Lipoprotein lipase degradation by adipocytes: receptor-associated protein (RAP)-sensitive and proteoglycan-mediated pathways

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Abstract Lipoprotein lipase (LPL), the major enzyme responsible for the hydrolysis of triglycerides, is primarily synthesized by adipocytes and myocytes. In addition to synthesis, degradation of cell surface-associated LPL is thought to be important in regulating production of the enzyme. We studied LPL metabolism in the LPL synthesizing adipocyte cell line BFC-1β and assessed the contributions of cell surface heparan sulfate proteoglycans (HSPG), low density lipoprotein receptor related protein (LRP), and glycosylphosphatidylinositol (GPI)-linked proteins to LPL uptake and degradation by these cells. Adipocytes degraded 10–12% of total cell surface ^{125}I-labeled LPL in 2 h and 23–28% in 4 h. In 1 h, 30–54% of the degradation was inhibited by the 39 kDa receptor associated protein (RAP), an inhibitor of ligand binding to LRP. At 4 h, only 19–23% of the LPL degradation was RAP inhibitable. This suggested that two pathways with different kinetics were important for LPL degradation. Heparinase/heparitinase treatment of cells showed that most LPL degradation required the presence of HSPG. Treatment with phosphatidylinositol-specific phospholipase C (PIPLC) inhibited ^{125}I-labeled LPL degradation by 13%. However, neither RAP nor PIPLC treatment of adipocytes significantly increased the amount of endogenously produced LPL activity in the media. To determine whether direct uptake of LPL bound to HSPG could account for the non-RAP sensitive LPL uptake and degradation, proteoglycan metabolism was assessed by labeling cells with $^{35}SO_4$. Of the total pericellular proteoglycans, 14% were PIPLC releasable; surprisingly, 30% were dissociated from the cells with heparin. The amount of labeled pericellular proteoglycans decreased 26% in 2 h and 50% in 8 h, rapid enough to account for at least half of the degradation of cell surface LPL. We conclude that adipocytes degrade a fraction of the cell surface LPL, and that this process is mediated by both proteoglycans and RAP-sensitive receptors.—**Obunike, J. C., P. Sivaram, L. Paka, M. G. Low, and I. J. Goldberg.** Lipoprotein lipase degradation by adipocytes: receptor-associated protein (RAP)-sensitive and proteoglycan-mediated pathways. *J. Lipid Res.* 1996. **37:** 2439–2449.

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Several studies of tissues obtained from humans and animals (1–5) have shown that much of the regulation of adipose tissue lipoprotein lipase (LPL) activity occurs at the post-translational level. Some of this regulation is due to loss of LPL enzymatic activity without a decrease of LPL protein (6). Loss of LPL catabolic function occurs when the purified enzyme is incubated for a short period at 37°C in buffers containing physiological amounts of salt and albumin (7). Thus, unless the enzyme is stabilized by a chaperone protein or by its attachment to cell surfaces (8), it rapidly loses its ability to hydrolyze lipoprotein triglyceride and is unable to assist with the uptake of lipid by adipose tissue.

A second level of LPL regulation may be the degradation of the protein by the adipocyte. The majority of newly synthesized active LPL is found on the cell surface. Support for this conclusion derives from studies of cultured adipocytes (9, 10); most LPL activity produced by these cells is not in the medium but is released from the cell surface by treatment with heparin (9). Some of the LPL remaining on the adipocyte cell surface is internalized and much of this internalized LPL is degraded (11). However, the relative importance of each of several identified LPL degradative pathways is incompletely understood.

Similar processes may occur in vivo. For LPL to interact with circulating lipoproteins, it must be released from the adipocyte surface, a step that allows it to transfer to its site of action on the endothelial luminal surface. This transfer will also prevent LPL degradation by cells. This is because adipocytes are much more effective than endothelial cells in LPL degradation (12–14).

Abbreviations: LPL, lipoprotein lipase; HSPG, heparan sulfate proteoglycans; LRP, LDL receptor related protein; GPI, glycosyl-phosphatidylinositol; RAP, receptor-associated protein; PIPLC, phosphatidylinositol-specific phospholipase C; BFC, brown fat cell; α_2M, alpha 2 macroglobulin; LDL, low density lipoprotein.

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LPL degradation may involve several pathways. LPL is a ligand for the low density lipoprotein receptor related protein (LRP) (15–17). LRP-mediated uptake of LPL alone or LPL associated with lipoproteins (16–23) leads to intracellular degradation. LRP is found on adipocytes and is regulated by insulin treatment (24). Alternative pathways not involving LRP have been found in LPL-mediated degradative processes in macrophages (18). Moreover, a recent report claimed that all LPL degradation by CHO cells was via a non-LRP, heparan sulfate proteoglycans (HSPG)-mediated process (25). To assess these pathways in adipocytes, we studied the involvement of LRP in the uptake and degradation of LPL by BFC-1β-adipocytes. In addition, we determined the importance of HSPG, alone or in combination with receptors, in the degradation of LPL.

MATERIALS AND METHODS

Brown fat cell cultures

Brown fat cells (BFC-1β) were a kind gift from Dr. C. Forest (Vanderbilt University, Nashville, TN). The cells were plated at 10^5 cells/35-mm well into collagen (type III, Sigma) coated 6-well tissue culture plates (Falcon, Becton Dickinson, Lincoln Park, NJ) and were incubated at 37°C in a humidified atmosphere containing 5% CO_2. The cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 15 mM HEPES buffer, 100 units/ml penicillin G, 100 μg/ml streptomycin, 17 μM pantothenate, 33 μM biotin, 1% glutamine, and 10% (v/v) fetal bovine serum (Gemini Bioproducts Inc., Calabasas, CA). Two days after seeding, the confluent cells were converted to adipocytes by incubation in differentiation medium (the same medium supplemented with 10 nM insulin and 2 nM triiodothyronine). The medium was changed every 1–2 days and the cells were used for experiments at 14–20 days after confluence. To confirm that the cells were producing LPL, LPL activity was measured before each experiment using a glycerol triolein emulsion (26) as previously described (9). As LPL is a marker for adipocyte differentiation, we routinely checked LPL activity to confirm preadipocyte to adipocyte differentiation.

Lipoprotein lipase

LPL was purified from fresh bovine milk as described by Saxena, Witte, and Goldberg (27), following the method of Socorro, Green, and Jackson (28), and stored at −70°C. Active LPL was iodinated using lactoperoxidase and glucose oxidase enzymes as previously described (27). ^{125}I-labeled LPL was stored in the presence of 1 mg/ml bovine serum albumin (BSA) at 70°C. The specific radioactivity of ^{125}I-labeled LPL ranged between 1000 and 1500 counts/min per ng.

Assay of LPL activity

For cell studies, LPL activity was measured by the method described by Hietanen and Greenwood (29). One hundred μl of the substrate emulsion containing 1.5 μmol of triolein (Nu-Chek Prep, Elysian, MN), 1.6 μCi of glycerol tri-9,10-3H oleate (Amersham Corp.), 9 μg of egg yolk phosphatidylcholine (Sigma), 67 μl of buffer (0.3 M Tris-HCl, 3% BSA, pH 8.6) and 33 μl of human serum that had been heated to 37°C was mixed with 100 μl of medium or cell surface samples. The enzymatic reaction was allowed to proceed for 1 h at 37°C. Released fatty acids were extracted as described by Belfrage and Vaughan (30). The radioactivity was determined as described earlier (9).

Receptor associated protein

RAP, an endogenous cellular ligand that inhibits binding of ligands to LRP, was produced as a fusion protein with glutathione-S-transferase in an expression system utilizing human placental RAP cDNA (31) and was provided by Dr. D. Strickland, American Red Cross, Rockville, MD.

Alpha 2-macroglobulin (α_2M)

Activated α_2M was kindly provided by Dr. F. R. Maxfield (Department of Cell Biology, Columbia University). α_2M* was iodinated by chloramine T method as described (32).

Enzymes

Phosphatidylinositol-specific phospholipase C (PIPLC) was purified from culture supernatant of *Bacillus subtilis* transfected with the PIPLC gene from *B. thuringiensis* (33). Heparinase, heparitinase, and chondroitinase ABC were obtained from Sekagaki America Inc., Bethesda, MD.

Cell surface binding, internalization, and degradation of ^{125}I-labeled LPL

Cell surface, intracellular, and degraded LPL were studied using BFC-1β. Two different protocols were used that assessed either cellular catabolism of medium LPL or the LPL that was on the cell surface. This latter protocol was also used to assess the importance of cell surface LPL binding molecules (e.g., proteoglycans) that were enzymatically removed but could then reappear on the cell surface during a 37°C incubation.

Metabolism of LPL in the medium. Differentiated adipocytes were incubated for 1 or 4 h at 37°C in 1 ml of DMEM containing 3% BSA (DMEM-BSA) and ^{125}I-labeled LPL (1 μg/ml) with or without RAP (5 μg/ml) or

heparin (10 units/ml). Media were collected and LPL degradation was assessed as the amount of trichloroacetic acid-soluble, non-iodine radioactivity in the medium (34). Parallel incubations in cell-free wells were performed to control for non-cell-mediated LPL degradation. Cell surface LPL was assessed by incubating the cells for 30 min at 4°C with DMEM-BSA containing 100 units/ml heparin. After the final washings, cells were then dissolved in 0.1 N NaOH, and the radioactivity in the NaOH fraction representing the amount of intracellular LPL was determined.

Metabolism of adipocyte cell surface LPL. ^{125}I-labeled LPL was allowed to bind to adipocytes at 4°C for 1 h. Unbound LPL was removed by washing two times with DMEM-BSA and once with DMEM. In some experiments, cells were first incubated for 1 h at 37°C in DMEM-BSA containing heparinase and heparitinase (2.5 units/ml each) or PIPLC (2 units/ml) before allowing ^{125}I-labeled LPL to bind to the cell surface. Control and enzyme-treated cells were then incubated at 37°C in fresh DMEM-BSA with or without RAP or heparin and cell surface, intracellular, and degraded LPL were determined over time as described above.

Endogenous LPL release from cells

LPL activity produced by BFC-1β was studied. Adipocytes were washed and incubated with DMEM-BSA alone or medium containing RAP (5 μg/ml), PIPLC (2 units/ml), heparin (10 U/ml) or PIPLC plus heparin for 1 or 4 h at 37°C. LPL released into the medium was collected and the cell surface LPL was released by incubating for 30 min in DMEM-BSA containing 100 units/ml of heparin.

Kinetics of proteoglycan turnover

Adipocyte proteoglycans were labeled by incubating the cells for 20 h in medium containing 50 μCi/ml of [^{35}S]sulfate as described by Edwards and Wagner (35). After removal of the radiolabeling media, the cells were washed and ^{35}S-labeled proteoglycans were then chased with fresh medium containing 1 mM Na_2SO_4. Media, pericellular (trypsin releasable), and intracellular proteoglycans were assessed in triplicate cultures during the ensuing 24 h as previously described (36). Total proteoglycan radioactivity was measured after precipitation using 3 times sample volume of absolute ethanol containing sodium acetate (0.8 g/L). Chondroitin sulfate was added as a carrier and the samples were incubated overnight in −20°C freezer. Samples were then spun at 2000 rpm for 1 h, supernatant was removed, and the pellet was solubilized in 0.5 N NaOH. The amount of HSPG was determined as that resistant to digestion by chondroitinase ABC. The radioactivity in 1 ml of the aqueous phase was determined using 5 ml of scintillation fluid (Hydrofluor, National Diagnostics, Manville, NJ) in a model 1800 liquid scintillation counter (Beckman Instruments, Palo Alto, CA).

RESULTS

Time course of LPL uptake and degradation

To determine the rate of LPL uptake and degradation by adipocytes, we performed the experiment shown in **Fig. 1.** ^{125}I-labeled LPL was first bound to adipocytes at 4°C for 1 h, after which the cells were washed and then incubated with fresh medium at 37°C. Cell surface, intracellular, and degraded LPL were assessed for up to 6 h. Cell surface LPL decreased about 80% in the first 2 h. At 6 h, <10% of the LPL remained on the cell surface. During the same time period, intracellular and degraded LPL increased. At 6 h, 25% of the LPL initially on the cell surface had been degraded. As shown in the inset, 59% of the cell surface LPL was in the medium. Thus, there is a pathway by which cell surface LPL is internalized and degraded in adipocytes (37) and it leads to degradation of approximately 25% of the cell surface LPL.

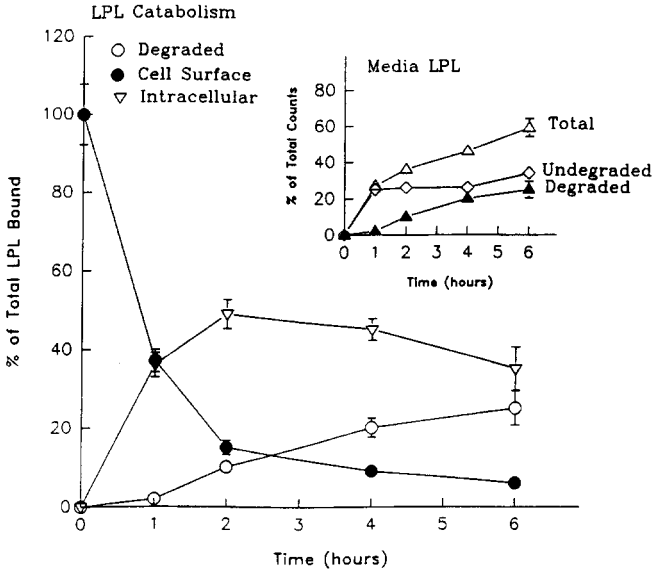


Fig. 1. Time course of cell surface LPL uptake and degradation. BFC-1β were incubated for 1 h at 4°C in medium containing ^{125}I-labeled LPL (1 μg/ml). The LPL-containing medium was removed, cells were washed and then incubated with fresh medium at 37°C to measure subsequent ^{125}I-labeled LPL degraded (open circles), within the cells (open triangles), and on the cell surface (solid dots). At the initial point (0 h), > 20 ng of the LPL was associated with the cells. Inset: Time course of total ^{125}I-labeled LPL released into medium (open triangles), undegraded (open diamonds), and degraded (filled triangles). Values presented are the percent of total counts at each time point and are the mean of triplicate determinations.

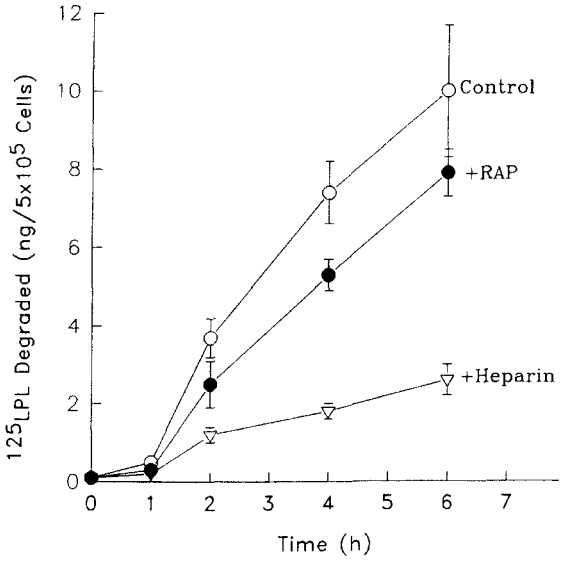


Fig. 2. Uptake and degradation of cell surface LPL: effects of RAP and heparin. ^{125}I-labeled LPL (1 μg/ml) was allowed to bind to BFC-1β at 4°C for 1 h. Cells were washed three times, incubated with fresh medium in the presence or absence of RAP (5 μg/ml) or heparin (10 units/ml) for various lengths of time, and LPL degraded was assessed. Shown are control cells (open circles) and cells incubated with RAP (solid dots) or heparin (open triangles). Values represent mean ± SD of triplicate determinations.

As LPL is a ligand for LRP (15–18) we tested whether RAP, an inhibitor of ligand binding to LRP, prevented degradation of LPL (**Fig. 2**). By 1 h, a significant reduction in degradation was found in the RAP-treated cells, 0.5 ± 0.05 ng versus 0.3 ± 0.04 ng, a 40% decrease. At 2 h, RAP decreased LPL degradation from 3.7 ± 0.5 ng to 2.5 ± 0.6 ng, a 32% decrease. Although a RAP-mediated decrease in degradation was still apparent at 6 h, the percent inhibition had declined to 21%. Heparin treatment decreased the amount of LPL degraded from 0.5 ± 0.05 ng to 0.2 ± 0.01 ng, a 60% decrease at 1 h. These data suggest that some LPL degradation by adipocytes is via a RAP-sensitive pathway, however, other mechanisms that are heparin-, but not RAP-, sensitive are also operative.

Effects of heparinase/heparitinase and RAP on the degradation of cell surface LPL

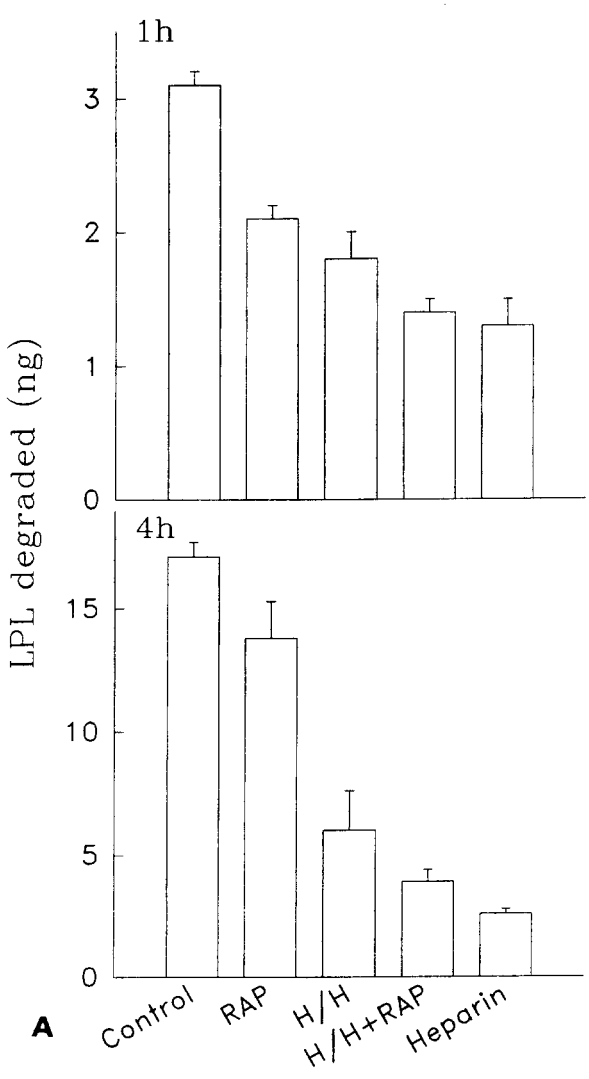
Our data suggest that most of the LPL degradation is heparin-sensitive. This probably includes both RAP-sensitive and non-RAP-sensitive pathways. Some ligands that associate with LRP require an initial interaction (or capture) by HSPG prior to binding to this receptor. LPL association with LRP also might require HSPG (15), therefore we assessed the effects of heparinase and heparitinase on LPL degradation. In parallel experiments, we also studied the effects of RAP and heparinase on the degradation of α_2M*, a LRP ligand that does not require HSPG binding for degradation (38, 39). Adipocytes were treated with heparinase and heparitinase at 37°C for 1 h and the cells were then cooled to 4°C. ^{125}I-labeled LPL (1 μg/ml) was allowed to associate with control and heparinase-treated adipocytes for 1 h at 4°C. Heparinase treatment decreased the amount of cell surface LPL by 70 ± 5%. The subsequent effects on LPL degradation were determined. As shown in **Fig. 3,** in this experiment RAP decreased LPL degradation to 68% of control at 1 h (3.1 ng in control vs 2.1 ng in RAP-treated cells) and to 82% of control at 4 h (from 17.1 ng to 13.8 ng). Heparinase/heparitinase decreased LPL degradation to 57 and 35% of control during the same time periods. When RAP was included in heparinase/heparitinase-treated cells, it further decreased LPL degradation to 46 and 23% of control at 1 h and 4 h, respectively (from 3.1 ng to 1.4 ng and from 17.1 ng to 3.9 ng). In a separate experiment to test the effect of different concentrations of RAP on LPL uptake and degradation, we found that LPL degradation decreased in a dose-dependent manner. At a RAP concentration of 40 μg/ml (1 μM), LPL degradation decreased by 67% at 1 h (from 36.4 ± 1.4 ng to 12.1 ± 1.8 ng).

These data provide further evidence that there are two pathways of LPL degradation. *1*) A RAP-sensitive, presumably LRP and LDL receptor, pathway. This pathway may involve both direct uptake of LPL by LRP or LDL receptor (inhibited by RAP alone) and a HSPG intermediate step, i.e., LPL first binds to HSPG and then to LRP (inhibited by heparinase and RAP). *2*) A RAP-insensitive, but heparinase-sensitive, presumably HSPG, pathway. This second pathway accounts for a greater percent of LPL degradation at the later, 4 h, time, suggesting that it is a slower metabolic process. As heparin decreased degradation by 60–85% (Fig. 3A) both HSPG and RAP pathways may be affected by heparin. However, these data suggest that the HSPG pathway (4 h) is relatively more sensitive to heparin than the RAP-sensitive pathway.

Metabolism of α_2M* was assessed under the same conditions. Binding, uptake, and degradation of α_2M* by adipocytes was inhibited 75–98% by RAP and was not affected by heparinase treatment (Fig. 3B). This suggested that cellular α_2M* metabolism, unlike that of LPL, is primarily via a RAP-sensitive pathway.

Degradation of LPL in the medium

In the previous experiments, degradation of cell surface LPL was specifically assessed by first allowing the LPL to bind to the cells at 4°C and then following degradation. LPL degradation may be a 2-step process: *1*) LPL binding to the cell surface, and *2*) LPL uptake and degradation. Because the HSPG and RAP-sensitive mechanisms appeared to have different kinetics, we as-



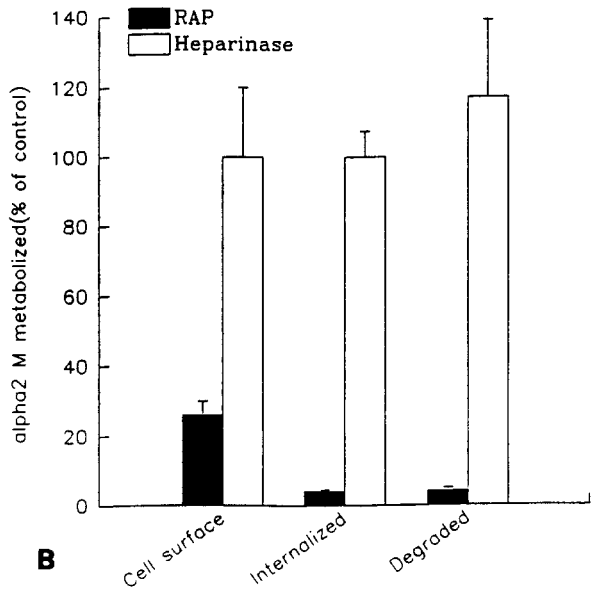


Fig. 3. Effects of heparinase/heparitinase, and RAP on the degradation of cell surface LPL and α_2M*. A: ^{125}I-labeled LPL: BFC-1β were incubated for 1 h at 37°C with medium alone or medium containing a combination of heparinase and heparitinase (H/H) (2.5 units/ml of each). Cells were washed, cooled to 4°C before adding ^{125}I-labeled LPL (1 μg/ml) and then incubated for 1 h. Unbound LPL was removed and the control and heparinase-treated cells were then incubated at 37°C in fresh medium alone or medium containing RAP (5 μg/ml) or heparin (10 units/ml). Degraded ^{125}I-labeled LPL was determined at 1 and 4 h. The effects of RAP and heparinase on cell surface, intracellular, and degraded α_2M* were determined (B). Values represent the mean ± SD of triplicate determinations. B: ^{125}I-labeled α_2M*: BFC-1β were incubated for 1 h at 37°C with medium alone or medium containing heparinase (5 units/ml). Cells were washed, cooled to 4°C and incubated with ^{125}I-labeled α_2M* (5 μg/ml) for 1 h. Unbound ^{125}I-labeled α_2M* was removed and control cells were incubated with medium containing RAP (5 μg/ml) and heparinase-treated cells were incubated with medium alone at 37°C for 1 h. Cell surface, internalized, and degraded α_2M* were determined.

sessed whether the RAP-sensitive process accounts for a different proportion of LPL degradation when LPL is in solution. To do this, cells were incubated with medium containing ^{125}I-labeled LPL at 37°C, and degraded LPL was assessed (Fig. 4). Again, heparin decreased degradation by >70% at 1 and 4 h. At 1 h, RAP decreased degradation by 64% of control (from 12.4 ng to 4.5 ng); at 4 h, however, only 22% of the degradation was RAP-sensitive (60.3 ng vs. 43.8 ng). Therefore, at the initial time point, more LPL was degraded via the RAP-sensitive process.

Effects of PIPLC on LPL uptake and degradation

Avian adipocytes (40), 3T3-L1 cells (41), and hepatocytes (42, 43) have cell surface HSPG that are GPI linked. We tested whether GPI-linked HSPG are involved in uptake and degradation of LPL by BFC-1β adipocytes. Cells were incubated with medium containing PIPLC (2 units/ml) for 1 h at 37°C, then LPL (1 μg/ml) was allowed to bind to cells for 1 h at 4°C and metabolism of LPL in control and enzyme-treated cells with and without RAP was assessed (**Fig. 5**). With cells treated with PIPLC, LPL degradation was decreased by 13 ± 2% (from 4.9 ng to 4.20 ng). RAP decreased LPL degradation to 70 ± 5% of control (4.9 ng vs. 3.43 ng). PIPLC and RAP decreased LPL degradation to 67 ± 1% of control (4.9 ng vs. 3.28 ng). This result suggests that some of the cell surface HSPG, as in avian adipocytes (40), are GPI anchored. The decrease in LPL degradation after removing these proteoglycans was commensurate with their effect on cell surface LPL binding.

Effect of RAP and PIPLC on endogenous LPL activity

Because newly synthesized LPL is present on the adipocyte surface, we tested whether treatment of adipocytes with RAP or PIPLC would increase media LPL. Because immunoprecipitating antibodies to mouse LPL

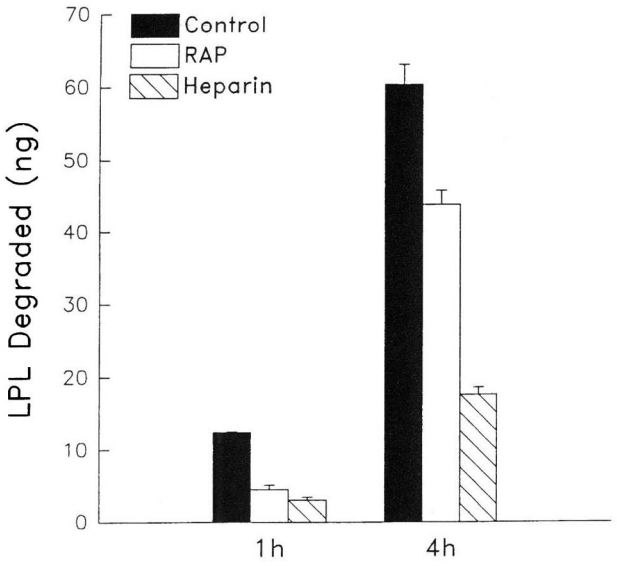


Fig. 4. Degradation of media LPL. Cells were incubated at 37°C for 1 or 4 h in medium containing ^{125}I-labeled LPL (1 μg/ml) in the presence of RAP (5 μg/ml, solid bar) or heparin (10 units/ml, open bar). Degraded ^{125}I-labeled LPL was measured. Values are the means ± SD of triplicate dishes.

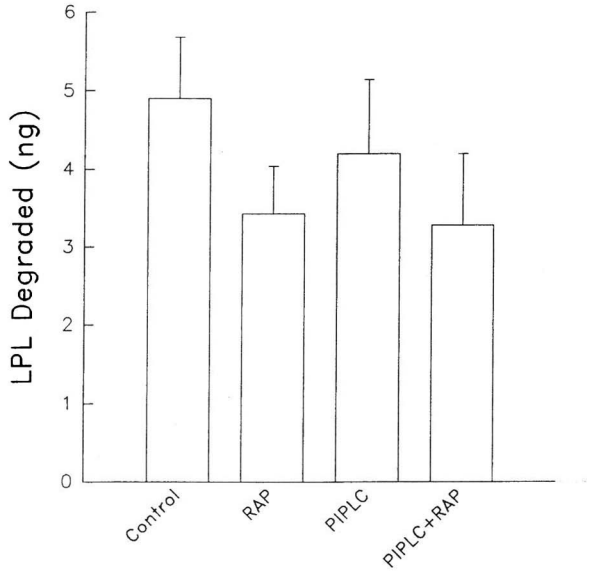


Fig. 5. Effects of PIPLC on LPL uptake and degradation. BFC-1β were incubated for 1 h at 37°C in medium with no enzyme or PIPLC (2 units/ml). Afterwards, cells were washed, cooled to 4°C before incubation with ^{125}I-labeled LPL (1 μg/ml) for 1 h. Unbound LPL was removed and cells were incubated at 37°C for 1 h in a fresh medium with or without RAP (5 μg/ml). Degraded ^{125}I-labeled LPL was determined. Values are the means ± SD of triplicate dishes.

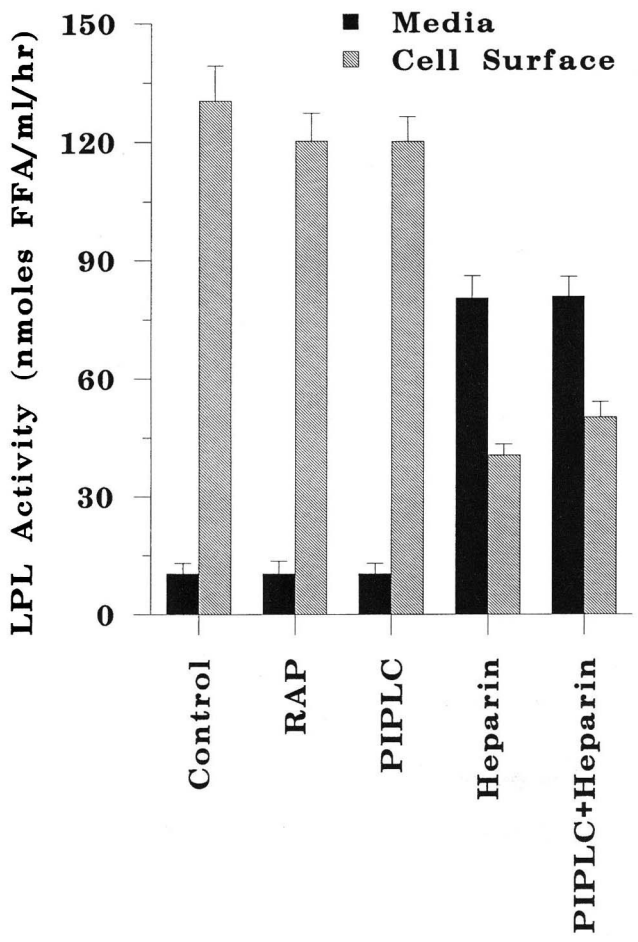


Fig. 6. Effect of RAP and PIPLC on endogenous LPL activity. BFC-1β were incubated for 1 h at 37°C in 1 ml of DMEM-BSA (control), DMEM-BSA containing RAP (5 μg/ml) or PIPLC (2 units/ml) or heparin (10 units/ml) or PIPLC and heparin. LPL activity in the media (solid bar) and on the cell surface (open bar) was measured. Surface LPL was released by incubating cells with 100 units/ml of heparin in DMEM-BSA at 4°C for 30 min. Values represent mean ± SD.

were not available, to test this, cells were incubated with or without RAP (5 μg/ml), PIPLC (2 units/ml), heparin (10 units/ml) or PIPLC plus heparin at 37°C for 1 h and media and cell surface LPL activities were assessed. As shown in **Fig. 6,** under control conditions, most of the LPL activity was present on the cell surface and very little was released into the medium. Addition of RAP had no effect on LPL release. Similarly, incubation of adipocytes with PIPLC did not release additional LPL activity into the medium (42 ± 10 for PIPLC compared to 47 ± 10 for controls). Heparin treatment increased LPL activity in the medium to >6-fold of control cells and markedly decreased cell surface LPL activity. As PIPLC did not release LPL, we tested whether this was due to reassociation of PIPLC-released LPL with cell surface HSPG. When cells were incubated in medium containing both heparin and PIPLC, no fur-

ther increase in media LPL activity was found compared to heparin alone. These data suggest that LPL activity is not preferentially found on GPI anchored proteoglycan and do not support the hypothesis that LPL is GPI anchored to the adipocyte cell surface.

HSPG turnover by BFC-1β adipocytes

The heparanase-sensitive and RAP-resistant LPL uptake and degradation, we postulated, was due, in part, to direct internalization of cell surface HSPG with bound LPL. Such a pathway would be reasonable only if the rate of cell surface HSPG uptake is similar to the rate of LPL uptake. The kinetics of HSPG turnover were examined in a pulse-chase experiment. Adipocytes were labeled with [^{35}S]sulfate and the amounts of total proteoglycans, CSPG, and HSPG were determined. Adipocyte proteoglycans are present in three pools, in the medium, inside the cells, and in a pericellular pool. The pericellular proteoglycans are those released with trypsin and include both integral membrane proteoglycans and extracellular proteoglycans that are not in solution. **Figure 7A** shows the total proteoglycan turnover. At 2 h, pericellular proteoglycans decreased 26%, and at 8 h approximately 50% of the pericellular proteoglycans remained. It should be noted that the trypsin-releasable proteoglycans are both on the cell surface and in the extracellular matrix. It is conceivable that much of this slowly turning-over pericellular pool is extracellular and not on the cell surface. Nonetheless, in 2 h an approximately 26% decrease was observed for pericellular proteoglycans and during that same period 38% of the LPL degradation occurred (Fig. 1). We, therefore, conclude that the proteoglycan pathway could account for much of the non-RAP-sensitive LPL degradation.

The effects of heparinases support the hypothesis that most cell surface LPL binds to HSPG. For this reason, the kinetics of HSPG and CSPG turnover were separately assessed (Fig. 7B). The amount of pericellular HSPG decreased fairly rapidly: a >25% decrease in 2 h, >40% in 4 h, and >60% at 24 h. Although we observed a similar decrease of pericellular CSPG, this decrease was slightly less rapid than the HSPG.

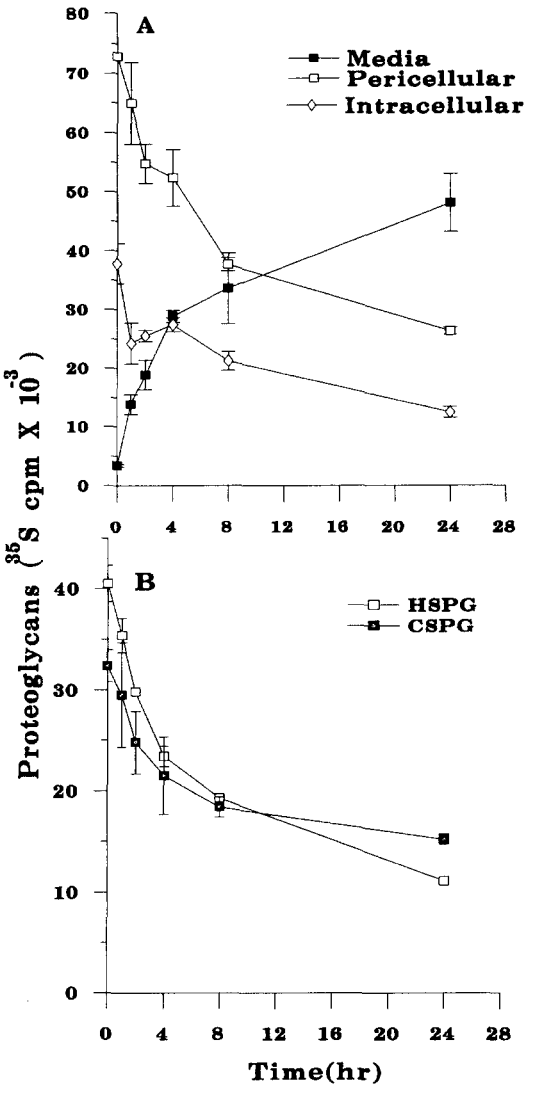


Fig. 7. Adipocyte proteoglycan metabolism. A: Turnover of total proteoglycans: BFC-1β were labeled with 50 μCi/ml [^{35}S]sulfate for 20 h. The labeling medium was removed, and the cells were incubated for indicated times in unlabeled medium containing 1 mM Na_2SO_4. Media (open circles), pericellular (trypsin releasable, solid circles), and intracellular (open triangles) ^{35}S-labeled proteoglycans were determined. B: Turnover of pericellular HSPG and CSPG: BFC-1β were labeled and chased as described in 7A. Pericellular proteoglycans were collected at each time point and the amounts of HSPG (open circles) and CSPG (closed circles) were determined.

Effect of PIPLC on adipocyte proteoglycan metabolism

To directly assess the amount of cell surface proteoglycans that were GPI anchored, cells were labeled with [^{35}S]sulfate for 16 h and then incubated in fresh medium with or without PIPLC for up to 1 h. Proteoglycans in the media were measured at 0, 10, 20, 30, 40, and 60 min (**Fig. 8A**). At each time point, ^{35}S-labeled proteoglycans in the medium were >3-fold higher in PIPLC-treated cells than in control cells. This confirms the presence of GPI anchored proteoglycans on these cells.

The percentage of pericellular proteoglycans in the different pools was assessed after a similar labeling experiment. Trypsin-releasable proteoglycans represent the total pericellular proteoglycans (Fig. 8B). Approximately 14% of the pericellular proteoglycans were released by PIPLC and 30% by heparin. This suggested that 14% of the pericellular HSPG are GPI anchored,

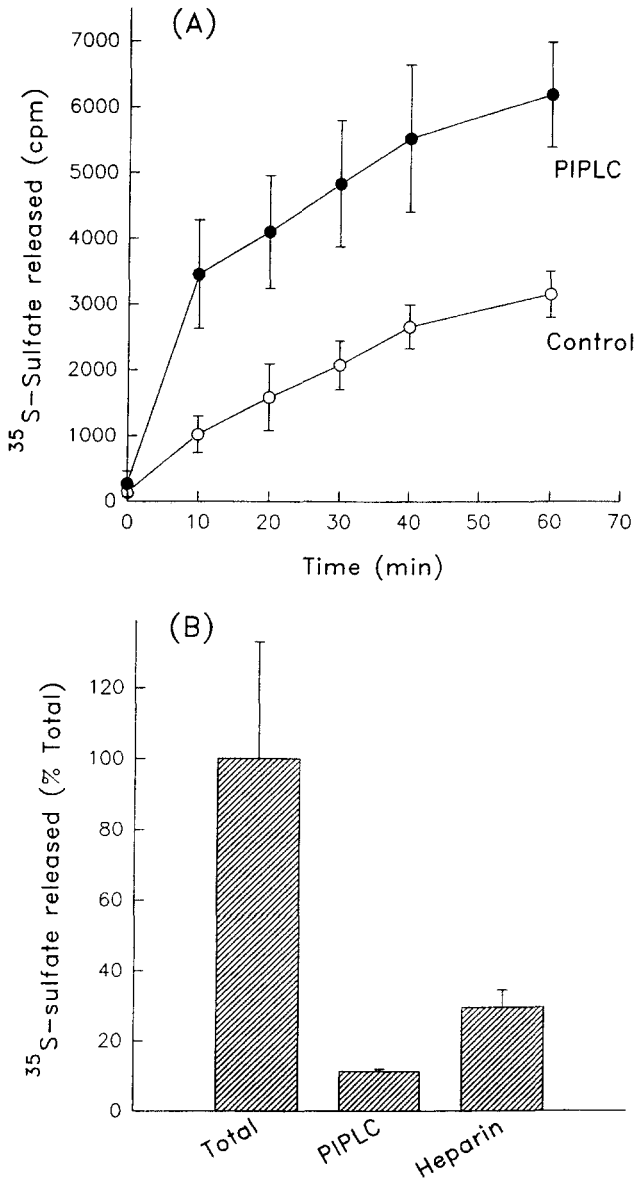


Fig. 8. Effect of PIPLC on adipocyte proteoglycan metabolism. A: Time course of proteoglycan release: BFC-1β were labeled with 50 μCi/ml [^{35}S]sulfate for 16 h. The labeling medium was removed and cells were incubated in fresh medium alone or medium with PIPLC (2 units/ml) for up to 1 h. Aliquots (100 μl) of media were collected at each time point and the amount of ^{35}S-labeled proteoglycans was measured. B: Percentage of pericellular proteoglycans in the different pools: ^{35}S-labeled adipocytes were incubated with PIPLC (2 units/ml) for 1 h at 37°C or with heparin (100 units/ml) for 15 min at 37°C and released proteoglycans were determined. Total proteoglycans represent those released by trypsin.

but the GPI-anchored HSPG do not appear to function in a metabolically more active manner. Moreover, a significant portion of the extracellular proteoglycans was released by heparin (44) and appear to be non-transmembrane proteoglycans.

DISCUSSION

The current studies were designed to investigate the biochemical mechanisms of LPL degradation by BFC-1β adipocytes. Our data are consistent with those of Cisar et al. (11). There appear to be two separate pathways for the uptake of cell surface LPL and, in our studies, 26% of the cell surface LPL and approximately 50% of the internalized LPL were degraded. Our data further suggest that adipocyte cell surface LPL is degraded by at least two mechanisms. One of these is sensitive to RAP, the 39 kDa inhibitor of LRP and VLDL receptor (45). The known interactions of LPL with LRP (16) and VLDL receptor and the findings that adipocytes contain both LRP and VLDL receptor (24, 46, 47) make it likely that RAP prevents LRP and VLDL receptor-mediated LPL degradation. RAP, however, is a multifunctional reagent and has also been shown to block the lipolysis-stimulated receptor (48), and, at a high concentration, the LDL receptor (49, 50). Recent studies showed that the LDL receptor can bind LPL (51). It is, therefore, likely that RAP inhibited internalization and degradation of LPL by LRP, VLDL receptors and LDL receptors.

There are two models for ligand interaction with LRP. In one, typified by α_2M, the ligand directly interacts with the receptor (52, 53). In the second model, typified by apolipoprotein E-containing lipoproteins (54, 55), hepatic lipase (56), and thrombospondin (57), the LRP ligand first binds to cell surface HSPG and is then internalized via the receptor. This is the process that appears to mediate much of the LRP-mediated uptake of lipoproteins. Most LPL-mediated lipoprotein degradation via LRP appears to require an initial interaction of LPL with HSPG (16). However, because RAP decreased LPL degradation in heparinase-treated cells, our data suggest that some LPL degradation was exclusive of HSPG. Thus, both HSPG and HSPG-independent pathways can degrade LPL.

There is a RAP-insensitive, but heparin- and heparinase-sensitive, pathway for LPL uptake and degradation by adipocytes. This pathway is likely to be similar to the HSPG internalization pathway in macrophages that degrades lipoproteins attached to the cell surface via an LPL anchor (18). The kinetic analyses showed that the turnover of cell surface HSPG was sufficiently rapid to account for much of the non-RAP-sensitive LPL degradation in the cells. Whether a subclass of HSPG accounts for most of this process was also assessed. We found that at least three pools of HSPG were present on the adipocyte surface, one of these pools is releasable with PIPLC and a second with heparin treatment of the cells. The majority of the cell surface HSPG are resistant to both of these treatments. PIPLC-sensitive

HSPG did not appear to be metabolically more active than other pericellular proteoglycans. Studies in progress will examine whether the relatively large pool of heparin-releasable HSPG is involved in LPL degradation.

Controversy exists in the literature as to whether LPL is anchored to adipocytes via a GPI anchor. The initial report that PIPLC released LPL from adipocytes (41) preceded the description by Misra et al. (40) that these cells have GPI-anchored HSPG. Recently two reports from a single laboratory have claimed to precipitate myo-[^{14}C]inositol-labeled LPL from adipocytes (58, 59). The specificity of the antibody used for these experiments was not reported and the size of the alleged precipitated LPL was identical to that of another GPI-anchored protein, the cAMP-binding ectoprotein. Recently, an analysis of LPL cDNA did not find a carboxyl-terminal signal sequence similar to that found in GPI anchored proteins (60). Our data are in agreement with those of Misra et al. (40) and show that in mouse adipocytes approximately 14% of the cell surface HSPG are GPI-anchored. After treating the cell with heparin, we were unable to measure any additional LPL activity released with PIPLC. This could be because the GPI-anchored LPL was inactive or because GPI-LPL is only found intracellularly. However, the most likely explanation for our results is that LPL is not a GPI-anchored protein.

LPL cellular metabolism may be a model for other cell surface HSPG-binding proteins. LPL is internalized and degraded by the cells by receptor-mediated and HSPG-mediated processes. Moreover, HSPG association promotes LPL degradation by receptor-mediated processes, while some degradation is mediated by direct interactions with the receptors. Degradation also occurs by a direct HSPG-mediated process. Recently, Berryman and Bensadoun (25) observed that most of the LPL degradation by wild type CHO cells transfected with human LPL was HSPG-mediated and not RAP-sensitive. In contrast, RAP significantly inhibited LPL degradation in a HSPG-deficient CHO cell line (25). Thus, differences in cell lines led to differences in LPL metabolism. For this reason, we believe that our data using adipocytes, which are consistent with observations made using hepatocytes (16), are more likely to reflect in vivo cellular degradative processes.

What is the importance of these degradative pathways to overall LPL metabolism? Several studies show that LRP levels can be regulated in vitro. Agents that stimulate LRP expression include phorbal esters, modified lipoproteins (61), lipopolysaccharide, interferon gamma (62), cAMP (63), and insulin (24). LRP regulators may, in part, control the amount of LPL on the adipocyte cell surface by affecting LPL degradation by adipocytes. In vivo, this would make more LPL available for transport to its physiologic site of action on the endothelium. However, it appears likely that degradation is but one of many regulatory steps that control LPL activity in vivo.

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REFERENCES

1. Ashby, P., D. P. Bennett, I. M. Spencer, and D. S. Robinson. 1978. Post-translational regulation of lipoprotein lipase activity in adipose tissue. *Biochem. J.* **176:** 865–872.
2. Simsolo, R. B., J. M. Ong, and P. A. Kern. 1992. Characterization of lipoprotein lipase activity, secretion, and degradation at different sites of post-translational processing in primary cultures of rat adipocytes. *J. Lipid Res.* **33:** 1777–17784.
3. Fried, S. K., C. D. Russell, N. L. Grauso, and R. E. Brolin. 1993. Lipoprotein lipase regulation by insulin and glucocorticoid in subcutaneous and omental adipose tissues of obese women and men. *J. Clin. Invest.* **92:** 2191–2198.
4. Simsolo, R. B., J. M. Ong, and P. A. Kern. 1993. The regulation of adipose tissue and muscle lipoprotein lipase in runners by detraining. *J. Clin. Invest.* **92:** 2124–2130.
5. Appel, B., and S. K. Fried. 1992. Effects of insulin and dexamethasone on lipoprotein lipase in human adipose tissue. *Am. J. Physiol.* **262:** E695–E699.
6. Kern, P. A., J. M. Ong, J. W. Goers, and M. E. Pedersen. 1988. Regulation of lipoprotein lipase immunoreactive mass in isolated human adipocytes. *J. Clin. Invest.* **81:** 398–406.
7. Fielding, C. J. 1973. Kinetics of lipoprotein lipase activity: effects of the substrate apoprotein on reaction velocity. *Biochem. Biophys. Acta.* **316:** 66–75.
8. Olivecrona T., G. Liu, M. Hultin, and G. Bengtsson-Olivecrona. 1993. Regulation of lipoprotein lipase. *Biochem. Soc. Trans.* **21:** 509–513.
9. Sasaki, A., and I. J. Goldberg. 1992. Lipoprotein lipase release from BFC-1 beta adipocytes. Effects of triglyceride-rich lipoproteins and lipolysis products. *J. Biol. Chem.* **267:** 15198–15204.
10. Ong, J. M., and P. A. Kern. 1989. Effect of feeding and obesity on lipoprotein lipase activity immunoreactive protein, and messenger RNA levels in human adipose tissue. *J. Clin. Invest.* **84:** 305–311.
11. Cisar, L. A., A. J. Hoogewerf, M. Cupp, C. A. Rapport, and A. Bensadoun. 1989. Secretion and degradation of lipoprotein lipase in cultured adipocytes. Binding of lipoprotein lipase to membrane heparan sulfate proteoglycans is necessary for degradation. *J. Biol. Chem.* **264:** 1767–1774.
12. 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. *Biochem. Biophys. Acta.* **963:** 183–191.
13. Saxena, U., M. G. Klein, and I. J. Goldberg. 1990. Metabolism of endothelial cell bound lipoprotein lipase. *J. Biol. Chem.* **265:** 12880–12886.
14. Hoogewerf, A. J., L. A. Cisar, D. C. Evans, and A. Bensadoun. 1991. Effect of chlorate on the sulfation of lipoprotein lipase and heparan sulfate proteoglycans. Sulfation of heparan sulfate proteoglycans affects lipoprotein lipase degradation. *J. Biol. Chem.* **226:** 16564–16571.
15. 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.
16. 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/alpha2-macroglobulin receptor binds and mediates catabolism of bovine milk lipoprotein lipase. *J. Biol. Chem.* **267:** 25764–25767.
17. 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.
18. Obunike, J. C., I. J. Edwards, S. C. Rumsey, L. K. Curtiss, W. D. Wagner, R. J. Deckelbaum, and I. J. Goldberg. 1994. Cellular differences in lipoprotein lipase-mediated uptake of low density lipoproteins. *J. Biol. Chem.* **269:** 13129–13135.
19. 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.
20. 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.
21. Blaner, W. S., J. C. Obunike, S. B. Kurlandsky, M. Al-Haideri, R. Piantedosi, R. J. Deckelbaum, and I. J. Goldberg. 1994. Lipoprotein lipase hydrolysis of retinyl ester. *J. Biol. Chem.* **269:** 16559–16565.
22. 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.
23. Mulder, M., P. Lombardi, H. Jansen, T. J. C. van Berkel, R. R. Frants, and L. M. Havekes. 1993. Low density lipoprotein receptor internalizes low density and very low density lipoproteins that are bound to heparan sulfate proteoglycans via lipoprotein lipase. *J. Biol. Chem.* **268:** 9369–9376.
24. Descamps, O., D. Bilheimer, and J. Herz. 1993. Insulin stimulates receptor-mediated uptake of apoE-enriched lipoproteins and activated alpha 2-macroglobulin in adipocytes. *J. Biol. Chem.* **268:** 974–981.
25. Berryman, D. E., and A. Bensadoun. 1994. Heparan sulfate proteoglycans are primarily responsible for the maintenance of enzyme activity, binding, and degradation of lipoprotein lipase in Chinese hamster ovary cells. *J. Biol. Chem.* **270:** 24535–24531.
26. Nilsson-Ehle, P., and M. C. Schotz. 1976. A stable, radioactive substrate emulsion for assay of lipoprotein lipase. *J. Lipid Res.* **17:** 536–541.
27. Saxena, U., L. D. Witte, and I. J. Goldberg. 1989. Release of endothelial lipoprotein lipase by plasma lipoproteins and fatty acids. *J. Biol. Chem.* **264:** 4349–4355.
28. Socorro, L., C. C. Green, and R. L. Jackson. 1985. Preparation of a homogeneous and stable form of bovine milk lipoprotein lipase. *Prep. Biochem.* **15:** 133–143.
29. Hietanen, E., and M. R. C. Greenwood. 1977. A comparison of lipoprotein lipase activity and adipocyte differentiation in growing male rats. *J. Lipid Res.* **18:** 480–490.
30. Belfrage, P., and M. Vaughan. 1969. Simple liquid–liquid partition system for isolation of labeled oleic acid from mixtures with glycerides. *J. Lipid Res.* **10:** 341–344.
31. 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.
32. Yamashiro, D. J., L. A. Borden, and F. R. Maxfield. 1989. Kinetics of alpha 2-macroglobulin endocytosis and degradation in mutant and wild-type Chinese hamster ovary cells. *J. Cell Physiol.* **139:** 377–382.
33. Low, M. G. 1992. Phospholipase that degrade the glycosylphosphatidylinositol anchor of membrane proteins. *In* Lipid Modification of Proteins: A Practical Approach. N. M. Hooper and A. J. Turner, editors. Oxford University Press, Oxford. 117–154.
34. Goldstein, J. L., S. K. Bau, and M. S. Brown. 1983. Receptor-mediated endocytosis of low-density lipoprotein in cultured cells. *Methods Enzymol.* **98:** 241–260.
35. Edwards, I. J., and W. D. Wagner. 1988. Distinct synthetic and structural characteristics of proteoglycans produced by cultured artery smooth muscle cells of atherosclerosis-susceptible pigeons. *J. Biol. Chem.* **263:** 9612–9620.
36. Edwards, I. J., and W. D. Wagner. 1992. Cell surface heparan sulfate proteoglycan and chondroitin sulfate proteoglycan of arterial smooth muscle cells. *Am. J. Pathol.* **140:** 193–205.
37. Cupp, M., A. Bensadoun, and K. Melford. 1987. Heparin decreases the degradation rate of lipoprotein lipase in adipocytes. *J. Biol. Chem.* **262:** 6383–6388.
38. Ji, Z-S., W. J. Brecht, R. D. Miranda, M. M. Hussain, T. L. Innerarity, and R. W. Mahley. 1993. Role of heparan sulfate proteoglycans in the binding and uptake of apolipoprotein E enriched remnant lipoproteins by cultured cats. *J. Biol. Chem.* **268:** 10160–10167.
39. Vassiliou G., and K. K. Stanley. 1994. Exogenous receptor-associated protein binds to two distinct sites on human fibroblasts but does not bind to the glycosaminoglycan residues of heparan sulfate proteoglycans. *J. Biol. Chem.* **269:** 15172–15178.
40. Misra, K. B., K. C. Kim, S. Cho, M. G. Low, and A. Bensadoun. 1994. Purification and characterization of adipocyte heparan sulfate proteoglycans with affinity for lipoprotein lipase. *J. Biol. Chem.* **269:** 23838–23844.
41. Chan, B. L., M. P. Lisanti, E. Rodriguez-Boulan, and A. R. Saltiel. 1988. Insulin-stimulated release of lipoprotein lipase by metabolism of its phosphatidylinositol anchor. *Science.* **241:** 1670–1672.
42. David, G., V. Lories, B. Decock, P. Marynen, J. J. Cassiman, and H. vanden Berghe. 1990. Molecular cloning of a phosphatidylinositol-anchored membrane heparan sulfate proteoglycan from human lung fibroblasts. *J. Cell Biol.* **111:** 3165–3176.

43. Ishihara, M., N. Fedarko, and H. E. Conrad. 1988. Involvement of phosphatidylinositol and insulin in the coordinate regulation of proteoheparan sulfate metabolism and hepatocyte growth. *J. Biol. Chem.* **262:** 4708–4716.
44. Sasaki, A., P. Sivaram, and I. J. Goldberg. 1993. Lipoprotein lipase binding to adipocytes: evidence for the presence of heparin sensitive binding protein. *Am. J. Physiol.* **265:** E880–E888.
45. Battey, F. D., M. E. Gafvels, D. J. FitzGerald, W. S. Argraves, D. A. Chappell, J. F. Strauss III, and D. K. Strickland. 1994. The 39-kDa receptor-associated protein regulates ligand binding by the very low density lipoprotein receptor. *J. Biol. Chem.* **269:** 23268–23273.
46. Argraves, K. M., F. D. Battey, C. D. MacCalman, K. R. McCrae, M. Gafvels, K. F. Kozarsky, D. A. Chappell, J. F. Strauss III, and D. K. Strickland. 1995. Very low density lipoprotein receptor mediates the cellular catabolism of lipoprotein lipase and pro urokinase activator/inhibitor complexes. *J. Biol. Chem.* **270:** 26550–26557.
47. Takahashi, S., Y. Kawarabaysi, 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.
48. Troussard, A. A., J. Khallou, C. J. Mann, P. Andre, D. K. Strickland, B. E. Bihain, and F. T. Yen. 1995. Inhibitory effect of the lipolysis-stimulated receptor of the 39-kDa receptor-associated protein. *J. Biol. Chem.* **270:** 17068–17070.
49. 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.
50. Mokuno, H., S. Brady, L. Kotite, J. Herz, and R. J. Havel. 1994. Effect of the 39 kDa receptor-associated protein on the hepatic uptake and endocytosis of chylomicron remnants and low density lipoproteins in the rat. *J. Biol. Chem.* **269:** 13238–13243.
51. Medh, J. D., S. L. Bowen, G. L. Fry, M. Pladet, M. Andracki, I. Inoue, J. M. Lalouel, D. K. Strickland, and D. A. Chappell. 1996. Lipoprotein lipase binds to low density lipoprotein receptor and induces receptor-mediated catabolism of very low density liproteins in vitro. *J. Biol. Chem.* **271:** 17073–17080.
52. Moestrup, S. K., and J. Gliemann. 1991. Analysis of ligand recognition by the purified α_2-macroglobulin receptor (low density lipoprotein receptor-related protein) *J. Biol. Chem.* **266:** 14011–14017.
53. Strickland, D. K., J. D. Ascom, S. Williams, F. Battey, E. Behre, K. McTigue, J. F. Battey, and W. S. Argraves. 1991. Primary structure of α_2-macroglobulin receptor-associated protein. *J. Biol. Chem.* **266:** 13364–13369.
54. Mahley, R. W., Z. S. Ji., W. J. Brecht., R. D. Miranda, and D. He. 1994. Role of heparan sulfate proteoglycans and the LDL receptor related protein in remnant lipoprotein metabolism. *Ann. NY Acad. Sci.* **737:** 39–52.
55. Ji, Z. S., D. A. Sanan, and R. W. Mahley. 1995. Intravenous heparinase inhibits remnant lipoprotein clearance from the plasma and uptake by the liver: in vivo role of heparan sulfate proteoglycans. *J. Lipid Res.* **36:** 583–592.
56. Kaunnas, M. Z., D. A. Chappell, H. Wong, W. S. Argraves, and D. K. Strickland. 1995. Low density lipoprotein receptor-related protein/α_2-macroglobulin receptor mediates the cellular internalization and degradation of hepatic lipase. *J. Biol. Chem.* **270:** 9307–9311.
57. Mikhailenko, I., M. Z. Kounnas, W. S. Argraves, and D. K. Strickland. 1995. Low density lipoprotein receptor-related protein/α_2-macroglobulin receptor mediates the cellular internalization and degradation of thrombospondin. *J. Biol. Chem.* **270:** 9543–9549.
58. Muller, G., E-M. Wetekam, C. Jung, and W. Bandlow. 1994. Membrane association of lipoprotein lipase and a cAMP-binding ectoprotein in rat adipocytes. *Biochemistry.* **33:** 12149–12159.
59. Muller, G., E. A. Dearey, A. Korndorfer, and W. Bandlow. 1994. Stimulation of a glycosylphosphatidylinositol-specific phospholipase by insulin and the sulfonylurea, glimepiride, in rat adipocytes depends on increased glucose transport. *J. Cell Biol.* **126:** 1267–1276.
60. Bruin, T., N. B. Groot, J. Jansen, and J. J. P. Kastelein. 1994. The C-terminus of lipoprotein lipase is essential for biological function but contains no domain for glycosylphosphatidylinositol anchoring. *Eur. J. Biochem.* **221:** 1019–1025.
61. Watanabe, Y., T. Inaba, H. Shimano, T. Gotoda, K. Yamamoto, H. Mokuno, H. Sato, Y. Yazaki, and N. Yamada. 1994. Induction of LDL receptor related protein during the differentiation of monocyte-macrophages. *Arterioscler. Thromb.* **14:** 1000–1006.
62. Lamarre, J., B. B. Wolf, E. L. Kittler, P. J. Quesenberry, and S. L. Gonias. 1993. Regulation of macrophage alpha 2-macroglobulin receptor/low density lipoprotein receptor related protein by lipopolysaccharide and interferon-gamma. *J. Clin. Invest.* **91:** 1219–1224.
63. Gafvels, M. E., G. Coukos, R. Sayegh, C. Coutifaris, D. K. Strickland, and J. F. Strauss 3d. 1992. Regulated expression of the trophoblast alpha 2-macroglobulin receptor/low density lipoprotein receptor-related protein. Differentiation and cAMP modulate protein and mRNA levels. *J. Biol. Chem.* **267:** 21230–21234.