

Lipolytic and ligand-binding functions of hepatic lipase protect against atherosclerosis in LDL receptor-deficient mice^S

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Abstract To elucidate the separate contributions of the lipolytic versus ligand-binding functions of hepatic lipase (HL) to lipoprotein metabolism and atherosclerosis, and to investigate the role of the low density lipoprotein receptor (LDLr) in these processes, we compared mice expressing catalytically active HL (HL-WT) with mice expressing inactive HL (HL-S145G) in a background lacking endogenous HL and the LDLr (LDLr-KO×HL-KO). HL-WT and HL-S145G reduced ($P < 0.05$ for all) cholesterol (55% vs. 20%), non-HDL-cholesterol (63% vs. 22%), and apolipoprotein B (apoB; 34% vs. 16%) by enhancing the catabolism of autologous ¹²⁵I-apoB-intermediate density lipoprotein (IDL)/LDL (fractional catabolic rate in day⁻¹: 6.07 ± 0.25 , LDLr-KO×HL-WT; 4.76 ± 0.30 , LDLr-KO×HL-S145G; 3.70 ± 0.13 , LDLr-KO×HL-KO); HL-WT had a greater impact on the concentration, composition, particle size, and catabolism of apoB-containing lipoproteins (apoB-Lps) and HDL. Importantly, consistent with the changes in apoB-Lps, atherosclerosis in LDLr-KO×HL-KO mice fed a regular chow diet (RCD) was reduced by both HL-WT and HL-S145G (by 71% and 51% in cross-sectional analysis, and by 85% and 67% in en face analysis; $P < 0.05$ for all). These data identify physiologically relevant but distinct roles for the lipolytic versus ligand-binding functions of HL in apoB-Lp metabolism and atherosclerosis and demonstrate that their differential effects on these processes are mediated by changes in catabolism via non-LDLr pathways. These changes, evident even in the presence of apoE, establish an antiatherogenic role of the ligand-binding function of HL in LDLr-deficient mice.—Freeman, L., M. J. A. Amar, R. Shamburek, B. Paigen, H. B. Brewer, Jr., S. Santamarina-Fojo, and H. González-Navarro. **Lipolytic and ligand-binding functions of hepatic lipase protect against atherosclerosis in LDL receptor-deficient mice.** *J. Lipid Res.* 2007. 48: 104–113.

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Hepatic lipase (HL) is a multifunctional protein with a complex role in lipoprotein metabolism. It is synthesized and secreted primarily by hepatocytes and functions not only as a lipolytic enzyme that hydrolyzes triglycerides (TGs) and phospholipids (PLs) in apolipoprotein B-containing lipoproteins (apoB-Lps) and HDL but also as a ligand or coreceptor that facilitates the cellular uptake of lipoproteins and lipoprotein lipids (1–9). These different functions of HL, which facilitate not only plasma lipoprotein metabolism but also cellular lipid uptake, may have variable effects on atherosclerosis; indeed, human and animal studies appear to support both a proatherogenic and an antiatherogenic role for HL (6, 7, 10–23).

The mechanisms underlying these variable and sometimes opposing atherosclerosis outcomes are not completely understood. The finding that HL in arterial wall lesions enhances atherosclerosis without altering plasma lipoprotein lipid levels (16, 24) provided partial insight into these discrepancies. Another possible yet not fully investigated explanation for these conflicting data may be the potentially differential effects of the lipolytic versus ligand-binding functions of HL on the plasma concentrations and subclass distributions of the different plasma lipoproteins, which in turn may differentially affect atherosclerosis. Initial adenoviral and transgenic overexpression of catalytically active and inactive HL in apolipoprotein E knockout (apoE-KO) versus low density lipoprotein receptor knockout (LDLr-KO) mice (5–8, 21) established a role for the ligand-binding function of HL in decreasing plasma levels of the proatherogenic apoB-Lps independent of lipolysis. These studies, although limited by the confounding effects of the fully active, endogenous mouse HL or by the transient, non-steady-state expression of catalytically active HL (HL-WT)

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or inactive HL (HL-S145G), also suggested different effects for the lipolytic versus ligand-binding functions of HL in apoB-Lp and HDL metabolism.

Recently, the separate roles of the ligand-binding versus lipolytic functions of HL in lipoprotein metabolism have been addressed more rigorously by stable expression of active HL-WT and inactive HL-S145G in transgenic mice lacking endogenous mouse HL (22, 23). In apoE-KO×HL-KO mice (22), both HL-WT and HL-S145G similarly enhanced the catabolism of apoB-Lps via low density lipoprotein receptor-related protein (LRP), significantly decreasing plasma apoB-Lp levels. In contrast, the lipid-lowering effects of HL-S145G were attenuated in LDLr-KO×HL-KO mice on a Western diet, despite the presence of intact LRP (21, 23). In both studies, the lipolytic and ligand-binding functions of HL had markedly different effects on atherosclerosis. Whereas HL-S145G protected against lesion development, HL-WT markedly enhanced atherosclerosis in apoE-KO×HL-KO mice (22). Conversely, HL-WT but not HL-S145G reduced aortic atherosclerosis in LDLr-KO×HL-KO mice fed a Western diet (23). These divergent results suggest that the impact of the ligand-binding function of HL on apoB-Lp metabolism and atherosclerosis varies with dietary and/or genetic differences, particularly with the presence of the LDLr and apoE.

To further elucidate the separate contributions of the lipolytic versus ligand-binding functions of HL to lipoprotein metabolism and atherosclerosis, and to investigate the role of the LDLr in these processes, we compared mice expressing active HL-WT with mice expressing inactive HL-S145G at physiological levels in liver of LDLr-KO mice lacking endogenous HL. This study delineates separate contributions of the lipolytic and ligand-binding functions of HL in apoB-Lp catabolism via non-LDLr pathways and establishes, for the first time, a protective role for the ligand-binding function of HL in LDLr-deficient mice.

METHODS

Mice and diets

Mice expressing human HL-S145G, generated as described previously (22), were cross-bred with HL-KO (25) and LDLr-KO (26) mice (C57Bl/6J background), generating HL-S145G transgenic mice homozygous for both mouse HL and LDLr deficiency. LDLr-KO mice expressing HL-WT were used as controls. Animals were fed a rodent autoclaved chow diet [regular chow diet (RCD)] (NIH-07 chow diet: 0.025% cholesterol, 4.5% fat; Ziegler Brothers, Inc., Gardner, PA). Studies were performed according to a research protocol approved by the Animal Care and Use Committee of the National Heart, Lung, and Blood Institute, National Institutes of Health.

Northern and Western analysis

RNA was isolated from mouse liver using Trizol (Invitrogen, Carlsbad, CA), and human liver poly(A⁺) RNA was purchased from Ambion, Inc. (Austin, TX). Northern blot analysis was performed using a digoxigenin-labeled human and mouse HL riboprobe (1). A ³²P-labeled 693 bp cyclophilin cDNA probe (Ambion) was used for standardization.

Analysis of plasma lipases and lipid profile

Postheparin plasma HL and LPL activities were determined using radiolabeled triolein (Amersham, Piscataway, NJ) (27). Plasma lipids were measured after a 4 h fast using enzymatic assays as described (16). HDL-cholesterol (HDL-C), determined after precipitation of apoB-Lps with dextran sulfate (Bayer Corp., Tarrytown, NY), was subtracted from total cholesterol (TC) to obtain non-HDL cholesterol (22). Plasma apolipoproteins were analyzed by sandwich ELISA using polyclonal antibodies against synthetic peptides of mouse apoA-I and apoB (8) and by 4–12% SDS-PAGE (0.2–1 μl of plasma), followed by immunoblotting with monospecific anti-mouse apoA-I, apoB, and apoE antibodies (Bioscience Resource Project, Maine, ME), followed by densitometric scanning (Personal Densitometer; Molecular Dynamics, Sunnyvale, CA), as reported (1). Lipoproteins were analyzed by fast-protein liquid chromatography (FPLC) (100 μl of pooled plasma; n = 5) as described (22).

Lipoprotein particle lipid composition and size analysis

VLDL, intermediate density lipoprotein (IDL)/LDL, and HDL were isolated from a single aliquot (2.5 ml) of pooled (n = 10 for each) mouse plasma by three consecutive sequential ultracentrifugations in KBr solutions adjusted for VLDL to d < 1.006 g/ml, for IDL/LDL to d = 1.006–1.063 g/ml, and for HDL to d = 1.063–1.21 g/ml; each of the three consecutive sequential ultracentrifugations was run at 5°C and 100,000 rpm for 5 h in a TL-100 ultracentrifuge (Beckman Instruments, Palo Alto, CA) (22). After dialysis against PBS, the lipid and apolipoprotein contents were measured as described above. For lipoprotein particle size, VLDL, IDL/LDL, and HDL fractions were analyzed by nondenaturing polyacrylamide gradient gel electrophoresis (22). Briefly, VLDL and IDL/LDL were electrophoresed (17 h at 35 V) on native Tris-glycine 4–12% polyacrylamide minigels (Invitrogen), and HDL was electrophoresed (24 h at 200 V) on native TBE 3–36% polyacrylamide gels (14 cm long). Gels were stained with Oil Red O and Coomassie Brilliant Blue R-250 to detect lipid and protein. The migration distances of particles were determined by densitometer scanning (FluorChem™ 8800 Imaging System; Alpha Innotech, Alexandria, VA), and the molecular diameter was calculated from a calibration curve generated from the migration distances of size standards of known diameter (22).

Metabolic studies

Lipoproteins were isolated by sequential ultracentrifugation as described above. Mouse ¹²⁵I-labeled apoB-IDL/LDL was prepared by a modification of the iodine monochloride method with an efficiency of 29–37% (28), reisolated by ultracentrifugation, and dialyzed overnight against phosphate-buffered saline. The integrity of the ¹²⁵I-labeled IDL/LDL was assessed by comparison with the particles before labeling using native agarose gel electrophoresis, FPLC analysis, and analysis of particle composition [total cholesterol (TC), free cholesterol (FC), cholesteryl ester (CE), phospholipid (PL), triglyceride (TG), and apolipoproteins]. Autologous mouse ¹²⁵I-labeled apoB-IDL/LDL (1 × 10⁶ dpm) was injected into the saphenous veins of animals in the three different mouse groups (n = 6 for each group), and mice were bled at different time points. After fractionation by SDS-PAGE (4–12%), ¹²⁵I-apoB bands were excised and counted in a γ counter (Cobra Auto-γ; Packard Instruments Co., Downers Grove, IL) (5). The fractional catabolic rate (FCR) was determined from the area under the apoB radioactivity curves using a multiexponential curve-fitting technique in the WinSAAM program (version 3.0.1).

Analysis of aortic lesions

For cross-sectional aortic lesion analysis, the heart and the attached section of the ascending aorta were prepared and analyzed as described (29). The methods for en face aortic lesion analysis to quantify atherosclerosis along the entire aorta were adapted from Teupser, Persky, and Breslow (30) and Jiang et al. (31). The mice were perfused with PBS for 3 min through a cannula inserted into the left ventricle of the heart followed by 30 min of perfusion with a fixative solution (4% paraformaldehyde, 5% sucrose, and 20 mM EDTA, pH 7.4). The aortas were then dissected from the origin of the heart to the ileal bifurcation. The tissue was then further fixed overnight with the same fixative solution, stained with Sudan IV solution for 25 min, destained for 25 min in 80% ethanol, and washed with water. The aortas were then cleaned using a microscope for visualization to remove any remaining fat attached to the outer surface, cut longitudinally, placed on a glass slide embedded with glycerin, covered by a microscope cover glass, and sealed with nail polish. Quantitation of plaques from the ileal bifurcation to the origin (not including branching vessels) was performed in a blinded manner and in triplicate using Image-Pro Plus version 4.1 software (Media Cybernetics, Inc.). Data are reported as the percentage of the aortic surface covered by lesions (total surface area of the atherosclerotic lesions divided by the total surface area of the aorta).

Statistical analysis

Data are presented as means \pm SEM. Unpaired Student's *t*-test was performed for analysis of plasma lipid concentrations and lipase activities. Nonparametric data were analyzed by the Mann-Whitney test (Instat Software; GraphPad, Inc., San Diego, CA).

RESULTS

Expression of HL-WT and HL-S145G decreases the plasma apoB-Lp concentrations in LDLr-KO \times HL-KO mice on a RCD

Northern analysis of RNA isolated from livers of LDLr-KO \times HL-WT and LDLr-KO \times HL-S145G mice demonstrated that HL-WT and HL-S145G were expressed at similar levels (see supplementary Fig. 1). Expression of HL-WT and HL-S145G in LDLr-KO mice lacking endogenous mouse HL decreased plasma lipids relative to those in control LDLr-KO \times HL-KO mice (Fig. 1A); however, the lipolytic activity of HL-WT was more effective at decreasing plasma lipids than HL-S145G (Fig. 1A). In female LDLr-KO \times HL-WT mice, the plasma concentrations of TC, TG, PL, CE, HDL-C, non-HDL-C, apoA-I, and apoB were reduced by 55, 50, 68, 50, 44, 63, 27, and 34%, respectively, compared with the corresponding lipid and lipoprotein concentrations in LDLr-KO \times HL-KO female mice (Fig. 1A). The lower plasma cholesterol levels were attributable mostly to reduced cholesterol in the FPLC fractions corresponding to IDL/LDL-C, although reduced VLDL-C and HDL-C were also evident by FPLC (Fig. 1B). In male LDLr-KO \times HL-WT mice, TC, TG, PL, CE, HDL-C, non-HDL-C, apoA-I, and apoB levels in plasma were reduced by 42, 29, 50, 39, 24, 48, 19, and 25%, respectively, compared with the plasma levels of the corresponding lipids in LDLr-KO \times HL-KO mice (data not shown). Inactive HL-S145G also decreased plasma TC, PL, CE, non-

HDL-C, and apoB in female LDLr-KO \times HL-S145G mice by 20, 12, 20, 22, and 16%, respectively, compared with levels in female LDLr-KO \times HL-KO mice (Fig. 1A); in male LDLr-KO \times HL-S145G mice, the plasma levels of TC, PL, CE, non-HDL-C, and apoB were reduced by 25, 15, 24, 28, and 17%, respectively, compared with the levels in male LDLr-KO \times HL-KO mice. HL-WT and HL-S145G reduced both apoB-100 and apoB-48 (Fig. 1A, inset). In contrast to HL-WT, HL-S145G did not alter plasma HDL-C or apoA-I levels (Fig. 1A). Plasma concentrations of apoE were similar in all three study groups (2.3 ± 0.3 , 2.5 ± 0.3 , and 2.6 ± 0.4 mg/dl, respectively). FPLC analysis revealed that most of the cholesterol reduction in LDLr-KO \times HL-S145G mice was attributable to decreased IDL/LDL-C (Fig. 1B).

Direct comparison of LDLr-KO \times HL-WT and LDLr-KO \times HL-S145G mice revealed not only lower plasma TC, TG, PL, CE, HDL-C, and apoA-I but also non-HDL-C and apoB levels in male and female mice expressing HL-WT ($P < 0.01$ for all). Thus, in contrast to apoE-KO \times HL-KO mice (15, 22), maximum decrease of apoB-Lp cholesterol and apoB in the LDLr-KO \times HL-KO mouse model requires the lipolytic function of HL.

Catalytically active HL-WT and inactive HL-S145G alter lipoprotein particle lipid content and size and enhance the catabolism of apoB-Lps

To evaluate the separate effects of the lipolytic and ligand-binding functions of HL on lipoprotein lipid content and size (22), we isolated VLDL, IDL/LDL, and HDL from LDLr-KO \times HL-KO, LDLr-KO \times HL-WT, and LDLr-KO \times HL-S145G mice by sequential density ultracentrifugation and determined the lipoprotein particle size and composition (Fig. 2A–C, Table 1) in each density fraction.

The combined lipolytic and ligand-binding functions of HL in LDLr-KO \times HL-WT mice reduced the concentrations of TC, TG, PL, FC, CE, and apoB in the VLDL and IDL/LDL density fractions and of all lipids and of apoA-I in the HDL fraction compared with LDLr-KO \times HL-KO mice (Table 1). To determine the contribution of the ligand-binding function of HL to changes in the lipid content of the different lipoproteins, a similar analysis was performed for particles isolated from LDLr-KO \times HL-S145G mice. HL-S145G also reduced the concentrations of TC, PL, FC, CE, and apoB in the VLDL and IDL/LDL fractions of LDLr-KO \times HL-KO mice, but to a lesser degree than HL-WT (Table 1). In contrast, HL-S145G had virtually no effect on the concentrations of the lipids and apoA-I in the HDL fraction (Table 1).

Changes in lipoprotein lipid and apolipoprotein content are anticipated to alter lipoprotein particle size. Therefore, we determined the sizes of VLDL, IDL/LDL, and HDL isolated from LDLr-KO \times HL-KO, LDLr-KO \times HL-WT, and LDLr-KO \times HL-S145G mice by native polyacrylamide gel electrophoresis (Fig. 2A–C; see supplementary Fig. 2). Expression of HL-WT and HL-S145G altered the abundance of the 370 Å particles in the VLDL

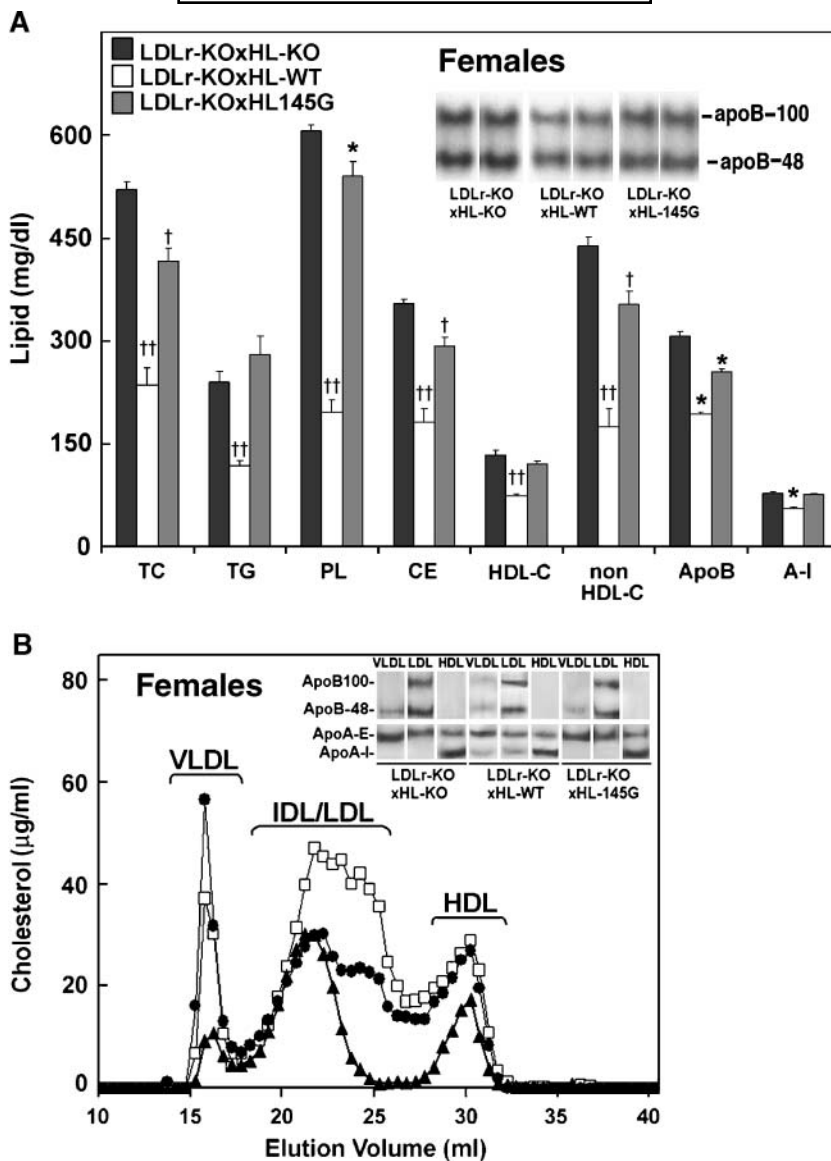


Fig. 1. HL-WT and HL-S145G plasma lipid, lipoprotein, and apolipoprotein concentrations in low density lipoprotein receptor knockout (LDLr-KO) × HL-KO mice on RCD. A: Plasma lipids, lipoproteins, and apolipoproteins of LDLr-KO×HL-KO (black bars; n = 11), LDLr-KO×HL-WT (white bars; n = 14), and LDLr-KO×HL-S145G (gray bars; n = 10) female mice. Inset: Immunoblot of plasma apolipoprotein B-100 (apoB-100) and apoB-48. B: Fast-protein liquid chromatography (FPLC) cholesterol profiles of pooled plasma (n = 5) from female LDLr-KO×HL-KO (open squares), LDLr-KO×HL-WT (closed triangles), and LDLr-KO×HL-S145G (closed circles) mice. Inset: Immunoblot of apoB-100, apoB-48, apoE, and apoA-I in FPLC fractions. Data are expressed as means ± SEM. * $P < 0.05$, [†] $P < 0.01$, ^{††} $P < 0.0001$ (compared with LDLr-KO×HL-KO). CE, cholesteryl ester; HDL-C, high density lipoprotein-cholesterol; IDL, intermediate density lipoprotein; PL, phospholipid; TC, total cholesterol; TG, triglyceride.

fraction and of the 360 and 210 Å lipoprotein particles in the IDL/LDL fractions of LDLr-KO×HL-KO mice (Fig. 2A, B, middle and bottom panels). However, HL-WT, which restored lipolytic activity, led to a greater reduction of these lipoprotein particles and resulted in decreased IDL/LDL particle size and heterogeneity (Fig. 2A, B, middle panels). These data indicate that both the lipolytic and ligand-binding functions of HL contribute separately to apoB-Lp remodeling; however, the metabolic impact of HL-WT is greater than that of HL-S145G in LDLr-

KO×HL-KO mice. Furthermore, the lipolytic function of HL is primarily responsible for HDL metabolism in LDLr-KO×HL-KO mice (Fig. 2C).

In LDLr-KO×HL-KO, LDLr-KO×HL-WT, and LDLr-KO×HL-S145G mice, most of the non-HDL cholesterol accumulates in IDL/LDL (Fig. 1B, Table 1). To investigate the in vivo mechanism by which the lipolytic versus ligand-binding functions of HL reduce the plasma concentrations of these proatherogenic apoB-Lps, we studied the plasma kinetics of autologous ¹²⁵I-labeled apoB-IDL/

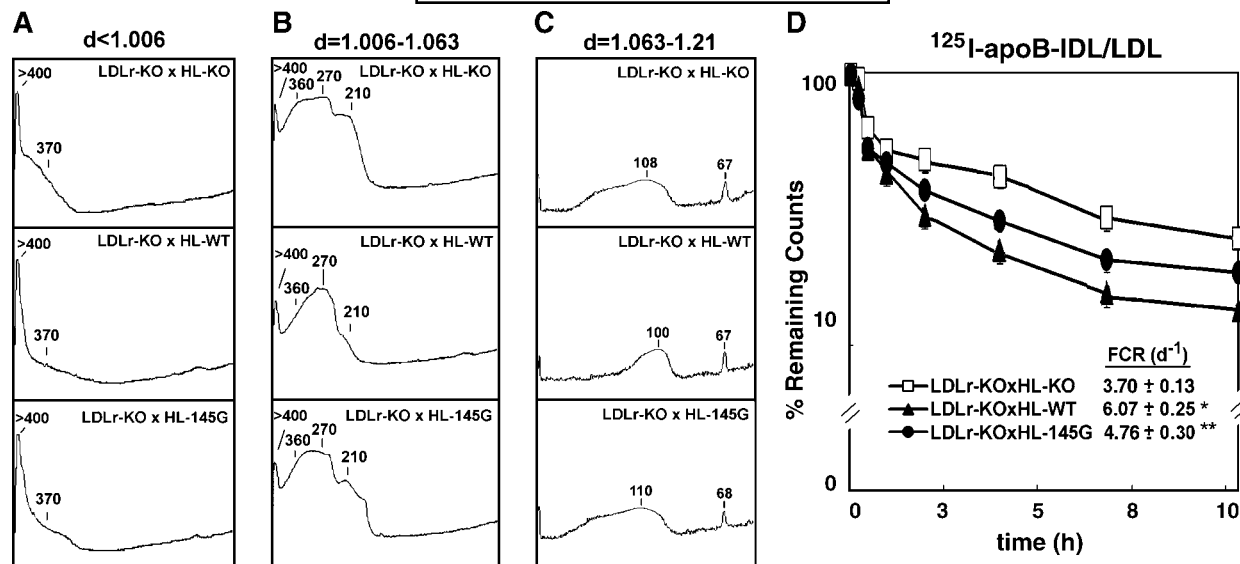


Fig. 2. Analysis of plasma lipoprotein particle size and plasma IDL/LDL kinetics of LDLr-KO×HL-KO, LDLr-KO×HL-WT, and LDLr-KO×HL-S145G mice on RCD. A–C: Analysis of VLDL ($d < 1.006$ g/ml) and IDL/LDL ($d = 1.006$ – 1.063 g/ml) fractions by native 4–12% gradient gel electrophoresis (A, B) and of the HDL ($d = 1.063$ – 1.21 g/ml) fraction by native 3–36% gradient gel electrophoresis (C) isolated from LDLr-KO×HL-KO, LDLr-KO×HL-WT, and LDLr-KO×HL-S145G mice. The diameters (in Å) of the major lipoprotein species are indicated. The ~67 Å peak in C is albumin (see supplementary Fig. II). D: Catabolism of autologous mouse 125 I-IDL/LDL in male LDLr-KO×HL-KO (open squares; $n = 6$), LDLr-KO×HL-WT (closed triangles; $n = 6$), and LDLr-KO×HL-S145G (closed circles; $n = 6$) mice. Fractional catabolic rate (FCR) values (day^{-1}) are shown. Data are expressed as means \pm SEM. * $P < 0.05$, ** $P < 0.01$ (compared with LDLr-KO×HL-KO).

LDL in the three mouse groups (Fig. 2D). Compared with LDLr-KO×HL-KO controls (FCR, day^{-1} : 3.70 ± 0.13), the catabolism of 125 I-labeled apoB-IDL/LDL was enhanced ($P < 0.05$ for both) by 1.6-fold in LDLr-KO×HL-WT (FCR, day^{-1} : 6.07 ± 0.25) and by 1.3-fold in LDLr-KO×HL-S145G (FCR, day^{-1} : 4.76 ± 0.30) female mice (Fig. 2D), thus providing an explanation for the 34% (HL-WT) and 16% (HL-S145G) reductions in apoB levels observed in

these two mouse groups (Fig. 1A). These findings indicate that both HL-WT and HL-S145G decrease apoB-Lps by enhancing their catabolism in plasma via non-LDLr-mediated lipoprotein uptake pathways in this mouse model and that, consistent with the changes in plasma lipids (Fig. 1A, B), maximal catabolism of IDL/LDL in LDLr-KO mice requires the lipolytic as well as the ligand-binding function of HL.

TABLE 1. Lipoprotein lipid concentrations in LDLr-KO×HL-KO, LDLr-KO×HL-WT, and LDLr-KO×HL-145G mice

Lipoprotein Lipid	Cholesterol	TG	PL	FC	CE	ApoB
<i>mg/dl</i>						
VLDL lipid ($d < 1.006$)						
LDLr-KO×HL-KO	166.3	212.5	128.6	57.3	109.1	79.3
LDLr-KO×HL-WT	45.0	95.8	36.9	14.2	30.8	52.6
LDLr-KO×HL-145G	108.3	195.8	88.0	35.7	72.6	53.8
	Cholesterol	TG	PL	FC	CE	ApoB
IDL/LDL lipid ($d = 1.006$ – 1.063)						
LDLr-KO×HL-KO	308.1	69.9	246.2	78.6	253.9	236.4
LDLr-KO×HL-WT	143.5	52.7	96.7	35.9	107.5	150.1
LDLr-KO×HL-145G	218.3	57.3	176.1	55.2	180.9	186.1
	Cholesterol	TG	PL	FC	CE	ApoA-I
HDL lipid ($d = 1.063$ – 1.21)						
LDLr-KO×HL-KO	113.7	23.5	189.5	23.1	102.2	53.0
LDLr-KO×HL-WT	81.2	11.2	128.2	13.6	74.4	31.0
LDLr-KO×HL-145G	128.0	20.8	211.4	26.0	115.0	56.0

ApoB, apolipoprotein B; CE, cholesteryl ester; FC, free cholesterol; IDL, intermediate density lipoprotein; PL, phospholipid; TG, triglyceride. The lipid and apolipoprotein concentrations of VLDL, IDL/LDL, and HDL fractions isolated by sequential ultracentrifugation from pooled ($n = 20$ for each group) LDLr-KO×HL-KO, LDLr-KO×HL-WT, and LDLr-KO×HL-145G mouse plasma are shown. Data shown are averages of at least two experiments.

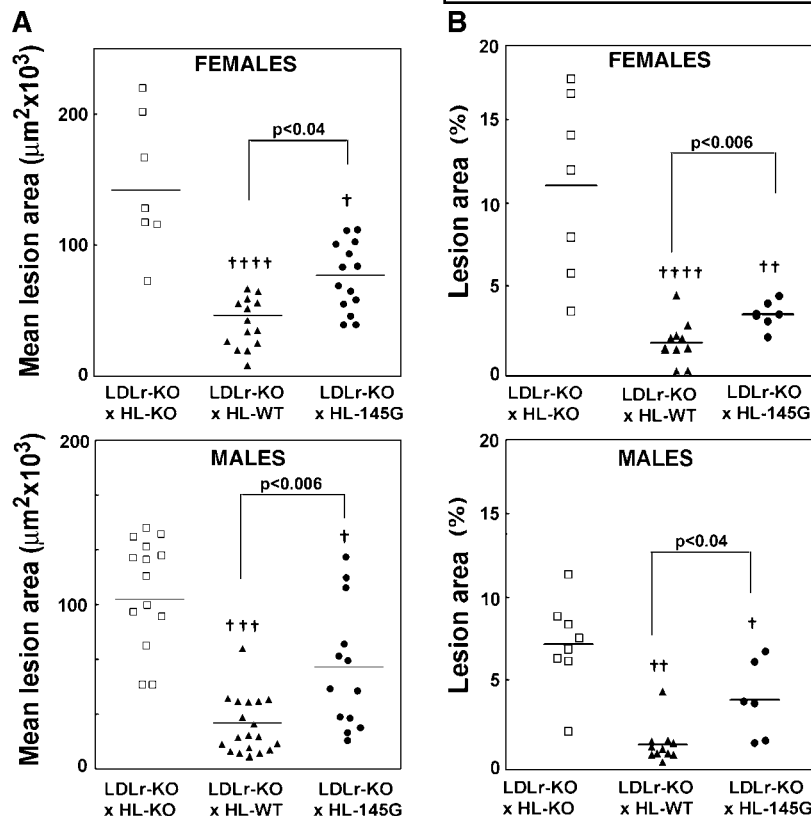


Fig. 3. Analysis of aortic atherosclerosis of LDLr-KO x HL-KO, LDLr-KO x HL-WT, and LDLr-KO x HL-S145G mice on RCD. A: Mean aortic lesion area was measured by cross-sectional analysis in 7.5 month old female (top panel) and 12 month old male (bottom panel) LDLr-KO x HL-KO (open squares), LDLr-KO x HL-WT (closed triangles), and LDLr-KO x HL-S145G (closed circles) mice on a RCD. B: Mean aortic lesion area was measured by en face analysis in 6.5 month old female (top panel) and 6.5 month old male (bottom panel) LDLr-KO x HL-KO (open squares), LDLr-KO x HL-WT (closed triangles), and LDLr-KO x HL-S145G (closed circles) mice on a RCD. Data are expressed as means \pm SEM. † $P < 0.035$, †† $P < 0.002$, ††† $P < 0.0001$, †††† $P < 2 \times 10^{-5}$ (compared with LDLr-KO x HL-KO mice).

Catalytically active HL-WT and inactive HL-S145G reduce the aortic atherosclerosis of LDLr-KO x HL-KO mice

We evaluated aortic lesion development by two independent analytical methods, cross-sectional proximal aortic analysis (Fig. 3A) and en face analysis of the entire aorta (Fig. 3B). On the RCD, expression of HL-WT significantly reduced proximal aortic atherosclerosis determined by cross-sectional analysis in LDLr-KO x HL-KO male (by 77%; $P < 0.0001$) and female (by 71%; $P < 2 \times 10^{-9}$) mice (Fig. 3A). Expression of HL-S145G also significantly decreased atherosclerosis in LDLr-KO x HL-KO male (by 50%; $P < 0.01$) and female (by 51%; $P < 0.01$) mice (Fig. 3A). However, consistent with the greater decrease of apoB-Lps by HL-WT (48% and 63%, respectively) versus HL-S145G (28% and 22%, respectively) in male and female LDLr-KO x HL-KO mice (Fig. 1A, B), HL-WT was more effective (females, 1.6-fold; $P < 0.04$; males, 2.2-fold; $P < 0.006$) than HL-S145G in reducing aortic lesion development determined by cross-sectional analysis (Fig. 3A). Quantitation of atherosclerosis by an independent method, en face analysis, yielded similar conclusions (Fig. 3B). Expression of HL-WT significantly reduced proximal aortic atherosclerosis in LDLr-KO x HL-KO male (by 87%; $P < 0.002$) and female (by 85%; $P < 5 \times 10^{-5}$) mice (Fig. 3B), whereas expression of HL-S145G significantly decreased atherosclerosis in LDLr-KO x HL-KO male (by 45%; $P < 0.035$) and female (by 67%; $P < 0.002$) mice (Fig. 3B). As in cross-sectional aortic analysis, HL-WT was found to be more effective than HL-S145G in reducing aortic lesion development in females (by 1.9-fold; $P < 0.006$) and males

(by 4.2-fold; $P < 0.04$) (Fig. 3B). These findings demonstrate that on a RCD, the lipolytic function of HL exerts a more protective effect than its ligand-binding function, but both HL-WT and catalytically inactive HL-S145G protect against the development of aortic atherosclerosis in LDLr-KO x HL-KO mice.

These findings thus establish a protective, antiatherogenic role of both the lipolytic and the ligand-binding functions of HL in LDLr-KO x HL-KO mice.

DISCUSSION

Recent studies have investigated the potential role of the lipolytic versus ligand-binding functions of HL in mediating the variable and sometimes opposing effects of HL on atherosclerosis. Using an atherosusceptible mouse model that does not express apoE or endogenous HL, we recently reported that despite similar decreases of the proatherogenic apoB-Lps, hepatic HL-S145G expression reduced atherosclerosis whereas hepatic HL-WT expression increased atherosclerosis (22). Because apoE plays a major role in facilitating the clearance of apoB-Lps, the absence of apoE in this mouse model may have potentially accentuated the effects of the ligand-binding function of HL on the plasma lipoproteins and atherosclerosis. Moreover, because both LDLr and LRP are intact, the role of LDLr in mediating the lipid-lowering effects of HL-WT and HL-S145G could not be addressed directly in apoE-KO x HL-KO mice. In this study, we investigated

the separate contributions of the lipolytic and ligand-binding functions of HL to lipoprotein metabolism and atherosclerosis in an alternative atherosusceptible mouse model (LDLr-KO×HL-KO), which lacks endogenous HL and is deficient in the LDLr but which does express apoE.

Both HL-WT and HL-S145G decreased the plasma concentrations of TC, PL, CE, non-HDL-C, and apoB; however, only HL-WT, and not HL-S145G, decreased plasma HDL, apoA-I, and TG levels in LDLr-KO×HL-KO mice. Similarly, both HL-WT and HL-S145G altered the TC, PL, FC, and CE lipid content of the apoB-Lps in LDLr-KO×HL-KO mice; again, the lipolytic function of HL had a significantly greater impact on the lipid composition, including TC, PL, and TG of IDL and LDL, than HL-S145G. Our findings agree with a recent report (23) indicating that active HL reduced the plasma TC by 55–65%, whereas catalytically inactive HL reduced TC by only 12–20% in LDLr-KO×HL-KO mice on a RCD.

Surprisingly, the reduction in plasma PL levels was greater than the decrease in plasma TG levels in mice expressing HL-WT, even though the TG lipase activity of HL-WT is greater than its phospholipase activity (32–34). The very low TG/PL ratio of HDL may account at least in part for this effect. In the LDLr-KO×HL-KO mouse model, however, the apoB-Lps are more abundant than HDL and HL-WT decreases PLs more than TGs in VLDL (high TG/PL ratio) and IDL/LDL (intermediate TG/PL ratio) as well as in HDL (low TG/PL ratio) (Fig. 1, Table 1). These findings are consistent with previous *in vitro* (35) and *in vivo* (16, 22, 36) studies that indicate that the TG lipase and phospholipase activities of HL on apoB-Lps may not differ greatly. Potential mechanisms for the preference of HL-WT for the lipolysis of PL versus TG in lipoproteins include accessibility (PLs are on the surface of lipoprotein particles, whereas TGs are buried within the core) as well as lipid and apolipoprotein content [shown to be important for HDL (37–42) and presumably for apoB-Lps as well]. Importantly, the decrease in plasma total TG and PL in LDLr-KO×HL-WT mice reflects not only the lipolytic function of HL-WT but also holoparticle uptake and/or selective lipid particle uptake of the apoB-Lps mediated by the ligand-binding function, which is present in HL-WT as well as HL-S145G mice. The parallel decrease in apoB and PL levels in apoB-Lps of LDLr-KO×HL-S145G versus LDLr-KO×HL-KO mice (Fig. 1, Table 1) indicates that the ligand-binding function removes apoB-Lp PLs by holoparticle uptake and clearance of apoB-Lps rather than by selective uptake of PLs from apoB-Lps (Table 1). In this case, the removal of TG and PL present in plasma apoB-Lps by the ligand-binding functions of HL-WT and HL-S145G would reflect the TG and PL contents in these particles. Our kinetic studies demonstrate that the FCR of ¹²⁵I-apoB IDL/LDL in LDLr-KO×HL-WT mice is even greater than that of ¹²⁵I-apoB IDL/LDL isolated from LDLr-KO×HL-S145G mice, indicating faster clearance of these particles and all of their lipids (including TG and PL) after lipolysis, which may diminish the effects of the preferential TG versus PL hydrolysis mediated by HL-WT

on plasma TG and PL levels. Finally, differences in particle composition at the time of secretion attributable to alterations in the metabolic steady state of the different mouse groups are another potential explanation for the preferential decrease in PLs versus TGs in LDLr-KO×HL-WT mice.

Our study also demonstrates that the lipolytic function of HL had a significantly greater impact on reducing the particle size of IDL and LDL, the major cholesterol-containing lipoproteins in LDLr-KO×HL-KO mice, than the ligand-binding function: mice expressing HL-S145G had larger, more heterogenous, more lipid-rich, and more abundant IDL/LDL particles than mice expressing HL-WT. Previous reports have shown that changes in LDL particle lipid composition can alter the exposure of apolipoprotein epitopes and, hence, alter the uptake of lipoproteins by cell surface receptors and proteoglycans (43–45). Thus, the relatively lipid-rich IDL/LDL generated in LDLr-KO×HL-S145G mice may be cleared by cell surface uptake pathways less effectively than the lipid-depleted IDL/LDL generated in LDLr-KO×HL-WT mice.

Our kinetic studies confirm that the contribution of the lipolytic function of HL to plasma IDL/LDL catabolism in LDLr-KO×HL-KO mice is greater (by ~30%) than that of its ligand-binding function (Fig. 2D). These data define the mechanism by which the lipolytic versus ligand-binding functions of HL differentially decrease the plasma levels of apoB-Lps in LDLr-KO mice and provide the first kinetic evidence that both active and inactive HL can enhance apoB-Lp catabolism *in vivo* via non-LDLr mechanisms.

The findings in LDLr-KO×HL-KO mice differ from previous results in E-KO×HL-KO mice, in which the ligand-binding function of HL accounted for most of the reduction in apoB-Lps (15, 22). Comparison of the kinetic parameters of the different types of lipoproteins that accumulate in these two mouse models provides an explanation for the greater impact of HL-S145G on apoB-Lp levels in E-KO×HL-KO versus LDLr-KO×HL-KO mice. In both mouse models, HL-WT had a greater effect on the FCR of LDL than HL-S145G (22). However, in E-KO×HL-KO mice, most of the apoB-Lp cholesterol is associated with VLDL and chylomicron remnants rather than LDL (22), whereas in LDLr-KO×HL-KO mice, much of the apoB-Lp cholesterol is present in LDL. Because LDL is the major cholesterol-carrying apoB-Lp in LDLr-KO×HL-KO mice, our kinetic data provide an explanation for the greater apoB-Lp cholesterol and apoB-lowering effect of HL-WT versus HL-S145G in LDLr-KO×HL-KO mice.

Our study also demonstrates that even in the absence of LDLr function and the presence of apoE, the lipolytic and ligand-binding functions of HL can enhance the FCR of apoB-Lps (by 1.6- and 1.3-fold, respectively). Thus, the contribution of the ligand-binding function of HL to apoB-Lp catabolism does not merely represent a minor pathway that becomes apparent only in the absence of the more robust uptake mechanism involving apoE

(22) but rather constitutes a physiologically relevant pathway with a significant impact on apoB-Lp catabolism even in the presence of apoE. In addition, these data demonstrate that pathways alternative to the LDLr can mediate the effects of both the lipolytic and the ligand-binding functions of HL on apoB-Lp clearance. These findings are consistent with a growing body of in vivo evidence supporting an important role for proteoglycans and LRP in mediating the HL-facilitated uptake of apoB-Lps (4, 22, 46, 47). Nonetheless, a role for the LDLr in HL-mediated apoB-Lp uptake has not been ruled out; in fact, infusion of anti-HL and anti-LDLr antibodies in mice with intact LDLr supports a role for LDLr in HL-mediated apoB-Lp catabolism (4). Altogether, these combined in vivo data implicate multiple pathways for the HL-facilitated uptake of apoB-Lps.


Finally, this study provides in vivo evidence for a protective, antiatherogenic role of both the lipolytic and the ligand-binding functions of HL in LDLr-KO×HL-KO mice. Both HL-WT and HL-S145G reduced proximal aortic atherosclerosis (Fig. 3). However, the lipolytic function of HL exerted a more protective effect than the ligand-binding function. Dichek, Qian, and Agrawal (23) also found that HL-WT was more atheroprotective than HL-S145G in LDLr-KO×HL-KO mice fed a Western diet for 12 weeks; however, they did not find the ligand-binding function of HL to confer atheroprotection. A key difference between the two studies (22, 23) relates to diet. As noted above, on the RCD, we (Fig. 1A, B) as well as Dichek, Qian, and Agrawal (23) noted significant decreases of TC and the proatherogenic non-HDL-C in LDLr-KO×HL-S145G mice. In our study, we performed cross-sectional and en face analyses of mouse aortic atherosclerosis on the RCD. In contrast, Dichek, Qian, and Agrawal (23) measured atherosclerosis at 12 weeks on the Western diet, when the reduction in plasma TC and non-HDL-C were no longer apparent in LDLr-KO×HL-S145G mice, rather than on the RCD or at 4 and 8 weeks, when differences in apoB-Lps were still evident in their study.

Although the lipolytic function of HL protects against lesion development in LDLr-KO×HL-KO mice (Fig. 3), HL-WT paradoxically increased atherosclerosis in E-KO×HL-KO mice (15, 22) despite decreasing apoB-Lp levels (22). This difference in the antiatherogenic versus proatherogenic effects of HL-WT may relate to the different types of lipoprotein particles that accumulate in the two mouse models. In E-KO mice, most of the apoB-Lp cholesterol is associated with VLDL and chylomicron remnants rather than LDL. E-KO×HL-KO mice also accumulate unique vesicular apoB-48-containing lipoproteins (48). Bergeron et al. (48) have suggested that the lipolytic function of HL may be particularly important in preventing the formation of these vesicular lipoproteins, which may not only have decreased atherogenic potential but may, in fact, facilitate reverse cholesterol transport. Thus, the increased atherosclerosis in E-KO×HL-WT versus E-KO×HL-KO mice may be attributable, at least in part, to its lipolytic role in preventing the formation of these

unique vesicular lipoproteins that are potentially atheroprotective. Additionally, the marked differences in the lipid and apoB contents of all apoB-Lps in E-KO×HL-WT versus E-KO×HL-S145G mice (22) may cause differential uptake of these lipoproteins by cell surface receptors and proteoglycans (43–45). In contrast to E-KO×HL-KO mice, atherosclerosis development paralleled the apoB-lowering effects of HL-WT versus HL-S145G in LDLr-KO×HL-KO mice. The paradoxical dissociation between the decrease of apoB-Lps and atherosclerosis in E-KO×HL-WT mice indicates that differences in particle type and composition caused by the presence or absence of lipolytic activity, rather than absolute plasma levels of the apoB-Lps, have a more significant impact on the development of aortic lesions in this mouse model. In contrast, this study demonstrates that decreases in plasma levels of apoB-Lps are the primary determinant of atherosclerosis in LDLr-KO×HL-WT mice.

Although a potential limitation of our studies is the use of endogenous mouse HL-WT in the LDLr-KO×HL-KO background, we note that in terms of the plasma lipid profile and the effect on atherosclerosis, very similar results were obtained between our endogenous mouse HL-WT and the transgenic human HL-WT used by Dichek, Qian, and Agrawal (23). Our findings seem to indicate that, in contrast to the E-KO mouse model (16), in the LDLr-KO mouse model HL expression in macrophages is not particularly important in the development of vascular disease.

The various studies conducted to date indicate that the impact of HL-WT and HL-S145G expression on lipoprotein metabolism and the development of atherosclerosis varies with dietary manipulation as well as genetic background and is attributable not only to the types but also to the compositions of the various lipoprotein particles that accumulate in the different mouse strains on different dietary regimes (21–23).

In summary, this study delineates the separate contributions of the lipolytic versus ligand-binding functions of HL to plasma lipoprotein composition and metabolism, identifying physiologically relevant but divergent roles for the lipolytic versus ligand-binding functions of HL in modulating the development of atherosclerosis in vivo and establishing, for the first time, an antiatherogenic role of the ligand-binding function of HL in LDLr-deficient mice. 

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