Hepatic triglyceride lipase promotes low density lipoprotein receptor-mediated catabolism of very low density lipoproteins in vitro

Jheem D. Medh,[1,*] Susan L. Bowen,[*] Glenna L. Fry,[*] Stacie Ruben,[*] John Hill,[†] Howard Wong,[†] and David A. Chappell[2,*]

Department of Internal Medicine,* University of Iowa College of Medicine, Iowa City, IA 52242, and Lipid Research Laboratory,† West Los Angeles VA Medical Center, and Department of Medicine,† University of California-Los Angeles, Los Angeles, CA 90073

Abstract **We demonstrate here that hepatic triglyceride lipase (HTGL) enhances VLDL degradation in cultured cells by a LDL receptor-mediated mechanism. VLDL binding at 4°C and degradation at 37°C by normal fibroblasts was stimulated by HTGL in a dose-dependent manner. A maximum increase of up to 7-fold was seen at 10 μg/ml HTGL. Both VLDL binding and degradation were significantly increased (4-fold) when LDL receptors were up-regulated by treatment with lovastatin. HTGL also stimulated VLDL degradation by LDL receptor-deficient FH fibroblasts but the level of maximal degradation was 40-fold lower than in lovastatin-treated normal fibroblasts. A prominent role for LDL receptors was confirmed by demonstration of similar HTGL-promoted VLDL degradation by normal and LRP-deficient murine embryonic fibroblasts. HTGL enhanced binding and internalization of apoprotein-free triglyceride emulsions, however, this was LDL receptor-independent. HTGL-stimulated binding and internalization of apoprotein-free emulsions was totally abolished by heparinase indicating that it was mediated by HSPG. In a cell-free assay HTGL competitively inhibited the binding of VLDL to immobilized LDL receptors at 4°C suggesting that it may directly bind to LDL receptors but may not bind VLDL particles at the same time. We conclude that the ability of HTGL to enhance VLDL degradation is due to its ability to concentrate lipoprotein particles on HSPG sites on the cell surface leading to LDL receptor-mediated endocytosis and degradation.**—Medh, J. D., S. L. Bowen, G. L. Fry, S. Ruben, J. Hill, H. Wong, and D. A. Chappell. **Hepatic triglyceride lipase promotes low density lipoprotein receptor-mediated catabolism of very low density lipoproteins in vitro.** ***J. Lipid Res.*** **1999.** 40: **1263–1275.**

Supplementary key words hepatic lipase • lipoprotein lipase • LDL receptor • LDL receptor-related protein • heparan sulfate proteoglycans

Hepatic triglyceride lipase (HTGL) is a 65 kDa glycoprotein synthesized by hepatocytes and localized on the luminal surface of hepatic endothelial cells (1, 2). Like lipoprotein lipase (LPL), HTGL catalyzes the hydrolysis of triglycerides and causes the conversion of triglyceride-rich chylomicrons and very low density lipoprotein (VLDL) particles into cholesterol-rich remnant particles (1–5). Both lipases are heparin binding proteins and are found in post-heparin plasma of humans and experimental animals. Early studies by various investigators have established a role for HTGL in remnant clearance. Inhibition of HTGL activity by injection of anti-HTGL IgG in rats (6), monkeys (7), or isolated perfused rat livers (8) impairs the hepatic uptake of radiolabeled remnant particles and causes an accumulation of plasma VLDL and low density lipoprotein (LDL) fractions (9, 10). In humans, the presence of familial HTGL deficiency results in elevated plasma levels of remnant-like particles containing cholesterol and triglycerides and the development of premature atherosclerosis (11–14). Expression of human HTGL in transgenic mice (15) and rabbits (16) results in a lowering of total plasma cholesterol. In HTGL knockout mice on a high-fat diet, the expression of human HTGL results in a correction of the abnormal lipid profile (17) and a significant decrease in the level of aortic cholesterol (15). These data support an anti-atherogenic role for HTGL.

In an attempt to understand the molecular mechanisms of HTGL function, several investigators have studied the effect of purified HTGL on remnant clearance by cultured cells (18–21). Sultan et al. (20) demonstrated that addition of partially purified hepatic lipase stimulates receptor-mediated uptake of remnant particles by freshly

Abbreviations: HTGL, hepatic triglyceride lipase; LPL, lipoprotein lipase; LDL, low density lipoproteins; VLDL, very low density lipoproteins; LPDS, lipoprotein-deficient serum; LRP, LDL receptor-related protein; HSPG, heparan sulfate proteoglycans; apo, apolipoprotein; BSA, bovine serum albumin; α_2M, α-2-macroglobulin; α_2M*, activated α_2M; RAP, receptor-associated protein.

[1] To whom correspondence should be addressed.

[2] Present address: 191 Lynch Creek Way, Suite 205, Petaluma, CA 94954.

isolated hepatocytes. Ji et al. (22) showed enhanced remnant binding and uptake by rat hepatoma cells transfected with the human HTGL cDNA. The initial cell-surface binding site for HTGL may be heparan sulfate proteoglycans (HSPG) as proteoglycan-deficient Chinese hamster ovary (CHO) cells are unable to degrade HTGL (21) and heparinase treatment inhibits HTGL-mediated chylomicron remnant uptake (22). Kounnas and her associates (21) demonstrated that HTGL binds to LRP in cultured cells and to purified LRP in a solid phase assay. These studies suggest that HTGL-mediated lipoprotein catabolism may occur via the LRP pathway.

It has not been determined whether LDL receptors also contribute to HTGL-mediated lipoprotein catabolism. We have demonstrated earlier that LDL receptors play a major role in LPL-promoted VLDL catabolism (23). The same may be true for HTGL as well. The present studies were aimed at determining whether LDL receptors contribute to the stimulation of VLDL catabolism by HTGL. We have used cultured cells and recombinant HTGL to demonstrate that HTGL-promoted VLDL catabolism proceeds via the LDL receptor pathway. We studied normal human foreskin fibroblasts (FSF cells) in which both LRP and LDL receptor-dependent pathways are active. Data were also obtained in LDL receptor-deficient (FH) human fibroblasts, HepG2 cells, and normal (MEF) and LRP-deficient (PEA13) murine embryonic fibroblasts. None of these cell lines is known to express GP330/LRP-2 or the VLDL receptor. Our results suggest a significant role for LDL receptors in HTGL-mediated binding and degradation of VLDL.

MATERIALS AND METHODS

Materials

VLDL particles with S_f 100–400 were isolated as described previously by ultracentrifugation of plasma from fasted normolipidemic human subjects with the most common apoE phenotype (E3/3) (24). Bovine milk LPL was isolated by heparin-Sepharose chromatography as described previously (25). Recombinant human HTGL was produced in CHO cells and purified from the culture media (26). Heparinase was purchased from Sigma Chemical Company (St. Louis, MO). Recombinant human receptor-associated protein (RAP) was produced as a fusion protein and purified as described previously (27). The carboxyl-terminal fragment of human LPL (amino acid residues 313–448) was produced in *E. coli* as a fusion protein with glutathione *S*-transferase (GST) as described previously (28) and designated as GST-LPLC. Using site-directed mutagenesis, tryptophan residues at positions 393 and 394 were changed to alanine to generate GST-$LPLC_{ww}$ (28, 29). Monoclonal antibody IgG-4A4 directed against the cytoplasmic terminal 14 amino acids of the LDL receptor was obtained as a hybridoma from the American Type Culture Collection (Rockville, MD). HTGL was iodinated using IODOGEN (Pierce) as previously described (30). VLDL particles were iodinated to specific activities of 300–500 cpm/ng by the iodine–monochloride method (31).

Preparation of apolipoprotein-free particles

Apoprotein-free particles with S_f 100–400 were isolated from a 10% Intralipid emulsion (Travenol) by ultracentrifugal flotation (24), and their triglyceride content was estimated by an enzymatic colorimetric assay (Sigma Chemical Co.) (32). They were labeled with [^{3}H]cholesteryl oleyl ether, a nondegradable marker of cellular uptake (33). A glass tube containing 0.5 ml of minimum essential medium (MEM), 4 mg/ml BSA, and 35 μCi [^{3}H]cholesteryl oleyl ether (Amersham) was sonicated for 10 min at room temperature. Intralipid particles with S_f 100–400 containing 3–4 mg triglycerides were added, and the mixture was incubated at 37°C for 20 min and then returned to room temperature. This treatment resulted in the incorporation of [^{3}H]cholesteryl oleyl ether in the emulsion. The final concentration of triglycerides and BSA was, respectively, ~5 mg/ml and ~1.5 mg/ml of the radiolabeled Intralipid suspension. The tritiated lipid emulsions were stored at 4°C overnight before use. The sizes of S_f 100–400 particles from plasma and those from Intralipid were similar as indicated by scanning electron microscopy on a Hitachi S-4000 instrument (data not shown) using previously described techniques (34).

Cell binding assays

Normal human foreskin fibroblasts (FSF cells) were cultured as described (35, 36). Mutant skin fibroblasts that do not express LDL receptors (FH cells) (37), were obtained from the National Institute of General Medical Sciences Human Genetic Mutant Cell Repository (GM00486A), Camden, NJ. Murine embryonic fibroblasts (MEF) and mutant MEF cells that lack LRP (PEA13) (38) were provided by Dr. Joachim Herz (Dallas, TX). HepG2 cells were obtained from American Type Culture Collection (ATCC, Rockville, MD). LDL receptors were up-regulated by incubation prior to the assay for 48 h with media containing 2 mg/ml lipoprotein-deficient serum (LPDS) and 24 h in the presence of 1 μg/ml of lovastatin (39, 40). In control cells (−lovastatin), LDL receptors were down-regulated by supplementing the incubation medium with 20 μg/ml LDL. In studies with murine fibroblasts, lovastatin was omitted and the duration of LPDS treatment was reduced to 24 h. Surface binding to metabolically inactive cells was studied after incubating cells with radiolabeled ligands for 3 h at 4°C as previously described (35, 36). Steady-state ligand internalization and degradation were measured after incubating cells with radiolabeled ligands at 37°C for 5 h (35, 36). Degradation was defined as the trichloroacetic acid-soluble radioactivity in the incubation medium. Surface binding and internalization were defined, respectively, as radioactivity released and remaining cell-associated after incubating cells at 4°C for 1 h in buffer containing 10 mg/ml tripolyphosphate. Total protein in each well as determined by the assay of Lowry et al. (41) varied by less than 15% within each experiment. Wells treated with lovastatin and/or LPDS contained ~60% of the protein amounts present in untreated wells. Results were corrected for cellular protein per well and are averages of duplicate (no error bars) or triplicate determinations. Figures shown are representative of 3 to 5 experiments.

Solid-phase binding assays

LDL receptors were partially purified by fractionation of total cell extracts over DE52-cellulose (Whatman) as described previously (42). Microtiter wells (96-well plates, immulon 2, Dynatech) were coated for 30 min at 37°C with 100 μl of buffer containing 3 μg of anti-LDL receptor antibody, IgG-4A4. The wells were then blocked with 1% BSA at 37°C for 1 h. LDL receptors from the LDL receptor preparations (DE52 eluants) were specifically immobilized by incubating IgG-coated wells for 16 h at 4°C with 0–200 μg DE52 eluant protein in 100 μl of binding buffer (50 mm Tris-HCl, pH 8.0, 2 mm $CaCl_2$, 0.5% BSA) (23, 43). Immobilized LDL receptors were incubated at 4°C for 3 h in binding buffer with 5 μg/ml ^{125}I-labeled VLDL in the ab-

sence or presence of HTGL. After three washes with binding buffer, the bound ligand was desorbed in 0.3 N NaOH and quantitated for radioactivity. All data points represent averages of triplicate determinations.

RESULTS

HTGL stimulates VLDL degradation by normal fibroblasts

We and others have reported earlier that LPL enhances VLDL degradation by cultured cells and that both LDL receptors and LRP are involved in this process (23, 29, 30, 44, 45). Here we investigated whether HTGL also stimulates VLDL catabolism and if the LDL receptor pathway is involved. Normal foreskin fibroblasts (FSF cells) which express both LDL receptors and LRP were used to compare the effects of HTGL and LPL on VLDL degradation (**Fig. 1A**). The cells were previously incubated with lovastatin as described in Methods to up-regulate their LDL receptor number. HTGL clearly stimulated VLDL catabolism by these cells in a dose-dependent manner. In the presence of 10 μg/ml HTGL VLDL degradation was stimulated almost 7-fold. Maximum stimulation of up to 12-fold was seen at 0.1 μg/ml LPL. Thus, HTGL was significantly less potent than LPL both in the dose required and the extent of maximum stimulation. The K_m for HTGL was ~1 μg/ml whereas for LPL it was 4 ng/ml. HTGL also significantly enhanced cell surface binding of VLDL at 4°C in control and lovastatin-treated normal fibroblasts by about 6.5-fold and 2.5-fold, respectively (Fig. 1B).

HTGL-stimulated VLDL binding is mediated by LDL receptors

To investigate the role of LDL receptors in HTGL-stimulated degradation of VLDL particles, we compared the effect of HTGL in FSF cells without (control) or with lovastatin treatment to increase their level of LDL receptor expression. As is well established, lovastatin treatment of FSF cells up-regulated their LDL receptors and increased ^{125}I-labeled LDL binding and degradation by 8- to 10-fold (data not shown) (23, 36). It has been shown previously that lovastatin does not effect LRP expression as indicated by catabolism of ^{125}I-labeled activated α_2M (α_2M*) (23, 46). As shown in Fig. 1B, HTGL enhanced cell-surface binding in both control and lovastatin-treated normal fibroblasts at 4°C. As HTGL has a heparin-binding domain, it is probable that a major fraction of the HTGL effect is due to binding to cell surface proteoglycans. However, HTGL-enhanced binding was two times that in lovastatin-treated cells indicating active participation of up-regulated LDL receptors in the process. Because the effect of HTGL and lovastatin was synergistic rather than just additive, we propose a role for LDL receptors in HTGL-mediated VLDL binding to the cell surface.

We next investigated the time-course of degradation in the presence and absence of HTGL in control and lovastatin-treated normal fibroblasts. HSPG-mediated internal-

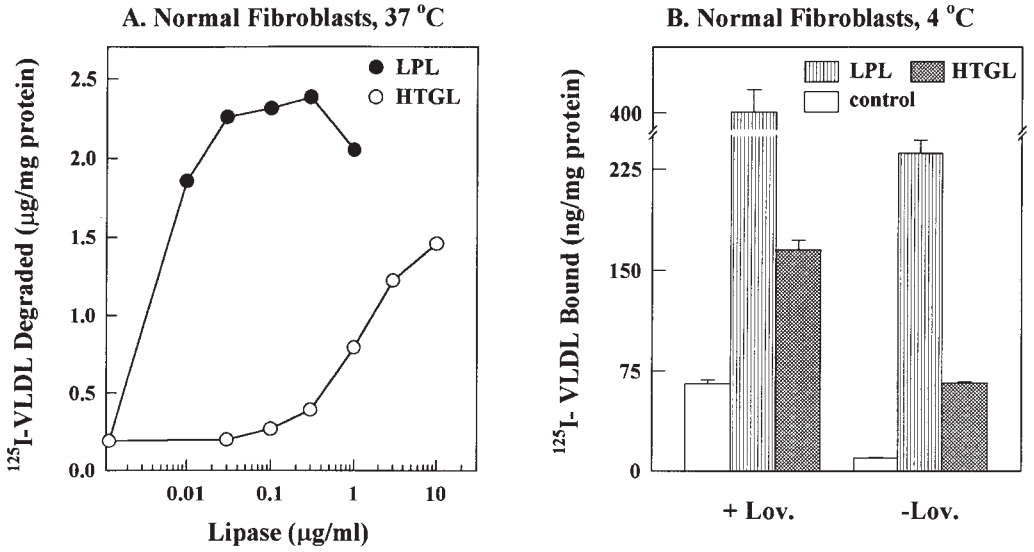

Fig. 1. Stimulation of ^{125}I-labeled VLDL binding and degradation in normal human fibroblasts by HTGL and LPL. Confluent normal human foreskin fibroblasts (FSF) were treated with either lipoprotein-deficient serum (LPDS) and lovastatin to up-regulate LDL receptors (A and + Lov. (B)) or maintained in lipoprotein-containing media (−Lov. (B)) as described under Methods. They were then incubated for (A) 5 h at 37°C or (B) 3 h at 4°C in media containing 5 μg/ml ^{125}I-labeled VLDL and increasing concentrations of (A) bovine LPL (closed circles) or human recombinant HTGL (open circles) or (B) media alone (open bars), 1 μg/ml LPL (hatched bars) or 3 μg/ml HTGL (filled bars). After washing as described, (A) degradation was measured as the radioactivity in the incubation medium that was soluble in 15% trichloroacetic acid. (B) Surface-bound radioactivity was dissociated by incubating cells for 1 h at 4°C in buffer containing 10 mg/ml polyphosphate. The amount of ligand was calculated using the specific radioactivity and corrected for cellular protein in each well.

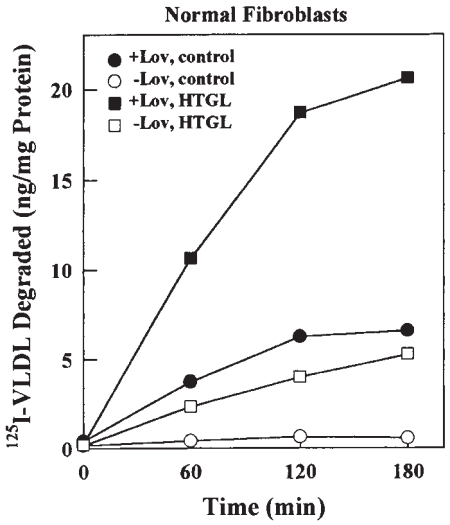

Fig. 2. Time-course of degradation of surface-bound ^{125}I-labeled VLDL. Normal fibroblasts were treated with LPDS and lovastatin (closed symbols) or with LDL (open symbols) as described in Fig. 1. They were incubated at 4°C for 3 h with 5 μg/ml ^{125}I-labeled VLDL in the absence (circles) or presence of 3 μg/ml HTGL (squares). Cells were then washed, and surface bound ligand was quantitated as in Fig. 1. The cells were then transferred to 37°C and degradation was determined as described in Fig. 1 at the indicated times.

ization has been shown to be significantly slower than receptor-mediated endocytosis. For the experiment shown in **Fig. 2**, ^{125}I-labeled VLDL was bound to the cell-surface at 4°C in the presence or absence of HTGL. The cells were washed to remove unbound ligand and then transferred to 37°C for various times prior to measurement of VLDL degradation. The relative amounts of VLDL degraded were consistent with surface binding represented in Fig. 1B. Degradation was highest in lovastatin-treated cells in the presence of HTGL and lowest in control cells in the absence of HTGL (Fig. 2). Within 1 h, degradation was dramatically higher in lovastatin-treated cells in the presence of HTGL. Degradation was significantly slower in control cells with fewer LDL receptors even in the presence of HTGL. The initial rates of degradation of prebound ^{125}I-labeled VLDL were 9.22, 1.91, 2.92, and 0.23 ng/h per mg protein, respectively, for the conditions of +lovastatin +HTGL, −lovastatin +HTGL, +lovastatin −HTGL, and −lovastatin −HTGL. By 3 h degradation under all conditions reached a plateau and the extent of degradation in the presence of both HTGL and lovastatin was 4-fold higher than in the presence of either one alone. Degradation in the absence of both agents was negligible. This experiment provides strong evidence for the involvement of the LDL receptor pathway in HTGL-stimulated degradation.

Relative contribution of LDL receptor versus LRP in HTGL-stimulated VLDL degradation

We compared the effect of HTGL concentration on VLDL degradation using FSF fibroblasts that express both LRP and LDL receptors and FH fibroblasts that lack LDL receptors. It has been reported previously that HTGL binds to LRP and may mediate VLDL catabolism via the LRP-pathway (19, 21). The presence of HTGL-enhanced VLDL degradation in both lovastatin-treated and control FSF cells (**Fig. 3A**) and the difference between the two curves increased with the amount of HTGL present. Maximum degradation was seen at 3–10 μg/ml HTGL with 4-fold higher degradation in lovastatin-treated cells. The K_m

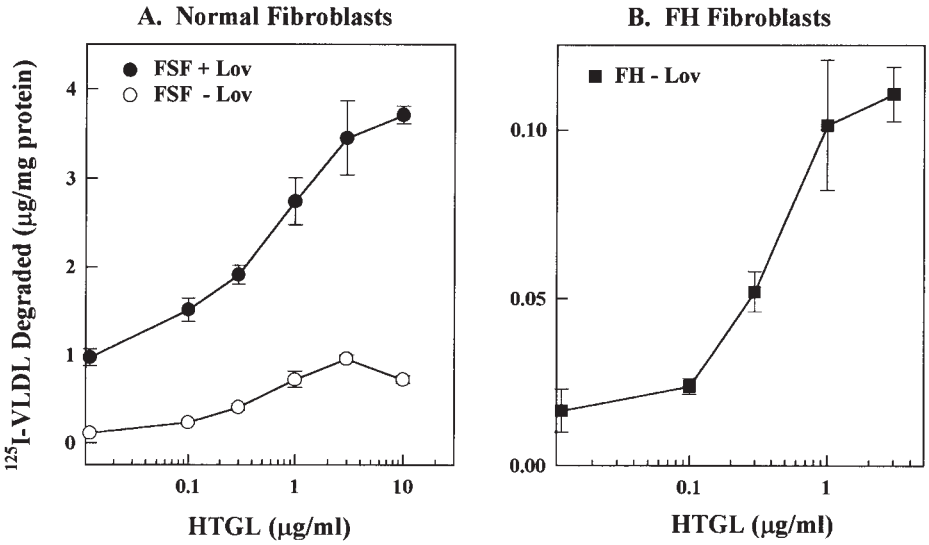

Fig. 3. HTGL-mediated ^{125}I-labeled VLDL degradation is lovastatin-dependent in normal fibroblasts and is higher in normal than in FH fibroblasts. (A) Normal human fibroblasts were treated with (closed circles) or without (open circles) lovastatin as described in the legend to Fig. 1. (B) FH fibroblasts lacking LDL receptors were cultured without lovastatin. The cells were incubated for 5 h at 37°C in media containing 5 μg/ml ^{125}I-labeled VLDL in the presence of increasing concentrations of HTGL. Degradation was measured as in Fig. 1.

values of HTGL were 0.5 μg/ml and 0.3 μg/ml, respectively, in lovastatin-treated and untreated normal fibroblasts. Because LRP expression is not regulated by lovastatin (23), the ability of HTGL to induce VLDL degradation after up-regulation of LDL receptors clearly points to the involvement of LDL receptors rather than LRP in the process. We found that VLDL degradation in FH fibroblasts was also significantly enhanced in the presence of HTGL (Fig. 3B). Similar to the observation in FSF cells, this effect was dependent on the concentration of HTGL. The K_m value of HTGL was 0.4 μg/ml in FH fibroblasts. In the absence of LDL receptors, degradation in these cells must be mediated by LRP. However, comparison of the absolute extent of VLDL degraded showed that degradation by FH fibroblasts was almost 10-fold and 40-fold lower, respectively, than the amount degraded by control and lovastatin-treated FSF cells. Both cell lines express similar levels of LRP as evidenced by their ability to bind α_2M* (data not shown) (23). Thus, even in cells expressing both LRP and LDL receptors, the latter pathway mediates a major fraction of total HTGL-stimulated VLDL degradation. This is consistent with our earlier finding that LPL-promoted degradation also prefers the LDL receptor pathway (23).

We next studied the catabolism of increasing concentrations of ^{125}I-labeled VLDL in the presence of a fixed HTGL concentration. At all concentrations of ^{125}I-labeled VLDL, degradation was enhanced several-fold by the presence of 3 μg/ml HTGL in FSF cells (**Fig. 4A**). Degradation was saturable at 12–15 μg/ml VLDL consistent with a receptor-mediated process. Both LDL receptors and LRP contribute to catabolism in the basal state. When LDL receptors were up-regulated by lovastatin treatment, degradation increased 6- to 8-fold. We interpreted the degradation data using Scatchard-type analysis. The ratio of degraded to free ligand was plotted against degraded ligand (graphs not shown). The analysis showed that HTGL promoted degradation by increasing the affinity for VLDL degradation by 5-fold in lovastatin-treated cells and by more than 25-fold in control cells without significantly increasing the number of degradation sites. On the other hand, degradation in the presence of HTGL was increased by lovastatin due to an increase in the number of degradation sites (30 fm/well vs. 100 fm/well high affinity sites). The curves for plus and minus lovastatin treatments were parallel indicating that the affinity was not significantly altered. Thus, LDL receptors mediate a majority of HTGL-dependent degradation of ^{125}I-labeled VLDL in cells expressing both LRP and LDL receptors.

HTGL-stimulated VLDL degradation via LRP is insignificant in murine embryonic fibroblasts

In another approach to determine the relative contributions of the two receptors in HTGL-promoted VLDL degradation, we compared degradation in normal (MEF) and LRP-deficient (PEA13) murine embryonic fibroblasts (38). In control experiments we determined that both cell lines degrade equal amounts of ^{125}I-labeled LDL, indicative of the similar levels of LDL receptor expression, but PEA13 cells did not degrade ^{125}I-labeled α_2M*, a ligand specific for LRP (23). If the LRP-pathway contributes significantly to HTGL-promoted VLDL degradation, one would expect degradation in MEF cells to be significantly higher than in PEA13 cells. However, as shown in Figs. 4B and 4C, the extent of degradation in the presence of HTGL was virtually identical in both cell lines. By Scatchard-type analysis, degradation sites in MEF cells were not significantly higher than in PEA13 cells (210 fm/well vs. 185 fm/well). Also, as in normal fibroblasts, the affinity for degradation by murine fibroblasts was not altered by lovastatin treatment; the stimulation in degradation by lovastatin was predominantly due to an increase in degrada-

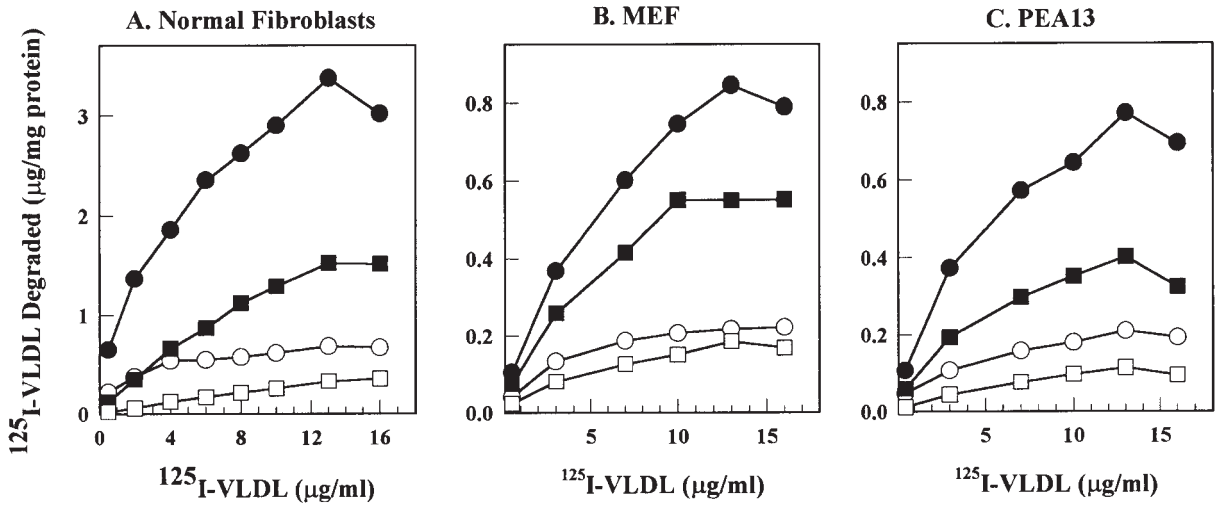

Fig. 4. Stimulation of ^{125}I-labeled VLDL catabolism by HTGL in MEF and PEA13 cells is similar. The experiment was performed as in Fig. 1 except that the cells were incubated for 5 h at 37°C in media containing increasing concentrations of ^{125}I-labeled VLDL in the absence (squares) or presence (circles) of 3 μg/ml HTGL. Degradation was measured as described in Fig. 1. Closed and open symbols represent, respectively, lovastatin-treated and untreated cells. (A) Normal human fibroblasts, (B) murine embryonic fibroblasts (MEF) and (C) mutant MEF cells lacking LRP (PEA13).

tion sites. A 5-fold increase in degradation sites was observed which was consistent with the 4- to 6-fold increase in LDL receptor number as determined by ^{125}I-labeled LDL degradation. In the absence of HTGL, degradation levels were slightly higher in MEF cells than in PEA13 cells. The reason for this is not clear as VLDL does not bind to LRP in the absence of exogenously added apoE or lipase (47). It is also evident from Fig. 4 that the murine fibroblasts are significantly less efficient in catabolizing VLDL than human fibroblasts. This could represent a difference between the two species or between neonatal and embryonic cells.

RAP partially inhibits but GST-LPLC$_{ww}$ completely inhibits HTGL-stimulated VLDL degradation

We assessed the ability of the 39 kDa receptor-associated protein (RAP) to inhibit HTGL-dependent VLDL degradation by cultured cells. RAP binds to LRP with a high affinity and, at nanomolar concentrations, completely inhibits the interactions of LRP with all of its known ligands (27, 48). We have shown previously that RAP also binds to LDL receptors but with a much lower affinity (43). As expected, 3 μg/ml HTGL stimulated VLDL degradation in normal fibroblasts and HepG2 cells (**Fig. 5A**). In the presence of 300 nm RAP, HTGL-promoted VLDL degradation was only partially inhibited in all cell types tested (Fig. 5A). At this concentration RAP totally inhibited the LRP pathway as determined by ^{125}I-labeled α_2M* degradation but only partially inhibited ^{125}I-labeled LDL degradation (data not shown). The inability of RAP to completely abolish HTGL-dependent VLDL degradation provides further evidence for a role for LDL receptors rather than LRP in the process. Here we also tested the effect of HTGL on VLDL degradation by HepG2 cells (Fig. 5A). HepG2 cells are a human hepatoma cell line and a good model system for studying hepatic function. Because the liver is the primary site of HTGL synthesis and function and also of VLDL uptake and catabolism, HepG2 cells provided a relevant system to confirm our observations. HepG2 cells also secrete HTGL but measurable amounts do not accumulate during the 5-h incubation for the degradation assay (49). Degradation was significantly less in HepG2 cells than in normal fibroblasts as reported earlier (50).

We also studied the effect of GST-LPLC$_{ww}$ on HTGL-stimulated VLDL degradation (Fig. 5B). GST-LPLC$_{ww}$ is a fusion protein of glutathione-S-transferase with the carboxyl-terminal non-catalytic fragment of LPL (amino acid residues 313–448) in which tryptophan residues at positions 393 and 394 are changed to alanine by site-directed mutagenesis (28, 29). As a result of these substitutions, GST-LPLC$_{ww}$ is unable to bind lipoproteins but retains its ability to bind to both LDL receptors and LRP. Thus it serves as a competitive inhibitor of receptor-mediated lipoprotein degradation (23). We found that GST-LPLC$_{ww}$ completely inhibited LDL catabolism (23) and HTGL-stimulated VLDL catabolism in FSF cells (Fig. 5B).

HTGL stimulates internalization of apoprotein-free intralipid emulsion

ApoE mediates the binding of VLDL particles to LDL receptors (47). To determine whether apoE was required for HTGL-dependent VLDL endocytosis, we isolated apoprotein-free particles with S_f 100–400 from a triglyceride-phospholipid emulsion and radiolabeled them as described in Methods. We found that HTGL stimulated binding (**Fig. 6A**) and internalization (Fig. 6B) of these apoprotein-free particles by normal fibroblasts in an HTGL dose-dependent manner with a K_m value of ~1 μg/ml HTGL for surface binding. Internalization was not saturated under the experimental conditions. The results with apoprotein-free particles indicate that apoproteins are not required for HTGL's stimulatory effect on lipid binding and internalization. As the radiolabel is non-degradable, it was not possible to investigate whether HTGL stimulates degradation of apoprotein-free lipid particles. Up-regulation of LDL receptors did not significantly increase binding or in-

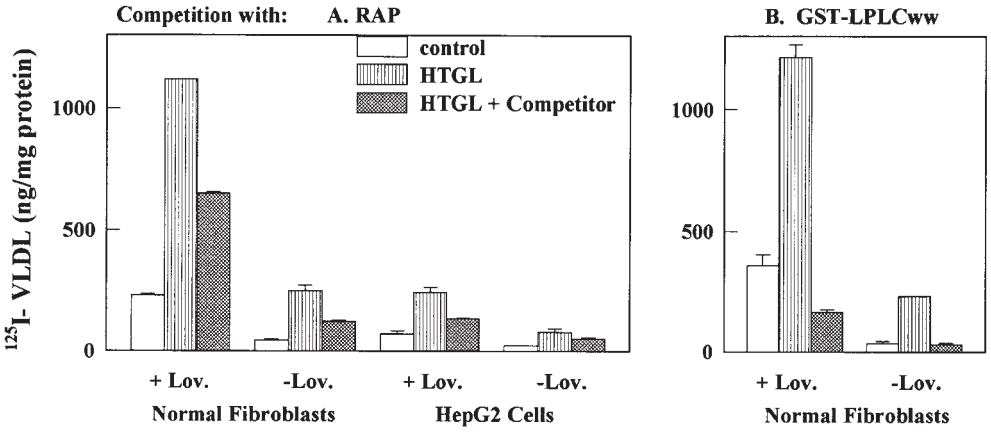

Fig. 5. HTGL-stimulated ^{125}I-labeled VLDL degradation is only partially inhibited by RAP. Normal fibroblasts or HepG2 cells were treated with or without lovastatin and incubated for 5 h at 37°C in media containing 5 μg/ml ^{125}I-labeled VLDL either alone (open bars), in the presence of 3 μg/ml HTGL (hatched bars), 3 μg/ml HTGL and 300 nM RAP (A, filled bars) or 3 μg/ml HTGL and 50 μg/ml GST-LPLC$_{ww}$ (B, filled bars). Ligand degradation was measured as described in Fig. 1.

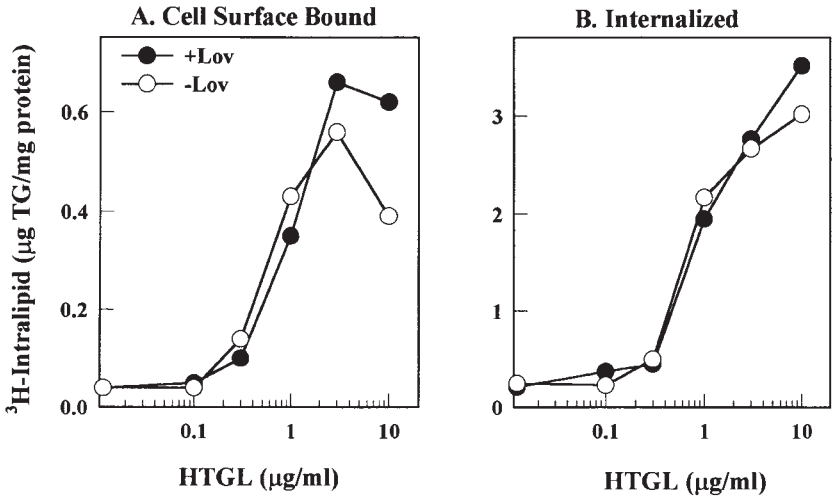

Fig. 6. HTGL promotes binding and internalization of protein-free triglyceride emulsions by normal fibroblasts. Normal human fibroblasts were incubated with (closed circles) or without (open circles) lovastatin as in Fig. 1. The cells were then incubated for 5 h at 37°C in media containing 100 μg/ml triglyceride in [^{3}H]cholesterol oleyl ether-labeled emulsions with S_f 100–400 in the presence of increasing concentrations of HTGL. Surface binding was determined as the radioactivity released by washing the cells with tripolyphosphate as described in Methods. Internalization was measured as the radioactivity still associated with cells after removing surface-bound ligand.

ternalization, suggesting that endocytosis of apoprotein-free particles is not LDL receptor-mediated. Cell surface heparan sulfate proteoglycans may be responsible for this receptor-independent binding and internalization (51).

Effect of heparinase treatment on HTGL-stimulated binding and degradation

The HTGL molecule has a heparin-binding domain that is mutually exclusive from its lipid binding domain (26). It is believed that HTGL may bind to cell surface HSPG via its heparin-binding domain. HSPG-mediated mechanisms can be inhibited by heparin or by digestion of HSPG with heparinase. We investigated the role of cell surface HSPG in mediating cell binding and internalization of HTGL–Intralipid complexes. Proteoglycans on the surface of lovastatin-treated normal fibroblasts were digested by treatment with 0.01 units/ml heparinase for 30 min at 37°C. The cells were washed and allowed to internalize [^{3}H]Intralipid as described in Methods in the presence of 10 μg/ml heparin. Control cells were treated identically but without heparinase or heparin. As expected, HTGL stimulated both surface binding and internalization of [^{3}H]Intralipid in the absence of heparinase/heparin. However, when HSPG-mediated mechanisms were inhibited by heparinase/heparin treatment, binding as well as internalization of apoprotein-free Intralipid emulsions were reduced to control levels seen in the absence of HTGL (**Fig. 7A**). This experiment supports a role for HSPG in mediating HTGL's stimulatory effect on binding and internalization of apoprotein-free triglyceride emulsions.

We also investigated the role of HSPG in mediating HTGL-stimulated degradation of normal human VLDL. The experiment described above was repeated using ^{125}I-labeled VLDL as the ligand. As shown in Fig. 7B, there was no effect of heparinase/heparin treatment on HTGL-stimulated ^{125}I-labeled VLDL binding or degradation at 37°C. Thus, LDL receptors, rather than HSPG, are responsible for mediating endocytosis and degradation of VLDL. However, cell surface binding at 4°C had a significant HSPG-mediated component. This is shown in **Fig. 8**. In this experiment, FSF cells were pretreated at 37°C with increasing concentrations of heparinase to digest cell surface HSPG. Cells were then allowed to bind ^{125}I-labeled VLDL for 3 h at 4°C in the presence or absence of HTGL. Results for each condition are presented as a percentage of the binding obtained in the absence of heparinase for that condition. The level of binding under each condition was similar to that represented in Fig. 1B. Heparinase treatment did not significantly reduce binding in the absence of HTGL. However, it abolished more than 80% of binding in the presence of HTGL in control cells (no lovastatin treatment) indicating that most of the binding was to HSPG. In cells treated with lovastatin and HTGL, heparinase treatment abolished 60–70% of the binding. The heparinase-resistant and HTGL-dependent binding may be direct binding of VLDL–HTGL complexes to LDL receptors. Thus HSPG are clearly important for HTGL's ability to stimulate cell surface VLDL binding at 4°C. Subsequent to binding HSPG, lipoprotein particles may be transferred to LDL receptors for receptor-mediated endocytosis and degradation (52).

HTGL inhibits VLDL binding to LDL receptors in solid-phase assays

It was demonstrated previously that HTGL binds to purified LRP in solid-phase assays (21). Here we tested the effect of HTGL on VLDL binding to immobilized LDL receptors in a similar cell-free assay. Microtiter plates coated

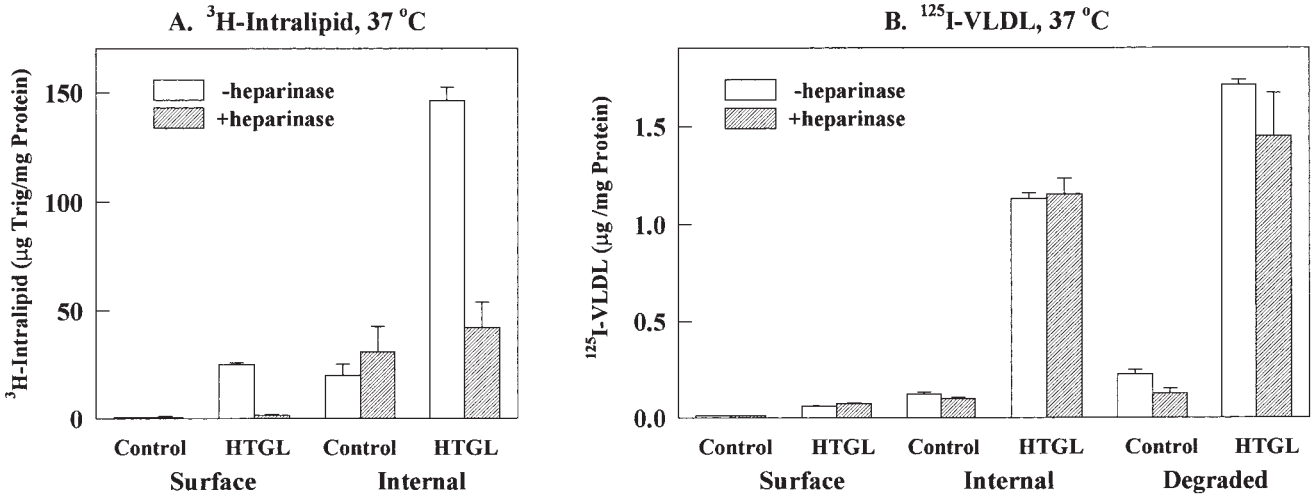

Fig. 7. Heparinase treatment inhibits HTGL-stimulated Intralipid uptake but not VLDL degradation at 37°C. Normal fibroblasts treated with lovastatin as in Fig. 1 were incubated for 30 min at 37°C in the presence (hatched bars) or absence (open bars) of 0.01 units/ml of heparinase. The cells were then washed and incubated in media containing (A) 100 μg/ml triglyceride in [^{3}H]Intralipid emulsion or (B) 5 μg/ml ^{125}I-labeled VLDL. Heparin (10 μg/ml) was added to media of cells treated with heparinase. After 5 h at 37°C, surface binding, internalization and degradation were measured as described in Methods. As [^{3}H]Intralipid is tagged using non-degradable cholesteryl olelyl ether, degradation could not be measured in A.

with 100 μg of LDL receptor preparation per well were incubated at 4°C with 5 μg/ml ^{125}I-labeled VLDL in the presence of increasing concentrations of HTGL. We expected HTGL to stimulate VLDL binding or have no effect if HTGL did not directly bind to LDL receptors. Surprisingly, we found that HTGL competed for VLDL binding to immobilized LDL receptors (**Fig. 9A**). Inhibition was almost complete at 10 μg/ml HTGL and half maximal at about 1 μg/ml. HTGL had no effect on background binding to BSA-coated wells. To confirm these results we studied the effect of 1 μg/ml HTGL on ^{125}I-labeled VLDL binding to increasing amounts of immobilized LDL receptors. Again, at each concentration of LDL receptor, binding was about 50% lower in the presence of HTGL (Fig. 9B). This is exactly the opposite of the results we obtained with LPL (23). The inhibition by HTGL of VLDL binding to LDL receptors suggests that HTGL is unable to bind to both VLDL particles and LDL receptors simultaneously as has been proposed for LPL.

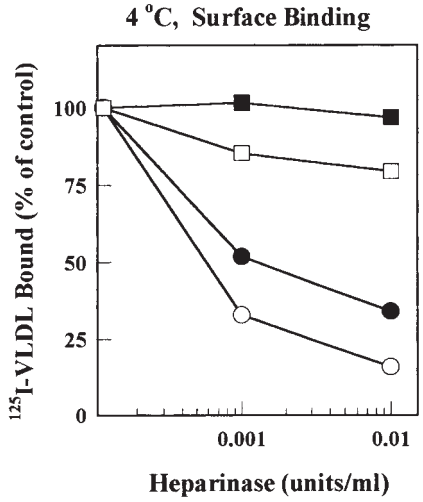

Fig. 8. Heparinase pretreatment inhibits HTGL-mediated cell surface ^{125}I-labeled VLDL binding at 4°C. FSF cells treated with (closed symbols) or without (open symbols) lovastatin as in Fig. 1 were incubated for 1 h at 37°C with the indicated concentrations of heparinase. The cells were then chilled, washed, and incubated for 3 h at 4°C with 5 μg/ml ^{125}I-labeled VLDL in the absence (squares) or presence of 3 μg/ml HTGL (circles). Cell surface binding was determined as in Fig. 1 and is represented as a percentage of binding measured in the absence of heparinase (100% binding at zero heparinase). The 100% binding under each condition is similar to that shown in Fig. 1B.

DISCUSSION

For a number of years HTGL has been known to influence remnant catabolism. HTGL is synthesized by hepatocytes and is present on the luminal surface of hepatic endothelial cells (53). Thus it is well positioned for a significant role in remnant catabolism by the liver. Several studies have attempted to investigate the role of HTGL in receptor-mediated lipoprotein catabolism using cultured cells. However, most studies have not compared the relative contributions of LRP and LDL receptor pathways. Sultan et al. (20) demonstrated that addition of partially purified hepatic lipase stimulates receptor-mediated uptake of remnant particles by freshly isolated hepatocytes. Ji et al. (22) showed enhanced remnant binding and uptake by rat hepatoma cells transfected with the human HTGL cDNA. They suggest that binding is to cell surface heparan sulfate proteoglycans as HTGL-mediated chylomicron remnant uptake is inhibited by heparinase treatment.

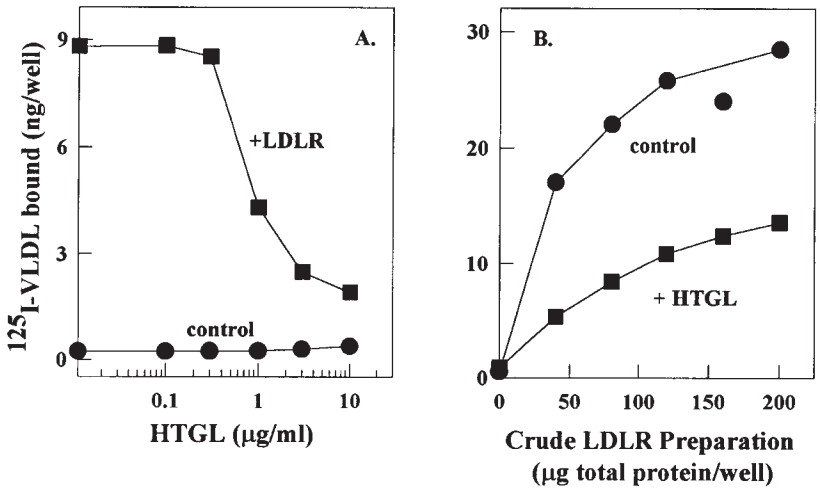

Fig. 9. HTGL inhibits ^{125}I-labeled VLDL binding to LDL receptors in solid-phase assays. Microtiter wells coated with 30 μg/ml of antibody direct against the cytoplasmic tail of LDL receptors (IgG-4A4) were incubated overnight at 4°C with 100 μl of buffer containing a crude LDL receptor preparation. Unoccupied sites were blocked with 1% BSA and immobilized receptor was incubated at 4°C for 3 h with 5 μg/ml ^{125}I-labeled VLDL in the presence or absence of HTGL. After washing unbound ligand, bound ligand was dissociated with 0.3 N NaOH and quantitated. (A) Zero (circles) or 100 μg total protein/well (squares) of LDL receptor preparation and indicated concentration of HTGL. (B) Indicated amounts of crude LDL receptor preparation, zero (circles) or 1 μg/ml HTGL (squares). Data points are averages of duplicate determinations and representative of two experiments.

However, they did not attempt to determine the receptor pathway involved in the endocytic process.

A few studies implicate LDL receptors in HTGL-stimulated catabolism of LDL but not of VLDL or remnant particles. Komaromy, Azhar, and Cooper (54) genetically engineered CHO cells to express a cell surface-anchored form of HTGL. These cells demonstrate increased LDL receptor-mediated LDL uptake but no alteration in VLDL catabolism. Choi et al. (55) studied CHO cells transfected with the cDNA for HTGL. They also found that HTGL-secreting cells stimulate LDL but not chylomicron remnant catabolism. Using specific antibodies, they determined that HTGL-stimulated degradation of LDL particles is mediated by the LDL receptor pathway. Aviram, Bierman, and Chait (56) have shown that modification of LDL particles by preincubation with hepatic lipase leads to increased LDL receptor-mediated uptake and cholesterol accumulation in monocyte-derived macrophages.

Other studies support a role for LRP in HTGL-stimulated remnant catabolism. Chang and Borensztajn (57) showed that uptake of hepatic lipase-treated chylomicrons by a murine hepatic cell line is inhibited by lactoferrin, a ligand for LRP. Krapp et al. (19) used normal and LDL receptor-deficient CHO cells to show that HTGL-stimulated VLDL catabolism proceeds via the LRP pathway. Kounnas et al. (21) showed that HTGL itself is a ligand for LRP and is internalized by the LRP pathway. To date no one has successfully demonstrated the role of LDL receptors in HTGL-stimulated VLDL catabolism.

The present data establish that HTGL stimulates VLDL catabolism via LDL receptors. HTGL induces catabolism of normal VLDL in FSF, HepG2, MEF, and PEA13 cells. PEA13 cells are LRP-deficient cells and both MEF and PEA13 cells do not express other remnant receptors including GP330 or VLDL receptors (38, 58). Thus, HTGL-dependent VLDL catabolism in PEA13 cells is probably mediated by LDL receptors. The level of degradation is similar to that in MEF cells which express both LRP and LDL receptors. This result confirms a role for LDL receptors rather than LRP in HTGL-mediated VLDL catabolism. This is supported by the great enhancement in VLDL catabolism seen when cells are treated with lovastatin to up-regulate their LDL receptor expression. The effects of lovastatin and HTGL are synergistic rather than additive, further suggesting the involvement of LDL receptors in HTGL-mediated catabolism. Lovastatin does not alter the expression of other known lipoprotein receptors including LRP, GP300, and VLDL receptors. Lovastatin increases HTGL-stimulated VLDL degradation in all four cell lines possessing an intact LDL receptor pathway, but not in FH fibroblasts that lack LDL receptors. We found that HTGL also stimulated VLDL catabolism in FH cells which lack a functional LDL receptor pathway, however, the amount of catabolism is 40-fold lower in these cells. The expression of LRP in FSF and FH cells is identical as determined by their ability to bind and internalize an LRP-specific ligand α_2M* (23). Thus, the significant difference between HTGL's effect in the two cell lines suggests a definite but relatively minor role for LRP and a dominant role for LDL receptors.

Studies with competitors provide further evidence that HTGL stimulates VLDL degradation via LDL receptors. Receptor-associated protein (RAP) is extensively used as a specific antagonist of LRP. RAP binds to LRP with high affinity and inhibits LRP's interactions with all of its ligands

(59, 60). We have shown earlier that RAP also binds to LDL receptors but with a 100-fold lower affinity (43). Here, we found that RAP only partially inhibited HTGL-stimulated VLDL catabolism at concentrations that inhibit catabolism of HTGL by LRP (21). We also studied inhibition by an antagonist of LDL receptors, GST-LPLC$_{ww}$. This mutant with tryptophan to alanine substitutions at positions 393 and 394 in carboxyl terminal fragment of LPL was generated by site-directed mutagenesis. These point mutations abolish GST-LPLC's binding to VLDL without affecting binding to LDL receptors or LRP (28, 29). GST-LPLC$_{ww}$ completely inhibited HTGL-stimulated VLDL catabolism. This result rules out the possibility that the RAP-resistant catabolism may be nonspecific. Thus, a major role for LDL receptors is established by several independent approaches. *i*) The effects of HTGL and lovastatin are synergistic; *ii*) the effect of HTGL is 40-fold higher in FSF than in FH fibroblasts; *iii*) the effect of HTGL is identical in MEF and PEA13 cells; and *iv*) RAP only partially inhibits HTGL's effect.

HTGL induced the binding and uptake of apoprotein-free triglyceride emulsions. This is consistent with the finding of Ji et al. (22) that apoE is not required for HTGL-mediated remnant uptake. They found that uptake was equally efficient for remnants from the apoE-deficient mice. We report that unlike VLDL uptake, binding and uptake of apoprotein-free Intralipid emulsion was not LDL receptor-dependent as it was not increased by lovastatin treatment. This suggests that in the absence of apoprotein components, HTGL–lipid complexes may not directly bind to LDL receptors in cultured cells. Instead, binding was to cell surface HSPG as evidenced by its sensitivity to heparinase treatment. We found that even when we used VLDL particles, which have both apoE and apoB-100, cell surface binding at 4°C was significantly inhibited by pretreatment of cells with heparinase. However, heparinase did not affect HTGL-stimulated VLDL degradation at 37°C. Thus, as reported earlier (19, 21, 22), cell surface proteoglycans may be the initial binding site for HTGL–lipid complexes. The VLDL particles (but not triglyceride emulsions) may then be transferred to neighboring LDL receptors accounting for the lovastatin effect.

To investigate the possibility that HTGL may directly bind to LDL receptors, we used cell-free solid-phase assays. LDL receptors from a crude cell membrane preparation were immobilized to microtiter wells using an anti-LDL receptor monoclonal antibody (IgG-4A4). We have shown earlier that only LDL receptors are specifically immobilized by this procedure (23, 43). Data obtained by the solid-phase assay suggest that HTGL may be a low affinity ligand for LDL receptors. VLDL binding to immobilized LDL receptors was inhibited by HTGL in a dose-dependent manner. The inhibitory effect may be due to HTGL binding to VLDL particles and quenching them from binding LDL receptors or HTGL directly binding LDL receptors and hindering the binding of VLDL particles. The latter possibility is more likely as we determined that this inhibition was not due to sequestration of VLDL particles by HTGL. HTGL inhibited VLDL binding even when the VLDL concentration was increased to 50 nm (data not shown). In fact, we found that the inhibition was greater at higher VLDL concentrations and that, in the presence of trace amounts of ^{125}I-labeled VLDL, HTGL did not inhibit VLDL binding to immobilized LDL receptors. This may be explained if LDL receptors at lower VLDL levels are in excess providing enough sites for both VLDL and HTGL binding. However, as VLDL levels are increased, they are unable to bind to sites occupied by HTGL. Thus HTGL's receptor-binding site appears to mask its lipid binding site preventing it from binding to both simultaneously. However, using ^{125}I-labeled HTGL, we were unable to demonstrate direct binding of HTGL to immobilized LDL receptors, indicating that in the absence of HSPG such an interaction may be of low affinity.

The inhibition of VLDL binding to immobilized LDL receptors by HTGL is the complete opposite of the effect of LPL. LPL enhanced VLDL binding even to immobilized LDL receptors (23). This property is attributed to its ability to simultaneously bind receptors and lipoproteins and form a bridge between the two. Salinelli et al. (61) have demonstrated that proper folding of LPL is essential for its bridging function. They found that cysteines at positions 216 and 239 as well as serine 172 were important in maintaining a structure capable of the bridging function. All three of these residues are conserved in HTGL (62). However, the tryptophans at 393 and 394 in LPL that are required to bind lipoprotein are not conserved in HTGL. Thus a relative shift in the positioning of the receptor and lipoprotein binding regions in HTGL compared to LPL may account for the absence of the bridging function in HTGL. However, HTGL is still capable of enhancing VLDL binding to the cell surface because of its ability to bind cell surface proteoglycans. The VLDL particles thus sequestered on the cell surface presumably are transferred to LDL receptors and LRP leading to enhanced receptor-mediated VLDL catabolism. HTGL may also activate apoE- and apoB-100-mediated binding by its lipolytic activity (63) but this is not required as HTGL also stimulates binding at 4°C when lipolysis would presumably be negligible.

Because LDL receptors far outnumber LRP in lovastatin-treated cells, the major contribution of LDL receptors is to be expected. It is not clear which receptor dominates in the in vivo situation where the LDL receptor may actually be down-regulated. Our results show that even down-regulated normal fibroblasts are capable of almost 10-fold greater HTGL-mediated VLDL catabolism than FH fibroblasts. However, hepatocytes may express greater LRP than LDL receptors. Thus, there may be a tissue-specific dominance of each receptor.

Preliminary studies using tetrahydrolipstatin (THL) indicate that lipolytic activity is not required for HTGL-stimulated VLDL binding and degradation (data not shown). THL inhibits lipase activity of all mammalian lipases by covalently binding to the active site serine residue (64). We found that HTGL that was treated with THL was equally capable of enhancing VLDL catabolism. However, we were unable to detect lipolytic activity in the preparation of HTGL used before or after treatment with THL. It

is possible that our HTGL preparation may have lost its catalytic activity during storage. Detailed studies are in progress to investigate the contribution, if any, of lipolysis on VLDL catabolism. Cell experiments at 4°C when enzymatic activity is negligible also support the idea that HTGL-promoted VLDL binding is independent of lypolytic activity. Thus, structural elements of HTGL may be more important than enzymatic activity. This is consistent with the demonstration by DeFaria et al. (65) that anti-rat HTGL antibody inhibited HTGL-stimulated remnant uptake in mice without inhibiting mouse HTGL lipolytic activity. Even though lipolysis may not be required for stimulation of VLDL catabolism, it has been demonstrated that lipolysis of VLDL enhances its ability to bind receptors due to the unmasking of apoE binding domains (63). This is similar to our earlier finding that the catalytically inactive fragment of LPL, LPLC, was only a third as potent as LPL at the same concentration.

HTGL-promoted VLDL degradation was 10-fold lower than that obtained for the same concentration of LPL. At least a 100-fold higher concentration of HTGL than LPL was required to induce comparable VLDL degradation. Physiologically, HTGL is present primarily on the endothelial cells lining hepatic sinusoidal cavities whereas LPL is present on the vascular endothelium. The concentrations of HTGL and LPL in human postheparin plasma are comparable (1). All of the plasma HTGL is recruited from the liver which has a blood volume less than 10% of the total body blood volume. Thus, locally, the concentration of HTGL in the liver may be significantly higher (by at least 10-fold) than that in post-heparin plasma. This is assuming that all of the plasma HTGL originates from the cell membrane bound pool. However, it is possible that heparin may induce HTGL secretion from intracellular storage vesicles. Martin et al. (66) purified 6.4 mg of HTGL from 8400 ml of post-heparin plasma with only 11% recovery. This translates to a concentration of 7 μg HTGL/ml in plasma. Thus, the concentration of 3 μg/ml used in most of our experiments is within this physiological range.

The physiological role of HTGL vis-à-vis that of LPL is not clear. It is generally believed that LPL is important for the generation of remnant particles whereas HTGL further hydrolyzes remnants to LDL and HDL (1). The contribution of lipases to receptor-mediated remnant clearance may be tissue specific. LPL is definitely a major player at the vascular wall but HTGL may play a significant role in the liver. Plasma HTGL levels are also thought to influence the development of atherosclerosis. HTGL's ability to promote VLDL clearance from the plasma should be antiatherogenic. It is known that HTGL levels are reduced in conditions that predispose to atherogenesis such as diabetes and hypothyroidism (2, 67, 68). Familial HTGL deficiency was associated with abnormal catabolism and accumulation of triglyceride-rich lipoproteins and the development of premature atherosclerosis (11, 12). Additional research is warranted to better understand the role of HTGL in lipoprotein catabolism in normal and pathophysiological states.

We thank Mary Lou Booth and Gregory Aylsworth for assistance with tissue culture. This work was supported by grant HL49264 from the National Institutes of Health and a grant-in-aid award from the American Heart Association.

Manuscript received 4 December 1998 and in revised form 12 March 1999.

REFERENCES

1. Olivecrona, T., and G. Bengtsson-Olivecrona. 1993. Lipoprotein lipase and hepatic lipase. *Curr. Opin. Lipidol.* **4:** 187–196.
2. Jansen, H., and W. C. Hulsmann. 1985. Enzymology and physiological role of hepatic lipase. *Biochem. Soc. Trans.* **13:** 24–26.
3. Bensadoun, A., and D. E. Berryman. 1996. Genetics and molecular biology of hepatic lipase. *Curr. Opin. Lipidol.* **7:** 77–81.
4. Beisiegel, U. 1996. New aspects on the role of plasma lipases in lipoprotein catabolism and atherosclerosis [Review]. *Atherosclerosis.* **124:** 1–8.
5. Olivecrona, G., and T. Olivecrona. 1995. Triglyceride lipases and atherosclerosis. *Curr. Opin. Lipidol.* **6:** 291–305.
6. Sultan, F., D. Lagrange, H. Jansen, and S. Griglio. 1990. Inhibition of hepatic lipase activity impairs chylomicron remnant-removal in rats. *Biochim. Biophys. Acta.* **1042:** 150–152.
7. Goldberg, I. J., N. A. Le, J. R. Paterniti, Jr., H. N. Ginsberg, F. T. Lindgren, and W. V. Brown. 1982. Lipoprotein metabolism during acute inhibition of hepatic triglyceride lipase in the cynomolgus monkey. *J. Clin. Invest.* **70:** 1184–1192.
8. 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.
9. Jansen, H., A. van Tol, and W. C. Hulsmann. 1980. On the metabolic function of heparin-releasable liver lipase. *Biochem. Biophys. Res. Commun.* **92:** 53–59.
10. Murase, T., and H. Itakura. 1981. Accumulation of intermediate density lipoprotein in plasma after intravenous administration of hepatic triglyceride lipase antibody in rats. *Atherosclerosis.* **39:** 293–300.
11. Connelly, P. W., G. F. Maguire, M. Lee, and J. A. Little. 1990. Plasma lipoproteins in familial hepatic lipase deficiency. *Arteriosclerosis.* **10:** 40–48.
12. Hegele, R. A., J. A. Little, C. Vezina, G. F. Maguire, L. Tu, T. S. Wolever, D. J. A. Jenkins, and P. W. Connelly. 1993. Hepatic lipase deficiency. Clinical, biochemical, and molecular genetic characteristics. *Arterioscler. Thromb.* **13:** 720–728.
13. 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.
14. Dugi, K. A., L. M. Feuerstein, S. Hill, J. Shih, S. Santamarina-Fojo, H. B. Brewer, and J. M. Hoeg. 1997. Lipoprotein lipase correlates positively and hepatic lipase inversely with calcific atherosclerosis in homozygous familial hypercholesterolemia. *Arterioscler. Thromb. Vasc. Biol.* **17:** 354–364.
15. Busch, S. J., R. L. Barnhart, G. A. Martin, M. C. Fitzgerald, M. T. Yates, S. J. Mao, C. E. Thomas, and R. L. Jackson. 1994. Human hepatic triglyceride lipase expression reduces high density lipoprotein and aortic cholesterol in cholesterol-fed transgenic mice. *J. Biol. Chem.* **269:** 16376–16382.
16. 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.
17. Applebaum-Bowden, D., J. Kobayashi, V. S. Kashyap, D. R. Brown, A. Berard, S. Meyn, C. Parrott, N. Maeda, R. Shamburek, H. B. Brewer, 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.
18. 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.
19. 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.
20. Sultan, F., D. Lagrange, X. Le Liepvre, and S. Griglio. 1989. Chylomicron-remnant uptake by freshly isolated hepatocytes. Effect of heparin and of hepatic triacylglycerol lipase. *Biochem. J.* **258:** 587–594.
21. 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.
22. 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.
23. Medh, J. D., S. L. Bowen, G. L. Fry, S. Ruben, M. Andracki, I. Inoue, J. M. Lalouel, D. K. Strickland, and D. A. Chappell. 1996. Lipoprotein lipase binds to low density lipoprotein receptors and induces receptor-mediated catabolism of very low density lipoproteins in vitro. *J. Biol. Chem.* **271:** 17073–17080.
24. Chappell, D. A. 1988. Pre-beta-very low density lipoproteins as precursors of beta-very low density lipoproteins. A model for the pathogenesis of familial dysbetalipoproteinemia (type III hyperlipoproteinemia). *J. Clin. Invest.* **82:** 628–639.
25. Iverius, P. H., and A. M. Ostlund-Lindqvist. 1976. Lipoprotein lipase from bovine milk. Isolation procedure, chemical characterization, and molecular weight analysis. *J. Biol. Chem.* **251:** 7791–7795.
26. Hill, J. S., R. C. Davis, D. Yang, J. Wen, J. S. Philo, P. H. Poon, M. L. Phillips, E. S. Kempner, and H. Wong. 1996. Human hepatic lipase subunit structure determination. *J. Biol. Chem.* **271:** 22931–22936.
27. Williams, S. E., J. D. Ashcom, W. S. Argraves, and D. K. Strickland. 1992. A novel mechanism for controlling the activity of alpha 2-macroglobulin receptor/low density lipoprotein receptor-related protein. Multiple regulatory sites for 39-kDa receptor-associated protein. *J. Biol. Chem.* **267:** 9035–9040.
28. Chappell, D. A., I. Inoue, G. L. Fry, M. W. Pladet, P. H. Iverius, J. M. Lalouel, and D. K. Strickland. 1994. The carboxy-terminal domain of lipoprotein lipase induces cellular catabolism of normal very low density lipoproteins via the low density lipoprotein receptor-related protein/alpha 2-macroglobulin receptor. *Ann. NY Acad. Sci.* **737:** 434–438.
29. Williams, S. E., I. Inoue, H. Tran, G. L. Fry, M. W. Pladet, P. H. Iverius, J. M. Lalouel, D. A. Chappell, and D. K. Strickland. 1994. The carboxyl-terminal domain of lipoprotein lipase binds to the low density lipoprotein receptor-related protein/alpha 2-macroglobulin receptor (LRP) and mediates binding of normal very low density lipoproteins to LRP. *J. Biol. Chem.* **269:** 8653–8658.
30. Chappell, D. A., I. Inoue, G. L. Fry, M. W. Pladet, S. L. Bowen, P. H. Iverius, J. M. Lalouel, and D. K. Strickland. 1994. Cellular catabolism of normal very low density lipoproteins via the low density lipoprotein receptor-related protein/alpha 2-macroglobulin receptor is induced by the C-terminal domain of lipoprotein lipase. *J. Biol. Chem.* **269:** 18001–18006.
31. Bilheimer, D. W., S. Eisenberg, and R. Levy. 1972. The metabolism of very low density lipoprotein proteins. I. Preliminary in vitro and in vivo observations. *Biochim. Biophys. Acta.* **260:** 212–221.
32. McGowan, M. W., J. D. Artiss, D. R. Strandbergh, and B. C. Zak. 1983. A peroxidase-coupled method for the colorimetric determination of serum triglycerides. *Clin. Chem.* **29:** 538–542.
33. 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.
34. Hamilton-Attwell, V. L., J. Du Plessis, and C. J. Van Wyk. 1986. A new scanning electron microscope (SEM) method for the determination of particle size in parenteral fat emulsions. *J. Microsc.* **145:** 347–349.
35. Chappell, D. A., G. L. Fry, M. A. Waknitz, and J. J. Berns. 1992. Evidence for isomerization during binding of apolipoproteinB-100 to low density lipoprotein receptors. *J. Biol. Chem.* **267:** 270–279.
36. Chappell, D. A., G. L. Fry, M. A. Waknitz, and J. J. Berns. 1991. Ligand size as a determinant for catabolism by the low density lipoprotein (LDL) receptor pathway. A lattice model for LDL binding. *J. Biol. Chem.* **266:** 19296–19302.
37. Hobbs, H. H., M. S. Brown, D. W. Russell, J. Davignon, and J. L. Goldstein. 1987. Deletion in the gene for the low-density lipoprotein receptor in a majority of French Canadians with familial hypercholesterolemia. *N. Engl. J. Med.* **317:** 734–737.
38. Willnow, T. E., and J. Herz. 1994. Genetic deficiency in low density lipoprotein receptor-related protein confers cellular resistance to Pseudomonas exotoxin A. Evidence that this protein is required for uptake and degradation of multiple ligands. *J. Cell Sci.* **107:** 719–726.
39. Brown, M. S., J. R. Faust, J. L. Goldstein, I. Kaneko, and A. Endo. 1978. Induction of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity in human fibroblasts incubated with compactin (ML-236B), a competitive inhibitor of the reductase. *J. Biol. Chem.* **253:** 1121–1128.
40. Goldstein, J. L., S. K. Basu, and M. S. Brown. 1983. Receptor-mediated endocytosis of low density lipoprotein in cultured cells. *Methods Enzymol.* **98:** 241–260.
41. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–276.
42. Van Driel, I. R., M. S. Brown, and J. L. Goldstein. 1989. Stoichiometric binding of low density lipoprotein (LDL) and monoclonal antibodies to LDL receptors in a solid phase assay. *J. Biol. Chem.* **264:** 9533–9538.
43. Medh, J. D., G. L. Fry, S. L. Bowen, M. W. Pladet, D. K. Strickland, and D. A. Chappell. 1995. The 39-kDa receptor-associated protein modulates lipoprotein catabolism by binding to LDL receptors. *J. Biol. Chem.* **270:** 536–540.
44. Beisiegel, U., A. Krapp, W. Weber, and G. Olivecrona. 1994. The role of alpha 2M receptor/LRP in chylomicron remnant metabolism. *Ann. NY Acad. Sci.* **737:** 53–69.
45. Nykjaer, A., G. Bengtsson-Olivecrona, A. Lookene, S. K. Moestrup, C. M. Petersen, W. Weber, U. Beisiegel, and J. Gliemann. 1993. The alpha 2-macroglobulin receptor/low density lipoprotein receptor-related protein binds lipoprotein lipase and beta-migrating very low density lipoprotein associated with the lipase. *J. Biol. Chem.* **268:** 15048–15055.
46. Kutt, H., J. Herz, and K. K. Stanley. 1989. Structure of the low-density lipoprotein receptor-related protein (LRP) promoter. *Biochim. Biophys. Acta.* **1009:** 229–236.
47. Chappell, D. A., G. L. Fry, M. A. Waknitz, L. E. Muhonen, and M. W. Pladet. 1993. Low density lipoprotein receptors bind and mediate cellular catabolism of normal very low density lipoproteins in vitro. *J. Biol. Chem.* **268:** 25487–25493.
48. Orlando, R. A., and M. G. Farquhar. 1994. Functional domains of the receptor-associated protein (RAP). *Proc. Natl. Acad. Sci. USA.* **91:** 3161–3165.
49. Busch, S. J., J. L. Krstenansky, T. J. Owen, and R. L. Jackson. 1989. Human hepatoma (HepG2) cells secrete a single 65 K dalton triglyceride lipase immunologically identical to postheparin plasma hepatic lipase. *Life Sci.* **45:** 615–622.
50. Lombardi, P., M. Mulder, E. de Wit, T. J. van Berkel, R. R. Frants, and L. M. Havekes. 1993. Low-density lipoproteins are degraded in HepG2 cells with low efficiency. *Biochem. J.* **290:** 509–514.
51. Ji, Z. S., S. Fazio, and R. W. Mahley. 1994. Variable heparan sulfate proteoglycan binding of apolipoprotein E variants may modulate the expression of type III hyperlipoproteinemia. *J. Biol. Chem.* **269:** 13421–13428.
52. Mulder, M., P. Lombardi, H. Jansen, T. J. 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.
53. Laposata, E. A., H. M. Laboda, J. M. Glick, and J. F. Strauss. 1987. Hepatic lipase. Synthesis, processing, and secretion by isolated rat hepatocytes. *J. Biol. Chem.* **262:** 5333–5338.
54. Komaromy, M., S. Azhar, and A. D. Cooper. 1996. Chinese hamster ovary cells expressing a cell surface-anchored form of hepatic lipase. Characterization of low density lipoprotein and chylomicron remnant uptake and selective uptake of high density lipoprotein-cholesteryl ester. *J. Biol. Chem.* **271:** 16906–16914.
55. 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.
56. Aviram, M., E. L. Bierman, and A. Chait. 1988. Modification of low density lipoprotein by lipoprotein lipase or hepatic lipase induces enhanced uptake and cholesterol accumulation in cells. *J. Biol. Chem.* **263:** 15416–15422.

57. Chang, S., and J. Borensztajn. 1995. Uptake of chylomicron remnants and hepatic lipase-treated chylomicrons by a non-transformed murine hepatocyte cell line in culture. *Biochim. Biophys. Acta.* **1256:** 81–87.
58. Takahashi, S., Y. Kawarabayasi, T. Nakai, J. Sakai, and T. Yamamoto. 1992. Rabbit very low density lipoprotein receptor: a low density lipoprotein receptor-like protein with distinct ligand specificity. *Proc. Natl. Acad. Sci. USA.* **89:** 9252–9256.
59. Strickland, D. K., J. D. Ashcom, S. Williams, F. Battey, E. Behre, K. McTigue, J. F. Battey, and W. S. Argraves. 1991. Primary structure of alpha 2-macroglobulin receptor-associated protein. Human homologue of a Heymann nephritis antigen. *J. Biol. Chem.* **266:** 13364–13369.
60. Moestrup, S. K., T. L. Holtet, M. Etzerodt, H. C. Thogersen, A. Nykjaer, P. A. Andreasen, H. H. Rasmussen, L. Sottrup-Jensen, and J. Gliemann. 1993. Alpha 2-macroglobulin-proteinase complexes, plasminogen activator inhibitor type-1-plasminogen activator complexes, and receptor-associated protein bind to a region of the alpha 2-macroglobulin receptor containing a cluster of eight complement-type repeats. *J. Biol. Chem.* **268:** 13691–13696.
61. Salinelli, S., J. Y. Lo, M. P. Mims, E. Zsigmond, L. C. Smith, and L. Chan. 1996. Structure-function relationship of lipoprotein lipase-mediated enhancement of very low density lipoprotein binding and catabolism by the low density lipoprotein receptor. Functional importance of a properly folded surface loop covering the catalytic center. *J. Biol. Chem.* **271:** 21906–21913.
62. Datta, S., C-C. Luo, W-H. Li, P. VanTuinen, D. H. Ledbetter, M. A. Brown, S-H. Chen, S-W. Liu, and L. Chan. 1988. Human hepatic lipase. *J. Biol. Chem.* **263:** 1107–1110.
63. 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.
64. Lookene, A., N. Skottova, and G. Olivecrona. 1994. Interactions of lipoprotein lipase with the active-site inhibitor tetrahydrolipstatin (Orlistat). *Eur. J. Biochem.* **222:** 395–403.
65. DeFaria, 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.
66. Martin, G. A., S. J. Busch, G. D. Meredith, A. D. Cardin, D. T. Blankenship, S. J. T. Mao, A. E. Rechtin, C. W. Woods, M. M. Racke, Schafer, M. P., M. C. Fitzgerald, D. M. Burke, M. A. Flanagan, and R. L. Jackkson. 1988. Isolation and cDNA sequence of human postheparin plasma hepatic triglyceride lipase. *J. Biol. Chem.* **263:** 10907–10914.
67. Staels, B., A. Van Tol, L. Chan, H. Will, G. Verhoeven, and J. Auwerx. 1990. Alterations in thyroid status modulate apolipoprotein, hepatic triglyceride lipase, and low density lipoprotein receptor in rats. *Endocrinology.* **127:** 1144–1152.
68. Hulsmann, W. C., M. C. Oerlemans, and M. M. Geelhoed-Mieras. 1977. Effect of hypothyroidism, diabetes and polyunsaturated fatty acids on heparin-releasable rat liver lipase. *Biochem. Biophys. Res. Commun.* **79:** 784–788.