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In vivo evidence of a role for hepatic lipase in human apoB-containing lipoprotein metabolism, independent of its lipolytic activity

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Abstract **Hepatic lipase (HL) is a key player in lipoprotein metabolism by modulating, through its lipolytic activity, the triglyceride (TG) and phospholipid content of apolipoprotein B (apoB)-containing lipoproteins and of high density lipoproteins (HDL), thereby affecting their size and density. A new and separate role has been suggested for HL in cellular lipoprotein metabolism, in which it serves as a ligand promoting cellular uptake of apoB-containing remnant lipoproteins and HDL. We tested the hypothesis that HL has both a lipolytic and a nonlipolytic role in human lipoprotein metabolism, by measuring lipid plasma concentrations, lipoprotein density distribution by density gradient ultracentrifugation, and lipoprotein composition, in three subjects with HL deficiency: two of the patients (S-1 and S-3) were characterized as having neither plasma HL activity nor detectable HL protein; the third subject (S-2) had no plasma HL activity but a detectable amount (35.5 ng/ml) of HL protein. All HL-deficient subjects showed a severalfold increase in lipoprotein TG content across the lipoprotein density spectrum [very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL), low density lipoprotein (LDL), and HDL] as compared with control subjects. They also had remarkably more buoyant LDL particles (LDL-R_f = 0.342–0.394) as compared with the control subjects (LDL-R_f = 0.303). Subjects S-1 and S-3 (no HL activity or protein) presented with a distinct increase in cholesterol and apoB levels in the IDL and VLDL density range as compared with patient S-2, with detectable HL protein, and the control subjects. This study provides evidence in humans that HL indeed plays an important role in lipoprotein metabolism independent of its enzymatic activity: in particular, inactive HL protein appears to affect VLDL and IDL particle concentration, whereas HL enzymatic activity seems to influence VLDL-, IDL-, LDL-, and HDL-TG content and their physical properties.** —Zambon, A., S. S. Deeb, A. Bensadoun, K. E. Foster, and J. D. Brunzell. **In vivo evidence of a role for hepatic lipase in human apoB-containing lipoprotein metabolism, independent of its lipolytic activity.** ***J. Lipid Res.*** **2000.** 41: **2094–2099.**

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Hepatic lipase (HL) is a 477-amino acid glycoprotein that plays a pivotal role in lipoprotein metabolism (1). The human HL gene spans more than 30 kb, is composed of nine exons and eight introns (2, 3), and is located on chromosome 15q21 (4). The majority of HL is synthesized and secreted by the liver and is bound to heparan sulfate proteoglycans on the surfaces of sinusoidal endothelial cells and external surfaces of microvilli of parenchymal cells in the space of Disse (5, 6).

HL catalyzes the hydrolysis of triglycerides (TG) and phospholipids of intermediate density lipoprotein (IDL) remnants, large buoyant low density lipoproteins (LDL), and high density lipoproteins (HDL), resulting in smaller, more dense lipoprotein particles. HL deficiency is rare, with only 12 patients described (1). The absence of HL activity leads to large buoyant, TG-enriched LDL particles (7). In normal males and those with coronary artery disease (CAD) HL activity is related to decreasing size and increasing density of both HDL and LDL particles (8).

In addition to its catalytic activity as a lipase, in vitro data suggested an additional and perhaps separate role for HL in cellular lipoprotein metabolism, in which it serves as a ligand promoting cellular uptake of apolipoprotein apoB -containing remnant lipoproteins and HDL (9–12). The enhancement of hepatic uptake of apoB-containing lipoproteins was independent of lipolytic activity and did not require apoE (10–12). These data have been confirmed by in vivo experiments showing that in transgenic mice overexpressing catalytically active human HL in the liver (13), both apoB-containing remnant lipo-

Abbreviations: apo, apolipoprotein; CAD, coronary artery disease; DGUC, density gradient ultracentrifugation; FFA, free fatty acid; HDL, high density lipoprotein; HDL-C, HDL cholesterol; HL, hepatic lipase; IDL, intermediate density lipoprotein; LDL, low density lipoprotein; LDL-C, LDL cholesterol; PHLA, postheparin lipolytic activity; PHP-LPL, postheparin plasma lipoprotein lipase; TG, triglyceride; VLDL, very low density lipoprotein.

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proteins and HDL were reduced, while in mice overexpressing catalytically inactive HL, the apoB-containing lipoproteins were reduced but HDL was minimally decreased. Further in vivo evidence (14) demonstrates that both lipolytic and nonlipolytic functions of HL are important for HDL metabolism by affecting distinct metabolic pathways involved in the catabolism of different HDL subclasses. At present, there is no published evidence of a potential role of HL in human lipoprotein metabolism, independent of its lipolytic activity.

In the present study, we investigated plasma lipid concentrations, lipoprotein density distribution, and lipoprotein composition in three subjects with HL deficiency: two of the patients were characterized as having neither plasma HL activity nor detectable HL protein; the third subject had no plasma HL activity but a detectable amount of HL protein. Our results support the hypothesis that HL affects human apoB-containing lipoprotein metabolism by both lipolytic and nonlipolytic pathways. Thus, we provide in vivo evidence of a role for HL in lipoprotein metabolism in humans, independent of lipolysis.

PATIENTS AND METHODS

Patients

Subject "Seattle 1" (S-1), a Hispanic male with HL deficiency, was born in 1936 (7). He is homozygous for an intron 1 acceptor splice site mutation (15). He was initially referred to the University of Washington Medical Center (Seattle, WA) for severe hypertriglyceridemia (4,000 mg/dl) and found to have also low levels of postheparin plasma lipoprotein lipase (PHP-LPL) activity [PHP-LPL = 102 nmol of free fatty acid (FFA) per min per ml] compatible with heterozygosity for a genetic defect of the LPL gene. The patient had no apoC-II deficiency as evaluated by our postheparin lipolytic activity (PHLA) assay. He was placed on clofibrate, 1 g twice a day, which decreased his TG and increased his postheparin plasma LPL activity into the normal range (see Table 2). The studies in this article were performed while S-1 was receiving fibrate therapy. He had myocardial infarction at age 41 years.

Subject "Seattle 2" (S-2), a Caucasian male with HL deficiency, was born in 1944. He is heterozygous for the missense mutation G225R (1). No other defect was found in HL exons 1 through 9. He was initially referred to the University of Washington Medical Center for severe hypertriglyceridemia (4,105 mg/dl) and found to have low levels of PHP-LPL activity (93 nmol of FFA per min per ml), compatible with heterozygosity for a genetic defect of the LPL gene. The patient had no apoC-II deficiency as evaluated by our PHLA assay. He was placed on clofibrate, 1 g twice a day, with a decrease in TG level and increase in PHP-LPL activity into the normal range (see Table 2). The studies in this report were performed while S-2 was receiving fibrate therapy. He had myocardial infarction at age 37. Subjects S-1 and S-2 were studied while receiving fibrate therapy to correct the defect associated with low PHP-LPL activity and severe hypertriglyceridemia, to better evaluate the residual effects of HL deficiency.

Because the first two HL-deficient patients presented with severe hypertriglyceridemia and low PHP-LPL activity, it was hypothesized that HL deficiency with modest hypertriglyceridemia and normal PHP-LPL activity might be more common (16). With examination of 84 middle-aged males with CAD and elevated plasma apoB levels in the Familial Atherosclerosis Treatment Study (17), a third patient with HL deficiency was discovered (1, 16). Subject "Seattle 3" (S-3), born in 1947, is a Caucasian male with HL deficiency. He is homozygous for a major deletion involving the HL gene promoter and exon 1. He had modest hypertriglyceridemia and normal PHP-LPL activity while not taking any medications. He had a myocardial infarction at age 42.

Thirty Caucasian men aged 33 ± 2 years were recruited as control subjects. None of these subjects had known lipid abnormalities, or concurrent disease affecting lipid metabolism. None of them was taking lipid-altering medication at the time of the study. The present study was approved by the human subjects review committee of the University of Washington, and informed consent was obtained from all subjects.

Blood collection

Blood specimens were collected in 0.1% ethylenediaminetetraacetic acid after a 12- to 14-h fast for lipid measurements and density gradient ultracentrifugation (DGUC) for evaluation of lipoprotein cholesterol distribution. An intravenous heparin bolus of 60 IU/kg was then administered, and after 10 min blood was collected in iced lithium-heparin tubes for measurement of HL and LPL activity. Blood samples were immediately processed by centrifugation at 4°C and stored at −70°C for determination of HL activity, LPL activity, lipoprotein distribution, and LDL peak buoyancy.

Lipid and lipoprotein determinations

Plasma, very low density lipoprotein (VLDL), IDL, LDL, HDL cholesterol, TG, apoB, apoA-I, and apoE phenotype as well as VLDL, IDL, and LDL phospholipids, and free and esterified cholesterol were measured at the Northwest Lipid Research Laboratories (Seattle, WA) as previously described (18, 19).

Density gradient ultracentrifugation for apoB-containing lipoproteins

Density gradient ultracentrifugation separates lipoprotein particles by the rate of flotation in a salt density gradient (20) and is designed to optimize the resolution of apoB-containing lipoproteins based on strategies previously described (21). A discontinuous salt gradient is produced by layering 1 ml of plasma adjusted to a density of 1.08 g/ml (total volume, 5 ml) below 12 ml of a 1.006-g/ml NaCl solution in a Sorvall TV 865 B tube (DuPont, Wilmington, DE). Samples are then centrifuged at 65,000 rpm for 90 min at 10°C. While maintaining temperature, centrifuge tubes are then placed in a tube fractionator (ISCO, Lincoln, NE), pierced, and drained from the bottom, using a P-1 peristaltic pump (Pharmacia, Piscataway, NJ) at a flow rate of 1.7 ml/min. Thirty-eight 0.45 ml fractions are collected. Cholesterol is measured in each fraction by enzymatic kit (Sigma Chemical, St. Louis, MO). Cholesterol recovery with this technique is 98 ± 4% (mean ± SD) of total plasma cholesterol. LDL relative flotation (R_f), a measure of LDL peak buoyancy, is calculated as the fraction number of the major peak of LDL divided by the total number of fractions collected. Each lipoprotein elution range was determined as previously described (18).

Postheparin plasma lipase activity and HL mass quantification

Total lipolytic activity was measured in plasma as previously described (22). Glycerol tri[1-^{14}C]oleate, emulsified with lecithin and Sigma (St. Louis, MO) albumin, was incubated with postheparin plasma for 60 min at 37°C and the liberated ^{14}C-labeled FFA were extracted and counted. HL activity, in nanomoles of fatty acids released per minute per milliliter of plasma, is defined as the activity remaining in the postheparin sample after incubation with a specific monoclonal antibody (5D2) that

TABLE 1. Lipids and apolipoproteins

	Chol	TG	VLDL-C	IDL-C	LDL-C	HDL-C	HDL-TG	ApoB	ApoA-I	ApoE Phenotype	Therapy
Control subjects (n = 30)	181 ± 6.3	88 ± 9.5	20 ± 1.9	14 ± 6.1	99 ± 4.6	50 ± 1.8	10 ± 3.5	99 ± 4.6	134 ± 3.1		No
S-1	196	107	50	45	72	42	45	177	154	E3/E3	Fibrate
S-2	231	221	23	18	133	64	52	122	171	E4/E3	Fibrate
S-3	337	475	84	60	145	45	52	243	146	E3/E3	No

Values are expressed in mg/dl (means ± SD).
VLDL, IDL, and LDL cholesterol values are obtained from DGUC profiles (see Patients and Methods).

selectively inhibits LPL (23, 24). HL mass was determined by enzyme-linked immunosorbent assay with monoclonal antibodies generated against human HL (25).

RESULTS

Lipids and apolipoproteins of HL-deficient patients and control subjects are presented in **Table 1**. Fibrate therapy lowered TG levels in subjects S-1 and S-2 from a baseline concentration of more than 4,000 mg/dl. An increase in total and LDL cholesterol (LDL-C), TG, VLDL-TG, and apoB was observed in the HL-deficient patient (S-3) not receiving lipid-lowering medication. All HL-deficient subjects showed a 2- to 5-fold TG increase across the lipoprotein density spectrum (VLDL, IDL, and LDL: see Table 3; HDL: Table 1) compared with the control subjects. In addition, HDL cholesterol (HDL-C) was higher in patient S-2 than in patients S-1 and S-3 (Table 1).

No postheparin HL activity was detected in the three HL-deficient subjects (**Table 2**). In addition, S-1 and S-3 had no detectable HL protein mass, while in S-2, HL mass was 35.5 ng/ml, about 20% of the normal value for HL mass in normolipidemic, healthy men (189 ± 70 ng/ml; Table 2). Postheparin LPL activity was similar among the HL-deficient patients at the time they were studied, and between patients and control subjects.

Lipoprotein distribution across a density gradient was evaluated by DGUC. Lipoprotein elution ranges were determined as previously described (18). All three HL-deficient patients had remarkably more buoyant LDL particles (LDL-R_f) as compared with control subjects (**Fig. 1**, and **Table 3**). In addition, subjects S-1 and S-3, with neither HL activity nor HL protein mass, presented with a distinct increase in cholesterol concentration in the IDL and VLDL density range as compared with patient S-2, with detectable HL protein, or the control subjects (Fig. 1).

Lipoprotein composition was evaluated by pooling fractions collected within each lipoprotein class density range as previously described (8, 18). Lipid and apoB concentrations in the pooled fractions were measured. To evaluate the lipid composition per lipoprotein particle, the lipid-to-apoB ratio was evaluated for each lipid component (Table 3). Confirming the data from the cholesterol distribution profiles (Fig. 1), subjects S-1 and S-3, with no HL activity or HL protein, had a 3- to 5-fold higher VLDL concentration, and a 2- to 3-fold higher IDL apoB concentration, than either subject S-2, with detectable, inactive HL protein, or control subjects (Table 3). A marked increase in the TG content per particle was observed in all HL-deficient patients across the apoB-containing lipoprotein density spectrum. In the HL-deficient patients, the VLDL-TG content per particle was 2- to 3-fold higher, the IDL-TG content was 3- to 4-fold higher, and the LDL-TG content was 4- to 5-fold greater compared with control subjects (Table 3).

TABLE 2. Plasma lipases

	LPL Activity	HL Activity	HL Mass
Control subjects (n = 30)	170 ± 12	263 ± 16	189 ± 70[a]
S-1	162	0	ND
S-2	160	7	35.5
S-3	146	5	ND

Plasma lipase activity is expressed as nmol/min/ml of released FFA (means ± SD).
HL mass is expressed in ng/ml. ND, not detected.
[a] n = 5 males.

DISCUSSION

Previous studies, both in vitro and in animal models, suggested that HL may play a significant role in lipoprotein metabolism independent of its lipolytic activity. This

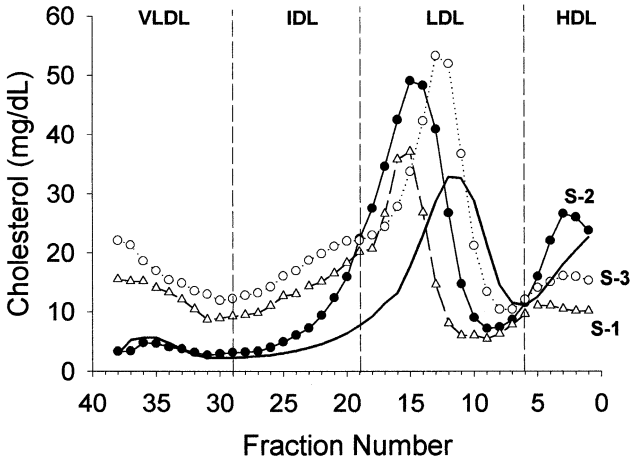

Fig. 1. DGUC lipoprotein cholesterol profiles for subjects with HL deficiency and 30 normolipidemic control subjects. Lipoprotein class elution ranges were defined as previously reported (18). Subject S-1, open triangles; subject S-2, solid circles; subject S-3, open circles; control subjects, solid line.

TABLE 3. VLDL, IDL, and LDL particle composition

	Control Subjects (n = 30)	S-1	S-2	S-3
VLDL				
ApoB	4 ± 0.4	10	3	14
FC/apoB	0.62 ± 0.03	0.8	0.73	0.71
CE/apoB	1.5 ± 0.09	1.73	1.68	1.58
TG/apoB	3.2 ± 0.2	6.8	8	6.9
PL/apoB	1.3 ± 0.2	2.1	2.3	1.92
IDL				
ApoB	5 ± 0.7	11	5	13
FC/apoB	0.47 ± 0.01	0.46	0.46	0.4
CE/apoB	1.6 ± 0.05	1.04	1.36	1.06
TG/apoB	0.8 ± 0.03	2.64	3	2.58
PL/apoB	1.2 ± 0.03	1.24	1.36	1.2
LDL				
ApoB	23 ± 1.4	24	26	29
FC/apoB	0.41 ± 0.01	0.41	0.45	0.36
CE/apoB	1.0 ± 0.03	0.76	0.88	0.79
TG/apoB	0.4 ± 0.01	1.72	1.93	1.74
PL/apoB	1.16 ± 0.03	0.94	1.16	1.01
LDL buoyancy (R_f)	0.303 ± 0.012	0.394	0.394	0.342

ApoB values are expressed as mg/dl. Lipid-to-apoB ratios are expressed as weight to weight. In control subjects values are expressed as means ± SD. Abbreviations: FC, free cholesterol; CE, cholesteryl esters; PL, Phospholipids.

study provides, for the first time in humans, evidence that HL indeed plays an important role in human lipoprotein metabolism independent of its enzymatic activity. In particular, inactive HL protein appears to affect VLDL and IDL particle concentration while HL enzymatic activity seems to influence VLDL-, IDL-, LDL-, and HDL-TG content and their physical properties.

The function of HL as a lipolytic enzyme in lipoprotein metabolism has been known for a long time (26, 27). By modulating the phospholipid and TG content of IDL, LDL, and HDL particles, HL contributes significantly to determining their lipid composition and thereby their density and size. Increased levels of HL activity lead to phospholipid- and TG-depleted LDL particles that are smaller and denser (8). Small, dense LDL are associated with an increased risk of cardiovascular disease (28). A prevalence of smaller, TG- and phospholipid-depleted HDL particles (HDL_3) has also been associated with increased HL activity levels (29). On the other hand, the absence of HL activity leads to the accumulation of TG within lipoproteins, resulting in large buoyant LDL (7) and HDL particles. The present study confirms and expands this observation by demonstrating that, regardless of the presence in plasma of HL-inactive protein, as in patient S-2, the absence of HL enzymatic activity is associated with remarkably more buoyant, TG-rich LDL as compared with the control subjects (Table 3 and Fig. 1). Auwerx et al. (7) demonstrated that these large LDL particles contain apoB-100 as their main apoprotein and little, if any, apoE, truly representing a "metabolic end-product" of the apoB-containing lipoprotein cascade in these HL-deficient patients. Moreover, incubation, in vitro, of these large, buoyant LDL particles from subject S-1 with human HL resulted in LDL of "normal" density and lipid composition (7). Studies of transgenic rabbits overexpressing HL confirm the importance of this enzyme in modulating LDL density, demonstrating, in addition, that HL also affects VLDL and IDL physical properties (30). These results suggest a potential effect of HL activity on VLDL and IDL lipid composition. Our study confirms this hypothesis by showing that HL enzymatic activity appears to be needed to prevent VLDL-, IDL-, and HDL-TG enrichment (Table 3), suggesting that HL activity modulates lipoprotein lipid composition across a broad lipoprotein density spectrum. A mild phospholipid enrichment in the VLDL particles was also observed in HL-deficient patients. Surprisingly, no phospholipid enrichment in the IDL or LDL particles was observed. The activity of other important enzymes involved in the phospholipid exchange between lipoproteins, such as the phospholipid transfer protein (31), may at least partly account for these findings, and specific studies need to be performed to address this hypothesis.

In addition to its function as plasma triacylglycerol hydrolase and phospholipase, studies both in vitro and in animal models have suggested a role for HL as a ligand that mediates the uptake of lipoproteins and/or lipoprotein lipids, by facilitating the interaction of lipoproteins with cell surface proteoglycans and/or receptors (9–14). HL appears to increase the binding and uptake of different lipoproteins, including chylomicron and VLDL remnants (9, 11), β-VLDL (10, 12), as well as HDL (12), by a number of different cells in culture. Definite in vivo evidence of a role of HL in lipoprotein metabolism independent of lipolysis has been provided by studies in which a catalytically inactive enzyme has been expressed in various animal models. Expression of both active and catalytically inactive HL in apoE-deficient mice decreased the apoB-containing lipoprotein levels by approximately 50% (13), indicating that HL may play a role in apoB-containing lipoprotein removal independent of its enzymatic activity. Our results confirm this observation in human subjects. Despite expressing a relatively small amount (20% of the HL protein found in normal subjects) of inactive protein, patient S-2 had less cholesterol in the VLDL and IDL elution range as compared with patients S-1 and S-3, who had neither HL activity nor protein (Fig. 1). Furthermore, VLDL and IDL apoB concentrations, reflecting the number of circulating VLDL and IDL particles, were severalfold higher in patients with no protein, while patient S-2 had IDL and VLDL apoB levels comparable to those found in normal control subjects. No major difference in the LDL apoB levels was observed. These data suggest that even small amounts of inactive HL protein may significantly affect human VLDL and IDL catabolism, as previously observed in vitro (10, 11) and in animal models (13). LDL catabolism appears not to be significantly influenced by the presence/absence of HL protein.

In patient S-2, with inactive HL protein, HDL-C was higher than in patients S-1 and S-3. This finding might be associated with the observed differences in TG-rich lipoprotein metabolism in the presence of catalytically inactive HL (S-2), and differs from previous observations in animal models, where expression of inactive HL had only

minimal effect on HDL levels (13) or was associated with a decrease in a specific subclass of HDL containing apoA-II (14). The potential effect of inactive HL on apoB-containing lipoproteins and HDL metabolism clearly points out the need for specific, in vivo, kinetic studies of these patients, which will provide crucial information to define the role of HL in lipoprotein metabolism, independent of its lipolytic activity. In addition, it would be potentially interesting to evaluate whether the abnormal lipid composition of these lipoproteins, and specifically the remarkable TG enrichment of IDL and HDL, alter their affinity as ligand for HL.

Lipoprotein lipase activity was similar in the HL-deficient patients and control subjects, when studied, indicating that the increased buoyancy of the LDL particles and the TG enrichment observed in all lipoprotein subclasses as well as the increased concentration of VLDL and IDL particles seen in S-1 and S-3 were not the result of differences in LPL activity. Patients S-1 and S-2 were being treated with a fibrate, which normalized their LPL activity. These lipid-lowering agents appear to increase LPL activity by binding and activating a specific transcription factor in the liver, the peroxisome proliferator-activated receptor α, that stimulates LPL expression (32). We considered whether pharmacological therapy with fibrate might interfere with cholesterol distribution as determined by DGUC, or with VLDL and IDL concentration. However, patients with neither HL activity nor HL protein had similar cholesterol distribution profiles in the VLDL and IDL elution range as well as similar VLDL and IDL apoB levels regardless of whether they received clofibrate (S-1) or not (S-3). In addition, both patient S-1 and S-2 were receiving therapy and yet their cholesterol distribution across the VLDL and IDL density range as well as their VLDL and IDL apoB levels were remarkably different. It appears therefore unlikely that the different results observed in the VLDL and IDL cholesterol distribution and concentration in the presence of inactive HL protein (S-3) might be attributable to drug treatment with fibrate.

The clinical implications of altered lipoprotein composition (i.e., TG enrichment) as well as abnormal VLDL and IDL catabolism for the pathophysiology of premature CAD are highlighted by the evidence that all three HL-deficient patients had myocardial infarction between the age of 37 and 42 years, as did most of the other nine patients with HL deficiency reported so far by other groups (1). The premature coronary disease that occurs in these individuals might reflect arterial wall uptake and retention of the increased numbers of apoB-containing lipoproteins of abnormal composition and/or HDL might be defective and unable to participate in reverse cholesterol transport because of the compositional abnormalities seen in these lipoproteins.

A potential limitation of this study arises from the small number of patients studied. HL deficiency is a rare disorder, with only 12 patients characterized so far in the world (1). However, although limited to only three patients, of which one has inactive HL protein, the results of this study fully support previously published evidence from both in vitro and in vivo animal model experiments.

In summary, the present study provides supportive in vivo evidence of a role for HL in human lipoprotein metabolism, independent of its lipolytic activity. We demonstrated that both lipolytic and nonlipolytic functions of HL are important for lipoprotein metabolism. In particular, HL lipolytic activity modulates lipoprotein lipid composition across all lipoprotein subclasses as well as LDL density. The presence of small amounts of inactive HL protein remarkably affects VLDL and VLDL remnant (IDL) plasma concentrations. The pathophysiological role that both lipolytic and ligand-binding functions of HL play in the development and progression of human atherosclerosis remains to be elucidated.

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