Endothelial Lipase: A Key Player in HDL Metabolism Modulates Inflammation and Atherosclerotic Risk

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Abstract: Endothelial Lipase (EL) is a newly identified member of the triacylglycerol lipase family. Recent studies suggested that EL may be an important determinant of HDL-metabolism and inflammation acting at the level of the vessel wall. The aim of this review is to summarize important facts derived from experimental approaches and from epidemiologic human studies to provide a comprehensive view on the role of EL in inflammation and atherogenesis as well as target for potential pharmaceutical interventions.

Key Words: HDL metabolism, atherosclerosis, inflammation.

INTRODUCTION

Human plasma lipases are water-soluble enzymes that play important roles in the metabolism of lipids. Lipids consist of glycerol esterified with variously occurring fatty acids (Fig. **1A**). Lipids and their components serve as essential structural elements of membranes and complex signal mediators or as ligands for receptors. One common source of lipids is diet. After dietary lipids are absorbed, they are packed and transported in the form of various lipoproteins that serve as vehicles. If needed, lipids are broken down to glycerol and fatty acids by lipases for further utilization. When exogenous lipids are lacking, endogenous fatty acids are synthesized de novo from carbohydrates during a complex and energy-consuming process. Free fatty acids occur in plasma and tissues in low concentrations in the absence of substrates or starvation. Most fatty acids, either saturated or unsaturated, circulate, bound to serum albumin, in the plasma. Some of the naturally occurring fatty acids are shown in Fig. (**1B**).

If fatty acids enter the intracellular space, the cell is forced to process them because their presence per se seems to be toxic to cellular integrity. Processing occurs as they are either acetylated and shuttled into the mitochondria for oxidation, or packaged as triacylglycerols (TG) for storing.

Lipids in the form of triacylglycerols are an important, high-caloric substrate of energy metabolism mostly stored in the white adipose tissue. Phospholipids have a phosphate group substituting for one of the three fatty acid chains (Fig. **3A**). Phospholipids are the building blocks of cellular membranes and therefore have hydrophilic heads (glycerol and phosphate) and hydrophobic tails (the non-polar fatty acids). One of the most important membrane components is the phospholipid phosphatidylcholine, also called lecithin (Fig. **3B**). The role of the lipase enzyme family is to hydrolyze the ester bonds of the water-insoluble lipid molecules (Fig. **2**), which releases fatty acids for direct utilization, re-packaging for transport to target organs, or storage purposes [1].

The need for an enzyme family of lipases is based upon the above mentioned heterogeneity of lipids. Enzymes belonging to the lipase family share significant structural homologies, but differ in their tissue distribution, substrate affinity, and in the structure of their active sites.

The best characterized members of human plasma lipase family comprise lipoprotein lipase (LPL) and hepatic lipase (HL) [2]. More recently, several other enzymes have been identified as members of the lipase gene family such as phosphatidylserine phospholipase A1 [3], lipase H [4] and endothelial lipase (EL) [5]. In this article we, especially focus on EL, a new member of the lipase enzyme family.

POSITION OF EL IN THE PLASMA LIPASE FAMILY

Endothelial lipase (EL), discovered in 1999, is a relatively new member of the human plasma lipase family. EL (encoded by the LIPG gene) is synthesized as a protein of 500 amino acids with a molecular mass of about 55 kDa. 18 amino acids from the primary protein are cleaved resulting in a mature protein of 482 amino acids [5]. EL has a 45% and 40% structural homology to LPL, and HL, and a 27% homology to pancreatic lipase, respectively [5]. Compared to the primary sequence of the other lipases, the catalytic serine, aspartic acid, and histidine residues as well as 10 cysteine disulfide bond formation residues are conserved [6]. The 19-residue lid formation of EL is shorter and less amphipathic as compared to the lid of LPL and HL, resulting in a different enzymatic activity. EL was given its name because of its structural homologies with members of the lipase enzyme family and its localization to vascular endothelial cells (endothelial lipase, EL). More recently, EL has shown

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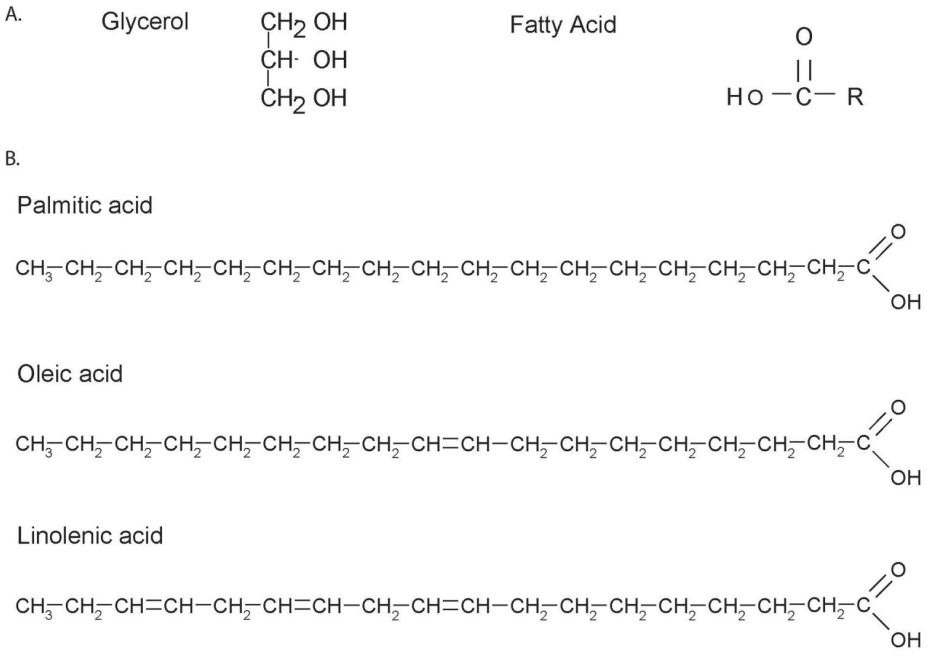

Fig. (1). A). Chemical structures of the components of neutral lipids. B). Chemical structure of some of the naturally occurring fatty acids, 3 of which are esterified with glycerol to build a neutral lipid.

to be expressed in cultured human hepatocytes, macrophages, and osteosarcoma cells, too [6, 7]. High levels of EL have been demonstrated in embryonic endothelial cells, but this expression level fades with maturation. In adult tissues, EL was reported to be expressed in coronary arteries, placenta, thyroid, liver, lung, adrenals, kidney, testis, and ovary [5, 6, 8]. Immunohistochemical and *in situ* hybridization data from liver, the organ that expresses the highest level of EL [6], show that EL is synthesized in endothelial cells and is virtually absent in parenchymal cells [9]. Northern blot and immunohistochemistry suggest that EL protein occurs in endothelial cells of organs where its mRNA is present and remains localized there to exert enzymatic activity [6, 9].

This site/mode of action clearly differentiates EL from LPL and HL. LPL is secreted by parenchymal cells of muscle, adipose, heart, brain and by macrophages [10]. Although LPL is expressed in the parenchymal cells of the organs, the transfer of the enzyme to the luminal surface of the endothelial cells, which requires heparan sulfate proteoglycans, is essential for LPL action [11]. HL is found in liver, adrenals, ovaries and macrophages [12, 13]. In rats and humans, HL is synthesized in hepatocytes and either remains located there or is transported to the luminal endothelial cells [9]. In mice, HL is found to circulate in plasma. Unlike EL, that is synthesized and acting in the same cell, LPL and HL are synthesized at one site to be transported to another site to exert their action. HL, LPL, and EL share the feature that they are all expressed in macrophages [8, 14, 13], suggesting that they all play a role in inflammation and atherosclerosis.

ROLE OF EL ON LIPID METABOLISM

Lipases in general and EL in particular, have been shown to influence HDL-cholesterol through modulating effects on HDL-particle size and metabolism. While the members of the lipase family share substantial sequence homologies, they differ in their lipolytic activity and substrate specificity. Lipoprotein lipase mainly hydrolyzes triglyceride-rich particles and is less active on those containing phospholipids. In contrast, different phospholipids are the preferred substrate for EL that has little activity on triglycerides. HL uses either

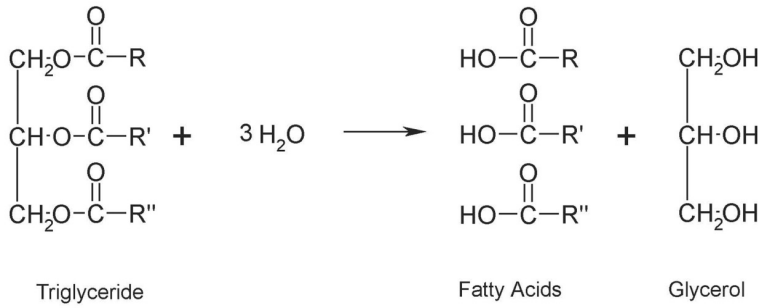

Fig. (2). Triglyceride hydrolysis reaction into fatty acids and glycerol.

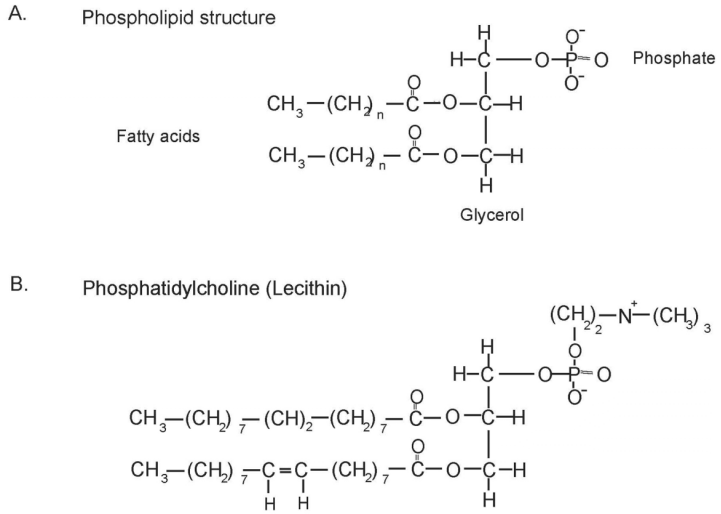

Fig. (3). A). Chemical structure of a phospholipid: glycerol esterified with 2 neutral fatty acids and phospholipids. B). The most abundant and important endogenous phospholipid Lecithin.

phospholipids or triglycerides equally effectively as substrates [15-18].

There is an inverse relationship between HDL-cholesterol levels and EL expression [19] and consequently, there is a positive correlation between the HDL-cholesterol clearance rate with EL catalytic capacity. This effect is not surprising as EL hydrolyzes predominately phospholipid-containing particles mostly occurring in HDL-cholesterol [18, 20]. Transgenic and adenoviral over-expression of EL has resulted in a marked reduction of HDL-cholesterol level in mice [5, 21]. Conversely, the HDL-cholesterol levels were up-regulated in EL knockout mice [19, 22]. Similarly, in a study where an antibody raised against EL was used to inhibit EL function, HDL-cholesterol increased significantly [21]. The results from these studies are presented in more detail further below.

Analysis of the gene encoding for EL (LIPG) revealed the existence of polymorphisms that are associated with HDL-cholesterol levels in large population based studies [23,24]. However, high HDL-cholesterol levels in individuals are not solely dependent on EL function or polymorphisms [25]. In fact, HDL-metabolism is incompletely understood. In addition to modifications of HDL-particles in the bloodstream mediated by lipases such as HL and LPL, HDL-particles are being taken up ubiquitously *via* specific HDL-receptors, such as scavenger receptor class B type 1 (SRB1) [26]. The major locations of HDL-particle catabolism are the liver and the kidney [27, 28]. While the function of liver EL has been subject of most studies, its role in kidney HDL-catabolism is probably underappreciated. It is known that, in the kidney, EL accelerates the HDL-catabolism by enhancing the uptake of apolipoprotein A-I that is lipid depleted by EL action [29]. In addition, EL may not only enhance the intracellular catabolism of reabsorbed HDL-particles, but also facilitate the binding and absorption of the entire HDL-particles.

It has been shown that EL may facilitate the binding and uptake of lipoproteins from the plasma stream *via* surface-binding to heparin sulfate proteoglycans and independently of the catalytic activity [30]. This represents a non-catalytic ability of EL to modify lipoprotein metabolism. Such functions have also been demonstrated for other members of the lipase family (LPL, HL) [31-33]. Overexpression of a non-catalytic mutant of EL in mouse livers using an adenoviral approach produced a slight decrease in HDL-cholesterol, an effect that was amplified in HL knockout mice, demonstrating that other lipases may compensate for diminished EL activity [34]. The non-catalytic process is called bridging. In addition to HDL-cholesterol, EL has a non-catalytic bridging interaction with apoB-containing lipoproteins, intermediate-density lipoproteins (IDL), low-density-lipoproteins (LDL) and very-low-density-lipoproteins (VLDL) as substrates. EL probably serves as a ligand for the lipoprotein particles and mediates their uptake *via* the cell surface receptors [30].

Another non-enzymatic mechanism by which the presence or absence of EL affects HDL-cholesterol is a specific influence on gene expression. The absence of EL, for instance, increases selected genes that are involved in HDL-formation such as apo-AI and apoE, two major apolipoprotein components of HDL-cholesterol, while HDL-cholesterol-accepting receptors that modify the uptake of HDL-cholesterol are unchanged [22]. Size and structural changes of the HDL-particle may also affect the absorbance of the particle by its receptors [35]. However, the HDL-particle size as well as the total cholesterol, free cholesterol and triacylglycerol content of HDL were not significantly altered after overexpression of catalytic-inactive EL [34].

The catalytic activity is the most important mechanism by which EL mediates changes in HDL-cholesterol levels [34]. This notion is also supported by the observation that other members of the lipase family are up-regulated when EL is missing, again probably due to compensatory mechanisms [22].

The importance of the catalytic action of EL on HDL-particles has convincingly been demonstrated by changes in HDL-cholesterol levels when EL is either knocked out or

overexpressed [19, 21]. These studies revealed a clear inverse relationship between EL function and HDL-cholesterol levels. In a mouse model that lacks EL expression, Ishida *et al*. [19] showed a 57% increase in HDL-cholesterol as compared to wild type littermate control animals. Vice versa, constitutive overexpression of EL in mice or using an adenovirus encoding for EL *in vivo* has shown to bring HDL-cholesterol levels down by 19% [19, 29].

Based on the catalytic activity of EL, one could also expect an inverse relationship between HDL-particle size and EL activity. However, this association has only been demonstrated for EL overexpression [29]. The reverse, an increase in HDL-particle size by EL inhibition has only been reported in HL knockout mice and not in wild type mice [21]. Jin *et al*. [21] speculated that HL and EL influence each other while remodeling the HDL-particle and that the influence of EL inhibition on HDL-particle size may depend on the metabolic milieu as well as the HDL-particle size at baseline. It is known for a long time that HL knockout mice have HDL-particles of larger size and increased phospholipid content [36]. These particles may represent better substrates for EL, although this has not been proven so far.

The structure-function relationship of EL has been studied extensively using site-directed mutagenesis [20]. Thereby, chimeric enzymes have been generated that combine known sequences from different related lipases for the identification of the specific sites of lipolytic action. These studies clearly demonstrate that HDL-particles are the preferred substrate for EL [20]. This finding is very important given the association between HDL-cholesterol and coronary heart disease [37]. When EL was inhibited in mice using a polyclonal antibody, HDL-cholesterol levels increased significantly (nearly 50%) 48 hours after injection together with a significantly slower HDL-catabolism as shown by a slower phospholipid turnover [21]. Studies of HDL-composition after the EL inhibition revealed that the composition of HDL did not change significantly during the treatment, and the change in the HDL-phospholipid fractional catabolic rate was too low (21%) to account for the increase of plasma HDL-cholesterol and phospholipids (both nearly 50%) [21]. These results suggest that the increase in HDL-cholesterol in knockout animals or after EL inhibition is mainly due to an increase of HDL-particle number and that secondary effects may promote the transfer of cholesterol and phospholipids from other lipoproteins or tissues to form HDL-cholesterol [21, 29]. The significance of these speculations is not clear and will be addressed by future studies.

Interestingly, EL is also capable of hydrolyzing both VLDL- and LDL-cholesterol, at least *in vitro* [16]. However, the exact role of EL on modulating apoB-containing lipoproteins *in vivo* is unclear. Most studies were performed in mice, a species that usually does not carry abundant apoB-containing lipoproteins in the serum. EL knockout mice did not show significant differences in apoB-containing lipoproteins, even when the mice were fed a high fat diet [21]. In addition, LDL-cholesterol levels were increased in male mice knocked out for EL [19]. The latter result could not be confirmed in another study using EL knockout mice [22]. In a study performed in mice with increased apoB-containing lipoproteins, hepatic expression of EL resulted in markedly decreased levels of VLDL/LDL-cholesterol, phospholipid, and apoB, accompanied by significantly increased LDL apolipoprotein and phospholipid catabolism [38].

In addition to these conflicting results from the animal studies, the role of EL cannot be assessed easily in humans because its catalytic activity is inhibited by apolipoprotein C-II in serum that makes it difficult to distinguish EL activity from LPL/HL-activity in human postheparin plasma [16].

Therefore, unlike HL or LPL, EL can only be approached using an ELISA to measure its concentration in plasma while activity is only measurable *in vitro* [16]. Further studies are needed to clarify the role of EL, especially in a clinical setting.

EL REGULATION

Little data is available regarding the regulation of EL expression. Increased gene expression of EL in different cell types has been described in inflammatory settings. Thus, EL expression may be mediated by cytokines. It is assumed that several transcription factors influence EL expression by binding to the EL promoter [24]. In addition, the expression of EL mRNA is regulated by physical forces such as stretch and tension by a so far not elucidated mechanism [39]. There are also posttranslational changes on EL that modify its function. N-glycosylation of EL at different sites of the enzyme modulates its catalytic activity, changes its bridging function, and also influences its substrate specificity [40-42]. On a functional level, EL catalytic activity is influenced by the apolipoprotein composition of the HDL-particle. It has been demonstrated *in vitro* and *in vivo* that apolipoprotein A-II has an inhibitory effect on HDL-cholesterol hydrolysis [43, 44]. Furthermore, angiopoetin-like protein 3 (ANGPTL3) inhibited the phospholipase activity of EL in an animal study [45]. An inhibitory effect of ANGPTL3 also already been demonstrated for LPL [46]. The mechanism for this inhibition is also not fully explained, but it was assumed that ANGPTL3 possesses a putative heparin binding-site that may capture the enzyme. Based on this finding one can speculate whether ANGPTL4 may also affect EL because this has been shown for LPL and HL [47], but not in EL so far. More recently, an effect of hepatic proconvertases on HDL-cholesterol has been demonstrated and has been linked with EL activity. Hepatic proconvertases are highly conserved endopeptidases that cleave proteins at specific recognition sites [48]. Jin *et al*. [49] demonstrated that hepatic proconvertases modulate HDL-cholesterol levels by direct inactivation of EL, and through the inactivation of ANGPTL3, the endogenous inhibitor of EL.

EL AND INFLAMMATION

Acute and chronic inflammatory states are known to be associated with unfavorable changes in lipid levels such as decreased HDL-cholesterol levels and hypertriglyceridemia (high levels of triacylglycerols) [50]. The mechanisms and the consequences of these inflammation-induced changes in lipid parameters are not exactly known.

Decreased HDL-cholesterol levels in inflammatory situations suggest a participation of EL in inflammation. EL is a major modulator of HDL-cholesterol, and while other members of the lipase family have been shown to be down-

regulated by inflammatory stimuli [14, 51, 52], EL is strongly up-regulated by inflammation [53]. Treatment with cytokines resulted in a robust increase of triglyceride- and phospholipase-activity in endothelial cells. The use of an antibody against EL demonstrated that both activities resulted from EL. Furthermore, it could be shown that cytokines such as tumor necrosis factor α (TNFα) or interleukin-1β (IL-1β) caused a dose-dependent up-regulation in endothelial cell-derived EL mRNA and protein *in vitro* [54, 55].

Mice that were treated with lipopolysaccharide (LPS), a wall component of gram-negative bacteria used to mimic sepsis in animals models, had increased EL mRNA and protein levels in aorta, lung, heart kidney, liver, and spleen, accompanied by an increase of EL mass in post-heparin plasma [53].

LPS-treatment induces many mediators of septic shock and activates a plethora of transcription factors. Therefore, the increase of EL during inflammatory conditions has been studied in more detail. Kempe *et al*. [55] were able to identify binding sites in EL that interact with the redox-sensitive, pro-inflammatory nuclear transcription factor κB (NFκB) *in vivo* and *in vitro*. These observations and studies performed by other authors [54] lead to the speculation that EL may be under control of transcription factors that are activated by acute and chronic inflammation.

It is therefore conceivable that the 11% decrease of HDL-cholesterol in mice treated with LPS [53] may be, at least in part, mediated by a cytokine-induced up-regulation of EL. The up-regulation of EL in the setting of an acute inflammation may reflect the increased need of energy substrates in the form of fatty acids in the vasculature at the site of the event. These fatty acids are probably necessary for the increased metabolism, and also provide substrates for the generation of transcription factors, involved in maintaining the transcriptional regulation of the endothelial barrier [56, 57]. Support for this speculation comes from the observation that EL was shown to possess a preference for docosahexaenoic acid-containing phospholipids at the sn-2 position [18], which makes it likely that EL plays an important role in the delivery of docosahexaenoic acid to the brain under normal physiologic conditions.

Increased EL activity in the vasculature leads to decreased HDL-mediated removal of cholesterol from the periphery and increased availability of hydrolyzed phospholipids and fatty acids in the vasculature. These effects may provide an increased supply of cholesterol esters as well as fatty acids from HDL-particles to cells affected by inflammation, and deficient in membrane components required for repair. A tissue repair mechanism relying on a similar mechanism has been proposed in a model of experimental kidney damage [58].

A related mechanism has been proposed for group IIA secretory phospholipase A2, that is also up-regulated under inflammatory conditions [59]. Fig. (**4**) shows a schematic view on how EL function may produce a shift in the supply of fatty acids to target organs under different conditions.

In addition, the action of EL on the vascular surface may be responsible for the adhesion of leukocytes and monocytes to the site of inflammation as shown in a study by Kojma *et al*. [53]. The role of macrophage EL itself has recently been addressed in this context and may provide additional data on the role of EL in inflammatory processes. Similar to endothelial cells, LPS treatment increased EL mRNA levels in macrophages *in vitro* [60, 61]. In macrophages from different sources, these authors could also demonstrate that EL expression is controlled by toll-like-receptor 4 (TLR4). An up-regulation of EL expression was abolished in cells derived from TLR4 knockout mice [61], and treatment of cells with ligands for TLR4 or TLR3 produced an increase of EL expression *in vivo* and *in vitro* in an TLR-dependent manner [60]. Vice versa, suppression of EL (and LPL) was shown to be associated with decreased proinflammatory cytokine expression [62]. These observations suggest that EL itself or EL activity may play a regulatory role. Another study showing that the enzymatic activity of EL hydrolyzing HDL-particles is required to modulate the balanced expression of inflammatory markers (such as IL-10 and 12) in macrophages supports this observation [60].

The increase of EL expression in macrophages was accompanied by an increased ability to bind and ingest LDL-particles [61, 63]. Treatment of macrophages with statins *in vivo* was reported to lower EL expression [64]. As a consequence, these observations provide a mechanism by which EL could modulate the development of vascular lesions, and provide at the same time a novel mechanism for the pleiotropic beneficial effects of statin treatment.

The existing data suggest how EL functions in the vasculature at the site of acute inflammation (Fig. **4**). The first phase of the EL response at the time of an acute event may be compensatory and therefore beneficial. However, little data is available regarding the role of EL during chronic inflammatory processes.

The metabolic syndrome can be considered a chronic low-grade inflammatory condition [65], and Badellino *et al*. [66] have demonstrated elevated plasma EL mass in patients with the metabolic syndrome versus normal controls. In addition, increased plasma concentrations of EL were found in subjects with high inflammatory markers [67], and were associated with increased amounts of visceral adipose tissue [68].

So far there are no data on the plasma levels of EL activity or mass in patient populations with chronic inflammatory diseases such as rheumatoid arthritis and others. However, the existing data lead us to speculate that EL could be constitutively up-regulated during chronic inflammation that accompanies the metabolic syndrome. Increased EL activity may decrease HDL-cholesterol levels in these conditions, and thereby accelerate the atherosclerotic process.

Chronic inflammation has also been linked to the development of cancer [69]. Several lines of evidence have shown that a reduction in the activity of LPL is involved in cachexia induction in cancer patients [70]. Given the increased need for energy supply of the cancerous cells and the excessive adjacent angiogenesis, perhaps EL antagonizes the effects of LPL. Increased expression of EL in the vasculature neighboring cancerous tissue could supply the growing needs of

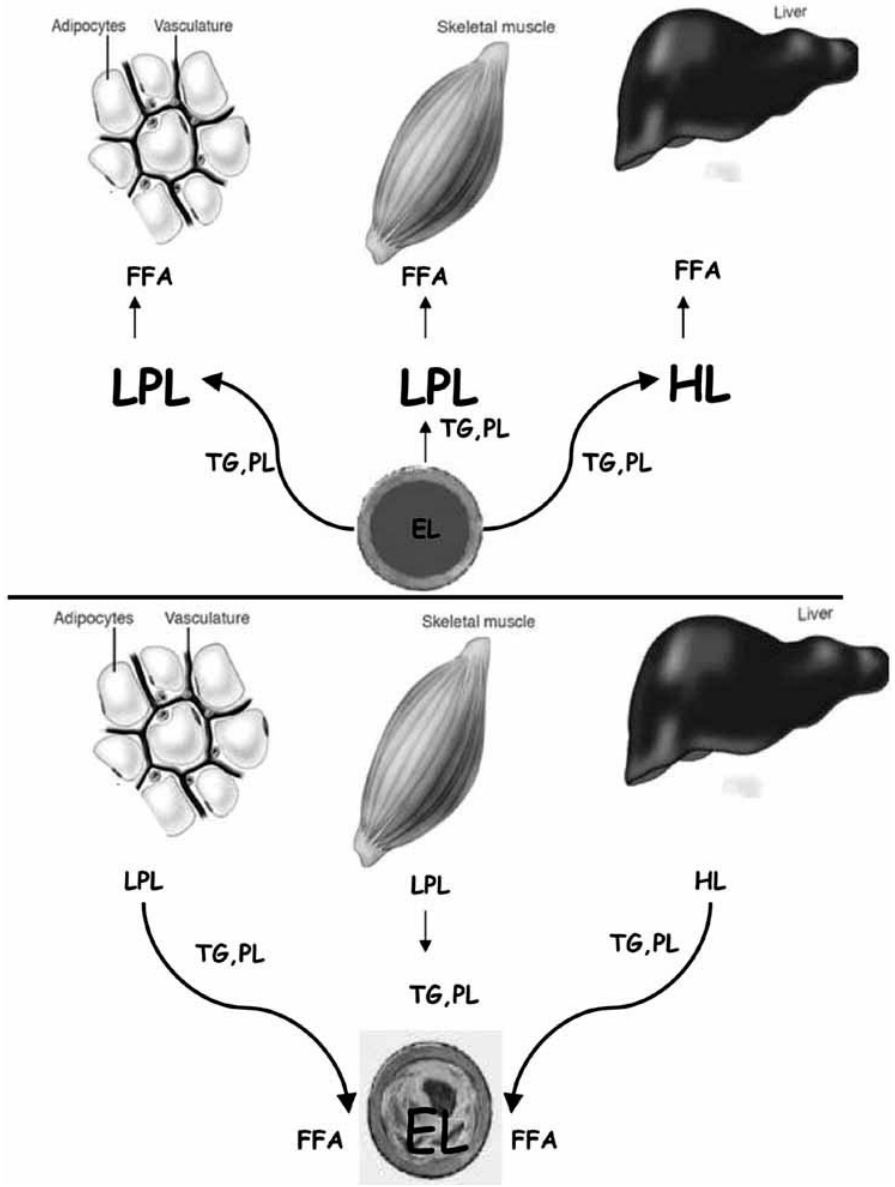

Fig. (4). Schematic view on the role of EL in inflammation: The upper part of the sketch shows the non-inflamed condition with high LPL and HL activity providing fatty acids to the peripheral organs. The lower part represents the inflammatory condition, in that increased EL provides fatty acids to the vasculature of affected organ systems. The size of the letters represents magnification of the enzyme activity.

these structures. These speculations certainly await further clarification.

EL AND ATHEROGENIC RISK

Results from several epidemiologic studies have clearly established an inverse relationship between HDL-cholesterol and atherosclerotic cardiovascular disease [71]. Because of these data, it is consistent to ask for the role of EL in atherosclerosis, which has already been partially addressed by some studies in mice and humans.

Recently, Badellino and colleagues showed that EL concentration in both pre- and post-heparin plasma was significantly correlated with all NCEP ATP III-defined metabolic syndrome factors that are all more or less strong risk factors for atherosclerosis [66]. Furthermore, EL mass was positively associated with coronary artery calcification, a measure of subclinical atherosclerosis in humans, even after controlling for cardiovascular risk factors, plasma lipids, and vasoactive medications [66]. In pathological sections from human coronary arteries, EL has been identified in the atheromatous plaques and its expression has also been demonstrated in macrophages and smooth muscle cells [8, 72]. In different rat models of hypertension, EL expression was upregulated in the aorta, heart and lungs, accompanied by significantly reduced HDL-cholesterol levels as compared to the controls [73].

These data suggest a negative impact of EL on several risk factors involved in the atherosclerotic process. These findings have to be expanded to the finding that overexpression of EL in animal models is associated with a marked

reduction of HDL-cholesterol levels [21]. Genetic variants in the EL gene that are associated with changes in HDL-cholesterol levels have been identified and an association of the same variants with fewer myocardial infarctions was suggested. However, statistically significant differences have not been observed after correction for multiple testing [22, 23]. A recent study on a Chinese population demonstrated a significant association of a common EL variant (EL584 C/T) with HDL-cholesterol levels. In this study, a similar association existed between the polymorphism and the incidence of myocardial infarction, but the reduced risk of myocardial infarction was independent of HDL-cholesterol [74]. This study was following another study in a Japanese cohort that provides basically the same results [75]. The independence of the EL-mediated changes in vascular disease risk from HDL-cholesterol *in vivo* suggests that this association could be mediated *via* the strong association between pro-inflammatory mediators and EL concentrations [67]. However, this association was only reported in subjects of Asian origin so far and has to be confirmed in different populations.

Two studies have addressed the role of EL in mouse models of atherosclerosis to obtain a deeper insight in the role of EL in atherosclerosis. These studies, however, provided conflicting results. Ishida and colleagues studied EL knockout mice that were crossed onto an apoE deficient background (double knockout) and compared them to their apoE knockout littermates. On the apoE deficient background, the atherosclerotic burden as assessed by quantification of lesions at the root of the aortic arch was decreased in the EL knockout mice. The animals were started at 4 weeks of age on either chow or a high fat diet. On chow diet, the lesion size was significantly decreased by about 70% in both male and female mice. After 12 weeks on high fat diet, the authors found decreased atherosclerotic lesions in the cross sections of the aortic roots of the EL knockout mice compared to their littermate controls. This difference was statistically significant in males and not in females when fed high fat diet [76].

Conversely, Ko and colleagues studied the role of EL in atherosclerosis in a different line of EL knockout mice and came to contrasting results. These authors investigated the role of EL in atherosclerosis in EL deficient mice that were either on the apoE knockout or the LDL receptor knockout background. In this experiment, EL and apoE double knockout mice as well as apoE knockout littermate control animals were evaluated for atherosclerosis at the aortic root after 26 weeks on chow diet. EL and LDL receptor double knockout mice and their LDL receptor deficient littermate controls were sacrificed after 18 weeks of high fat feeding. In both lines of this experiment, the authors did not find any significant difference in the level of atherosclerosis between the EL knockout mice and the control animals [77].

It is unclear why the two studies resulted in contrary results. The animals used in the two studies were on a pure C57BL/6J background. Therefore, background strain differences may not account for the different results.

Interestingly, the amount of atherosclerotic burden in the littermate apoE knockout control mice on chow diet differed significantly between the two studies. The techniques used to quantify the atherosclerotic burden in the two studies were similar, but not identical. This and other factors could partly explain why there were more lesions in younger animals on chow diet in the study by Ishida and colleagues [76].

A feasible approach to define the role of EL in the development of atherosclerosis in mice would be to conduct an atherosclerosis study using the *en face* analysis in addition to the described aortic sinus assay.

In addition to methodological problems, Ko *et al*. [77] found dramatically increased LDL-cholesterol on a high fat diet that may have obliterated any beneficial effect of higher HDL-cholesterol in the EL knockout mice on the LDL receptor knockout background. Based on the known catalytic effect of EL on apoB-containing lipoproteins [38] this observation cannot be unexpected, and, in fact, provides evidence for a potential pro-atherogenic effect of EL inhibition. Some support for a pro-atherogenic phenotype of EL inhibition also comes from the EL-ANGPTL3 pathway. ANGPTL3 acts as endogenous inhibitor of EL, and thus regulates HDL-cholesterol levels as mentioned above [45]. Therefore, one could expect some protective effects by ANGPTL3 mediated inhibition of EL. On the other hand, decreased expression of ANGPTL3 itself has shown to be protective against atherosclerosis [78].

At this point we raise the important clinical question whether a potential benefit of a pharmacologic intervention on EL could be countervailed by undesired changes in lipoproteins other than HDL-cholesterol or even through unrelated effects. Furthermore, some experts believe that it is the lipid-poor pre-beta-HDL that confers most of the anti-atherogenic effect of the HDL-fraction [79]. However, the role of pre-beta-HDL in atherosclerotic process is not fully elucidated as there are also studies showing the opposite, such as increased smaller-size HDL-particles in coronary artery disease [80, 81]. This increase could derive from either delayed maturation of pre-beta-HDL into alpha-migrating HDL or activation of the ABCA1 pathway in atherosclerotic lesions [82]. Pre-beta-HDL is primarily composed of apo-AI and phospholipids and it has little core lipid. Because of these facts it would be reasonable to study the specific role of EL in pre-beta-HDL-maturation.

These concerns in general can be extended to the question whether a drug-induced raise of HDL-cholesterol levels will really proof beneficial. The recently published trial results of torcetrapib (a CETP inhibitor) may serve as an example [83]. We must finally not forget that the proof of principle of the beneficial effect of raising HDL-cholesterol levels on vascular disease has yet to be shown [79]. In conclusion, the importance of the role of EL in atherosclerosis in clinical practice and pharmacotherapy is yet to be determined.

CONCLUDING REMARKS

EL is a new member of the triglyceride lipase family. With its predominant phospholipase activity EL has a profound effect on HDL-cholesterol concentration. Recent work revealed a role of EL in pathological processes such as atherosclerosis and inflammation. Besides structural homologies with other members of the lipase gene family, there are

marked functional differences between EL and other lipases, of which some have been already described, and many others await to be elucidated. We think that EL activity is involved in pathological processes by increasing substrate supply at the site of an injury. However, a lasting up-regulation of EL in chronic diseases seems to provoke side effects. Furthermore, EL is involved in inflammatory processes as it is up-regulated by inflammation and also influences cytokine expression *via* its HDL-cholesterol-modulating effect. The present knowledge identifies EL as an interesting candidate for pharmaceutical intervention, especially regarding its influence on HDL-cholesterol levels and inflammation. However, additional work is needed to further clarify the role of EL in health and disease.

ABBREVIATIONS

EL	=	Endothelial lipase
TG	=	Triacylglycerol
HL	=	Hepatic lipase
LPL	=	Lipoprotein lipase
IDL	=	Intermediate-density lipoproteins
VLDL	=	Very-low-density-lipoproteins
LDL	=	Low-density-lipoproteins
LPS	=	Lipopolysaccharide
TLR	=	Toll-like-receptor
NFκB	=	Nuclear transcription factor κB
IL	=	Interleukin
ANGPTL	=	Angiopoietin-like-protein
CETP	=	Cholesterol-esther-transfer-protein

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Received: 13 November, 2007 Revised: 02 December, 2007 Accepted: 03 December, 2007