux C from fibroblasts via the scavenger receptor class BI was decreased by 90%, while ABCAI-mediated efflux from macrophages was increased by 63% compared with wild-type mice (20). The impact of inhibiting EL activity or expression in mice on HDL metabolism is consistent with data derived from models in which EL was overexpressed. Indeed, inhibiting EL through immunoprecipitation or by knocking out the EL gene in mouse models led to significantly increased plasma HDL-C and apoA-I concentrations (11-13,23) and HDL diameter (11,13,23). These observations are consistent with the reduced catabolism of HDL observed in mice lacking EL (13,23). It is interesting to note that the combined absence of EL and scavenger receptor class BI has been associated with a 90% inhibition of endogenous lecithin C acyltransferase activity in mice (11). Thus, it...
has been proposed that inactivation or absence of EL could interfere with the reverse C transport through significant alterations in HDL structure and function and/or indirectly through inhibition of lecithin C acyltransferase activity (11). Finally, Broedl et al (5) have hypothesized that EL may modulate HDL metabolism in vivo independent of its lipolytic function. Their data suggested that when catalytically inactive EL was expressed in the livers of HL knockout mice, moderate transient reductions in C, phospholipid and HDL-C were obtained, thus demonstrating that, in particular circumstances, bridging can be a minor but significant mechanism through which EL mediates a reduction in HDL. However, their data suggested that lipolytic activity of EL appeared as the main determinant for its effects on HDL metabolism.

**Evidence from studies in humans**

Studies investigating the role of plasma EL concentrations on HDL in humans are scarce. Among a simple of 800 patients from the SIRCA, in a cohort of asymptomatic individuals with at least one family member experiencing early-onset coronary artery disease, an increased EL concentration was negatively associated with plasma HDL-C concentrations (8). In our sample of 80 moderately obese, but otherwise healthy men, those characterized by higher plasma EL concentrations had reduced HDL₂-C, HDL phospholipid and HDL₃ phospholipid compared with men that had lower EL concentrations (14). Future studies are required to fully appreciate the role of EL in modulating HDL metabolism in humans, and to understand its contribution (positive or negative) to the antiatherogenic properties of HDL.

**Genetic studies**

To date, a number of single nucleotide polymorphisms (SNPs) in the EL gene (LIPG) have been identified. However, only a few of these variants have been shown to be related to variations in plasma HDL-C concentrations. Among six potentially functional SNPs discovered by deLemos et al (24), the T111I variant was the most common but no significant differences in HDL-C concentrations in carriers of this variant and noncarriers were found (24,25). On the other hand, the T111I variation in the 372 patients from the Lipoprotein and Coronary Atherosclerosis Study (LCAS) was associated with significant variations in plasma HDL-C and apoC-III concentrations, as well as with the ratios of HDL-C to LDL-C and apoA-I to apoB. The authors (13) noted that the T111I gene variant was not associated with differences in the progression of coronary atherosclerosis or changes in HDL-C in response to fluvastatin therapy in the 2.5-year prospective LCAS study. Halverstadt et al (26) studied 83 sedentary, healthy 50- to 75-year-old patients with a constant weight before and after exercise training. They found that carriers of the rare allele of the 2237G/A variant were more than twice that observed among carriers of the wild-type allele (T111T) in the promoter region had elevated serum HDL-C concentrations (P=0.003) (27). Finally, a variation in both the EL promoter (−384A/C) and the 3′ untranslated region (2373G/A) were significantly associated with variations in serum HDL-C concentrations among healthy, school-aged Japanese children. More precisely, carriers of the rare allele in the promoter region had elevated serum HDL-C concentrations compared with children with the wild-type allele. On the other hand, children with the rare allele of the 2237G/A variant had lower serum HDL-C concentrations compared with those that had the more frequent allele (28).

**EL: A PRO- OR ANTIATHEROGENIC ENZYME?**

The relatively limited information on EL from in vitro and in vivo studies as well as from only a few studies in humans demonstrates that it should definitely be considered as an important enzyme in the etiology of atherosclerosis, largely through its impact on apparently all subclasses of lipoproteins. Other mechanisms may also be implicated. Indeed, a few studies have recently attributed a potential role of EL in modulating lipoprotein metabolism in proinflammatory states, including atherosclerosis. Hirata et al (7) first showed that EL mRNA concentrations were increased in cells by inflammatory cytokines implicated in vascular disease such as tumour necrosis factor-alpha and interleukin-1-beta. Subsequently, Jin et al (6) demonstrated that the TG lipase and phospholipase activities observed from endothelial cells in response to cytokines are primarily a result of upregulation of EL. In lipopolysaccharide-induced mouse models of inflammation, EL mRNA and protein concentrations were markedly increased in the aorta, lung, heart, kidney, liver and spleen. This upregulation in mouse tissues was accompanied by an increased EL activity in postheparin plasma. In vitro data suggest that EL may also promote monocyte adhesion to the vascular endothelium through an interaction with heparan sulphate proteoglycans (29). This upregulation of EL by proinflammatory cytokines is in sharp contrast to the downregulation observed with the other two major members of the lipase gene family, LPL (30) and HL (31), in vivo.

The extent to which the data are related to human physiology remains to be established. However, preliminary results from SIRCA support the hypothesis linking EL and inflammation by showing a positive correlation between plasma EL concentrations and interleukin-6 and C-reactive protein concentrations. These observations, along with data from our own group that have related elevated plasma EL concentrations to a high risk lipoprotein-lipid profile including the presence of small dense LDL (14) and the metabolic syndrome (15), suggest that EL may have proatherogenic properties. However, the direct role of EL in promoting the appearance of an atherogenic lipid profile remains to be firmly established. Data also suggest that EL may be an important modulator of energy homeostasis and possibly host defense in states of acute and chronic inflammation (32).
Paradis and Lamarche

PERSPECTIVES

The importance of EL in modulating HDL metabolism among animals is now supported by many studies. However, its role in regulating the metabolism of all lipoproteins remains to be demonstrated in healthy individuals as well as in dyslipidemic, diabetic and cardiac patients. Preliminary data in humans suggest that the impact of EL on HDL and other classes of lipoproteins may be more subtle than initially expected based on early data from in vitro and in vivo animal studies and based on reports showing relatively weak associations among several SNPs in the EL gene and plasma lipoprotein concentrations.

The potential link between inflammation and EL is also of interest. This association is particularly important in light of the accumulating evidence relating inflammation and CVD risk. Additional data from fundamental, clinical and epidemiological studies are, therefore, clearly warranted to further investigate the direct and indirect role of EL in proinflammatory states associated with an increased risk of CVD.

CONCLUSION

Data from studies reviewed are inconsistent in demonstrating an impact of EL on apoB-containing lipoprotein metabolism, while the impact of EL on HDL metabolism appeared to be more consistent. Recent investigations have reported simultaneous elevation of EL and of proinflammatory cytokines. Taken together, these observations support a role of EL in atherosclerosis.

SUPPORT: Benoit Lamarche is a Chair Professor in Nutrition, Functional Foods and Cardiovascular Health from the Canada Research Chair Program. Marie-Eve Paradis is the recipient of a studentship from Le Fonds de la recherche en santé du Québec.