Lipoprotein lipase enhances removal of chylomicrons and chylomicron remnants by the perfused rat liver

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Abstract Lipoprotein lipase has been found to efficiently mediate binding of lipoproteins to cell surfaces and to the low density lipoprotein (LDL) receptor-related protein (LRP) under cell culture conditions (Beisiegel et al. 1991. *Proc. Natl. Acad. Sci. USA.* **88:** 8242–8346). This supports the previously proposed idea that the lipase could have a role in receptor-mediated uptake of chylomicron remnants in the liver. We have investigated the effects of lipoprotein lipase on the clearance of chylomicrons during perfusions of rat livers. The chylomicrons were doubly labeled in vivo with [^{14}C]retinol (in retinyl esters) and with [^{3}H]oleic acid (in triacylglycerols) and were collected from lymph. In the absence of any lipase the clearance of chylomicron label from the perfusion medium was slow. Addition of lipoprotein lipase caused lipolysis of chylomicron triacylglycerols as evidenced by increased levels of ^{14}C-labeled fatty acids in the perfusate. Simultaneously, the level of [^{14}C]retinyl esters in the perfusate decreased dramatically, indicating core-particle removal. Similar effects were seen with an unrelated lipase from *Pseudomonas fluorescens.* To discriminate between the effects of lipolysis and a true liganding effect of the lipoprotein lipase protein, the active site inhibitors tetrahydrolipstatin^{R} and hexadecylsulfonylfluoride were used to reduce or totally inhibit the catalytical activity. With lipase covalently inhibited by the latter inhibitor, lipolysis during perfusions was low or absent. Nonetheless, the inhibited enzyme had a clear effect on the removal of chylomicrons by the liver. With 1.2 μg of inhibited lipase/ml perfusate, about 70% of the core label had been removed after 15 min as compared to about 20% in perfusions without lipase. With identical amounts of active lipoprotein lipase protein, more than 90% of the label was removed. We conclude that any lipase causing lipolysis of chylomicrons can stimulate their clearance by the liver, but that lipoprotein lipase has an additional effect on the removal, which is not dependent on its catalytic activity.—**Skottova, N., R. Savonen, A. Lookene, M. Hultin, and G. Olivecrona.** Lipoprotein lipase enhances removal of chylomicrons and chylomicron remnants by the perfused rat liver. *J. Lipid Res.* 1995. **36:** 1334–1344.

Supplementary key words lipolysis • remnant uptake • lipase inhibitors • retinyl ester

Lipolysis of chylomicrons by the enzyme lipoprotein lipase (LPL) is the initial step in their catabolism and results in formation of chylomicron remnants (1). Chylomicron remnants are known to be rapidly taken up in the liver by receptor-mediated processes (2–5). In contrast, intact chylomicrons are cleared slowly from the circulation (6, 7).

A candidate chylomicron remnant receptor was proposed by Hertz et al. (8). Due to its close structural resemblance to the LDL receptor it was named the LDL receptor-related protein (LRP). LRP binds apolipoprotein E (apoE) and apoE-containing lipoproteins such as β-VLDL (9–11). In addition to lipoproteins, LRP binds a number of other ligands in vitro and is now also known as the catabolic receptor for protease-activated α2-macroglobulin and several other protease-inhibitor complexes (12, 13).

Chylomicrons with the truncated form of apolipoprotein B (apoB-48), lacking the LDL receptor-binding domain, bind to the LDL receptor via apoE. The relative contribution of the LDL receptor and of LRP for chylomicron remnant removal is a matter of debate. Remnant clearance appears to be normal in familial hypercholesterolemia (14), suggesting that, in the absence of functional LDL-receptors, remnants can be efficiently removed by LRP and/or by other mechanisms. Kita et al. (15) found no abnormalities of remnant clearance in WHHL rabbits, whereas Mamo et al. (16) found a marked retardation of the clearance of an injected "chylomicron-like" emulsion and also of chylomicron remnants in WHHL rabbits (17). Their results were, however, questioned by Demacker, van Heijst, and Stalenhoef (18).

Abbreviations: apoB, apolipoprotein B; apoE, apolipoprotein E; BSA, bovine serum albumin; FFA, free fatty acids; HDS, hexadecylsulfonylfluoride; HEPES, 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid; HL, hepatic lipase; LDL receptor, low density lipoprotein receptor; LPL, lipoprotein lipase; LRP, low density lipoprotein receptor-related protein; RE, retinyl esters; TCA, trichloroacetic acid; THL, tetrahydrolipstatin, Orlistat^{R}; TG, triacylglycerols; Tris, 2-amino-2(hydroxymethyl)-1,3-propandiol.

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Recent studies in mice support the view that both the LDL receptor and LRP are engaged in remnant removal (5, 19, 20).

LPL is bound to the vascular side of the vessel walls via interaction with cell surface proteoglycans (21, 22). There is also some LPL in the circulating blood (23, 24), but its concentration is kept low due to efficient uptake in the liver (25, 26). Most of the circulating LPL is bound to lipoproteins (24, 27). It had been proposed by Felts, Itakura, and Crane (28) that LPL attached to chylomicron remnants might be the signal for recognition of these particles by the liver. Following this assumption, Beisiegel, Weber, and Bengtsson-Olivecrona (29) demonstrated that LPL markedly increased binding of apoE-containing lipoproteins to cell surfaces. This effect was independent of catalytic activity as it was also obtained with LPL inhibited by the active site inhibitor tetrahydrolipstatin. Furthermore, by chemical cross-linking they showed that LPL was bound to LRP and that binding of apoE to LRP was greatly stimulated by the presence of LPL. These initial observations were later confirmed and extended (30–32). In purified systems LPL was shown to mediate binding of β-VLDL to LRP (33) and the site in LPL for binding to LRP was localized to the C-terminal folding domain of the enzyme (33–35).

A direct effect of LPL on remnant uptake by the liver has not yet been demonstrated under conditions approximating the in vivo situation. The aim of the present study was to investigate, using the perfused rat liver, whether LPL, in addition to its lipolytic effect, can mediate binding of remnants to the intact liver. To enable distinction between lipolysis-induced remnant removal and LPL-mediated remnant removal, we have studied the effects of active site-inhibited variants of LPL as well as of a non-related bacterial lipase with activity against lipoproteins.

MATERIALS AND METHODS

Reagents

Hypnorm was from Janssen, Bersee, Belgium; Stesolid was from Kabi-Pharmacia Parenterals, Sweden; Dormicum was from Roche, Switzerland; Garamycin (10 mg/ml) was from Essex Läkemedel AB, Sweden, and Intralipid (20%) was from Kabi Pharmacia, Sweden. [^{14}C]oleic acid ([1-^{14}C]oleic acid, 50–60 mCi/mmol) and [^{3}H]retinol ([11,12(n)-^{3}H]vitamin A, 40–60 Ci/mmol) were purchased from Amersham, UK. Eagle's medium (minimal essential medium Eagle modified with Earle's salts and 2.0 g Na-bicarbonate/l, without methionine and glutamine) was obtained from Flow Laboratories, Scotland. Bovine serum albumin (BSA fraction V), insulin (from bovine pancreas), L-glutamine and Na-deoxycholate were from Sigma Chemicals, St. Louis, MO. HEPES (4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid), bis-Tris and Tris (2-amino-2(hydroxymethyl)-1,3-propandiol) were purchased from Boehringer Mannheim, Germany. Linoleic acid was from B.D.H. Laboratory Chemicals Division, England. D-glucose was from Kebo Lab AB, Sweden; sodium dodecylsulfate (SDS) was from BDH Biochemicals, England. EDTA dinatrium salt (Triplex III) and trichloroacetic acid (TCA) were from Merck, Germany. Tetrahydrolipstatin (THL, Orlistat^{R}) was a generous gift from Professor A. Fischli (F. Hoffman La Roche LTD, Basel, Switzerland) and bacterial lipase (from *Pseudomonas fluorescens*) was a kind gift from Amano Pharmaceuticals, Nagoya, Japan. HDS (hexadecylsulfonylfluoride) was a kind gift from Drs. A. Slootbom and H. Verheij, Utrecht, Holland. Oxygen with 5% CO_2 was from AGA, Sweden.

Animals

Male Sprague-Dawley rats were supplied by Moellegard Breeding Center, Denmark. The animals were fed a standard pellet diet with free access to water. All animal procedures were approved by the local ethics committee.

For liver perfusions, rats weighing 180–240 g were fasted overnight. For chylomicron preparations, non-fasted rats weighing 200–230 g were used. The rats were anesthetized with Hypnorm (50 μl/100 g body weight) and Stesolid or Dormicum (50 μl/100 g body weight).

Lipases

Lipoprotein lipase was purified from bovine milk (36). The THL-inhibited LPL was prepared by incubation of LPL (0.3–0.5 mg/ml) in 4 mM deoxycholate/10 mM Tris-HCl, pH 8.5, with a 10-fold molar excess of THL, for 30 min at room temperature (37). THL-inhibited LPL was purified by adsorption to heparin-Sepharose and eluted with 1.5 M NaCl in 10 mM bis-Tris buffer, pH 6.5. HDS-LPL was prepared as follows: LPL (0.4–0.6 mg/ml) was dialyzed in 5 mM deoxycholate/10 mM Tris/0.1 mM linoleic acid, pH 8.5, at 4°C. HDS from a 20 mM stock solution in dimetylsulfoxate was added in 10-fold molar excess over LPL monomers and incubated for at least 6 h at 25°C. Remaining catalytic activity in HDL-LPL was determined using tributurin emulsified by gum arabic as substrate (37). This activity never exceeded 1.4% of that of native LPL. HDS-LPL was purified from unreacted HDS by adsorption to heparin-Sepharose and was then eluted either with a salt gradient or directly with 1.6 M NaCl in 10 mM bis-Tris, pH 6.5. Preparations of HDS-LPL were stored at 4°C and used within 24 h. ^{125}I-labeled LPL was prepared by the lactoperoxidase method and repurified as previously described (26). ^{125}I-labeled THL-LPL and HDS-LPL were prepared by inhibition of ^{125}I-labeled LPL mixed with unlabeled LPL, as described above for the unlabeled preparations. Bacterial lipase was dissolved in 20 mM Tris-Cl, 5 mM deoxycholate, 0.1 mM SDS, pH 8.5, to a final concentration of 1.0 mg/ml.

Lipoproteins

To obtain doubly labeled chylomicrons, the thoracic duct of a rat was cannulated (38, 39). After intragastric infusion of 1.0 ml 20% Intralipid containing 100–150 μCi of [^{14}C]oleic acid and 100–150 μCi of [^{3}H]retinol, 10 ml of lymph was collected during 4–6 h into 50 μl of Garamycin and 100 μl EDTA (10% w/v). Chylomicrons were isolated by centrifugation of lymph through 0.15 M NaCl at 15°C in a SW 60 rotor (L8-M Ultracentrifuge, Beckman) for 60 min at 27,000 rpm. The doubly labeled chylomicrons had a specific radioactivity of ^{3}H about 400 dpm/nmol TG and of ^{14}C about 500 dpm/nmol TG. The molar ratio of TG/cholesterol was 26.20 ± 1.60.

Liver perfusions

Livers were perfused by the method of Vilaró et al. (40), modified as follows. Briefly, the portal vein was cannulated and perfusion was started at a rate of 1.5–2.0 ml/min per g liver with medium A (136 mM NaCl, 5.4 mM KCl, 0.81 mM $MgSO_4$, 0.98 mM $MgCl_2$, 0.44 mM KH_2PO_4, 1.33 mM Na_2HPO_4, 1.3 mM $CaCl_2$, 5.5 mM glucose, 10 mM HEPES, and 1% albumin, pH 7.4, gassed with a mixture of 95% O_2 and 5% CO_2) for 10 min to wash out residual blood. During the first 3–4 min of this time the liver was excised from the rat and placed in the perfusion chamber (37°C). Care was taken not to damage any part of the liver or to cause infarctions during extirpation. Only visibly undamaged and well-perfused livers were allowed to proceed to experiments. After this washing in a single pass mode, the perfusion medium was changed to 14.0 ml gassed medium B (Eagle's medium, supplemented with 5% albumin, 5 mM glutamine, and 0.1 μM insulin) which was allowed to circulate for 5 min before start of the experiment. All solutions were warmed to 37°C before use. At the start of the experiment 1.0 ml of test medium, which had been previously incubated for 5 min at 37°C, was added to the circulating medium B. Test medium contained, unless otherwise stated, 300 μl rat serum (inactivated for 30 min at 56°C) as source of apolipoprotein C-II, chylomicrons corresponding to 5 mg triacylglycerols, bovine LPL, bacterial LPL, THL-LPL or HDS-LDL (or just 1.6 M NaCl in 10 mM bis-Tris in controls) and finally 0.15 M NaCl to a total volume 1.0 ml. Samples of 200 μl were taken from the perfusion media at the times indicated in the figures. ^{3}H- or ^{14}C-

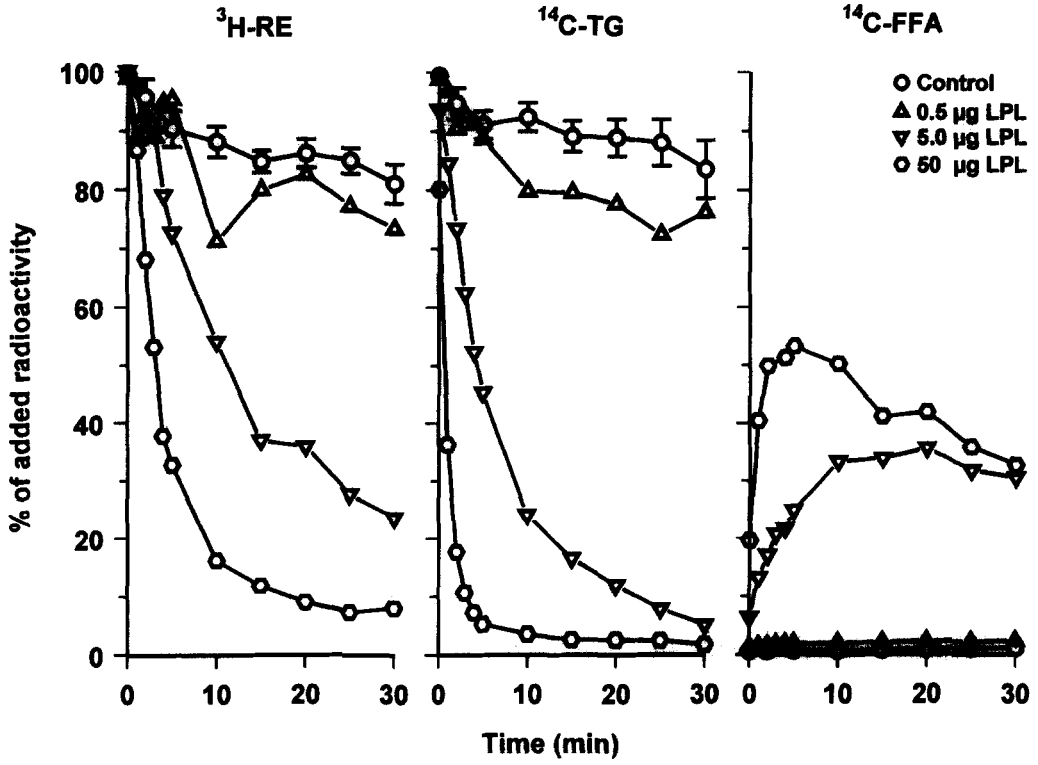

Fig. 1. Effect of bovine lipoprotein lipase on the levels of chylomicron [^{3}H]RE, [^{14}C]TG, and [^{14}C]FFA in medium during liver perfusions. Doubly labeled chylomicrons (corresponding to 5 mg TG) were preincubated for 5 min with the indicated amounts of catalytically active bovine LPL in the presence of heat-inactivated rat serum (300 μl) in a total volume of 1 ml at 37°C. Each mixture was added to 14 ml of medium B and was then perfused through an excised rat liver in a recirculating system. More details are given in the Methods section. Samples of 200 μl were withdrawn at the indicated times. The data points represent a mean of data from two parallel perfusions for each curve. Data for perfusions in the absence of LPL (control) are means ± SE for six perfusions.

radioactivity in samples of chylomicron preparations or of perfusion media was determined by liquid scintillation counting in a LKB Wallac beta-counter, after extraction according to the method of Borgström (41) as modified by Vilaró et al. (40) for ^{3}H- and ^{14}C-radioactivity present in TG and FFA fractions. RE were recovered in the organic phase together with the TG. Samples of 100 μl were taken from perfusions with ^{125}I-labeled lipases. These samples were measured for ^{125}I-radioactivity in a Wallac 1480 WIZARD gamma counter (Turku, Finland) and were then precipitated with 400 μl ice-cold 10% (w/v) TCA. After 30 min at 4°C the samples were centrifuged for 10 min at 3000 rpm in a Hereaus Minifug T and 250 μl of each supernatant was transferred to new tubes for counting of acid-soluble radioactivity. Reported radioactivities are those calculated for the precipitates. The level of free radiolabeled iodine in ^{125}I-labeled LPL preparations was less than 5% in the starting materials as judged from precipitation with TCA. At the end of the perfusion experiments the medium was changed to medium A in a single pass mode for 5 min. Then, the whole liver, or pieces of it, was directly taken for determination of ^{125}I-radioactivity or for determination of ^{3}H- and ^{14}C-radioactivity after homogenization and lipid extraction (40).

Biochemical analyses

TG and cholesterol were assayed by enzymatic methods using kits from Boehringer Mannheim, Germany.

RESULTS

Effects of different amounts of LPL and of bacterial lipase on the levels of chylomicron [^{3}H]retinyl esters, [^{14}C]triacylglycerols, and [^{14}C]oleic acid in the perfusion media

Rat chylomicrons radiolabeled in vivo with [^{3}H]retinol (in retinyl esters (RE)) and with [^{14}C]oleic acid (in triacylglycerols (TG)) were passed through rat livers in a recirculating perfusion system. In the absence of any added lipase the disappearance of chylomicron constituents was slow (**Fig. 1**). About 80% of both [^{3}H]RE and of [^{14}C]TG remained in the media after perfusion for 30 min. Significant levels of ^{14}C-labeled free fatty acids (FFA) were detected only at the longest perfusion times and amounted to less than 3% of the radioactivity in glycerides. Addition of 0.5 μg LPL to the chylomicrons before perfusion caused a small rise in the level of ^{14}C-labeled FFA and also an increase in the removal of both [^{14}C]TG and of [^{3}H]RE. These effects were more pronounced with higher

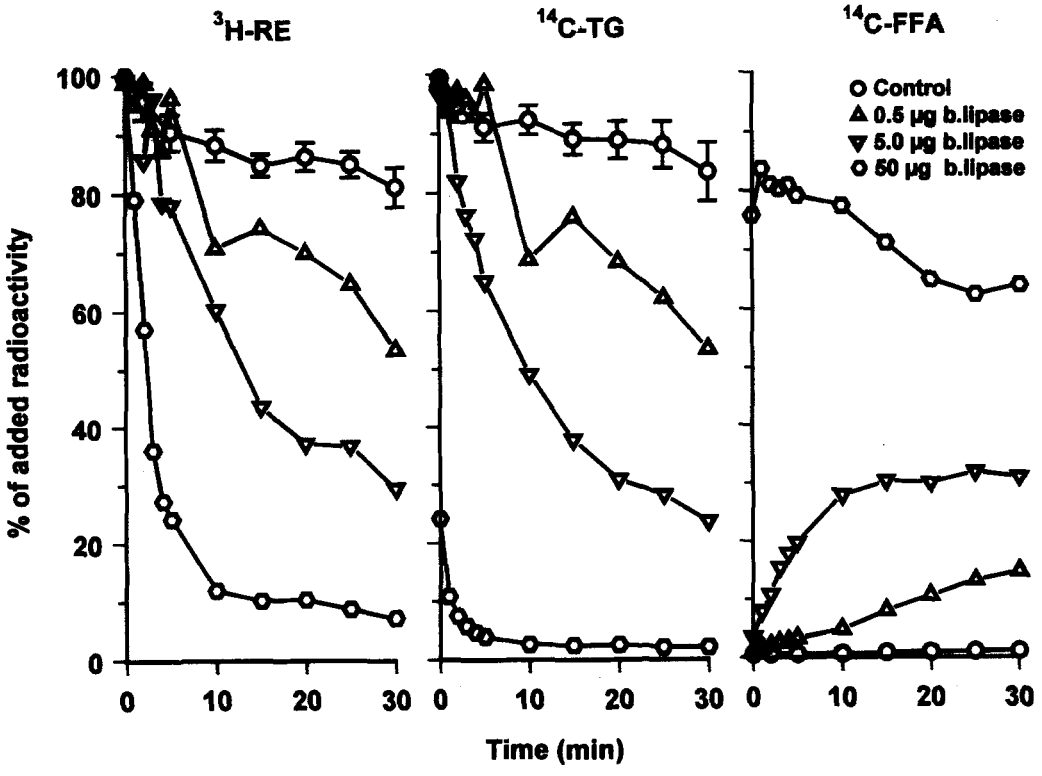

Fig. 2. Effect of bacterial lipase on the levels of chylomicron [^{3}H]RE, [^{14}C]TG, and [^{14}C]FFA in medium during liver perfusions. Conditions were as in Fig. 1 but the chylomicrons were preincubated with the indicated amounts of lipase from *Pseudomonas fluorescens*. Data points represent a mean of the results from two parallel perfusions for each curve. The data for controls are the same as in Fig. 1. The two experiments were conducted with the same batch of chylomicrons.

amounts of LPL (5 and 50 μg/liver). With 50 μg LPL about 20% of the fatty acids had been released as FFA into the medium already at the earliest time point and the chylomicron remnant constituents disappeared quickly from the perfusion medium. Thus, lipolysis appeared to be an important determinant for particle removal.

To investigate whether this was specific for LPL, or whether any triglyceride lipase could accomplish the same effects, chylomicrons were incubated with a lipase from *Pseudomonas fluorescens*. This enzyme was chosen because it has similar activity against emulsified triacylglycerol as LPL (42), it does not hydrolyze phospholipids (A. Lookene and G. Olivecrona, unpublished results) and it is active against lipoproteins (29). **Figure 2** shows that the bacterial lipase caused more lipolysis based on enzyme protein than did LPL. At 50 μg/liver about 80% of the ^{14}C label appeared in FFA. As with LPL, [^{3}H]RE disappeared rapidly from the medium in a dose-dependent manner, indicating particle removal.

Effects of LPL inhibited by tetrahydrolipstatin on lipolysis and chylomicron/chylomicron remnant removal during perfusion

To study whether catalytically inactive LPL had any effect on chylomicron removal we used the active-site inhibitor tetrahydrolipstatin (THL). This inhibitor had previously been shown to inhibit LPL in such a way that the lipase could still mediate binding of lipoproteins to cells and to LRP (29, 33). With increasing doses of THL-LPL added to the chylomicrons before perfusion, the removal rates for labeled chylomicron constituents were increased (**Fig. 3**). However, ^{14}C-labeled FFA appeared in the perfusate in a dose-dependent manner, indicating that some of the inhibited LPL had regained activity. As THL-LPL was not fully re-activated, 10-fold more THL-LPL had to be added to reach about the same lipolysis as with uninhibited LPL. Under such conditions, the higher amount of the inhibited lipase protein caused a more rapid particle removal than what was obtained with the 10-fold lower amount of uninhibited LPL (compare 5 μg LPL in Fig. 1 with 50 μg THL-LPL in Fig. 3). Furthermore, when the effects of THL-LPL and of bacterial lipase on the removal of chylomicron constituents were compared at the same level of lipolysis (compare 5 μg bacterial lipase in Fig. 2 with 50 μg THL-LPL in Fig. 3), THL-LPL caused a more rapid removal of both [^{14}C]TG and [^{3}H]RE than did bacterial lipase. With 10 μg of THL-LPL less lipolysis occurred than with 5 μg of the bacterial lipase, but still the removal rate for chylomicron constituents was higher.

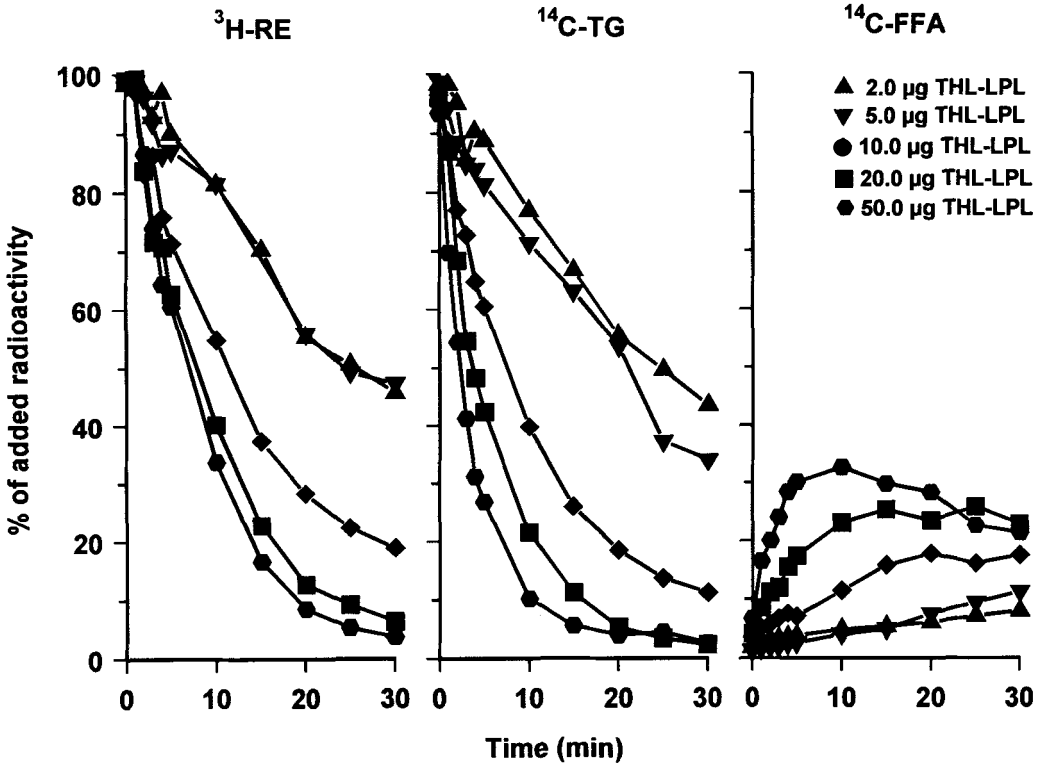

Fig. 3. Effect of THL-LPL on the levels of chylomicron [^{3}H]RE, [^{14}C]TG, and [^{14}C]FFA levels in medium during liver perfusions. Conditions as in Fig. 1 but the chylomicrons were preincubated with the indicated amounts of THL-LPL. The data points for each curve are from single perfusions.

Effects of LPL inhibited by the irreversible inhibitor hexadecylsulfonylfluoride on the removal of chylomicron constituents

The previous experiments showed that lipolysis is an important determinant for chylomicron removal by the liver. They also indicated that LPL might have additional, specific liganding effects. To study these further, we used the irreversible active site inhibitor hexadecylsulfonylfluoride (HDS). After incubation of LPL with a 10-fold molar excess of HDS, and removal of unreacted inhibitor, less than 1.4% of the original catalytic activity remained. With this inhibitor no reactivation was seen under any condition. With HDS-LPL added to chylomicrons in liver perfusion experiments little lipolysis occurred as judged from the low level of ^{14}C-labeled oleic acid in the perfusate (**Fig. 4**). In comparison with control perfusions without LPL, the removal of both [^{14}C]TG and [^{3}H]RE were accelerated, strongly indicating that with HDS-LPL essentially nonlipolyzed chylomicron particles were removed by the liver. After perfusion for 15 min with 20 μg of HDS-LPL, about 30% of the [^{3}H]RE remained in the circulating media. In controls without LPL about 80% remained and in the presence of 20 μg active LPL less than 10% remained. The increased removal was paralleled by increased levels of ^{3}H-radioactivity in livers perfused with LPL or with HDS-LPL for 30 min as compared to livers perfused without lipase (**Fig. 5**). The values shown were obtained after the livers had been washed in a non-circulating mode for 5 min.

Time-course for removal of THL-LPL and of HDS-LPL from the perfusates

It was previously known that LPL is rapidly removed by the liver both in vivo and during perfusions (25, 26, 40, 43, 44). The effects of LPL on chylomicron removal could be accomplished either by circulating LPL or by LPL already bound in the liver, or most likely by both. Perfusion of livers with ^{125}I-labeled preparations of THL-LPL and of HDS-LPL showed that the inhibited LPL variants were removed somewhat more rapidly than was normal LPL (**Fig. 6**). More than 90% of their radioactivity was found in livers after 30 min of perfusion, while corresponding numbers for uninhibited LPL were about 80%. Thus, during the main part of the perfusions with chylomicrons, most of the LPL or the LPL variants were already bound in the liver. Additional experiments (**Table 1**) demonstrated that loading of the livers with LPL by perfusion with lipase before perfusion with chylomicrons led to lipolysis and increased particle removal in a manner similar to that when the chylomicrons were preincubated with the lipase before perfusion.

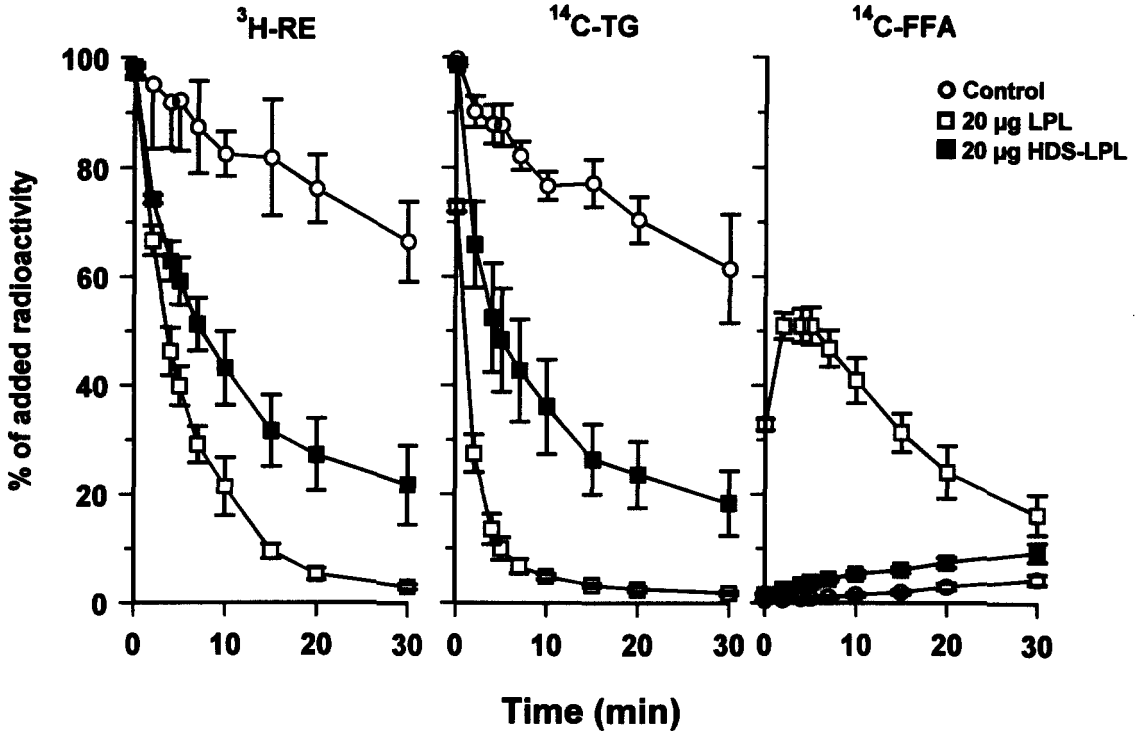

Fig. 4. Effect of catalytically inactive HDS-LPL on the levels of chylomicron [^{3}H]RE, [^{14}C]TG, and [^{14}C]FFA in medium during liver perfusions. Conditions were the same as in Fig. 1. The chylomicrons were preincubated with the indicated amounts of LPL or of HDS-LPL. Data points are means ± SE of five identical perfusions. The experiment was conducted over 4 days and perfusions of each of the three kinds were run each day.

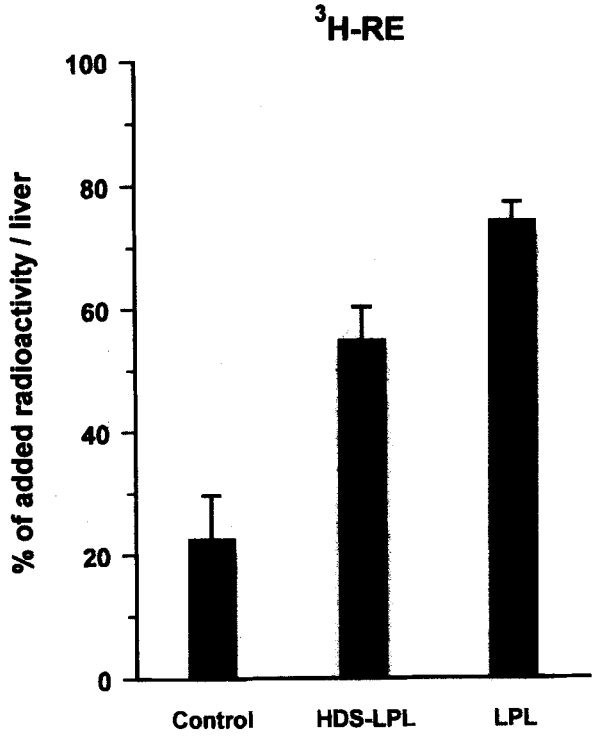

Fig. 5. Amounts of [^{3}H]RE in livers after perfusion of doubly labeled chylomicrons in the presence of catalytically active LPL or of inactive HDS-LPL. After the perfusions in Fig. 4 the livers were washed for 5 min in a single pass mode with medium A. The whole livers were then homogenized and the [^{3}H]RE were extracted and counted as described in the Methods section. In comparable experiments, less than 1% of the ^{3}H radioactivity was found as retinol in the water-soluble phase, even in the presence of active LPL. The bars represent means ± SE of five livers each.

DISCUSSION

The aim of this study was to evaluate the role of LPL for chylomicron and chylomicron remnant removal by the perfused rat liver following the assumption that LPL might serve as a link between the lipoproteins and hepatic binding sites (28, 29).

Initial experiments showed that lipolysis was the most important determinant for chylomicron clearance in accord with previous investigations (6, 7). We show here that the necessary lipolysis could be accomplished by pure bovine LPL in vitro, but a similar effect was also obtained with a lipase from *Pseudomonas fluorescens*. This lipase differs from LPL in that it does not bind to heparin (G. Olivecrona, unpublished results), does not bind to LRP (33) and does not hydrolyze phospholipids (A. Lookene and G. Olivecrona, unpublished results). As expected, the bacterial lipase did not mediate binding of lipoproteins to cells or to isolated LRP (29, 33). Borensztajn, Getz, and Kotlar (45) reported that phospholipid hydrolysis is an important determinant for chylomicron remnant recognition by the liver. Nevertheless, in our study chylomicron remnants generated by the bacterial lipase alone were cleared by the liver almost as efficiently as remnants generated by LPL. Using HL, Brasaemle, Cornely, Moss, and Bensadoun (46) demonstrated that hydrolysis of triacylglycerols and phospholipids in rat chylomicrons led to increased exposure of determinants in apoE to immunoglobulins. A likely conclusion from several studies is that lipolysis and accumulation of lipolysis products, from triacylglycerols or from phospholipids, changes the physical structure of the lipoprotein surface so that the particle is recognized by the liver. This effect can probably be accomplished by any triacylglycerol lipase with activity against chylomicrons.

The focus in the present study was to explore whether LPL had any effect other than lipolysis on chylomicron remnant removal. This was postulated based on the ability of LPL to mediate binding of lipoproteins both to cell surfaces and directly to purified LRP (29–33, 47–50). In these systems lipolysis was not a prerequisite for binding (29, 30, 48, 50). As the overwhelming effect of LPL on chylomicron clearance was on lipolysis, two additional approaches were used to distinguish between lipolysis-induced particle removal and LPL-mediated removal. One was a comparison of the effects of LPL to those of the bacterial lipase at the same degree of lipolysis. The other was to use inhibited variants of LPL. The active-site inhibitor THL was previously shown to inhibit LPL at very low concentrations (37) and the inhibited LPL retained ability to interact both with lipoproteins (29) and with LRP (33). A drawback is that the inhibited enzyme can regain activity by slow hydrolysis of the inhibitor (37) and that reactivation is more rapid in the presence of other lipid substrates than in buffer only (37). This was also the case in the present experiments with chylomicrons, as evidenced by the appearance of labeled FFA in the perfusate. Both the comparison with the effects of the bacterial lipase and the comparison between active LPL and partially inactivated THL-LPL indicated that the presence of LPL protein as such had a promoting effect on chylomicron remnant removal. Furthermore, in direct comparisons between THL-LPL and the bacterial lipase at a similar degree of lipolysis, particle removal was always more rapid with THL-LPL. These results indicated an additional role of the LPL protein in clearance of chylomicron remnants by the liver.

Due to the complexity of the system we had to find an irreversible active site inhibitor for LPL. In our hands phenylmethylsulfonylfluoride (PMSF) reacts poorly with LPL even in millimolar concentrations. In contrast, the derivative hexadecylsulfonylfluoride (HDS) caused near complete inhibition of LPL when added in only a 10-fold molar excess. This inhibitor had previously been successfully used with other lipases (51, 52). HDS-LPL was purified from excess inhibitor by chromatography on

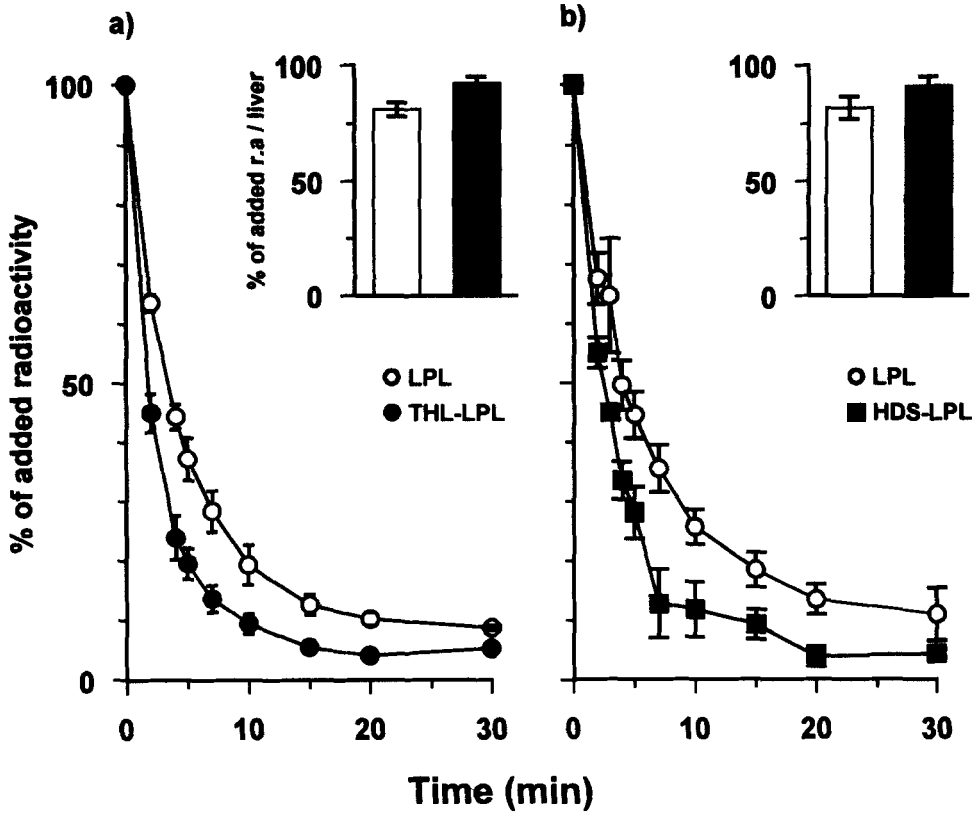

Fig. 6. Disappearance of ^{125}I-labeled catalytically active LPL, of ^{125}I-labeled THL-LPL, and of ^{125}I-labeled HDS-LPL from medium during liver perfusion. a) ^{125}I-labeled catalytically active LPL (50 μg of protein, sp act 25,000 cpm/μg) and ^{125}I-labeled THL-LPL (50 μg of protein, sp act 20,000 cpm/μg) were each mixed with 300 μl heat-inactivated rat serum. b) ^{125}I-labeled catalytically active LPL and ^{125}I-labeled HDS-LPL (50 μg of protein, sp act 20,000 cpm/μg) were each mixed with 300 μl inactivated rat serum. After 5 min of preincubation at 37°C the mixtures were added to 14 ml of medium B and perfused through liver for 30 min in a recirculating system. Inset: Liver uptake of ^{125}I-labeled catalytically active LPL (unfilled bars) and of a) ^{125}I-labeled THL-LPL (filled bars) or b) ^{125}I-labeled HDS-LPL (filled bars) after 30 min perfusion followed by an additional 5 min wash with medium A. Data are means from three parallel perfusions. Three representative samples were taken from each liver for determination of radioactivity.

heparin-Sepharose and was eluted from the column at high salt concentration, indicating that its overall structure was similar to that of catalytically active LPL dimers. Furthermore, iodine-labeled HDS-LPL and THL-LPL were both cleared from the perfusion media by the liver in a fashion similar to that previously found for catalytically active LPL (40). Thus, HDS-LPL could be considered as a good model for native LPL. Interestingly, HDS-LPL was able to markedly promote removal of marginally lipolyzed chylomicrons by the perfused liver and it increased several fold the amount of [^{3}H]RE taken up in the liver as compared to control perfusions without any added lipase. This is the first time that a liganding function of LPL has been demonstrated under conditions approximating the in vivo situation.

TABLE 1. Content of radioactivity from [^{3}H]retinyl ester and [^{14}C]triacylglycerol in livers perloaded with lipoprotein lipase and then perfused for 60 min with doubly labeled chylomicrons

	% of Added Radioactivity	
Condition	^{3}H/Liver	^{14}C/Liver
Control	26.40 ± 4.88	20.80 ± 4.36
With LPL	94.48 ± 7.27	55.22 ± 5.73

Livers were first perfused for 10 min with 5 ml of recirculating medium B containing 50 μg of LPL or with medium only (control). After a wash for 15 min in a single pass mode with medium A, the livers were perfused with doubly labeled chylomicrons in medium B for 60 min in a recirculating mode. After a final wash for 5 min with medium A, the livers were homogenized and extracted for determination of radioactivities. Values are means ± SE from two perfusions with active LPL and from six perfusions without LPL.

Hepatic lipase is structurally and functionally related to LPL (22, 53). It is mainly localized in the liver and several lines of evidence indicate that LPL and HL have partly overlapping functions, HL being involved mainly in remodelling of remnant lipoproteins. A role of HL in chylomicron remnant uptake by the liver has been suggested based on studies with specific antibodies (54, 55) and by detachment of HL from its binding sites by heparin (54). Belcher et al. (56) found that the main fraction

of HL in the liver is located in endosomes and suggested that continued lipolysis of the remnants by HL may occur in the endocytosed vesicles. A striking fact, known for long (7), is that although the perfused rat livers are fully loaded with HL, essentially no lipolysis and very slow uptake of chylomicrons occurs. Thus, HL cannot accomplish the necessary rapid lipolysis and particle uptake on its own even though HL was recently shown to bind to LRP (35, 50). Furthermore, a pivotal role of HL in remnant metabolism can be questioned on the basis that several animal species have low or almost absent levels of HL in their livers (22).

LPL mediates binding of lipoproteins not only to LRP; the main interaction on cell surfaces appears to be to heparan sulfate proteoglycans (22, 31, 48–50). As LPL binds to the proteoglycans, and as both apoE and apoB are heparin-binding proteins, an initial contact between the lipoprotein and LPL can mediate a multipoint cooperative attachment of the lipoprotein to the cell surface. It has been proposed that this initial interaction brings the lipoprotein in close proximity with the cell surface and may facilitate the subsequent interaction with specific receptors. An important factor in this interaction appears to be apoE which is provided with the particle or acquired from the cell surface (57). Moreover, LPL can mediate binding of lipoproteins to extracellular matrix (58–60). Our current data on LPL-mediated binding of chylomicrons to the perfused liver do not allow us to speculate on which interactions are increased by LPL. If the effect is not primarily on binding to endocytic receptors, the increased concentration of chylomicrons on the cell surfaces could, in a second step, be followed by increased receptor-mediated uptake (13).

In conclusion, our study has indicated two independent determinants of chylomicron particle clearance by the liver: *1)* lipolysis, which can probably be accomplished by any triglyceride lipase with activity against lipoproteins and which may not involve increased binding either to proteoglycans or to LRP; and *2)* a liganding effect of the LPL protein that can mediate clearance of both remnants and essentially unlipolyzed chylomicrons. The present data do not resolve by which mechanism this occurs.

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REFERENCES

1. Mjøs, O. D., O. Faergeman, R. L. Hamilton, and R. J. Havel. 1975. Characterization of remnants produced during the metabolism of triglyceride-rich lipoproteins of blood plasma and intestinal lymph in the rat. *J. Clin. Invest.* **56:** 603–615.
2. Havel, R. J., and R. L. Hamilton. 1988. Hepatocytic lipoprotein receptors and intracellular lipoprotein catabolism. *Hepatology.* **8:** 1689–1704.
3. Mahley, R. W., and M. M. Hussain. 1991. Chylomicron and chylomicron remnant catabolism. *Curr. Opin. Lipidol.* **2:** 170–176.
4. Windler, E. E., J. Greeve, W. H. Daerr, and H. Greten. 1988. Binding of rat chylomicrons and their remnants to the hepatic low-density-lipoprotein receptor and its role in remnant removal. *Biochem. J.* **252:** 553–561.
5. Choi, S. Y., and A. D. Cooper. 1993. A comparison of the roles of the low density lipoprotein (LDL) receptor and the LDL receptor-related protein/α2-macroglobulin receptor in chylomicron remnant removal in the mouse in vivo. *J. Biol. Chem.* **268:** 15804–15811.
6. Redgrave, T. G. 1970. Formation of cholesteryl ester-rich particulate lipid during metabolism of chylomicrons. *J. Clin. Invest.* **49:** 465–471.
7. Noel, S. P., P. J. Dolphin, and D. Rubinstein. 1975. An in vitro model for the catabolism of rat chylomicrons. *Biochem. Biophys. Res. Commun.* **63:** 764–772.
8. Herz, J., U. Hamann, S. Rogne, O. Myklebost, H. Gausepohl, and K. K. Stanley. 1988. Surface location and high affinity for calcium of a 500-kD liver membrane protein closely related to the LDL-receptor suggests a physiological role as lipoprotein receptor. *EMBO J.* **7:** 4119–4127.
9. Beisiegel, U., W. Weber, G. Ihrke, J. Herz, and K. K. Stanley. 1989. The LDL-receptor-related protein, LRP, is an apolipoprotein E-binding protein. *Nature.* **341:** 162–164.
10. Kowal, R. C., J. Herz, J. L. Goldstein, V. Esser, and M. S. Brown. 1989. Low density lipoprotein receptor-related protein mediates uptake of cholesteryl esters derived from apoprotein E-enriched lipoproteins. *Proc. Natl. Acad. Sci. USA.* **86:** 5810–5814.
11. Kowal, R. C., J. Herz, K. H. Weisgraber, R. W. Mahley, M. S. Brown, and J. L. Goldstein. 1990. Opposing effects of apolipoproteins E and C on lipoprotein binding to low density lipoprotein receptor-related protein. *J. Biol. Chem.* **265:** 10771–10779.
12. Brown, M. S., J. Herz, R. C. Kowal, and J. L. Goldstein. 1991. The low-density lipoprotein receptor-related protein: double agent or decoy? *Curr. Opin. Lipidol.* **2:** 65–72.
13. Krieger, M., and J. Herz. 1994. Structures and functions of multiligand lipoprotein receptors: macrophage scavenger receptors and LDL receptor-related protein (LRP). *Annu. Rev. Biochem.* **63:** 601–637.
14. Goldstein, J. L., and M. S. Brown. 1989. Familial hypercholesterolemia. *In* The Metabolic Basis of Inherited Disease. C. R. Scriver, A. L. Beaudet, W. S. Sly, and D. Valle, editors. McGraw-Hill, New York. 1215–1250.
15. Kita, T., J. L. Goldstein, M. S. Brown, Y. Watanabe, C. A. Hornick, and R. J. Havel. 1982. Hepatic uptake of chylomicron remnants in WHHL rabbits: a mechanism genetically distinct from the low density lipoprotein receptor. *Proc. Natl. Acad. Sci. USA.* **79:** 3623–3627.
16. Mamo, J. C. L., A. Bowler, C. L. Elsegood, and T. G. Redgrave. 1991. Defective plasma clearance of chylomicron-like lipid emulsions in Watanabe heritable hyperlipidemic rabbits. *Biochim. Biophys. Acta.* **1081:** 241–245.
17. Bowler, A., T. G. Redgrave, and J. C. Mamo. 1991. Chylomicron-remnant clearance in homozygote and heterozygote Watanabe-heritable-hyperlipidaemic rabbits is defective. Lack of evidence for an independent chylomicron-remnant receptor. *Biochem. J.* **276:** 381–386.

18. Demacker, P. N. M., P. J. van Heijst, and A. F. H. Stalenhoef. 1992. A study of the chylomicron metabolism in WHHL rabbits after fat loading. Discrepancy between results based on measurement of apoprotein B-48 or retinyl palmitate. *Biochem. J.* **285:** 641–646.
19. Choi, S. Y., L. G. Fong, M. J. Kirven, and A. D. Cooper. 1991. Use of an anti-low density lipoprotein receptor antibody to quantify the role of the LDL receptor in the removal of chylomicron remnants in the mouse in vivo. *J. Clin. Invest.* **88:** 1173–1181.
20. Willnow, T. E., Z. Sheng, S. Ishibashi, and J. Herz. 1994. Inhibition of hepatic chylomicron remnant uptake by gene transfer of a receptor antagonist. *Science.* **264:** 1471–1474.
21. Cryer, A. 1981. Tissue lipoprotein lipase activity and its action in lipoprotein metabolism. *Int. J. Biochem.* **13:** 525–541.
22. Olivecrona, T., and G. Bengtsson-Olivecrona. 1993. Lipoprotein lipase and hepatic lipase. *Curr. Opin. Lipidol.* **4:** 187–196.
23. Peterson, J., T. Olivecrona, and G. Bengtsson-Olivecrona. 1985. Distribution of lipoprotein lipase and hepatic lipase between plasma and tissues: effect of hypertriglyceridemia. *Biochim. Biophys. Acta.* **837:** 262–270.
24. Vilella, E., J. Joven, M. Fernández, S. Vilaró, J. D. Brunzell, T. Olivecrona, and G. Bengtsson-Olivecrona. 1993. Lipoprotein lipase in human plasma is mainly inactive and associated with cholesterol-rich lipoproteins. *J. Lipid Res.* **34:** 1555–1564.
25. Wallinder, L., G. Bengtsson, and T. Olivecrona. 1979. Rapid removal to the liver of intravenously injected lipoprotein lipase. *Biochim. Biophys. Acta.* **575:** 166–173.
26. Wallinder, L., J. Peterson, T. Olivecrona, and G. Bengtsson-Olivecrona. 1984. Hepatic and extrahepatic uptake of intravenously injected lipoprotein lipase. *Biochim. Biophys. Acta.* **795:** 513–524.
27. Goldberg, I. J., J. J. Kandel, C. B. Blum, and H. N. Ginsberg. 1986. Association of plasma lipoproteins with postheparin plasma lipase activities. *J. Clin. Invest.* **78:** 1523–1528.
28. Felts, J. M., H. Itakura, and R. T. Crane. 1975. The mechanism of assimilation of constituents of chylomicrons, very low density lipoproteins and remnants—a new theory. *Biochem. Biophys. Res. Commun.* **66:** 1467–1475.
29. Beisiegel, U., W. Weber, and G. Bengtsson-Olivecrona. 1991. Lipoprotein lipase enhances the binding of chylomicrons to low density lipoprotein receptor-related protein. *Proc. Natl. Acad. Sci. USA.* **88:** 8342–8346.
30. Chappell, D. A., G. L. Fry, M. A. Waknitz, P-H. Iverius, S. E. Williams, and D. K. Strickland. 1992. The low density lipoprotein receptor-related protein/α2-macroglobulin receptor binds and mediates catabolism of bovine milk lipoprotein lipase. *J. Biol. Chem.* **267:** 25764–25767.
31. Chappell, D. A., G. L. Fry, M. A. Waknitz, L. E. Muhonen, M. W. Pladet, P-H. Iverius, and D. K. Strickland. 1993. Lipoprotein lipase induces catabolism of normal triglyceride-rich lipoproteins via the low density lipoprotein receptor-related protein/α2-macroglobulin receptor in vitro. A process facilitated by cell-surface proteoglycans. *J. Biol. Chem.* **268:** 14168–14175.
32. Willnow, T. E., J. L. Goldstein, K. Orth, M. S. Brown, and J. Herz. 1992. Low density lipoprotein receptor-related protein and gp330 bind similar ligands, including plasminogen activator-inhibitor complexes and lactoferrin, an inhibitor of chylomicron remnant clearance. *J. Biol. Chem.* **267:** 26172–26180.
33. Nykjær, A., G. Bengtsson-Olivecrona, A. Lookene, S. K. Moestrup, C. M. Petersen, W. Weber, U. Beisiegel, and J. Gliemann. 1993. The α2-macroglobulin receptor/low density lipoprotein receptor-related protein binds lipoprotein lipase and β-migrating very low density lipoprotein associated with the lipase. *J. Biol. Chem.* **268:** 15048–15055.
34. 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/α_2-macroglobulin receptor (LRP) and mediates binding of normal very low density lipoproteins to LRP. *J. Biol. Chem.* **269:** 8653–8658.
35. Nykjær, A., M. Nielsen, A. Lookene, N. Meyer, H. Roigaard, M. Etzerodt, U. Beisiegel, G. Olivecrona, and J. Gliemann. 1994. A C-terminal fragment of lipoprotein lipase binds to LDL receptor-related protein and inhibits lipase mediated uptake of lipoprotein in cells. *J. Biol. Chem.* **268:** 31747–31755.
36. Bengtsson-Olivecrona, G., and T. Olivecrona. 1991. Phospholipase activity of milk lipoprotein lipase. *Methods Enzymol.* **197:** 345–356.
37. Lookene, A., N. Skottova, and G. Olivecrona. 1994. Interactions of lipoprotein lipase with the active-site inhibitor tetrahydrolipstatin (Orlistat)^R. *Eur. J. Biochem.* **222:** 395–403.
38. Waynforth, H. B. 1980. Experimental and Surgical Technique in the Rat. Academic Press, London.
39. Chevreuil, O., M. Hultin, P. B. Østergaard, and T. Olivecrona. 1993. Biphasic effects of low-molecular-weight and conventional heparins on chylomicron clearance in rats. *Arterioscler. Thromb.* **13:** 1397–1403.
40. Vilaró, S., M. Llobera, G. Bengtsson-Olivecrona, and T. Olivecrona. 1988. Lipoprotein lipase uptake by the liver: localization, turnover, and metabolic role. *Am. J. Physiol.* **254:** G711–G722.
41. Borgström, B. 1952. Investigation of lipid separation methods. *Acta Physiol. Scand.* **25:** 101–110.
42. Bengtsson, G., and T. Olivecrona. 1980. Lipoprotein lipase. Mechanism of product inhibition. *Eur. J. Biochem.* **106:** 557–562.
43. Vilaró, S., and M. Llobera. 1988. Uptake and metabolism of Intralipid by rat liver: an electron-microscopic study. *J. Nutr.* **118:** 932–940.
44. Chajek-Shaul, T., G. Friedman, E. Ziv, H. Bar-On, and G. Bengtsson-Olivecrona. 1988. Fate of lipoprotein lipase taken up by the rat liver: evidence for a conformational change with loss of catalytic activity. *Biochim. Biophys. Acta.* **963:** 183–191.
45. Borensztajn, J., G. S. Getz, and T. J. Kotlar. 1988. Uptake of chylomicron remnants by the liver: further evidence for the modulating role of phospholipids. *J. Lipid Res.* **29:** 1087–1096.
46. 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.
47. Friedman, G., T. Chajek-Shaul, O. Stein, T. Olivecrona, and Y. Stein. 1981. The role of lipoprotein lipase in the assimilation of cholesteryl linoleyl ether by cultured cells incubated with labeled chylomicrons. *Biochim. Biophys. Acta.* **666:** 156–164.
48. Eisenberg, S., E. Sehayek, T. Olivecrona, and I. Vlodavsky. 1992. Lipoprotein lipase enhances binding of lipoproteins to heparan sulfate on cell surfaces and extracellular matrix. *J. Clin. Invest.* **90:** 2013–2021.
49. Mulder, M., P. Lombardi, H. Jansen, T. J. C. Van Berkel, R. R. Frants, and L. M. Havekes. 1992. Heparan sulphate

proteoglycans are involved in the lipoprotein lipase-mediated enhancement of the cellular binding of very low density and low density lipoproteins. *Biochem. Biophys. Res. Commun.* **185:** 582–587.
50. Beisiegel, U., A. Krapp, W. Weber, and G. Olivecrona. 1994. The role of α_2M receptor/LRP in chylomicron remnant metabolism. *Ann. NY Acad. Sci.* **737:** 53–69.
51. Horrevoets, A. J. G., H. M. Verheij, and G. H. De Haas. 1991. Inactivation of *Eschericia coli* outer-membrane phospholipase A by the affinity label hexadecanesulfonyl fluoride. Evidence for an active-site serine. *Eur. J. Biochem.* **198:** 247–253.
52. Leuveling Tjeenk, M., Y. B. M. Bulsink, A. J. Slotboom, H. M. Verheij, G. H. De Haas, G. Damleitner, and F. Götz. 1994. Inactivation of *Staphylococcus hyicus* lipase by hexadecylsulfonyl fluoride: evidence for an active site serine. *Prot. Engineer.* **7:** 579–583.
53. Taskinen, M-R., and T. Kuusi. 1987. Enzymes involved in triglyceride hydrolysis. *In* Clinical Endocrinology and Metabolism. Lipoprotein Metabolism. J. Shepherd, editor. W. B. Saunders, London, UK. 639–666.
54. 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.
55. 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.
56. Belcher, J. D., R. L. Hamilton, S. E. Brady, and R. J. Havel. 1988. Hepatocytic endosomes have hepatic lipase. *Circulation.* **II:** -145.
57. Ji, Z-S., S. Fazio, Y-L. Lee, and R. W. Mahley. 1994. Secretion-capture role for apolipoprotein E in remnant lipoprotein metabolism involving cell surface heparan sulfate proteoglycans. *J. Biol. Chem.* **269:** 2764–2772.
58. Chajek-Shaul, T., G. Friedman, G. Bengtsson-Olivecrona, I. Vlodavsky, and H. Bar-Shavit. 1990. Interaction of lipoprotein lipase with subendothelial extracellular matrix. *Biochim. Biophys. Acta.* **1042:** 168–175.
59. Saxena, U., M. G. Klein, T. M. Vanni, and I. J. Goldberg. 1992. Lipoprotein lipase increases low density lipoprotein retention by subendothelial cell matrix. *J. Clin. Invest.* **89:** 373–380.
60. Tabas, I., Y. Li, R. W. Brocia, S. W. Xu, T. L. Swenson, and K. J. Williams. 1993. Lipoprotein lipase and sphingomyelinase synergistically enhance the association of atherogenic lipoproteins with smooth muscle cells and extracellular matrix. A possible mechanism for low density lipoprotein and lipoprotein[a] retention and macrophage foam cell formation. *J. Biol. Chem.* **268:** 20419–20432.