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Overexpression of Hepatic Lipase in Transgenic Mice Decreases Apolipoprotein B-containing and High Density Lipoproteins

EVIDENCE THAT HEPATIC LIPASE ACTS AS A LIGAND FOR LIPOPROTEIN UPTAKE*

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To determine the mechanisms by which human hepatic lipase (HL) contributes to the metabolism of apolipoprotein (apo) B-containing lipoproteins and high density lipoproteins (HDL) *in vivo*, we developed and characterized HL transgenic mice. HL was localized by immunohistochemistry to the liver and to the adrenal cortex. In hemizygous (*hHLTg*$^{+/0}$) and homozygous (*hHLTg*$^{+/+}$) mice, postheparin plasma HL activity increased by 25- and 50-fold and plasma cholesterol levels decreased by 80% and 85%, respectively. In mice fed a high fat, high cholesterol diet to increase endogenous apoB-containing lipoproteins, plasma cholesterol decreased 33% (*hHLTg*$^{+/0}$) and 75% (*hHLTg*$^{+/+}$). Both apoB-containing remnant lipoproteins and HDL were reduced. To extend this observation, the HL transgene was expressed in human apoB transgenic (*huBTg*) and apoE-deficient (*apoE*$^{-/-}$) mice, both of which have high plasma levels of apoB-containing lipoproteins. (Note that the *huBTg* mice that were used in these studies were all hemizygous for the human apoB gene.) In both the *huBTg,hHLTg*$^{+/0}$ mice and the *apoE*$^{-/-}$*,hHLTg*$^{+/0}$ mice, plasma cholesterol decreased by 50%. This decrease was reflected in both the apoB-containing and the HDL fractions. To determine if HL catalytic activity is required for these decreases, we expressed catalytically inactive HL (HL-CAT) in *apoE*$^{-/-}$ mice. The postheparin plasma HL activities were similar in the *apoE*$^{-/-}$ and the *apoE*$^{-/-}$*,HL-CAT*$^{+/0}$ mice, reflecting the activity of the endogenous mouse HL and confirming that the HL-CAT was catalytically inactive. However, the postheparin plasma HL activity was 20-fold higher in the *apoE*$^{-/-}$*,hHLTg*$^{+/0}$ mice, indicating expression of the active human HL. Immunoblotting demonstrated high levels of human HL in postheparin plasma of both *apoE*$^{-/-}$*,hHLTg*$^{+/0}$ and *apoE*$^{-/-}$*,HL-CAT*$^{+/0}$ mice. Plasma cholesterol and apoB-containing lipoprotein levels were ~60% lower in *apoE*$^{-/-}$*,HL-CAT*$^{+/0}$ mice than in *apoE*$^{-/-}$ mice. However, the HDL were only minimally reduced. Thus, the catalytic activity of HL is critical for its effects on HDL but not for its effects on apoB-containing lipoproteins. These results provide evidence that HL can act as a ligand to remove apoB-containing lipoproteins from plasma.

Hepatic lipase (HL)[1] is a 66-kDa lipolytic enzyme that is synthesized and secreted exclusively by the liver (1–4). It is anchored via heparan sulfate proteoglycans (HSPG) to the surface of hepatocytes and liver sinusoid endothelial cells (5–8). In lipoprotein metabolism, HL functions both as an acylglycerol hydrolase, hydrolyzing triglycerides in chylomicron remnants, intermediate density lipoproteins (IDL), and high density lipoproteins (HDL) (1–4, 9–12), and as a phospholipase, converting the phospholipid-rich HDL_2 to HDL_3 (1–4, 9, 13–16). The HDL_3 then serve as acceptors for cholesterol in peripheral tissues. In steroidogenic tissues such as the adrenal glands, HL-mediated hydrolysis of HDL_2 phospholipids may facilitate transfer of core cholesteryl esters to steroidogenic cells (17). Although HL is not produced in the adrenal glands, HL activity and HL protein have been demonstrated in homogenates of rat adrenal tissue. However, its distribution within that tissue is unknown (18–21) .

A role for HL in HDL metabolism was first demonstrated in rats (9, 16, 22–24). Infusions of HL antibodies to inhibit enzyme activity resulted in the accumulation of HDL_2 in plasma, reflecting the lack of lipolytic processing of HDL_2 to HDL_3 (9, 16, 22, 23). Expression of human HL in transgenic or adenoviral vector-transduced mice significantly decreased plasma HDL (25, 26). A potential role for HL in the processing of apolipoprotein (apo) B-containing lipoproteins (very low density lipoproteins (VLDL), IDL, and low density lipoproteins (LDL)) was suggested by significantly increased levels of apoB-containing lipoproteins resulting from the infusion of HL antibodies into rats or cynomolgus monkeys (9–11). Consistent with these roles, HL-deficient patients are hypercholesterolemic and hypertriglyceridemic and accumulate β-migrating very low density lipoprotein (β-VLDL) remnants, triglyceride-rich LDL, and HDL (27–33) .

Recent *in vitro* data suggest that HL may serve as a ligand to mediate the binding and uptake of lipoproteins via the HSPG/LDL receptor-related protein (LRP) pathway (34–38). In these *in vitro* studies, enhancement of lipoprotein uptake was independent of catalytic activity and apoE but required the enzyme

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[1] The abbreviations used are: HL, hepatic lipase; apo, apolipoprotein; HSPG, heparan sulfate proteoglycans; HL-CAT, catalytically inactive HL; HDL, high density lipoproteins; IDL, intermediate density lipoprotein(s); LDL, low density lipoprotein(s); LRP, LDL receptor-related protein; VLDL, very low density lipoprotein(s); kb, kilobase(s); FPLC, fast performance liquid chromatography.

to be bound to HSPG (34, 38). The HSPG-bound HL facilitates the uptake of both human chylomicrons and rabbit β-VLDL *in vitro.* This enhancement occurs in part via the LRP (34, 36, 38). Direct binding of HL to the LRP has been demonstrated by several groups, including ours (35–38) .

In addition to enhancing remnant uptake, HL also enhances the *in vitro* cellular uptake of human ^{125}I-LDL (34, 38, 39). This uptake may be mediated in part via the HSPG/LRP pathway (34, 38). These *in vitro* data and the fact that apoB-containing lipoproteins accumulate in HL-deficient patients indicate that HL also facilitates the clearance of apoB-containing lipoproteins.

To establish the mechanisms by which HL contributes to the metabolism of apoB-containing lipoproteins and HDL *in vivo*, we developed and characterized HL transgenic mice. Because mouse plasma has low levels of apoB-containing lipoproteins and high levels of HDL, we first determined the effects of HL on HDL. To determine the effects of HL on apoB-containing lipoproteins, we induced higher levels of these lipoproteins by diet. We also induced high levels of apoB-containing lipoproteins by breeding HL transgenic mice onto the human apoB transgenic and the apoE-deficient backgrounds (40, 41). To establish whether the effects of HL resulted from increased catalysis or whether it also can act as a ligand, we expressed a catalytically inactive HL (HL-CAT) on the apoE-deficient background.

MATERIALS AND METHODS

Transgenic Mice—ICR mice were purchased from Charles River Breeding Laboratories (Wilmington, DE). The transgenes were based on the plivhHL1 construct described by Fan *et al.* (42), which contained either the human HL cDNA or the human HL-CAT cDNA (38). The HL-CAT cDNA was generated by polymerase chain reaction-based site-directed mutagenesis and introduced an A → G nucleotide transition at position 559 in the human HL cDNA (43, 44). This resulted in replacement of the catalytic serine by a glycine at amino acid 145 of the human HL enzyme. These constructs were designed for liver-specific expression and contained the following sequences of the human apoE gene: 3 kilobases (kb) of 5′-flanking sequence, the first exon, the first intron, six nucleotides of the second exon, a polylinker site for insertion of the cDNA, 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. Transgenic mice expressing the unmodified human HL (*hHLTg*) and the mutant (*HL-CAT*) cDNAs were generated with established techniques (45). Founder pups were identified by Southern blot analysis of tail DNA with a radioactively labeled HL cDNA probe (44, 46, 47). Transgenic founders were mated with nontransgenic mice to obtain hemizygous mice; hemizygous littermates were mated to obtain homozygous animals. The HL transgenic mice were bred with human apoB transgenic mice (*huBTg*) (40) and with apoE-deficient ($apoE^{-/-}$) mice (41) to generate *huBTg,hHLTg* and $apoE^{-/-}$,*hHLTg* transgenic mice. Transgenic mouse plasma was screened for human apoB by a Western blot assay (48) with a ^{125}I-labeled monoclonal antibody to human apoB (C1 4) (49, 50) and for the absence of apoE with a polyclonal rabbit anti-mouse apoE antibody (GMoE) developed in this laboratory.

There were no significant differences in body weight between the nontransgenic and transgenic mice. All mice were fed regular mouse chow (4.5% fat, Purina 5001) unless otherwise specified. All experiments used female mice except for those involving $apoE^{-/-}$ mice, which used male mice. Littermates served as controls for all lines studied.

Purification of HL and Antibody Production—Human HL was purified from conditioned medium from McA-RH7777 rat hepatoma cells that had been stably transfected with the HL cDNA (34, 44). Cells were grown in Dulbecco's modified Eagle's medium (Life Technologies, Inc.) containing 2 units/ml heparin (Elkins-Sinn, Cherry Hill, NJ) in 7% CO_2. After a 48-h incubation, 2200 ml of conditioned medium was harvested in the presence of 30% glycerol (v/v) to preserve enzyme activity. The medium was applied to a human antithrombin III antibody affinity column (3.5-ml bed volume) (Sigma) in series with a heparin-Sepharose CL6B affinity column (60-ml bed volume) (Pharmacia, Uppsala, Sweden). The equilibration and wash buffers consisted of 10 mM sodium phosphate, pH 7.5, 30% glycerol (v/v), 0.4 M NaCl, and 0.1% cholamidopropyldiethyl ammoniopropane sulfonate (w/v). The HL was subsequently eluted from the heparin-Sepharose column with a linear gradient (0.4–2 M) of NaCl. Conductivity measurements of the eluted fractions established the linearity of the gradient. As a control, medium from nontransfected McA-RH7777 cells was subjected to the same purification procedures but was not applied to the antithrombin III antibody affinity column.

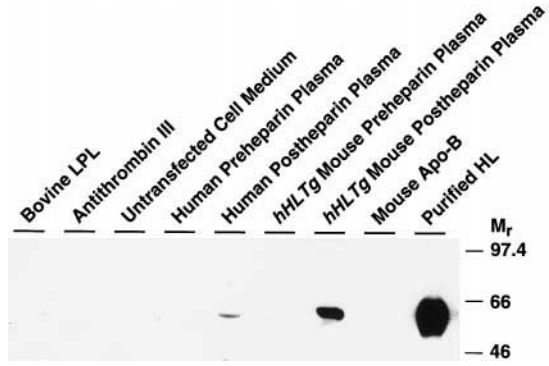

FIG. 1. **Western blot assay demonstrating monospecificity of the rabbit anti-human HL antibody toward human HL.** Rat antithrombin III (0.5 μg) (Sigma A6397); bovine lipoprotein lipase (1 μg) (gift from Dr. Ira Goldberg, Columbia University); purified, lyophilized protein (1 μg) from nontransfected McA-RH7777 cell medium; human and HL transgenic mouse pre- and postheparin plasma (1 μl each); and mouse apoB (3.7 μg) and purified HL standard (0.8 μg) were resolved by electrophoresis on 3–20% polyacrylamide-sodium dodecyl sulfate gradient gels, transferred to nitrocellulose, and incubated with the human HL antibody.

Fractions (4.3 ml each) were collected into glass tubes containing 10 units of heparin (1000 units/ml) and monitored for both protein elution (by absorbance at 280 nm) and peak HL activity (51). Fractions corresponding to eluted HL activity were pooled and dialyzed against four changes of 4 liters of 10 mM ammonium bicarbonate, pH 8.2, for 12 h at 4 °C, followed by lyophilization and storage at −70 °C.

Two female New Zealand White rabbits were immunized with 0.5 mg of partially purified human HL antigen three times at 2-week intervals (52). The antiserum was rendered monospecific for human HL by absorption with proteins purified from medium of nontransfected McA-RH7777 cells. Residual antithrombin III antibodies were removed by incubation with agarose-cross-linked bovine plasma antithrombin III (Sigma A8293). The specificity of the human HL antibody was demonstrated in a Western blot assay (Fig. 1).

Human HL Expression—Total RNA was isolated (53) from 10 tissues and analyzed by RNase protection with an antisense HL probe as described (42). Total liver RNA from nontransgenic and HL transgenic mice was analyzed by Northern blot assay (54). Nontransgenic and transgenic mouse plasma was collected into EDTA-containing tubes before and 10 min after intravenous injection of heparin (150 units/kg of body weight) and analyzed for protein expression. Western blots of pre- and postheparin plasma were performed with the monospecific anti-human HL antibody described above. Since more than one-half of mouse HL circulates in plasma, we would expect to detect any cross-reactivity with the mouse HL in the mouse preheparin plasma sample (55). The catalytic activity of HL in pre- and postheparin plasma was determined with glycerol [1-^{14}C]trioleate as a substrate in the presence of 1 M NaCl as described (51).

Lipoprotein Analysis—Total plasma cholesterol and triglyceride levels were determined with standard enzymatic assays (cholesterol: Abbott Spectrum, Abbott Park, IL; triglycerides: GPO-PAP kit, Boehringer Mannheim). Plasma was obtained by tail bleeding mice after a 4-h fast. Mouse plasma lipoproteins were fractionated by fast performance liquid chromatography (FPLC) gel filtration on a Superose 6 HR 10/50 column (Pharmacia Biotech Inc.) (56, 57). For these analyses, 60–100 μl of plasma was mixed with phosphate-buffered saline containing 10 mM EDTA to a total volume of 260 μl. The column was eluted with phosphate-buffered saline/EDTA at a flow rate of 0.4 ml/min, and 60 0.5-ml fractions were collected. Standard enzymatic assays (100-μl aliquots of each fraction) were used to measure cholesterol and triglyceride levels (see above). All determinations were corrected for plasma cholesterol and triglyceride recoveries, which ranged from 70 to 100%.

Diet Study—To induce high levels of endogenous apoB-containing lipoproteins, control and HL transgenic mice were placed on a high fat, high cholesterol diet consisting of 15.8% (w/w) fat, 1.25% (w/w) cholesterol, and 0.5% (w/w) sodium cholate for 8 weeks (TD 88051, Harlan Teklad, Madison, WI) (58). Fasting plasma total and lipoprotein cho-

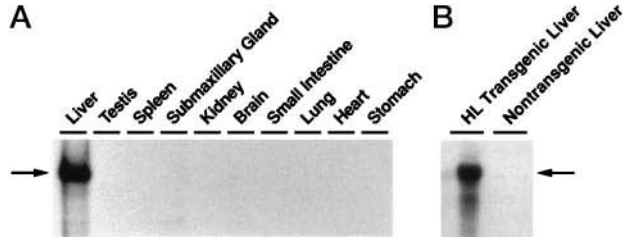

FIG. 2. *A*, human HL mRNA shown only in the liver of human HL transgenic mice by RNase protection assay. Total RNA (10 μg/tissue) from an F1 transgenic mouse was hybridized to a ^{32}P-labeled human HL antisense probe, digested with RNase, resolved by gel electrophoresis, and detected by autoradiography. *B*, Northern blot analysis of total RNA isolated from nontransgenic and human HL transgenic mouse livers. The RNA (20 μg) was separated by electrophoresis on a 1.1% agarose gel in the presence of 6% formaldehyde.

lesterol and triglyceride concentrations were determined as described above.

Immunohistochemical Studies—After a 4-h fast, nontransgenic and human HL transgenic mice were anesthetized by intraperitoneal injection of avertin (20 μl/g of body weight) (59). Through a laparotomy, 100 μl of saline or saline containing 5 units (150 units/kg) of heparin (Elkins-Sinn) was injected into the portal vein with a 30-gauge needle. The needle was removed, the laparotomy incision was covered, and the mouse was kept warm until 10 min after the injection. A thoracotomy was performed, and the apex of the beating heart was punctured; the left ventricle was cannulated with a 25-gauge angiocatheter (Deseret, Becton Dickinson, Sandy, UT), the inferior vena cava was severed, blood was flushed by infusing phosphate-buffered saline through the angiocatheter, and perfusion fixation was performed with 3% paraformaldehyde in phosphate-buffered saline at room temperature. The livers were cut into pieces, fixed overnight in paraformaldehyde at 4 °C, and frozen at −70 °C in Tissue-Tek compound (Sakura Finetek, Torrance, CA) or embedded in paraffin. Adrenal tissues were processed similarly. Paraffin-embedded liver specimens were stained with Alcian blue to visualize the extracellular matrix (60) .

Quantitation of apoB in Plasma from ApoE$^{-/-}$,ApoE$^{-/-}$,hHLTg, and ApoE$^{-/-}$,HL-CAT Mice—Plasma from three female mice from each of two groups (*apoE*$^{-/-}$ or *apoE*$^{-/-}$,*hHLTg*) was pooled (2 μl) and applied in triplicate to replicate 3–8% polyacrylamide-sodium dodecyl sulfate gels. After electrophoresis, one gel was silver-stained (61) and the other was blotted to a nitrocellulose membrane (Schleicher & Schuell). The membrane was incubated with ^{125}I-labeled rabbit anti-mouse apoB antibody (62) that reacts with both apoB-48 and apoB-100 and washed, and the radioactivity was quantitated with a PhosphorImager. A standard curve of appropriate dilutions of the labeled antibody was analyzed in parallel.

In a separate experiment, plasma from three groups of three male mice (*apoE*$^{-/-}$, *apoE*$^{-/-}$,*hHLTg*, or *apoE*$^{-/-}$,*HL-CAT*) was pooled, separated on a 4% polyacrylamide-sodium dodecyl sulfate gel, and transferred to nitrocellulose membrane. The membrane was incubated with the rabbit anti-mouse apoB antibody (62), reacted with a biotinylated second antibody, and developed with an ECL kit (Amersham, Buckinghamshire, United Kingdom). Immunoblots were analyzed by gel densitometry on a Umax Powerlook II system with Sigma Gel gel analysis software (Jandel Corp., San Rafael, CA).

Statistical Analyses—The *t* test for unequal variances was used to determine the statistical significance of differences.

RESULTS

Characterization of hHLTg Transgenic Mice

Tissue Distribution of Human HL mRNA—RNase protection assays of 10 different tissues from a single line of transgenic mice showed expression of the human HL transgene solely in the liver (Fig. 2*A*). Northern blots of total RNA from nontransgenic and transgenic liver demonstrated a 1.6-kb human HL mRNA, corresponding to its expected size, in transgenic mice only (Fig. 2*B*).

Expression of Human HL Protein in Transgenic Mouse Plasma—Western blot analysis of postheparin mouse plasma revealed a gene dose-dependent increase in HL levels in the *hHLTg*$^{+/0}$ and *hHLTg*$^{+/+}$ mice (Fig. 3). The postheparin

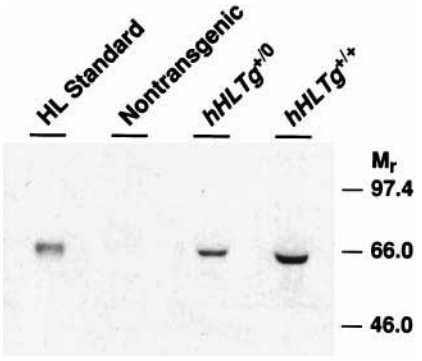

FIG. 3. **Western blot assay of postheparin plasma from nontransgenic, *hHLTg*$^{+/0}$, and *hHLTg*$^{+/+}$ mice.** Plasma (1 μl) from each mouse and purified human HL as a standard (0.03 μg) were separated on an 8% polyacrylamide-sodium dodecyl sulfate gel, transferred to nitrocellulose, and incubated with the rabbit anti-human HL antibody and a secondary horseradish peroxidase-conjugated goat anti-rabbit antibody. Antibody binding was visualized with an electrochemiluminescence assay.

TABLE I
HL activity in pre- and postheparin plasma from nontransgenic and hHLTg mice

FFA, free fatty acid.

Genotype	*n*	HL activity	
		Preheparin	Postheparin
		μEq FFA/ml/h	
Nontransgenic	4	9.6 ± 1.2	16.9 ± 4.0
hHLTg$^{+/0}$	4	8.0 ± 0.9	416.0 ± 86[a]
hHLTg$^{+/+}$	3	11.1 ± 0.9	838.0 ± 157[a]

[a] $p < 0.01$ compared with postheparin plasma HL activity in nontransgenic mice.

plasma HL activities were ~25-fold and ~50-fold higher in the *hHLTg*$^{+/0}$ and *hHLTg*$^{+/+}$ mice, respectively, than in nontransgenic mice (Table I). There was no difference in HL activity between nontransgenic and transgenic mice before heparin injection. Therefore, we conclude that all of the human HL was bound to cell-surface HSPG. These results demonstrate that human HL does not circulate in plasma in these mice, whereas more than one-half of mouse HL is free in circulation, consistent with a previous report (55) .

Immunohistochemical Localization of Human HL in Transgenic Mouse Liver—To determine the specific location of bound human HL in the liver, nontransgenic and *hHLTg* mice were injected with either saline or heparin followed by perfusion-fixation and immunostaining of the livers with the HL antibody (Fig. 1). In the saline-infused mice, HL immunofluorescence revealed a sinusoidal distribution (Fig. 4*A*). In the heparin-infused mice, immunofluorescence was markedly attenuated (Fig. 4*B*), consistent with release of the human HL from extracellular or cell-surface HSPG by heparin. Heparin-releasable immunofluorescence also surrounded the central veins and was colocalized with the extracellular matrix as shown by Alcian blue staining (data not shown). No specific immunofluorescence was observed in the nontransgenic mouse liver (Fig. 4, *C* and *D*) or in the spleen and kidney of *hHLTg* or nontransgenic mice (data not shown).

Since HL-like activity and protein have been demonstrated in homogenates of human, hamster, and rat adrenal tissue (18–21) and since HL may play a role in providing exogenous cholesterol for adrenal steroidogenesis, we analyzed adrenal tissue for the presence of HL (Fig. 5). Immunohistochemical staining of adrenal tissue from *hHLTg* mice demonstrated human HL in the *zona fasciculata* and the *zona reticularis* and no significant signal in the *zona glomerulosa* or in the adrenal

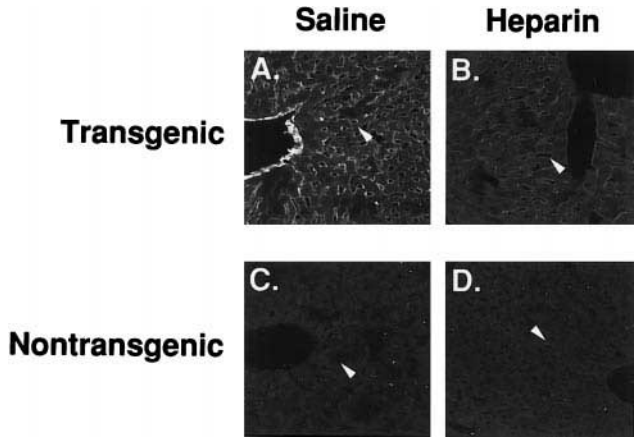

FIG. 4. **Immunohistochemical localization of human HL in *hHLTg*$^{+/0}$ or nontransgenic mouse livers 10 min after portal vein infusion of equal volumes of saline (*A* and *C*) or 150 units/kg heparin in saline (*B* and *D*).** The livers were perfusion-fixed with 3% paraformaldehyde in phosphate-buffered saline. Frozen tissue was cut with a cryostat into 10–15 μm sections and mounted on glass slides. Immunostaining was performed at room temperature with the monospecific anti-human HL antibody (1:50) for 1 h, with biotinylated goat anti-rabbit secondary antibody (1:500) (Zymed, South San Francisco, CA) for 1 h, and with fluorescein streptavidin (1:1000) (Amersham) for 1 h. The stained slides were evaluated with a Bio-Rad MCR 600 confocal microscope. *Arrowheads* indicate the liver sinusoids.

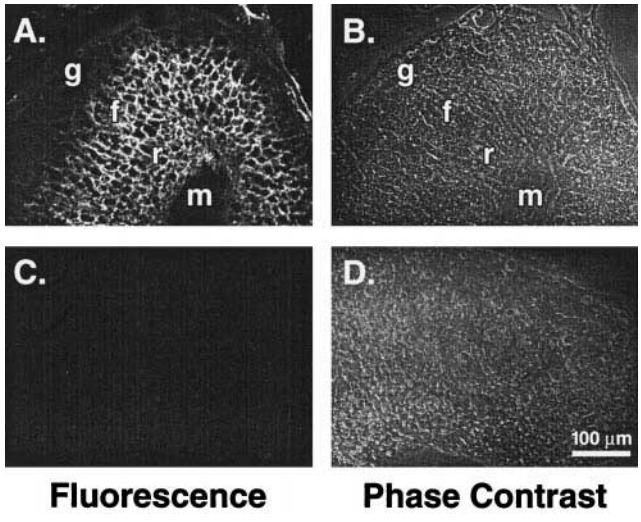

FIG. 5. **Immunohistochemical localization of HL in adrenal tissue from *hHLTg* (*A*) and nontransgenic (*C*) mice.** The adrenals were fixed with 3% paraformaldehyde in phosphate-buffered saline, frozen, and stored at −70 °C. Frozen sections (10–15 μm) were reacted as described in Fig. 4 except for tyramide signal amplification with biotinylated secondary antibody and streptavidin-horseradish peroxidase to deposit fluorescein-tyramide. The stained slides were evaluated as in Fig. 4. In parallel, adjacent tissue sections were examined for intact adrenal tissue morphology by phase-contrast microscopy (*B* and *D*). *g*, *zona glomerulosa*; *f*, *zona fasciculata*; *r*, *zona reticularis; m*, medulla.

medulla (Fig. 5*A*). No human HL was detected in adrenal tissue from nontransgenic mice (Fig. 5*C*). Normal adrenal tissue morphology was verified by examining stained and adjacent sections simultaneously by phase-contrast microscopy (Fig. 5, *B* and *D*).

Plasma Lipid and Lipoprotein Analyses in Nontransgenic and Transgenic Mice on a Chow Diet—Increased expression of HL in chow-fed transgenic mice decreased plasma cholesterol by 80–85% and plasma triglyceride by ~35% in both the *hHLTg*$^{+/0}$ and the *hHLTg*$^{+/+}$ mice (Table II). These findings suggested that the mechanisms by which HL reduced plasma

TABLE II
Plasma cholesterol and triglyceride concentrations

Genotype	*n*	Sex	Diet	Plasma cholesterol	Plasma triglycerides
				mg/dl ± *S.D.*	
Nontransgenic	4	F	Chow	65 ± 5	46 ± 20
hHLTg$^{+/0}$	5	F	Chow	12 ± 3[a]	28 ± 10[b]
hHLTg$^{+/+}$	3	F	Chow	10 ± 4[a]	32 ± 2
Nontransgenic	3	F	High fat	225 ± 22	32 ± 19
hHLTg$^{+/0}$	4	F	High fat	150 ± 17[a]	26 ± 6
hHLTg$^{+/+}$	3	F	High fat	56 ± 19[a]	21 ± 3
huBTg$^{+/0}$	7	F	Chow	82 ± 14	68 ± 20
huBTg$^{+/0}$,*hHLTg*$^{+/0}$	5	F	Chow	44 ± 10[c]	44 ± 13[d]
apoE$^{-/-}$	4	F	Chow	569 ± 59	89 ± 22
apoE$^{-/-}$,*hHLTg*$^{+/0}$	4	F	Chow	272 ± 67[e]	49 ± 17[f]
apoE$^{-/-}$,*hHLTg*$^{+/+}$	3	F	Chow	202 ± 28[e]	19 ± 8[f]
apoE$^{-/-}$	7	M	Chow	560 ± 94	124 ± 92
apoE$^{-/-}$,*hHLTg*$^{+/0}$	3	M	Chow	251 ± 40[e]	20 ± 9[f]
apoE$^{-/-}$,*HL-CAT*	5	M	Chow	240 ± 72[e]	65 ± 8

[a] $p < 0.001$ *versus* nontransgenic mice.
[b] $p < 0.05$ *versus* nontransgenic mice.
[c] $p < 0.005$ *versus huBTg* mice.
[d] $p < 0.05$ *versus huBTg* mice.
[e] $p < 0.005$ *versus apoE*$^{-/-}$ mice.
[f] $p < 0.05$ *versus apoE*$^{-/-}$ mice.

cholesterol and triglyceride were operating at their maximal capacity in the hemizygous mice.

Plasma lipoprotein profiles were determined by FPLC in at least three mice from each group. The lipoprotein cholesterol profile in a nontransgenic chow-fed mouse was characterized by a small deviation in apoB-containing LDL and a prominent peak for HDL (Fig. 6*A*). In contrast, the *hHLTg* mice had reductions in both of these lipoprotein fractions (~85%) (Fig. 6, *B* and *C*).

Plasma Lipid and Lipoprotein Analyses in Nontransgenic and Transgenic Mice on a High Fat Diet—To investigate further the observation that HL reduces apoB-containing lipoproteins as well as HDL in chow-fed mice, we induced higher levels of apoB-containing lipoproteins by feeding the mice a high fat diet. Analysis of the plasma lipid and lipoprotein levels showed that the plasma cholesterol increased in all mice on a high fat diet, mainly as a result of increased apoB-containing lipoproteins. However, overexpression of HL in these mice lowered the cholesterol by 33% in the *hHLTg*$^{+/0}$ mice and by 75% in the *hHLTg*$^{+/+}$ mice (Table II). Plasma triglyceride levels decreased by 20–35% in the transgenic mice. The FPLC profiles are illustrated in Fig. 6 (*D–F*). The apoB-containing lipoproteins (VLDL, IDL, and LDL) decreased in both hemi- and homozygous *hHLTg* mice (Fig. 6, *E* and *F*). Additionally, HL overexpression dramatically reduced HDL levels in both hemi- and homozygous mice. The peak in the profile after the HDL cholesterol peak is probably not triglyceride but glycerol. As summarized in Table III, the VLDL cholesterol decreased by 28% and 85%, respectively, in the *hHLTg*$^{+/0}$ and *hHLTg*$^{+/+}$ transgenic mice fed a high fat diet. The IDL and LDL fractions decreased by ~25% in the *hHLTg*$^{+/0}$ and by ~70% in the *hHLTg*$^{+/+}$ mice. As expected, the HDL levels were also reduced: by ~60% in the *hHLTg*$^{+/0}$ and by ~70% in the *hHLTg*$^{+/+}$ mice. Thus, in mice fed a high fat diet, HL lowers cholesterol and triglycerides by decreasing plasma levels of both apoB-containing lipoproteins and HDL.

Characterization of the huBTg,hHLTg Double-transgenic Mice

To extend the observation that HL lowers apoB-containing lipoproteins, we examined the effect of increased HL expression in human apoB transgenic (*huBTg*) mice. These transgenic

mice have elevated plasma levels of apoB-containing lipoproteins mainly reflected as elevated levels of triglyceride-rich LDL (40). Breeding the *hHLTg* mouse onto the apoB background increased postheparin plasma HL activity by 50-fold (7.4 ± 1 and 358 ± 114 μEq of free fatty acid/ml/h in *huBTg* and *huBTg,hHLTg*$^{+/0}$ mice, respectively) and in the double-transgenic mice decreased plasma total cholesterol by 50% and plasma triglyceride levels by 35% (Table II). Plasma FPLC fractions showed significant decreases in both cholesterol and triglyceride in the IDL and LDL fractions; however, despite a substantial reduction in LDL triglyceride, these particles remained triglyceride-rich (Table IV and Fig. 7). This finding indicates that HL alone cannot hydrolyze the excess triglycerides in this particle and suggests that other factors contribute to the processing of triglyceride-rich LDL. As expected, a large reduction in HDL (62%) was observed in the double-transgenic mice compared with the *huBTg*$^{+/0}$ mice.

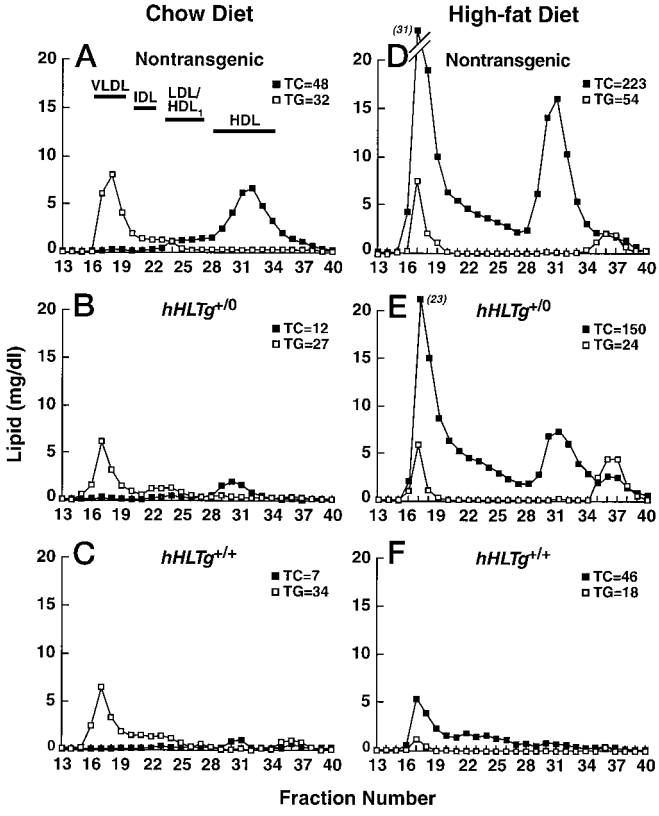

FIG. 6. **FPLC profiles of plasma from fasted nontransgenic (*A*), *hHLTg*$^{+/0}$ (*B*), and *hHLTg*$^{+/+}$ (*C*) female mice on a chow diet and from fasted nontransgenic (*D*), *hHLTg*$^{+/0}$ (*E*), and *hHLTg*$^{+/+}$ (*F*) female mice after 8 weeks on a high fat, high cholesterol diet.** Plasma (100 μl) from each animal was fractionated by Superose 6 chromatography, and fractions were assayed in duplicate for cholesterol and triglycerides with standard colorimetric assays. The fractions containing VLDL, IDL, LDL/HDL$_1$, and HDL are indicated with *horizontal bars* in *A*. *TC*, total cholesterol; *TG*, triglycerides.

Characterization of the ApoE$^{-/-}$ *and ApoE*$^{-/-}$*,hHLTg Mice*

In vitro, HL enhances the cellular uptake of remnants (in the form of β-VLDL) independently of apoE (34). To test whether this is also true *in vivo*, we bred the *hHLTg* mice onto the apoE-deficient background (41). Postheparin plasma HL activity in the resulting mice was ~25-fold higher than in nontransgenic apoE-deficient littermates. Plasma cholesterol and triglyceride levels in *apoE*$^{-/-}$*,hHLTg*$^{+/0}$ mice are summarized in Table II. Plasma cholesterol and triglyceride decreased ~50% in the *apoE*$^{-/-}$*,hHLTg*$^{+/0}$ mice. Plasma lipoprotein profiles were determined by FPLC, and lipoprotein classes were quantitated for at least three mice from each group of *apoE*$^{-/-}$ and *apoE*$^{-/-}$*,hHLTg*$^{+/0}$ mice on the chow diet. HL expression decreased all apoB-containing lipoprotein fractions ~50–60% and reduced HDL ~30–40% in the *apoE*$^{-/-}$*,hHLTg*$^{+/-}$ mice (Table V and Fig. 8, *A* and *C*). The triglyceride was reduced by about half in all apoB-containing lipoproteins. These findings suggest that HL mediates the clearance of remnant lipoproteins in the absence of apoE. To confirm this observation, we quantitated the apoB levels in the plasma of *apoE*$^{-/-}$ and *apoE*$^{-/-}$*,hHLTg*$^{+/0}$ mice. In the *apoE*$^{-/-}$*,hHLTg*$^{+/0}$ mice, apoB-48 plasma levels decreased 56%, while the apoB-100 levels did not change significantly. The significant decrease in apoB-48 in the *apoE*$^{-/-}$*,hHLTg*$^{+/0}$ mice suggests that HL has a prominent effect, primarily on remnant lipoproteins.

Characterization of the apoE$^{-/-}$*,HL-CAT*$^{+/0}$ *Mice*

In vitro, HL reduces remnants and remnant-like particles independently of catalytic activity (34, 38, 63), indicating a ligand function for HL. To investigate this phenomenon *in vivo*, we expressed HL-CAT on the remnant-rich *apoE*$^{-/-}$ background. The *apoE*$^{-/-}$*,HL-CAT*$^{+/0}$ transgenic mice were characterized with respect to their plasma lipid and lipoprotein concentrations and compared with age- and sex-matched *apoE*$^{-/-}$ and *apoE*$^{-/-}$*,hHLTg*$^{+/0}$ mice (Table II). The postheparin plasma lipase activities were 14 ± 2, 24 ± 2, and 568 ± 10 μEq of free fatty acid/ml/h in the *apoE*$^{-/-}$, *apoE*$^{-/-}$*,HL-CAT*$^{+/0}$, and *apoE*$^{-/-}$*,hHLTg*$^{+/0}$ mice, respectively. The activity in the *apoE*$^{-/-}$*,HL-CAT* mice was thus ~4% that in the *apoE*$^{-/-}$*, hHLTg*$^{+/0}$ mice. The HL immunoreactivity (as quantitated by

TABLE III
Lipoprotein cholesterol and triglyceride concentrations in plasma from nontransgenic and hHLTg mice

Lipoprotein	Chow diet			High fat diet		
	Nontransgenic (n = 4)	*hHLTg*$^{+/0}$ (n = 5)	*hHLTg*$^{+/+}$ (n = 3)	Nontransgenic (n = 3)	*hHLTg*$^{+/0}$ (n = 4)	*hHLTg*$^{+/+}$ (n = 3)
Cholesterol (mg/dl ± S.D.)						
VLDL	<1	<1	<1	61 ± 12	44 ± 11	9 ± 4[a]
IDL	<1	<1	<1	18 ± 6	14 ± 4	4 ± 2[c]
LDL/HDL$_1$	6 ± 2	2 ± 1[a]	1 ± 0.6[b]	17 ± 3	12 ± 4	7 ± 1[a]
HDL	39 ± 4	5 ± 1[b]	6 ± 4[b]	73 ± 15	31 ± 16[a]	20 ± 16[a]
Triglyceride (mg/dl ± S.D.)						
VLDL	17 ± 4	15 ± 7	3 ± 4	11 ± 3	5 ± 3[d]	3 ± 1[d]
IDL	4 ± 3	<1	7 ± 1	<1	<1	<1
LDL/HDL$_1$	7 ± 4	<1	4 ± 1	<1	<1	<1
HDL	3 ± 2	<1	2 ± 2	<1	<1	<1

[a] $p < 0.02$ *versus* nontransgenic mice.
[b] $p < 0.01$ *versus* nontransgenic mice.
[c] $p = 0.063$ *versus* nontransgenic mice.
[d] $p < 0.05$ *versus* nontransgenic mice.

TABLE IV
Lipoprotein cholesterol and triglyceride concentrations in plasma from nontransgenic, huBTg, and huBTg,hHLTg mice on a chow diet

Lipoprotein	Nontransgenic (n = 4)	*huBTg* (n = 7)	*huBTg,hHLTg*$^{+/0}$ (n = 5)
Cholesterol (mg/dl ± S.D.)			
VLDL	<1	2 ± 1	2 ± 1
IDL	<1	3 ± 0.5	2 ± 0.3[a]
LDL/HDL$_1$	6 ± 2	17 ± 4	11 ± 3[b]
HDL	39 ± 4	32 ± 10	12 ± 2[a]
Triglyceride (mg/dl ± S.D.)			
VLDL	17 ± 4	17 ± 9	14 ± 8
IDL	4 ± 3	6 ± 2	2 ± 1.5[a]
LDL/HDL$_1$	7 ± 4	21 ± 6	13 ± 6[b]
HDL	3 ± 2	2 ± 1	2 ± 2

[a] $p < 0.005$ *versus huBTg* mice.
[b] $p < 0.04$ *versus huBTg* mice.

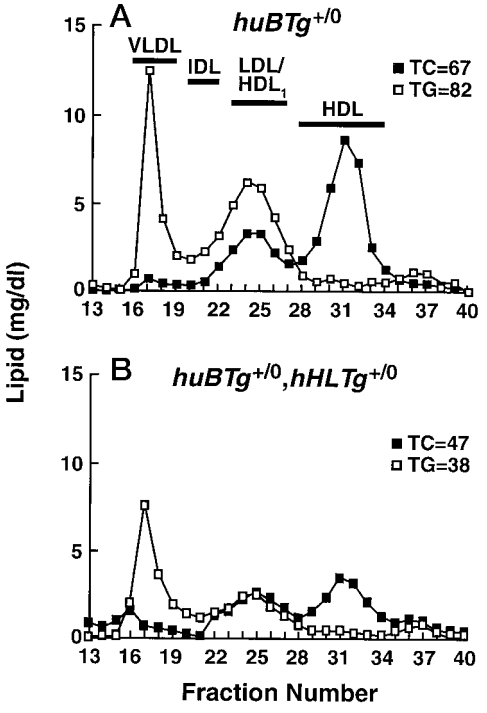

FIG. 7. **FPLC profiles of plasma from fasted *huBTg*$^{+/0}$ (*A*) and *huBTg*$^{+/0}$, *hHLTg*$^{+/0}$ (*B*) female mice on a chow diet.** Plasma (100 μl) from each animal was fractionated by Superose 6 chromatography, and fractions were assayed in duplicate, for cholesterol and triglyceride with standard colorimetric assays. The fractions containing VLDL, IDL, LDL/HDL$_1$, and HDL are indicated with *horizontal bars* in *A*. *TC*, total cholesterol; *TG*, triglycerides.

Western blots) of postheparin plasma from the *apoE*$^{-/-}$,*HL-CAT*$^{+/0}$ mice was ~30% of that seen in *apoE*$^{-/-}$,*hHLTg*$^{+/0}$ mice (6148 ± 1369 *versus* 19,017 ± 3508 arbitrary units). As predicted from the *in vitro* data (34, 38, 63), HL-CAT reduced both total and apoB-containing lipoprotein cholesterol ~60% compared with the level in *apoE*$^{-/-}$ mice (Tables II and V, Fig. 8*B*). Despite a significantly lower level of HL expression in *HL-CAT*$^{+/0}$ than in *hHLTg*$^{+/0}$ mice, there were similar decreases in VLDL, IDL, and LDL cholesterol, demonstrating a ligand activity for HL and the lack of a necessity for catalytic activity. However, the *apoE*$^{-/-}$, *HL-CAT*$^{+/0}$ mice had only a small (not statistically significant) decrease in HDL cholesterol, suggesting that catalytic activity is important at least for part of the HDL-reducing effect of HL.

Western blots of plasma for apoB levels in the male *apoE*$^{-/-}$, *apoE*$^{-/-}$,*hHLTg*$^{+/0}$, and *apoE*$^{-/-}$,*HL-CAT*$^{+/0}$ mice were quantitated by gel scanning and revealed similar decreases of apoB-48 in the *apoE*$^{-/-}$,*hHLTg*$^{+/0}$ and *apoE*$^{-/-}$,*HL-CAT*$^{+/0}$ transgenic mice when compared with *apoE*$^{-/-}$ mice (5489 ± 547, 5600 ± 1022, and 10,598 ± 1096 arbitrary units, respectively). Thus, these results demonstrate that HL can reduce total and apoB-containing lipoprotein cholesterol independently of its catalytic activity. These data also indicate that apoB48-containing remnant lipoproteins in the *apoE*$^{-/-}$ mice are cleared from the plasma similarly by HL with or without catalytic activity.

TABLE V
Lipoprotein cholesterol concentrations in apoE$^{-/-}$, *apoE*$^{-/-}$,*hHLTg*$^{+/0}$, *and apoE*$^{-/-}$,*HL-CAT*$^{+/0}$ *mice on a chow diet*

Lipoprotein	*ApoE*$^{-/-}$		*ApoE*$^{-/-}$,*hHLTg*$^{+/0}$		*ApoE*$^{-/-}$ *HL-CAT*$^{+/0}$
	Male (n = 7)	Female (n = 4)	Male (n = 3)	Female (n = 4)	Male (n = 5)
Cholesterol (mg/dl ± S.D.)					
VLDL	176 ± 44	233 ± 31	71 ± 5[a]	92 ± 29[a]	64 ± 15[a]
IDL	84 ± 19	86 ± 7	35 ± 5[a]	42 ± 12[b]	35 ± 15[a]
LDL/HDL$_1$	84 ± 44	80 ± 14	35 ± 5[b]	41 ± 10[b]	43 ± 21[c]
HDL	42 ± 13	34 ± 16	24 ± 7[c]	23 ± 5	35 ± 6

[a] $p < 0.005$ *versus apoE*$^{-/-}$ male and female mice.
[b] $p < 0.01$ *versus apoE*$^{-/-}$ male and female mice.
[c] $p < 0.05$ *versus apoE*$^{-/-}$ male and female mice.

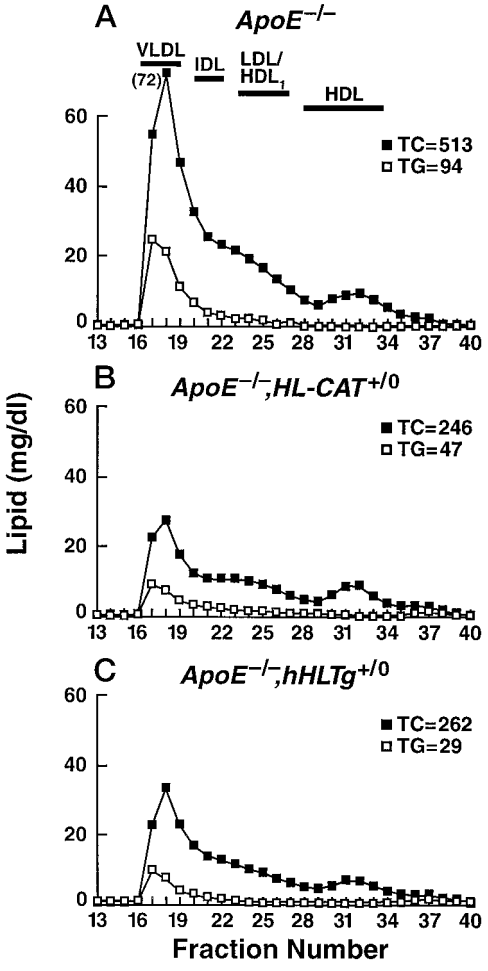

FIG. 8. **FPLC profiles of plasma from fasted *apoE*$^{-/-}$ (*A*), *apoE*$^{-/-}$, *HL-CAT*$^{+/0}$ (*B*) and *apoE*$^{-/-}$,*hHLTg*$^{+/0}$ (*C*) male mice on a chow diet.** Plasma from each animal was fractionated and assayed as in Fig. 7. *TC*, total cholesterol; *TG*, triglycerides.

DISCUSSION

To establish the mechanisms by which HL contributes to the metabolism of apoB-containing lipoproteins and HDL *in vivo*,

we developed and characterized transgenic mice expressing wild-type human HL or HL-CAT. These studies demonstrated that HL markedly reduced plasma total cholesterol by lowering both apoB-containing lipoproteins and HDL. Hepatic lipase lowered apoB-containing lipoproteins independently of both its catalytic activity and apoE; however, HL catalytic activity was required for maximal HDL reduction.

Evidence of a significant role for HL in lipid metabolism comes from studies using anti-HL antibody infusions in monkeys and rats (9–11) and from studies of HL-deficient patients (27–33). All of these studies reported increased plasma lipids and accumulation of apoB-containing lipoproteins and HDL, indicating that these effects are directly related to the lack of HL activity. Conversely, overexpression of HL in HL transgenic rabbits and mice reduced plasma lipids (25, 42) and apoB-containing lipoproteins (42). In the rabbits, HL was expressed uniquely in the liver as shown by immunohistochemistry: human HL was present on the sinusoidal endothelial cell surfaces and associated with the matrix in the space of Disse and microvillar surfaces of hepatocytes (5). Hepatic lipase expression markedly lowered plasma cholesterol and triglycerides by decreasing both IDL and HDL in the rabbits (42) and also reduced total cholesterol and triglycerides in the mice (25). In contrast to our findings, HL had no significant effects on remnants or LDL in those mice, even after 8 weeks on a high fat diet (25). The lack of an effect on the apoB-containing lipoproteins might have resulted from a lower HL level (a 7-fold increase over endogenous mouse HL *versus* a 20-fold increase in our mice) or a reduction in the effective HL concentration in the liver, as HL was also expressed in other tissues in these mice. Thus, although these previous studies demonstrated that HL expression reduces plasma lipids, they did not uniformly establish a role for HL in the metabolism of apoB-containing lipoproteins.

In our current study, overexpression of human HL markedly decreased plasma apoB-containing lipoproteins in two animal models that have elevated levels of apoB-containing VLDL, IDL, and LDL: mice lacking apoE and mice fed a high fat diet. In addition, *huBTg* mice, which have high levels of triglyceride-rich LDL (40, 64), displayed reduced levels of these lipoproteins when crossed with mice overexpressing HL. We hypothesized that this decrease resulted from both the hydrolytic and the direct ligand-binding functions of HL. Evidence for a direct ligand-binding function of HL derives from *in vitro* studies of McA-RH7777 cells stably transfected with human HL. In the HL-transfected cells, the binding and uptake of β-VLDL remnant lipoproteins and LDL were 3- and 7-fold higher, respectively, than in nontransfected cells (34). In similar studies, addition of HL to remnant-like particles increased their binding to hepatic plasma membranes and their uptake by hepatocytes (63). Thus, HL appears to have a direct ligand-binding function.

To examine this possibility, we determined whether HL catalytic activity was required to decrease levels of apoB-containing lipoproteins. Because *in vitro* studies had indicated that HL can enhance remnant uptake independently of catalytic activity (34), we generated mice that were transgenic for HL-CAT. Expression of HL-CAT on an $apoE^{-/-}$ genetic background lowered plasma cholesterol levels to the levels in wild-type (catalytically active) human HL transgenic mice on the $apoE^{-/-}$ background despite the lower levels of immunoreactive HL in the $apoE^{-/-}$*,HL-CAT* mice. Thus, as predicted, catalytic activity was not required to decrease the levels of apoB-containing lipoproteins. It is reasonable to postulate a smaller decrease in apoB-containing lipoproteins at lower levels of HL expression. In addition, because apoB-containing lipoproteins are markedly increased in human HL deficiency and in HL knockout mice, it is reasonable to expect that this effect (to decrease apoB lipoproteins) is physiological (27, 31, 33, 58). These results provide two important pieces of information: HL can clear lipoproteins independently of its catalytic activity and can also do so in the absence of apoE (the ligand that normally mediates remnant uptake), perhaps by functioning as a ligand.

The proposed ligand function of HL depends in part on its heparin-binding function and requires binding to HSPG (34). To bind proteoglycans, positively charged residues within HL, which confer heparin-binding properties, interact with negatively charged residues in the proteoglycans. These interactions may stabilize the enzyme, properly orient it toward its lipoprotein substrate/ligand, or anchor it in proximity to receptors for uptake. The contribution of proteoglycan binding, and therefore heparin-binding properties, to the ligand function of HL was demonstrated *in vitro* by treating HL-transfected cells with heparinase to degrade extracellular proteoglycans (34). Heparinase decreased the HL-mediated binding and uptake of β-VLDL and LDL ~90% compared with untreated cells. Thus, heparin binding of HL (to HSPG) mediated the uptake of both types of lipoproteins. When we examined the HL ligand function by treating cells with the 39-kDa protein to block the LRP-mediated pathway, only ~40% of β-VLDL binding and uptake and only a minimal portion of LDL binding and uptake were inhibited (38). Because it completely blocked the binding and degradation of ^{125}I-α_2-macroglobulin, the 39-kDa protein was completely functional (65, 66). We conclude that the major part of the proposed ligand function of HL occurs via binding to HSPG.

In the present study, expression of human HL in transgenic mice also reduced HDL levels. This reduction in HDL had been reported previously for HL transgenic rabbits and HL transgenic and HL adenovirus-transduced mice (25, 26, 42). The decrease in HDL was expected because HL has considerable phospholipase activity and HDL are a preferred, phospholipid-rich substrate of HL (12–15, 22–24). The HDL-lowering mechanisms in this model probably involve both the catalytic and ligand-binding activities of HL. Previous studies indicated that HL enhances the selective uptake of HDL-derived cholesteryl esters (67–69). In addition, we recently demonstrated that HL enhances the selective uptake of HDL-derived cholesteryl ether *in vitro* (38). We hypothesized that one mechanism by which HL enhances selective uptake is by hydrolyzing the phospholipids on the particle surface, thereby releasing core cholesteryl esters for uptake, possibly in association with the recently described scavenger receptor B1 pathway (70). This enhancement of selective uptake and the presence of HL in the corticosteroid-producing zones of the adrenal cortex suggest that HL facilitates delivery of lipoprotein cholesterol to cells for steroidogenesis.

To establish whether the catalytic function of HL was an absolute requirement for HDL reduction *in vivo*, we expressed HL-CAT in the $apoE^{-/-}$ mice and measured the plasma HDL levels. The HDL levels were slightly lower in the $apoE^{-/-}$*,HL-CAT*$^{+/0}$ mice than in $apoE^{-/-}$ mice (but this difference did not reach statistical significance). Thus, HL appears to reduce HDL levels mainly by its catalytic function. However, we cannot rule out the possibility of a small reduction in HDL levels through its ligand function.

Our data demonstrate the powerful cholesterol-lowering properties of HL in several different transgenic mouse models. In addition, our studies of both wild-type HL and HL-CAT expressed on the $apoE^{-/-}$ background indicate that HL functions both as a hydrolytic enzyme and as a ligand to mediate enhanced sequestration and/or receptor-mediated lipoprotein clearance. Our report constitutes the first *in vivo* evidence that

HL can act as a ligand to remove lipoproteins and supports a role for HL in the nonenzymatic clearance of apoB-containing lipoproteins.

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