Relevance of hepatic lipase to the metabolism of triacylglycerol-rich lipoproteins

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Abstract
HL (hepatic lipase) is a glycoprotein that is synthesized and secreted by the liver, and which binds to heparan sulphate proteoglycans on the surface of sinusoidal endothelial cells and on the external surface of parenchymal cells in the space of Disse. HL catalyses the hydrolysis of triacylglycerols and phospholipids in different lipoproteins, contributing to the remodelling of VLDL (very-low-density lipoprotein) remnants, as well as IDL, LDL and HDL (intermediate-, low- and high-density lipoprotein respectively). HL deficiency in humans is associated with diminished conversion of VLDL remnants into IDL and a near-complete absence of IDL-to-LDL conversion. Remnant lipoproteins and IDL are major determinants of coronary artery disease risk, and accumulation of these lipoproteins in the presence of low HL activity might lead to increased atherosclerosis. In addition to and independently of its lipolytic activity, HL participates as a ligand in promoting the hepatic uptake of remnants and IDL particles, and the latter may represent an additional mechanism linking low HL levels to plasma accumulation of these atherogenic lipoproteins. On the other hand, high HL activity may also result in an increased atherogenic risk by promoting the formation of atherogenic small, dense LDL particles. Finally, HL is also synthesized by human macrophages, suggesting that, at the arterial wall site, HL may also contribute locally to promote atherosclerosis by enhancing the formation and retention in the subendothelial space of the arterial wall of VLDL remnants, IDL and small, dense LDL. In conclusion, by interfering with the metabolism of apolipoprotein B100-containing lipoproteins, HL may have pro- as well as anti-atherogenic effects. The anti- or pro-atherogenic role of HL is likely to be modulated by the concurrent presence of other lipid abnormalities (i.e. LDL-cholesterol levels), as well as by the genetic regulation of other enzymes involved in lipoprotein metabolism.

Introduction
Lipases are water-soluble enzymes that hydrolyse the ester bonds of water-insoluble substrates such as triacylglycerols (triglycerides), phospholipids and cholesteryl esters. The lipase gene family originally included LPL (lipoprotein lipase), HL (hepatic lipase) and pancreatic lipase. The genes encoding these lipases have similar exon/intron boundaries, and the proteins have significant amino acid sequence similarity, which led to the proposal that they have evolved from a common ancestor. Recent studies have implicated two other lipolytic enzymes as closely related members of this lipase gene family, namely endothelial lipase and phosphatidylserine phospholipase A1 [1] (Figure 1).

The human HL gene (LIPC) is located on chromosome 15q21, comprises nine exons, spans 135 kb of DNA and encodes a protein of 449 amino acids [1]. HL is a glycoprotein that catalyses the hydrolysis of triacylglycerols, phosphatidylethanolamines and phosphatidylcholines and the proteins have significant amino acid sequence similarity, which led to the proposal that they have evolved from a common ancestor. Recent studies have implicated two other lipolytic enzymes as closely related members of this lipase gene family, namely endothelial lipase and phosphatidylserine phospholipase A1 [1] (Figure 1).

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Table 1 | Human lipases involved in lipoprotein metabolism
EL, endothelial lipase; TG, triacylglycerol; PL, phospholipid.

<table>
<thead>
<tr>
<th>LPL</th>
<th>HL</th>
<th>EL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromosome</td>
<td>8p22</td>
<td>15q41</td>
</tr>
<tr>
<td>Substrate specificity</td>
<td>TG &gt; PL</td>
<td>TG = PL</td>
</tr>
<tr>
<td>Cofactor</td>
<td>Yes: apo CII</td>
<td>No</td>
</tr>
<tr>
<td>Preferred substrate</td>
<td>Chylomicrons, VLDL</td>
<td>IDL, HDL, LDL</td>
</tr>
<tr>
<td>Tissue localization</td>
<td>Adipose tissue, skeletal muscle, liver and steroid</td>
<td>Liver and steroid hormone-producing glands, Endothelium (liver, lung, testis, thyroid, macrophages)</td>
</tr>
<tr>
<td>Regulation</td>
<td>Rapid; fasting, feeding, exercise</td>
<td>Slower; endocrine</td>
</tr>
<tr>
<td>Half-life (h)</td>
<td>&lt;2</td>
<td>&gt;4</td>
</tr>
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Figure 1 | Evolutionary relationships of the triacylglycerol lipase gene family
Relationships are based on gene sequence identity. EL, endothelial lipase; PS-PLA₁, phosphatidylserine phospholipase A₁; PLRP, pancreatic lipase-related protein; PL, pancreatic lipase.

Role of HL in chylomicron/VLDL remnant and LDL metabolism
Plasma levels of TRLPs (triacylglycerol-rich lipoproteins) are determined by their rates of secretion from intestine (chylomicrons) and liver (VLDL), as well as by their rate of catabolism. The latter includes both triacylglycerol hydrolysis and particle uptake by cells. Catabolism of TRLP begins with the hydrolysis of core triacylglycerols by the enzyme LPL, which is synthesized primarily in adipose tissue and muscles. LPL, bound to endothelial heparan sulphate proteoglycans, interacts with TRLPs on the luminal surface of vascular endothelial cells. Thereby the initial steps of TRLP lipolysis take place in the capillary beds of adipose tissue and muscles. The smaller chylomicron and VLDL remnants resulting from the initial lipolysis by LPL are then able to permeate the fenestrate endothelium that separates the hepatocyte surface and the space of Disse from the circulation [5]. LDL receptors and the LDL receptor-related protein on the surface of the hepatocytes clear the remnant lipoproteins via an interaction with apo E (apolipoprotein E) on the chylomicron remnants and with either apo E or apo B100 on the VLDL remnants [5]. Proteoglycans on the cell surface also play an important role in TRLP remnant uptake by the liver [6]. Hepatic clearance seems to be the final step in chylomicron metabolism, while both hepatic clearance and conversion into IDL (intermediate-density lipoprotein) and LDL are alternative pathways for the metabolism of VLDL remnants [7]. HL plays a key role in both the uptake of TRLP remnants and their conversion into IDL and LDL, acting both as a ligand and as an enzyme in these processes.

Effects of HL catalytic activity
Human as well as animal studies have shown that HL is involved in the metabolism of apo B100-containing lipoproteins. Multiple lines of evidence suggest that human HL modulates the lipid composition of VLDL remnants and large, buoyant LDL, resulting in denser lipoprotein particles.
both in patients with cardiovascular disease and in normal subjects [8,9]. Direct evidence that HL plays a key role in human lipoprotein metabolism is obtained from studies of individuals with HL deficiency. Few patients with true HL deficiency have been identified [10], but a detailed study of the lipoprotein phenotype in these patients and their family members supports the concept that HL activity is essential for normal lipoprotein metabolism. Patients with complete HL deficiency present with elevated plasma cholesterol and triacylglycerol concentrations, and with a lipoprotein profile characterized by the presence of large \( \beta \)-VLDL, indicating a defect in VLDL remnant metabolism, as well as large, buoyant triacylglycerol- and phospholipid-enriched LDL and HDL (Figure 2). These large LDL particles contain apo B100 as their main apoprotein and very little, if any, apo E, truly representing a 'metabolic end-product' of the apo B-containing lipoprotein cascade in these HL-deficient patients. Moreover, incubation of these large, buoyant LDL-like particles from an HL-deficient subject with active human HL results in the formation of LDL of 'normal' density and lipid composition [11]. This enzyme appears to be rate limiting for the hydrolysis of triacylglycerol in these lipoproteins. Demant and co-workers [12], employing stable isotope techniques, reported in HL-deficient humans a 50% diminished conversion of small VLDL (VLDL remnants) into IDL and a near-complete absence of IDL-to-LDL conversion. This is consistent with the development of a type III-like lipoprotein profile described in HL-deficient subjects [10]. IDL-like lipoproteins also accumulate in conditions characterized by decreased HL activity, such as hypothyroidism [13].

IDL is a major determinant of CAD (coronary artery disease) risk [14], and accumulation of these lipoproteins in the presence of low HL activity might lead to increased atherosclerosis. An additional mechanism by which HL, by modulating TRLP metabolism, may affect atherosclerosis is postprandial lipid clearing. Chylomicron remnants are considered to be highly atherogenic. HL may promote postprandial lipid (chylomicron-remnant) clearing via several mechanisms, both dependent on and independent of its lipolytic activity [15]. In addition, species with low HL activity are prone to develop dietary hyperlipidaemia. Rabbits, for example, readily develop diet-induced hypercholesterolaemia, which is attenuated by overexpression of HL [16]. The influence of HL on these processes may reflect an anti-atherogenic potential of the protein.

On the other hand, HL also promotes the formation of atherogenic small, dense LDL particles: the higher the HL activity, the smaller, denser and more atherogenic the LDL particles [8]. LDL triacylglycerols originate from triacylglycerol-rich VLDL in exchange for cholesteryl esters under the influence of the cholesteryl ester transfer protein. Hydrolysis of these LDL triacylglycerols by HL results in the formation of small, dense LDL. In hypertrygliceridaemic men with CAD and with a family history of CAD who participated in the FATS study, a decrease in HL activity associated with big, buoyant LDL particles correlated with a decrease in coronary stenosis [17]. It thus appears that, in humans, HL activity is not related unambiguously to the risk, presence or progression of CAD.

**HL as a ligand for lipoprotein uptake**

Recent evidence, in humans, provides support for the concept that HL indeed plays an important role in lipoprotein metabolism independent of its enzymic activity [18]. That study evaluated three patients with complete HL deficiency. All three patients had buoyant LDL compared with normal
individuals. Two of the patients were characterized by having neither plasma HL activity nor detectable HL protein ($H_{Lact}^−/HLprot^−$); the third subject had no plasma HL activity, but did have a detectable amount (35.5 ng/ml) of HL protein ($H_{Lact}^−/HLprot^+$) (Figure 3). Despite expressing a relatively small amount of inactive protein (20% of the HL protein mass found in normal subjects), the $H_{Lact}^−/HLprot^+$ patient had less cholesterol in the VLDL and IDL elution range compared with the $H_{Lact}^−/HLprot^−$ patients.

Furthermore, the VLDL and IDL apo B concentrations, reflecting the number of circulating VLDL and IDL particles, were several-fold higher in the $H_{Lact}^−/HLprot^−$ patients. These data suggest that even small amounts of inactive HL protein may significantly affect human VLDL and IDL catabolism, as observed previously in vitro and in animal models. The $H_{Lact}^+/HLprot^+$ patient (with the inactive protein) had higher levels of HDL-cholesterol than the $H_{Lact}^-/HLprot^-$ patients. This finding differs from previous observations in animal models, where expression of inactive HL had only a minimal effect on HDL levels [19] or was associated with a decrease only in the apo AI-containing subclass of HDL [20]. All HL-deficient subjects showed a several-fold increase in lipoprotein triacylglycerol content across the lipoprotein density spectrum (VLDL, IDL, LDL and HDL) as compared with control subjects. Therefore inactive HL protein appears to affect VLDL and IDL particle concentration, while HL enzymic activity seems to influence VLDL, IDL, LDL and HDL triacylglycerol content and physical properties [18].

Recently, evidence has been published on the presence and synthesis of HL in human macrophages [21], a finding that may help to clarify the role of HL in atherogenesis, with implications for VLDL remnant and IDL particle metabolism. Macrophage expression of a related lipase, LPL, has been suggested to play an essential role in the progression of atherosclerotic lesions by promoting lipoprotein internalization and lipid accumulation by macrophages [22]. Like LPL, macrophage-synthesized HL may also contribute to foam cell formation and promote atherosclerosis by enhancing monocyte recruitment and retention in the arterial wall and facilitating the retention of VLDL remnants, IDL and small, dense LDL in the subendothelial space. In addition, HL synthesized in macrophages may enhance the uptake of these atherogenic lipoproteins into macrophages, suggesting a ‘localized’ pro-atherogenic function for HL in the arterial wall.

**TRLPs and atherosclerosis: clinical implications of HL-mediated metabolism**

TRLPs have been implicated in clinical coronary disease events for over 40 years. Triacylglycerol-enriched apo B-containing lipoproteins have been isolated from human atherosclerotic lesions [23]. Evidence for toxicity in endothelial cells of lipolytic products of TRLPs has been reported by Chung and Segrest [24]. More recently, these investigators have identified liposomal particles within arterial lesions that have characteristics similar to those of phospholipid- and apo AI-enriched particles formed during the in vitro lipolysis of TRLPs [25]. These observations raise the possibility that the HL-mediated uptake or intra-arterial formation (see discussion of macrophage-synthesized HL above) of such lipolytic remnants could contribute to the development of atherosclerotic disease. Studies of the transport of lipoprotein particles into the rabbit aorta have demonstrated that, whereas influx was greatest for small lipoproteins, the retention of lipoproteins within arterial tissue was greatest for VLDL particles of up to 750 Å in size, a range that includes LDL, IDL and larger VLDL species [26]. Although findings in HL-deficient patients indicate that the lipolytic action of this enzyme contributes to modulation of the atherosclerotic process, recent evidence [21] supports its role in the retention/formation of VLDL remnants, IDL and possibly small, dense LDL by the arterial wall [27]. Experimental evidence strongly supports a similar role for macrophage-derived LPL [28]. Therefore the interaction of arterial HL with TRLPs may also promote retention of these particles and their lipolytic products.

Remnant lipoprotein and IDL metabolism is related to human atherosclerosis in vivo. In the Monitored Atherosclerosis Regression Study (MARS), an association between coronary artery lesion progression and the mass of all major lipoprotein fractions was observed across the VLDL–IDL spectrum, as measured by analytical ultracentrifugation [29]. In contrast, no significant relationship was found between coronary artery lesion progression and the bulk of LDL mass. A more recent analysis from the MARS trial has shown that the progression of carotid artery intima-media thickness was significantly correlated with on-trial levels of IDL mass, but not with levels of the VLDL, LDL or HDL fractions [30]. Moreover, in multivariate models that included other lipoprotein predictors of carotid intima-media
thickness progression, including total/HDL cholesterol ratio, plasma apo B and plasma apo E, IDL mass was the only variable that showed a significant relationship.

TRLPs are associated with CAD events and with the progression/regression of coronary atherosclerosis. TRLP remnants and IDL appear to be most closely related to changes in mild/moderate lesions. It appears that clinical events most commonly derive from lesions that are initially of mild or moderate severity and which suddenly undergo a transformation to a severe ‘culprit’ lesion [31]. Thus impaired TRLP catabolism, as occurs in the presence of low HL activity, may be particularly important in the progression to clinical CAD events. Accumulation of TRLPs is typically associated with other lipoprotein abnormalities, specifically the presence of small, dense LDL and lower HDL-cholesterol levels, which are commonly found in association with an increased risk of CAD.

Conclusions
By modulating the metabolism of apo B100-containing lipoproteins, HL may have pro- as well as anti-atherogenic effects. Either enzymically or as a binding protein, it may change the level, the composition and/or the metabolism of lipoproteins in a more or a less atherogenic direction. In the presence of hypertriglyceridaemia or an increased LDL concentration, the pro-atherogenic effect of high HL activity (the formation of small, dense LDL) may prevail: the presence of high concentrations of small, dense and atherogenic LDL (the formation of small, dense LDL) may prevail: the presence of high HL activity, may be particularly important in the progression to clinical CAD events. Accumulation of TRLPs is typically associated with other lipoprotein abnormalities, specifically the presence of small, dense LDL and lower HDL-cholesterol levels, which are commonly found in association with an increased risk of CAD.

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