thematic review

Hepatic lipase: a pro- or anti-atherogenic protein?

Hans Jansen,[1,*,†,§] Adrie J. M. Verhoeven,[*] and Eric J. G. Sijbrands[†]

Departments of Biochemistry,[*] Internal Medicine,[†] and Clinical Chemistry,[§] Erasmus MC, Erasmus University Rotterdam, Rotterdam, The Netherlands

Abstract **Hepatic lipase (HL) plays a role in the metabolism of pro- and anti-atherogenic lipoproteins affecting their plasma level and composition. However, there is controversy regarding whether HL accelerates or retards atherosclerosis. Its effects on different lipoprotein classes show that, potentially, HL may promote as well as decrease atherogenesis. Studies in animals with genetically modulated HL expression show that it depends on the model used whether HL acts pro- or anti-atherogenic. In humans, HL activity seems to correlate inversely with atherosclerosis in (familial) hypercholesterolemia, and positively in hypertriglyceridemia. In normolipidemia, HL activity is weakly associated with coronary artery disease (CAD). Genetically low or absent HL activity is usually associated with increased CAD risk, especially if plasma lipid transport is impaired due to other factors. Since HL promotes the uptake of lipoproteins and lipoprotein-associated lipids, HL may affect intracellular lipid content. We hypothesize that the prime role of HL is to maintain, in concert with other factors (e.g., lipoprotein receptors), intracellular lipid homeostasis. This, and the uncertainties about its impact on human atherosclerosis, makes it difficult to predict whether HL is a suitable target for intervention to lower CAD risk. First, the physiological meaning of changes in HL activity under different conditions should be clarified.**—Jansen, H., A. J. M. Verhoeven, and E. J. G. Sijbrands. **Hepatic lipase: a pro- or anti-atherogenic protein?** ***J. Lipid Res.*** 43: **1352–1362.**

Supplementary key words coronary heart disease • risk factor • lipoprotein metabolism • lipid homeostasis • liver • hyperlipidemia • obesity

Many factors contribute to the development and progression of atherosclerosis. There are good reasons to believe that hepatic lipase (HL) is one of these factors. HL plays a role in the metabolism of several pro- and anti-atherogenic lipoproteins. In humans, a change in HL activity is often associated with changes in the plasma level and composition of different lipoproteins, and with an increased or lowered risk of coronary artery disease (CAD). However, despite of extensive research during the last decade and the use of genetically modified animal models, the questions of whether HL is a pro- or anti-atherogenic enzyme, and whether we can or should modulate HL to decrease CAD risk, have not been unambiguously answered. In vitro and in vivo studies suggest the pro- as well as anti-atherogenic potential of HL (**Table 1**). In recent studies, HL was identified as focal point in the development of CAD. However, the investigators came to opposite conclusions with regard to the influence of HL on atherogenesis. While Zambon and coworkers (1) concluded "regression of coronary atherosclerosis results from . . . a new pathway of HL-mediated (read decreased HL) improvement in LDL buoyancy," Dugi and coworkers (2) stated "Low hepatic lipase activity is a novel risk factor for coronary artery disease."

In this review, we will summarize evidence that connects HL to CAD. We will discuss the potential impact of HL on CAD based on its proposed roles in (lipoprotein) metabolism. Finally, we will try to answer the question of whether or not modulation of HL expression/activity is an option to reduce atherosclerotic risk. To limit the number of literature references, we will as much as possible refer to recent reviews in which more detailed information can be found.

HEPATIC LIPASE

The nature and molecular characteristics of HL are reviewed in an accompanying review in this issue (3). Therefore, we summarize only major characteristics here.

The gene encoding the HL glycoprotein is a member of the lipase gene family. This gene, indicated as *LIPC*, is expressed, although to very different levels, in (almost) all species. Once glycosylated, HL has a molecular mass around 60 kDa. The protein is primarily synthesized in the liver and bound extracellularly to parenchymal cell surfaces in the space of Disse. In mice, guinea pigs, and golden hamsters, part of its activity circulates in the

Abbreviations: apo, apolipoprotein; CAD, coronary artery disease; FH, familial hypercholesterolemia; HL, hepatic lipase; RCT, reverse cholesterol transfer; LDLR, LDL receptor.

[1] To whom correspondence should be addressed.
e-mail: jansen@bc1.fgg.eur.nl

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TABLE 1. Arguments for pro- and anti-atherogenic potential of hepatic lipase

Impact on Human Lipoprotein Metabolism	
Pro-atherogenic	Decreases LDL size
Anti-atherogenic	Stimulates HDL cholesterol (ester) uptake (reverse cholesterol transport)
	Stimulates post-prandial lipid clearing
	Stimulates IDL clearing
	Stimulates preβ HDL, HDL3 formation
Animal Studies	
Pro-atherogenic	Deficiency attenuates atherosclerosis in apoE k.o. mice
	Overexpression augments plaque size in rabbits
Anti-atherogenic	Over-expression in mice decreases aortic cholesterol deposition
	Inhibition of activity in apoA-II overexpressing mice increases aortic cholesterol deposition
	Animals low in HL show diet-induced hyperlipidemia
Associations with Human Lipoprotein Profile	
Pro-atherogenic	Activity inversely correlated with HDL (2)
	Activity inversely correlated with LDL size
Anti-atherogenic	Activity inversely correlated with post-prandial lipids
	Activity inversely correlated with LpCIII:B
Association with Human Atherosclerosis Promoting Diseases and Other Conditions	
Pro-atherogenic	Activity high in males compared to females
	Activity positively associated with insulin-resistance and high in type 2 diabetes
	Activity positively correlated with omental fat mass, fasting insulin
	Activity high in FH
Anti-atherogenic	Activity low in hypothyroidism
Association with Human CAD	
Pro-atherogenic	Decrease in activity during hypolipidemic drug treatment associated with increased LDL size and decreased CAD
Anti-atherogenic	Activity low in CAD patients (if accompanied by low CETP)
	Activity inversely associated with calcification in homozygote FH
	Activity predictor of CAD regression after dietary intervention
	Deficiency associated with increased CAD risk

The table summarizes arguments in favor of a pro- or anti-atherogenic potential of HL based on different experimental approaches.

plasma. In humans, its activity can be measured in the blood plasma after intravenous heparin injection to release the enzyme from the hepatic extracellular matrix. In addition to liver, HL is found in the adrenal glands and ovaries. The liver-associated HL activity represents more than 95% of the total activity and is therefore the most important activity for plasma lipoprotein metabolism. Recently, HL expression was detected in macrophages. Although macrophage-derived HL may be important for the atherosclerotic process, we will not discuss it, because little is known about this activity at the moment.

HL affects the phospholipid, triglyceride, and cholesterol content of several lipoprotein classes by its phospholipase A-1 and triglyceridase activities. Additionally, HL may influence lipoprotein metabolism by bridging lipoproteins to lipoprotein receptors or other cell surface components. As depicted in **Fig. 1**, HL is involved in the metabolism of HDL, apolipoprotein (apo)B-100, and apoB-48 containing lipoproteins [recently reviewed in (4–6)].

ASSOCIATION WITH ATHEROSCLEROSIS

Human studies

As shown in Table 1, HL may be linked to atherosclerosis in many ways. In humans, we have to rely on its activity in post-heparin plasma or the presence of functional genetic variants to assess the relation of HL with CAD.

In conditions with increased atherosclerotic risk, HL activity is often high. Males exhibit higher HL activity than premenopausal women, HL is lowered upon physical activity (7), and increased by smoking (8). In addition, HL activity increases with the degree of insulin-resistance in type 2 diabetes (9) and with omental fat mass in women (10). In familial combined hyperlipidemia (FCH) (11) and type 2 diabetes, HL may contribute to the development of the atherogenic lipid profile, characterized by low HDL cholesterol (HDL-C) levels and the presence of small-dense LDL (9). Increased HL activity is also reported in familial hypercholesterolemia (FH), a condition with greatly enhanced atherogenic risk (12). In homozygote FH patients, however, HL activity is strongly inversely associated with the extent of coronary calcification (13).

Low HL activity has been reported in patients with clinically overt CAD (2, 14, 15). In a population of 200 men undergoing elective coronary angiography, the extent of CAD correlated inversely with HL activity ($r = -0.19$, $P < 0.001$), indicating that approximately 4% of the variance in CAD could be explained by HL. However, 45% of these men were on lipid-lowering drugs. This may have influenced the association as lipid-lowering drugs are reported to reduce HL activity (16, 17). In 720 normolipidemic

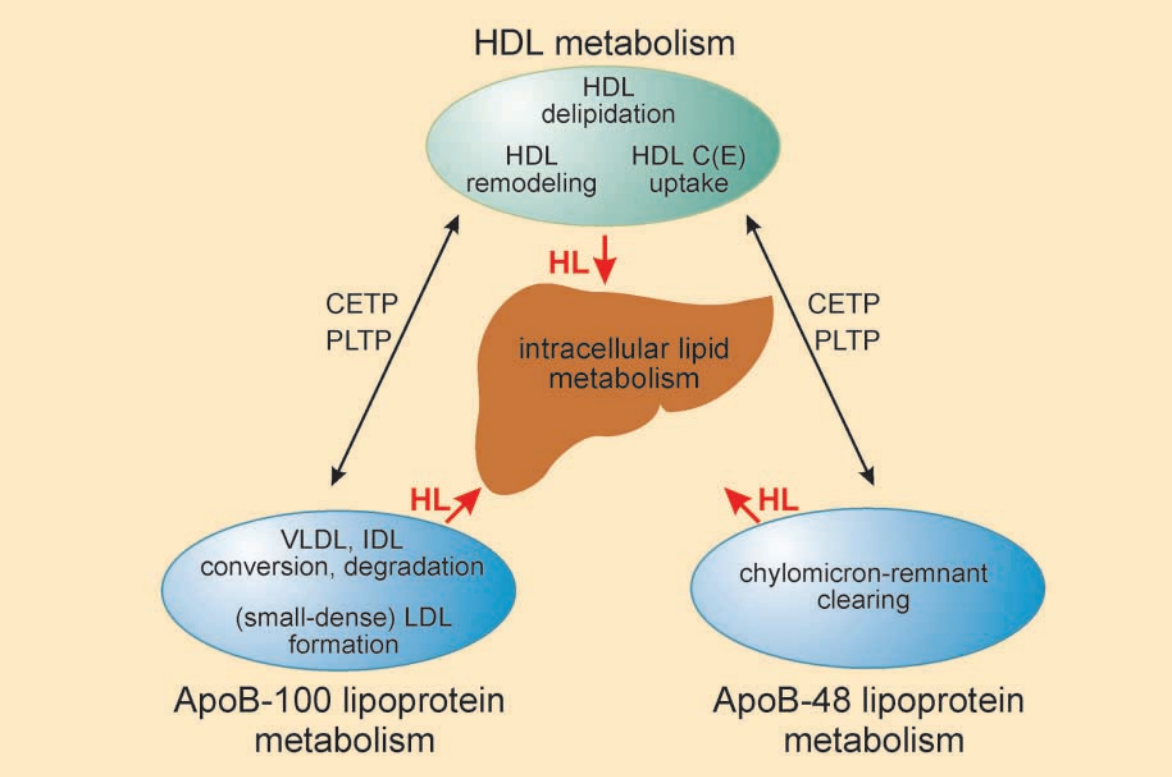

Fig. 1. Central role of hepatic lipase (HL) in lipid metabolism. The oval panels represent the major pathways of plasma lipid transport in which HL is proposed to play a role. Within the panels, processes affected by HL are given. Between the lipoproteins of the different pathways, lipids are exchanged under the influence of transfer proteins, cholesterol ester transfer protein (CETP), and phospholipid transfer protein (PLTP) as represented by double headed arrows. HL interacts with the different pathways by hydrolyzing lipids, notably phospholipids and triglycerides, or by bridging the lipoprotein to lipoprotein receptors or heparan sulphates on hepatocytes. In this way, HL connects plasma lipid transport to intracellular lipid metabolism in the liver.

or slightly hyperlipidemic males with angiographically proven CAD (REGRESS study), HL activity was not different from age-matched controls (18).

The relationship between HL activity and changes in CAD during intervention was studied in the Leiden Intervention Trial. Subjects with severe CAD were subjected to a strictly vegetarian diet for 2 years. HL activity determined at the end of the study period correlated positively with regression of coronary atherosclerotic lesion size ($r = -0.55$) (14); it appeared to be the most important predictor of regression. However, during hypolipidemic treatment of hypertriglyceridemic CAD patients with a familial history of CAD who participated in the FATS study, Zambon and coworkers (19) found that a decrease in HL activity correlated with a decrease in coronary stenosis ($r = 0.57$). It thus appears that in humans, HL activity is not unambiguously related to the risk, presence, or progression of CAD.

Correlation between HL activity and CAD does not necessarily reflect a causal relationship. Association of genetically induced variation of HL expression with CAD is more likely to be indicative for a contribution of HL to CAD risk.

HL deficiency is generally associated with increased CAD risk [reviewed by Connelly and Hegele (20)]; however, the number of affected individuals is low, so the contribution to CAD is difficult to quantify. For the general population, a common functional variant in the *LIPC* promoter [*HL*-C480T (18) or *LIPC*-514C>T (21)] is of interest. This base substitution is linked with three other base variants in the proximal *LIPC* promoter (21). The collective presence of the linked variants represents two alleles that will be indicated as *LIPC* C- and T-allele. The T-allele leads to a diminished HL promoter activity and is associated with a 15–30% lower post-heparin HL activity in humans. Two studies compared the occurrence of the T-allele in normolipidemic subjects with and without CAD (18, 22). In both studies, the frequency of the T-allele tended to be slightly higher among the CAD patients than in the controls. Recently, Andersen and coworkers reported in the Copenhagen Heart Study a 1.7- (95% CI: 1.2–2.4) fold higher risk of CAD in homozygote T-allele carriers relative to homozygote C-allele carriers (23). In healthy, mildly hypercholesterolemic T-allele carriers, Fan et al. (24) found a reduced adenosine-stimulated coronary blood flow, an early sign of coronary dysfunction. In men with suspected CAD, Dugi and coworkers (2) found that the T-allele was significantly associated with more severe CAD. Hokanson and coworkers (25) found the T-allele associated with coronary calcification in type 1 diabetes. These data show that a genetic variant leading to a 15–30% lowering of HL activity may increase

atherosclerotic risk, but that it does not constitute a major risk factor.

In their review on HL deficiency, Connelly and Hegele (20) proposed that HL deficiency increases atherogenic risk, especially in the presence of a second genetic or environmental factor affecting lipoprotein levels. Data of Hirano and coworkers (26) support this idea. They found that subjects with low HL activity exhibited increased CAD only in the presence of genetically determined low cholesteryl ester transfer protein (CETP) activity. We recently obtained further support for this view by comparing the presence of CETP and LIPC variants in men with atherosclerosis (REGRESS study) with non-symptomatic controls. The CAD patients were 7.16 more often carrier of the combined *LIPC* T/T (low HL) and *CETP* B2/B2 (low CETP) genotype than the controls. Moreover, the combined homozygosity for the *LIPC* T-allele and the *CETP* B2 allele associated within the REGRESS group with a larger progression of CAD compared with other allelic combinations (unpublished). It thus appears that while a slightly reduced HL expression in itself has little effect on CAD, it may significantly increase CAD risk in the presence of other lipid abnormalities. The apparent potentiation of the impact of HL on CAD by other factors is further illustrated by the difference in association of HL with CAD in two studies by Dugi and coworkers in homozygote FH and other patients. HL activity and CAD were stronger correlated in the homozygous FH patients than in the other patients (2, 13). In the FH patients, HL activity and lipoprotein lipase (LPL) dimer mass together accounted for 85% of the variability in coronary calcification ($r = 0.92$, $P = 0.0005$).

If interaction with environmental and other genetic factors is important, the *LIPC* C>T variation might affect lipid metabolism and CAD risk differently in different populations; and vice versa, the atherogenicity of other factors may be modulated by differences in the presence the *LIPC* C>T genotype as well. In this respect it is of interest that the frequency of the C>T polymorphism varies greatly among different ethnic groups. While the T-allele is the common allele in African Americans with a frequency of 0.53 (27), in Caucasians the T-allele frequency is approximately 0.20 (18, 21). Subjects of East Asian descent show intermediate values (28). This means that when allele frequencies in populations are compared with the established effect of an HL variant on CAD risk, the ethnic background and other confounding factors should be taken into account.

Animal models

Causality between HL overexpression or deficiency and atherosclerosis has been studied in genetically modified models. In cholesterol-fed transgenic mice, overexpression of HL led to a diminished aortic cholesterol content in spite of reduced HDL levels (29). Inhibition of HL activity in mice by overexpression of apoA-II increased HDL levels but accelerated atherosclerosis (30, 31). In atherogenic apoE deficient female mice, however, HL deficiency led to smaller aortic plaque size (32). In cholesterol-fed rabbits, HL overexpression attenuated the rise in plasma lipids, but increased lesion thickness (33). These data clearly support the view that the effect of HL on atherogenesis is dependent on the context in which it is studied.

Taken together, the human and animal studies show that HL variably associates with atherosclerosis and CAD risk. In humans, the strongest, but opposite, correlations between HL activity and CAD exist in homozygous FH and hypertriglyceridemia. Genetically decreased HL in humans carrying the *LIPC* T-allele tends to increase CAD risk, but only to a limited extent. In conjunction with variants in other genes (and/or environmental factors), the effect of the T-allele may be augmented.

HL presumably affects CAD through its effects on lipoprotein metabolism. In the following sections, we will discuss the potential consequences of these effects for atherosclerosis.

ASSOCIATION WITH PRO- AND ANTI-ATHEROGENIC LIPOPROTEINS

Role in HDL metabolism and reverse cholesterol transport

HL activity is usually inversely correlated with HDL-C levels. Therefore, high HL activity is often considered to increase CAD risk. However, this association may be caused by several distinct mechanisms. Opposite changes in HL activity and apoA-I synthesis or lecithin:cholesterol transferase (LCAT) activity (34) may result in an inverse correlation between HDL and HL without reflecting a causal relationship (5). Still, there are good reasons to assume a role of HL in HDL metabolism. In humans, the HL gene locus is associated with 25% of the variation in HDL-C levels (35). The *LIPC* T-allele increases HDL-C, although this effect differs greatly among different populations and is sometimes even absent (36) [for a review see Cohen (6)]. It is generally agreed that HL is involved in the interconversion of HDL subclasses by hydrolyzing phospholipids and triglycerides [reviewed by Navab et al. (37)]. Since HL is a membrane bound enzyme, this may lead to uptake of the hydrolysis products into the cell. In 1980, we proposed a role for HL in reverse cholesterol transport (RCT) based on the assumption that degradation of HDL phospholipids renders the particle more prone to deliver cholesterol (ester) to the lipase-containing tissues (38). It has now been well established by in vitro and in vivo studies that HL promotes the uptake of HDL-C (ester) (39, 40), either directly and/or by facilitating uptake via lipoprotein receptors such as the putative HDL receptor (HDLR), scavenger receptor class B type I (SR-BI) (41, 42). By stimulating HDL-C (ester) uptake, HL may lower HDL-C levels. In all animal studies in which HL expression was modulated, HDL levels and HL expression changed in the opposite direction. A decrease in HDL as occurred in HL overexpressing transgenic mice (29) was also observed upon hepatic overexpression of SR-BI in mice liver (43). In both conditions, the low HDL-C level may be associated with an accelerated RCT.

Delipidation of HDL by HL leads to the generation of smaller HDL subclasses (e.g., HDL3 and/or preβ HDL). These subclasses stimulate cholesterol efflux from cells efficiently [reviewed by Fielding and Fielding (44)]. Cholesterol efflux from peripheral tissues represents the first step in RCT. Thus, potentially, HL may stimulate RCT by promoting HDL-C (ester) uptake in the liver as well as by affecting HDL functionality. Involvement of HL in RCT is regarded a major mechanism by which HL may influence CAD risk [reviews by von Eckerdstein et al. (45, 46) and Hill and MacQueen (47)].

In addition, changes in HL activity may alter the (anti) atherogenic properties of HDL in different ways. In apoE deficient mice, HL deficiency increased the cholesterol efflux inducing capacity of HDL (32). In apoA-II overexpressing mice, however, where HL activity is inhibited, HDL lost its anti-inflammatory capacity and became proinflammatory (31). This, and the fact that HL affects the metabolism of other lipoproteins as well, explains why increased HDL levels due to lowered HL activity do not always decrease peripheral lipid deposition. It illustrates once more that the HDL level is often a poor indicator of atherogenic risk in animal models. Nofer et al. (48) reviewed the role of HDL in atherosclerosis in genetically modified mouse models. It appeared that only in 19 out of the 31 strains, HDL was inversely associated with atherosclerosis.

In humans, peripheral cholesterol once taken up by HDL and esterified by LCAT may be transferred to the liver via two distinct routes (**Fig. 2**). Both routes are connected via lipid transfer proteins. In the direct route, which involves HL, the liver takes up HDL-C directly. In the indirect route, HDL-C ester is first transferred to the apoB-100 or apoB-48 containing lipoproteins, and is finally taken up by receptor-mediated endocytosis (e.g., LDLR) [for reviews see (46, 47)]. In the absence of CETP, the direct route represents the major RCT pathway.

As discussed above, in experimental animals that lack CETP, HL is often inversely associated with atherosclerosis. In humans with low CETP, HL activity also seems inversely associated with CAD. Hence, the anti-atherogenic properties of HL seem to prevail when the indirect route of RCT is (relatively) blocked. This may occur in situations with low CETP as well as in situations with decreased LDLR activity, such as FH. If cholesterol ester transfer to apoB-containing lipoproteins and LDLR activity are high, the HL-mediated pathway may be of less importance for RCT.

The presence of CETP has, however, additional consequences for the role of HL in HDL metabolism. Cholesterol esters are transferred to apoB containing lipoproteins in exchange for triglycerides. During cholesterol ester transfer, HDL is depleted of cholesterol ester and enriched in triglyceride. This explains the generally observed inverse

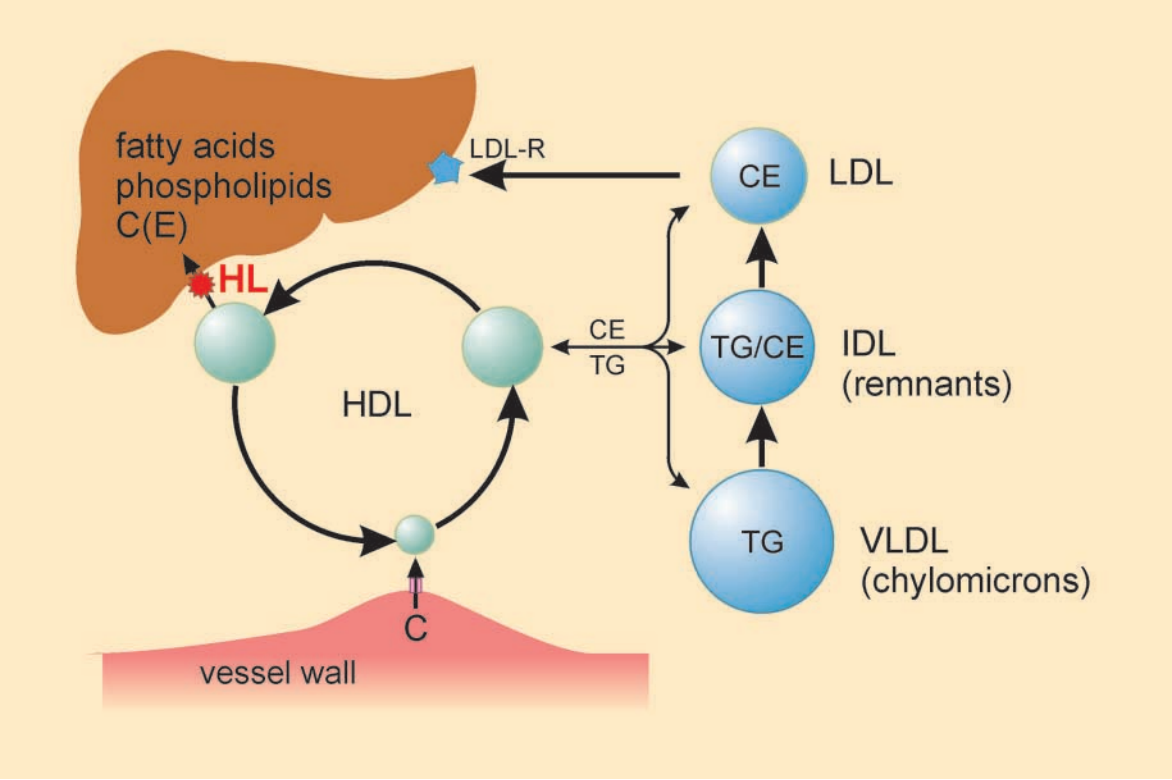

Fig. 2. Direct and indirect routes of reverse cholesterol transport in humans. Cholesterol from peripheral tissues is transported to the liver by two distinct mechanisms [for reviews see (46, 47)]. In the direct route, which involves HL (represented by the red symbol), the liver takes up HDL cholesterol (HDL-C) via mechanisms in which lipoprotein receptors also, like the scavenger receptor class B type I (SR-BI), may take part. In the indirect route, HDL-C ester is first transferred to apolipoprotein (apo)B containing lipoproteins in exchange for triglycerides. The apoB containing lipoproteins are taken up via receptor-mediated endocytosis.

correlation between plasma triglyceride and HDL-C levels. HDL triglycerides are substrate for HL. Hydrolysis of the HDL triglycerides and phospholipids by HL results in delipidation of HDL. Since delipidated HDL is more prone to degradation than lipid-rich HDL, HDL degradation increases. To what extent cholesterol esters are exchanged for triglycerides depends on the triglyceride concentration. In hypertriglyceridemic conditions, such as type 2 diabetes, more HDL-C will be transferred to the apoB containing lipoproteins and HDL will become triglyceride-enriched (49). HL-mediated hydrolysis of triglyceride in the cholesterol ester poor HDL will not contribute greatly to HDL-C ester uptake and RCT, but to an accelerated degradation of apoA-I. This suggests that during hypertriglyceridemia, the effect of HL on HDL metabolism may be rather pro- than anti-atherogenic.

In conclusion, HL may affect HDL metabolism, levels, and composition in several ways.

With regard to RCT, the effect of HL on HDL metabolism may differ under different conditions. Possibly, it is beneficial during hypercholesterolemia, but unfavorable during hypertriglyceridemia.

Role in IDL/LDL metabolism

Human as well animal studies have shown that HL also affects the metabolism of apoB-100 containing lipoproteins. Demant and coworkers (50), employing stable isotope techniques, reported in HL deficient humans a 50% diminished conversion of small VLDL to IDL and a near complete absence of IDL to LDL conversion. This was consistent with the development of a type III-like lipoprotein profile described in HL deficient subjects (20). We found that the T-allele, which lowers HL expression partially, dose dependently increased LpCIII:B levels in healthy young participants of the EARS-II study (51). IDL-like lipoproteins accumulate also in conditions with decreased HL activity, such as hypothyroidism (52).

IDL is a major determinant of CAD risk (53). Therefore, low HL activity might lead to increased atherosclerosis due to the accumulation of IDL. On the other hand, in humans, HL may also promote the generation of atherogenic small-dense LDL. LDL triglycerides may be derived from triglyceride-rich VLDL in exchange for cholesterol ester under influence of CETP. Hydrolysis of these LDL triglycerides by HL results in the generation of small-dense LDL [reviewed by Havel (54)]. Zambon and coworkers (19) showed in hypertriglyceridemic males with CAD that intensive lipid lowering resulted in increased LDL buoyancy, which was strongly ($r = 0.79$) correlated with decreased HL activity. The increased LDL size was the major determinant of regression of atherosclerosis. The decrease in HL may thus lead to regression of CAD during treatment of such patients. Therefore, Zambon and coworkers proposed HL as a focal point in the development of atherosclerosis and as a potential site of treatment (1).

However, HL is not always a determinant of LDL size. Neither in a study on 126 dizygote female twins, nor in a genetic linkage study in 19 families, was the HL gene locus linked to LDL size (55, 56). In the normolipidemic REGRESS population, we found that HL associated with the presence of the LDL subclass pattern B, representing small-dense LDL. This relationship disappeared in multivariate analysis when triglycerides and HDL were taken into account (57). It may be that only if LDL is greatly enriched in triglycerides, as in hypertriglyceridemia, HL activity becomes rate-limiting in the hydrolysis of LDL triglycerides and thus in the generation of small-dense LDL. In non-hypertriglyceridemic conditions, the amount of VLDL triglycerides exchanged may be limiting and determine the LDL size.

Taken together, by interfering with the metabolism of apoB-100 containing lipoproteins, HL may have pro- as well as anti-atherogenic effects. During hypertriglyceridemia (its role in the formation of small-dense LDL) the pro-atherogenic effect of high HL may prevail over the potentially beneficial effects on IDL metabolism.

Role in postprandial lipid clearing

The third major mechanism by which HL 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 [reviewed by Yu and Cooper (58)]. In addition, species with low HL are prone to develop dietary hyperlipidemia. Rabbits, for example, readily develop diet-induced hypercholesterolemia, which is attenuated by overexpression of HL (59).

The influence of HL on these processes clearly represents an anti-atherogenic potential of the protein.

ADDITIONAL MECHANISMS

Interaction with carbohydrate metabolism and insulin-resistance

While the role of HL in lipoprotein metabolism is considered to be the major mechanism by which it may affect atherosclerosis, novel evidence suggests that HL may interfere with glucose homeostasis as well.

Pihlajamaki and coworkers (60) studied healthy controls and members of families with familial combined hyperlipidemia in a Finnish population. They showed that the *LIPC* T-allele was not only associated with dyslipidemia but also with insulin-resistance.

In the EARS-II study population, consisting of healthy young males, we found that interaction of the *LIPC* T-allele with the *APOC3*-482T variant resulted in a significantly increased glucose and insulin response during oral glucose tolerance testing (61). In this light, it is of interest that the omental fat mass, a determinant of insulin-sensitivity, is associated with HL activity (10, 62, 63).

Although little is known about the consequences of these findings, the link between HL expression and glucose homeostasis constitutes an exciting new field of research and may represent a novel link between HL and CAD.

IMPORTANCE OF HL IN (PLASMA) LIPID METABOLISM

Importance of HL and interaction with other factors of lipid metabolism

While HL may affect the metabolism of several lipoproteins and CAD risk, its quantitative contribution to these processes is not clear. HL activity is often only weakly correlated to lipoprotein levels and to CAD risk. This does not disqualify HL as an important player in these processes. A number of factors may obscure the relationship between HL and lipoproteins and atherosclerosis. HL activity may be stimulated or inhibited by apoA-I, apoA-II, apoA-IV, apoC-III, and apoE . In situ, the nature of its lipoprotein substrate may vary depending on the composition of lipoproteins as discussed above for HDL and LDL. CETP may play an important role in this.

Similarly, phospholipid transfer protein (PLTP) facilitates the transfer of phospholipids [recently reviewed by Huuskonen and Ehnholm (64) and van Tol (65)]. If HDL phospholipids are degraded by HL, HDL phospholipids may be replenished under the influence of PLTP by, for example, the phospholipid-rich surface fragments generated during the lipoprotein lipase-mediated degradation of triglyceride-rich lipoproteins. The in situ HL phospholipase activity is thus dependent on LPL and PLTP activities, and on the amount of triglyceride-rich lipoproteins.

Therefore, the impact of HL on lipoprotein metabolism will vary depending on the amount and nature of potential substrates, and on its activity relative to that of the transfer proteins. Consequently, in post-heparin plasma-measured HL activity may only remotely reflect its in situ activity.

HL linking intracellular lipid metabolism to plasma lipid transport

HL is usually studied in relation to its role in plasma lipoprotein transport. Very little is known about the impact that HL may have on intracellular lipid homeostasis. Its tissue-bound character and exclusive localization in tissues with an active cholesterol metabolism, however, point to involvement in intracellular lipid homeostasis. While different pathways of plasma lipoproteins are connected by lipid transfer proteins, HL, in concert with lipoprotein receptors, links the plasma lipid transport to intracellular lipid metabolism (Fig. 1). In fact, the main function of the enzyme is probably the maintenance of intracellular lipid homeostasis. The influence on plasma lipoprotein transport may be secondary to this role. **Figure 3** shows HL as one of the factors that affects intracellular lipid metabolism. There are good indications that HL is a key player in intracellular cholesterol homeostasis in different tissues. Gene-targeted inactivation of HL expression in mice, and acute inhibition of HL activity in rat adrenals, resulted in a greatly stimulated adrenal SR-BI expression (66, 67). In rat liver and ovaries, inactivation of HL activity by antibod-

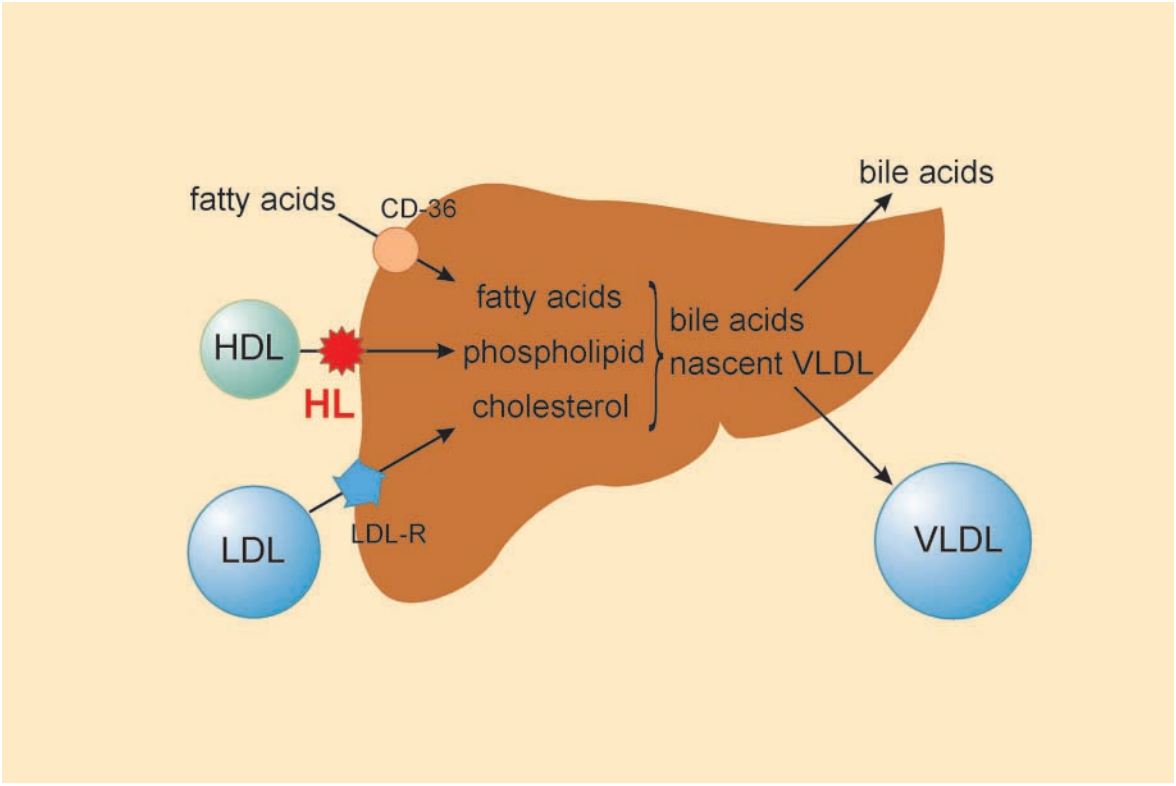

Fig. 3. HL as a factor in hepatic intracellular lipid homeostasis. Lipids are taken up by the liver via several mechanisms. Lipoprotein receptors mediate in the endocytosis of whole lipoproteins. HL (red symbol) may modulate the binding of lipoproteins (e.g., chylomicron-remnants) to receptors (58), or stimulate the uptake of HDL-C (ester) directly, or facilitate selective uptake via SR-BI (41, 42).

ies led to stimulation of de novo cholesterol synthesis (68, 69). Likely, the stimulation of SR-B1 and cholesterol synthesis takes place to maintain intracellular cholesterol levels. The occurrence of similar compensatory mechanisms in the liver might explain the rather faint phenotypic effects of HL deficiency.

Cholesterol homeostasis is maintained through coordinate regulation of the expression of many genes. If HL plays a role in cholesterol homeostasis, regulation of HL expression should fit within the same framework. Busch and coworkers (70) showed that in HepG2 cells treated with the HMG-CoA reductase inhibitor mevinolin, the addition of 25-hydroxycholesterol decreased HMG-CoA reductase expression and increased HL expression. They suggested that an intermediate of the de novo cholesterol-synthesizing pathway affects HL expression (71). We (72) recently found that the activity of HL promoter-reporter constructs in HepG2 cells is reduced by co-transfection with an SREBP-2 expression vector. SREBP-2 is a transcription factor that plays a central role in cholesterol homeostasis (73). A number of observations in humans support the hypothesis that HL expression is regulated as an integral part of cholesterol homeostasis. In FH, for example, HL activity is relatively high (12, 17). Moriguchi et al. (12) suggested that this reflects an adaptation to the low LDLR activity to increase the intracellular cholesterol pool. Cholesterol synthesis inhibitors that decrease plasma cholesterol by increasing LDLR activity (by increasing SREBP-2) may decrease HL expression (28). Hormonal regulation of HL expression by sex hormones fits in the same picture. Estrogens stimulate LDLR expression and decrease HL activity, while androgens have the opposite effect (74).

Concomitant (opposite) changes in LDLR and HL activity may profoundly affect plasma lipoprotein metabolism. As discussed, LDLR, CETP, and HL activity determine for a large part the efficiency of two different routes of RCT. Opposite changes in LDLR and HL expression (e.g., by estrogens/androgens, statins) may result in efficient RCT via either pathway. If both LDLR activity and HL are decreased (e.g., during hypothyroidism), both pathways are blocked resulting in a highly increased CAD risk. In this view, RCT is influenced as a consequence of (hormonal, dietary) alterations in hepatic cholesterol homeostasis with pro- or anti-atherogenic consequences.

The role of HL in intracellular lipid metabolism may not be limited to cholesterol homeostasis. By hydrolyzing HDL phospholipids and triglycerides, and by stimulating chylomicron-remnant uptake and IDL metabolism, HL contributes to the uptake of phospholipids and fatty acids by the liver (Fig. 3). In this way, HL enhances the amount of lipid components, which may be used for VLDL synthesis. The major precursors for VLDL triglycerides are, however, fatty acids derived from omental fat stores. Fatty acid addition to HepG2 cells was found to stimulate HL expression (72, 75). A similar stimulatory effect of fatty acids on HL expression in situ would explain the positive correlation between HL activity and omental fat mass, and the high HL activity in conditions with a high flux of fatty acids to the liver (obesity, insulin-resistance, type 2 diabetes). In this way, HL appears to be coordinately regulated with VLDL triglyceride synthesis. We found that in HepG2 cells, HL expression is stimulated by upstream stimulatory factor and inhibited by SREBPs, transcription factors central in fatty acid metabolism in the liver (73, 76). This supports such coordinate regulation.

We can only speculate about the physiological meaning of coordinate regulation. Connelly (5) suggested that HL is essential for the delivery of certain phospholipids to the liver. HL, in mediating the uptake of HDL phospholipids, may thus contribute to the pool of phospholipids in the liver (Fig. 3).

We hypothesize that in conditions with a high fatty acid flux to the liver, when hepatic lipids are exported by enhanced VLDL secretion, HL is stimulated to maintain intracellular lipid levels (phospholipids, cholesterol) in the liver.

Obviously, these considerations are highly speculative at the moment, as quantitative data on the impact of HL on different metabolic processes are missing. However, these mechanisms have important consequences if intervention at the site of HL is considered.

Should or can we affect HL activity to lower CAD risk?

From these data and considerations, it appears that there are many uncertainties about the exact role of HL in lipid metabolism and its impact on atherogenesis. It seems that the relation of HL with lipid metabolism is very complex. This limits the feasibility to use HL as a target for intervention to lower CAD risk. Changes in HL activity seem to be part of the coordinate regulation of genes involved in lipid metabolism. This predicts that any intervention in HL expression may result in, aside from an effect on plasma lipoprotein transport, a change in the expression of other genes and of intracellular lipid homeostasis. Lowering HL activity during hypertriglyceridemia may decrease the atherogenic risk due to an improved lipid profile, notably an increased LDL size. However, the RCT via the "direct" pathway and post-prandial lipid clearing may become impaired unless, at the same time, LDLR activity is stimulated; vice versa, stimulation of HL expression in FH could result in a further down-regulation of LDLR activity and increased plasma LDL levels. The net effect that such changes on atherosclerosis is unpredictable.

Another major complication in manipulating HL expression is that we do not know the exact activity of HL in vivo. We lack knowledge on the predominance of the in situ phospholipase or triglyceridase activity and how these activities vary under different conditions. By non-specifically altering both activities, unexpected side effects may occur.

Finally, it should be realized that there are indications that the atherogenic potential of HL varies with the (genetic) status of other genes (e.g., *CETP*). Increasing or decreasing HL expression may have varying effects in different individuals.

EPILOGUE

HL is clearly a protein with many faces that affects the metabolism of many lipoproteins. Either enzymatically or as a binding protein, it may change the level, the composition, and metabolism of lipoproteins into a more or less atherogenic direction. The question of whether HL is a pro- or anti-atherogenic protein cannot be answered without taking the context in which it is studied into account.

In examining the available data, it appears that high HL activity is anti-atherogenic in (familial) hypercholesterolemia and pro-atherogenic in hypertriglyceridemia. In normolipidemia, HL seems to have little effect on CAD risk.

We hypothesize that, aside from plasma lipoprotein metabolism, HL is an important factor in intracellular lipid homeostasis of the liver, adrenals, and ovaries. Elucidation of this role is of great importance.

In view of its apparent involvement in the complex lipid homeostasis and association with glucose homeostasis, intervention in HL expression may have unexpected effects. Before intervention in normally regulated HL expression is considered, the metabolic meaning of changes in HL expression under different conditions should be known.

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