Lipoprotein Lipase Binds to Low Density Lipoprotein Receptors and Induces Receptor-mediated Catabolism of Very Low Density Lipoproteins in Vitro*

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Lipoprotein lipase (LPL), the major enzyme responsible for the hydrolysis of plasma triglycerides, promotes binding and catabolism of triglyceride-rich lipoproteins by various cultured cells. Recent studies demonstrate that LPL binds to three members of the low density lipoprotein (LDL) receptor family, including the LDL receptor-related protein (LRP), GP330/LRP-2, and very low density lipoprotein (VLDL) receptors and induces receptor-mediated lipoprotein catabolism. We show here that LDL receptors also bind LPL and mediate LDL-dependent catabolism of large VLDL with S, 100-400. Up-regulation of LDL receptors by lovastatin treatment of normal human foreskin fibroblasts (FSF cells) resulted in an increase in LPL-induced VLDL binding and catabolism to a level that was 10-15-fold greater than in LDL receptor-negative fibroblasts, despite similar LRP activity in both cell lines. This indicates that the contribution of LRP to LDL-dependent degradation of VLDL is small when LDL receptors are maximally up-regulated. Furthermore studies in LR-deficient murine embryonic fibroblasts showed that the level of LPL-dependent degradation of VLDL was similar to that in normal murine embryonic fibroblasts. LPL also promoted the internalization of protein-free triglyceride emulsions; lovastatin-treatment resulted in 2-fold higher uptake in FSF cells, indicating that LPL itself could bind to LDL receptors. However, the lower induction of emulsion catabolism as compared with native VLDL suggests that LPL-induced catabolism via LDL receptors is only partially dependent on receptor binding by LPL and instead is primarily due to activation of apolipoproteins such as apoE. A fusion protein between glutathione S-transferase and the catalytically inactive carboxy-terminal domain of LPL (GST-LPLC) also induced binding and catabolism of VLDL. However GST-LPLC was not as active as native LPL, indicating that lipolysis is required for a maximal LPL effect. Mutations of critical tryptophan residues in GST-LPLC that abolish binding to VLDL converted the protein to an inhibitor of lipoprotein binding to LDL receptors. In solid-phase assays using immobilized receptors, LDL receptors bound to LPL in a dose-dependent manner. Both LPL and GST-LPLC promoted binding of VLDL to LDL receptor-coated wells. These results indicate that LPL binds to LDL receptors and suggest that the carboxy-terminal domain of LPL contributes to this interaction.

Lipoprotein lipase (LPL) catalyzes the hydrolysis of triglycerides in plasma chylomicrons and very low density lipoproteins (VLDL) (1–4). In humans, deficiency of either LPL or apolipoprotein (apo) CII, an essential cofactor for LPL’s lipolytic activity, results in severe elevation in plasma chylomicrons and large VLDL (5). In addition to its lipolytic actions, LPL is also a ligand for receptors belonging to the family of low density lipoprotein (LDL) receptors, including LDL receptor-related protein (LRP)/α2-macroglobulin (α2-M) receptor, GP330/LRP-2, and VLDL receptors (6–13). LPL can influence lipoprotein catabolism by simultaneously binding to both lipoproteins and cell surface receptors (6–13). LPL promotes VLDL catabolism in LDL receptor-lacking mutant fibroblasts, and ligands specific for LRP inhibit LPL-stimulated VLDL degradation (9). LPL promotes lipoprotein binding to purified LRP (9) and GP330/LRP-2 (11) in solid-phase assays. Argraves et al. (12) demonstrated that transfection of PEA13 cells with VLDL receptors enhances their ability to internalize and degrade 125I-LPL. Takahashi et al. (13) showed that in Chinese hamster ovary cells co-transfected with the VLDL receptor and LPL, binding and degradation of 125I-VLDL is much greater than in cells transfected with VLDL receptor alone. These studies demonstrate that LPL-dependent VLDL catabolism is mediated by LRP (6–10), GP330/LRP-2 (10, 11), and VLDL receptors (12, 13). LPL also binds to cell surface heparan sulfate proteoglycans (3, 14–16). LPL binding to either heparan sulfate proteoglycans or receptors can be separated from its lipolytic activity as indicated by studies with LPL, the carboxy-terminal, non-catalytic domain of LPL (8, 17, 18).

There are conflicting data regarding the ability of LPL to promote lipoprotein catabolism via LDL receptors (19–23). LDL receptors are the major mediators of LDL clearance from plasma, but they have also been implicated as mediators of VLDL clearance via apoE (24). Aviram et al. (19) concluded that LPL enhances LDL uptake and degradation in both fibro-

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1 The abbreviations used are: LPL, lipoprotein lipase; LPLC, carboxy-terminal domain of LPL; LDL, low density lipoproteins; VLDL, very low density lipoproteins; LPDS, lipoprotein deficient serum; LRP, LDL receptor-related protein; GST, glutathione S-transferase; apo, apolipoprotein; BSA, bovine serum albumin; α2-M, α2-macroglobulin; α2-M*, activated α2-M; RAP, α2-M receptor-associated protein; HUVECs, human umbilical vein endothelial cells; MEF, murine embryonic fibroblast.
blasts and human monocyte-derived macrophages via LDL receptors. They suggest that LPL's lipolytic activity is necessary for its ability to enhance LDL degradation, but they did not study the effect of LPL on the catabolism of VLDL, which is a better substrate for LPL than LDL (25). Several investigators determined that LPL-mediated LDL degradation is independent of the LDL receptor and is not regulated by factors affecting LDL receptor expression (20–22). These studies also focused on the effect of LPL on LDL rather than VLDL catabolism. Mulder et al. (23) concluded that internalization of LPL-VLDL complexes in HepG2 cells was mediated by LDL receptors, since it was negligible in LDL receptor-lacking fibroblasts. However, they did not study lipoprotein degradation or the ability of LPL itself to bind to LDL receptors. The relative contributions of LRP versus LDL receptors to the effects of LPL have not been clearly shown in cells expressing both receptors.

The present studies were aimed at resolving these discrepancies. Normal human foreskin fibroblasts (FSF cells) were studied in which both LR P and LDL receptor-dependent pathways are active. Data were also obtained in LDL receptor-lacking (FH) human fibroblasts, HepG2 cells, and normal (MEF) and LRP-deficient (PEA13) murine embryonic fibroblasts. None of these cell lines is known to express GP330/LRP-2, but normal human foreskin fibroblasts and human monocyte-derived macrophages via LDL receptors. They suggest that LPL's lipolytic activity is necessary for its ability to enhance LDL degradation, but they did not study the effect of LPL on the catabolism of VLDL, which is a better substrate for LPL than LDL (25). Several investigators determined that LPL-mediated LDL degradation is independent of the LDL receptor and is not regulated by factors affecting LDL receptor expression (20–22). These studies also focused on the effect of LPL on LDL rather than VLDL catabolism. Mulder et al. (23) concluded that internalization of LPL-VLDL complexes in HepG2 cells was mediated by LDL receptors, since it was negligible in LDL receptor-lacking fibroblasts. However, they did not study lipoprotein degradation or the ability of LPL itself to bind to LDL receptors. The relative contributions of LRP versus LDL receptors to the effects of LPL have not been clearly shown in cells expressing both receptors.

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1. **Stimulation of \(^{125}\text{I}-\text{VLDL} \)** binding and degradation in normal human fibroblasts by LPL or GST-LPLC. Confluent fibroblasts were treated with either lipoprotein-deficient serum and lovastatin (closed symbols) or maintained in lipoprotein-containing media (open symbols) as described under “Experimental Procedures.” They were then incubated for 3 h at 4°C (A) or 5 h at 37°C (B) in media containing 35 nm \(^{125}\text{I}-\text{VLDL} \) and increasing concentrations of LPL (circles) or GST-LPLC (squares). After washing as described, surface-bound radioactivity was dissociated by incubating cells for 30 min at 4°C in buffer containing 10 mg/ml polyethylene glycol (A). B, degradation was measured as the radioactivity in the incubation medium that was soluble in 15% trichloroacetic acid. The moles of ligand were calculated from the radioactivity and corrected for cellular protein in each well. Results are averages of duplicate measurements.

Interpretation. This was true in the presence or absence of lovastatin/LPS treatment. Half-maximal LPL-induced \(^{125}\text{I}-\text{VLDL} \) binding to cell surfaces at 4°C shifted to a 5-fold lower LPL concentration when LDL receptors were up-regulated by lovastatin (Fig. 1A). This surprising result suggests that binding affinity was dependent on the number of LDL receptors, as would be expected if LPL simultaneously interacted with multiple LDL receptors. Another interesting observation in Fig. 1A is that GST-LPLC, a fusion protein containing the carboxy-terminal segment (residues 313–448) of LPL, was also able to enhance VLDL binding in a dose-dependent and lovastatin-regulated manner, albeit with a lower maximal binding. This indicates that the carboxyl-terminal domain of LPL, which lacks catalytic activity, contributes to LPL’s interaction with LDL receptors. LPL also significantly promoted degradation of \(^{125}\text{I}-\text{VLDL} \) by LDL receptors in a dose-dependent manner (Fig. 1B). Up-regulation of LDL receptors enhanced LPL-dependent degradation up to 4-fold. Either with or withoutLovastatin treatment, significantly higher concentrations of GST-LPLC than LPL were required to enhance \(^{125}\text{I}-\text{VLDL} \) degradation, and the magnitude of stimulation was much lower than that obtained with LPL.

To further examine the contribution of LDL receptors to LPL-dependent VLDL degradation, studies were performed in cells lacking LRP. Results in Fig. 2 show degradation of \(^{125}\text{I}-\text{LDL} \) and \(^{125}\text{I}-\text{a-M} \) by wild-type murine embryonic fibroblasts (MEF cells) and by mutant MEF cells lacking LRP (PEA13 cells). Unlike MEF cells, PEA13 cells are completely devoid of LRP activity and are unable to catabolize \(^{125}\text{I}-\text{a-M} \) (Fig. 2A). In contrast, \(^{125}\text{I}-\text{LDL} \) degradation is comparable in both cell lines and is stimulated 5–7-fold by lovastatin treatment (Fig. 2B). This indicates that the lack of LRP in PEA13 cells does not affect the level of LDL catabolism or its up-regulation by lovastatin. As expected, catabolism of \(^{125}\text{I}-\text{a-M} \) was not affected by lovastatin treatment.

We compared the effect of LPL and Lovastatin treatment in normal (FSF) and LDL receptor-lacking (FH) human fibroblasts and wild-type (MEF) and LRP-deficient (PEA13) murine embryonic fibroblasts (Fig. 3). LPL promoted \(^{125}\text{I}-\text{VLDL} \) degradation in a dose-dependent manner in untreated FSF cells. LPL stimulated VLDL degradation up to 15-fold in Lovastatin-treated normal fibroblasts. In three separate experiments, up-regulation of LDL receptors with Lovastatin resulted in a 4–5-fold increase in LPL-induced \(^{125}\text{I}-\text{VLDL} \) degradation by FSF cells (Fig. 3A). In control experiments (data not shown), catabolism of \(^{125}\text{I}-\text{LDL} \) in the absence of LPL was increased by 8–10-fold by Lovastatin treatment. These data suggest that LDL receptors mediate the vast majority of LPL-dependent \(^{125}\text{I}-\text{VLDL} \) degradation in Lovastatin-treated cells.

Previously, we showed that LPL-induced uptake of \(^{125}\text{I}-\text{VLDL} \) via LRP (7, 9), and this finding was confirmed here (Fig. 3B) with a 10–20-fold induction of \(^{125}\text{I}-\text{VLDL} \) catabolism by gradually increasing the concentration of LPL. However, compared with normal fibroblasts, the magnitude of \(^{125}\text{I}-\text{VLDL} \) degradation was 10-fold lower and not significantly altered by Lovastatin treatment. Even in the absence of Lovastatin treatment, normal fibroblasts displayed 2.5–4-fold higher catabolism in the presence of LPL than FH cells (Fig. 3, A and B). This difference probably reflects degradation by the LDL-receptor pathway, since degradation of \(^{125}\text{I}-\text{a-M} \) by normal and FH cells was nearly identical, indicating that both cell lines express similar levels of LRP (data not shown). LPL-dependent \(^{125}\text{I}-\text{VLDL} \) degradation by LRD-deficient PEA13 cells was up-regulated by Lovastatin treatment (Fig. 3C). In the presence or absence of Lovastatin treatment, LPL-induced \(^{125}\text{I}-\text{VLDL} \) degradation by PEA13 cells was comparable with that in MEF cells. Taken together, these results suggest that a majority of LPL-dependent LDL catabolism occurs via the LDL receptor pathway.

We next studied the catabolism of increasing concentrations of \(^{125}\text{I}-\text{VLDL} \) in the presence of a fixed LPL concentration (Fig. 4). At all concentrations of \(^{125}\text{I}-\text{VLDL} \), degradation was enhanced 50-fold by the presence of 1 µg/ml LPL in FSF, HUVECs, MEF, and PEA13 cells. LPL-dependent degradation of \(^{125}\text{I}-\text{VLDL} \) was strongly up-regulated by Lovastatin treatment in all four cell lines, suggesting a role for LDL receptors. Unlike normal fibroblasts, HUVECs express very low levels of LRP and are unable to catabolize significant amounts of \(^{125}\text{I}-\text{a-M} \) (data not shown and Ref. 12). Although HUVECs express VLDL receptors, these receptors are not up-regulated by Lovastatin. The up-regulation of LPL receptors in HUVECs with Lovastatin caused an increase in LPL-dependent VLDL catabolism that was similar to that in the other cell lines. LPL-induced VLDL catabolism in PEA13 cells was very similar to that in MEF cells despite the absence of LRP in the former.

Relative Contribution of LDL Receptor Versus LRP-mediated Catabolism of LDL in the Basal State—In the basal state, LRP expression is constitutive and LDL receptor expression is low but not absent. To measure the relative contribution of LRP and LDL receptors to LPL-induced LDL catabolism under basal conditions, we compared wild-type murine fibroblasts to the LRP-deficient PEA13 cells.
deficient PEA13 cells (Fig. 5). When cells were incubated with 20 \(\mu\)g/ml LDL to down-regulate LDL receptors prior to the assay, MEF cells displayed \(-30\%\) greater LDL-induced VLDL degradation (Fig. 5). This agrees with results shown in Fig. 3C. But in Fig. 4, C and D, LDL-induced VLDL degradation was slightly greater in PEA13 cells than in MEF cells. Thus the difference between MEF and PEA13 was too small to be seen in all experiments, despite the fact that in most experiments MEF cells displayed slightly greater LDL-dependent VLDL catabolism than PEA13 cells.

The receptor-mediated component of VLDL catabolism was measured by the ability of RAP to compete for the effects of LPL. RAP has previously been shown to inhibit catabolism via all members of the LDL receptor family (46, 48–50). As shown in Fig. 5, RAP inhibited \(>50\%\) of the catabolism in both MEF and PEA13 cells. The ability of RAP to inhibit degradation in LRP-lacking PEA13 cells provides evidence that the LDL receptor pathway contributes to LPL-dependent VLDL degradation, because no other members of the LDL receptor family are expressed by these cells.

In control experiments (data not shown) LPL-stimulated 125I-VLDL degradation was partially inhibited by anti-LDL receptor monoclonal antibody IgG-C7, anti-LPL antibody mAb7, and RAP, but not by nonimmune murine IgG or anti-LRP antibody Rb777. However, Rb777 completely inhibited degradation of 125I-\(\alpha_2\)M*.

LPL-stimulated Degradation of Protein-free Intralipid Emulsion Is Mediated by LDL Receptors—To determine if the ability of LPL to induce 125I-VLDL catabolism via LDL receptors could be due to a direct interaction between LPL and LDL receptors, we studied catabolism of protein-free triglyceride and phospholipid emulsions. In these experiments, neither apoE nor apo B100 were present. Likewise, the activator of LPL, apoc-II, was not present. The emulsion was subjected to ultracentrifugation to isolate particles with S\(_{\text{f}}\) 100–400, which were labeled with 125I-cholesterol oleyl ether, a nondegradable marker of cellular binding or uptake. Results in Fig. 6 show that in FSF cells, basal uptake of the emulsion was low, but LPL promoted the internalization of these particles in a dose-dependent manner, even in the absence of any apolipoproteins. This suggests a direct interaction between LPL and cell surface receptors. FSF cells treated with lovastatin were able to take up almost
lipoprotein lipase (LPL) binds to LDL receptors in solid-phase assays. Microtiter plates coated with increasing concentrations of LDL receptors were used to immobilize LPL. The amount of LPL bound was quantitated using a monoclonal antibody against LPL (mAb7). The presence of LDL receptors, the amount of LPL bound increased with the dose of LPL added; binding of LPL in the absence of LDL receptors was significantly lower.

We investigated the ability of LPL or GST-LPLC to promote binding of **125I**-VLDL to LDL receptors in solid-phase assays (Fig. 9). These assays were done at 4°C, a temperature which inhibits lipolytic activity. In the absence of either protein, **125I**-VLDL bound to LDL receptor-coated wells (Fig. 9A), as shown previously (24). Both LPL and GST-LPLC stimulated the binding of **125I**-VLDL to immobilized LDL receptors (Fig. 9A). Non-specific binding to BSA-coated wells was <5% and has been subtracted from these data. In Fig. 9B, wells coated with anti-LDL receptor antibody IgG-444 were used to immobilize increasing amounts of LDL receptors from DE52 eluates (LDL receptor preparation) of normal fibroblasts. At a fixed concentration of 125I-VLDL, binding was dependent on the amount of LDL receptors added and was enhanced by 2-3-fold in the presence of LPL (Fig. 9B).

**DISCUSSION**

It is well established that LPL promotes the catabolism of triglyceride-rich lipoproteins by a variety of cultured cells. Pre-
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Fig. 9. LPL mediates $^{125}$I-VLDL binding to immobilized LDL receptors at $4^\circ$C. Microtiter wells coated with 30 μg/ml IgG-4A4 were incubated with 100 μl of buffer containing 100 μg (A) or zero to 75 μg of DE52 eluant (B) as described under "Experimental Procedures." Wells were then incubated with buffer containing either 0.2 to 70 nM (A) or 3.5 nM $^{125}$I-VLDL in the absence (closed squares) or presence of 12 μg/ml GST-LPLC (open circles) or 10 μg/ml LPL (closed circles) (B). In A nonspecific binding in the absence of LDL receptors is subtracted for each data point.

Previous studies show that LPL promotes lipoprotein degradation via LRP (6–10). The VLDL receptor and GP330-LRP are implicated in the process as well (10–13). Here we demonstrate that LDL receptors also contribute to the LPL-dependent enhancement of lipoprotein catabolism.

The present data establish that LPL stimulates VLDL catabolism via LDL receptors and binds directly to LDL receptors. LPL induces catabolism of normal VLDL in FSF, FH, HUVECs, MEF, and PEAS cells. Since PEAS cells do not express either LRP, GP330, or VLDL receptors (39, 51), LPL-dependent VLDL catabolism by these cells is probably mediated by LDL receptors. Treatment with lovastatin to up-regulate LDL receptors greatly enhances LPL-stimulated VLDL degradation in all four cell lines possessing an intact LDL receptor pathway. Up-regulation of LDL receptors results in a 2-fold increase in LPL-dependent binding and uptake of protein-free intralipid emulsion by macrophages. GST-LPLC, a fusion protein containing the carboxyl-terminal noncatalytic domain of LPL, also stimulates $^{125}$I-VLDL binding at $4^\circ$C and degradation at $37^\circ$C as was shown previously in studies on LRP (17, 18). However, GST-LPLC was significantly less potent than LPL. This may be explained by the inability of GST-LPLC to undergo dimerization (17) or optimal protein folding. Nykjaer et al. (52) showed that dimeric LPL is much more potent than monomeric LPL at stimulating $^{125}$I-VLDL binding to immobilized LRP. The effect of LPL appears to depend on the ratio of VLDL to LPL. Thus, increasing concentrations of LPL to $30\;\text{nm}$ or GST-LPLC to $700\;\text{nm}$ induces degradation of 3.5 nm VLDL in a dose-dependent manner. At higher concentrations, when LPL and GST-LPLC are present in even larger molar excess over VLDL, they behave as competitive inhibitors of LDL receptor-mediated catabolism (data not shown) as observed previously in studies of LRP (18).

Despite the fact that lipolysis is not required for LPL-dependent VLDL catabolism, it clearly enhances catabolism. Lipolysis may expose or activate the receptor-recognition domains of apoE or apoB100 and contribute to LPL's effect (53–57). This is consistent with the finding that the lipid component of lipoprotein particles may modulate the reactivity of the apoprotein component (58). Also, Takahashi et al. (12) showed that the structurally unrelated Pseudomonas lipase can enhance VLDL catabolism via the VLDL receptor. Thus, GST-LPLC's lack of catalytic activity may be partly responsible for its relatively lower potency than LPL to the stimulate VLDL catabolism at $37^\circ$C. In our study, internalization of protein-free emulsion increased only 2-fold following up-regulation of LDL receptors in FSF cells, whereas under similar conditions VLDL catabolism was increased up to 5-fold. Thus, the magnitude of LPL-induced catabolism of the emulsion is less than that obtained with native VLDL. This suggests that the effect of LPL on VLDL catabolism is only partially dependent on direct interaction of LPL with the receptor.

Our studies indicate that the affinity of LPL for LDL receptors is lower than its affinity for LRP, GP330-LRP-2, or VLDL receptors. The $K_D$ values reported for these receptors are, respectively, 34, 6, and 1 nm (11, 12, 17). However, due to the low affinity of LPL for LDL receptors as compared with its high nonspecific binding, we could not accurately determine its affinity in solid-phase or cell surface assays. Also, we were unable to demonstrate LPL binding to LDL receptors by ligand blotting due to high nonspecific binding of LPL to the membrane support at the concentrations of LPL required.

Our studies indicate a major role for LDL receptors as compared with LRP in LPL-dependent VLDL catabolism. By comparing FSF and MEF cells, which express both LDL receptors and LRP, to their mutant counterparts that lack either LDL receptors or LRP, we were able to demonstrate that LDL receptors contribute to the majority of LPL-dependent VLDL catabolism. Only when LDL receptors were not up-regulated could a significant contribution of LRP to LPL-dependent VLDL catabolism be demonstrated. However, this may reflect the situation in vivo where the LDL receptor pathway is usually down-regulated (59).

Although we studied VLDL, it is likely that these observations apply to chylomicron catabolism as well, because the remnants of large VLDL and chylomicrons appear to share the same catabolic pathway (60). The relative contributions of LDL receptors and LRP to remnant catabolism in vivo are not negligible. Moreover, LPL promotes the internalization of protein-free intralipid emulsions that are devoid of apoC-II, an essential co-factor for LPL's lipolytic activity. Rumsey et al. (21) also showed that LPL enhances the internalization of protein-free triglyceride emulsion particles by macrophages. GST-LPLC, a fusion protein containing the carboxyl-terminal noncatalytic domain of LPL, also stimulates $^{125}$I-VLDL binding at $4^\circ$C and degradation at $37^\circ$C as was shown previously in studies on LRP (17, 18). However, GST-LPLC was significantly less potent than LPL. This may be explained by the inability of GST-LPLC to undergo dimerization (17) or optimal protein folding. Nykjaer et al. (52) showed that dimeric LPL is much more potent than monomeric LPL at stimulating $^{125}$I-VLDL binding to immobilized LRP. The effect of LPL appears to depend on the ratio of VLDL to LPL.
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