Hepatic lipase facilitates the selective uptake of cholesteryl esters from remnant lipoproteins in apoE-deficient mice

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Abstract **We have investigated the role of hepatic lipase (HL) in remnant lipoprotein metabolism independent of lipolysis by using recombinant adenovirus to express native and catalytically inactive HL (HL-145G) in apolipoprotein (apo)E-deficient mice characterized by increased plasma concentrations of apoB-48-containing remnants. In the absence of apoE, the mechanisms by which apoB-48-containing remnants are taken up by either low density lipoprotein (LDL)-receptor or LDL-receptor-related protein (LRP) remain unclear. Overexpression of either native or catalytically inactive HL in apoE-deficient mice led to similar reductions ($P > 0.5$) in the plasma concentrations of cholesterol (41% and 53%) and non high density lipoprotein (HDL)-cholesterol (41% and 56%) indicating that even in the absence of lipolysis, HL can partially compensate for the absence of apoE in this animal model. Although the clearance of [^{3}H]cholesteryl ether from VLDL was significantly increased (approximately 2-fold; $P < 0.02$) in mice expressing native or inactive HL compared to luciferase controls, the fractional catabolic rates (FCR) of [^{125}I-labeled] apoB- very low density lipoprotein (VLDL) in all three groups of mice were similar ($P > 0.4$, all) indicating selective cholesterol uptake. Hepatic uptake of [^{3}H]cholesteryl ether from VLDL was greater in mice expressing either native HL (87%) or inactive HL-145G (72%) compared to luciferase controls (56%). Our combined findings are consistent with a role for HL in mediating the selective uptake of cholesterol from remnant lipoproteins in apoE-deficient mice, independent of lipolysis. These studies support the concept that hepatic lipase (HL) may serve as a ligand that mediates the interaction between remnant lipoproteins and cell surface receptors and/or proteoglycans. We hypothesize that one of these pathways may involve the interaction of HL with cell surface receptors, such as scavenger receptor (SR)-BI, that mediate the selective uptake of cholesteryl esters.—**Amar, M. J. A., K. A. Dugi, C. C. Haudenschild, R. D. Shamburek, B. Foger, M. Chase, A. Bensadoun, R. F. Hoyt, Jr., H. B. Brewer, Jr., and S. Santamarina-Fojo. **Hepatic lipase facilitates the selective uptake of cholesteryl esters from remnant lipoproteins in apoE-deficient mice.** ***J. Lipid Res.*** **1998.** 39: **2436–2442.**

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Hepatic lipase (HL) is a member of a family of structurally and functionally related enzymes that include pancreatic lipase and lipoprotein lipase (LPL). It is synthesized by hepatocytes and binds to endothelial cells lining the adrenals, ovaries, and the sinusoids of the liver (1, 2). HL hydrolyzes triglycerides and phospholipids present in intermediate density lipoproteins (IDL) and high density lipoproteins (HDL) and thus, through its function as a lipolytic enzyme, plays a major role in the metabolism of circulating plasma lipoproteins.

In addition to its enzymatic action as a lipase, several groups have proposed an additional role for HL in cellular lipoprotein metabolism. In 1975, Felts, Itakura, and Crane (3) first suggested that LPL might provide a recognition site for lipoprotein removal by a remnant receptor. Subsequent studies have provided in vitro evidence supporting a role for both LPL (4–7) and HL (8–19) in mediating the interaction of different lipoproteins with cell surface receptors and/or proteoglycans as well as facilitating the cellular uptake of lipoproteins and/or lipoprotein lipid. In LPL, this interaction appears to be independent of the catalytic activity of the enzyme (20).

HL has been shown to enhance the binding and/or uptake of chylomicrons, chylomicron remnants, or β-VLDL by isolated rat hepatocytes (9), HL-secreting hepatoma

Abbreviations: HL, hepatic lipase; LPL, lipoprotein lipase; pfu, plaque-forming units; HDL, high density lipoprotein; LDL, low density lipoprotein; IDL, intermediate density lipoprotein; VLDL, very low density lipoprotein; FCR, fractional catabolic rate; SR-BI, scavenger receptor BI; LRP, LDL receptor-related protein; apo, apolipoprotein.

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cells (11, 15), perfused rat liver (10) as well as human hepatoma, Chinese hamster ovary (CHO) (14, 16), and human fibroblast cells (14). The process appears to require proteoglycans (12, 14). HL-mediated enhanced uptake of these lipoproteins may, at least in part, be mediated by the low density lipoprotein receptor-related protein (LRP) which has been shown to bind to HL (12, 14). In addition, HL appears to promote the selective uptake of cholesteryl ester from HDL by perfused rat liver (8, 18), CHO cells expressing HL (13), and hepatoma cells (17).

Although these combined studies provide strong evidence for a role of HL and LPL in cellular lipoprotein metabolism, the in vivo relevance of the lipase-receptor/proteoglycan interactions have not been completely elucidated. In the present manuscript we evaluate the potential in vivo role for HL in remnant lipoprotein metabolism by expressing a catalytically inactive HL (HL-145G) in apoE-deficient mice (21) using recombinant adenovirus. We demonstrate that both native HL and HL-145G reduce the plasma concentrations of cholesterol present in circulating remnant lipoproteins, providing new evidence for an in vivo role of HL in lipoprotein metabolism independent of lipolysis. In addition, our studies indicate that HL facilitates the clearance of cholesteryl esters from the remnant lipoproteins, which accumulate in apoE-deficient mice, primarily through a selective uptake pathway.

MATERIALS AND METHODS

Generation of recombinant adenovirus

A full-length human native HL, mutant HL-145G (containing AGT→GGT substitution in the codon for the active serine 145) and luciferase cDNAs were subcloned into a shuttle vector (pAdl2-HL) containing CMV enhancer and promoter elements, as well as SV40 polyadenylation signal (22). Recombinant adenovirus was generated after cotransfection of pAdl2-HL and pJM17 (Ad5 genome) in 293 cells (23, 24), propagated in 293 cells and purified by cesium chloride density ultracentrifugation. The purified virus was then titered and diluted in 0.2% mouse albumin (Sigma Chemical Co., St. Louis, MO) before infusion into the animals.

ApoE-deficient mice

Male apoE-deficient mice (3 months of age, 25–30 g) obtained from the University of North Carolina (21) were bred and housed at the National Institutes of Health (NIH) under protocols approved by the Animal Care and Use Committee of the National Heart, Lung, and Blood Institute. Control mice received 8×10^8 pfu/ml of rLucif-AdV and experimental mice received 2.4×10^8 pfu/ml of rHL-AdV and 2.0×10^8 pfu/ml of rHL-145G-AdV by saphenous vein injection. Sham-injected animals, used as controls for the metabolic studies, received 50 μl of phosphate-buffered saline (PBS). After a 4-h fast, blood samples were collected from the retro-orbital plexus at days 0, 2, 4, 7, 10, 14, and 21 relative to adenovirus infusion. Blood samples were centrifuged at 2,500 *g* for 20 min at 4°C. Plasma was removed, aliquoted, immediately frozen on dry ice, and stored at −70°C.

Determination of HL and LPL mass and activity

Enzymatic activities were quantitated in triplicate using ^{14}C-labeled triolein (25) as previously described. HL mass was determined in triplicate by a sandwich enzyme-linked immunosorbent assay (26).

Plasma lipid, lipoprotein, and apolipoprotein analyses

Total cholesterol, triglycerides, phospholipids, and free cholesterol were assayed using enzymatic kits from Sigma Diagnostics (St. Louis, MO) and Wako Chemicals USA, Inc. (Richmond, VA) as described (27). HDL-cholesterol was determined as the cholesterol remaining in the plasma after precipitation of apoB-containing lipoproteins with either dextran sulfate (Ciba-Corning, Oberlin, OH) or heparin-calcium (28). Mouse apoA-I was quantitated by a sandwich ELISA using microtiter immunoassay plates (Immunlon 1, Dynatech Labs, Chantilly, VA) in which a rabbit anti-mouse apoA-I polyclonal antibody (ABE 1, University of Ottawa Heart Institute) was used for capture and a horseradish peroxidase-conjugated rabbit anti-mouse polyclonal antibody was used for detection. Quantitation of plasma apoB concentrations was performed in three separate studies by Western blotting followed by densitometric scanning. Briefly, 1 and 3 μl of individual ($n = 5$) mouse plasma as well as plasma pooled from 5 mice from each study group were applied to 4–20% Tris-glycine PAGE gels (Novex, San Diego, CA) in duplicate and transferred to Immobilon-P membranes (Millipore, Bedford, MA). The membranes were then incubated with a polyclonal rabbit anti-mouse apoB antibody (27) that reacts with both apoB-48 and apoB-100 followed by incubation with biotinylated goat anti-rabbit antibody (Vector Labs, Burlingame, CA) and developed by immunohistochemical staining (ABC Vectastain kit, Vector Labs, Burlingame, CA). Densitometric scanning was performed using the Molecular Dynamics Personal Densitometer SI (Molecular Dynamics, Sunnyvale, CA). Analyses of plasma lipoproteins by fast protein liquid chromatography (FPLC) were performed as described (27). In all studies, 50 μl of mouse pooled plasma was applied to FPLC. The elution volumes of the plasma lipoproteins separated by FPLC were VLDL, 15.0–16.0 ml; IDL/LDL, 20.0–24.0 ml; HDL, 30.0–31.0 ml.

VLDL metabolic studies

[^{3}H]CE-VLDL was generated as previously described (29) with the following modifications. VLDL ($d > 1.006$) was isolated from 2.5 ml of mouse plasma using a TLA-100.2 rotor in a TL-100 ultracentrifuge (Beckman Instruments, Palo Alto, CA) at 5°C, 95,000 rpm for 4 h. One hundred μCi of cholesteryl hexadecyl ether (cholesteryl-[1,2, ^{3}H (N)]palmityl ether) (40–60 Ci/mmol; New England Nuclear, Boston, MA) was dried under nitrogen, resuspended in 10 μl of absolute ethanol, and added dropwise over 3 min to the lipoprotein solution (1.05 mg/ml protein concentration) while gently shaking with interruptions every 45 sec for brief (1 sec) vortexing as previously described (29). ^{125}I-labeled mouse apoB was prepared by a modification of the iodine monochloride method (30) and 0.7–1.0 mg of VLDL was labeled with ^{125}I with an efficiency of 29–37%. Approximately 0.5 mol of iodine was incorporated per mol of protein. [^{3}H]CE-VLDL and [^{125}I]-labeled apoB-VLDL were then reisolated by ultracentrifugation ($d < 1.006$) and dialyzed overnight against $1 \times$ PBS, 0.01% EDTA. The final lipoprotein sample preparations were then analyzed by FPLC and agarose gel electrophoresis to ensure integrity of the VLDL particle. Approximately 47% of the [^{3}H]CE was recovered in the VLDL after labeling. Homogeneous labeling of VLDL was ascertained by superimposing radioactivity and chemistry profiles obtained by FPLC and agarose gels. [^{3}H]CE-VLDL (8×10^5 dpm) and ^{125}I-labeled apoB-VLDL (1×10^6 dpm) were injected into the saphenous veins of two different groups of apoE-deficient mice 5 days after infusion of either rHL-AdV, rHL-145G-AdV, or rLucif-AdV. For quantitation of ^{125}I-labeled apoB-VLDL, 3 μl of mouse plasma was fractionated by SDS-

PAGE using 4–15% gradient gels (31) and the apoB bands were excised and analyzed for their radioactive content (Cobra Autogama, Packard Instrument Co., Downers Grove, IL). Greater than 95% of counts remained associated with VLDL 5 min, 24 h, and 48 h after injection of ^{125}I-labeled apoB-VLDL as determined by FPLC analysis of plasma lipoproteins. Plasma decay curves for [^{3}H]CE-VLDL were generated by dividing the plasma radioactivity at each time point by the plasma radioactivity at the initial 1-min time point, which was the same among the study groups (all; $P > 0.4$). The fractional catabolic rate was determined from the area under the plasma radioactivity curves using a multiexponential curve fitting technique on the SAAM program (32).

After the [^{3}H]CE VLDL study was completed, a subset of mice was perfused with cold 0.15 M NaCl and livers, heart, lung, spleen, kidneys, adrenals, and ovaries were harvested, extracted in 20 volumes of chloroform–methanol 2:1 (v/v) and the phases were separated by the addition of water (33). Aliquots of the lower phase were counted in a Tri-Carb 2500 TR liquid scintillation counter (Packard Instrument Co., Downers Grove, IL) as previously described (34). Mean recoveries at 60 min were >95% of counts for all groups with no statistical differences among the groups ($P > 0.5$). Plasma and liver counts accounted for >90% of total tissue plus plasma radioactivity.

Statistical analysis

Values are reported as mean ± SEM. Comparisons between groups of mice were made using the Student's *t* test for independent samples (two-tailed).

RESULTS

Table 1 summarizes the plasma lipid, lipoprotein, apolipoprotein, and human HL concentrations as well as HL activity in apoE-deficient mice before and 4 days after infusion of recombinant adenovirus. Infusion of rHL-AdV and rHL-145G-AdV resulted in the expression of similar levels (8 ± 1 μg/ml) of human HL in apoE-deficient mice. As expected, animals injected with rHL-AdV but not rHL-145G-AdV had an increase in plasma post-heparin HL activity. Mice expressing similar levels of either native HL or catalytically inactive HL-145G had significant reductions ($P < 0.001$) in their baseline levels of cholesterol, triglycerides, phospholipids, and cholesteryl esters. However, plasma HDL-cholesterol and apoA-I were reduced ($P < 0.001$) only in mice expressing the native HL and not the catalytically inactive HL-145G ($P > 0.5$) indicating a different effect of these two functionally distinct lipases on HDL-cholesterol metabolism. Expression of luciferase had no effect on the plasma lipid profile of apoE-deficient mice.

Analysis of the plasma lipoproteins by FPLC (**Fig. 1**) demonstrated that the majority of the reduction in the plasma total cholesterol concentrations reflected a decrease in the cholesterol present in the VLDL, IDL, and LDL of mice expressing either the native HL (panel A) or catalytically inactive HL-145G (panel B). However, analysis of the plasma apoB concentrations by immunoblotting, followed by scanning and densitometric quantitation, demonstrated no significant decrease ($P > 0.08$) in the plasma apoB concentrations in apoE-deficient mice after infusion of either rHL-AdV, rHL-145G-AdV, or rLucif-AdV (day 0; 10 ± 2, 11 ± 2, 10 ± 5 and day 4; 11 ± 5, 9 ± 1, 12 ± 2 arbitrary units, respectively). These data indicate that expression of either active HL or catalytically inactive HL-145G in apoE-deficient mice resulted in a selective decrease in the lipids present in the apoB-containing lipoproteins.

To further investigate these findings, the clearance of autologous ^{125}I-labeled apoB-VLDL in mice 4 days after infusion of recombinant adenovirus was determined. As illustrated in **Fig. 2A**, no significant difference ($P > 0.4$) was observed in the removal of ^{125}I-labeled apoB-VLDL among mice expressing native-HL, catalytically inactive HL-145G, and luciferase (FCR in d^{-1} = 2.15 ± 0.16, 2.14 ± 0.11, and 1.99 ± 0.13, respectively; $P > 0.4$ all). Thus, consistent with the plasma apoB quantitation data, expression of either active or inactive HL in apoE-deficient mice appeared to have no significant impact on the clearance of apoB-VLDL.

In contrast, the plasma decay curves of mice injected with [^{3}H]VLDL-cholesteryl ether demonstrated enhanced clearance of the radiolabeled lipid in mice expressing native-HL and inactive HL-145G compared to that of the luciferase controls (Fig. 2B). The FCR of [^{3}H]VLDL-cholesteryl ether in apoE-deficient mice expressing either native

TABLE 1. Concentration of the plasma lipids, lipoproteins, apolipoproteins, and HL in apoE-deficient mice before and after infusion of recombinant adenovirus

	HL Activity	HL Concentration	TC	TG	PL	CE	Non HDL-C	HDL-C	ApoA-I
	μmol/min/ml	*μg/ml*				*mg/dl*			
rHL-Adv (n = 12)									
Pre-Tx	0.6 ± 0.03	—	769 ± 20	207 ± 22	431 ± 12	517 ± 19	743 ± 23	26 ± 3	46 ± 4
Post-Tx	10 ± 4	8 ± 1	452 ± 21[a]	53 ± 8[a]	221 ± 14[a]	311 ± 14[a]	438 ± 22[a]	14 ± 2[a]	18 ± 2[a]
rHl145G-Adv(n = 10)									
Pre-Tx	0.4 ± 0.04	—	663 ± 20	227 ± 34	379 ± 26	441 ± 21	641 ± 22	22 ± 4	33 ± 14
Post-Tx	0.7 ± 0.01	8 ± 1	310 ± 26[a]	64 ± 16[a]	193 ± 11[a]	199 ± 19[a]	285 ± 31[a]	25 ± 3	43 ± 13
rLucif-Adv (n = 11)									
Pre-Tx	0.6 ± 0.02	—	717 ± 44	231 ± 38	440 ± 29	476 ± 29	697 ± 44	20 ± 3	29 ± 4
Post-Tx	0.6 ± 0.02	—	735 ± 58	236 ± 14	471 ± 44	471 ± 44	711 ± 58	24 ± 4	32 ± 3

TC, total cholesterol; TG, triglycerides; PL, phospholipids; CE, cholesteryl ester; HDL-C, high density lipoprotein cholesterol; nonHDL-C, non-high density lipoprotein cholesterol. Data are expressed as mean ± SEM.

[a] $P < 0.001$ (compared to Pre-Tx).

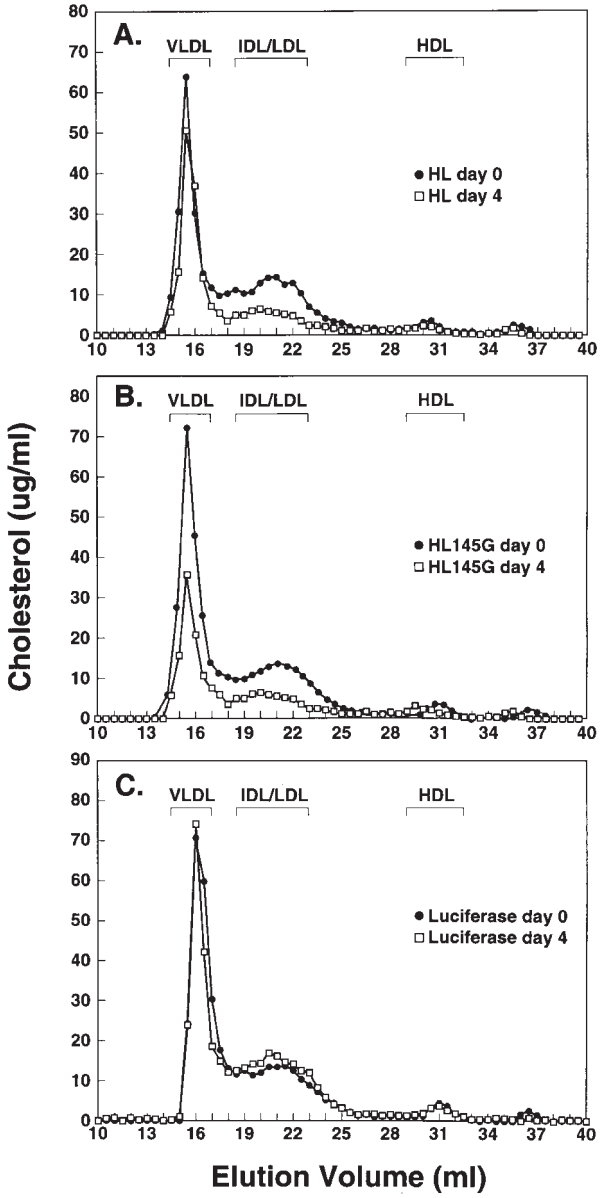

Fig. 1. FPLC analysis of the plasma lipoproteins in apoE-deficient mice before and 4 days after infusion of recombinant adenovirus. The cholesterol distribution in the plasma lipoproteins of apoE-deficient mice before (Pre-Tx; ●) and after (□) injection of recombinant adenovirus expressing either native HL (panel A), catalytically inactive HL-145G (panel B), and luciferase (panel C) is illustrated. For each of the three study groups, 50 μl of pooled plasma from five mice (10 μl each) was applied to the FPLC.

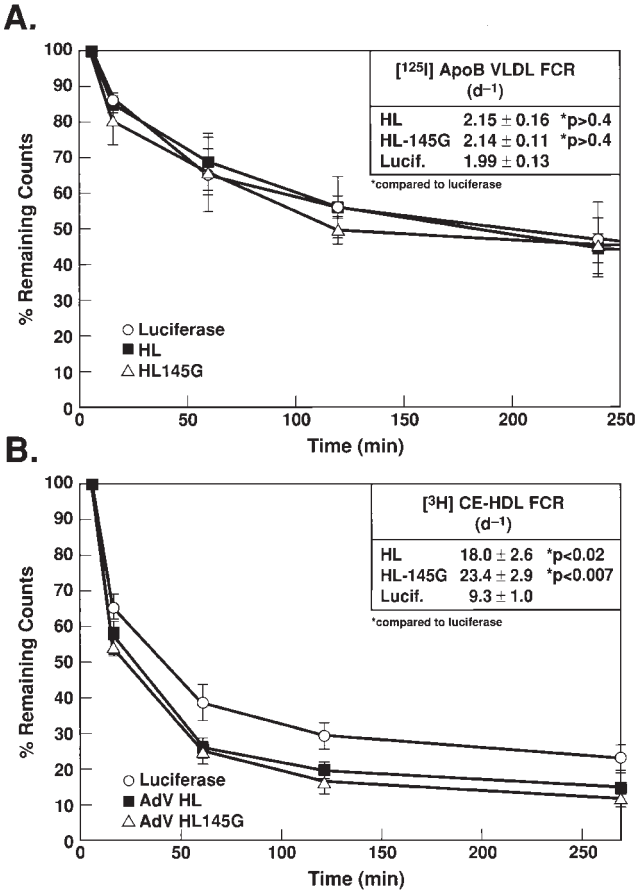

Fig. 2. In vivo metabolism of ^{125}I-labeled apoB-VLDL and [^{3}H]CE-VLDL in apoE-deficient mice injected with rHL-AdV, rHL-145G-AdV, and rLucif-AdV. Autologous apoE deficient mouse VLDL was isolated and radiolabeled, as described in Methods. The clearance of ^{125}I-labeled apoB-VLDL (panel A; n = 9 each group)and of [^{3}H]CE-VLDL (panel B; n = 5 each group) in apoE-deficient mice 4 days after infusion of recombinant adenovirus expressing native HL (■), catalytically inactive HL (△), and luciferase (○) are illustrated. FCR are shown in the insets.

HL or catalytically inactive HL-145G was increased by approximately 2-fold ($P < 0.02$, all) over that of mice expressing luciferase (FCR in d^{-1} = 18.0 ± 2.6, 23.4 ± 2.9, and 9.3 ± 1.0, respectively). In the luciferase expressing group, the percent of remaining counts in plasma for [^{3}H]CE was less (40%) than for ^{125}I-labeled apoB (70%). These findings are consistent with the faster FCR of cholesteryl ester (35) compared to that of apoB-containing lipoproteins (36) which reflects the independent metabolic pathway of cholesteryl ester and apoB-containing particles.

To control for potential effects on the metabolism of VLDL by the systemic infusion of recombinant adenovirus, we performed similar turnover studies in sham-injected mice infused with PBS as well as mice injected with rLucif-AdV. The clearance of both, ^{125}I-labeled apoB-VLDL and [^{3}H]VLDL-cholesteryl ether was similar in mice receiving rLucif-AdV or PBS (**Fig. 3**, panels A and B) demonstrating that infusion of adenovirus alone did not alter VLDL metabolism.

Finally, the accumulation of [^{3}H]cholesteryl ether in the liver of mice injected with either rHL-AdV or rHL-145G-AdV (87 ± 2% and 72 ± 2% of total plasma and liver counts, respectively) was increased ($P < 0.0005$) compared to that of mice injected with rLucif-AdV (56 ± 1% of total counts). Conversely, a larger quantity of [^{3}H]VLDL-cholesteryl ether ($P < 0.0005$) remained in the plasma of mice expressing luciferase (44 ± 1%) compared to mice expressing either native HL (13 ± 2%) or HL-145G (28 ± 2%) indicating enhanced hepatic delivery of cholesteryl ether from the VLDL of mice expressing either enzyme compared to luciferase controls.

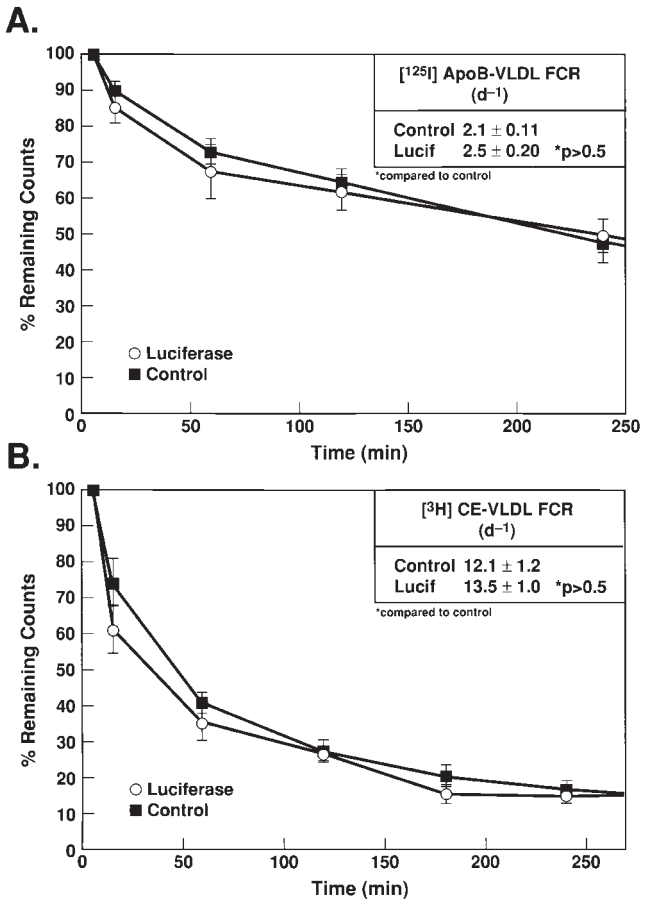

Fig. 3. The clearance of ^{125}I-labeled apoB-VLDL (panel C; n = 3 each group) and of [^{3}H]CE-VLDL (panel D; n = 3 each group) in apoE-deficient mice that were either sham-injected with PBS (panel A; ■) or infused with rLucif-AdV (panel B; ○) is illustrated. Fractional catabolic rates (FCR) are shown in the insets.

DISCUSSION

In addition to its classic function as a lipolytic enzyme, HL has been proposed to play a major role in cellular lipoprotein metabolism by mediating the interaction of different lipoproteins with cell surface receptors and/or proteoglycans. Although in vitro studies provide evidence supporting a ligand role for HL in cellular lipoprotein metabolism, the physiological relevance of HL-mediated uptake of lipoproteins and/or lipoprotein lipids to lipoprotein metabolism in vivo remains to be elucidated. We have recently demonstrated that transient expression of different lipases using recombinant adenovirus permits the investigation of specific biochemical pathways in vivo (27, 37, 38). In the present study we evaluate the potential in vivo role of HL in remnant lipoprotein metabolism, independent of its classic lipolytic function, by utilizing recombinant adenovirus to express native and catalytically inactive HL in apoE-deficient mice.

ApoE-deficient mice accumulate cholesterol-rich remnants that contain primarily apoB-48 as their major apolipoprotein (21). We thus investigated whether in the absence of apoE, HL could mediate the clearance of apoB-48-containing remnants, in vivo. Overexpression of native HL decreased the baseline total cholesterol and nonHDL-cholesterol by 41% and 42%, respectively, indicating that HL can partially compensate for the absence of apoE. Furthermore, the lipolytic function of HL was not required for the reduction in remnant lipoprotein cholesterol observed in apoE-deficient mice. Thus, compared to mice expressing native HL, mice injected with the catalytically inactive rHL-145G-AdV had a similar reduction in plasma cholesterol (41% vs. 53%) and non-HDL-cholesterol (42% vs. 55%) concentrations. These combined results indicate that HL may not only enhance remnant uptake by exposing apoE as a ligand for receptor-mediated uptake as previously proposed (39), but in addition, the enzyme may play a more direct role in remnant lipoprotein metabolism that is independent of both lipolysis and apoE. Although hydrolysis of triglycerides by HL appears to play an important role in chylomicron remnant metabolism (40–43), lipolysis may not be as important for the clearance of the cholesterol-rich and triglyceride-poor apoB-48-containing remnants that accumulate in apoE-deficient mice (21). In contrast, catalytic activity appears to be critical for HL-mediated HDL metabolism in vivo, as in the absence of lipolysis HL-145G could not induce plasma HDL-cholesterol lowering. These findings have been confirmed in an independent mouse model, the HL-deficient mouse, which accumulates HDL instead of remnant lipoproteins (44) as well as in transgenic apoE-deficient mice that overexpress catalytically inactive HL-145G (45).

The mechanism leading to HL-mediated enhanced remnant cholesterol clearance in apoE-deficient mice was further investigated. Analysis of the plasma lipid profile indicated that despite significant reduction in the baseline non-HDL-cholesterol concentrations, plasma apoB levels were not decreased by expression of either native or catalytically inactive HL, a finding that suggests HL-mediated selective lipid uptake. This was confirmed by metabolic studies that determined the plasma clearance of ^{125}I-labeled apoB-VLDL and [^{3}H]CE-VLDL. The FCRs of apoB-VLDL in mice expressing either native or catalytically inactive HL were similar to those of mice expressing the irrelevant luciferase transgene or sham-injected apoE-deficient mouse controls. In contrast, the clearance of [^{3}H]cholesteryl ether from VLDL was increased in mice expressing either native or catalytically inactive HL compared to luciferase controls. As a result of these changes the liver uptake of [^{3}H]cholesteryl ether from VLDL was significantly greater in mice expressing native HL (87%) and inactive HL-145G (72%) compared to luciferase controls (56%). Thus, in the absence of lipolysis and significant reduction in plasma apoB concentrations, HL enhanced the delivery of VLDL-cholesteryl ester to the liver in apoE-deficient mice.

The ability to detect HL-mediated selective lipid uptake from remnant lipoproteins was, most likely, enhanced by our selection of the apoE-deficient animal model for these studies. In the absence of apoE, the clearance of apoB-48-containing remnant lipoproteins by either the LDL receptor or LRP should be impaired and the relative

contribution of other uptake pathways may be increased. Previous studies have demonstrated that HL may enhance the cellular uptake of remnants by LRP in vitro (9–11, 15). Our findings are most consistent with an important role of HL in facilitating the selective uptake of cholesteryl ester from remnant lipoproteins in apoE-deficient mice. The selective removal of cholesteryl ester from apoB-containing lipoproteins has been demonstrated in different cultured cell systems (46–50). However, HL has not been previously implicated in this process. HL-mediated selective cholesterol uptake may thus represent an additional and important pathway for remnant lipoprotein cholesterol metabolism in apoE-deficient mice.

One potential mechanism by which HL may enhance the selective uptake of cholesterol as well as other lipids from remnant lipoproteins is by interacting with the recently described scavenger receptor BI (SR-BI) (51). SR-BI has been shown to mediate cellular selective lipid uptake from HDL (52) and to bind with high affinity not only to HDL but also to modified as well as native LDL (52). Some (53) but not all of the specific ligand(s) for SR-BI have been identified. It is possible that, similar to LRP, HL may interact with SR-BI, and thus facilitate the selective uptake of cholesterol from remnant lipoproteins.

Interestingly, both active and inactive HL reduced the plasma concentrations of phospholipids and triglycerides, in addition to cholesteryl esters, in apoE-deficient mice. These findings are consistent with the concept that the uptake of cholesteryl ester, as mediated by selective receptors such as SR-BI, may also be accompanied by the uptake of other lipoprotein lipids as demonstrated by SR-BI-mediated enhanced DiI-HDL lipid uptake (52, 54). Alternatively, HL-mediated selective cholesteryl ester uptake may lead to enhanced lipolysis of remodeled remnants by endogenous mouse HL and LPL, a process that would result in alteration of the lipid content of the particles without changes in the clearance of apoB-containing remnants. Unlike remnant lipoproteins, the inability of the catalytically inactive HL-145G to lower HDL cholesterol levels suggests that lipolysis may be a prerequisite for HL-mediated enhanced selective uptake of HDL cholesteryl esters by SR-BI in apoE-deficient mice. In addition, other as yet undescribed selective lipid uptake receptors, with different lipoprotein specificities, could also be implicated.

Our combined findings are consistent with a role of HL in mediating the selective uptake of lipids from remnant lipoproteins in apoE-deficient mice independent of lipolysis. These studies support the concept that in addition to its function as a lipolytic enzyme, HL serves as a ligand that mediates the interaction between different lipoproteins and cell surface receptors and/or proteoglycans. We hypothesize that one of these pathways may involve the interaction of HL with cell surface receptors, such as SR-BI, that mediate the selective uptake of lipoprotein lipids.

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REFERENCES

1. Kuusi, T., E. A. Nikkla, I. Virtanen, and P. K. Kinnunen. 1979. Localization of the heparin-releasable lipase in situ in the rat liver. *Biochem. J.* **181:** 245–256.
2. Doolittle, M. H., H. Wong, R. C. Davis, and M. C. Schotz. 1987. Synthesis of hepatic lipase in liver and extrahepatic tissues. *J. Lipid Res.* **28:** 1326–1334.
3. Felts, J. M., H. Itakura, and R. T. Crane. 1975. The mechanism of assimilation of constituents of chylomicrons, very low density lipoproteins and remnants—a new theory. *Biochem. Biophys. Res. Commun.* **66:** 1467–1475.
4. Beisiegel, U., W. Weber, and G. Bengtsson-Olivecrona. 1991. Lipoprotein lipase enhances the binding of chylomicrons to low density lipoprotein receptor-related protein. *Proc. Natl. Acad. Sci. USA.* **88:** 8342–8346.
5. Chappell, D. A., G. L. Fry, M. A. Waknitz, L. E. Muhonen, M. W. Pladet, P. H. Iverius, and D. K. Strickland. 1993. Lipoprotein lipase induces catabolism of normal triglyceride-rich lipoproteins via the low density lipoprotein receptor-related protein/α_2-macroglobulin receptor in vitro. *J. Biol. Chem.* **268:** 14168–14175.
6. Mulder, M., P. Lombardi, H. Jansen, T. J. C. Van Berkel, R. R. Frants, and L. M. Havekes. 1993. Low density lipoprotein receptor internalizes low density and very low density lipoproteins that are bound to heparan sulfate proteoglycans via lipoprotein lipase. *J. Biol. Chem.* **268:** 9369–9375.
7. Rumsey, S. C., J. C. Obunike, Y. Arad, R. J. Deckelbaum, and I. J. Goldberg. 1992. Lipoprotein lipase-mediated uptake and degradation of low density lipoproteins by fibroblasts and macrophages. *J. Clin. Invest.* **90:** 1504–1512.
8. Marques-Vidal, P., C. Azéma, X. Collet, C. Vieu, H. Chap, and B. Perret. 1994. Hepatic lipase promotes the uptake of HDL esterified cholesterol by the perfused rat liver: a study using reconstituted HDL particles of defined phospholipid composition. *J. Lipid Res.* **35:** 373–384.
9. Diard, P., M-I. Malewiak, D. Lagrange, and S. Griglio. 1994. Hepatic lipase may act as a ligand in the uptake of artificial chylomicron remnant-like particles by isolated rat hepatocytes. *Biochem. J.* **299:** 889–894.
10. Shafi, S., S. E. Brady, A. Bensadoun, and R. J. Havel. 1994. Role of hepatic lipase in the uptake and processing of chylomicron remnants in rat liver. *J. Lipid Res.* **35:** 709–720.
11. Ji, Z-S., S. J. Lauer, S. Fazio, A. Bensadoun, J. M. Taylor, and R. W. Mahley. 1994. Enhanced binding and uptake of remnant lipoproteins by hepatic lipase-secreting hepatoma cells in culture. *J. Biol. Chem.* **269:** 13429–13436.
12. Kounnas, M. Z., D. A. Chappell, H. Wong, W. S. Argraves, and D. K. Strickland. 1995. The cellular internalization and degradation of hepatic lipase is mediated by low density lipoprotein receptor-related protein and requires cell surface proteoglycans. *J. Biol. Chem.* **270:** 9307–9312.
13. Komaromy, M., S. Azhar, and A. D. Cooper. 1996. Chinese hamster ovary cells expressing a cell surface-anchored form of hepatic lipase. *J. Biol. Chem.* **271:** 16906–16914.
14. Krapp, A., S. Ahle, S. Kersting, Y. Hua, K. Kneser, M. Nielsen, J. Gliemann, and U. Beisiegel. 1996. Hepatic lipase mediates the uptake of chylomicrons and β-VLDL into cells via the LDL receptor-related protein (LRP). *J. Lipid Res.* **37:** 926–936.
15. Ji, Z-S., H. L. Dichek, R. D. Miranda, and R. W. Mahley. 1997. Heparan sulfate proteoglycans participate in hepatic lipase and apolipoprotein E-mediated binding and uptake of plasma lipoproteins, including high density lipoproteins. *J. Biol. Chem.* **272:** 31285–31292.
16. Choi, S. Y., M. C. Komaromy, J. Chen, L. G. Fong, and A. D. Cooper. 1994. Acceleration of uptake of LDL but not chylomicrons or chylomicron remnants by cells that secrete apoE and hepatic lipase. *J. Lipid Res.* **35:** 848–859.
17. Bamberger, M., J. M. Glick, and G. H. Rothblat. 1983. Hepatic lipase stimulates the uptake of high density lipoprotein cholesterol by hepatoma cells. *J. Lipid Res.* **24:** 869–876.
18. Kadowaki, H., G. M. Patton, and S. J. Robins. 1992. Metabolism of high density lipoprotein lipids by the rat liver: evidence for participation of hepatic lipase in the uptake of cholesteryl ester. *J. Lipid Res.* **33:** 1689–1698.
19. Collet, X., B. P. Perret, G. Simard, C. Vieu, and L. Douste-Blazy. 1990. Behaviour of phospholipase-modified HDL towards cultured hepatocytes. I. Enhanced transfers of HDL sterols and apoproteins. *Biochim. Biophys. Acta.* **1043:** 301–310.

20. Krapp, A., H. Zhang, D. Ginzinger, M-S. Liu, A. Lindberg, G. Olivecrona, M. R. Hayden, and U. Beisiegel. 1995. Structural features in lipoprotein lipase necessary for the mediation of lipoprotein uptake into cells. *J. Lipid Res.* **36:** 2362–2373.
21. Zhang, S. H., R. L. Reddick, J. A. Piedrahita, and N. Maeda. 1992. Spontaneous hypercholesterolemia and arterial lesions in mice lacking apolipoprotein E. *Science.* **258:** 468–471.
22. McKinnon, R. D., S. Bacchetti, and F. L. Graham. 1982. Tn5 mutagenesis of the transforming genes of human adenovirus type 5. *Gene.* **19:** 33–42.
23. McGrory, W. J., D. S. Bautista, and F. L. Graham. 1988. A simple technique for the rescue of early region 1 mutations into infectious human adenovirus type 5. *Virology.* **163:** 614–617.
24. Harrison, T., F. L. Graham, and J. Williams. 1977. Host-range mutants of adenovirus type 5 defective for growth in HeLa cells. *Virology.* **77:** 319–329.
25. Iverius, P-H., and J. D. Brunzell. 1985. Human adipose tissue lipoprotein lipase: changes with feeding and relation to postheparin plasma enzyme. *Am. J. Physiol.* **249:** E107–E114.
26. Bensadoun, A. 1996. Sandwich-immunoassay for the measurement of human hepatic lipase. *Methods Enzymol.* **263:** 333–338.
27. Dugi, K. A., B. L. Vaisman, N. Sakai, C. L. Knapper, S. M. Meyn, H. B. Brewer, Jr., and S. Santamarina-Fojo. 1997. Adenovirus-mediated expression of hepatic lipase in LCAT transgenic mice. *J. Lipid Res.* **38:** 1822–1832.
28. Burstein, M., H. R. Scholnick, and R. Morfin. 1970. Rapid method for the isolation of lipoproteins from human serum by precipitation with polyanions. *J. Lipid Res.* **11:** 583–595.
29. Shamburek, R. D., P. G. Pentchev, L. A. Zech, J. Blanchette-Mackie, E. D. Carstea, J. M. VandenBroek, P. S. Cooper, E. B. Neufeld, R. D. Phair, H. B. Brewer, Jr., R. O. Brady, and C. C. Schwartz. 1997. Intracellular trafficking of the free cholesterol derived from LDL cholesteryl ester is defective in vivo in Niemann-Pick C disease: insights on normal metabolism of HDL and LDL gained from the NP-C mutation. *J. Lipid Res.* **38:** 2422–2435.
30. McFarlane, A. S. 1958. Efficient trace-labeling of proteins with iodine. *Nature.* **182:** 53–57.
31. Brousseau, M. E., S. Santamarina-Fojo, B. L. Vaisman, D. Applebaum-Bowden, A. M. Berard, G. D. Talley, H. B. Brewer, Jr., and J. M. Hoeg. 1997. Overexpression of human lecithin:cholesterol acyltransferase in cholesterol-fed rabbits: LDL metabolism and HDL metabolism are affected in a gene dose-dependent manner. *J. Lipid Res.* **38:** 2537–2547.
32. Guyton, J. R., G. H. Dahlen, W. Patsch, J. A. Kautz, and A. M. Gotto, Jr. 1985. Relationship of plasma lipoprotein Lp[a] levels to race and to apolipoprotein B. *Arteriosclerosis.* **5:** 265–272.
33. Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226:** 497–509.
34. Foger, B., S. Santamarina-Fojo, R. D. Shamburek, C. L. Parrott, G. D. Talley, and H. B. Brewer, Jr. 1997. Plasma phospholipid transfer protein: adenovirus-mediated overexpression in mice leads to decreased plasma high density lipoprotein (HDL) and enhanced hepatic uptake of phospholipids and cholesteryl esters from HDL. *J. Biol. Chem.* **43:** 27393–27400.
35. Schwartz, C. C., L. A. Zech, J. M. VandenBroek, and P. S. Cooper. 1993. Cholesterol kinetics in subjects with bile fistula: positive relationship between size of the bile acid precursor pool and bile acid synthetic rate. *J. Clin. Invest.* **91:** 923–938.
36. Ikewaki, K., M. Nishiwaki, T. Sakamoto, T. Ishikawa, T. Fairwell, L. A. Zech, M. Nagano, H. Nakamura, H. B. Brewer, Jr., and D. J. Rader. 1995. Increased catabolic rate of low density lipoproteins in humans with cholesteryl ester transfer protein deficiency. *J. Clin. Invest.* **96:** 1573–1581.
37. Applebaum-Bowden, D., J. Kobayashi, V. S. Kashyap, D. R. Brown, A. M. Berard, S. Meyn, C. Parrott, N. Maeda, R. D. Shamburek, H. B. Brewer, Jr., and S. Santamarina-Fojo. 1996. Hepatic lipase gene therapy in hepatic lipase-deficient mice: adenovirus-mediated replacement of a lipolytic enzyme to the vascular endothelium. *J. Clin. Invest.* **97:** 799–805.
38. Kobayashi, J., D. Applebaum-Bowden, K. A. Dugi, D. Brown, V. Kashyap, C. Parrott, N. Maeda, H. B. Brewer, Jr., and S. Santamarina-Fojo. 1996. Analysis of protein structure–function in vivo: adenovirus-mediated transfer of lipase mutants in hepatic lipase deficient mice. *J. Biol. Chem.* **271:** 26296–26301.
39. Brasaemle, D. L., K. Cornely-Moss, and A. Bensadoun. 1993. Hepatic lipase treatment of chylomicron remnants increases exposure of apolipoprotein E. *J. Lipid Res.* **34:** 455–465.
40. Breckenridge, W. C., J. A. Little, P. Alaupovic, C. S. Wang, A. Kuksis, G. Kakis, F. Lindgren, and G. Gardiner. 1982. Lipoprotein abnormalities associated with a familial deficiency of hepatic lipase. *Atherosclerosis.* **45:** 161–179.
41. Demant, T., L. A. Carlson, L. Holmquist, F. Karpe, P. Nilsson-Ehle, C. J. Packard, and J. Shepherd. 1988. Lipoprotein metabolism in hepatic lipase deficiency: studies on the turnover of apolipoprotein B and on the effect of hepatic lipase on high density lipoprotein. *J. Lipid Res.* **29:** 1603–1611.
42. Auwerx, J. H., C. A. Marzetta, J. E. Hokanson, and J. D. Brunzell. 1989. Large buoyant LDL-like particles in hepatic lipase deficiency. *Arteriosclerosis.* **9:** 319–325.
43. Fan, J., J. Wang, A. Bensadoun, S. J. Lauer, Q. Dang, R. W. Mahley, and J. M. Taylor. 1994. Overexpression of hepatic lipase in transgenic rabbits leads to a marked reduction of plasma high density lipoproteins and intermediate density lipoproteins. *Proc. Natl. Acad. Sci. USA.* **91:** 8724–8728.
44. Dugi, K. A., C. L. Knapper, D. Applebaum-Bowden, A. Bensadoun, S. M. Meyn, T. T. Le, N. Maeda, H. B. Brewer, Jr., and S. Santamarina-Fojo. 1996. In vivo evidence for a nonlipolytic role of hepatic lipase in the metabolism of high density lipoprotein. *Circulation.* **94:** I-398(Abstr.).
45. Dichek, H. L., W. Brecht, J. Fan, Z-S. Ji, S. P. A. McCormick, H. Akeefe, L. A. Conzo, D. A. Sanan, K. H. Weisgraber, S. G. Young, J. M. Taylor, and R. W. Mahley. 1998. Overexpression of hepatic lipase in transgenic mice decreases apolipoprotein B-containing and high density lipoproteins. *J. Biol. Chem.* **273:** 1896–1903.
46. Swarnakar, S., M. E. Reyland, J. Deng, S. Azhar, and D. L. Williams. 1998. Selective uptake of low density lipoprotein-cholesteryl ester is enhanced by inducible apolipoprotein E expression in cultured mouse adrenocortical cells. *J. Biol. Chem.* **273:** 12140–12147.
47. Green, S. R., and R. C. Pittman. 1991. Selective uptake of cholesteryl esters from low density lipoproteins in vitro and in vivo. *J. Lipid Res.* **32:** 667–678.
48. Brissette, L., and L. Falstrault. 1994. Analysis of the selective uptake of the cholesteryl ester of human intermediate density lipoproteins by HepG2 cells. *Biochim. Biophys. Acta Lipids Lipid Metab.* **1213:** 5–13.
49. Brissette, L., L. Falstrault, J. Lafond, and L. Izem. 1996. The selective uptake of the cholesteryl esters of low density lipoproteins parallels the activity of protein kinase C. *Biochem. Arch.* **1301:** 133–140.
50. Rinninger, F., M. Brundert, S. Jackle, T. Kaiser, and H. Greten. 1995. Selective uptake of low-density lipoprotein-associated cholesteryl esters by human fibroblasts, human HepG2 hepatoma cells and J774 macrophages in culture. *Biochim. Biophys. Acta.* **1255:** 144–153.
51. Acton, S., A. Rigotti, K. T. Landschulz, S. Xu, H. H. Hobbs, and M. Krieger. 1996. Identification of scavenger receptor SR-BI as a high density lipoprotein receptor. *Science.* **271:** 518–520.
52. Acton, S. L., P. E. Scherer, H. F. Lodish, and M. Krieger. 1994. Expression cloning of SR-BI, a CD36-related class B scavenger receptor. *J. Biol. Chem.* **269:** 21003–21009.
53. Xu, S., M. Laccotripe, X. Huang, A. Rigotti, V. I. Zannis, and M. Krieger. 1997. Apolipoproteins of HDL can directly mediate binding to the scavenger receptor SR-BI, an HDL receptor that mediates selective lipid uptake. *J. Lipid Res.* **38:** 1289–1298.
54. Kozarsky, K. F., M. H. Donahee, A. Rigotti, S. N. Iqbal, E. R. Edelman, and M. Krieger. 1997. Overexpression of the HDL receptor SR-BI alters plasma HDL and bile cholesterol levels. *Nature.* **387:** 414–417.