Role of Hepatic Lipase and Endothelial Lipase in High-Density Lipoprotein–Mediated Reverse Cholesterol Transport

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Abstract Reverse cholesterol transport (RCT) constitutes a key part of the atheroprotective properties of high-density lipoproteins (HDL). Hepatic lipase (HL) and endothelial lipase (EL) are negative regulators of plasma HDL cholesterol levels. Although overexpression of EL decreases overall macrophage-to-feces RCT, knockout of both HL and EL leaves RCT essentially unaffected. With respect to important individual steps of RCT, current data on the role of EL and HL in cholesterol efflux are not conclusive. Both enzymes increase hepatic selective cholesterol uptake; however, this does not translate into altered biliary cholesterol secretion, which is regarded the final step of RCT. Also, the impact of HL and EL on atherosclerosis is not clear cut; rather it depends on respective experimental conditions and chosen models. More mechanistic insights into the diverse biological properties of these enzymes are therefore required to firmly establish EL and HL as targets for the treatment of atherosclerotic cardiovascular disease.

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Introduction

Despite the widespread use of low-density lipoprotein (LDL) reduction therapies, atherosclerotic cardiovascular disease (CVD) remains a leading cause of morbidity and mortality in developed countries. Treatment with statins (HMG-CoA reductase inhibitors), for example, only decreases the risk of major coronary events by 15% to 40% even though these drugs consistently and efficiently lower LDL cholesterol [1]. This substantial residual risk indicates a potential clinical benefit of novel therapeutic strategies that look beyond LDL and focus on high-density lipoproteins (HDL). Plasma HDL cholesterol levels are strongly, inversely, and independently related to cardiovascular disease risk [2]. A major part of this atheroprotective potential of HDL conceivably consists of its key role in reverse cholesterol transport (RCT, see below for details) [2]. Among the factors modulating HDL metabolism, lipases such as hepatic lipase (HL) and endothelial lipase (EL) are of prime importance. HL and EL both belong to the triacylglycerol lipase family, which also includes lipoprotein lipase (LPL), but members of this family differ in their respective hydrolytic activities. Whereas LPL is almost exclusively a triglyceridase, HL uses both phospholipids and triglycerides as substrates, whereas EL possesses predominantly phospholipase and very little triglyceridase activity (Table 1) [3]. By decreasing the triglyceride and phospholipid content of HDL, HL and EL are both significant negative regulators of plasma HDL levels [2]. Therefore, pharmacologic inhibition of these lipases might represent a valid approach to increase HDL cholesterol. This review focuses on the impact of both HL and EL on HDL metabolism and RCT, but also addresses the consequences of modulating HL and EL expression and activity for atherosclerotic lesion development.

Table 1 Characteristics of hepatic lipase and endothelial lipase

	Hepatic lipase	Endothelial lipase
Structural features	65-kD glycoprotein	68-kD glycoprotein
Tissue expression	Hepatocytes, macrophages	Endothelial cells, macrophages, thyroid gland, hepatocytes, kidney, lung, ovary, testis, and placenta
Lipolytic activity	TG > PL	PL > TG
Substrates	All lipoproteins	HDL
Impact on plasma HDL cholesterol levels	Knockout ↑	Knockout ↑
	Overexpression ↓	Overexpression ↓
Impact on cholesterol efflux	Knockout ↑ ↔	Knockout ↓ ↑
		Overexpression ↑ ↓
Impact on hepatic uptake of HDL cholesterol	Knockout ↓	Knockout ↓
	Overexpression ↑	Overexpression ↑
Impact on biliary cholesterol secretion	Knockout ↔	Overexpression ↔
Impact on fecal neutral sterol and bile acid excretion	Knockout ↔	Overexpression ↔
Impact on macrophage-to-feces RCT	Knockout ↔	Knockout ↔
		Overexpression ↓
Impact on atherosclerosis	Knockout ↑ ↓	Knockout ↓ ↔
	Overexpression ↓	
	Macrophage expression ↑	
	Macrophage knockout ↑	

TG triglycerides, *PL* phospholipids, *RCT* reverse cholesterol transport; ↑ = increased; ↓ = decreased; ↔ = not affected. Multiple arrows per condition indicate divergent reports in literature

Role of High-Density Lipoproteins in Reverse Cholesterol Transport

HDL plays a central role in RCT (Fig. 1) [2]. The first step in RCT involves the efflux of cholesterol from peripheral cells, most importantly macrophage foam cells within atherosclerotic plaques, toward either apolipoprotein (apo) A-I, which is mainly ATP-binding cassette transporter A1 (ABCA1)-mediated, or HDL, a process mainly driven by ABCG1. Lecithin:cholesterol acyltransferase (LCAT) then catalyzes the formation of mature spherical HDL by converting free cholesterol to apolar cholesterol ester, which moves to the core of the HDL particle. In addition to ABCA1 and ABCG1, the scavenger receptor class B type I (SR-BI) might also contribute to net cholesterol efflux. The cholesterol originating from peripheral cells is subsequently taken up into the liver in several distinct pathways that are all relevant for RCT: 1) selective uptake mediated by SR-BI, 2) holoparticle uptake involving the extracellularly localized β-chain of the mitochondrial F1 ATPase as well as P2Y13 receptors, and 3) in species such as humans, rabbits, hamsters, and monkeys, but not mice and rats, transfer to apoB-containing lipoproteins by cholesteryl ester transfer protein (CETP) followed by uptake via LDL receptors. The major pathway for disposal of hepatic cholesterol from the body is generally believed to

be its secretion into bile in the form of either free cholesterol or bile acids. Eventually, neutral sterols (cholesterol and its metabolites) and bile acids are excreted via the feces. The RCT pathway has been reviewed in more detailed elsewhere [2].

Hepatic Lipase

HL is a 65-kD glycoprotein synthesized and secreted primarily by hepatocytes and to a lesser extent by macrophages (Table 1) [4]. In the liver, HL is anchored via heparan sulfate proteoglycans to hepatocytes in the Space of Disse and to the sinusoidal endothelial cell surface [4]. Virtually all lipoprotein subclasses are substrates for HL, resulting in the generation of smaller and denser particles (Table 1) [5]. In addition, independent of its catalytic activity, HL anchored to cell surface proteoglycans also has a bridging function promoting receptor-mediated uptake of lipoproteins [4].

HL and HDL Metabolism

By hydrolyzing triglycerides and phospholipids, HL converts lipid-rich large HDL_2 to smaller, more dense HDL_3. Mice lacking HL characteristically display increased plasma levels of larger, phospholipid- and triglyceride-enriched
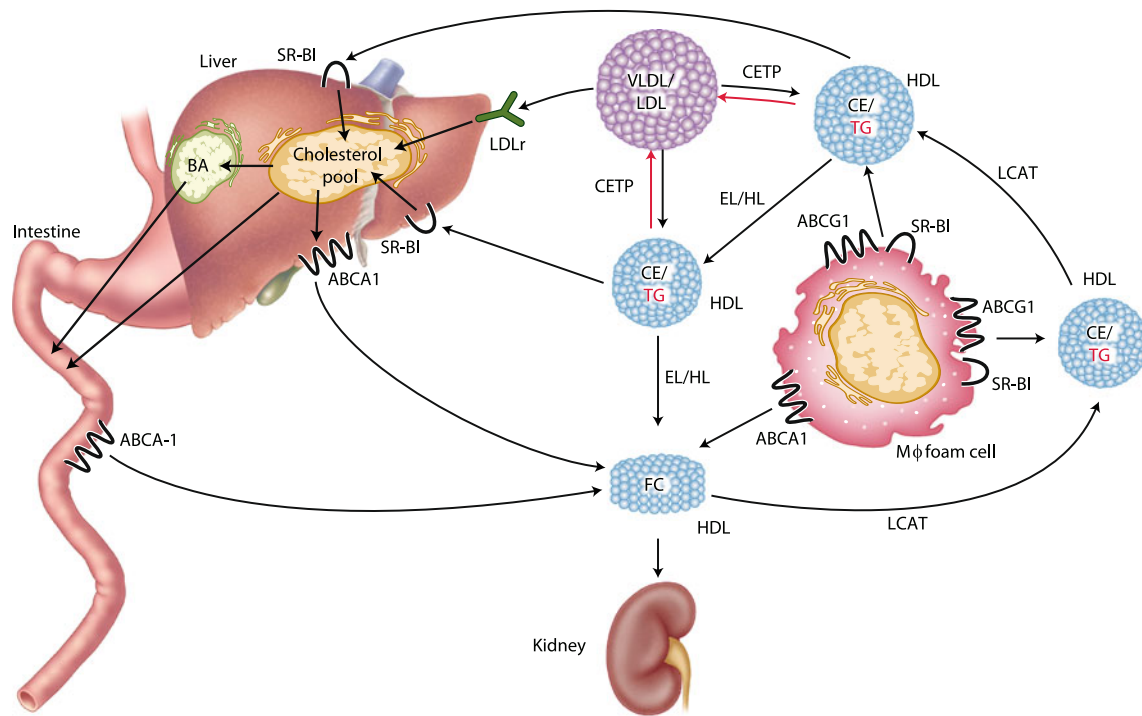
Fig. 1 Schematic depicting the impact of hepatic lipase (HL) and endothelial lipase (EL) on reverse cholesterol transport (RCT). Small discoidal high-density lipoprotein (HDL) particles are generated by the liver (about 70% contribution) and by the intestine (about 30% contribution). Free cholesterol (FC) from macrophage foam cells is effluxed toward these particles by ATP-binding cassette transporter A1 (ABCA1). Through the action of lecithin:cholesterol acyltransferase (LCAT), these particles mature and become spherical; ABCG1 and scavenger receptor class B type I (SR-BI) add more cholesterol onto these larger HDL. EL and HL hydrolyze HDL phospholipids and phospholipids/triglycerides (TG), respectively, thereby destabilizing the particle, resulting in shedding of poorly lipidated apolipoprotein (apo) A-I that is subject to clearance by the kidneys. Cholesteryl ester from these HDL particles becomes more susceptible towards SR-BI—mediated selective uptake. In turn, selective uptake is also required for HDL remodeling by these lipases to continue. Via SR-BI, HDL cholesterol enters the hepatic cholesterol pool and can either be directly secreted as free cholesterol into bile and feces or after metabolic conversion into bile acids. Cholesteryl ester transfer protein (CETP), expressed in humans but not in mice and rats, mediates the hetero-exchange of cholesteryl ester (CE) originating from HDL with triglycerides originating from apoB-containing lipoproteins. TG-enrichment of HDL makes the particle a better substrate for HL. On the other hand, cholesterol transferred to apoB-containing lipoproteins can be taken up into the liver via low-density lipoprotein receptors (LDLR) and then also enters the hepatic cholesterol pool. EL and HL expression consistently results in lower plasma HDL levels, increased hepatic cholesterol content, but unchanged biliary cholesterol secretion rates. Results regarding the impact of these lipases on cholesterol efflux are variable. The net effect of the absence of HL and EL on overall RCT is minimal, whereas overexpression of EL has been shown to decrease RCT. VLDL—very low-density lipoprotein

HDL (Table 1) [6•]. In turn, in mice and in rabbits, overexpression of human HL resulted in a marked decrease in HDL cholesterol due to an increased catabolic rate [5]. HL can also impact on HDL metabolism independent of its lipolytic properties, as adenovirus-mediated overexpression of catalytically inactive HL in HL knockout mice led to a reduction in plasma HDL levels, though to a lesser extent than observed in mice overexpressing similar levels of wild-type HL [5]. HL deficiency in humans also results in elevated levels of larger HDL_2 particles, an enrichment of HDL with triglycerides, and hyperalphalipoproteinemia attributable to slower catabolism of apoA-I [7]. Consistently, certain polymorphisms in the HL gene such as S267F, C-480T, and C-514T that result in lower HL expression and activity are firmly associated with higher plasma HDL cholesterol [8, 9]. These combined data support a key role for HL as a negative regulator of plasma HDL levels.

Impact of HL on Selected Key Steps in RCT

Cholesterol efflux is a critical first step in RCT [2]. Plasma as well as HDL from HL-deficient mice has an increased capacity to elicit cholesterol efflux from lipid-laden macrophages in vitro (Table 1), which was ascribed to phospholipid enrichment of HDL from the HL knockouts [6•]. However, in humans with a complete deficiency of HL, the ability of plasma or isolated HDL to promote cholesterol efflux from normal human skin fibroblasts was not altered [7]. Thus, the impact of HL on macrophage cholesterol efflux remains to be elucidated. It would be interesting to also study the effect of HL overexpression, because HL increases the formation of preβ-HDL, an important mediator of ABCA1-mediated macrophage cholesterol efflux [5]. In addition, the impact of macrophage HL on cholesterol efflux has not been explored yet.

Another important step in RCT is hepatic uptake of HDL cholesterol, and several lines of evidence indicate that HL stimulates this process (Table 1). HL increases the selective uptake of HDL cholesteryl ester (CE) in hepatic and non-hepatic cell lines [4]. Moreover, in vivo hepatic selective uptake of HDL-CE was impaired in HL knockout mice [6•] and significantly higher in mice overexpressing human HL [5]. Both the hydrolytic activity of HL and its heparan sulfate proteoglycan-dependent bridging properties contribute to the stimulating effect of HL on SR-BI—mediated selective uptake [5].

Although HL facilitates the uptake of HDL cholesterol into the liver, this does not appear to translate into increased biliary sterol secretion (Table 1), a third important step in RCT. The biliary secretion rates of cholesterol and bile acids were identical in HL-deficient mice and wild-type controls, even when fed cholesterol-enriched or lithogenic diets [10]. Consequently, HL deficiency did not change fecal neutral sterol or bile acid excretion (Table 1) [10].

HL and Macrophage-Specific RCT

Even though RCT from macrophages to feces represents only a small fraction of the total cholesterol pool, this flux is nevertheless considered highly relevant for the development of atherosclerosis. When mice were injected with ^{3}H cholesterol-labeled macrophages into the peritoneal cavity and the appearance of label in plasma and feces was followed for 48 h, plasma levels of ^{3}H-sterol were higher in mice deficient in HL compared to controls. However, overall macrophage-to-feces RCT determined by excretion of macrophage-derived label into the feces over 48 h remained essentially unaffected in HL knockouts (Table 1) [6•]. On the other hand, the effects of HL overexpression have not been addressed thus far. Also, HL expression by macrophages might have an impact on RCT that has not been experimentally explored yet.

HL and Atherosclerosis

Several studies have thus far addressed the role of HL in atherosclerosis development in different rodent models (Table 1) and humans but without reaching conclusive results. In LDL receptor–knockout mice, aortic atherosclerotic lesion formation was accelerated when endogenous HL was also absent [11], whereas transgenic overexpression of human HL reduced atherosclerosis by 40% to 70% [12, 13]. Overexpressing the catalytically inactive variant of human HL was atheroprotective in chow-fed LDL receptor–knockout mice lacking endogenous HL [13, 14], whereas no effect on atherosclerosis development was seen in the same model on a Western diet [12]. In contrast, HL deficiency in apoE knockout mice on a regular chow diet reduced aortic lesion size by 75% despite increased circulating levels of atherogenic apoB-containing lipoproteins. Decreased atherosclerosis development in this mouse model was proposed to be due to higher HDL levels and an increased capacity of HDL to elicit cholesterol efflux [15]. Furthermore, HL deficiency delayed the onset of occlusive atherosclerosis, resulting in myocardial infarction and cardiac dysfunction in SR-BI/apoE double-knockout mice [16]. However, the impact of HL on atherosclerotic lesion formation in an experimental animal model expressing CETP has not been reported. Bone marrow transplantation studies showed that restricting HL expression to macrophages enhanced the formation of early atherosclerotic lesions in both apoE knockout mice and LCAT transgenic mice without changing plasma lipoprotein levels [17]. On the other hand, a more recent publication demonstrated that deficiency of leukocyte-derived HL reduced circulating HDL cholesterol levels and increased the extent of aortic atherosclerosis in LDL receptor—knockout mice expressing human CETP [18]. Whether the experimental design or the mouse model used offers the explanation for these divergent results remains to be seen.

Complete HL deficiency in humans is a rare condition; however, some of these patients develop premature coronary artery disease despite increased plasma HDL cholesterol levels [19]. Likewise, a lower post-heparin plasma HL activity has been related to a higher extent of coronary artery disease in 200 men undergoing coronary angiography [20]. In contrast, human studies investigating the relationship between polymorphisms in the HL gene, which supposedly resulted in reduced HL activity, and atherosclerotic cardiovascular disease did not provide a clear picture (summarized in Table 2). In an autopsy cohort of 700 middle-aged Finnish men, the HL C-480T promoter polymorphism was related to a larger coronary atherosclerotic plaque area [21], and in a large population-based Danish study homozygosity for three common single nucleotide polymorphisms in the HL promoter (−216A, −480T, and −729G) was associated with an increased risk of ischemic heart disease despite higher plasma HDL cholesterol [22]. Furthermore, the prevalence of the C to T substitution at position 514 of the HL gene was significantly higher in 490 middle-aged male Caucasian Australian patients with coronary heart disease compared with 330 controls [23]. Controversially, in the almost 9000 participants of the Copenhagen City Heart study, no association between risk for ischemic cardiovascular disease and six genetic variants of the HL gene was detectable [8]. Importantly, the frequency of the −514T allele of HL was identical between men with documented coronary artery disease and healthy controls [24]. Other researchers found in a multiethnic population that individuals with the CC

Table 2 Selected hepatic lipase and endothelial lipase gene polymorphisms and their association with atherosclerosis and cardiovascular disease

Lipase	Gene polymorphism	Number of subjects	Impact on lipase expression and activity	Impact on HDL cholesterol levels	Outcome for atherosclerosis or CVD	Reference
HL	S267F, C-480T	2879 cases and 7735 controls	ND	Increased	No association with risk of ischemic CVD	[8]
	C-514T	501	ND	ND	Increased coronary plaque area in carriers	[21]
	G-216A, C-480T, A-719G	1741 cases and 7948 controls	ND	Increased	Increased risk of ischemic heart disease in homozygotes for all 3 variants	[22]
	C-514T	562 cases and 642 controls	ND	Increased	Increased risk of cardiovascular heart disease in male carriers but not in female carriers	[23]
	C-514T	496 cases and 294 controls	Decreased HL activity	Increased or not affected	No association with risk of CVD	[24]
	C-480T	87	ND	ND	Increased carotid intima-media wall thickness in homozygous carriers	[25]
EL	Thr111Ile[a]	107 cases and 107 controls	ND	Not affected	Decreased risk of acute myocardial infarction in carriers	[47]
	Thr111Ile	265 cases and 265 controls	ND	Increased	Decreased risk of coronary artery disease in carriers	[48]
	Thr111Ile	1501 cases and 2639 controls	ND	Not affected	No association with risk of coronary heart disease	[49]
	Thr111Ile	607 cases and 1271 controls	ND	Increased	No association with risk of coronary artery disease	[50]

CVD cardiovascular disease, HL hepatic lipase, EL endothelial lipase, ND not determined

[a] Please note that functional studies indicated that the Thr111Ile variant of endothelial lipase does not differ in expression and activity from wild-type endothelial lipase [34••]

genotype of the C-480T variant, who have higher HL activity, had a 13% greater carotid intima wall-thickness [25]. Taken together, these data stress the need for further carefully conducted studies that, in addition to genotyping the participating subjects, also aim to correlate HL mass as well as activity with relevant hard clinical end points of atherosclerotic CVD.

Endothelial Lipase

EL, encoded by the *LIPG* gene, is a 68-kD glycoprotein that was cloned independently in 1999 by two groups (Table 1) [26, 27]. The amino acid sequence of EL is 41% homologous to HL. Nevertheless, EL is distinct from other members of the triacylglycerol family because it is synthesized and secreted by endothelial cells. In addition, expression of EL has been detected in human placenta, thyroid gland, lung, liver, kidney, ovary, testis, and macrophages in vitro as well as in foam cells within human atherosclerotic plaques (Table 1) [3, 26, 27]. EL has primarily *sn*-1-phospholipase activity toward all lipoprotein subclasses, however, the preferred substrate is apparently phospholipids within HDL (Table 1) [3]. Similar to HL, EL mediates bridging between lipoproteins and cell surface heparin sulfate proteoglycans [3].

EL and HDL Metabolism

EL is a negative regulator of plasma HDL cholesterol levels (Table 1). Adenovirus-mediated overexpression of EL resulted in a marked decrease in plasma HDL cholesterol and apoA-I levels due to a dose-dependent increase in the catabolic rate of HDL apolipoproteins as well as HDL-CE [27, 28•, 29]. These metabolic effects are almost entirely dependent on the catalytic activity of EL and are not so much mediated by its bridging function, as overexpression of catalytically inactive EL had discernable effects on plasma HDL cholesterol only in HL-deficient mice but not in wild-type or apoA-I transgenic mice [3]. Conversely, loss-of-function studies in mice using either specific EL knockout models or antibody-mediated EL inhibition showed increased plasma levels of phospholipids, apoA-I, and HDL cholesterol due to a slower catabolic rate [3, 6•]. Also a shift in the HDL size toward larger particles was noted [3]. Interestingly, inhibition of EL activity [3] or EL knockout [6•] in HL-deficient mice resulted in a further significant increase in plasma HDL cholesterol levels, indicating an independent and additive role of these lipases in HDL metabolism.

Based on these experimental findings, we have proposed a model in which hydrolysis of HDL phospholipids by EL destabilizes the HDL particle, resulting in shedding of

poorly lipidated apoA-I molecules that are rapidly cleared by the kidneys (Fig. 1) [3, 29]. Concomitantly, the CE within the remainder of the HDL particle is rendered more susceptible toward SR-BI—mediated selective uptake, a mechanism that is also a prerequisite for EL-induced HDL remodeling to continue [28•, 29]. This mechanism results in an increased hepatic cholesterol content without translating into altered biliary cholesterol secretion [30].

To put these data from experimental mouse models into perspective, an important question is if EL has a similar impact on HDL metabolism in humans and thereby might represent a potential therapeutic target. In a sample of 510 healthy individuals with a family history of premature coronary artery disease, post-heparin plasma mass levels of EL were positively correlated with small HDL and negatively with large HDL particles [31]. Selected common polymorphisms of EL were also found to be associated with HDL phenotypes [32, 33]. However, the strongest data supporting a negative impact of EL on plasma HDL cholesterol in humans come from a recent report demonstrating that the low-frequency Asn396Ser *LIPG* variant results in reduced lipolytic activity and is strongly associated with increased plasma HDL cholesterol levels [34••]. Another rare mutation in the EL gene, G26S, impairs secretion of the EL protein, leading to reduced EL plasma levels and thereby also to elevated plasma HDL cholesterol in African-American carriers [35]. However, the more common Thr111Ile EL variant, which had previously been variably and inconsistently associated with plasma HDL cholesterol phenotypes [36, 37], did not impact on EL function in vitro and failed to correlate with plasma HDL cholesterol levels in a comprehensive meta-analysis of five cohort studies [34••].

Impact of EL on Selected Key Steps of RCT

Only a few studies have examined the effect of EL on HDL-mediated cellular cholesterol efflux (the first step in RCT), and none have achieved conclusive results (Table 1). Decreased EL expression in vitro in macrophages caused a decline in efflux toward apoA-I (i.e., the part of the pathway mainly mediated by ABCA1), whereas overexpression of EL or addition of exogenous EL had an opposite effect [38]. Notably, both abolishing the catalytic activity or the bridging function of EL partially inhibited macrophage cholesterol efflux [38], indicating that these two properties of EL are relevant in this process. In contrast, using modification of the efflux acceptors by EL, one study noted no effect on the ABCA1-dependent pathway [39], whereas another group reported a 63% increase in ABCA1-mediated efflux toward HDL from EL-overexpressing mice [40]. However, increased efflux

toward plasma and HDL from EL knockout mice has also been observed, and an additive effect was seen in EL/HL double-knockout mice [6•]. With respect to SR-BI–mediated efflux, a reduced capacity of EL-modified HDL [39] or of serum from EL-overexpressing mice [40] was described.

Although it variably modulates cholesterol efflux, EL was consistently reported to enhance the second step in the RCT pathway, which is the uptake of HDL cholesterol into the liver (Table 1). While diminished selective uptake of CE was observed using HDL$_3$ from EL-knockout mice [6•], SR-BI—mediated selective uptake was enhanced from HDL from EL-overexpressing mice in vitro and in vivo [28•, 30]. This increase in selective uptake is dependent upon both the catalytic activity and the bridging function of EL [41]. Consequently, these properties of EL resulted in vivo in a significant increase in hepatic cholesterol content following EL overexpression [28•, 30].

However, the major pathway of cholesterol excretion from the body, namely biliary sterol secretion either as free cholesterol or after metabolic conversion into bile acids, remained essentially unchanged in EL-overexpressing mice (Table 1). Correspondingly, EL overexpression did not affect the mass fecal output of neutral sterols and bile acids (Table 1) [30].

EL and Macrophage-Specific RCT

To date, two studies have explored the effect of EL on macrophage-specific RCT using ^{3}H-cholesterol-labeled macrophage foam cells (Table 1). EL overexpression significantly decreased in vivo RCT and the same effect was observed with overexpression of profurin [42]. Profurin inhibits proprotein convertases, which in general mediate site-specific proteolysis and have specifically been shown to impact plasma HDL cholesterol levels via specific cleavage of EL resulting in inactivation of this lipase [42]; therefore, profurin overexpression has a similar metabolic effect than EL overexpression. In addition, angiopoietin-like 3 (ANGPTL3) has been demonstrated to be an endogenous inhibitor of EL, and consequently ANGPTL3 knockout mice have low plasma HDL cholesterol levels [3]. Therefore, the effect on RCT in ANGPTL3 knockouts would be expected to be the same as in mice overexpressing EL (namely, decreased), but this has not been experimentally tested thus far. On the other hand, overall RCT is unchanged in EL knockout mice [6•]. However, EL knockouts had increased plasma levels of cholesterol originating from macrophages during the time course of the study, and this increase was significantly greater than in HL knockout mice [6•]. No additive effect of the absence of both EL and HL on plasma counts or on overall RCT was observed [6•], indicating that the effects

of absence of EL and HL on overall RCT in mice are minor.

EL and Atherosclerosis

Studies exploring the role of EL in experimental atherogenesis have not yielded conclusive results thus far (Table 1). One study reported that targeted disruption of EL in apoE knockout mice attenuated atherosclerotic lesion formation by approximately 70% on chow and to a lesser degree on a Western-type diet [43]. Interestingly, the lack of EL resulted in increased plasma levels of antiatherogenic HDL as well as proatherogenic very low-density lipoprotein and LDL cholesterol [43]. Therefore, the authors rather proposed reduced monocyte adhesion to the vascular wall via heparan sulfate proteoglycans as an underlying mechanism for decreased atherosclerosis development in apoE/ EL double knockout mice [43]. On the contrary, in another comprehensive study, no differences in atherosclerotic lesion size and lesion macrophage content were detected between mice expressing or lacking EL either on the apoE knockout or the LDL-receptor knockout background [44]. Also, this report noted a slight but consistent increase in plasma levels of both cholesterol within HDL as well as apoB-containing lipoproteins [44].

Inflammation plays an important role in the development of atherosclerosis, and a link between EL and inflammation has been suggested. Expression of EL is substantially upregulated in cultured endothelial cells in response to proinflammatory cytokines and in humans during acute experimental inflammation [3]. Although EL has been shown to enhance monocyte adhesion to the vessel wall [43], another report suggested that EL decreases endothelial adhesion molecule expression by generating peroxisome proliferator—activated receptor α (PPARα) ligands from HDL phospholipids [3]. Therefore, although EL expression is upregulated by inflammatory stimuli, it is currently unclear if EL itself exerts anti- or proinflammatory activity. In analogy to other secreted phospholipases [45], however, it could also be conceivable that EL expression induces endothelial dysfunction and thereby the susceptibility to atherosclerotic CVD, although this concept has not been experimentally addressed thus far.

In humans, the association between atherosclerotic cardiovascular disease and EL levels or variants of the EL gene has been investigated (summarized in Table 2). As indirect evidence, a significant positive correlation was found between coronary artery calcification scores and EL mass levels in pre-heparin (odds ratio=1.67) and post-heparin (odds ratio=2.42) plasma among apparently healthy individuals that persisted even after adjustment for plasma lipids, vasoactive medication, and established risk factors

[31]. A report on a relatively small group of patients with end-stage renal disease on hemodialysis suggested that during a 2-year follow-up, patients experiencing CVD events had higher serum EL levels compared with event-free patients [46]. Two small case-control studies from Japan and China noted that the common Thr111Ile EL variant was related to a decreased risk of acute myocardial infarction and coronary artery disease, respectively [47, 48]. However, the Thr111Ile variant was shown in in vitro assays to be indiscernible from wild-type EL [34••]. Subsequently, three larger Caucasian study populations could not confirm an impact of the Thr111Ile variant on cardiovascular risk [49]. More recent data from two independent, prospective, nested-control studies reported that out of five single nucleotide polymorphism in *LIPG*, only the Thr111Ile variant was modestly associated with higher HDL cholesterol, higher apoA-I levels, and a higher concentration of larger HDL particles, whereas none of the EL variants tested proved to be a risk factor for atherosclerotic cardiovascular disease [50]. Clearly, more studies are needed that relate plasma levels and/or activity of EL to hard endpoints of CVD.

Conclusions

HL and EL are both negative regulators of plasma HDL cholesterol levels. For both lipases, variable effects on cholesterol efflux, which is the first step in RCT, have been reported. HL and EL increase hepatic cholesterol content, but this does not impact biliary sterol secretion. Overall, EL overexpression decreases macrophage-to-feces RCT, whereas EL as well as HL knockout did not affect RCT. In terms of establishing these lipases as targets for therapeutic intervention, increases in RCT upon inhibition are not likely to be expected; however, a decrease in RCT might not occur either. More work seems required on the impact of HL and EL on atherosclerotic disease, including measurements of plasma mass and activity. In addition, exploring other biological properties of these lipases, such as their role in modulating inflammatory pathways, might prove useful in deciding whether HL and EL are suitable targets for pharmacologic inhibition as a therapeutic strategy against atherosclerotic CVD.

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