The bridging function of hepatic lipase clears plasma cholesterol in LDL receptor-deficient “apoB-48-only” and “apoB-100-only” mice

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Abstract Hepatic lipase clears plasma cholesterol by lipolytic and nonlipolytic processing of lipoproteins. We hypothesized that the nonlipolytic processing (known as the bridging function) clears cholesterol by removing apoB-48- and apoB-100-containing lipoproteins by whole particle uptake. To test our hypotheses, we expressed catalytically inactive human HL (ciHL) in LDL receptor deficient “apoB-48-only” and “apoB-100-only” mice. Expression of ciHL in “apoB-48-only” mice reduced cholesterol by reducing LDL-C (by 54%, 46 ± 6 vs. 19 ± 8 mg/dl, $P < 0.001$). ApoB-48 was similarly reduced (by 60%). The similar reductions in LDL-C and apoB-48 indicate cholesterol removal by whole particle uptake. Expression of ciHL in “apoB-100-only” mice reduced cholesterol by reducing IDL-C (by 37%, 61 ± 19 vs. 38 ± 12 mg/dl, $P < 0.003$). Apo-B100 was also reduced (by 27%). The contribution of nutritional influences was examined with a high-fat diet challenge in the “apoB-100-only” background. On the high fat diet, ciHL reduced IDL-C (by 30%, 355 ± 72 vs. 257 ± 64 mg/dl, $P <$ 0.04) but did not reduce apoB-100. The reduction in IDL-C in excess of apoB-100 suggests removal either by selective cholesteryl ester uptake, or by selective removal of larger, cholesteryl ester-enriched particles. Our results demonstrate that the bridging function removes apoB-48- and apoB-100-containing lipoproteins by whole particle uptake and other mechanisms.—Dichek, H. L., K. Qian, and N. Agrawal. **The bridging function of hepatic lipase clears plasma cholesterol in LDL receptor-deficient “apoB-48-only” and “apoB-100-only” mice.** ***J. Lipid Res.*** **2004.** 45: **551–560.**

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Human hepatic lipase (HL) is a central component of lipoprotein metabolism (1, 2). HL is synthesized and secreted by the liver, where it is anchored to the surface of hepatocytes and sinusoidal endothelial cells via heparan sulfate proteoglycans (HSPGs) (3–6). HL hydrolyzes triglycerides and phospholipids in remnants (chylomicron remnants and IDL) and LDL to yield particles that are depleted in triglycerides and phospholipids and are more optimal for receptor-mediated uptake (2, 7–9). HL may also play a role in reverse cholesterol transport by hydrolyzing phospholipids in HDL, which converts HDL2 to HDL3 (10, 11).

The significant role of HL in lipoprotein metabolism is apparent from human studies and data generated in animals. For example, plasma of HL-deficient patients contains high levels of apolipoprotein B (apoB)-containing lipoproteins and HDL (12–17). Infusion of anti-HL antibodies in rats and monkeys increases levels of apoB-containing lipoproteins and HDL (18–20). Expression of moderate and high levels of wild-type HL in mice and rabbits reduces levels of apoB-containing lipoproteins and HDL (6, 21–24). Taken together, these studies indicate a major role for HL in determining the plasma levels of apoB-containing lipoproteins and HDL.

HL regulates plasma levels of apoB-containing lipoproteins using both catalytic and bridging functions (6, 25). In particular, wild-type HL (reflecting both catalytic and bridging functions) reduces plasma levels of both apoB-48- and apoB-100-containing lipoproteins. However, the contribution of the bridging function to their reduction is not clear. Previous in vitro studies demonstrated roles for the bridging function in the cellular uptake of chylomicrons, remnants, and LDL. Cells transfected with wild-type HL and studied at 4°C to abolish catalytic activity showed enhanced uptake of human chylomicrons, and cells incubated in the presence of heat-inactivated HL displayed increased binding and uptake of remnants (26, 27). Cells transfected with a mutant, catalytically inactive hepatic li-

Abbreviations: apoB, apolipoprotein B; ciHL, catalytically inactive hepatic lipase; FPLC, fast-protein liquid chromatography; HDL-C, HDL-cholesterol; HL, hepatic lipase; HSPG, heparan sulfate proteoglycan; IDL-C, IDL-cholesterol; LDL-C, LDL-cholesterol; LDLR, LDL receptor; LRP, LDLR-related protein; SR-BI, scavenger receptor BI.

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pase (ciHL) displayed more association with ^{125}I-labeled LDL than did control transfected cells, thus suggesting that the bridging function facilitated LDL receptor (LDLR)-mediated uptake of LDL (26, 28).

The effect of the bridging function on plasma levels of apoB-containing lipoproteins has also been studied in vivo in apoE-deficient and LDLR-deficient mice. In apoE-deficient mice (which have high levels of apoB-48-containing lipoproteins), overexpression of ciHL reduced levels of VLDL, remnants, and LDL (6, 29). In LDLR-deficient mice (which have high levels of both apoB-48- and apoB-100-containing lipoproteins), overexpression of ciHL also reduced remnants and LDL (30). This latter finding indicated that the bridging function uses an LDLR-independent pathway to reduce apoB levels, inasmuch as both apoB-48- and apoB-100-containing lipoproteins are catabolized in part via the LDLR (which is absent in LDLR-deficient mice) (31).

On the basis of this information, we hypothesized that the bridging function of HL facilitates removal of both apoB-48- and apoB-100-containing lipoproteins. Furthermore, we hypothesized that the bridging function facilitates particle removal by whole-particle uptake. To test our hypotheses, we expressed ciHL in LDLR-deficient mice that were genetically modified to express only apoB-48 or apoB-100 and determined the effect of the bridging function on levels of apoB-100- and apoB-48-containing lipoproteins (31). We also assessed the dependence of the bridging function on particle composition. To do this, we examined the response of plasma lipids and apolipoproteins to a cholesterol-enriched, high-fat (Western) diet. To control for any contribution from endogenous mouse HL, we also expressed ciHL in LDLR-deficient mice that lacked mouse HL.

MATERIALS AND METHODS

Genetically modified mice

Mice that were genetically modified to express only mouse apoB-100 (*apob$^{100/100}$*) (31) (a gift from Dr. Stephen G. Young, Gladstone Institute of Cardiovascular Disease, San Francisco, CA) were bred with *Ldlr$^{-/-}$apob$^{+/+}$* mice (that have the wild-type mouse apoB gene), and the resulting offspring were bred with each other to yield LDLR-deficient mice that were homozygous for apoB-100 and that no longer had the wild-type mouse apoB gene. The resulting *Ldlr$^{-/-}$apob$^{100/100}$* mice were bred with *Ldlr$^{-/-}$apob$^{+/+}$* mice that are transgenic for a human ciHL (HLS145G). Also, LDLR-deficient mice that were genetically modified to express only mouse apoB-48 (*Ldlr$^{-/-}$apob$^{48/48}$*) (31) (also a gift from Dr. Stephen G. Young) were bred with *Ldlr$^{-/-}$apob$^{+/+}$* mice that are transgenic for human ciHL (*Ldlr$^{-/-}$apob$^{+/+}$ HL^{S145G}*). The resulting littermates were bred to achieve homozygosity for both the genetically modified mouse apoB gene (to yield either *Ldlr$^{-/-}$apob$^{48/48}$* or *apob$^{100/100}$*) and the gene-targeted mouse LDLR gene as well as heterozygosity for the ciHL transgene (30). The wild-type mouse apoB gene was absent in these mice. The ciHL transgene was reported previously and consists of a mutant human HL cDNA in which a glycine replaces serine at position 145 in the catalytic triad (HLS145G), resulting in expression of ciHL (6).

Ldlr$^{-/-}$apob$^{+/+}$ HL^{S145G} mice were crossed with gene-targeted mouse HL-deficient mice (*hl$^{-/-}$*) (32), and the resulting littermates were bred to yield homozygosity for the gene-targeted mouse LDLR and HL genes and heterozygosity for the ciHL transgene (*Ldlr$^{-/-}$hl$^{-/-}$apob$^{+/+}$ HL^{S145G}*).

Background strains were identical within each genotype group, but differed between genotypes, so that *Ldlr$^{-/-}$apob$^{48/48}$* background had 75% C57Bl/6 and 25% SJL, *Ldlr$^{-/-}$apob$^{100/100}$* background had 81.25% C57Bl/6 and 18.75% SJL, and the *Ldlr$^{-/-}$hl$^{-/-}$apob$^{+/+}$* background had 96% C57/Bl6, 1.5% SJL, and 1.5% ICR.

Liver-specific expression of the HLS145G transgene was conferred by sequences in 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 locus.

Presence of the ciHL transgene was determined by polymerase chain reaction (PCR) analysis of tail DNA using the primers H-26 (5′-AGC-CAT-TTG-GAA-GAA-GAG-CTC-AAG-CT-3′) and H-36 (5′-GGA-TCT-TTA-ACT-GGT-AAT-GAT-AAA-CTT-TGA-3′) in the following program: an initial 3 min denaturation at 92°C followed by 30 cycles of a 1 min denaturation at 92°C, a 2 min annealing at 60°C, and 3 min elongation at 72°C, ending with a 2 min elongation at 72°C. This yielded a 987 bp fragment of the human HL transgene. Homozygosity for the *hl$^{-/-}$* background was determined by PCR of tail DNA using the primers hl-433 (5′-TTC-TCG-GAG-CAA-AGT-TCA-CCT-AAT-TGG-3′), hl-508 (5′CTG-TGA-TTC-TTC-CAA-TCT-TGT-TCT-TCC-3′) and upper neo (5′-GAT-TGG-GAA-GAC-AAT-AGC-AGG-CAT-GC-3′) in the following program: an initial 5 min denaturation at 92°C, followed by 30 cycles of a 1 min denaturation at 92°C, 2 min annealing at 60°C, and 2 min elongation at 72°C, ending with a 5 min elongation at 72°C. This yielded a 200 bp fragment of the mouse HL knockout gene and a 100 bp product of the wild-type mouse HL gene.

Homozygosity for the gene-targeted LDLR gene in *Ldlr$^{-/-}$apob$^{+/+}$* mice was determined by PCR (33). Homozygosity for the *apob$^{+/+}$* and *apob$^{48/48}$* backgrounds was confirmed by PCR analysis of tail DNA (using a protocol developed by Dr. Stephen G. Young and colleagues) as follows: primers M49mus 5′-CTG-AAT-GCA-TCT-GAC-TGG-GAG-AGA-3′ and M50mus 5′-CGG-ATA-TGA-TAC-TGT-TCA-TCA-AGA-A-3′ in a program consisting of 15 s denaturation at 94°C, 30 s annealing at 60°C, and 60 s extension at 72°C for 30 cycles, yielding a 281 bp fragment (wild-type *apob$^{+/+}$*). Digestion with SpeI cleaves the fragment into a 181 bp and a 100 bp fragment in the presence of homozygosity for the *apob$^{48/48}$* genotype. Digestion of heterozygous mouse DNA results in three bands: 281 bp, 181 bp, and 100 bp. Expression of only apoB-48 in *Ldlr$^{-/-}$apob$^{48/48}$* mice, and both apoB-48 and apoB-100 in *Ldlr$^{-/-}$hl$^{-/-}$apob$^{+/+}$* mice was confirmed by Western blot analysis of mouse plasma using a rabbit anti-mouse apoB antibody that detects mouse apoB-48 and mouse apoB-100 (34) (a gift from Dr. Stephen G. Young). Mice that were homozygous for the *apob$^{100/100}$* genotype were identified as those expressing only apoB-100 in plasma, as determined by Western analysis with the rabbit anti-mouse apoB antibody (34).

All mice were male. Groups of littermates were analyzed within each of the *Ldlr$^{-/-}$apob$^{48/48}$*, *Ldlr$^{-/-}$apob$^{100/100}$*, and *Ldlr$^{-/-}$hl$^{-/-}$apob$^{+/+}$* genotypes. After weaning at 21 days, mice were fed a chow diet and were housed in a full-barrier facility with a 12 h light-dark cycle. All studies were approved by the Institutional Animal Care and Use Committee of the University of Washington.

Expression of human ciHL

Plasma samples from mice fed a chow diet were collected in tubes containing ethylene diaminetetraacetic acid prior to and

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10 min after tail vein injection of heparin (150 U/kg body weight) and were kept frozen at −80°C until analysis for protein expression. Western blot analysis of pre- and postheparin plasma was performed with a monospecific polyclonal rabbit anti-human HL antiserum (6).

Lipase assays

Triglyceride lipase activities were quantitated in two separate assays in triplicate with glycerol [^{1}-14C]trioleate-labeled triolein emulsion as a substrate in the presence of 1 M NaCl (35).

Lipoprotein analysis

Plasma was obtained by orbital vein bleeding after a 4 h fast. Mouse plasma lipoproteins were fractionated by fast-protein liquid chromatography (FPLC) on a Superose 6 HR 10/30 column (Amersham Pharmacia Biotech, Piscataway, NJ) as described previously (6). The cholesterol and triglyceride levels in whole plasma as well as in eluted fractions of plasma were determined with standard enzymatic assays (cholesterol: Abbot Spectrum, Abbott Park, IL; triglycerides: GPO-PAP kit, Boehringer Mannheim, Indianapolis, IN). The cholesterol and triglyceride concentrations in the eluted fractions (of plasma) were then corrected against the cholesterol and triglyceride concentrations in whole plasma to account for variability in the efficiency of the Superose 6 column (recovery). The recovery ranged from 70% to 100%.

Lipoprotein cholesterol and triglyceride concentrations were obtained by adding the concentrations of fractions 16–19 (VLDL), 20–22 (IDL), 23–27 (LDL), and 28–34 (HDL). Because cholesterol and triglyceride concentrations in the fractions (after correction for recovery) are calculated numbers, whereas plasma total cholesterol and triglycerides are actual measured numbers, only approximate comparisons can be made between total and lipoprotein lipids.

Western analysis of plasma apoB-48 and apoB-100

Plasmas from 4–5 mice of each genotype were pooled and subjected to Western blot analysis. The pooled plasmas were applied in quadruplicate or quintuplicate to 4% polyacrylamide-sodium dodecyl sulfate gels. Three to four individual gels were run (duplicate gels run for the *Ldlr*$^{-/-}$*apob*$^{48/48}$ mice on a chow diet) to facilitate comparisons between genotypes and to ensure reproducibility. After blotting, nitrocellulose membranes were incubated with a rabbit anti-mouse apoB antibody that reacts with both apoB-48 and apoB-100 (34), incubated with biotinylated second antibody, and developed with an ECL kit (Amersham Pharmacia Biotech). Immunoblots were analyzed by densitometry on a GelDoc 2000 (Bio-Rad, Hercules, CA) using the Quantity One software package (Bio-Rad). Reproducibility of densitometry results was assessed by repeat measurements of individual blots.

Western analysis of plasma apoE and apoA-I

Plasma from 4–7 mice of each genotype was pooled, and the pooled plasmas were applied in quadruplicate or quintuplicate and fractionated on 12% polyacrylamide-sodium dodecyl sulfate gels (Bio-Rad) and transferred to nitrocellulose membranes. Three to four individual gels were run to facilitate comparisons between genotypes and to ensure reproducibility. The membranes were incubated with a goat anti-mouse apoE antibody (that also reacts with mouse apoA-I) (a gift from Dr. Karl H. Weisgraber, Gladstone Institute of Cardiovascular Disease), reacted with biotinylated second antibody, and analyzed as above.

Selective cholesteryl ester uptake assessment

The presence of a greater decrease in cholesterol than in apolipoprotein was used as an approximate measure of selective cholesteryl ester uptake. Thus, selective cholesteryl ester uptake was assumed to occur in VLDL, IDL, and LDL when lipoprotein cholesterol decreased to a greater extent than apoB-48 and/or apoB-100 levels. Likewise, HDL-selective cholesteryl ester uptake was assumed to occur when HDL-cholesterol (HDL-C) decreased but apoA-I levels remained virtually unchanged.

Diet study

To assess the dependence of the bridging function on lipoprotein lipid composition, six to nine animals of each of the *Ldlr*$^{-/-}$*apob*$^{100/100}$ and *Ldlr*$^{-/-}$*hl*$^{-/-}$*apob*$^{+/+}$ genotypes were fed a cholesterol-enriched, high-fat (Western) diet [21% (w/w) fat and 0.15% (w/w) cholesterol] (TD 88137, Harlan, Teklad, Madison, WI) for 2 weeks. Fasted plasma lipoproteins were separated by FPLC, and plasma apolipoprotein levels were analyzed by Western blotting as described.

Statistical analysis

Data are presented as the mean ± SD. Student's *t*-test for unequal variances was used to determine the statistical significance of differences.

RESULTS

Expression of the human ciHL transgene

PCR analysis for the ciHL transgene demonstrated a fragment of the expected 987 bp size in transgenic mice and its absence in nontransgenic mice. Western analysis of postheparin plasma confirmed the presence of the HL protein and demonstrated similar amounts of immunoreactive human HL in all transgenic mice (data not shown). The amounts of ciHL in postheparin plasma of all three genotypes were similar to the amounts of ciHL in the previously reported *Ldlr*$^{-/-}$*apob*$^{+/+}$ *HL*^{S145G} mice. There was no human HL in the nontransgenic mice. Absence of human HL catalytic activity in postheparin plasma was verified by the lack of increased HL activity (above that of the endogenous mouse HL) in all genotypes (**Table 1**). Only background activity was present in the *Ldlr*$^{-/-}$*hl*$^{-/-}$*apob*$^{+/+}$*HL*^{S145G} mice.

Plasma lipids and lipoproteins in *Ldlr*$^{-/-}$*apob*$^{48/48}$, *Ldlr*$^{-/-}$*apob*$^{100/100}$, and *Ldlr*$^{-/-}$*hl*$^{-/-}$*apob*$^{+/+}$ mice with and without ciHL on a chow diet

Ldlr$^{-/-}$*apob*$^{48/48}$ *background.* Expression of ciHL reduced cholesterol levels by 25% in *Ldlr*$^{-/-}$*apob*$^{48/48}$ *HL*^{S145G} mice when compared with *Ldlr*$^{-/-}$*apob*$^{48/48}$ mice [171 ± 4 mg/dl in *Ldlr*$^{-/-}$*apob*$^{48/48}$ (n = 4) vs 129 ± 27 mg/dl in *Ldlr*$^{-/-}$*apob*$^{48/48}$ *HL*^{S145G} mice (n = 6), $P < 0.02$]. The triglyceride levels were not changed significantly (**Table 2**). Plasma lipoprotein profiles were determined by FPLC (**Fig. 1**). The FPLC cholesterol profiles in *Ldlr*$^{-/-}$*apob*$^{48/48}$ mice showed minimal elevations of VLDL and IDL and major peaks of LDL and HDL.

In ciHL-expressing mice, LDL-cholesterol (LDL-C) and HDL-C concentrations were reduced by 54% (46 ± 6 vs 19 ± 8 mg/dl, $P < 0.002$) and 21% (85 ± 8 vs 67 ± 16 mg/dl, $P < 0.05$), respectively (Fig. 1A and Table 2). The FPLC triglyceride profiles displayed a prominent peak in VLDL and moderate elevations in IDL and LDL. Expression of ciHL did not affect lipoprotein triglyceride levels significantly (Fig. 1D and Table 2).

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TABLE 1. Plasma HL activities in male mice

Lipase Activity[a,b]	$Ldlr^{-/-}$ $apob^{48/48}$ (n = 3)	$Ldlr^{-/-}$ $apob^{48/48}$ HL^{S145G} (n = 3)	$Ldlr^{-/-}$ $apob^{100/100}$ (n = 3)	$Ldlr^{-/-}$ $apob^{100/100}$ HL^{S145G} (n = 3)	$Ldlr^{-/-}hl^{-/-}$ $apob^{+/+}$ (n = 3)	$Ldlr^{-/-}hl^{-/-}apob^{+/+}$ HL^{S145G} (n = 3)
Preheparin	6 ± 1	7 ± 0	10 ± 1	7 ± 0	1 ± 0	1 ± 1
Postheparin	15 ± 0	19 ± 1	19 ± 1	17 ± 0	4 ± 1	4 ± 1

HL, hepatic lipase; LDLR, LDL receptor.
[a] Lipase activities are expressed in μEq FFA per ml/h.
[b] Values given as mean ± SE.

$Ldlr^{-/-}apob^{100/100}$ background. We also uncovered a cholesterol-reducing effect of ciHL on apoB-100-containing lipoproteins. Thus, expression of ciHL reduced cholesterol levels by 18% in $Ldlr^{-/-}apob^{100/100}$ HL^{S145G} mice when compared with $Ldlr^{-/-}apob^{100/100}$ mice [286 ± 42 mg/dl in $Ldlr^{-/-}apob^{100/100}$ (n = 13) vs 235 ± 33 mg/dl in $Ldlr^{-/-}apob^{100/100}$ HL^{S145G} mice (n = 10) $P < 0.003$] (Table 2). The triglyceride levels were also decreased, although this decrease was not statistically significant (Table 2). The FPLC cholesterol profiles in $Ldlr^{-/-}apob^{100/100}$ mice showed a minimal elevation in VLDL, a prominent IDL/LDL peak, and a moderate elevation of HDL (Fig. 1B).

In ciHL-expressing mice, IDL-cholesterol (IDL-C) concentrations were reduced by 38% (61 ± 19 vs 38 ± 12 mg/dl, $P < 0.003$) (Fig. 1B and Table 2). There was also a trend toward reduction of LDL-C (Table 2). HDL-C levels were reduced by 30% (36 ± 6 vs 25 ± 7 mg/dl, $P < 0.003$) (Table 2).

The FPLC triglyceride profiles in $Ldlr^{-/-}apob^{100/100}$ mice displayed a small peak in VLDL and a prominent IDL/LDL peak (Fig. 1E). Expression of ciHL reduced triglyceride concentrations within the IDL subfraction by ∼20% (Fig. 1E and Table 2).

$Ldlr^{-/-}hl^{-/-}apob^{+/+}$ background. To eliminate endogenous murine HL activity as a confounding factor, we studied double knockout mice that were deficient in mouse HL and LDLR. Expression of ciHL reduced cholesterol by 24% in $Ldlr^{-/-}hl^{-/-}apob^{+/+}$ HL^{S145G} mice when compared with $Ldlr^{-/-}hl^{-/-}apob^{+/+}$ mice [427 ± 60 mg/dl in $Ldlr^{-/-}hl^{-/-}apob^{+/+}$(n = 9) vs 326 ± 38 mg/dl in $Ldlr^{-/-}hl^{-/-}apob^{+/+}HL^{S145G}$ mice (n = 6), $P < 0.002$]. The triglyceride levels were not changed significantly (Table 2). The FPLC cholesterol profiles in $Ldlr^{-/-}hl^{-/-}apob^{+/+}$ mice demonstrated a moderate peak in VLDL, a prominent IDL/LDL peak, and a prominent HDL peak (Fig. 1C).

In ciHL-expressing mice, LDL-C concentrations were reduced by 30% (172 ± 38 vs 120 ± 25 mg/dl, $P < 0.01$) (Fig. 1F and Table 2). Remnant (IDL) and HDL-C concentrations were not reduced significantly.

The FPLC triglyceride profiles in $Ldlr^{-/-}hl^{-/-}apob^{+/+}$ mice showed a marked peak in VLDL and a prominent IDL/LDL peak (Fig. 1F). Expression of ciHL did not affect lipoprotein triglyceride levels significantly (Fig. 1F and Table 2).

Plasma lipids and lipoproteins in $Ldlr^{-/-}apob^{100/100}$ and $Ldlr^{-/-}hl^{-/-}apob^{+/+}$ mice with and without ciHL on a Western diet

$Ldlr^{-/-}apob^{100/100}$ background. The 2 week Western diet challenge increased plasma cholesterol substantially (3.5-

TABLE 2. Plasma total and lipoprotein cholesterol and triglyceride concentrations on a chow diet

Lipids	$Ldlr^{-/-}apob^{48/48}$ (n = 4)	$Ldlr^{-/-}$ $apob^{48/48}$ HL^{S145G} (n = 5)	$Ldlr^{-/-}$ $apob^{100/100}$ (n = 13)	$Ldlr^{-/-}$ $apob^{100/100}$ HL^{S145G} (n = 10)	$Ldlr^{-/-}hl^{-/-}$ $apob^{+/+}$ (n = 9)	$Ldlr^{-/-}hl^{-/-}$ $apob^{+/+}$ HL^{S145G} (n = 6)
TC	171 ± 4	129 ± 27[a]	286 ± 42	235 ± 33[d]	427 ± 60	326 ± 38[f]
TG	97 ± 57	78 ± 33	123 ± 30	102 ± 27	183 ± 43	214 ± 43
VLDL-C	7 ± 2	7 ± 4	9 ± 4	9 ± 2	31 ± 10	23 ± 11
IDL-C	10 ± 2	7 ± 3	61 ± 19	38 ± 12[e]	58 ± 26	40 ± 13
LDL-C	46 ± 6	19 ± 8[b]	148 ± 27	124 ± 31	172 ± 38	120 ± 25[g]
HDL-C	85 ± 8	67 ± 16[c]	36 ± 6	25 ± 7[e]	125 ± 24	107 ± 17
VLDL-T	34 ± 34	20 ± 8	23 ± 9	22 ± 9	68 ± 48	82 ± 23
IDL-T	16 ± 14	10 ± 5	38 ± 15	30 ± 10	36 ± 16	49 ± 14
LDL-T	25 ± 18	15 ± 9	57 ± 23	62 ± 25	58 ± 17	62 ± 17
HDL-T	18 ± 12	16 ± 10	6 ± 4	4 ± 3	15 ± 9	12 ± 9

IDL-C, IDL-cholesterol; IDL-T, IDL-triglyceride; HDL-C, HDL-cholesterol; HDL-T, HDL-triglyceride; LDL-C, LDL-cholesterol; LDL-T, LDL-triglyceride; TC; plasma total cholesterol; TG, plasma total triglyceride; VLDL-T, VLDL-triglyceride. Plasma TC, TG, and lipoprotein and concentrations are in mg/dl. Values given as mean ± SD.
[a] $P < 0.02$ versus $Ldlr^{-/-}apob^{48/48}$ mice.
[b] $P < 0.001$ versus $Ldlr^{-/-}apob^{48/48}$ mice.
[c] $P < 0.05$ versus $Ldlr^{-/-}apob^{48/48}$ mice.
[d] $P < 0.003$ versus $Ldlr^{-/-}apob^{100/100}$ mice.
[e] $P < 0.003$ versus $Ldlr^{-/-}apob^{100/100}$ mice.
[f] $P < 0.002$ versus $Ldlr^{-/-}hl^{-/-}apob^{+/+}$ mice.
[g] $P < 0.01$ versus $Ldlr^{-/-}hl^{-/-}apob^{+/+}$ mice.

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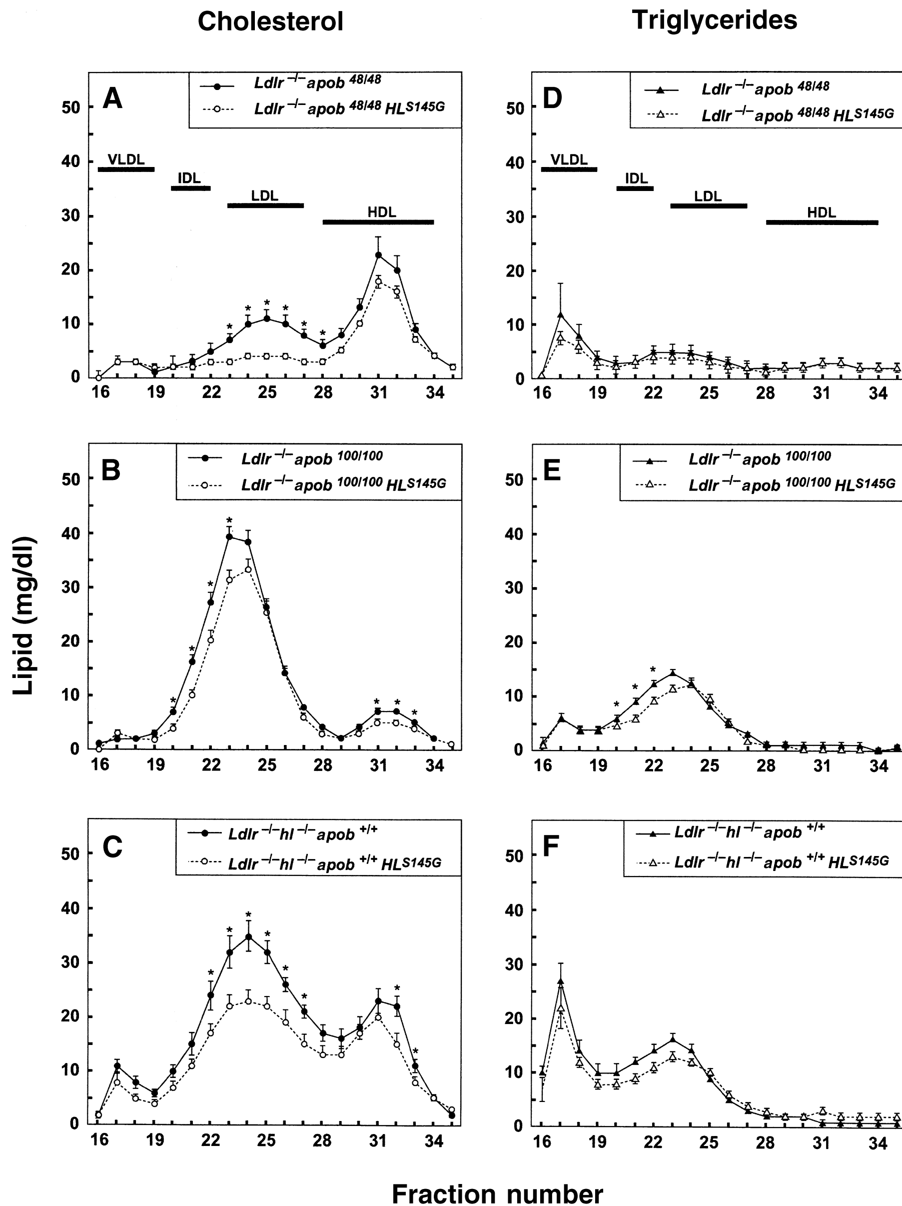

Fig. 1. Fast-protein liquid chromatography (FPLC) profiles of plasma from fasted *Ldlr*$^{-/-}$*apob*$^{48/48}$ (n = 4) and *Ldlr*$^{-/-}$*apob*$^{48/48}$ *HL*^{S145G} (n = 5), *Ldlr*$^{-/-}$*apob*$^{100/100}$ (n = 10) and *Ldlr*$^{-/-}$*apob*$^{100/100}$ *HL*^{S145G} (n = 13), and *Ldlr*$^{-/-}$*hl*$^{-/-}$*apob*$^{+/+}$ (n = 9) and *Ldlr*$^{-/-}$*hl*$^{-/-}$*apob*$^{+/+}$ *HL*^{S145G} (n = 6) male mice on a chow diet. A–C: Cholesterol concentrations; D–F: triglyceride concentrations. Plasma (100 μl) from each mouse was fractionated by Superose 6 chromatography, and fractions were assayed for cholesterol and triglycerides with standard colorimetric assays. Lipoprotein distributions are indicated with horizontal bars. Tracings represent the average ± SD of between four and thirteen individual FPLCs of each genotype.

fold) in all mice on the *Ldlr*$^{-/-}$*apob*$^{100/100}$ mice background. However, expression of ciHL reduced plasma cholesterol by 20% in *Ldlr*$^{-/-}$*apob*$^{100/100}$ *HL*^{S145G} mice compared with *Ldlr*$^{-/-}$*apo*$^{100/100}$ mice [1,019 ± 138 mg/dl in *Ldlr*$^{-/-}$*apob*$^{100/100}$ (n = 6) vs 808 ± 186 mg/dl in *Ldlr*$^{-/-}$*apob*$^{100/100}$ *HL*^{S145G} mice (n = 6), $P < 0.05$] (**Table 3**). The triglyceride levels did not change significantly (Table 3). Analysis of FPLC cholesterol profiles revealed a moderate VLDL peak, a large IDL/LDL peak, and a small HDL peak in *Ldlr*$^{-/-}$*apob*$^{100/100}$ mice (**Fig. 2A**).

In ciHL-expressing mice, IDL-C concentrations were reduced by 28% ($P < 0.04$) (Fig. 2A and Table 3). The FPLC triglyceride profile revealed a moderate peak in VLDL only (Fig. 2B). Lipoprotein triglyceride levels did not change significantly in *Ldlr*$^{-/-}$*apob*$^{100/100}$ *HL*^{S145G} mice (Fig. 2B and Table 3).

Ldlr$^{-/-}$*hl*$^{-/-}$ *apob*$^{+/+}$ *background.* The 2 week Western diet challenge increased plasma cholesterol 4–5-fold in all mice on the *Ldlr*$^{-/-}$*hl*$^{-/-}$*apob*$^{+/+}$ background (Table 3). Surprisingly, expression of ciHL had no significant effect on plasma cholesterol levels. The triglyceride levels were not changed significantly. The FPLC cholesterol profiles revealed a sharp, prominent VLDL cholesterol peak and a prominent IDL/LDL-C peak Expression of ciHL did not affect the lipo-

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TABLE 3. Plasma total and lipoprotein cholesterol (C) and triglyceride (T) concentrations on a Western diet

Lipids	$Ldlr^{-/-}apob^{100/100}$ (n = 6)	$Ldlr^{-/-}apob^{100/100}$ HL^{S145G} (n = 6)	$Ldlr^{-/-}hl^{-/-}$ (n = 7)	$apob^{+/+}Ldlr^{-/-}hl^{-/-}apob^{+/+}$ HL^{S145G} (n = 9)
TC	1019 ± 138	808 ± 186[a]	1778 ± 243	1563 ± 236
TG	218 ± 125	186 ± 93	490 ± 143	499 ± 134
VLDL-C	210 ± 42	149 ± 57	538 ± 122	595 ± 165
IDL-C	355 ± 72	257 ± 64[b]	334 ± 48	347 ± 69
LDL-C	279 ± 44	260 ± 42	446 ± 67	432 ± 61
HDL-C	70 ± 15	70 ± 14	123 ± 15	150 ± 13[d]
VLDL-T	158 ± 101	127 ± 53	339 ± 131	409 ± 151
IDL-T	29 ± 16	25 ± 12	37 ± 13	59 ± 17[c]
LDL-T	22 ± 14	24 ± 11	31 ± 11	51 ± 14[c]
HDL-T	3 ± 2	4 ± 1	6 ± 3	12 ± 4 d

Plasma TC, TG, and lipoprotein (C) and (T) concentrations are in mg/dl. Values given as mean ± SD.
[a] $P < 0.05$ vs $Ldlr^{-/-}apob^{100/100}$ mice.
[b] $P < 0.04$ vs $Ldlr^{-/-}apob^{100/100}$ mice.
[c] $P < 0.04$ vs $Ldlr^{-/-}hl^{-/-}apob^{+/+}$ mice.
[d] $P < 0.005$ vs $Ldlr^{-/-}hl^{-/-}apob^{+/+}$ mice.

protein distribution significantly, but was associated with an increased concentration of HDL-C (Fig. 2C, D and Table 3). The FPLC triglyceride profile revealed a prominent VLDL peak. Expression of ciHL did not decrease the VLDL triglyceride peak. In fact, triglyceride levels were generally higher in the $Ldlr^{-/-}hl^{-/-}apob^{+/+}$ HL^{S145G} mice when compared with $Ldlr^{-/-}hl^{-/-}apob^{+/+}$ mice.

Plasma apoB-48 and apoB-100 levels

We next sought to estimate the respective contributions of whole-particle uptake (reflected by similar decreases in both cholesterol and apoB-48 or apoB-100) and selective cholesteryl ester uptake (reflected by a decrease in cholesterol but minimal or no decrease in apoB-48 or apoB-100) to the ciHL-mediated lipoprotein reduction. To do so, we assessed plasma apoB-48 and apoB-100 levels by densitometric scanning of Western blots (**Table 4**). The changes in apoB-48 and apoB-100 were then compared with the changes in lipoprotein cholesterol.

$Ldlr^{-/-}apob^{48/48}$ background. Expression of ciHL reduced plasma apoB-48 levels by ~60% ($P < 0.01$) in chow-fed $Ldlr^{-/-}apob^{48/48}$ HL^{S145G} mice (in the presence of endogenous mouse HL). As expected, there was no apoB-100 in plasma from mice on the $Ldlr^{-/-}apob^{48/48}$ genetic background (Table 4).

$Ldlr^{-/-}apob^{100/100}$ background. Expression of ciHL reduced plasma apoB-100 by 27% ($P < 0.03$) in chow-fed $Ldlr^{-/-}apob^{100/100}$ HL^{S145G} mice (in the presence of endogenous mouse HL) (Table 4). Interestingly, expression of ciHL did not reduce apoB-100 levels in Western diet-fed $Ldlr^{-/-}apob^{100/100}$ HL^{S145G} mice (data not shown). [In fact apoB-100 levels were increased by ~16% (data not shown) despite a significant reduction in the IDL-C level (Table 3)]. As expected, there was no apoB-48 in plasma from mice on the $Ldlr^{-/-}apob^{100/100}$ genetic background.

$Ldlr^{-/-}hl^{-/-}apob^{+/+}$ background. Expression of ciHL reduced plasma apoB-48 levels by 14% ($P < 0.01$) in chow-fed $Ldlr^{-/-}hl^{-/-}apob^{+/+}$ HL^{S145G} mice (in the absence of endogenous HL) but did not affect apoB-48 levels in Western diet-fed $Ldlr^{-/-}hl^{-/-}apob^{+/+}$ HL^{S145G} mice (Table 4).

Plasma apoB-100 levels were not reduced significantly on either chow or Western diets in these mice (Table 4 and data not shown).

Plasma apoE levels

Next, using apoE as a marker of whole-particle uptake, we examined whether ciHL reduces cholesterol by mediating lipoprotein uptake.

$Ldlr^{-/-}apob^{48/48}$ background. Expression of ciHL reduced plasma apo-E levels (by ~30%, $P < 0.005$) in chow-fed $Ldlr^{-/-}apob^{48/48}$ HL^{S145G} mice (Table 4). However, this effect was lost when mice were fed a Western diet (data not shown).

$Ldlr^{-/-}apob^{100/100}$ background. Expression of ciHL also reduced plasma apoE levels (by 14%, $P < 0.03$) in chow-fed $Ldlr^{-/-}apob^{100/100}$ HL^{S145} mice (Table 4). However this effect was lost when mice were fed a Western diet (data not shown).

$Ldlr^{-/-}hl^{-/-}apob^{+/+}$ background. Likewise, expression of ciHL reduced plasma apoE levels (by 16%, $P < 0.01$) in chow-fed $Ldlr^{-/-}hl^{-/-}apob^{+/+}$ HL^{S145G} mice (Table 4). However, this effect was lost when mice were fed a Western diet (data not shown).

Plasma apoA-I levels

To estimate whether the ciHL-mediated reduction in HDL-C occurred by whole-lipoprotein uptake (reflected by simultaneous reductions in cholesterol and apoA-I) or by selective cholesteryl ester uptake (reflected by reduction in cholesterol and minimal or no reduction in apoA-I), we assessed plasma apoA-I levels by densitometric scanning of Western blots.

$Ldlr^{-/-}apob^{48/48}$ background. Expression of ciHL reduced plasma apoA-I by 40% in chow-fed $Ldlr^{-/-}apob^{48/48}$ mice (Table 4).

$Ldlr^{-/-}apob^{100/100}$ background. However, apoA-I was only slightly decreased in chow fed $Ldlr^{-/-}apob^{100/100}$ HL^{S145G} mice (Table 4). Western diet feeding did not reduce apoA-I levels in ciHL-expressing $Ldlr^{-/-}apob^{100/100}$ HL^{S145G} mice (data not shown).

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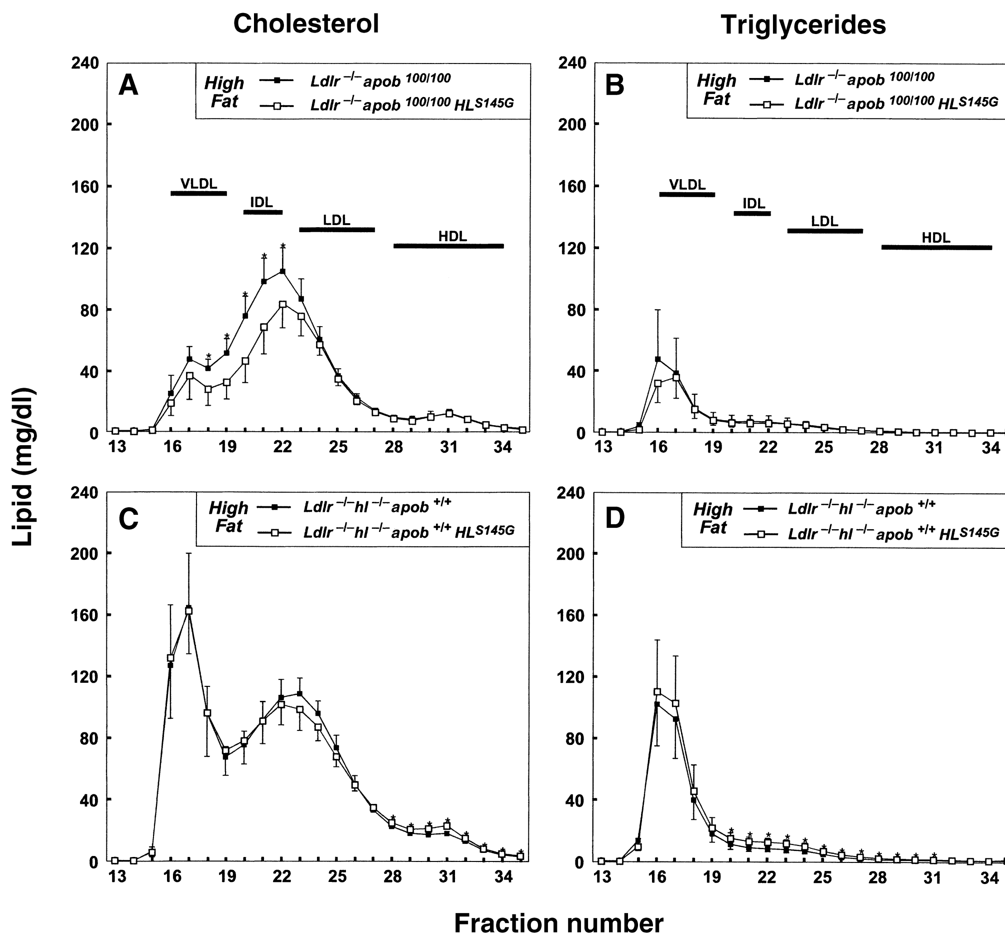

Fig. 2. FPLC profiles of plasma from fasted *Ldlr*$^{-/-}$*apob*$^{100/100}$ (n = 6) and *Ldlr*$^{-/-}$*apob*$^{100/100}$ *HL*^{S145G} (n = 6) and *Ldlr*$^{-/-}$*hl*$^{-/-}$ *apob*$^{+/+}$ (n = 7) and *Ldlr*$^{-/-}$*hl*$^{-/-}$*apob*$^{+/+}$ *HL*^{S145G} (n = 9) male mice on a cholesterol-enriched, high-fat (Western) diet. A and C: Cholesterol concentrations; B and D: triglyceride concentrations. Plasma (100 μl) from each mouse was fractionated by Superose 6 chromatography, and fractions were assayed for cholesterol and triglycerides with standard colorimetric assays. Lipoprotein distributions are indicated with horizontal bars. Tracings represent the average ± SD of between six and nine individual FPLCs of each genotype.

Ldlr$^{-/-}$*hl*$^{-/-}$*apob*$^{+/+}$ *background.* Likewise, apoA-I was only slightly decreased in chow-fed *Ldlr*$^{-/-}$*hl*$^{-/-}$*apob*$^{+/+}$ *HL*^{S145G} mice (Table 4). Western diet feeding did not reduce apoA-I levels in ciHL-expressing *Ldlr*$^{-/-}$*hl*$^{-/-}$*apob*$^{+/+}$ *HL*^{S145G} mice (data not shown).

DISCUSSION

Our studies in genetically modified mice demonstrate that the bridging function of HL enhances uptake of both apoB-48- and apoB-100-containing lipoproteins and suggest that this occurs by several mechanisms, including whole-particle uptake and selective cholesteryl ester uptake. In addition, our studies indicate that the bridging function is modulated by diet and endogenous (murine) HL in these mouse models.

In LDLR-deficient apoB-48-only mice, LDL-sized lipoproteins and plasma apoB-48 were each reduced by ~50–60%. Thus, apoB-48-containing lipoproteins are indeed substrates for ciHL. These results are consistent with and extend previous studies in apoE-deficient mice, which have high levels of apoB-48 (6, 29). Overexpression of ciHL (either transgenically or by gene transfer) in those

TABLE 4. Plasma apolipoprotein decrease in mice on a chow diet

Apolipoprotein	*Ldlr*$^{-/-}$*apob*$^{48/48}$ *HL*^{S145G}	*Ldlr*$^{-/-}$*apob*$^{100/100}$ *HL*^{S145G}	*Ldlr*$^{-/-}$*hl*$^{-/-}$*apob*$^{+/+}$ *HL*^{S145G}
apoB-48	60[b]	ND	14[f]
apoB-100	ND	27[d]	14
apoE	30[a]	14[e]	16[f]
apoA-I	40[c]	2	10

Data are expressed as percent of nontransgenic mice (matched for genetic background). ND, not detected.
[a] $P < 0.005$ vs *Ldlr*$^{-/-}$*apob*$^{48/48}$ mice.
[b] $P < 0.01$ vs *Ldlr*$^{-/-}$*apob*$^{48/48}$ mice.
[c] $P < 0.03$ vs *Ldlr*$^{-/-}$*apob*$^{48/48}$ mice.
[d] $P < 0.01$ vs *Ldlr*$^{-/-}$*apob*$^{100/100}$ mice.
[e] $P < 0.03$ vs *Ldlr*$^{-/-}$*apob*$^{100/100}$ mice.
[f] $P < 0.01$ vs *Ldlr*$^{-/-}$*hl*$^{-/-}$*apob*$^{+/+}$ mice.

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mice dramatically reduced levels of remnant- and LDL-sized lipoproteins (6, 29).

The similar reductions in lipoprotein-cholesterol and apoB-48 suggest removal by whole-lipoprotein uptake. One mechanism by which ciHL mediates whole-lipoprotein uptake may involve (ciHL-mediated) contact between apoB-48-containing lipoproteins and the LDLR-related protein (LRP), which then removes the lipoprotein via endocytosis (36, 37). This mechanism is supported by a simultaneous decrease in plasma apoE (by ~30%, $P < 0.005$), (another ligand for the LRP), in the *Ldlr*$^{-/-}$*apob*$^{48/48}$ *HL*^{S145G} mice.

The LRP removes mainly apoB-48-containing lipoproteins (38). In several animal models, expression of receptor-associated protein (which inhibits LRP-mediated endocytosis) resulted in massive accumulation of apoB-48-containing lipoproteins (31, 39). The removal of apoB-48-containing lipoproteins via the LRP is mediated by apoE (37). Consistent with this, we demonstrated similar decreases in apoE and apoB-48 in both *Ldlr*$^{-/-}$*apob*$^{48/48}$ *HL*^{S145G} mice and *Ldlr*$^{-/-}$*hl*$^{-/-}$*apob*$^{+/+}$ *HL*^{S145G} mice. Lipoprotein removal may also be enhanced by HSPG binding of lipoproteins (which serves both to concentrate them and to place them in proximity to receptors for uptake) (26, 40, 41). The HSPG-mediated removal mechanism of apoB-48-containing lipoproteins may include binding by apoE on the lipoprotein (37, 42). In addition to promoting receptor-mediated uptake, ciHL-mediated retention of lipoproteins at the cell surface likely facilitates selective cholesteryl ester uptake by the scavenger receptor BI (SR-BI) (43–46). Finally, an intriguing possibility is that cholesterol depletion (via selective cholesteryl ester uptake) optimizes the particle for removal, thus accounting for the similar reductions in cholesterol and apoB-48 in *Ldlr*$^{-/-}$*apob*$^{48/48}$ *HL*^{S145G} mice.

A majority of apoB-100-containing lipoproteins are removed via the LDLR. However, in the course of experiments with apoB-100-only mice, we uncovered a ciHL-mediated mechanism for the removal of apoB-100-containing lipoproteins that is independent of the LDLR. Also, unlike the situation in LDLR-mediated whole-particle removal, the LDL-R-independent, ciHL-mediated removal reduces cholesterol to a greater extent than apoB-100. Potential mechanisms to explain why the ciHL-mediated cholesterol reduction exceeds the apoB-100 reduction include preferential removal of larger, lipid-rich subclasses of lipoproteins (that have higher cholesteryl ester:apoB-100 ratios) and preferential removal of lipoproteins with a high cholesteryl ester:cholesterol ratio. These mechanisms would yield increased removal of cholesteryl ester relative to apoB-100. Although the apoB-100-containing particles also contain apoE, (and therefore can be removed by the LRP pathway), the contribution of apoE to the removal is small in this animal model. Specifically, apoE decreased by 14% (on chow diet) or not at all (on Western diet), whereas IDL-C decreased by 37% (on chow diet) and by 20% (on Western diet) in ciHL-expressing *Ldlr*$^{-/-}$*apob*$^{100/100}$ *HL*^{S145G} mice.

An additional mechanism of LDLR-independent lipoprotein removal is by selective cholesteryl ester uptake. Selective cholesteryl ester uptake is suggested as a one of the potential mechanisms here by findings in chow-fed *Ldlr*$^{-/-}$ *apob*$^{100/100}$ *HL*^{S145G} mice, in which the combined reduction of remnant- and LDL-C (by 54%) was twice that of the reduction in plasma apoB-100 (reduced by 27%). Also, in Western diet-fed *Ldlr*$^{-/-}$*apob*$^{100/100}$ *HL*^{S145G} mice, IDL-C decreased by 20%, whereas apoB-100 remained virtually unchanged. It is suggested that the apoB-100-containing lipoproteins (bound to cell surface HSPG) are presented to the SR-BI for selective cholesteryl ester uptake. This is a reasonable possibility, because SR-BI binds both native and modified LDL and mediates selective cholesterol uptake from those lipoproteins (47). The cholesterol-depleted particles may then be improved substrates for receptor-mediated or other uptake, thus explaining the reduced apolipoprotein levels.

Another explanation for the LDLR-independent lipoprotein removal is that ciHL-mediated retention of apoB-100-containing lipoproteins at the cell surface facilitates their removal by an HSPG-mediated pathway. This is supported by in vitro studies demonstrating that cells expressing either syndecan-1 (an HSPG core protein) or perlecan (a genetically distinct HSPG core protein) bound and internalized modified LDL (40, 41).

HDL-C levels were also reduced by ciHL, and this effect was modulated by endogenous mouse HL. In particular, ciHL expression in the presence of mouse HL reduced HDL-C levels in *Ldlr*$^{-/-}$*apob*$^{48/48}$ *HL*^{S145G} mice (by ~20%) and in *Ldlr*$^{-/-}$*apob*$^{100/100}$ *HL*^{S145G} mice (by 30%). It is possible that the greater HDL-C reduction in *Ldlr*$^{-/-}$ *apob*$^{100/100}$ *HL*^{S145G} mice reflects differences in particle affinity for ciHL; for instance, if apoB-48 has higher affinity for ciHL than apoB-100, there would be less ciHL available to bind (and remove) HDL in apoB-48-only mice than in apoB-100-only mice.

Surprisingly, ciHL expression in the absence of mouse HL failed to significantly reduce HDL-C in *Ldlr*$^{-/-}$*hl*$^{-/-}$ *apob*$^{+/+}$ *HL*^{S145G} mice. These results suggest a role for lipolytic modification (by endogenous mouse HL) in ciHL-mediated HDL removal.

Interestingly, apoA-I levels were reduced in *Ldlr*$^{-/-}$ *apob*$^{48/48}$ *HL*^{S145G} mice (by 40%) but not in *Ldlr*$^{-/-}$ *apob*$^{100/100}$ *HL*^{S145G} mice. Because HDL-C was reduced in both genetic backgrounds, these results indicate two different mechanisms for HDL removal for the two different genetic backgrounds, whole-particle uptake for the "apoB-48-only" mice and selective cholesterol uptake for the "apoB-100-only" mice.

We propose that cholesterol depletion of HDL by selective uptake may produce particles that are cleared more efficiently through other pathways (reflected by reductions in both HDL-C and apoA-I). These in vivo results confirm in vitro findings in several cell lines, including McArdle7777 rat hepatoma cells and HEK 293 cells, of a 3-fold increase in ^{125}I-labeled HDL uptake in the presence of ciHL, as compared with in its absence (28, 46). These results differ from the report of ciHL-mediated HDL reduction in (mouse) HL-deficient mice, in which high levels of ciHL were achieved by adenoviral-mediated gene

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transfer (48). However, these differences may be explained by background strain differences or by the additional deficiency of the LDLR in our study.

Our results also demonstrate that endogenous mouse HL is not absolutely necessary for the effect of ciHL on apoB-containing lipoproteins. We observed a 30% reduction in LDL-C and also a trend toward reduction in remnant-sized lipoproteins in $Ldlr^{-/-}hl^{-/-}apob^{+/+}$ HL^{S145G} mice (in the absence of endogenous mouse HL activity) (Tables 2–4). Thus, catalytic modification was not required for ciHL-mediated removal of LDL-C in this genetic background.

The LDL reduction observed in $Ldlr^{-/-}hl^{-/-}apob^{+/+}$ HL^{S145G} mice is compatible with human data in which LDL was not reduced in the presence of (reduced levels of) a naturally occurring mutant ciHL compared with in its absence (49). Although human HL deficiency is characterized by increased apoB-containing lipoproteins (and HDL) (and one might have expected reductions in these lipoproteins in the presence of ciHL compared with in its complete absence), the naturally occurring ciHL was reduced to one-fifth that of normal levels, which might explain the lack of an effect in these patients. Because in our study ciHL was increased (by ~20-fold) compared with normal levels, and because this increased ciHL reduced LDL, we conclude that ciHL-mediated LDL removal requires high levels of ciHL.

Our results demonstrate that the bridging function facilitates removal of both apoB-48- and apoB-100-containing lipoproteins and that their removal occurs by several different mechanisms. These mechanisms include whole-particle uptake and selective cholesteryl ester uptake. Also, our results show that the bridging function is modified by endogenous (murine) HL activity and diet.

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