Hepatic lipase mediates the uptake of chylomicrons and β-VLDL into cells via the LDL receptor-related protein (LRP)

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Abstract The uptake of triglyceride-rich lipoproteins has been described as being mediated by apolipoprotein E and lipoprotein lipase (LpL). Proteoglycans, the LDL-receptor, and the LDL receptor-related protein (LRP) are the cellular acceptors. In addition to LpL, hepatic lipase (HL) has been shown to bind to LRP. In this study, the role of HL in lipoprotein uptake was investigated. Human chylomicrons and rabbit β-VLDL were used as ligands for human hepatoma cells, primary human hepatocytes, normal and proteoglycan-deficient Chinese hamster ovary (CHO) cells, and normal and LDL receptor-deficient human fibroblasts. We show that HL induces stimulation of the uptake of chylomicrons and β-VLDL into the different cell lines. HL is known to bind to heparan sulfate, and experiments on normal and proteoglycan-deficient CHO cells showed that cell surface proteoglycans are essential for HL-mediated uptake of lipoproteins. To exclude LDL receptor-mediated uptake, we performed experiments on LDL receptor-deficient fibroblasts that demonstrated that the LDL receptor was not important for the HL-mediated uptake of lipoproteins. Crosslinking experiments confirmed the binding of HL to LRP on the cell surface. To identify the region of HL involved in the interaction with LRP, we used a C-terminal fragment of LpL, known to inhibit LpL-mediated uptake. HL-mediated lipoprotein uptake was suppressed by this fragment. Our experiments indicate that HL, like LpL, can mediate the uptake of lipoproteins into cells, most probably via a C-terminal binding site. The uptake, initiated by proteoglycan binding, is mediated by LRP.—**Krapp, A., S. Ahle, S. Kersting, Y. Hua, K. Kneser, M. Nielsen, J. Gliemann, and U. Beisiegel.** Hepatic lipase mediates the uptake of chylomicrons and β-VLDL into cells via the LDL receptor-related protein (LRP). *J. Lipid Res.* 1996. **37:** 926–936.

Supplementary key words hepatic lipase • LDL receptor-related protein • lipoprotein lipase • human chylomicrons • proteoglycans

The function of HL as a lipolytic enzyme in lipoprotein metabolism has been known for a long time (1). HL catalyzes the hydrolysis of triacylglycerides, acyl-CoA thioesters, and phospholipids (2–4). Chylomicron remnants and intermediate density lipoproteins have been described as substrates (5, 6). HL is also important in the metabolism of high density lipoprotein (HDL), as it seems to be responsible for the conversion of HDL_2 to HDL_3 (7, 8). HL is synthesized in hepatocytes and located on the sinusoidal endothelium of the liver (9). Recently, a truncated form of HL has been described that is synthesized in rat adrenals (10). HL defects have been identified in several families (11, 12). Individuals with HL-deficiency are characterized by elevated levels of triglycerides and cholesterol as well as the production of abnormal LDL particles (13). Mice lacking the HL gene due to targeted gene disruption developed mild hyperlipidemias (14).

HL belongs to the lipase gene family (15), along with lipoprotein lipase (LpL) and pancreatic lipase (PL). Human HL has a 44% homology with LpL and about 27% with PL at the amino acid sequence level (16–18). PL is the only member of this family for which the three-dimensional crystallographic structure has been elucidated (19). It shows two distinct domains: the active site in the N-terminal folding domain between amino acids 1 and 335 and a C-terminal domain spanning amino acids 336–448. The amino acid sequence homologies and the similarities in the gene structure suggest a similar folding pattern for the other two lipases (16, 20). HL and LpL are both able to hydrolyze plasma lipoproteins whilst attached to the endothelium via proteoglycans. Their structural and functional features have been discussed in several recent reviews (21–23).

Abbreviations: HL, hepatic lipase; LRP, low density lipoprotein receptor-related protein; LpL, lipoprotein lipase; PL, pancreatic lipase; FH, familial hypercholesterolemia; CHO, Chinese hamster ovary cells.

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LpL has been shown to bind to LRP (24–26) and to mediate the binding of lipoproteins to this receptor. Also, it can facilitate the uptake of chylomicrons and VLDL into cells (24, 27) and enhances the removal of chylomicrons and chylomicron remnants by the perfused rat liver (28). LRP has been described as a receptor for chylomicron remnants (29). It is a multifunctional receptor that has been shown in parallel to bind apoE (30) and the activated form of $\alpha_2$macroglobulin (31, 32). Several other unrelated substances have subsequently been described as ligands for LRP (33), as well as a 39–40 kDa protein which copurifies with the receptor and has been termed 'receptor-associated protein' or RAP (34, 35). LRP belongs to the LDL receptor gene family which also includes the LDL receptor, gp 330 (36) and the VLDL receptor (37).

The function of LpL in mediating the binding and uptake of chylomicrons and other lipoproteins into different cell lines via LRP in vitro has been extensively characterized. It is independent of the catalytic activity but dependent on the C-terminal domain of the protein (24, 38–40).

HL has also been described as binding to LRP (39, 41) and found to be involved in chylomicron remnant catabolism. It could be shown that HL is able to mediate the binding (42) and uptake (43) of lipoproteins into cells. On the other hand, it has been suggested that HL may promote hepatic clearance of apoE-containing lipoproteins, either by hydrolysis and exposure of an apoE-receptor binding site (44) or by enhancing cell association via binding to cell-surface proteoglycans (45).

Our studies show for the first time that HL can mediate the uptake of human chylomicrons and rabbit β-VLDL into human hepatocytes and human hepatoma cells. The uptake is initiated by binding of the lipoproteins to proteoglycans on the cell surface, and facilitated by LRP. Experiments with LDL receptor-deficient human fibroblasts indicated that the LDL receptor is not necessary for HL-mediated uptake of chylomicrons and β-VLDL.

METHODS

Cells

Human hepatoma cells (Hep3b, HuH_7) and fibroblasts (normal and LDL receptor-deficient) were grown in Dulbeccos's modified Eagle's medium (DMEM; Gibco) with 10% fetal calf serum, penicillin/streptomycin (Gibco) at 37°C in 5% CO_2/95% air.

CHO cells (normal [K1] and PG-deficient [745]) were grown in Nutrient Mixture (F12 HAM, Gibco) with 10% fetal calf serum, penicillin/streptomycin (Gibco) under the same conditions.

The Hep3b cells were obtained from the American Type Culture Collection (ATCC HB 8064). The HuH_7 cells were a kind gift from Dr. Hans Will (Hamburg, Germany) (46). The normal fibroblasts are cultured from a skin biopsy of a control person. The FH cells were derived from a French Canadian patient, homozygous for the >15-kilobase deletion that eliminates the promotor and the first exon of the LDL-receptor gene (47); these cells were a kind gift from Dr. Davignon (Montreal, Canada). No LDL receptor can be detected in these cells. The CHO cells we kindly obtained from Dr. Esko (Birmingham, AL) (48).

Isolation and culture of human hepatocytes

Human liver tissue was obtained from patients with liver tumors who underwent resection operations in the surgical clinic of the University Hospital Eppendorf. The experimental protocol was approved by the Committee for Medical Ethics of the Aerztekammer Hamburg. Only liver segments devoid of tumor tissue were used for cell preparations. Hepatocytes were isolated and cultured as described by Galle et al. (49). Briefly, liver tissue was preperfused with MEM (Gibco) supplemented with 0.5 mM EGTA (Sigma) 25 U/ml heparin (Roche), 20 mM HEPES (Gibco) for 15 min. at 37°C. The perfusion medium was William's (Gibco) supplemented

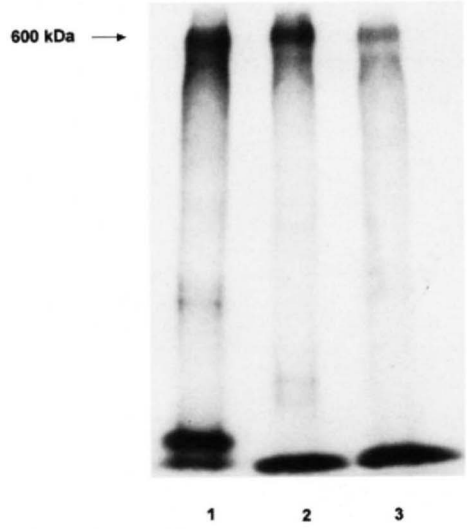

Fig. 1. Crosslinking of HL, LpL, and a C-terminal LpL fragment to Hep3b. Binding experiments were performed on Hep3b cells with ^{125}I-labeled LpL (lane 1), ^{125}I-labeled HL (lane 2) and ^{125}I-labeled LpL fragment (lane 3). Incubation was performed for 30 min at 4°C and subsequently the bound ligand was chemically linked to the receptor by 1-ethyl-3(3-dimethylaminopropyl)-carbodiimide/N-hydroxysuccinimide. After crosslinking, the cells were solubilized and applied to an SDS-PAGE, and electroblotted onto nitrocellulose. The radiolabeled bands were detected on X-ray film after 5 days of exposure. All lanes show a band at 600 kDa and the corresponding labeled lipase on the bottom. Shown is supernatant 2, which contains most of the radioactivity.

with 0.5 mg/ml collagenase (Type IV, Sigma), 3 mM CaCl2 and 20 mM HEPES (Sigma) foThroughout preperfusion and perfusion, media were gassed with carbogen (95% O2, 5% CO2). After perfusion, cells were isolated by shaking and rinsing the tissue, followed by filtration and several washing and centrifugation steps (50 *g*, 5 min). The hepatocytes were plated in 35-mm plastic tissue culture wells (Nunc) at a density of 2×10^6. The medium was William's medium supplemented with 10% FCS, 0.7 μg/ml insulin (Sigma), 0.065 μg/ml glucagon (Sigma), triiodothyronine 0.065 μg/ml (Sigma), 4.0 μg/ml dexamethasone (Serva), 100 U/ml penicillin/streptomycin, 2 mM glutamin (Gibco) and 20 mM HEPES at 37°C in 5% CO_2/95% air.

Lipid and protein quantification

Cholesterol and triglycerides in the lipoproteins were determined with enzymatic colorimetric assays from Boehringer Mannheim. The protein content of the lipoproteins and the cell proteins was determined by the Lowry technique, modified for lipid containing samples by addition of 0.1% SDS.

Rabbit β-VLDL and human chylomicrons

β-VLDL (d < 1.006 g/ml) was isolated from the blood of cholesterol-fed rabbits (50). The protein content of the different preparations was 0.74 ± 0.46 mg of protein per ml. The ICI method was used for iodination (51). The sodium iodine Na ^{125}I was purchased from Amersham Buchler. The protein content of the different ^{125}I preparations was 0.34 ± 0.12 mg of protein per ml. The specific radioactivity in the protein was between 7.3 ± 1.8×10^4 cpm/μg; 30 ± 5% of the ^{125}Iodine was found in the lipid faction.

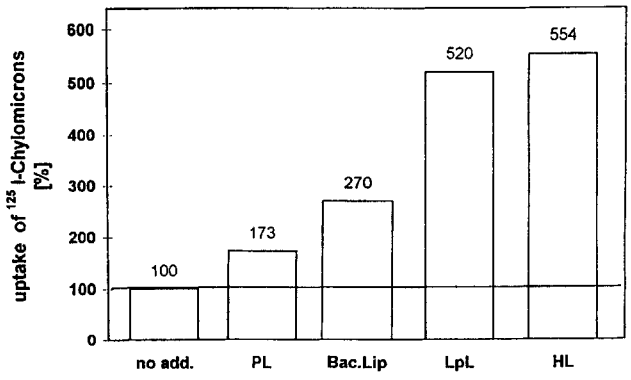

Fig. 2. Effect of different lipases on the uptake of ^{125}I-labeled chylomicrons into Hep3b cells. PL, bacterial lipase, LpL, and HL were added to Hep3b cells in a concentration of 0.1 μg/ml. The uptake experiment was performed at 37°C for 90 min using ^{125}I-labeled chylomicrons as ligand. Uptake was calculated in ng chylomicrons/mg cell protein. The results are the average of duplicate samples. In absence of lipases the uptake is 16 ng/mg cell protein and this is expressed as 100%.

Chylomicrons were obtained from LpL-deficient or apoC-II-deficient patients. For isolation of the chylomicrons, the plasma was layered under 10% sucrose (Sigma) and centrifuged at 280,000 *g* for 45 min at 4°C. Chylomicrons were taken from the top of the sucrose, resuspended, and a second sucrose spin was performed to purify the chylomicrons from contamination of serum proteins. The apolipoprotein content of the different preparations was 0.60 ± 0.25 mg of protein per ml. Iodination was performed as described for β-VLDL. The protein content of the different ^{125}I preparations was 0.26 ± 0.07 mg of protein per ml. The specific radioactivity in the protein was between $5.8 \pm 2.3 \times 10^4$ cpm/μg; 38 ± 9% of the ^{125}Iodine was found in the lipid faction.

Bovine LpL, PL, and bacterial lipase

Bovine LpL was purified from milk as described previously (52). The specific activity of the preparations, using a phospholipid-stabilized emulsion of soy bean oil (Intralipid; Kabi-Nutrition, Stockholm) at pH 8.5 and 25°C, with human serum as source of apoC-II, was 570 units/mg. One unit corresponds to 1 μmol of fatty acids released per ml per min. Protein concentrations were calculated using the absorbency at 280 nm and the absorption coefficient ($A_{1\%}$) 16.8 cm^{-1}.

Pancreatic lipase from porcine pancreas was obtained from Sigma and bacterial lipase derived from *Pseudomonas fluorescens* was a gift from Amano Pharmaceutical (Nagoya, Japan).

Isolation of HL from HuH_7 supernatants

HuH_7 cells were grown in 45 ml of the medium described above, containing 7 U/ml heparin (Roche) and 10 ng/ml TPCK (Boehringer) in 500 qcm Triple-Flask (Nunc). The medium was exchanged every day and stored at -80°C. To isolate HL, 500 ml of the medium was applied to a heparin-Sepharose High-Trap column (Pharmacia) after addition of sodium chloride to a final concentration of 0.3 M, and HL was eluted using 10 mM BisTris (Roth), pH 7.4, containing 1 M NaCl and 10% glycerol (Merck). The eluate was concentrated by dialysis against 3.4 M ammonium sulfate (Serva). HL was stored as a precipitate at -20°C and resuspended immediately before being used in the assay.

Isolation of HL from human postheparin plasma

HL was purified from postheparin plasma as described previously (53). In brief, fresh postheparin plasma was adjusted to 0.4 M NaCl and mixed with heparin-Sepharose for 2 h at 4°C. After washes of heparin-Sepharose, elution was performed with 0.7 M NaCl in 10 mM phosphate-buffer, pH 7.4. HL was eluted under this condition whereas LpL was retained. To remove possible traces of LpL in the eluate, the prepa-

ration was immunoprecipitated with rabbit IgG raised against LpL. Then the eluate was adjusted to 0.2 M NaCl and applied to a Sepharose column with immobilized, modified (N-desulfated and N-acetylated) heparin (50). After washing with 0.2 M NaCl, HL was eluted with 0.9 M NaCl.

Assay of HL activity

The activity of HL was measured using an emulsion of gum arabic-stabilized radiolabeled [^{14}C]trioleyl glycerol (Amersham) (54). The lipid emulsion was prepared by mixing 50 mg of unlabeled triolein (Fluka) and 2.5 μCi [^{14}C]trioleyl glycerol, followed by sonicating in a buffer containing 0.2 M Tris, 0.5% gum arabic, 1 M NaCl, 5% albumin. To each sample 0.2 ml of this emulsion was added and incubation was performed for 30 min at 28°C. The enzymatic reaction was stopped adding a mixture of chloroform–n-heptane–methanol 75:60:85 (v/v/v). The radioactivity of free fatty acids was measured in the aqueous phase after extraction. To inhibit HL activity, we used the lipase inhibitor Orlistat Orlistat (tetrahydrolipstatin, THL (Orlistat); La Roche), which was shown to be a general inhibitor for mammalian lipases (55). The HL activity was inhibited completely in our assay system.

Preparation of the C-terminal fragment of LpL

Expression of a fusion protein containing human LpL residues 378–448 was performed as described previously (39). Purification of the hexa-histidine-tagged protein was performed as described (56) except that 2 mM glutathione/0.2 mM oxidized glutathione was added to allow disulfide reshuffling.

Iodination of HL, LpL, and the LpL fragment

HL and the LpL fragment were iodinated using Iodobeads (Pierce) following the manufacturer's instructions. To one Iodobead in 200 μl PBS containing 0.003% sodium azide, 0.5 mCi ^{125}I was added. After 5 min, 5 to 10 μg HL or LpL-fragment was added. Incubation was for 10 min. Free iodine was removed from iodinated protein by running over a PD 10 column (Pharmacia) equilibrated with 3% BSA. Fractions containing ^{125}I-labeled HL or ^{125}I-labeled LpL-fragment were collected. The labeled preparations contained approximately 2 μg/ml protein and had a specific activity of 10–20 × 10^6 cpm/μg.

LpL was iodinated using lactoperoxidase and glucose oxidase and was repurified on heparin-Sepharose (57). The labeled LpL preparations contained approximately 1 μg/ml protein and had a specific activity of 10–30 × 10^6 cpm/μg.

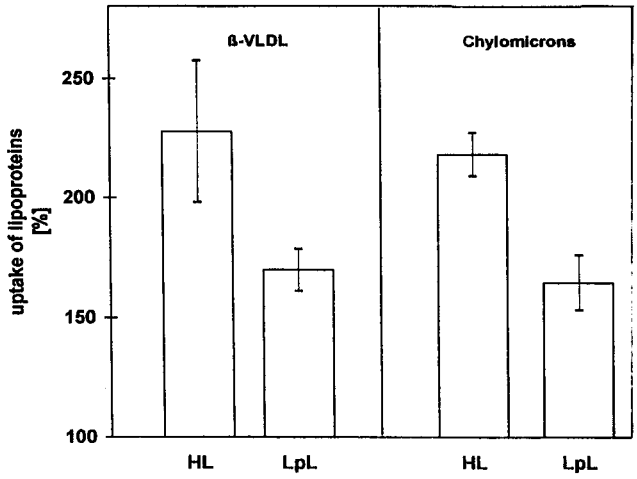

Fig. 3. Effect of HL and LpL on the uptake of ^{125}I-labeled chylomicrons and ^{125}I-labeled β-VLDL into Hep3b cells. Uptake experiments with addition of HL and LpL were performed with ^{125}I-labeled chylomicrons and ^{125}I-labeled β-VLDL as ligands. Experimental details are described in Fig. 2. The final concentration of LpL was 0.2 μg/ml; the concentration of HL was 0.1 μg/ml. The mean uptake of lipoproteins without lipase addition was 16 ng/mg cell protein for chylomicrons and 30 ng/mg cell protein for β-VLDL. These values were set to 100%. Shown is always the mean of three experiments.

Uptake experiments

For the uptake experiments, the Hep3b or HuH_7 cells, normal or PG-deficient CHO cells, and normal or LDL receptor-deficient fibroblasts were grown in 24-well plates from Nunc. Cells (1 × 10^6/ml) were seeded at day 0 and used as confluent monolayers at day 2. Human hepatocytes were plated in 6-well plates (Nunc) at a density of 2 × 10^6/ml at day 0 and used between days 2 and 4. The experiments were performed in 1 ml (24-well) or 2 ml (6-well) of DMEM containing 5% BSA (fraction V, Sigma) and 0.02 M HEPES (pH 7.4), with 2 μg of ^{125}I-labeled β-VLDL protein or 1 μg ^{125}I-labeled chylomicron protein per ml. Incubations were performed for 90 min at 37°C. To avoid lipolysis, the active site inhibitor (55) was present in the incubation medium (25 μg/ml). To determine the fraction of lipoprotein taken up, the cells were washed several times with phosphate-buffered saline (PBS), pH 7.4, containing 2 mg/ml BSA. The first and last wash were performed in the absence of BSA. Surface-bound lipoproteins were then released by PBS containing 770 U of heparin/ml (Liquemin, Roche). After washing, the cell monolayers were dissolved in 0.1 M NaOH and radioactivity and cell protein were measured.

Crosslinking experiments

Cells were grown in 6-cm petri dishes for crosslinking experiments. Binding was performed following the pro-

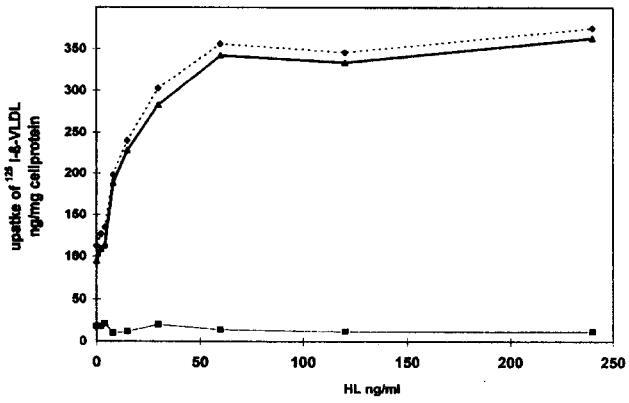

Fig. 4. Saturation curve for the HL-mediated uptake of human chylomicrons. Uptake of ^{125}I-labeled β-VLDL into Hep3b cells in the presence of increasing amounts of HL is shown. Concentration of HL was between 0 and 250 ng/ml. The experiment was performed as described in Fig. 2. Shown is the total (-◆-), unspecific (-■-), and specific (-▲-), uptake for the different concentrations of HL. Each point represents the mean of duplicate measurements.

tocol of uptake experiments except that the incubation was for 30 min at 0°C. After the final wash with PBS, pH 7.4, cells were washed once with PBS, pH 7.0. Subsequently, the bound ligand was linked to its receptor by the chemical crosslinker, 1-ethyl-3(3-dimethylaminopropyl)-carbodiimid in combination with N-hydroxysulfosuccinimide (Pierce) at final concentrations of 1 mg/ml in PBS, pH 7.0. The cells were incubated with the crosslinking reagents for 30 min at room temperature, followed by a wash with PBS. The cells were then scraped from the plastic dish, centrifuged at 400 *g* for 10 min, and solubilized in 20 mM Tris-HCl (Roth), pH 7.5/150 mM NaCl/2 mM $MgCl_2$/1% Nonident P-40 (Serva) (supernatant 1). The insoluble proteins were removed by centrifugation and solubilized in 5% SDS (supernatant 2). Solubilized proteins were applied to a SDS polyacrylamide gel (5%, SDS-PAGE) with subsequent electroblotting to nitrocellulose (0.45 μg, Schleicher & Schuell), which was exposed to X-ray film (Kodak, Biomax MR).

RESULTS

Production of HL with human hepatoma cells (HuH_7)

We isolated HL from the human hepatoma cell line HuH_7 (46), which does not secrete LpL. HuH_7 cells were kept in culture for up to 2 months without trypsination. The production of HL was monitored by its enzyme activity. The activity of HL increased in day 6 to a level of about 15 mU/ml and stayed at this level until the end of the experiment (around 30 days).

Because there was no ELISA available for HL, the mass was measured semi-quantitatively using enzyme activity and immunoblotting. The preparations were compared to the HL isolated from postheparin plasma and quantified via the known specific activity. The concentration of HL in our experiments was always approximately 0.1 μg/ml, a concentration at which the uptake mediated by HL was found to be saturated (Fig. 4).

Binding and crosslinking experiments with HL on Hep3b cells

Binding experiments were performed with ^{125}I-labeled HL and a specific binding of HL to the cells could be shown. To identify the binding protein for HL on the cell surface, we performed crosslinking experiments with ^{125}I-labeled HL in comparison with ^{125}I-labeled LpL. EDC-sulfo-NHS [1-ethyl-3(3-dimethyl-aminopropyl)-carbodiimide/N-hydroxy-succinimide] was used as a crosslinking agent. The autoradiography of the experiment is shown in **Fig. 1.** Lanes 1 and 2 represent the crosslinking of ^{125}I-labeled LpL and ^{125}I-labeled HL, respectively, to Hep3b cells. A major band of approximately 600 kDa was detected. This corresponds to the mobility of crosslinked LRP as demonstrated by Western blotting in parallel (data not shown). These experiments demonstrate that HL, like LpL, is able to bind to LRP on the cell surface. No other dominant band appears on the autoradiography. The radioactivity at the bottom of the lanes represents the labeled lipases.

Comparison of different lipases in uptake experiments on hepatoma cells and hepatocytes

Similarly to earlier experiments with LpL (42), we performed uptake experiments on Hep3b cells to test the ability of different lipases to mediate the uptake of ^{125}I-labeled chylomicrons into the cells. The uptake was defined as the cell-associated radioactivity after a 90-min incubation at 37°C and after a heparin release of surface-bound radioactivity. Specific uptake is shown in all figures, calculated by subtraction of unspecific uptake (in presence of a surplus of unlabeled ligand) from the total uptake. LpL and HL, but not PL and bacterial lipase can efficiently mediate the uptake of ^{125}I-labeled chylomicrons into hepatoma cells (**Fig. 2**). The residual effect of the PL and the noticeable increase in uptake in the presence of bacterial lipase can be explained by their enzyme activity as discussed later.

To verify the use of HUH_7 cell-derived HL instead of human plasma HL, we compared both in uptake experi-

TABLE 1. Effect of HL on lipoprotein uptake into human hepatoma cells and hepatocytes

Cells	% of β-VLDL Uptake (n)	% of Chylomicron Uptake (n)
Hep3b	191 ± 41 (8)	220 ± 51 (5)
HuH_7	167 ± 24 (3)	176 ± 48 (4)
Hepatocytes	166/120	156/209

Uptake experiments were performed as described in Material and Methods and in the legend to Fig. 2. Specific uptake was determined in presence and absence of HL on the different cell lines, and the values for the lipoprotein uptake without HL were set to 100%. The number of experiments included in this table is given in parentheses; two experiments were performed on hepatocytes and both values are presented.

ments on HUH_7 cells. Using 0.1 μg/ml of either HL preparation, we found the binding of chylomicrons increased from 7.6 ng/mg cell protein, without addition of lipase, to 24 ng/ml with the plasma HL and 22 ng/ml with HuH_7 HL. The uptake was increased from 109 ng/ml to 244 and 247 ng/ml, respectively.

The effect of LpL and HL on the uptake of ^{125}I-labeled β-VLDL and ^{125}I-labeled chylomicrons was compared on Hep3b cells. As shown in **Fig. 3,** HL (0.1 μg/ml) and LpL (0.2 μg/ml) both increased the basic uptake of the two ligands. Specific uptake of ^{125}I-labeled β-VLDL and ^{125}I-labeled chylomicrons without addition of lipases was set to 100%. The data indicate that HL facilitates the uptake of lipoproteins more efficiently than LpL, as 0.1 μg/ml HL induces a higher increase than 0.2 μg/ml of the LpL (see also discussion).

In the subsequent studies we focused on HL and investigated its function in lipoprotein uptake on different cell types. With increasing amounts of HL the uptake into Hep3b cells was increased correspondingly up to 60 ng/ml. The addition of 120 or 240 ng HL/ml did not further increase the uptake (**Fig. 4**). To work at a saturation level we used approximately 100 ng HL/ml in the experiments.

Comparison between hepatoma cells and fresh hepatocytes

To elucidate possible functional differences between human hepatoma cells (Hep3b and HuH_7) and fresh human hepatocytes, we performed uptake experiments with both ligands. There was no significant difference between the uptake of β-VLDL or chylomicrons into the three cell lines (**Table 1**).

HL-mediated uptake of lipoproteins is dependent on proteoglycans

LpL and HL bind with high affinity to cell surface proteoglycans (21). To elucidate the influence of proteoglycans on the HL-mediated uptake of lipoproteins, we performed experiments on normal [K1] and proteoglycan-deficient [745] CHO cells. **Figure 5** shows experiments using both ^{125}I-labeled β-VLDL and ^{125}I-labeled chylomicrons as ligands. HL increased the uptake of β-VLDL into normal CHO cells in two independent experiments to 339% and 439% and of chylomicrons to 349% and 320%. (The β-VLDL uptake without addition of HL was set too 100%). The uptake into proteoglycan-deficient CHO cells was, however, only 109% and 119% for β-VLDL, and 110% and 102% for chylomicrons in the same experiments. Thus proteoglycan-deficient cells did not allow any significant HL-mediated uptake of lipoproteins. The binding to proteoglycans seems, therefore, to be a necessary step in the uptake mechanism.

HL-mediated uptake into normal and LDL receptor-deficient human fibroblasts

We performed experiments on normal and LDL receptor-deficient (FH) human fibroblasts to investigate whether the HL-mediated uptake of β-VLDL or chylomicrons might be facilitated by the LDL receptor. As shown in **Fig. 6,** HL-mediated uptake of both ligands is increased on fibroblasts independent of the presence of the LDL receptor. Only one representative experiment is shown; however, all experiments performed on these cell lines showed comparable HL-mediated uptake.

C-terminal region of HL is involved in the binding to LRP

To analyze which domain in HL might be involved in the interaction with LRP, we took advantage of the

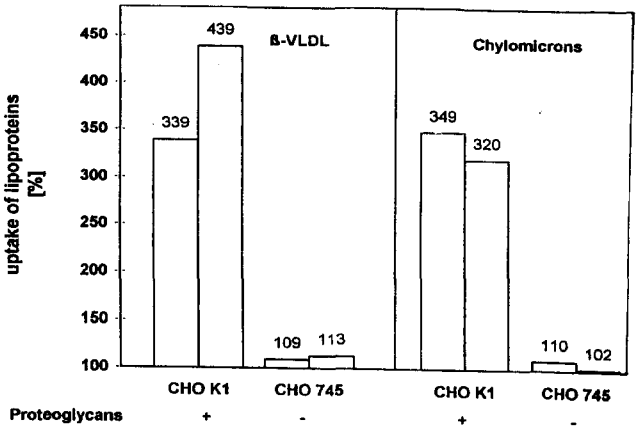

Fig. 5. Effect of proteoglycans on HL-mediated uptake of ^{125}I-labeled chylomicrons and ^{125}I-labeled β-VLDL. Two independent uptake experiments were performed as described for Fig. 2 on normal (K) and proteoglycan-deficient (745) CHO cells performed with ^{125}I-labeled chylomicrons and ^{125}I-labeled β-VLDL as ligands. The final concentration of HL was 0.1 μg/ml. The mean uptake of chylomicrons was 50 ng/mg cell protein and 48 ng/mg cell protein for CHO (K1) and CHO (745), respectively. The uptake of β-VLDL without addition of HL was 70 ng/mg cell protein and 40 ng/mg cell protein for CHO (K1) and CHO (745). These values were set to 100%.

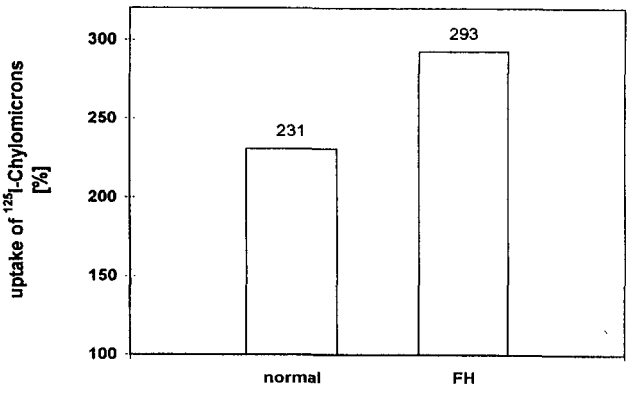

Fig. 6. Role of the LDL receptor for HL-mediated uptake of ^{125}I-labeled chylomicrons. An uptake experiment with addition of HL (0.1 μg/ml) was performed on normal and LDL receptor-deficient (FH) fibroblasts with ^{125}I-labeled chylomicrons as described in Fig. 2. The uptake of lipoproteins without lipase addition was 18 ng/mg cell protein for normal fibroblasts and 15 ng/mg cell protein for FH fibroblasts. The uptake of lipoproteins without lipase addition was set to 100%. Earlier experiments performed under slightly different conditions confirmed these results.

sequence homology with LpL. A C-terminal fragment of LpL (amino acids 378–448) had been produced, and shown to bind to LRP (39, see also Fig. 1). This fragment had been used to inhibit the LpL-mediated uptake of lipoproteins into cells. To verify whether the C-terminal domain in HL is also responsible for this function, we used this LpL fragment in further studies with HL-mediated uptake. A 50-fold excess of the C-terminal LpL fragment clearly inhibited the HL-mediated uptake of ^{125}I-labeled β-VLDL into Hep3b cells (**Fig. 7**). The mean uptake mediated by addition of HL was 182% (the β-VLDL uptake without HL was set to 100%), whereas in presence of the fragment it was only 72%. This means a total inhibition of HL-mediated β-VLDL uptake by the LpL fragment, and a partial inhibition of the HL-independent uptake. We have no explanation for the later observation at present. In general, these results indicate that LpL and HL bind to the same epitope on the cell surface and to LRP.

Degradation of lipoproteins taken up via HL

As a measure for intracellular catabolism, the degradation of apolipoproteins has been used in many uptake experiments, particularly for the LDL receptor-mediated uptake (58). We tried to determine the degradation of both β-VLDL and chylomicrons after HL-mediated uptake. However, the apolipoproteins were only degraded to a minimal extent in the chosen time of 4 h. This is in accordance with data from Tabas et al. (59) and will be discussed later.

DISCUSSION

The role of HL in lipoprotein metabolism is not yet fully understood. We studied this protein with reference to its role in remnant catabolism. As HL enzyme activity was inhibited by Orlistat, the use of this agent allowed us to focus on the structure of HL. HL can bind to cells via proteoglycans and to lipoproteins via lipids. In addition to these features it is thought that it may bind to cellular receptors that facilitate its uptake into the cell (39, 43). Kounnas et al. (41) have shown that one of these receptors can be LRP. We confirmed these data with binding studies and crosslinking experiments using ^{125}I-labeled HL on human hepatoma cells. Similarly to LpL, the HL was linked only to LRP, no other binding protein was significantly labeled by the ^{125}I-labeled lipases on the surface of Hep3b cells. Direct binding of HL to LRP has also been shown earlier with isolated LRP in slot-blot experiments (39). Moreover, recent data from our laboratory (60) on LRP-deficient cells show that HL-mediated uptake is facilitated mainly by LRP.

Previous studies have shown that LpL mediates binding and uptake of lipoproteins into different cell lines (24, 25, 27). Within the lipase gene family LpL and HL show the highest homology to each other and are the only two members of the family that bind to heparin and proteoglycans. Therefore, both bind to the endothelium of the vessel walls.

We studied the role of HL in lipoprotein uptake in analogy to the data on LpL. It was shown that HL, but not PL, and a bacterial lipase can efficiently mediate the uptake of lipoproteins to hepatoma cells (Fig. 2; 42). These results reflect the ability of HL to bind to proteoglycans and subsequently facilitate the uptake via a receptor-mediated process. Both PL and the bacterial lipase, which do not bind to proteoglycans, cannot mediate the uptake in a comparable way. However, they induce a slight increase in uptake which may be due to the fact that residual lipolysis will produce remnants that express different apolipoprotein structures on the surface, and expose the apoE as ligand for receptor-mediated uptake (28). This could be true in the case of the PL even in the presence of Orlistat, a potent, but reversible, inhibitor of PL (55), LpL (61) and HL activity. The activity of bacterial lipase is not affected by this compound, which explains the more pronounced effect compared to PL. Recent liver perfusion studies also demonstrated that the bacterial lipase can induce chylomicron clearance due to its hydrolytic activity (28). The data with PL suggest that the presence of Orlistat might not always completely inhibit the HL activity in our experiments.

Human plasma HL and HL from HUH_7 cells were shown to mediate the uptake of chylomicrons in a identical fashion. For this reason we decided to use the

HL isolated from HuH_7 cells, which was easier to obtain and free of LpL, in all further experiments. The uptake of both ligands, human chylomicrons and β-VLDL, was stimulated by HL in a more effective way than by LpL. To determine whether the HL-mediated uptake is saturable and to find the appropriate level of HL for the experiments, we performed a saturation curve for β-VLDL uptake (Fig. 4). Based on the described semiquantitative measurement of HL we found that 0.1 μg/ml is sufficient to reach a maximal effect. These data were derived from human hepatoma cells, but parallel experiments on freshly isolated human hepatocytes gave the same results (Table 1). Earlier studies with LpL revealed a saturation level of chylomicron uptake into human hepatocytes of 0.3 μg LpL/ml (42). The comparison of the saturation levels leads to the conclusion that the mediation of lipoprotein uptake by HL seems to be more efficient than that by LpL.

The importance of proteoglycans for the uptake was investigated on normal and proteoglycan-deficient CHO cells. The increase in the uptake of chylomicrons and β-VLDL mediated by addition of HL on normal CHO cells was nearly completely abolished on proteoglycan-deficient CHO cells. These results indicate that a fast and efficient binding of HL to the cellular proteoglycans is the initial step in the uptake mechanism. The uptake is, however, receptor-dependent as discussed above.

To exclude a role for the LDL receptor in the HL-mediated uptake of chylomicrons, normal and LDL receptor-deficient fibroblasts were used. HL-mediated uptake of chylomicrons into normal and LDL receptor-deficient fibroblasts was similar, indicating that the LDL receptor is, in vitro, not essential for HL-mediated lipoprotein uptake. Moreover, there was no band detectable at the molecular weight of the LDL receptor in crosslinking experiments using ^{125}I-labeled HL as a ligand on Hep3b cells. Only LRP was labeled by the ^{125}I-labeled HL.

To investigate the region of HL that might be involved in the interaction between HL and LRP, we again followed the experiments done for LpL. In LpL the C-terminal domain is responsible for this function (38–40) and a C-terminal fragment of LpL (amino acids 378–448, (39)) has been shown to bind to LRP. This fragment competitively inhibited the uptake mediated by LpL and we therefore looked for its ability to interfere with the HL-mediated uptake of lipoproteins. The LpL fragment inhibited the HL-mediated uptake of β-VLDL when added in a 50-fold excess to HL. With this experiment we present evidence that the C-terminus of HL might be important for the function of HL in the mediation of lipoprotein uptake. The actual binding site remains to be determined; however, due to the high

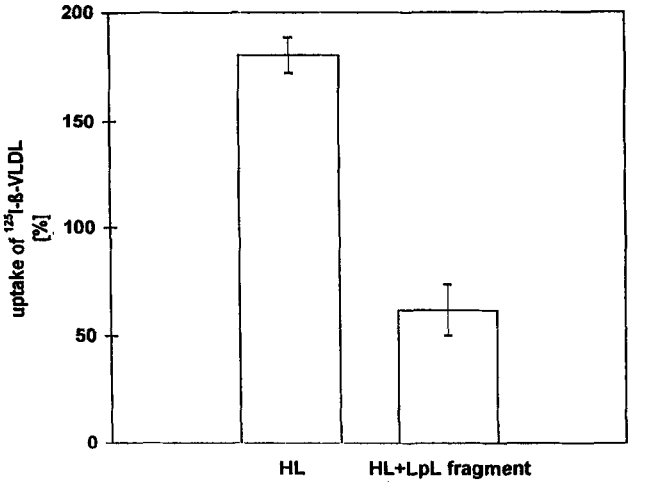

Fig. 7. Inhibition of HL-mediated uptake using a C-terminal LpL fragment. Uptake experiments with addition of HL (0.1 μg/ml) were performed on Hep3b cells with ^{125}I-labeled β-VLDL as described in Fig. 2. A 50-fold molar excess of a C-terminal fragment of LpL was used for inhibition of HL-mediated uptake. The mean of four independent experiments is shown. The mean uptake of β-VLDL without lipase addition was 19 ng/mg cell protein. The uptake without lipase addition was set to 100%. The fragment partly inhibited the HL independent uptake.

homology between HL and LpL at the C-terminal end, it is most likely that both proteins bind to LRP via a structurally similar site. In earlier studies we were able to demonstrate with slot-blotting that LpL inhibits the binding of ^{125}I-labeled LRP to human HL, indicating that both proteins share a common binding site to the receptor. In the cell culture experiments, however, we cannot exclude that both enzymes also compete for the binding to the lipoproteins. In recent experiments we confirmed the data published by Kounnas et al. (41) that HL binding to LRP is inhibited by RAP (data not shown).

We were not able to demonstrate appreciable amounts of degradation of apolipoproteins after HL-mediated uptake. Similar observations were published by Tabas et al. for β-VLDL (59). We have a whole series of data showing that human chylomicrons after LpL-mediated uptake are only partly degraded (62), but most of the protein-bound radioactivity is resecreted into the medium. These data on the retro-endocytosis of chylomicron constituents indicate that the lipase-mediated uptake of lipoproteins follows an intracellular route distinct from the LDL catabolism.

Whether the similar function of LpL and HL in facilitating lipoprotein uptake in vitro represents in vivo a backup system between the two enzymes, or whether they have an additive effect, remains to be elucidated. Individuals with an LpL deficiency accumulate chylomicrons in plasma, e.g., HL cannot replace the catalytic

function of LpL, as unhydrolyzed chylomicrons are too large to enter the space of Disse where HL is located. HL-deficient individuals are often characterized by elevated levels of triglycerides and cholesterol and do not show the severe accumulation of chylomicrons or chylomicron remnants that LpL deficiency does. Therefore, it seems possible that LpL can partly substitute for HL in these patients.

We have shown that HL can mediate the uptake of rabbit β-VLDL and human chylomicrons into cells in vitro. The mechanism of this uptake is not dependent on the enzyme activity but is most probably due to the C-terminal structure of the molecule. The uptake seems to function in two steps, the fast and effective binding to proteoglycans on the cell surface and a second receptor-mediated step leading to internalization. The only receptor recognized to date as an HL binding protein is the LRP.

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