

Review

Hepatic Lipase: a Comprehensive View of its Role on Plasma Lipid and Lipoprotein Metabolism

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Hepatic lipase (HL) is a key enzyme catalyzing the hydrolysis of triglycerides (TG) and phospholipids (PLs) in several lipoproteins. It is generally recognized that HL is involved in the remodeling of remnant, low-density lipoprotein (LDL), high-density lipoprotein (HDL) and the production of small, dense low-density lipoproteins (sd-LDLs).

On the other hand, it is unclear whether HL accelerates or retards atherosclerosis. From the clinical point of view, HL deficiency may provide useful information on answering this question, but the rarity of this disease makes it impossible to conduct epidemiological study.

In this review, we describe a comprehensive and updated view of the clinical significance of HL on lipid and lipoprotein metabolism.

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Key words: HDL, EL, LPL, HL deficiency, Atherosclerosis

Structural and Functional Relationships of HL

HL (EC:3.1.1.3) is a member of the lipase gene family, which includes pancreatic lipase, HL, and endothelial lipase (EL)^{1,2}. The HL gene is located on chromosome 15 (q15–q22) in humans and on chromosome 9 in mice³⁻⁵. It spans over 60 kb with eight introns and nine exons accounting for 1.6 kb.

HL is a 65-kD glycoprotein synthesized and primarily secreted by hepatocytes and to a lesser extent by macrophages⁶. HL can hydrolyze triglycerides (TG) and phospholipids (PL) in several lipoproteins but is predominant in the conversion of intermediate-density lipoproteins (IDLs) to LDLs and the conversion of post-prandial triglyceride-rich HDL into the post-absorptive TG-poor HDL. Conventional view of the role of HL on plasma lipid and lipoprotein metabo-

lisms is shown in **Fig. 1**.

The enzyme can be divided into an NH₂-terminal domain containing the catalytic site joined by a short spanning region to a smaller COOH-terminal domain. The NH₂-terminal portion contains an active site serine⁷ in a pentapeptide consensus sequence, Gly-Xaa-Ser-Xaa-Gly, as part of a classic Ser-Asp-His catalytic triad, and a putative hinged loop structure covering the active site. *In vitro* and *in vivo* analysis revealed that the loop structure is an important determinant of the substrate specificity of lipoprotein lipase (LPL) and HL^{8,9}. The COOH-terminal domain is suggested to contain a putative lipoprotein-binding site^{10,11}.

HL and Lipoprotein Metabolism

It has been well recognized that remnant lipoproteins and sd-LDL are important risk factors in the development of cardiovascular diseases. A number of previous studies suggest that HL is mainly involved in the metabolism of remnant lipoproteins as well as HDL. In 1990, researchers started to pay close attention to ligand function as well as enzymatic activities

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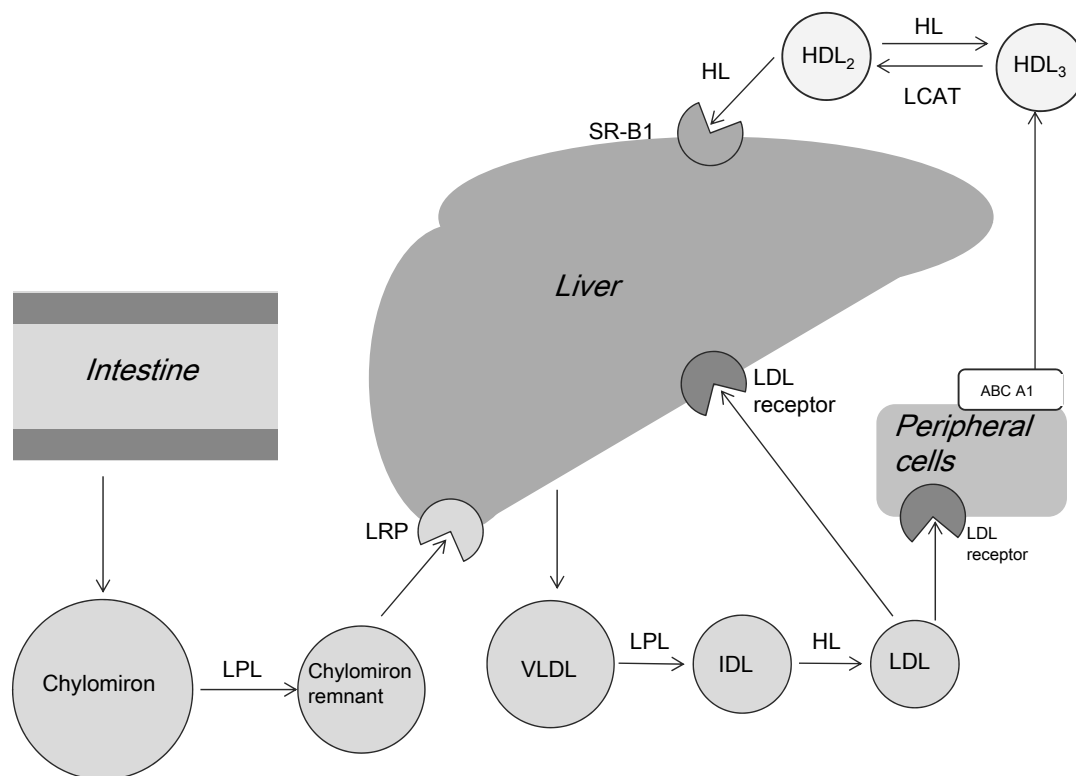


Fig. 1. Schematic illustration of the multiple roles of hepatic lipase (HL) in lipoprotein metabolism. HL, presents in the basolateral surface of hepatocytes and the luminal and subluminal surfaces of endothelial cells or freely circulates in the bloodstream. It hydrolyzes triglycerides and phospholipids present in circulating plasma lipoproteins, including IDL, chylomirion remnants, and HDL₂ (Ref 6).

of HL¹²⁻¹⁵). Sultan *et al.* showed that the inhibition of HL activity using a specific goat antibody against rat HL impairs chylomirion remnant removal in rats. They also suggested that HL facilitates the uptake of chylomirion remnant-like particles, not only as a lipolytic enzyme but also as a ligand anchored to extracellular glycosaminoglycans in isolated rat hepatocytes¹³).

Ji and colleagues proposed using rat hepatoma cells transfected with a human HL cDNA that HL contributes to the enhanced cell association of specific types of remnant lipoproteins by initiating their binding to cell-surface HSPG¹⁴. Another group in USA reported that in mice anti-HL antibody caused a small but significant delay in the remnant removal from plasma and a larger decrease in hepatic uptake, independent of the lipolytic function of HL¹⁵). Studies in animals with genetically modified HL expression showed how HL affects lipid and lipoprotein metabolism *in vivo*. It was reported that in HL deficient mice, total cholesterol levels in plasma were increased by approximately 30% compared with those in wild type animals. Plasma levels of PL and HDL-C were also

increased, but plasma levels of TG were not altered¹⁶. We showed that adenovirus-mediated replacement of the HL gene in HL deficient mice was associated with a drastic reduction in levels of TC, HDL-C, and PL and a moderate reduction in TG levels¹⁷) (**Table 1**).

The same group also generated HL overexpressing human HL to further clarify the role of HL in lipid and lipoprotein metabolism *in vivo*¹⁸). They found that in those mice, there was a substantial decrease in lipids among IDL, LDL, and HDL fractions. Dichek and coworkers¹⁹) expressed catalytically inactive HL in apoE^{-/-} mice and found that plasma cholesterol and apoB-containing lipoprotein levels were approximately 60% lower in these mice than in apoE^{-/-} mice, suggesting that HL works as a ligand rather than an enzyme for the removal of apoB-containing lipoproteins from plasma. In a gene transfer study using recombinant adenovirus to express native and catalytically inactive HL (HL-145G) in apo E-deficient mice, Amar *et al.* suggested that HL serves as a ligand that mediates the interaction between remnant lipoproteins and cell surface receptors and/or proteoglycans²⁰).

Table 1. Plasma lipids and lipoproteins in HL-deficient mice before and after infusion of HL-rAdV

	TC	TG	PL	CE	HDL-C
Day 0	176 ± 9	58 ± 4	314 ± 12	122 ± 8	129 ± 9
Day 4	35 ± 6	31 ± 11	73 ± 10	13 ± 6	21 ± 4
Day 8	94 ± 10	51 ± 5	207 ± 16	35 ± 12	62 ± 8
controls	101 ± 2	63 ± 2	211 ± 4	66 ± 2	78 ± 3

TC, total cholesterol; TG, triglycerides; PL phospholipid; CE, cholesteryl ester; HDL, high-density lipoprotein
 Values are shown in mg/dL.
 This data is from Ref.17

Studies using rabbits were also reported. Fan and coworkers²¹⁾ reported that the overexpression of HL in rabbits, the majority of which were associated with hepatocyte surfaces²²⁾, leads to a marked reduction in plasma HDL and IDL levels. Another study²³⁾ showed that HL transgenic rabbits had substantial reductions in medium and small VLDL and IDL fractions but not in larger VLDL fractions. And LDL levels were also reduced, with a shift from larger, more buoyant to smaller, denser particles.

A human study showed that women with more sd-LDL had a higher HL activity and lower HDL₂-C levels in a cohort of 120 normolipidemic, nondiabetic, premenopausal women²⁴⁾. We established a novel and simple method of measuring post-heparin plasma (PHP)-HL activity without using isotopes^{25, 26)}. Using this method, we found that in middle-age obese or overweight American men or postmenopausal women HL activity in PHP had no association with RLP-TG, RLP-TC, or sd-LDL but had an inverse association with plasma HDL-C levels²⁷⁾. Thus we suggest that other than the effect of HL on HDL, clinical significances of HL on remnant and sd-LDL need to be analyzed.

Relevance of HL Deficiency to Atherosclerosis

One approach for understanding how HL affects the development and progression of atherosclerosis is to clarify whether or not complete HL deficiency is an atherogenic disease, but it seems to be hard to recruit a large number of study subjects because of the rarity of this disease. A few reports on this issue exist; they are from North America and Northern Europe. Canadian investigators reported that β -VLDL in compound heterozygotes for HL mutations (S267F/T383M) readily induced cholesteryl ester accumulation in J774²⁸⁾. The same group suggested that human HL deficiency in the context of a second factor causing hyperlipidemia is strongly associated with premature coronary

artery disease in^{29, 30)}. In contrast to these studies on human, Mezdour and colleagues suggested using mice lacking both HL and apoE and that HL deficiency increases plasma cholesterol but reduces susceptibility to atherosclerosis³¹⁾. Although homozygous or compound heterozygous deficiency of HL is rare, it is presumed that considerable number of individuals with heterozygous HL deficiency may exist in general population. Practically it does not appear easy to detect heterozygous HL deficiency in general population because the abnormality of lipid and lipoprotein profiles is not expected to be prominent. Investigators in Canada³²⁾ mentioned that 3 subjects with S267F/T383M showed myocardial infarction at their 50s whereas other subjects with either S267F or T383M alone did not have coronary artery disease even after their 60s. This report implies that the severity of HL deficiency could be related to the development of atherogenic disease. Authors from Finland³³⁾ reported that a moderate elevation of total TG, IDL, LDL, and HDL₂ and HDL₃-TG levels was observed in heterozygous HL deficiency with R186H or L334F in a Finnish pedigree.

As mentioned briefly above, we have established a novel method for measuring HL activity in PHP^{25, 26)}. This method is easy, reliable, and gives us important information on how HL activity associates with a series of lipoproteins. Our method for measuring HL activity will easily enable us to detect individuals with low HL activity, leading to the identification of either heterozygous or homozygous HL deficiency. To the best of our knowledge, there have been no reports on HL deficiency in the Japanese population.

Recently we established a new ELISA method for measuring human HL protein mass in PHP³⁴⁾ (**Fig. 2**). HL concentration in PHP from 124 American volunteers was 172 ± 147 ng/mL with a range of 42–1200 ng/mL. HL concentration had a strong correlation with HL activity ($r=0.778$, $p<0.001$) measured by the method we previously reported²⁶⁾. Using this method, we investigated the relation between PHP-HL protein

mass and several lipoproteins and EL mass (**Fig. 3**). There was an inverse correlation between PHP-HL protein mass and serum HDL-C levels ($r=-0.23$, $p=0.011$) whereas there was no correlation between HL protein mass and LDL-C, sdLDL, TG, RLP-C, or RLP-TG. Interestingly a positive correlation was observed between HL and EL concentrations ($r=0.22$, $p=0.013$).

Polymorphism of HL Gene

Studies have shown that the LIPC promoter -514C>T polymorphism is associated with a mild reduction in HL activity and an elevation in HDL-C levels³⁵⁻³⁹. We previously reported that this polymorphism had a weak effect on increasing HDL-C levels in the Japanese population⁴⁰ but did not have a significant association with coronary artery disease in Japanese FH individuals⁴¹. There is a study showing that in men with coronary artery disease (CAD), HL activity in PHP was 15–20% lower in heterozygotes and 30% lower in homozygotes for the -514T allele⁴². Hokanson and colleagues⁴³ reported that the LIPC-480C>T polymorphism (the same as -514T allele) was associated with subclinical CAD in type 1 diabetes. These reports imply that individuals with genetically low HL are associated with atherogenic disease.

HL in Various Conditions

The effects of sex steroids have been studied in various conditions and upon replacement therapy. Native or alkylated estrogens are known to depress HL activity, whereas progestagens with androgen property or anabolic steroids increase it^{44, 45}. In line with this, HL activity is lower in pre-menopausal women than in men, which increases after menopause. Glucocorticoids have also been involved in the regulation of HL. In rats, corticotrophin-induced hypercorticism resulted in a decrease of hepatocyte HL-activity⁴⁶. In humans, corticotrophin but not glucocorticoid treatment significantly decreased HL activity⁴⁷. HL activity also varies with the levels of thyroid hormones, as observed in hypo- or hyperthyroid states, or following thyroxin administration⁴⁸. HL activity moderately increased in response to physiological concentrations of triiodo-thyronine, but no modifications in HL-mRNA or protein were recorded⁴⁹.

The relation of HL to insulin is not as clear as that of LPL. In human studies, HL activity is increased in individuals with insulin resistance⁵⁰, type 2 diabetes⁵⁰, and high abdominal fat mass⁵¹, all of which are closely related to the development of atherosclerotic

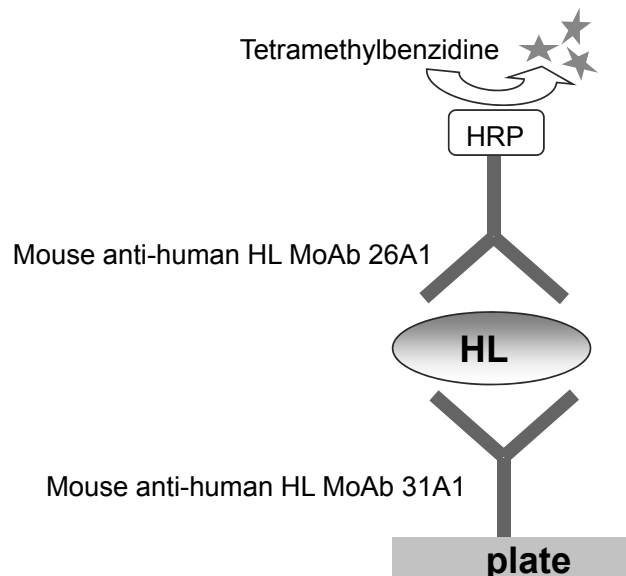


Fig. 2. HL sandwich ELISA

Each well of the microtiter plates (96 wells) was coated with 100 μ L of 100 mmol/L concentrated carbonate buffer (pH 9.5) containing 0.25 μ g purified 31A1 mouse monoclonal IgG followed by an overnight incubation at 4°C. The plates were then washed with PBS-T and blocked with 200 μ L of 1% (wt/vol) BSA in PBS containing 0.05% NaN₃/well overnight at 4°C. After washing twice with PBS-T, 100 μ L of both the serially diluted standard of recombinant human HTGL-471 and the 8-fold plasma samples with 1% BSA in PBS-T were added into each well of the coated microtiter plates in duplicate and incubated overnight at 4°C. After washing 4 times with PBS-T, 100 μ L of HRP-conjugated 26A1 mouse IgG Fab was added into each well and the samples were incubated for 30 min at 4°C. The wells were washed 8 times with PBS-T, and 100 μ L of tetramethyl benzidine solution (Kem-En-Tec) was added into each well as a substrate followed by incubation in the dark for 30 min at room temperature. The reaction was stopped by adding 100 μ L of 0.5 mol/L H₂SO₄. We measured the absorbance of the solution at 450 nm by means of an ELISA reader (E-Max; Molecular Devices).

disease. These aspects of HL appear to be quite opposite to LPL. Unlike HL, LPL activity or mass decreases in individuals with insulin resistance^{52, 53}, type 2 diabetes^{54, 55}, intra-abdominal visceral fat accumulation^{56, 57}, or metabolic syndrome⁵⁸.

Related to these findings, in the previous study on 55 hyperlipidemic Japanese men, we found that LPL activity showed a positive relationship [$r=0.345$, $p=0.010$] to serum levels of adiponectin an anti-atherogenic adipocytokine⁵⁹⁻⁶¹, whereas HL activity showed an inverse relationship [$r=-0.365$ $p=0.006$]⁶². Researchers in Europe also reported that PHP-HL activity is inversely⁶³ and LPL is positively⁶⁴ associated with plasma adiponectin levels, independent of insulin resistance represented by the homeostasis model assess-

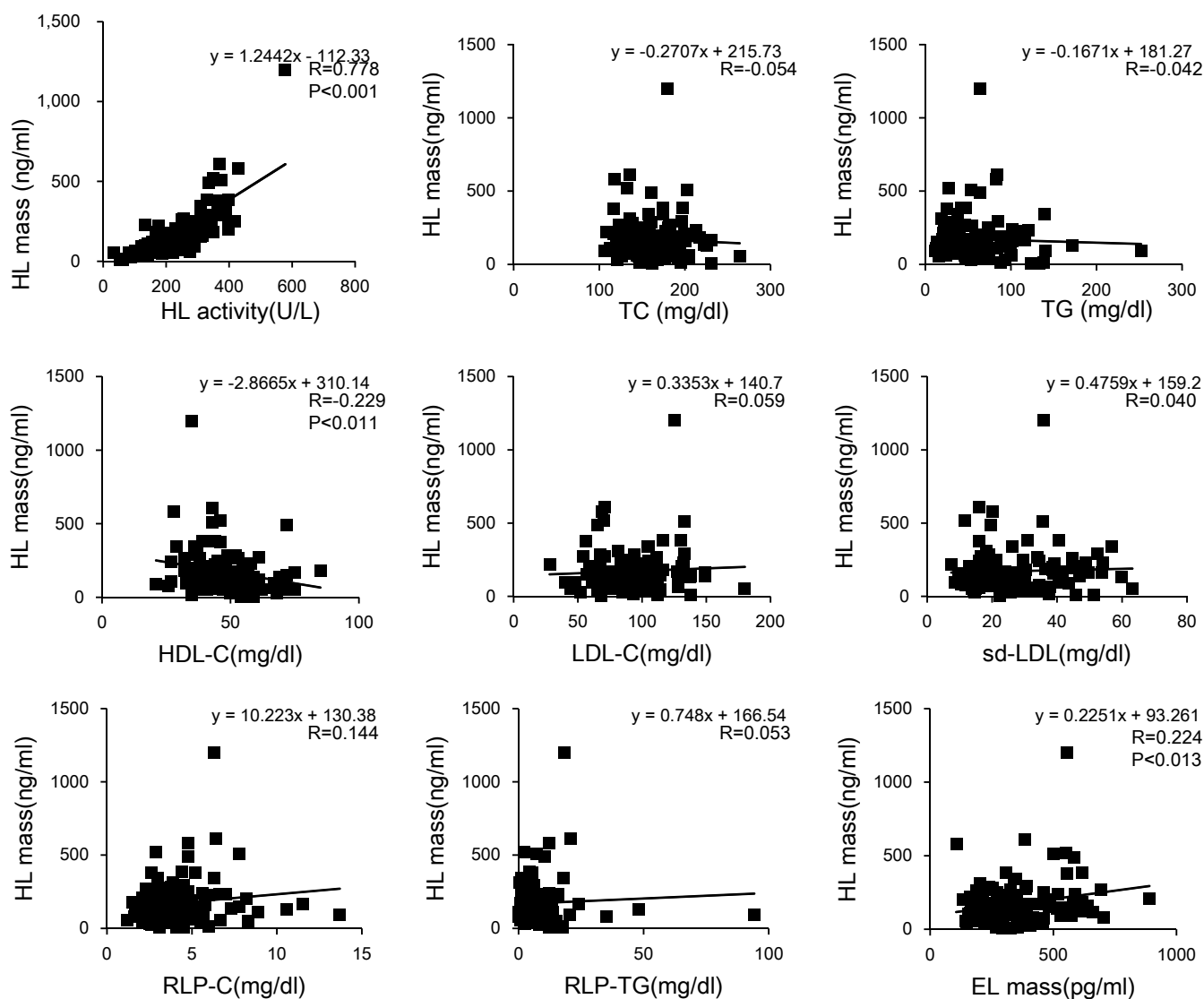


Fig. 3. Correlation between HL concentration and other plasma proteins in post-heparin plasma. We assayed HL mass, HL activity, TG, HDL-C, LDL-C, sdLDL, RLP-C, RLP-TG, and EL mass in PHP from 123 human subjects. The values in the upper column show the correlation coefficient between the other assay values and HL concentration assay values. Data are from Ref. 34

ment of insulin resistance and inflammation. These reports suggest that HL increases in atherogenic condition, which is quite opposite to LPL.

Studies show that HL activity increases or tends to increase in individuals with fatty liver⁶⁵⁻⁶⁷. Whether or not HL is simply associated with these disorders is an interesting topic. A report by authors from USA showed that unlike wild type mice, HL KO mice showed no development of fat accumulation in the liver even after high fat diet being loaded⁶⁸. This finding suggests that HL is not simply associated with the development of fatty liver but may have causal effect

on the development of this disease. Given this fact and combined with the fact that fat accumulation of the liver is responsible for the development of diabetes⁶⁹, HL may indirectly contribute to the formation of atherogenic disease. In line with these, a recent study in Argentina suggests that high HL activity is related to the formation of non-alcoholic fatty liver disease beyond insulin resistance⁷⁰. We investigated the correlation between liver enzyme and HL in American volunteers. The correlation coefficient between alanine aminotransferase (ALT) and PHP-HL activity was 0.185 ($p < 0.05$) whereas that between ALT and PHP-

HL mass was (0.285 $p < 0.01$). This suggest that HL mass could be superior to HL activity for detecting fatty liver.

Source of Materials for HL Determination

To clarify whether HL is clinically pro- or anti-atherogenic, it would be desirable that HL is measurable in serum as a material without heparin injection. So far HL protein mass is measurable only in PHP as materials, which is in contrast to LPL protein mass being measurable in serum as well as PHP. Indeed, in the EPIC-Norfolk population cohort who developed fatal or nonfatal CAD during 7 years of follow-up, reduced levels of serum LPL concentration were associated with an increased risk of future coronary artery disease⁷¹. Similar epidemiologic studies on HL would become possible to be conducted if HL protein were measured in serum as materials.

HL and Angiopoietin-Like Protein 3 (ANGPTL3)

Considerable numbers of studies using mice suggest that ANGPTL3 is involved in plasma TG metabolism through the inhibition of LPL activities⁷²⁻⁷⁴. However, Shimamura *et al.*⁷⁵ and Moon *et al.*⁷⁶ found that plasma ANGPTL3 levels did not correlate with TG levels in human plasma unlike in mice and were shown to be more strongly associated with HDL metabolism. We recently reported that in American overweight or obese subjects, ANGPTL3 concentration inversely correlated with HL activities in PHP²⁷. This data suggest that ANGPTL3 is involved in HDL metabolism through the inhibition of HL activity in human.

HL and EL

It is shown that plasma endothelial lipase (EL) activity inversely correlated with HDL-C levels, and EL activity in CAD patients was significantly higher than in non CAD patients⁷⁷. Several studies show that EL levels are increased in a metabolic syndrome^{78, 79}. And it is observed that inflammation is associated with the elevation of EL⁷⁹. In relation to this fact, it is reported that there is a weak but significant inverse correlation between EL and adiponectin levels⁸⁰. However, studies, including our own, show that inverse correlations exist between adiponectin levels and HL activity^{81, 82}. Moreover, it is recognized that HL activity is increased in a metabolic syndrome or an insulin resistance state⁸³⁻⁸⁵. These previous finding suggest that there is a similarity between changes in EL and HL in meta-

bolic disorders, and we found that there was a positive correlation between EL and HL concentrations³⁴.

Treatment of HL Deficiency

To the best of our knowledge, the reports on HL deficiency treatment are very limited. In earlier studies, investigators in Canada reported³² the effect of lovastatin or gemfibrozil on plasma lipid in one subject with S267F/T383M. There were reductions in plasma cholesterol by lovastatin and those in TG by gemfibrozil.

Other investigators in Canada reported the effect of fenofibrate on plasma lipids and lipoproteins in HL deficiency with A174T/T383M⁸⁶ (**Table 2**). There were marked reductions in plasma TC, TG, apoB, apo C-III, VLDL-C, VLDL-TG, LDL-TG, and HDL-TG levels after treatment without any changes in PHP-HL activities or protein mass.

In contrast there were significant increases in the LPL protein mass. The authors concluded that these changes may be due to the activation of PPAR- α by fenofibrate.

More recently, a 38-year-old Arab man with complete HL deficiency was reported⁸⁷. His parents are second cousins. He had BMI of 28.2 kg/m² and had no xanthomas or xanthelasmas. Cardiovascular exam was unremarkable. Sequencing of his genomic DNA detected one homozygous coding mutation (L334F). Treatment with rosuvastatin (20 mg per day) showed considerable reductions in plasma total cholesterol (279 \rightarrow 151 mg/dL) and TG (1000 \rightarrow 177 mg/dL).

Conclusion

HL is a multifactorial enzyme with lipolytic and ligand function and is involved in various kinds of lipid and lipoprotein metabolism. Although various animal and clinical studies were conducted, it is still too early to conclude whether this enzyme is pro- or anti-atherogenic. The number of the reported HL deficiency cases is too scarce to draw a conclusion whether or not this disease is pro-atherogenic. Alternative approach to answer this question is to clarify the relation between HL and the incidence of atherosclerotic disease in cohort studies with large number of subjects. To achieve this, PHP is not an ideal material because of the complexity of the procedure. We are looking forward to the establishment of a high-sensitivity ELISA system for serum HL protein mass, which makes it possible to conduct large scale population studies on the correlation between the enzyme mass

Table 2. Effect of fenofibrate (160 mg/day for 6 months) on plasma lipoproteins in HL deficient patients

	control (n=5)	complete HL deficiency (n=2)		% changes
		before treatment	after treatment	
age, y	39 ± 4	36 ± 1	37 ± 1	+3
BMI, kg/m ²	29 ± 4.9	31.4 ± 1.3	30 ± 1.2	-4
WC, cm	99 ± 13.5	105 ± 2.8	102 ± 7.1	-3
Plasma				
cholesterol, mg/dL	199 ± 34.7	296 ± 1.54	152 ± 4.24	-49
triglyceridesl, mg/dL	140 ± 67.3	635 ± 331	116 ± 17.7	-82
apoB, mg/dL	102 ± 24	163 ± 18	74 ± 6	-55
apoC-III, mg/dL	16.8 ± 5.9	28.6 ± 1.3	10.6 ± 1.2	-63
VLDL				
cholesterol, mg/dL	21.9 ± 14.2	144 ± 59.7	8.9 ± 3.08	-94
triglyceridesl, mg/dL	102.7 ± 63.7	429 ± 330	27.4 ± 9.74	-94
LDL				
cholesterol, mg/dL	128 ± 29.3	107 ± 48.9	90.1 ± 3.08	-16
triglyceridesl, mg/dL	18.6 ± 4.425	130 ± 20.4	53.1 ± 8.85	-59
LDLsize, Å	256 ± 3	252 ± 1.8	264 ± 0.7	+5
HDL				
cholesterol, mg/dL	48.1 ± 11.6	45.4 ± 12.3	53.5 ± 10.8	+18
triglyceridesl, mg/dL	18.6 ± 1.77	75.2 ± 20.4	35.4 ± 0.89	-53
HDLsize, Å	83.1 ± 0.9	108 ± 1.7	104 ± 1.1	-3
CETP mass, µg/mL	1.42 ± 0.19	1.8 ± 0.56	1.52 ± 0.14	-15
HL mass, ng/mL	416 ± 53	17 ± 4.2	14 ± 0.1	-18
HL activity, µmol/mL/h	20.5 ± 4.7	0.44 ± 0.61	0.34 ± 0.03	-23
LPLactivity, µmol/mL/h	5.1 ± 2.1	3.2 ± 0.9	5.2 ± 1.4	+62

Data are quoted and modified from Ref 86

and the occurrence of cardiovascular diseases.

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Ethical Approval

Not applicable

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Conflicts of Interest

None.

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