Hepatic lipase overexpression lowers remnant and LDL levels by a noncatalytic mechanism in LDL receptor-deficient mice

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Abstract **To address the role of the noncatalytic ligand function of hepatic lipase (HL) in low density lipoprotein (LDL) receptor-mediated lipoprotein metabolism, we characterized transgenic mice lacking the LDL receptor (LDLR) that express either catalytically active (*Ldlr*$^{-/-}$*HL*) or inactive (*Ldlr*$^{-/-}$*HL*^{S145G}) human HL on both chow and high fat diets and compared them with nontransgenic *Ldlr*$^{-/-}$ mice. In mice fed a chow diet, apolipoprotein (apo)B-containing lipoprotein levels were 40–60% lower in *Ldlr*$^{-/-}$*HL* and *Ldlr*$^{-/-}$*HL*^{S145G} mice than in *Ldlr*$^{-/-}$ mice. This decrease was mainly reflected by decreased apoB-48 levels in the *Ldlr*$^{-/-}$*HL* mice and by decreased apoB-100 levels in *Ldlr*$^{-/-}$*HL*^{S145G} mice. These findings indicate that HL can reduce apoB-100-containing lipoproteins through a noncatalytic ligand activity that is independent of the LDLR. Cholesterol enrichment of the apoB-containing lipoproteins induced by feeding *Ldlr*$^{-/-}$*HL* and *Ldlr*$^{-/-}$*HL*^{S145G} mice a cholesterol-enriched high fat (Western) diet resulted in parallel decreases in both apoB-100 and apoB-48 levels, indicating that HL is particularly efficient at reducing cholesterol-enriched apoB-containing lipoproteins through both catalytic and noncatalytic mechanisms. These data suggest that the noncatalytic function of HL provides an alternate clearance pathway for apoB-100- and apoB-48-containing lipoproteins that is independent of the LDLR and that contributes to the clearance of high density lipoproteins.**—Dichek, H. L., S. M. Johnson, H. Akeefe, G. T. Lo, E. Sage, C. E. Yap, and R. W. Mahley. **Hepatic lipase overexpression lowers remnant and LDL levels by a noncatalytic mechanism in LDL receptor-deficient mice.** ***J. Lipid Res.*** **2001.** 42: **201–210.**

Supplementary key words noncatalytic function • ligand function • apoB-100 • FPLC • Western diet • phospholipase A1 activity

Hepatic lipase (HL) plays a central role in lipoprotein metabolism by regulating plasma lipoprotein levels (1–4). It is produced and secreted by hepatocytes and translocated to heparan sulfate proteoglycans (HSPG) at the surface of hepatocytes and endothelial cells in liver sinusoids (5, 6). HL hydrolyzes triglycerides and phospholipids in the apolipoprotein (apo)B-containing intermediate density lipoproteins (IDL) and low density lipoproteins (LDL) to yield smaller particles (1–4). The role for HL in apoB-containing lipoprotein metabolism is suggested by the increased levels of β-very low density lipoproteins (VLDL), IDL, and LDL in HL-deficient patients (7–13), as well as by the increased levels of IDL and LDL resulting from infusion of anti-HL antibodies into rats or cynomologous monkeys (14–16). In addition, antibody inhibition studies (17) as well as studies of HL-deficient mice (18) and humans (7–9, 13, 19) demonstrate that HL hydrolyzes phospholipids and triglycerides in class 2 high density lipoproteins (HDL) to yield HDL_3, which remove cholesterol from peripheral tissues through the process of reverse cholesterol transport (1–4). In rodents, HL may also facilitate the delivery of lipoprotein cholesterol to steroidogenic tissues by hydrolyzing phospholipids on the lipoprotein surface, thereby facilitating transfer of free cholesterol and core cholesterol esters to cells for steroid hormone synthesis (20, 21).

Recent in vitro evidence suggests that cell-surface HL can act as a ligand for receptor-mediated endocytosis of lipoproteins independently of its catalytic activity (22–26). We have shown that overexpression of human HL in transgenic mice decreases the plasma levels of lipoproteins containing apoB and reduces plasma HDL levels (6). In particular, overexpression of human HL in *Apoe*$^{-/-}$ mice profoundly

Abbreviations: apo, apolipoprotein; ECL, electrochemiluminescence; FFA, free fatty acids; FPLC, fast performance liquid chromatography; HDL, high density lipoprotein; HDL-C, HDL cholesterol; HL, hepatic lipase; HSPG, heparan sulfate proteoglycans; IDL, intermediate density lipoprotein; LDL, low density lipoprotein; LDLR, LDL receptor; LRP, LDLR-related protein; PLA1, phospholipase A1; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; VLDL, very low density lipoprotein.

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reduces plasma levels of apoB-48-containing remnant lipoproteins. The HL-mediated apoB-48 reduction can occur in the absence of catalytic activity because both wild-type catalytically active HL and mutant catalytically inactive HL (HL^{S145G}) were equally effective in clearing apoB-48-containing remnants in the $Apoe^{-/-}$ mice. The effective clearance of remnants by both wild-type and catalytic inactive HL was also demonstrated in a recent study using adenoviral vector-mediated gene delivery to $Apoe^{-/-}$ mice (27, 28). However, the conclusions from those experiments were limited to HL's effects on remnant cholesterol and apoB-48 levels and could not address the effects on apoB-100 levels because of the presence of high levels of apoB-48 and near absence of apoB-100 in $Apoe^{-/-}$ mice. Previously, in vitro studies addressed the possibility that HL serves as a ligand in the clearance of apoB-100-containing lipoproteins (26). In those studies, ^{125}I-labeled human LDL were incubated for 2 h at 37°C with rat hepatoma cells transfected with HL or HL^{S145G} (26). Direct binding and uptake of ^{125}I-labeled human LDL were similarly increased in HL- and HL^{S145G}-transfected cells, indicating that HL mediates LDL uptake independently of catalytic activity (26). The increased LDL uptake observed in those in vitro studies suggests that HL (attached to HSPG) functions by concentrating the apoB-100-containing lipoproteins in proximity to the LDL receptor (LDLR), which then mediates their uptake. Another intriguing possibility is that HL (attached to HSPG) directly mediates the uptake of apoB-100-containing lipoproteins independently of the LDLR by using HSPG alone as a receptor.

Although the in vitro data have generated excitement about the existence of a HL-mediated noncatalytic clearance pathway for apoB-100-containing lipoproteins (including IDL and LDL), in vivo evidence for such a pathway is lacking. To date, all in vivo data on the effects of catalytically inactive HL^{S145G} have been derived from experiments in $Apoe^{-/-}$ mice in which apoB-100 levels are very low. Therefore, we examined whether HL^{S145G} also reduces apoB-100-containing lipoproteins in vivo and assessed the dependence of this clearance on the LDLR pathway in LDLR-deficient ($Ldlr^{-/-}$) mice (29). These mice are ideally suited for such studies because they accumulate apoB-100-enriched lipoproteins (primarily IDL and LDL) that are normally cleared by the LDLR. In this study, we bred transgenic mice expressing catalytically active HL (reflecting the combined catalytic and ligand functions of HL) and catalytically inactive HL^{S145G} (reflecting only the ligand function of HL) with $Ldlr^{-/-}$ mice and determined the effect of HL or HL^{S145G} expression on plasma lipoprotein profiles and plasma apolipoprotein content in mice fed a chow diet or a cholesterol-enriched high fat (Western) diet.

MATERIALS AND METHODS

Transgenic mice

Gene-targeted C57Bl/6 mice lacking the endogenous mouse LDLR gene ($Ldlr^{-/-}$) (29) were crossed with transgenic mice expressing wild-type catalytically active human HL (6) or a mutant catalytically inactive human HL (HL^{S145G}) to achieve homozygosity for the gene-targeted mouse LDLR gene and heterozygosity for the HL or HL^{S145G} transgene. Liver expression of the HL and HL^{S145G} transgenes was achieved with sequences from the human apoE gene as described (6): 3 kb of the 5′-flanking sequence, the first exon, the first intron, and the first six untranslated nucleotides of the second exon; a polylinker for cDNA insertion; the nontranslated portion of the fourth exon; 0.1 kb of 3′-flanking sequence; and the first hepatic control region of the apoE gene locus. The transgenic mice expressing HL and HL^{S145G} were previously backcrossed to achieve 97.5% C56Bl/6 background. Homozygosity for the $Ldlr^{-/-}$ genotype was determined by polymerase chain reaction analysis of tail DNA (30). The human HL transgene was detected by Southern blotting with a radioactively labeled human HL cDNA probe (6, 31–33).

Human HL expression

Plasma from $Ldlr^{-/-}$, $Ldlr^{-/-}HL$, and $Ldlr^{-/-}HL^{S145G}$ mice fed either chow or high fat cholesterol-enriched (Western) diets were collected into ethylenediaminetetraacetic acid (EDTA)-containing tubes before and 10 min after intravenous administration of heparin (150 U/kg body weight) and kept frozen at −80°C until analyzed for protein expression. Western blots of pre- and postheparin plasma were performed with a monospecific polyclonal rabbit anti-human HL antiserum (6). Triglyceride lipase activities were quantitated in duplicate with glycerol (1-^{14}C trioleate)-labeled triolein emulsion as a substrate in the presence of 1 M NaCl (34). Phospholipase A1 (PLA1) activities were quantitated in duplicate using 1,2 di[1-^{14}C]oleoyl-L-3-phosphatidylcholine (^{14}C-DOPC)-labeled mixed liposomes that were synthesized by a modification of the triolein emulsion as described (35, 36). Dioleoylphosphatidylcholine (20 mg/ml, Sigma) was used in place of egg yolk extract. Labeled triolein was substituted with ^{14}C-DOPC (Amersham Pharmacia Biotech) at an activity of 0.16 μCi / ml of substrate. Substrate (200 μl) was added to 10 μl of postheparin plasma in a final volume of 330 μl [150 mM NaCl, 100 mM Tris-HCl, pH 8, 3% bovine serum albumin (Pentex, Kankakee, IL), 2 Units/ml heparin (Elkins-Sinn, Cherry Hill, NJ)]. The samples were incubated at 33°C for 2 h, followed by oleic acid extraction and scintillation counting (37).

Lipoprotein analysis

Blood was collected by orbital vein bleeding after a 4-h fast and placed in tubes containing 5 μl of 0.5 M EDTA, pH 8.0, 2 μl of aprotinin, and 2 μl of 0.09% benzamidine. Plasma was separated by centrifugation at 14,000 rpm for 12 min at 4°C. Plasma total cholesterol and triglyceride concentrations were measured with standard enzymatic assays (cholesterol: Abbott Spectrum, Abbott Park, IL; triglycerides: GPO-PAP kit, Boehringer-Mannheim). Plasma lipoproteins were fractionated by fast performance liquid chromatography (FPLC) gel filtration on a Superose 6HR 10/30 column (Pharmacia Biotech) (6, 38, 39). For these analyses, 100 μl of plasma was mixed with 160 μl of phosphate-buffered saline (PBS) containing 10 mM EDTA. The elution position for LDL in FPLC fractions 23–27 was confirmed by analyzing the elution position for human LDL (d = 1.02–1.05 g/ml) prepared by ultracentrifugation from the plasma of a normolipidemic subject. The column was eluted with PBS/EDTA at a flow rate of 0.4 ml/min, and 60 0.5-ml fractions were collected. Cholesterol and triglyceride levels were determined enzymatically as described above. Plasma cholesterol and triglyceride recovery was 70–100%. Comparisons between groups were evaluated using a one-way analysis of variance (ANOVA) followed by pairwise multiple comparison (Tukey's test).

Quantitation of apoB-100 and apoB-48 in plasma from mice fed a chow diet

Plasma samples from five female *Ldlr*$^{-/-}$ mice, five female *Ldlr*$^{-/-}$*HL*^{S145G} mice, and three female *Ldlr*$^{-/-}$*HL* mice were pooled separately, and each pool was applied in four aliquots to two 4% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gels. Separated plasma proteins were transferred to a nitrocellulose membrane (Bio-Rad Laboratories, Hercules, CA) for Western blot analysis. The membrane was incubated with a monospecific rabbit anti-mouse apoB antibody (1:7,500) that binds both apoB-100 and apoB-48 (40), reacted with goat anti-rabbit-horseradish peroxidase conjugate (1:25,000), and developed with an electrochemiluminescence kit (ECL; Amersham, Buckinghamshire, UK). Immunoblots were analyzed by gel densitometry with a Multi-Image Light Cabinet and Alphaimager 2000 system (version 3.3; Alpha Innotech, San Leandro, CA). Data were analyzed using Student's *t*-test for unequal variances.

Quantitation of apoA-I in plasma from mice fed a chow diet

Plasma was pooled as described above, fractionated by SDS-PAGE on 12% gels, and transferred to a nitrocellulose membrane. The membrane was incubated with a polyclonal rabbit anti-mouse apoA-I antiserum, which also detects mouse apoE (6). The second antibody incubation, ECL development, and densitometric scanning were performed as described for apoB. Data were analyzed with a *t*-test for unequal variances.

Diet study

To expand our evaluation of the catalytic and ligand effects of HL in lipid metabolism to include the effects on cholesterol-enriched lipoproteins (containing high levels of apoB-48 in addition to apoB-100), *Ldlr*$^{-/-}$, *Ldlr*$^{-/-}$*HL*, and *Ldlr*$^{-/-}$*HL*^{S145G} mice were fed a Western diet of 21% (w/w) fat and 0.15% (w/w) cholesterol (TD 88137, Harlan Teklad, Madison, WI) for 2 weeks. Fasting plasma total and lipoprotein cholesterol and triglyceride concentrations were determined as described above. Comparisons between groups were evaluated using a one-way ANOVA followed by pairwise multiple comparison (Tukey's test). To investigate the possibility that the high fat diet modified the expression of either the endogenous or the transgenic HL, pre- and postheparin plasmas were obtained for lipase activity determinations (to detect changes in both the endogenous mouse HL and the transgenic human HL expression) and Western blot analysis for HL immunoreactivity determinations (to detect changes in the human HL transgenes). Results were analyzed by Student's *t*-test for unequal variances.

Quantitation of plasma apoB-100, apoB-48, and apoA-I in mice fed a high fat diet

For quantitation of apoB-100 and apoB-48, plasma samples from four mice of each genotype were pooled separately, and each pool was applied in four aliquots to two 4% gels for fractionation of plasma proteins by SDS-PAGE. The transfer, antibody incubations, blot development, and image analyses were performed as described above. For quantitation of apoA-I, pooled plasma was applied in triplicate to a 12% SDS-PAGE gel for Western blot analysis, as described above. Data were analyzed with a *t*-test for unequal variances.

RESULTS

Expression and characterization of the human HL protein

Similar expression levels of immunoreactive human HL in *Ldlr*$^{-/-}$*HL* and *Ldlr*$^{-/-}$*HL*^{S145G} were established semiquantitatively by Western blot and scanning densitometry analysis of postheparin plasma (**Fig. 1**). The expression levels of human HL were not changed by Western diet (data not shown), suggesting the absence of dietary regulation of the transgenes. No immunoreactivity was detected in the *Ldlr*$^{-/-}$ mice, reflecting the lack of cross-reactivity of the HL antibody with mouse HL. Preheparin plasma from *Ldlr*$^{-/-}$*HL* and *Ldlr*$^{-/-}$*HL*^{S145G} lacked immunoreactive HL, showing the absence of circulating trans-

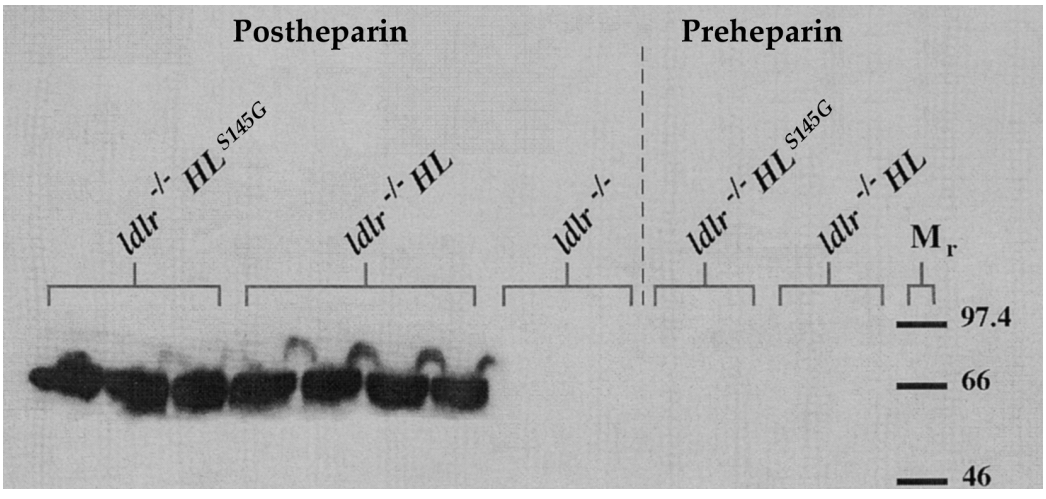

Fig. 1. HL expression demonstrated by Western blot analysis. Western blot assay of pre- and postheparin plasma demonstrating similar levels of human HL expression in *Ldlr*$^{-/-}$*HL* and *Ldlr*$^{-/-}$*HL*^{S145G} mice. Preheparin plasma samples (2 μl) from two *Ldlr*$^{-/-}$*HL* and two *Ldlr*$^{-/-}$*HL*^{S145G} mice and postheparin plasma samples (2 μl) from two *Ldlr*$^{-/-}$, four *Ldlr*$^{-/-}$*H*, and three *Ldlr*$^{-/-}$*HL*^{S145G} mice were separately pooled, fractionated by SDS-PAGE on 12% gels, transferred to nitrocellulose, and probed first with a rabbit anti-human HL antibody and then with horseradish peroxidase-conjugated goat anti-rabbit antibody. Antibody binding was visualized with an ECL assay.

TABLE 1. Plasma HL triglyceride lipase activity in female mice fed chow and Western diets

	Chow Diet			Western Diet		
		HL Activity[a]			HL Activity[a]	
Genotype	n	Preheparin	Postheparin	n	Preheparin	Postheparin
		μEq FFA per ml/h			*μEq FFA per ml/h*	
Ldlr$^{-/-}$	7	9 ± 3	16 ± 5	3	8 ± 1	12 ± 2
Ldlr$^{-/-}$*HL*	8	8 ± 1	412 ± 142[b]	5	11 ± 3	430 ± 111[c]
Ldlr$^{-/-}$*HL*^{S145G}	9	8 ± 2	18 ± 4	3	8 ± 1	16 ± 3

[a] Values given as means ± SD.
[b] $P < 0.005$ vs. chow-fed *Ldlr*$^{-/-}$ postheparin plasma.
[c] $P < 0.00005$ vs. Western diet–fed *Ldlr*$^{-/-}$ postheparin plasma.

gene-derived HL and indicating that HL is bound to cell-surface HSPG.

The preheparin plasma HL activity was similar in all three genotypes (**Table 1**). The postheparin plasma HL activity in *Ldlr*$^{-/-}$*HL* mice was approximately 20-fold higher than in either *Ldlr*$^{-/-}$*HL*^{S145G} mice or *Ldlr*$^{-/-}$ mice, indicating a robust expression of the catalytically active HL and verifying the absence of catalytic activity of HL^{S145G}. Western diet treatment did not affect either pre- or postheparin plasma triglyceride lipase activities, indicating the absence of dietary regulation of the transgene expression (Table 1). The preheparin plasma PLA1 activities were similar in all three genotypes (**Table 2**). The postheparin plasma PLA1 activities in the *Ldlr*$^{-/-}$*HL* mice were 2-fold higher than in either *Ldlr*$^{-/-}$*HL*^{S145G} mice or *Ldlr*$^{-/-}$ mice, verifying the absence of PLA1 activity of HL^{S145G} (Table 2). The relatively small increase in PLA1 activity in *Ldlr*$^{-/-}$*HL* mice may result from the presence of plasma inhibitors that interfere with the PLA1 assay (1). One possible inhibitor may be mouse HDL phospholipid. The mouse HDL phospholipid may compete with the radiolabeled substrate for HL (which then hydrolyzes the unlabeled HDL phospholipid), thus decreasing the apparent PLA1 activity.

Plasma cholesterol and triglyceride levels in mice on chow and Western diets

On a chow diet, increased expression of catalytically active HL reduced plasma cholesterol by ~60% in the *Ldlr*$^{-/-}$*HL* mice, whereas increased expression of catalytically inactive HL reduced plasma cholesterol by 35% in the *Ldlr*$^{-/-}$*HL*^{S145G} mice compared with *Ldlr*$^{-/-}$ mice

TABLE 2. Plasma PLA1 activity in female mice

		PLA1 Activity[a]	
Genotype	n	Preheparin	Postheparin
		μEq free fatty acid per ml/h	
Ldlr$^{-/-}$	3	3.8 ± 0.6	6.2 ± 0.2
Ldlr$^{-/-}$*HL*	6	3.6 ± 0.1	12.9 ± 3.1[b]
Ldlr$^{-/-}$*HL*^{S145G}	3	3.6 ± 0.1	6.8 ± 0.3

[a] Values given as means ± SD.
[b] $P < 0.002$ vs. *Ldlr*$^{-/-}$ postheparin plasma.

TABLE 3. Total plasma cholesterol and triglyceride concentrations in female mice on a chow diet

	Ldlr$^{-/-}$ (n = 6)	*Ldlr*$^{-/-}$*HL* (n = 9)	*Ldlr*$^{-/-}$*HL*^{S145G} (n = 8)
		mg/dl	
Total cholesterol	223 ± 47	86 ± 28[a]	143 ± 29[a,b]
Total triglycerides	74 ± 37	30 ± 15[c]	58 ± 24

Values given as means ± SD.
[a] $P < 0.001$ vs. *Ldlr*$^{-/-}$ mice.
[b] $P < 0.01$ vs. *Ldlr*$^{-/-}$*HL* mice.
[c] $P < 0.01$ vs. *Ldlr*$^{-/-}$ mice.

(**Table 3**). Plasma triglycerides decreased by 60% in the *Ldlr*$^{-/-}$*HL* mice, whereas no significant decrease occurred in the *Ldlr*$^{-/-}$*HL*^{S145G} mice (Table 3). The decreases in cholesterol, occurring in both the presence and absence of exogenous HL catalytic activity, indicate that human HL overexpression can reduce cholesterol independently of increased catalytic activity. Furthermore, because the mice lack the LDLR, the HL-mediated cholesterol reduction is also independent of the LDLR.

We next examined the catalytic and ligand functions of HL in *Ldlr*$^{-/-}$, *Ldlr*$^{-/-}$*HL*, and *Ldlr*$^{-/-}$*HL*^{S145G} mice fed a cholesterol-enriched high fat (Western) diet for 2 weeks. As expected, the Western diet increased cholesterol levels substantially (3.5–4-fold) in all mice. However, plasma cholesterol levels were 53% ($P < 0.003$) lower in *Ldlr*$^{-/-}$*HL* mice and 31% ($P < 0.003$) lower in *Ldlr*$^{-/-}$*HL*^{S145G} mice than in *Ldlr*$^{-/-}$ mice (**Table 4**). Similarly, plasma triglyceride levels were 66% ($P < 0.003$) lower in *Ldlr*$^{-/-}$*HL* mice and ~20% ($P < 0.01$) lower in *Ldlr*$^{-/-}$*HL*^{S145G} mice than in *Ldlr*$^{-/-}$mice. Taken together, the results from both chow-fed and Western diet-fed mice demonstrate that decreases in plasma cholesterol levels occur both in the presence (*Ldlr*$^{-/-}$*HL* mice) and absence (*Ldlr*$^{-/-}$*HL*^{S145G} mice) of HL catalytic activity. Also, because the decreases in cholesterol occur independently of the LDLR, this finding supports the existence of the postulated ligand function of HL.

Lipoprotein cholesterol and triglyceride levels in mice on chow and Western diets

Plasma lipoprotein profiles of at least six mice of each genotype on a chow diet were determined by FPLC. The

TABLE 4. Total plasma cholesterol and triglyceride concentrations in female mice on a Western diet

	Ldlr$^{-/-}$ (n = 6)	*Ldlr*$^{-/-}$*HL* (n = 4)	*Ldlr*$^{-/-}$*HL*^{S145G} (n = 4)
		mg/dl	
Total cholesterol	786 ± 96	361 ± 76[a]	537 ± 69[a,b]
Total triglycerides	121 ± 22	41 ± 15[a]	73 ± 21[c,d]

Values given as means ± SD.
[a] $P < 0.003$ vs. *Ldlr*$^{-/-}$ mice.
[b] $P < 0.04$ vs. *Ldlr*$^{-/-}$*HL* mice.
[c] $P < 0.01$ vs. *Ldlr*$^{-/-}$ mice.
[d] $P = 0.084$ vs. *Ldlr*$^{-/-}$*HL* mice.

lipoprotein cholesterol profiles of the *Ldlr*$^{-/-}$ mice were characterized by a small elevation in apoB-containing VLDL and prominent peaks in apoB-containing IDL and LDL as well as in HDL (**Fig. 2A**). The lipoprotein triglyceride profile of the *Ldlr*$^{-/-}$ mice was characterized by peaks in the VLDL, IDL, and LDL fractions (Fig. 2A). As expected, overexpression of catalytically active HL in the *Ldlr*$^{-/-}$*HL* mice reduced cholesterol concentrations in all apoB-containing lipoproteins compared with the levels in *Ldlr*$^{-/-}$ mice (Fig. 2B). The greatest reductions in cholesterol occurred in IDL (70% decrease, $P < 0.003$), LDL (67% decrease, $P < 0.003$), and HDL (54% decrease, $P < 0.003$). The triglyceride levels were also reduced in VLDL and IDL; however, the greatest reduction occurred in the LDL triglyceride level, which was 73% lower ($P < 0.003$) than in *Ldlr*$^{-/-}$ mice (Fig. 2B). Overexpression of catalytically inactive HL in the *Ldlr*$^{-/-}$*HL*^{S145G} mice reduced the cholesterol concentration in all apoB-containing lipoproteins (Fig. 2C, **Table 5**), with the most significant reduction occurring in LDL cholesterol, which was 34% lower ($P < 0.003$) than in *Ldlr*$^{-/-}$ mice. These results indicate that HL reduces LDL by a noncatalytic mechanism, presumably by

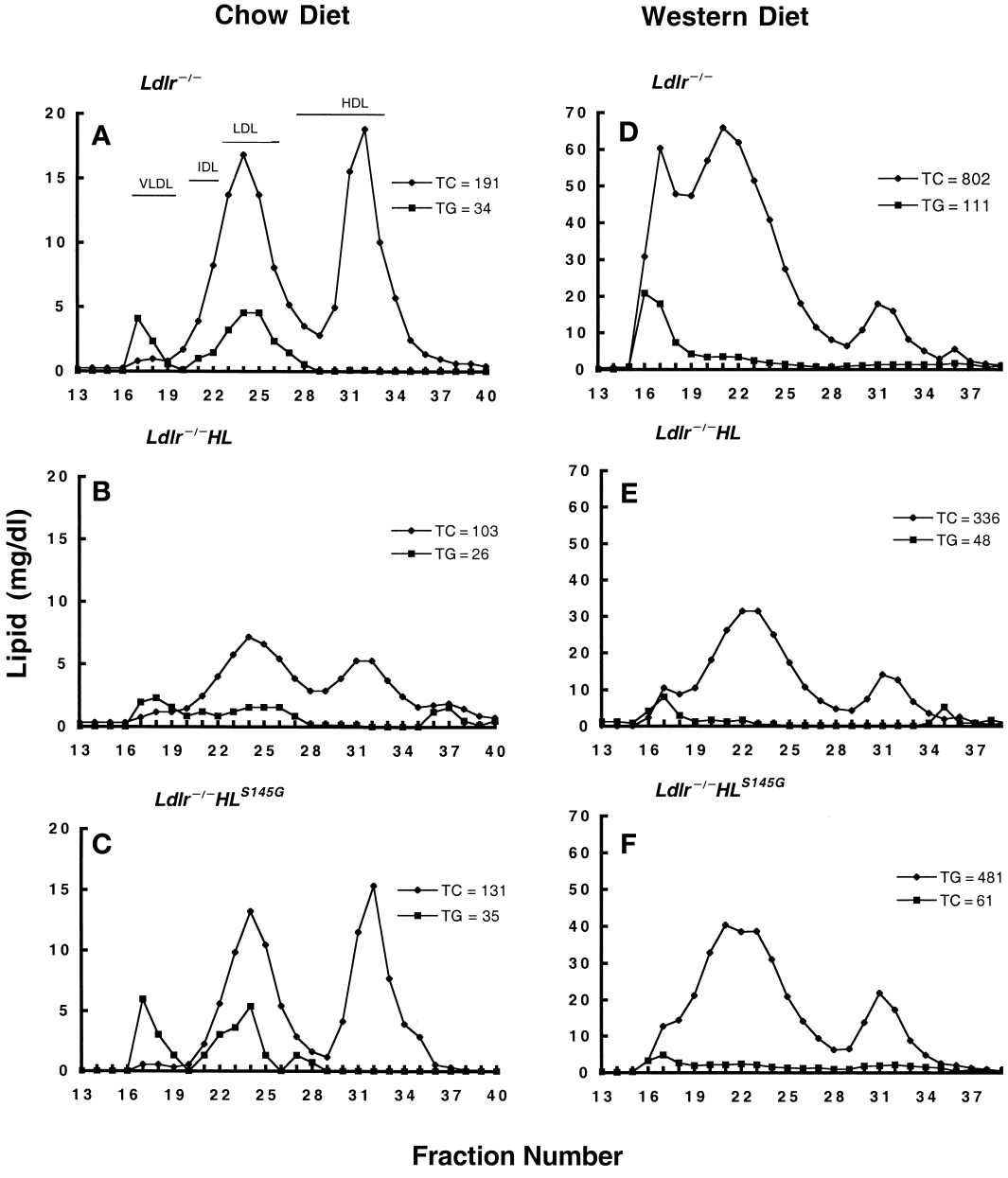

Fig. 2. Plasma lipid profiles. FPLC profiles of plasma from fasted *Ldlr*$^{-/-}$ (A), *Ldlr*$^{-/-}$*HL* (B), and *Ldlr*$^{-/-}$*HL*^{S145G} (C) female mice on a chow diet, and from fasted *Ldlr*$^{-/-}$ (D), *Ldlr*$^{-/-}$*HL* (E), and *Ldlr*$^{-/-}$*HL*^{S145G} (F) female mice after 2 weeks on a Western diet. Plasma (100 μl) from each mouse was fractionated by Superose 6 chromatography, and fractions were assayed for cholesterol and triglycerides with standard colorimetric assays. The fractions containing VLDL, IDL, LDL, and HDL are indicated with horizontal bars in (A). TC, total cholesterol; TG, triglycerides.

TABLE 5. Plasma lipoprotein cholesterol (C) and triglyceride (T) concentrations in female mice on a chow diet

Lipoprotein	$Ldlr^{-/-}$ (n = 6)	$Ldlr^{-/-}HL$ (n = 9)	$Ldlr^{-/-}HL^{S145G}$ (n = 8)
		mg/dl	
VLDL-C	9 ± 11	2 ± 1	4 ± 2
IDL-C	20 ± 10	6 ± 3[a]	11 ± 5[b]
LDL-C	67 ± 10	23 ± 9[a]	44 ± 12[a,c]
HDL-C	61 ± 25	28 ± 10[a]	40 ± 12
VLDL-T	15 ± 7	10 ± 5	12 ± 8
IDL-T	8 ± 7	3 ± 2	6 ± 5
LDL-T	19 ± 8	5 ± 4[a]	18 ± 5[c]
HDL-T	7 ± 9	3 ± 5	3 ± 2

Values given as means ± SD.
[a] $P < 0.003$ vs. $Ldlr^{-/-}$ mice.
[b] $P < 0.05$ vs. $Ldlr^{-/-}$ mice.
[c] $P < 0.003$ vs. $Ldlr^{-/-}HL$ mice.

TABLE 6. Plasma lipoprotein cholesterol (C) and triglyceride (T) concentrations in female mice on a Western diet

Lipoprotein	$Ldlr^{-/-}$ (n = 6)	$Ldlr^{-/-}HL$ (n = 4)	$Ldlr^{-/-}HL^{S145G}$ (n = 4)
		mg/dl	
VLDL-C	165 ± 22	40 ± 20[a]	68 ± 23[a]
IDL-C	178 ± 24	76 ± 15[a]	111 ± 9[a]
LDL-C	155 ± 14	90 ± 17[a]	126 ± 13[b,d]
HDL-C	88 ± 10	64 ± 10[c]	95 ± 14[d]
VLDL-T	47 ± 11	15 ± 9[a]	21 ± 13[c]
IDL-T	12 ± 1	4 ± 2[a]	7 ± 2[c]
LDL-T	10 ± 2	2 ± 2[a]	8 ± 2
HDL-T	11 ± 4	3 ± 3[b]	11 ± 12

Values given as means ± SD.
[a] $P < 0.005$ vs. $Ldlr^{-/-}$ mice.
[b] $P < 0.01$ vs. $Ldlr^{-/-}$ mice.
[c] $P < 0.05$ vs. $Ldlr^{-/-}$ mice.
[d] $P < 0.02$ vs. $Ldlr^{-/-}HL$ mice.

acting as a ligand, thereby facilitating whole lipoprotein uptake as well as selective cholesterol uptake. The LDL triglyceride concentrations were 3-fold higher in the $Ldlr^{-/-}HL^{S145G}$ mice compared with the $Ldlr^{-/-}HL$ mice ($P < 0.003$), whereas they were virtually unchanged compared with $Ldlr^{-/-}$mice. The triglyceride concentrations were unchanged in all other lipoprotein fractions (Table 5). The HDL cholesterol (HDL-C) level in $Ldlr^{-/-}HL^{S145G}$ mice fed a chow diet was reduced (by 33%, $P = 0.06$), and although the reduction was not statistically significant, it suggests that HL may also reduce HDL by acting as a ligand.

To characterize the changes in lipoprotein composition induced by Western diet treatment, we analyzed plasma lipoprotein profiles from at least four mice of each genotype by FPLC. As expected, the Western diet resulted in marked cholesterol enrichment of all apoB-containing lipoproteins (VLDL, IDL, and LDL) and a modest increase in plasma HDL in all mice (Fig. 2D, **Table 6**). Overexpression of the catalytically active HL in $Ldlr^{-/-}HL$ mice resulted in major cholesterol reductions in apoB-containing lipoproteins and HDL compared with $Ldlr^{-/-}$ mice (Fig. 2E). Cholesterol decreased by 75% in VLDL, by 57% in IDL, by 42% in LDL (all $P < 0.001$), and by 27% in HDL ($P < 0.04$). The triglyceride levels in apoB-containing lipoproteins and HDL also decreased (by ~70–80%), suggesting that all of the lipoprotein triglycerides are suitable substrates for HL catalysis. Overexpression of catalytically inactive HL in the $Ldlr^{-/-}HL^{S145G}$ mice decreased both cholesterol and triglyceride in apoB-containing VLDL and IDL (Fig. 2F, Table 6). In VLDL, cholesterol decreased by 58% ($P < 0.003$) and triglyceride by 55% ($P < 0.05$). In IDL, cholesterol decreased by 37% ($P < 0.005$) and triglyceride by 42% ($P < 0.05$). The triglyceride reductions in VLDL and IDL may result from hydrolytic modification of the lipoproteins by the endogenous mouse HL. Cholesterol decreased by ~20% in LDL ($P < 0.01$), perhaps reflecting selective uptake following phospholipid hydrolysis by the endogenous mouse HL, but was unchanged in HDL. Compared with $Ldlr^{-/-}$ mice, triglyceride concentrations were unchanged in both LDL and HDL.

Quantitation of apoB-100 and apoB-48 in plasma from mice fed chow and Western diets

To further examine whether HL reduces both apoB-100- and apoB-48-containing lipoproteins in chow-fed mice, we assessed plasma apoB-100 and apoB-48 levels by densitometric scanning of Western blots (**Fig. 3, A and B**). Plasma apoB-100 and apoB-48 levels were 15% and 53% lower (both $P < 0.002$), respectively, in $Ldlr^{-/-}HL$ mice than in $Ldlr^{-/-}$ mice. Thus, lipoproteins containing both types of apoB were reduced, but apoB-48-containing lipoproteins were the preferred substrate for catalytically active HL. In contrast, plasma apoB-100 levels in $Ldlr^{-/-}HL^{S145G}$ mice decreased by 23% ($P < 0.02$), but apoB-48 levels were unchanged, as compared with those in $Ldlr^{-/-}$ mice. Thus, in chow-fed mice, apoB-100-containing particles are the preferred substrate for catalytically inactive HL.

To determine the extent to which catalytically active HL reduces apoB-100- and apoB-48-containing lipoproteins in mice fed a Western diet, we compared plasma apoB-100 and apoB-48 levels in $Ldlr^{-/-}$ and $Ldlr^{-/-}HL$ mice (Fig. 3, C and D). In $Ldlr^{-/-}HL$ mice, plasma apoB-100 levels were 52% lower ($P < 0.0002$) and apoB-48 levels were 38% lower ($P < 0.02$) than in $Ldlr^{-/-}$ mice. These results suggest that the catalytic activity of HL is associated with a reduction of both apoB-100- and apoB-48-containing lipoproteins. Likewise, catalytically inactive HL^{S145G} also reduced both apoB-100- and apoB-48-containing lipoproteins (Fig. 3, C and D). Plasma apoB-100 levels decreased by 33% ($P < 0.02$) and apoB-48 levels by 43% ($P < 0.05$) in the $Ldlr^{-/-}HL^{S145G}$ mice. Thus, in mice fed a Western diet, apoB-containing lipoproteins can be effectively reduced by catalytically inactive HL.

Quantitation of apoA-I in mice fed chow and Western diets

To distinguish between the catalytic and noncatalytic (ligand) effects of HL on HDL, we determined whether the decrease in HDL-C in the chow-fed mice was accompanied by a decrease in plasma apoA-I. In the absence of increased catalytic activity beyond that of the endogenous HL, con-

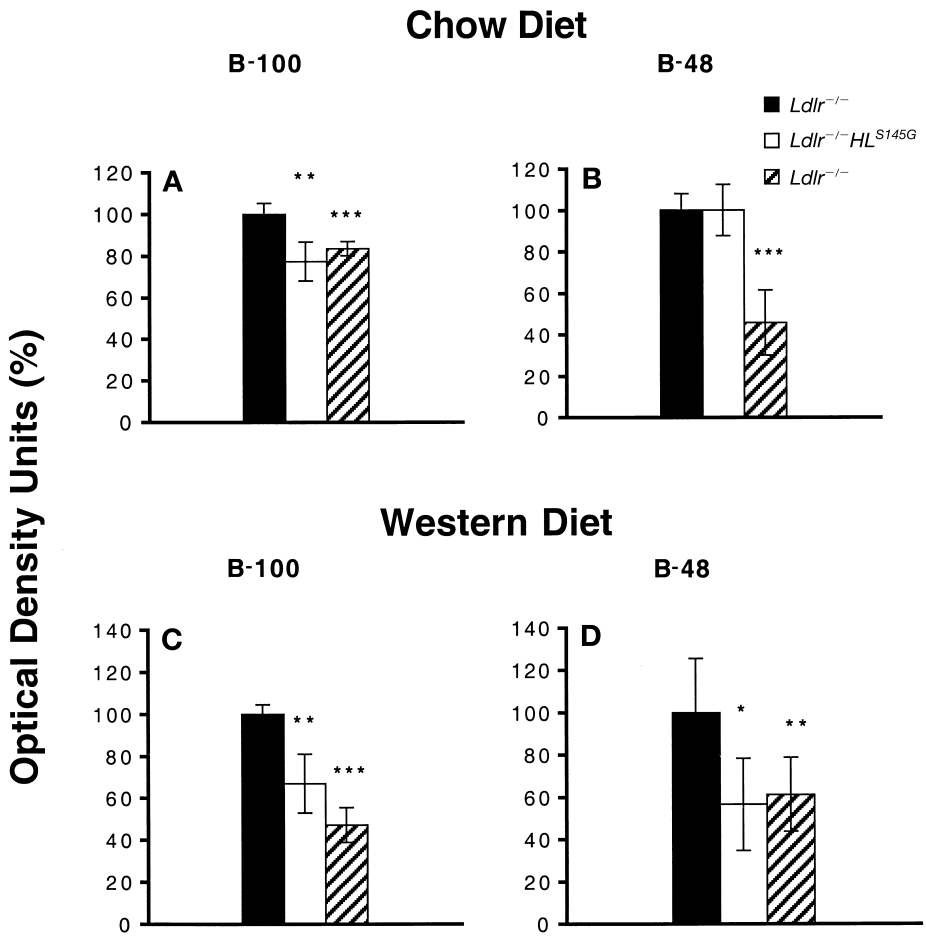

Fig. 3. Plasma apoB levels in female mice on a chow diet or after 2 weeks on a Western diet. Plasma apoB-100 and apoB-48 content in *Ldlr*$^{-/-}$, *Ldlr*$^{-/-}$*HL*^{S145G}, and *Ldlr*$^{-/-}$*HL* female mice on a chow diet or after 2 weeks on a Western diet. Plasma samples from three to five *Ldlr*$^{-/-}$, *Ldlr*$^{-/-}$*HL*^{S145G}, and *Ldlr*$^{-/-}$*HL* mice were separately pooled, fractionated in quadruplicate by SDS-PAGE on 4% gels, transferred to nitrocellulose, and incubated first with a rabbit anti-mouse apoB-100 and apoB-48 antibody and then with horseradish peroxidase-conjugated goat anti-rabbit antibody. Antibody binding was visualized with an ECL assay. (* $P < 0.05$ vs. *Ldlr*$^{-/-}$ mice; ** $P < 0.02$ vs. *Ldlr*$^{-/-}$ mice; *** $P < 0.002$ vs. *Ldlr*$^{-/-}$ mice.)

comitant decreases in both HDL-C and apoA-I would indicate uptake of whole lipoprotein particles. Plasma apoA-I levels were assessed by densitometric analysis of Western blots (**Fig. 4A**). Overexpression of catalytically active HL decreased plasma apoA-I by 40% ($P < 0.02$), whereas overexpression of catalytically inactive HL^{S145G} decreased plasma apoA-I by 26% ($P = 0.08$). These results raise the possibility that both the catalytic and ligand functions of overexpressed HL contribute to the HDL reduction in the *Ldlr*$^{-/-}$*HL* mice, whereas only the ligand function of the overexpressed HL contributes to the reduction of HDL and apoA-I in the *Ldlr*$^{-/-}$*HL*^{S145G} mice. Thus, in chow-fed mice, HL appears to reduce HDL-C through both its catalytic and ligand functions.

Finally, we compared plasma apoA-I levels in these mice on the Western diet by densitometric scanning of Western blots (Fig. 4B). Plasma apoA-I was substantially decreased (by ~40%, $P < 0.05$) in *Ldlr*$^{-/-}$*HL* mice but was only slightly decreased in *Ldlr*$^{-/-}$*HL*^{S145G} mice. Thus, in mice fed a Western diet, maximal reduction of HDL requires catalytically active HL.

DISCUSSION

This study demonstrates that high levels of HL reduce plasma cholesterol levels including apoB-containing lipoproteins in the absence of the LDLR. Furthermore, HL reduces cholesterol independently of catalytic activity. Maximal reduction (50–70% reduction of VLDL, IDL, and LDL) occurred in mice expressing catalytically active HL. However, marked reductions of the apoB-containing lipoproteins (20–60%) occurred even in mice expressing catalytically inactive HL. Specifically, expression of catalytically inactive HL reduced apoB-100-containing lipoproteins in *Ldlr*$^{-/-}$ mice fed a chow or Western diet and reduced apoB-48-containing lipoproteins in *Ldlr*$^{-/-}$ mice fed a Western diet. Catalytically inactive HL could serve as a ligand, con-

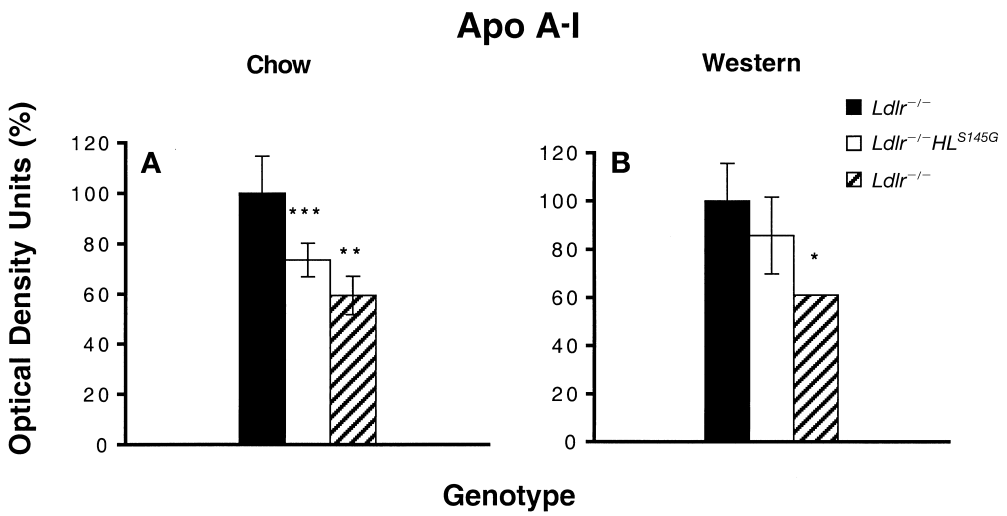

Fig. 4. Plasma apoA-I content in *Ldlr*$^{-/-}$, *Ldlr*$^{-/-}$*HL*^{S145G}, and *Ldlr*$^{-/-}$*HL* female mice. Plasma samples from three to five *Ldlr*$^{-/-}$, *Ldlr*$^{-/-}$*HL*^{S145G}, and *Ldlr*$^{-/-}$*HL* mice were separately pooled, fractionated by SDS-PAGE on 12% gels, transferred to nitrocellulose, and incubated first with a polyclonal rabbit anti-mouse antiserum and then with horseradish peroxidase-conjugated goat anti-rabbit antibody. Antibody binding was visualized with an ECL assay. (* $P < 0.02$ vs. *Ldlr*$^{-/-}$ mice fed a Western diet; ** $P < 0.03$ vs. *Ldlr*$^{-/-}$mice fed a chow diet; *** $P = 0.08$ vs. *Ldlr*$^{-/-}$ mice fed a chow diet.)

centrating the apoB-containing lipoproteins on the cell surface and facilitating uptake by the HSPG/LDLR-related protein (LRP) pathway or by HSPG alone acting as a receptor. Thus, in these studies, the high level of HL expression served to reveal an LDLR-independent clearance pathway for apoB-100-containing lipoproteins.

HDL-C and apoA-I levels were also reduced by overexpression of HL in *Ldlr*$^{-/-}$ mice. A marked reduction was noted in those mice expressing catalytically active HL and fed either a chow or a Western diet (HDL-C decreased 30–50%; apoA-I decreased ~40%). Furthermore, catalytically inactive HL reduced HDL-C and apoA-I (~30%, did not reach statistical significance) in *Ldlr*$^{-/-}$ mice on a chow diet but had no effect on these levels in *Ldlr*$^{-/-}$ mice on a Western diet. The catalytically inactive HL could affect HDL levels of the chow-fed mice by serving as a ligand, concentrating the particles at the cell surface and facilitating direct uptake (via HSPG-syndecan-1) (28, 41, 42) or selective uptake mediated by the B1 scavenger receptor (43, 44). It is conceivable that the absence of reduced HDL levels in Western diet-fed mice reflects displacement of HDL from HL^{S145G} by high levels of apoB-containing lipoproteins, as has been described for the homologous enzyme lipoprotein lipase (45). The displaced HDL would not be available for HL-mediated removal, and the HDL levels would remain unchanged.

Previous in vitro studies (using McArdle 7777 rat hepatoma cells that were stably transfected with human HL) demonstrated that HL expression enhanced remnant (β-VLDL) uptake (26). The relevance of these in vitro findings to the in vivo situation was initially examined in apoE-deficient mice (in which apoB-48-containing remnants accumulate) expressing either catalytically active HL (*Apoe*$^{-/-}$*HL*) or catalytically inactive HL^{S145G} (*Apoe*$^{-/-}$*HL*^{S145G}) (6). In both types of mice, plasma cholesterol decreased by ~50%, with the decrease mainly occurring in the apoB-48-containing remnant fraction (6). Thus, catalytically inactive HL^{S145G} cleared apoB-containing lipoproteins as effectively as catalytically active HL, indicating that HL can serve as a ligand to mediate the clearance of apoB-48-containing remnant lipoproteins in the *Apoe*$^{-/-}$ mice. Because apoE is absent in *Apoe*$^{-/-}$ mice and present in *Ldlr*$^{-/-}$ mice, our current findings indicate that the presence of apoE in the *Ldlr*$^{-/-}$ mice somehow interferes with the full ligand activity of the HL^{S145G}, perhaps by competing for binding sites on cell surface HSPG, LRP, or other receptors.

In our current study, HL^{S145G} did not reduce apoB-48-containing lipoproteins in chow-fed *Ldlr*$^{-/-}$ mice, but did reduce these lipoproteins in the Western diet-fed *Ldlr*$^{-/-}$ mice. Because of the similar lipoprotein composition in the Western diet-fed *Ldlr*$^{-/-}$ mice and the *Apoe*$^{-/-}$ mice, it is possible that the origin and chemical composition of the apoB-48 lipoproteins may modify the ligand function of catalytically inactive HL.

Our data suggest that HL at high levels provides an alternate clearance pathway for apoB-100-containing lipoproteins that is not dependent on the LDLR. Our studies demonstrate that both catalytically active and catalytically inactive HL can reduce these lipoproteins very significantly in *Ldlr*$^{-/-}$ mice, regardless of the type of diet. Although the *Ldlr*$^{-/-}$ mice express the endogenous mouse HL, our studies indicated similar activity levels in preheparin plasma in all three genotypes (*Ldlr*$^{-/-}$, *Ldlr*$^{-/-}$*HL*, and *Ldlr*$^{-/-}$*HL*^{S145G}). Furthermore, these preheparin plasma activity levels remained unchanged with Western diet treatment. The similar endogenous HL activity levels in all three genotypes indicate that the lipid and lipoprotein changes observed in the transgenic mice most likely result from the pharmacologic levels of expression of the

HL and HL^{S145G} transgenes. Therefore, it is unlikely that catalytic inactive HL augments the action of the endogenous HL. However, it is possible that the background mouse HL activity optimizes the lipoproteins as substrates for the transgenically expressed HL transgenes. Ideally, the physiologic relevance of HL's ligand function should be determined in HL knockout mice that express physiologic levels of HL^{S145G}.

Thus, the in vivo data demonstrate that HL and HL^{S145G} can function as ligands to mediate the clearance of remnants and LDL. We hypothesize that in the *Ldlr*$^{-/-}$*HL* and *Ldlr*$^{-/-}$*HL*^{S145G} mice, the apoB-containing lipoproteins are reduced either by receptor-mediated endocytosis or by sequestration at the cell surface through lipoprotein binding to HL-HSPG complexes that, in turn, act as receptors, eventually inducing endocytosis of the bound lipoprotein. Fuki et al. (46) described an analogous mechanism in vitro. In those studies, lipoproteins enriched in lipoprotein lipase, which is structurally and functionally similar to HL (31, 47, 48), were internalized through a process mediated by the proteoglycan syndecan-1.

Our current in vivo results demonstrate that catalytically active HL reduces both HDL-C and apoA-I. These results most likely reflect processing of HDL particles by elevated lipolytic activity (achieved by high transgene expression). The lipolytic processing, in turn, leads to dissociation of apoA-I and loss of protein from the circulation independently of selective uptake.

In addition, our results suggest that HL^{S145G} can reduce both HDL-C (by 33%, $P = 0.06$) and apoA-I (by 26%, $P = 0.08$) despite its lack of catalytic activity. Although the decreases in HDL-C and apoA-I did not reach statistical significance in this study, our findings are in apparent agreement with the findings of Dugi et al. (49) in which high levels of HL^{S145G} (achieved by adenoviral gene transfer in HL knockout mice) reduced both HDL-C (by 42%) and apoA-I (by 21%). Thus, our results are similar despite the presence of endogenous HL in our model system and its absence in the model system used by Dugi et al. (49). Their study was expanded to include measurements of apoA-II levels as well as turnover studies of apoA-I and apoA-II; they concluded that the major effect of HL^{S145G} was to decrease HDL-C and apoA-II with only a minor effect on apoA-I (49). The catalytically inactive HL^{S145G} did not significantly reduce HDL in mice fed a Western diet. This finding may reflect a diet-induced difference in HDL particle size and apoA-I conformation as well as particle composition that, taken together, may render it a poor substrate for HL-mediated uptake.

In summary, our data demonstrate that high levels of catalytically inactive HL can lower plasma cholesterol levels very significantly in *Ldlr*$^{-/-}$ mice, presumably by serving as a ligand for clearance. In *Ldlr*$^{-/-}$*HL*^{S145G} mice fed a chow diet, cholesterol lowering mediated by catalytically inactive HL resulted primarily from LDLR-independent clearance of apoB-100-containing lipoproteins. When *Ldlr*$^{-/-}$*HL*^{S145G} mice were fed a Western diet, which increases the levels of all apoB-containing lipoproteins, the levels of cholesterol-enriched apoB-48- and apoB-100-containing lipoproteins were markedly reduced. Our results raise the interesting possibility that upregulation of HL expression in hetero- and homozygous LDLR-deficiency states could serve to lower LDL cholesterol levels. These results also suggest the possible therapeutic benefit of overexpressing catalytically inactive HL^{S145G} to lower LDL cholesterol with minimal lowering of HDL. In summary, these data extend our knowledge of the importance of HL in the clearance of apoB-containing lipoproteins through a mechanism that is independent of both catalytic activity and the LDLR.

We thank Savvas Papanicolaou and Christina Aguilar for technical assistance, Stephen G. Young and Robert O. Ryan for helpful discussions, Gary Howard and Stephen Ordway for editorial assistance, and Betsy Lathrop for secretarial support. This work was supported, in part, by National Institutes of Health grant KO8 HL 04031 (to H.L.D.)

Manuscript received 16 March 2000, in revised form 31 August 2000, and in re-revised form 3 October 2000.

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ERRATA

In the article "Hepatic lipase overexpression lowers remnant and LDL levels by a noncatalytic mechanism in LDL receptor-deficient mice" by Helén L. Dichek et al., published in the February 2001 issue of the *Journal of Lipid Research* (Volume **42**, pages 201–210), the authors would like to change the acknowledgment section to now read: "We thank Savvas Papanicolaou and Christina Aguilar for technical assistance, Stephen G. Young, Ron M. Krauss, and Robert O. Ryan for helpful discussions, Gary Howard and Stephen Ordway for editorial assistance, and Betsy Lathrop for secretarial support. This work was supported, in part, by National Institutes of Health Grant KO8 HL03041 (to H.L.D.)."