Removal of chylomicron remnants in transgenic mice overexpressing normal and membrane-anchored hepatic lipase

Sung-Joon Lee,[†,§] Sujata Kadambi,[*] Kenneth C-W. Yu,[1,*] Christopher David,[*] Salman Azhar,[**] Allen D. Cooper,[*,†] and Sungshin Y. Choi[2,*]

Research Institute,[*] Palo Alto Medical Foundation, Palo Alto, CA; Department of Medicine,[†] Stanford University, Stanford, CA; Division of Food Sciences,[§] Korea University, Seoul, Korea; and Geriatric Research, Education, and Clinical Center,[**] Department of Veterans Affairs Palo Alto Health Care System, Palo Alto, CA

Abstract **The LDL receptor and the LDL receptor-related protein (LRP) mediate the removal of chylomicron remnants. The LRP pathway involves sequestration of particles in the space of Disse. It has been proposed that either alone or in combination with other factors, such as apolipoprotein E and proteoglycans, hepatic lipase (HL) may contribute to the sequestration of chylomicron remnants. To test this hypothesis, we generated two lines of transgenic mice producing rat HL as a native or as a membrane-anchored form. These animals express HL at levels similar to normal rat. Chylomicron remnants were perfused in a single nonrecirculating pass into the livers of the rat HL transgenic, HL-deficient, and wild-type (WT) mice for 20 min, and the rate of chylomicron remnant removal was measured. Chylomicron remnants were removed at a rate of ~50% per pass in WT mice. It was slightly increased in both transgenic mice and reduced in HL-deficient mice compared with the WT mice. Confocal microscopy of liver sections showed that a modest amount of HL colocalized with chylomicron remnant clusters in the transgenic mice, suggesting that HL is a component of the LRP-proteoglycan clusters. These data suggest that HL helps to direct cholesterol to the tissues in which it is localized by a nonenzymatic mechanism.**—Lee, S-J., S. Kadambi, K. C-W. Yu, C. David, S. Azhar, A. D. Cooper, and S. Y. Choi. **Removal of chylomicron remnants in transgenic mice overexpressing normal and membrane-anchored hepatic lipase.** ***J. Lipid Res.*** **2005.** 46: **27–35.**

Supplementary key words liver perfusion • in vivo • glycophosphatidylinositol • immunohistochemistry • low density lipoprotein receptor • low density lipoprotein receptor-related protein

Hepatic lipase (HL) is synthesized only in hepatic parenchymal cells and functions primarily as an endothelium-bound enzyme within the liver sinusoids (1–3). It hydrolyzes triglycerides and phospholipids in HDL, thus converting HDL_2 to HDL_3, and also functions in the conversion of intermediate density lipoproteins to LDL. It has also been proposed that by nonenzymatic actions the HL protein has a role in lipoprotein uptake. This has been established using both in vivo and in vitro systems. Injection of antibodies into mice and rats reduced the hepatic uptake of chylomicron remnants (4, 5). Transgenic rabbits expressing HL showed a marked reduction of HDL and intermediate density lipoprotein (6). In transgenic mice expressing HL, plasma lipoproteins including LDL, chylomicron remnants, and HDL were reduced compared with those in wild-type (WT) mice (7). The enhanced uptake of apolipoprotein B (apoB)-containing lipoproteins was mediated by a specific interaction of the enzyme with apoB (8) and was independent of the catalytic activity (9). In mice lacking HL, clearance of remnants was reduced but that of chylomicrons was unimpaired (10). Evidence for a direct role of HL in the hepatic uptake, however, is still not clearly defined because it is difficult to isolate the effect of HL in the liver from other components of the removal mechanism with in vivo models, whereas cultured cells lack the complexity of the intact liver. In this study, we used an isolated liver perfusion technique (11) to study the role of rat HL in the uptake of lipoproteins by the liver.

Unlike human HL and rat HL, mouse HL binds poorly to the endothelial cell surface and circulates in the blood after being synthesized by the hepatocytes as a result of

Manuscript received May 12, 2004 and in revised form 9 August 2004 and in re-revised form 29 September 2004 and in re-re-revised form 19 October 2004.

Published, JLR Papers in Press, November 1, 2004.
DOI 10.1194/jlr.M400184-JLR200

Abbreviations: apoB, apolipoprotein B; DiD, 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindodicarbocyanine percholate; GPI, glycophosphatidylinositol; HSPG, heparan sulfate proteoglycan; LRP, low density lipoprotein receptor-related protein; OG, Oregon Green; RAP, receptor-associated protein; WT, wild-type.

[1] Current address of K. C-W. Yu: School of Biological Sciences, Nanyang Technological University, Singapore.

[2] To whom correspondence should be addressed.
e-mail: chois@pamfri.org

the low binding affinity of the mouse enzyme to glycosaminoglycans (12). Thus, we generated transgenic mice that express the rat enzyme, which readily binds to endothelial cells. In addition, we prepared mice that express rat HL as a membrane-bound form using a glycophosphatidylinositol (GPI) as an anchor. Our laboratory previously prepared cell lines that express the native or GPI-anchored rat HL (13).

MATERIALS AND METHODS

Preparation of lipoproteins

Rat chylomicron remnants were obtained in vivo by injecting chylomicrons intravenously into functionally hepatectomized rats as described previously (14, 15). The remnants were then radiolabeled with carrier-free $Na^{125}I$ (Amersham Life Sciences, Arlington Heights, IL) by the iodine monochloride method of McFarlene (16) as previously described (17). Plasma LDL and HDL fractions were isolated by density gradient ultracentrifugation (18).

Preparation of receptor-associated protein

A plasmid containing the cDNA for human receptor-associated protein (RAP) fused with glutathione *S*-transferase was a gift from Dr. Dudley Strickland (Department of Biochemistry, American Red Cross, Rockville, MD). The protein was purified in our laboratory as previously described (19).

Transgenic mice

Animals were kept at 21–25°C and had free access to water and standard chow. All experiments were performed under protocols approved by the Committee on Animal Experimentation of the Palo Alto Medical Research Foundation. WT C57BL/6J mice and mice deficient in hepatic lipase ($HL^{-/-}$) were purchased from Jackson Laboratories (Bar Harbor, ME). We generated two lines of transgenic mice expressing rat HL as a native form ($mHL^{-/-}/rHL^{+/+}$) or a membrane-bound form using a GPI anchor ($mHL^{-/-}/gpi\text{-}rHL^{+/+}$) using FVB mice (Taconic, Germantown, NY). The rat HL constructs contained a vector designed for the liver-specific expression of the enzyme (7) and rat HL cDNA. The vector contained sequences from the human apoE gene and the hepatic control region of the apoE/C-I gene locus (20) and was a generous gift from Dr. J. Taylor (Gladstone Institute of Cardiovascular Disease, San Francisco, CA). Dichek et al. (7) and Fan et al. (6) previously used this vector to express HL in mouse and rabbit and reported that the animals overexpressed HL only in the liver. To generate the transgenic mice producing GPI-anchored rat HL, HL cDNA was modified as described previously (13). Briefly, the signal sequence for the addition of a GPI anchor was inserted into the rat HL cDNA followed by the addition of cloning sites by site-directed mutagenesis. The GPI anchor signal was obtained from human decay-accelerating factor, which has been characterized previously (21). We screened the pups by PCR using primers 5′-CTATGCTATTGCCGTGCGCA-3′ and 5′-CCTGTGTTCTGCAGGTTGCT-3′. Transgenic founders were mated with WT FVB mice, and the hemizygous littermates were mated for approximately seven to eight generations to obtain homozygous animals in the FVB background. The animals used in the experiments were all homozygotes. Lipolytic activity of the plasma samples was assayed as described previously in the presence of 1 M NaCl to suppress lipoprotein lipase activity (22).

Plasma lipid analysis

Blood was drawn from the mice after overnight fasting, and plasma cholesterol and triglycerides were measured using an enzymatic method (Sigma Chemical, St. Louis, MO). HDL cholesterol levels were measured in the supernatant after precipitating apoB-containing lipoproteins with HDL cholesterol reagent (Sigma). Phospholipid concentrations were measured using a kit from Wako (Richmond, VA). ApoA-I and apoB concentrations were determined by SDS-PAGE of VLDL and HDL fractions. Briefly, lipoproteins were isolated from the fasting plasma samples by density-gradient ultracentrifugation. The samples were then applied to either 4% or 7.5% SDS-PAGE. After staining with Coomassie Brilliant Blue, gels were then scanned and the density of apoB and apoA-I bands was determined using NIH Image J 1.3v software.

Liver perfusion

The livers of 15 week old mice were perfused using the single-pass nonrecirculating procedure previously described by our laboratory (11). The perfusate solution contained rat erythrocytes (20% hematocrit) in DMEM and was gassed with O_2. The liver was thermostatically maintained at 37°C. After a 5 min perfusion to remove blood, the perfusate solution containing chylomicron remnants labeled with ^{125}I or the fluorescent carbocyanine dye 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindodicarbocyanine perchlolate (DiD) (Molecular Probes, Inc., Eugene, OR) (23) was perfused into the liver via the portal vein for 20 min at 0.5 ml/min. For kinetic experiments, the samples were collected at 1 min intervals for the measurement of radioactivity. The radioactivity was counted using a γ counter.

Immunohistochemistry

Immunofluorescence colocalization of rat HL in mouse and rat livers was performed on liver sections (8 μm thick) as described previously (11). To stain for HL, liver sections were incubated with polyclonal rabbit anti-rat HL antiserum for 3 h followed by incubation with rhodamine-labeled sheep anti-rabbit IgG for 1 h. To stain for endothelial cells, liver sections were incubated with rabbit anti-human von Willebrand factor IgG for 1.5 h followed by incubation with FITC-labeled goat anti-rabbit IgG for 45 min. Rhodamine and FITC were excited at 568 and 488 nm, respectively. Emissions were collected at 600 ± 20 nm and 500 ± 30 nm for rhodamine and FITC, respectively.

In perfusion experiments, DiD-labeled remnants at 4 μg/ml were perfused for 20 min and then 0.9% NaCl solution was perfused for 5 min after the 20 min perfusion. After the perfusion with DiD-labeled remnants, the livers were sliced into small pieces and fixed in PBS with 4% paraformaldehyde for 15 min and in PBS plus 20% sucrose for 16 h. Tissue blocks were embedded in OCT, and 8 μm sections were cut and placed on glass slides.

The liver sections were incubated first in PBS plus 0.2% Triton X-100 for 5 min, next in PBS plus 5% BSA plus 5% rabbit serum for 30 min, and then with antibodies. After incubation of rabbit anti-LDL receptor-related protein (LRP) antibodies, the sections were incubated with Oregon Green (OG)-labeled goat anti-rabbit IgG (Molecular Probes). Digital images of the stained sections were obtained using a Molecular Dynamics Multiprobe confocal laser microscope (Sunnyvale, CA). DiD was excited at 644 nm and OG was excited at 488 nm. A greater than 660 nm filter and a 500–560 nm filter were used to collect DiD emission (channel 1: red) and OG emission (channel 2: green), respectively. Determination of the colocalized pixels, and counting of the number of clusters, were performed as described previously (24).

Western blot analysis and real-time PCR

Total membranes were prepared from mouse livers as previously described (24). Briefly, 1 g of minced mouse liver was washed by centrifugation with PBS. The pellet was suspended

with 4 ml of CM buffer (10 mM Tris, 150 mM NaCl, and 11 mM $CaCl_2$) containing protease inhibitor cocktail (Sigma) and then homogenized with a Polytron. After centrifugation at 8,000 rpm for 10 min, the supernatant was saved and centrifuged at 105,000 *g* for 1 h. The pellet was resuspended in 600 μl of Tris-maleate buffer (pH 6.0) and then sonicated briefly. This was followed by the addition of 600 μl of water including 300 mM CHAPS and 50 mM NaCl and then stirring on ice for 10 min. The solution was spun at 100,000 *g* for 1 h, and the supernatant containing the liver membrane protein fraction was collected. Twenty micrograms of protein was applied to 6% SDS-PAGE analysis and transferred to a nitrocellulose membrane. The membrane was then incubated with either anti-LDL receptor antibodies (1:2,000) (25) or anti-rat LRP antibodies (1:2,000; generously provided by Dr. J. Herz, University of Texas Southwestern Medical Center, Dallas, TX) (26) followed by incubation with HRP-labeled anti-rabbit IgG (1:10,000) as a secondary antibody. The LDL receptor or the LRP present in the samples was then visualized by ECL (Amersham Life Sciences).

For real-time PCR analysis, total RNA was prepared from the mouse liver using a kit from Qiagen, Inc. (Valencia, CA), and reverse transcription was performed on 1 μg of total RNA using random hexamer primers and Taqman reverse transcriptase (Applied Biosystems, Foster City, CA). The DNA fragments were purified, and a standard curve was prepared by carrying out real-time PCR with known amounts of cDNA and the probes. Real-time PCR was performed using 40 amplification cycles (95°C for 15 s, 55°C for 1 min, and 72°C for 30 s). The primers and probe for the LDL receptor were designed using Primer Express 1.5 (Applied Biosystems). Primers corresponded to nucleotides +461 to +481 and +572 to +589 of the mouse LDL receptor gene. DNA sequences from +483 to +504 were used as the specific probe. The probe was labeled with a reporter dye (FAM) and quencher dye (TAMRA). This technique measures the absolute amount of RNA present. In all samples, GAPDH mRNA levels were measured to provide an internal standard.

Northern blot analysis

Total RNA was prepared from the livers of WT, native rat HL, and GPI rat HL transgenic mice using a kit from Qiagen. Twenty micrograms of total RNA was electrophoresed on a 1% agarose gel containing formaldehyde and blotted. The blot was probed with cDNAs for ^{32}P-labeled rat hepatic lipase and for rat GAPDH as an internal control. The film was scanned using a PhosphorImager (Molecular Dynamics). The intensity of [^{32}P]HL was normalized by [^{32}P]GAPDH.

Data analysis

The data are expressed as means ± SEM. Student's *t*-test was performed for two-group comparisons. Values were considered statistically significant at $P < 0.05$.

RESULTS

Characterization of rat HL in transgenic mouse lines

We generated lines of transgenic mice that express either a native form of rat HL ($mHL^{-/-}/rHL^{+/+}$) or a form of HL that is bound to the hepatocyte membrane via a GPI anchor ($mHL^{-/-}/gpi\text{-}rHL^{+/+}$). The constructs contain part of the human apoE sequence for liver-specific expression. Northern blot analysis showed that both transgenic lines expressed rat HL at a similar level (**Fig. 1**). As previously reported by our laboratory (13), Western blot analysis showed that the molecular weight of the rat HL produced by the transgenic mouse liver was virtually identical to that of the enzyme produced by the rat liver (data not shown). Postheparin plasma samples obtained from mice that express the native form of rat HL showed that these animals express rat HL at a level of HL activity similar to the normal rat; when measured using [^{3}H]triolein as a substrate, the relative activities of the three groups were 100 ± 2.3%, 212 ± 9.5%, and 156 ± 3.5% for WT mice, transgenic mice ($mHL^{-/-}/rHL^{+/+}$), and WT rats. The GPI-anchored rat HL may be active, but because of the limitations of current methods the question of activity could not be addressed in this study. Thus, these mice will allow study of the role of rat HL at a physiologic concentration in contrast to mice that overexpress human HL.

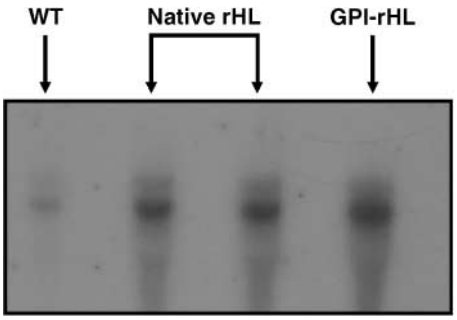

Fig. 1. Northern blot analysis of hepatic lipase (HL) transgenic mice. Total RNA was prepared from the livers of wild-type (WT), native rat HL (rHL) ($mHL^{-/-}/rHL^{+/+}$), and glycophosphatidylinositol (GPI) rat HL ($mHL^{-/-}/gpi\text{-}rHL^{+/+}$) transgenic mice. Twenty micrograms of total RNA was subjected to electrophoresis on a 1% agarose gel containing formaldehyde and blotted as described in Materials and Methods. The blot was probed with ^{32}P-labeled cDNA for rat HL.

Immunostaining of the liver sections with anti-HL antibodies showed that HL is present in both hepatocytes and endothelial cells in transgenic mice expressing rat HL. In contrast, only small amounts of the enzyme were detected in hepatocytes of WT animals (**Fig. 2**). In the livers of $mHL^{-/-}/gpi\text{-}rHL^{+/+}$, there was less colocalization with endothelial cells. Fasting plasma lipid levels were measured in both types of HL transgenic and HL knockout mice ($HL^{-/-}$) (**Fig. 3**). Triglyceride levels in rat HL transgenic mice were modestly but not significantly reduced compared with FVB WT mice. However, consistent with previous reports (10, 27), levels in $HL^{-/-}$ mice were significantly increased compared with C57BL/6J mice. Both plasma and HDL cholesterol levels showed a similar trend, with the difference between rat HL transgenic and control mice more prominent and significantly different. The lipid profile of transgenic mice was similar to that previously described for human HL transgenic mice (9). However, the differences between our rat HL transgenic and control mice were modest as a result of mild rat HL overexpression in our mouse compared with that reported by others (9).

Western blot analysis of the LDL receptor

The LDL receptor and LRP levels in mouse livers were analyzed by Western blotting using specific antibodies. Ex-

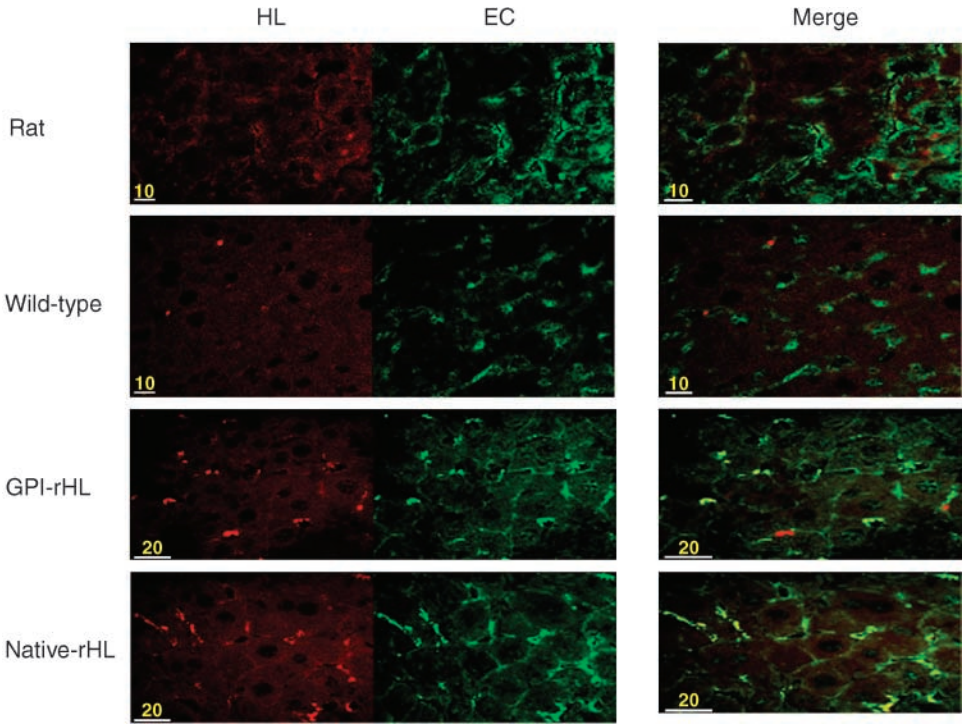

Fig. 2. Immunohistochemical staining of livers. Frozen liver sections (8 μm thick) were prepared from normal rats, WT mice, transgenic mice expressing a GPI-anchored rat HL ($mHL^{-/-}/gpi\text{-}rHL^{+/+}$), or the native rat HL ($mHL^{-/-}/rHL^{+/+}$) and immunostained using an anti-HL antibody for HL and anti-von Willebrand factor IgG for endothelial cells (EC). Bars indicate micrometers.

pression of the LDL receptor was reduced in animals expressing rat HL compared with WT mice (**Fig. 4A**). Conversely, $HL^{-/-}$ mice showed slightly higher levels of LDL receptor compared with WT animals. Similar results were obtained when the LDL receptor expression levels were determined by RT-PCR (Fig. 4B). These data suggest that the presence of rat HL is associated with greater hepatic cholesterol uptake, although total hepatic cholesterol content is not affected. In contrast, the LRP levels were not different in these animals.

Removal of remnants in mice expressing rat HL

To determine the capacity of livers from rat HL transgenic ($mHL^{-/-}/rHL^{+/+}$ and $mHL^{-/-}/gpi\text{-}rHL^{+/+}$) and $HL^{-/-}$ mice to remove chylomicron remnants, radiolabeled remnants (^{125}I-chylomicron remnants) were perfused through the livers using a single noncirculating perfusion. The nonspecific uptake measured by radiolabeled BSA was ~10% per pass (28). When chylomicron remnants (4 μg protein/ml perfusate) were perfused into livers of control, $mHL^{-/-}/rHL^{+/+}$ (**Fig. 5A**), and $mHL^{-/-}/gpi\text{-}rHL^{+/+}$ (Fig. 5B) mice, the removal of ^{125}I was ~55–60% per pass and the uptake of chylomicron remnants was slightly greater in rat HL transgenic livers than in control livers. Total liver uptake was also significantly higher in transgenic animals than in controls ($P < 0.05$) (**Fig. 6**). Removal of remnants appeared to be greater in mice expressing $mHL^{-/-}/rHL^{+/+}$ than in mice expressing $mHL^{-/-}/gpi\text{-}rHL^{+/+}$, but the difference was not significant (Fig. 5A, B). Surprisingly, the removal of chylomicron remnants was only slightly reduced in $HL^{-/-}$ mice (Fig. 5C). The difference was significant during later time points (18–20 min), but total liver uptake was not significantly different between $HL^{-/-}$ mice and controls (Fig. 6). This is consistent with the low level of HL normally present in the mouse line and suggests that HL levels are comparable to those of normal rats and humans, which facilitate remnant uptake.

Role of the LRP in HL enhancement of remnant removal

The effects of blocking the LRP-mediated pathway on remnant uptake in both lines of rat HL transgenic and $HL^{-/-}$ mice were examined. RAP (4 μg/ml) was added to the perfusate containing ^{125}I-remnants (11). This RAP concentration specifically inhibits the LRP but not the LDL receptor. The addition of RAP reduced remnant removal in both transgenic lines of rat HL transgenic mice to the levels of control and $HL^{-/-}$ mice (Fig. 5A, B). These results suggest that rat HL expression enhances LRP-mediated remnant uptake. Consistent with this, RAP did not have a significant effect on chylomicron remnant removal by $HL^{-/-}$ mice (Fig. 5C) or on hepatic uptake (Fig. 6).

Confocal microscopy of DiD remnant uptake

Chylomicron remnants cluster with the LRP and heparan sulfate proteoglycans (HSPGs) in the space of Disse in mouse livers (24). This is most noticeable when the LDL receptor is absent. Since previous experiments were done with normal mice in which the HL level is low, they were

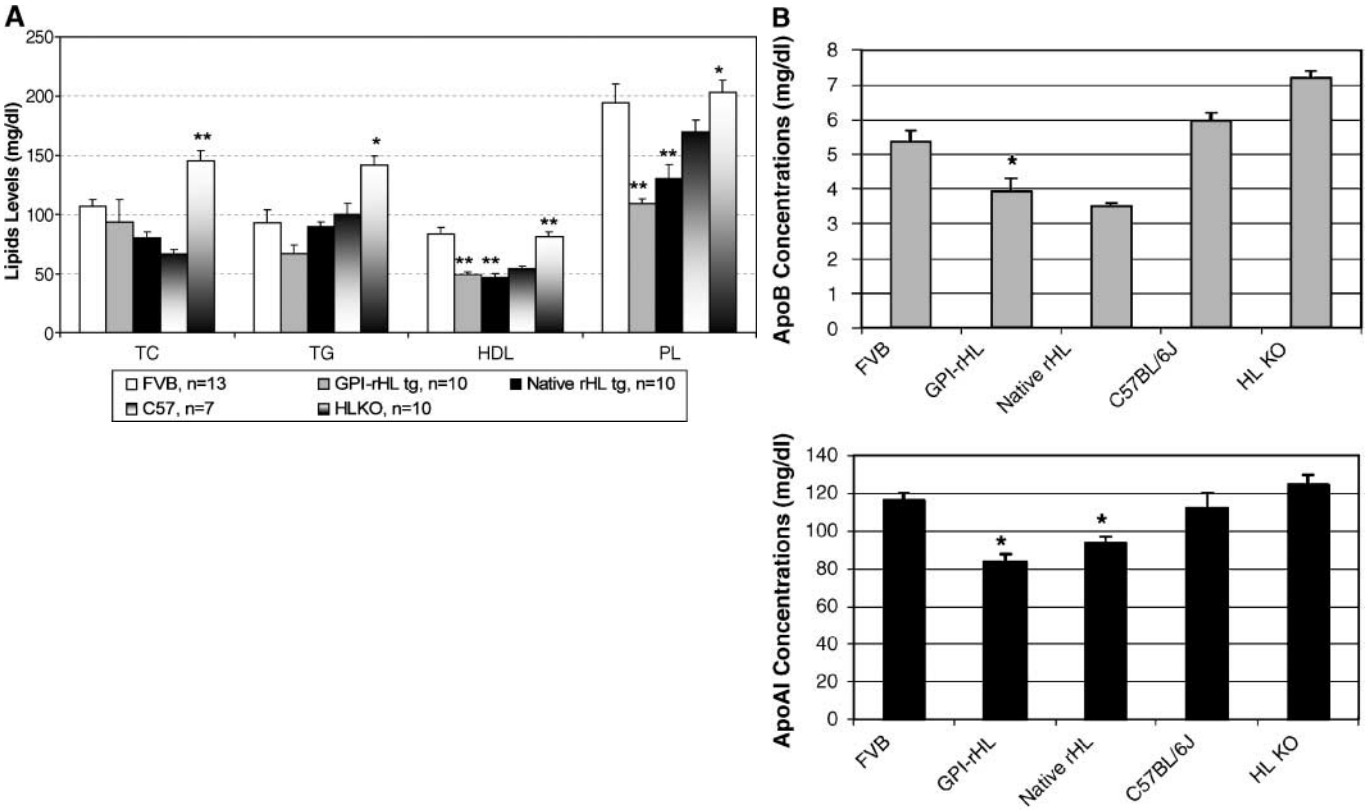

Fig. 3. Plasma lipid concentrations in $HL^{-/-}$, $mHL^{-/-}/rHL^{+/+}$, and $mHL^{-/-}/gpi\text{-}rHL^{+/+}$ mice. Mice were fasted overnight before the blood draw. A: Triglycerides (TG), cholesterol (TC), and phospholipids (PL) were measured as described in Materials and Methods. B: Apolipoprotein B (apoB) and apoA-I were measured by SDS-PAGE as described in Materials and Methods. WT FVB mice were used as controls for both native HL ($mHL^{-/-}/rHL^{+/+}$) and GPI HL transgenic (tg) ($mHL^{-/-}/gpi\text{-}rHL^{+/+}$) mice. C57BL/6J mice were used as controls for HL knockout (HLKO) ($HL^{-/-}$) mice. * $P < 0.05$; ** $P < 0.01$.

repeated with rat HL transgenic mice because rat HL participates in LRP remnant clusters. The livers of rat HL transgenic mice were perfused with DiD-labeled remnants and then stained with either anti-HL or anti-LRP antibody. Because these mice express the LDL receptor, the number of LRP remnant clusters was only 20–30% of those in the LDL receptor-deficient mice but was greater than in normal or $HL^{-/-}$ mice (**Fig. 7A**). This confirmed that most of the remnants were removed by the LDL receptor even in the presence of rat HL in mouse livers, as previously reported (29). When stained with anti-HL antibody, the LRP remnant clusters did colocalize with rat HL (Fig. 7B).

These results provide direct evidence that HL can participate in remnant uptake via the LRP pathway, and this is consistent with the data from the experiments with RAP.

DISCUSSION

The results of the current studies further establish and help to clarify the role of rat HL in the removal of lipoproteins and lipoprotein lipids by the liver. Specifically, they demonstrate that HL has a significant role in the LRP-mediated removal of chylomicron remnants in the intact liver. Mice that expressed the rat HL in both its native form and in a form that anchors the HL to the surface of the expressing cell by use of a GPI anchor were generated. Rat HL was used because, like the human form and unlike the mouse form, it is anchored to the cell surface of hepatocytes. Mice with a level of expression similar to normal rats or humans were chosen. Previous studies with mice expressing higher levels of HL have been reported (7, 9), but because of the very high level of expression, the results could not be applied to the role of physiologic levels.

In studies of patients with HL deficiency (5), it was speculated that some of the observed lipoprotein abnormalities could be attributable to the impaired removal of chylomicron remnants. Infusion of an anti-HL antibody that inhibited HL activity resulted in a delay in remnant clearance (4). Inhibition of HL reduced the rate of uptake of chylomicron remnants by the isolated perfused rat liver and augmented the endocytosis of those remnants that were taken up by the liver (30). In cell culture experiments, the presence of HL on McA-RH7777 cells accelerated the removal of both remnants and LDL (31, 32), whereas in studies in our laboratory using CHO cells that expressed HL, it was found that LDL but not chylomicron remnant uptake was enhanced (33). This was somewhat paradoxical in that other studies from this laboratory clearly documented the ability of HL to bind both apoB-100 and apoB-48 (8). The present results show that the

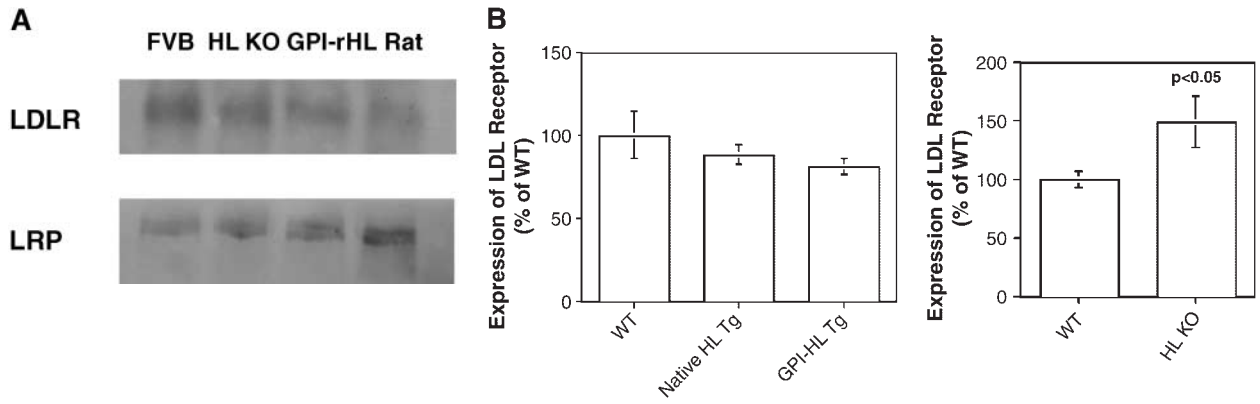

Fig. 4. Expression of the LDL receptors and the LDL receptor-related protein (LRP). A: Western blot analysis of the LDL receptor and the LRP. Twenty micrograms of total liver membrane prepared from FVB, HL knockout (HLKO) *(HL*$^{-/-}$*)*, GPI HL (*mHL*$^{-/-}$*/gpi-rHL*$^{+/+}$), and normal rat were separated by electrophoresis using 6% SDS-polyacrylamide gels and transferred to a nitrocellulose membrane followed by incubation with either polyclonal rabbit anti-LDL receptor (1:2,000 dilution) or anti-LRP antibody (1:2,000). Antibodies bound to the LDL receptor or the LRP were visualized using the ECL method. B: Level of LDL receptor mRNA in liver of rat HL transgenic (Tg) and deficient mice. mRNA was extracted as described in Materials and Methods, and then real-time PCR was carried out to quantify the expression of the LDL receptor gene. GAPDH was used as the internal standard. Data are expressed as means ± SEM (n = 5). The *y* axis indicates the relative percentage expression using FVB and C57BL/6J as references.

presence of rat HL at a physiologically relevant level increases the rate of removal of chylomicron remnants by the liver. Because mouse enzyme does not bind to cell surface proteoglycans, there is no significant difference in remnant removal between the normal and *HL*$^{-/-}$ mouse. In the transgenic mouse, remnant uptake is greater than in the normal mouse. Furthermore, it is at least marginally greater if the enzyme is present on both the hepatocytes and endothelial cell surface compared with the hepatocyte surface only, as in the case of the GPI-anchored rat HL.

The effect of HL seems to be, at least in part, if not exclusively, on the LRP-mediated remnant removal. RAP, an inhibitor of the LRP, eliminated the enhanced removal seen in the transgenic mice. As in previous experiments (11, 29), RAP had little effect on remnant removal in the normal mice, consistent with the postulate that in normal mouse, remnant removal is mediated by the LDL receptor. Additionally, Rohlmann et el. (34) previously reported that inactivation of the LRP gene in mice that expressed functional LDL receptors did not result in the accumulation of remnant lipoproteins, suggesting that the LDL receptor can functionally fully compensate for LRP.

The role of rat HL in the LRP-mediated removal was further confirmed by the confocal microscopy experiments, in which the number of clusters in the space of Disse in rat HL transgenic mice appeared to be greater than in the normal mouse liver. HL was clearly part of the cluster with the LRP and the remnants. Thus, HL, along with the HSPGs, may play an important role in enhancing the affinity of the LRP for remnants, allowing clustering in the space of Disse. It also suggests that in our previous cell culture experiments with nonhepatic CHO cells there may have been less potential for the LRP-mediated uptake of remnants, perhaps because of the other components of the cell surface. In contrast, in liver-derived McA-RH7777 cells, the LRP may be able to play a more prominent role; thus, HL enhances remnant removal in the latter cells but not in CHO cells. Interestingly, in recent studies of apoE isoforms, we concluded that humans may rely more heavily on LRP for remnant removal than do mice (S-J. Lee, S. Y. Choi, and A. D. Cooper, unpublished data), and one might speculate that the presence of HL in humans may contribute to this.

The GPI-anchored rat HL does not seem to be enzymatically active when the cell is intact (S. Y. Choi and S. Kadambi, unpublished data), although it is active when membranes are prepared from cells that express this form of lipase (13). If this is correct, then the enhancement of remnant removal is a nonenzymatic effect of rat HL. This agrees with data from several other reports that suggest that enhanced lipoprotein removal does not require HL activity (8, 35, 36).

The sum of the activities of rat HL is to considerably enhance the ability of an organ to remove lipoproteins and lipoprotein lipids from the circulation. This should not be surprising, because the enzyme is concentrated in the liver, the adrenal gland, and the ovary, organs that are highly active in cholesterol metabolism. The net result, however, may contribute to the "proatherogenic" activity that has been attributed to HL (37, 38). Indeed, presumably because the livers of mice expressing rat HL were taking up more lipoprotein and lipoprotein cholesterol, they had a lower level of LDL receptor expression. This, in turn, would lead to slower LDL removal and thus to higher LDL levels, with the attendant increased risk of atherosclerosis.

In summary, by studying the hepatic uptake of chylomicron remnants in transgenic mice producing rat HL in its native form or as a membrane-anchored form, the role of this molecule in hepatic lipid metabolism has been further established. HL is responsible for enhancing the LRP-mediated uptake of chylomicron remnants.

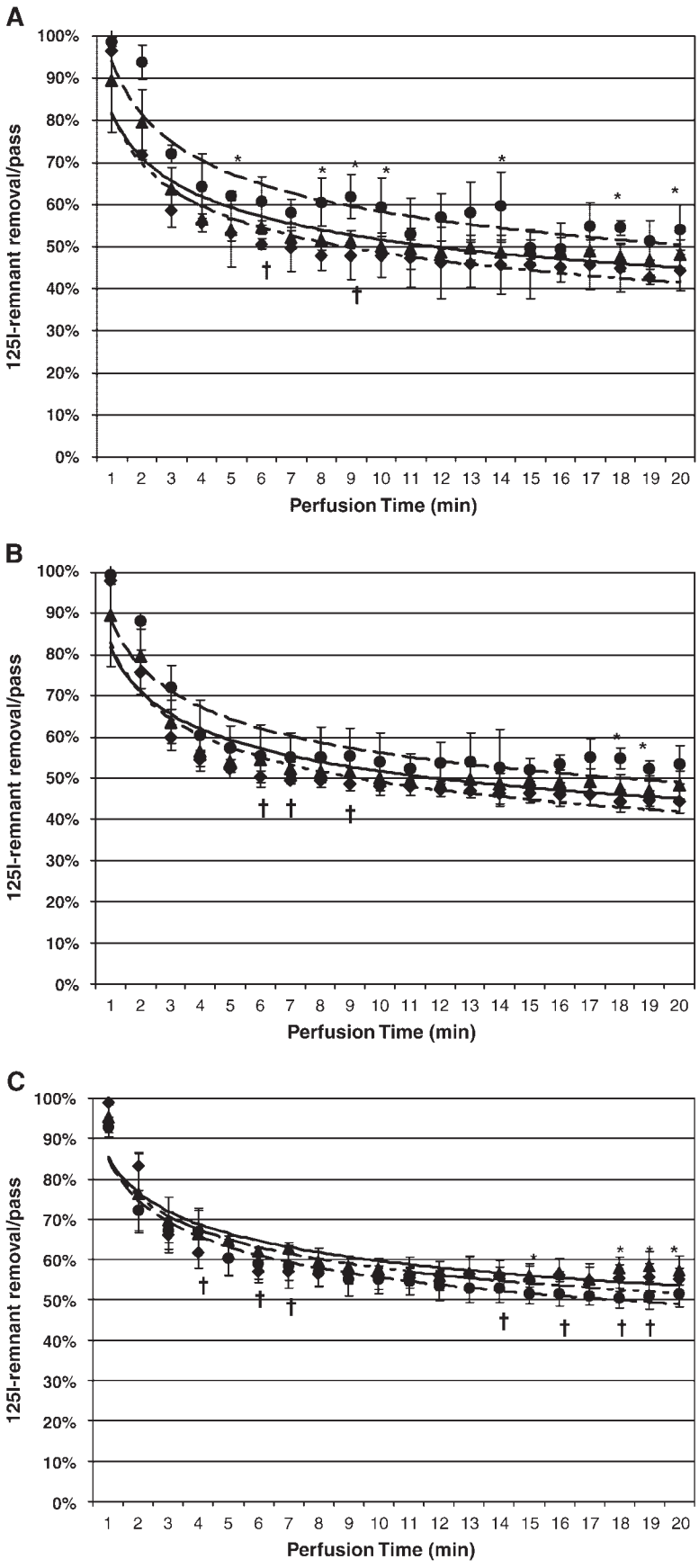

Fig. 5. Removal of chylomicron remnants by rat HL transgenic, knockout, and WT mouse livers. ^{125}I-labeled chylomicron remnants (4 μg/ml perfusate) were added to the perfusate of the isolated livers, and aliquots of the perfusate leaving the liver were collected at 1 min intervals for 20 min. The amount of remnants removed by the liver from the perfusate per pass is expressed as the percentage of ^{125}I-labeled remnants removed per pass, which is calculated by subtracting the radioactivity in a sample of perfusate that left the liver from the initial radioactivity in the perfusate, divided by the initial radioactivity in the perfusate, and multiplying by 100. A: FVB mice (n = 4), native rat HL transgenic (*mHL*$^{-/-}$*/rHL*$^{+/+}$) mice (n = 3), native rat HL transgenic mice in the presence of receptor-associated protein (RAP; n = 5). B: FVB mice (n = 4), GPI-anchored rat HL transgenic (*mHL*$^{-/-}$*/gpi-rHL*$^{+/+}$) mice (n = 3), GPI-anchored rat HL transgenic mice in the presence of RAP (n = 4). C: C57BL/6J mice (n = 4), HL knockout (*HL*$^{-/-}$) mice (n = 4), HL knockout mice in the presence of RAP (n = 4). * $P < 0.05$ between the WT and either HL transgenic or knockout mice in the absence of RAP. † $P < 0.05$ between WT and HL transgenic or knockout mice in the presence of RAP. Lines are power trend curves generated by Excel software (Microsoft). Each data point represents the mean ± SD.

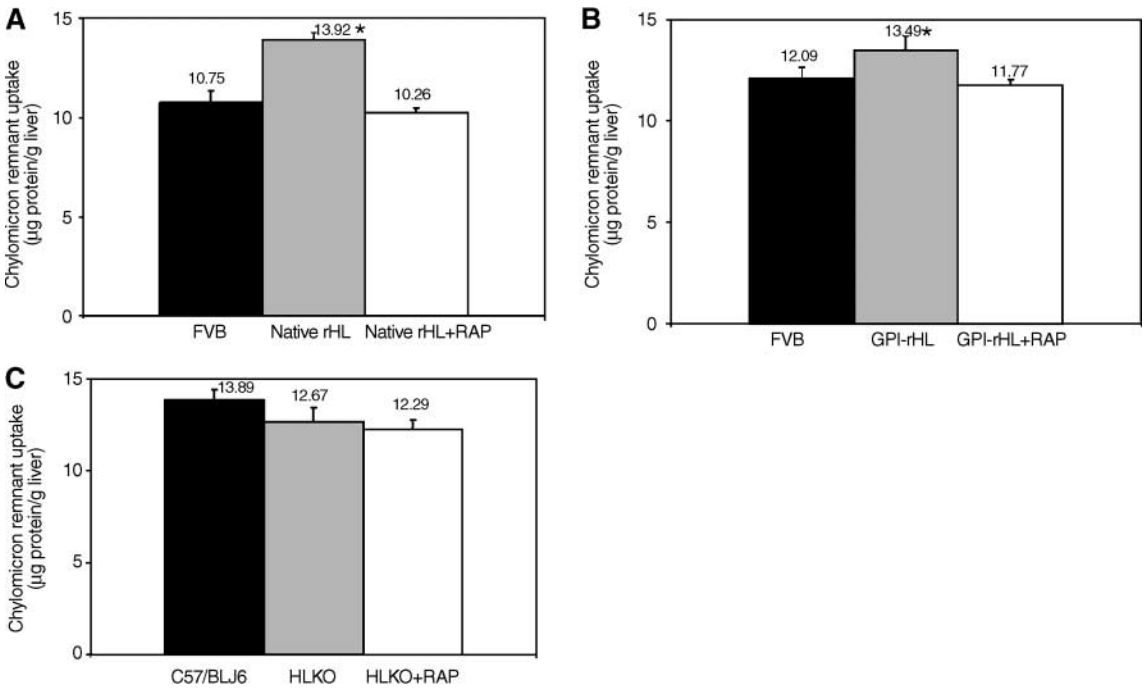

Fig. 6. Uptake of chylomicron remnants by the total liver. Livers of mice in the experiment described for Fig. 5 were removed after perfusion. Total radioactivity present was counted. The total amount of remnant uptake (μg) per gram of liver was calculated by dividing total radioactivity (cpm) per gram of dried liver by the specific radioactivity of the remnants. Black bars represent the remnant uptake in WT mouse livers (FVB or C57BL/6J); gray bars represent remnant uptake in native rat HL ($mHL^{-/-}/rHL^{+/+}$) (A), GPI rat HL ($mHL^{-/-}/gpi\text{-}rHL^{+/+}$) (B), and HL knockout (HLKO) ($HL^{-/-}$) (C). White bars represent the hepatic uptake of remnant in the presence of RAP in those animals. * $P < 0.05$ compared with FVB. Means ± SEM are shown. N is as in Fig. 5.

This work was supported by National Institutes of Health Grant HL-58037 (S.Y.C.), a Grant-in-Aid from the American Heart Association, Western Affiliate (S.Y.C.), the Stanford Digestive Disease Research Center (Grants DK-38318 and DK-56339) (A.D.C.), a Korea University grant for young scientist (S-J.L.), and the Office of Research and Development, Medical Research Service, Department of Veterans Affairs (S.A.). The authors are indebted to Dr. John Taylor (Gladstone Institute of Cardiovascular Disease, San Francisco, CA) for kindly providing a vector for liver-specific expression of the protein. The authors also thank Dr. Itamar Grosskopf and Jean Chen for scientific advice and help.

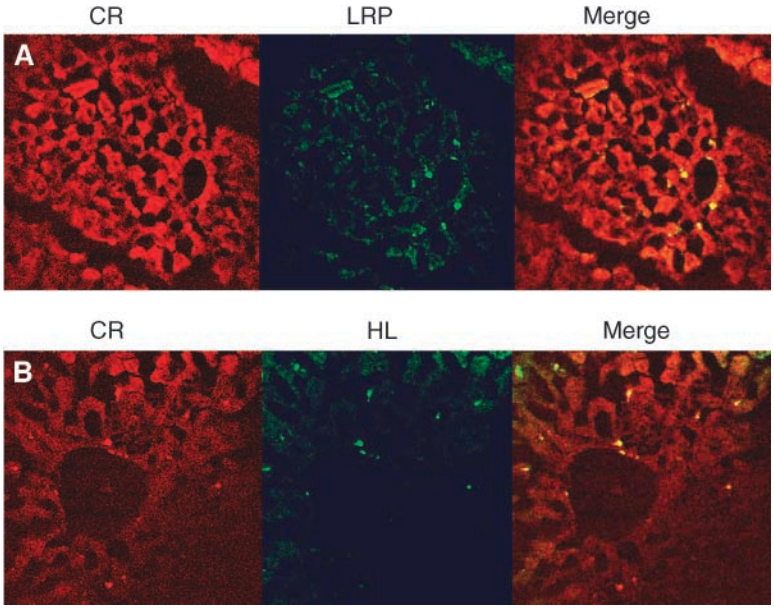

Fig. 7. Hepatic lipase colocalization in livers perfused with chylomicron remnants (CR). Livers from control and transgenic mice were perfused with 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindodicarbocyanine percholate (DiD)-labeled remnants (4 μg/ml, 20 min), and cut sections were immunostained with either rabbit anti-LRP antibody or rabbit anti-HL antibody followed by Oregon Green-labeled goat anti-rabbit IgG. A: Clustering of DiD remnants (red) that were colocalized with the LRP (green). B: Colocalization of DiD remnant and the HL (red) with HL (green). Merged images are shown at right.

REFERENCES

1. Jensen, G. L., D. L. Baly, P. M. Brannon, and A. Bensadoun. 1980. Synthesis and secretion of lipolytic enzymes by cultured chicken hepatocytes. *J. Biol. Chem.* **255:** 11141–11148.
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. Jansen, H., T. J. Van Berkel, and W. C. Hulsmann. 1980. Properties of binding of lipases to non-parenchymal rat liver cells. *Biochim. Biophys. Acta.* **619:** 119–128.
4. De Faria, E., L. G. Fong, M. Komaromy, and A. D. Cooper. 1996. Relative roles of the LDL receptor, the LDL receptor-like protein, and hepatic lipase in chylomicron remnant removal by the liver. *J. Lipid Res.* **37:** 197–209.
5. Sultan, F. D., D. E. Lagrange, H. Jansen, and S. Griglio. 1990. Inhibition of hepatic lipase activity impairs chylomicron remnant-removal in rats. *Biochim. Biophys. Acta.* **1042:** 150–152.
6. 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.
7. Dichek, H. L., W. Brecht, J. Fan, Z. S. Ji, S. P. A. McCormick, H. Akeefe, L. 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.
8. Choi, S. Y., I. J. Goldberg, L. K. Curtiss, and A. D. Cooper. 1998. Interaction between apoB and hepatic lipase mediates the uptake of apoB-containing lipoproteins. *J. Biol. Chem.* **273:** 20456–20462.
9. Dichek, H. L., S. M. Johnson, H. Akeefe, G. T. Lo, E. Sage, C. E. Yap, and R. W. Mahley. 2001. Hepatic lipase overexpression lowers remnant and LDL levels by a noncatalytic mechanism in LDL receptor-deficient mice. *J. Lipid Res.* **42:** 201–210.
10. Qiu, S., N. Bergeron, L. Kotite, R. M. Krauss, A. Bensadoun, and R. J. Havel. 1998. Metabolism of lipoproteins containing apolipoprotein B in hepatic lipase-deficient mice. *J. Lipid Res.* **39:** 1661–1668.
11. Yu, L. W., Y. Jiang, W. Chen, and A. D. Cooper. 1999. Evaluation of the components of the chylomicron remnant removal mechanism by use of the isolated perfused mouse liver. *J. Lipid Res.* **40:** 1899–1910.
12. Peterson, J., G. Bengtsson-Olivecrona, and T. Olivecrona. 1986. Mouse preheparin plasma contains high levels of hepatic lipase with low affinity for heparin. *Biochim. Biophys. Acta.* **878:** 65–70.
13. Komaromy, M. C., S. Azhar, and A. D. Cooper. 1986. Chinese hamster ovary cells expressing a cell surface-anchored form of hepatic lipase. *J. Biol. Chem.* **271:** 16906–16914.
14. Cooper, A. D. 1977. The metabolism of chylomicron remnants by isolated perfused rat liver. *Biochim. Biophys. Acta.* **488:** 464–474.
15. Redgrave, T. G., and G. Martin. 1977. Effects of chronic ethanol consumption on the catabolism of chylomicron triacylglycerol and cholesteryl ester in the rat. *Atherosclerosis.* **28:** 69–80.
16. McFarlene, A. S. 1958. Efficient trace-labeling of proteins with iodine. *Nature.* **182:** 53–57.
17. Kris-Etherton, P. M., and A. D. Cooper. 1980. Studies on the etiology of the hyperlipemia in rats fed an atherogenic diet. *J. Lipid Res.* **21:** 435–442.
18. Havel, R. C., H. A. Eder, and H. J. Bragdon. 1955. Distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J. Clin. Invest.* **34:** 1345–1353.
19. Fujioka, Y., A. D. Cooper, and L. G. Fong. 1998. Multiple processes are involved in the uptake of chylomicron remnants by mouse peritoneal macrophages. *J. Lipid Res.* **39:** 2339–2349.
20. Simonet, W. S., N. Bucay, S. J. Lauer, and J. M. Taylor. 1993. A far-downstream hepatocyte-specific control region directs expression of the linked human apolipoprotein E and C-I genes in transgenic mice. *J. Biol. Chem.* **268:** 8221–8229.
21. Caras, I., G. N. Weddell, M. A. Davitz, V. Nussenzweig, and D. W. Martin, Jr. 1987. Signal for attachment of a phospholipid membrane anchor in decay accelerating factor. *Science.* **238:** 1280–1283.
22. Nilsson-Ehle, P., and M. C. Schotz. 1976. A stable and radioactive substrate emulsion for assay of lipoprotein lipase. *J. Lipid Res.* **17:** 536–541.
23. Pitas, R. E., T. L. Innerarity, J. N. Weinsterin, and R. W. Mahley. 1981. Acetoacetylated lipoproteins used to distinguish fibroblasts from macrophages in vitro by fluorescence microscopy. *Atherosclerosis.* **1:** 177–185.
24. Yu, K. W., W. Chen, and A. D. Cooper. 2001. LDL receptor-related protein mediates cell-surface clustering and hepatic sequestration of chylomicron remnants in LDLR-deficient mice. *J. Clin. Invest.* **107:** 1387–1394.
25. Cooper, A. D., R. Nutik, and J. Chen. 1987. Characterization of the estrogen-induced lipoprotein receptor of rat liver. *J. Lipid Res.* **28:** 59–68.
26. Herz, J., R. C. Kowal, Y. K. Ho, M. S. Brown, and J. L. Goldstein. 1990. Low density lipoprotein receptor-related protein mediates endocytosis of monoclonal antibodies in cultured cells. *J. Biol. Chem.* **265:** 21355–21362.
27. Homanics, G. E., H. V. de Silva, J. Osada, S. H. Zhang, H. Wong, J. Borensztajn, and N. Maeda. 1995. Mild dyslipidemia in mice following targeted inactivation of the hepatic lipase gene. *J. Biol. Chem.* **270:** 2974–2980.
28. Yu, K. C., J. Jiang, W. Chen, and A. D. Cooper. 2000. Rapid initial removal of chylomicron remnants by the mouse liver does not require hepatically localized apolipoprotein E. *J. Lipid Res.* **41:** 1715–1727.
29. Choi, S. Y., and A. D. Cooper. 1993. A comparison of the roles of the low density lipoprotein (LDL) receptor and the LDL receptor-related protein/α2 macroglobulin receptor in chylomicron remnant removal in the mouse in vivo. *J. Biol. Chem.* **268:** 15804–15811.
30. 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.
31. Ji, Z-S., S. J. Lauer, S. Fazio, A. Bensadoun, J. M. Taylor, and R. W. Mahley. 1993. Enhanced binding and uptake of remnant lipoproteins by hepatic lipase-secreting hepatoma cells in culture. *J. Biol. Chem.* **269:** 13429–13436.
32. Lauer, S. J., Z-S. Ji, S. Fazio, A. Bensadoun, J. M. Taylor, and R. W. Mahley. 1993. Human hepatic lipase secretion by transfected McA-RH7777 cells results in enhanced binding and uptake of remnant lipoproteins. *Circulation.* **88:** I-321.
33. 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.
34. Rohlmann, A., M. Gotthardt, R. E. Hammer, and J. Herz. 1998. Inducible inactivation of hepatic LRP gene by Cre-mediated recombination confirms role of LRP in clearance of chylomicron remnants. *J. Clin. Invest.* **101:** 689–695.
35. Zambon, A., S. S. Deeb, A. Bensadoun, K. E. Foster, and J. D. Brunzell. 2000. In vivo evidence of a role for hepatic lipase in human apo B-containing lipoprotein metabolism, independent of its lipolytic activity. *J. Lipid Res.* **41:** 2094–2099.
36. 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.
37. González-Navarro, H., N. Zengxuan, L. Freeman, A. Bensadoun, K. Peterson, and S. Santamarina-Fojo. 2002. Identification of mouse and human macrophages as a site of synthesis of hepatic lipase. *J. Lipid Res.* **43:** 671–675.
38. Zambon, A., J. E. Hokansan, B. G. Brown, and J. D. Brunzell. 1999. Evidence for a new pathophysiological mechanism for coronary artery disease regression: hepatic lipase-mediated changes in LDL density. *Circulation.* **99:** 1959–1964.