Dissociation of LPL and LDL: effects of lipoproteins and anti-apoB antibodies

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Abstract We have shown previously that the activity of lipoprotein lipase (LPL), the major enzyme responsible for hydrolysis of triglyceride contained in circulating lipoproteins, is associated with lipoproteins in postheparin plasma. In other studies, microtiter plate assays showed that LPL interaction with low density lipoprotein (LDL) and very low density lipoprotein (VLDL) was decreased by antibodies to apolipoprotein (apo)B. To test whether antibodies to apoB affected LPL–LDL association in solution, two types of assays were performed, gel filtration and coprecipitation. First we showed that LPL activity and immunoreactive mass co-eluted during gel filtration of normal postheparin plasma, approximately with the peak of low density lipoproteins. Then LPL was used for gel filtration studies in the presence and absence of LDL and anti-apoB monoclonal antibodies. LPL association with LDL was diminished by antibodies to the amino-terminal region of apoB; antibodies to the carboxyl-terminal LDL receptor binding region of apoB were less effective. LDL binding to LPL containing heparin-agarose was also disrupted by the amino-terminal antibodies to apoB. To determine the LPL–lipoprotein association in situations in which the distribution of plasma lipoproteins was altered, we studied plasma from two types of subjects with dyslipidemias. The addition of ^{125}I-labeled LPL to type 1 postheparin plasma produced two peaks of radioactivity, one peak eluted in the void volume of the column (with the chylomicrons) and a second peak eluted just prior to the normal elution of low density lipoproteins. In postheparin plasma from an abetalipoproteinemic subject, LPL eluted with HDL. We conclude that LPL associates primarily with apoB-containing lipoproteins. The reason for this appears to be that LPL interacts with the apoB.—**Choi, S. Y., L. Pang, P. A. Kern, H. J. Kayden, L. K. Curtiss, T. M. Vanni-Reyes, and I. J. Goldberg.** Dissociation of LPL and LDL: effects of lipoproteins and anti-apoB antibodies. *J. Lipid Res.* 1997. **38:** 77–85.

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Since the 1940s it has been known that the injection of heparin into the circulation of humans and animals leads to hydrolysis of triglyceride in circulating chylomicrons via release of lipoprotein lipase (LPL) into the bloodstream (1). A severe deficiency in this activity results from the genetic disorder type 1 hyperlipoproteinemia, characterized by fasting chylomicronemia and episodes of pancreatitis (2). A second lipolytic enzyme, hepatic triglyceride lipase (HTGL), is also released into the circulation by heparin and is responsible for a large percentage of the neutral triglyceride lipolytic activity in postheparin plasma from normal humans (2, 3). This enzyme will hydrolyze triglyceride and phospholipid in a number of lipoproteins including small very low density lipoproteins (VLDL), low density lipoproteins (LDL), and high density lipoproteins (HDL) (4). HTGL deficiency in humans in associated with hypertriglyceridemia due to an increase in the plasma levels of small VLDL, intermediate density lipoproteins (IDL), and HDL (5).

In addition to its roles as a lipase, LPL anchors lipoproteins to cells and matrix (6). Because this enzyme interacts with both heparan sulfate proteoglycans (HSPG) and lipoproteins, it can form a bridge between these two molecules. LPL is also a ligand for the LDL receptor related protein (7–10). Therefore, via this receptor and via direct internalization of cell surface HSPG (11) LPL promotes cellular uptake of lipoproteins.

Previous studies from our laboratory demonstrated the association of both LPL and HTGL activities with

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Abbreviations: apo, apolipoprotein; LPL, lipoprotein lipase; LDL, low density lipoprotein; VLDL, very low density lipoprotein; HDL, high density lipoprotein; IDL, intermediate density lipoprotein; HTGL, hepatic triglyceride lipase; HSPG, heparan sulfate proteoglycans; ELISA, enzyme-linked immunosorbent assay; TBS, Tris-buffered saline; BSA, bovine serum albumin.

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lipoproteins in postheparin plasma (12). After gel filtration, LPL activity eluted with lipoproteins that contained apolipoproteins (apo) B and E and that were slightly larger than LDL. In the presence of hypertriglyceridemia, some LPL was also found in the void volume of the column; thus, this LPL co-eluted with large VLDL and chylomicrons. Because of the cellular studies showing that LPL augmented lipoprotein uptake and the possibility that LPL could serve as a ligand for the uptake of lipoproteins in the circulation, a renewed interest in LPL–lipoprotein interaction has occurred and, as initially proposed by Felts, Itakura, and Crane (13), the hypothesis that plasma LPL serves as the ligand for receptor-mediated lipoprotein degradation is again in vogue (14).

Recently, we made the unexpected observation that a protein with homology to the amino-terminal region of apoB was on the surface of cultured endothelial cells and was able to bind LPL on ligand blots (15). This observation and other data showing that LPL preferentially binds to VLDL and LDL rather than HDL (16) suggested that the protein component of lipoproteins modulates LPL–lipoprotein interaction. We now report studies using anti-apoB monoclonal antibodies to interrupt the interaction of LPL and LDL in solution. These data further support our hypothesis that the amino-terminal region of apoB facilitates LPL–LDL interactions in vivo. This interaction could mediate the retention of LDL in LPL-containing regions of atherosclerotic vessels.

METHODS

Human postheparin plasma

Blood was obtained from normal subjects and patients with lipoprotein abnormalities 15 min after an intravenous infusion of 60 units per kg body weight of heparin (Riker Lab, Inc., Northridge, CA) using a protocol approved by the Institutional Review Board of Columbia-Presbyterian Medical Center. The blood was placed on ice and the plasma was separated from the cells by centrifugation. The sample was frozen within 60 min and stored at −70°C until assayed.

Measurement of cholesterol

Cholesterol was determined by a specific enzymatic method using an ABA-100 analyzer (Abbott Laboratories, Chicago, IL).

Enzymatic assays of postheparin plasma

LPL and HTGL activities were measured using the emulsion described by Nilsson-Ehle and Schotz (17). Measurements of LPL activity were obtained by using a specific antiserum to HTGL (18), and HTGL activity was measured after inhibition of LPL activity by using an emulsion containing 1.25 M NaCl.

LPL immunoreactive mass measurements

Immunoreactive LPL protein was measured by a double antibody sandwich enzyme-linked immunosorbant assay (ELISA) using immunoaffinity-purified chicken anti-bovine milk LPL antibodies. This assay has previously been reported (19). In brief, affinity-purified anti-bovine LPL antibodies were bound to microtiter wells, and sample or bovine milk LPL standards were then added in buffer containing 1.0 M NaCl, 0.01% Triton X-100, 0.1% bovine serum albumin (BSA), and protease inhibitors (1 mM phenylmethylsulfonyl fluoride, 1 mM benzamidine, 1 mM EDTA, and 0.05 mM aprotinin). The wells were washed, and biotinylated affinity-purified antibody was added, followed by addition of streptavidin-peroxidase (Bethesda Research Labs, Bethesda, MD). The peroxidase reaction was developed and read in an ELISA plate reader.

Gel filtration chromatography of postheparin plasma

Postheparin plasma was defrosted and 2 ml was used for gel filtration chromatography on a 1 × 100 cm column containing 6% agarose (A5M, Bio-Rad, Rockville Center, NY) in 0.15 M NaCl, 0.01 M sodium phosphate, 0.01% ethylenediaminetetraacetate, pH 7.4 buffer (PBS). The void and elution volumes of LDL and HDL cholesterol and albumin were determined for each column as described previously (12). Studies were performed using postheparin plasma from patients with type 1 hyperlipoproteinemia (N = 2) and a patient with abetalipoproteinemia. To determine the elution of LPL in postheparin plasma from the subjects with type 1 hyperlipoproteinemia, ^{125}I-labeled bovine milk LPL, radioiodinated using lactoperoxidase and glucose oxidase as described previously (17), was added to the postheparin plasma prior to gel filtration. The radioactivity in the column fractions obtained after gel filtration of the plasma was determined. Approximately 85% of the added radioactivity was recovered from the column.

Gel filtration studies of LPL and LDL

Ten µg of purified bovine LPL and/or 0.2 mg LDL in 0.5 ml of buffer (PBS–0.3% BSA) was applied to a 1.5 × 42 cm column containing 6% agarose. To prevent aggregation which occurred when LPL was gel-filtered alone without added lipoproteins, 2 units/ml of heparin was added to the LPL. In some studies, monoclonal antibodies against apoB were added to the LPL and LDL prior to gel filtration. Antibodies used included MB47, directed against the LDL receptor binding region, and MB3 directed against domains within the amino terminal 1/3 of the apoB molecule (20).

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Coprecipitation of LDL with LPL

Interaction between ^{125}I-labeled LDL and LPL was assessed in solution using heparin-agarose (Bio-Rad Laboratories, Hercules, CA) saturated with LPL. In these experiments 0.1 ml of heparin agarose was incubated with 1 ml (approximately 400 μg) of purified bovine LPL overnight at 4°C on a rocker. The unbound LPL was then removed and the gel was washed three times with Tris-buffered saline (TBS; 150 mM NaCl with 10 mM Tris, pH 7.4). Human LDL was isolated and iodinated using iodogen (21). The radiolabeled LDL had a specific activity of 250 cpm/ng. One μg of ^{125}I-labeled LDL was incubated with 10 μl of control or LPL-bound heparin gel in 140 μl of TBS containing 3% BSA. Fifty μg of unlabeled LDL was added in some experiments to correct for the nonspecific or background binding. After 3 h incubation at 4°C the gel was precipitated by centrifugation for 1 min at 6000 rpm and the pellet was washed 5 times with TBS. All the experimental procedures were carried out at 4°C. The ^{125}I-labeled LDL precipitated with LPL was then counted in a gamma counter. Experiments were carried out in triplicate.

The role of anti-apoB antibodies was then assessed in the coprecipitation experiments. To do this, 50 μl of monoclonal anti-apoB antibodies (MB3, MB19 and MB47, ascites) was used to determine whether blocking regions of apoB affected the ability of LPL to co-precipitate ^{125}I-labeled LDL.

For calculations of the affinity of LPL–LDL interaction, 0–900 μM of radiolabeled LDL in 50 μl of TBS containing 3% BSA was incubated with 50 μl of LPL-saturated heparin agarose gel. The experimental protocol used in this experiment was identical to the antibody experiment described above. Nonspecific binding was corrected by adding 50-fold excess amount of unlabeled LDL. Data were analyzed by Scatchard analysis and the K_d was determined using the LIGAND software program (22).

RESULTS

Chromatographic characterization of LPL activity and protein from normal subjects

In our previous report (12) we questioned whether the elution of LPL protein might be different from that of LPL activity during gel filtration. Such a finding would occur if an inactive, non-lipoprotein binding form of LPL existed in postheparin plasma. To answer this question, fractions obtained during gel filtration of postheparin plasma from a normal subject were assayed for LPL mass using an ELISA. As shown in **Fig. 1,** LPL immunoreactive protein and LPL enzymatic activity

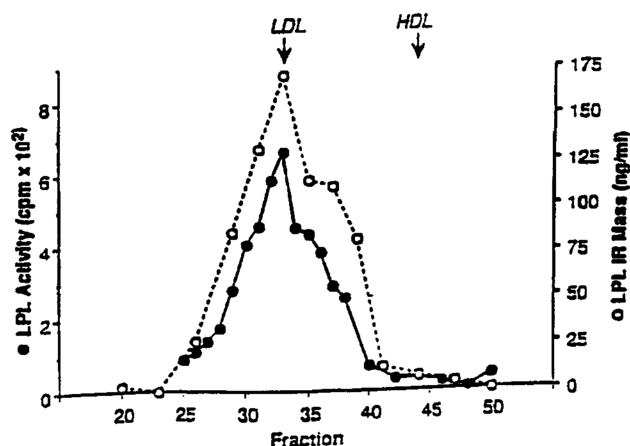

Fig. 1. Coelution of LPL protein and activity during gel filtration. LPL immunoreactive (IR) mass elution during gel filtration of normal postheparin plasma. Postheparin plasma (2 ml) was chromatographed on 6% agarose, and LPL activity and LPL immunoreactive mass by ELISA were determined on the column fractions as described in Methods.

eluted coincidentally during this study. Therefore, all the LPL activity and LPL protein recognized by a polyclonal antibody appeared to be associated with lipoproteins. A similar observation using a different type of LPL immunoassay has been reported by others (23).

Effects of monoclonal anti-apoB antibodies on LPL association with LDL

We next assessed whether monoclonal antibodies to apoB would affect the association of LPL and LDL. To do this, LPL in buffer containing 2 units/ml of heparin was incubated for 2 h at 4°C in the presence or absence of LDL and then gel-filtered. Shown in **Fig. 2A** are results of an experiment in which LPL was gel-filtered in the presence of LDL. Most LPL associated with the added LDL and co-eluted with the added lipoproteins. Because in the presence of MB3 the LPL eluted in a narrow peak, the amount of LPL activity in the column fractions was greater than in the gel filtrations (A and C) in which a more diffuse elution occurred. Addition of MB3 markedly decreased the association between LPL and LDL (Fig. 2B); MB47 addition had less effect (Fig. 2C). Therefore, the chromatographic association of LPL and LDL was disrupted by a monoclonal antibody to an amino-terminal domain of apoB.

Coprecipitation of LDL with LPL

To further confirm the interaction of LDL with LPL in solution, [125]-labeled LDL was precipitated with control or LPL-saturated heparin agarose and the amount of radioactivity associated with the gel was measured. In the absence of LPL, only 2.5% of the [125]-labeled LDL bound to the heparin gel. Thus, under the conditions

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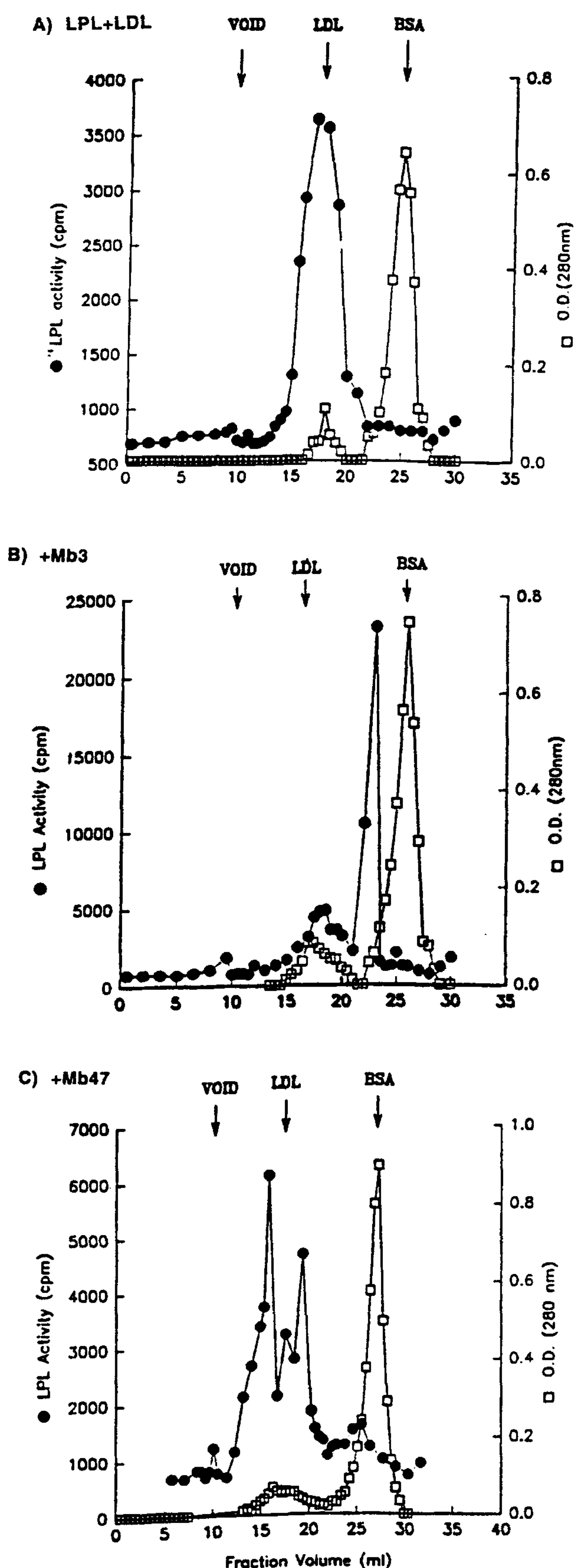

Fig. 2. Effect of anti-apoB monoclonal antibodies on gel filtration of LPL with LDL. Purified LPL (10 μg) was incubated in the presence or absence of 0.2 mg of LDL in PBS containing 3% BSA and 2 units/ml of heparin for 2 h at 4°C and gel-filtered on a 1.5 × 42 cm column (A5m, Bio-Rad) containing 6% agarose. LPL activity was assayed as described in Methods. The O.D. readings at 280 nm for each column fraction shows the elution of LDL and BSA. Panel A shows LDL and LPL; panel B shows the same experiment as panel A but with addition of monoclonal antibody MB3; and panel C represents the addition of MB47.

of this experiment (i.e., in 150 mM NaCl) very little LDL associated with heparin. Only in the presence of LPL did a significant amount of LDL associate with the heparin-containing gel. When the agarose contained LPL, the amount of ^{125}I-labeled LDL bound to the gel increased by approximately 11-fold (**Fig. 3A**). Nonspecific binding of ^{125}I-labeled LDL to heparin gel was determined by adding excess unlabeled LDL (50 μg) and was subtracted from the total binding.

We then studied whether antibodies to apoB would alter LDL association with LPL. As shown in Fig. 3B, both MB3 and MB19 blocked the association of LDL with LPL by approximately 70%, whereas MB47 produced a much smaller effect. In other experiments this amount of MB47 completely blocked LDL binding to the LDL receptor (11), suggesting that sufficient antibodies were present to interact with each LDL. These data demonstrate that the association of LPL with LDL is inhibited by antibodies that react with the amino-terminal region of apoB.

Scatchard analysis of LPL–LDL interaction

We performed the kinetic analysis of LDL binding to LPL in solution. Data shown in **Fig. 4** were obtained using a wide range of LDL concentrations (i.e., 0–900 nM). The data are expressed in terms of nanomoles of ^{125}-labeled LDL bound to 1 ml of heparin agarose gel saturated with LPL. At each concentration of ^{125}I-labeled LDL, nonspecific binding to heparan agarose gel was minimal (<10%) and subtracted. As shown in panel A, at low concentrations ^{125}I-labeled LDL bound to LPL-saturated gel with a high affinity (K_d = 3.76 nM, B_{max} = 8.52 × 10^{-3} nmol/ml gel). The rate of increase of additional LDL binding to LPL above this concentration was much less steep as shown in panel B with an average K_d of 236.97 nM and B_{max} of 121 × 10^{-3} nmol/ml gel. The present data agree with our previous report (16) in which we observed both low and high affinity processes in a solid phase assay.

Gel filtration of LPL in type 1 postheparin plasma

LPL activity in normal human postheparin plasma elutes just prior to the peak of LDL cholesterol (12). In that initial report and in subsequent studies by others

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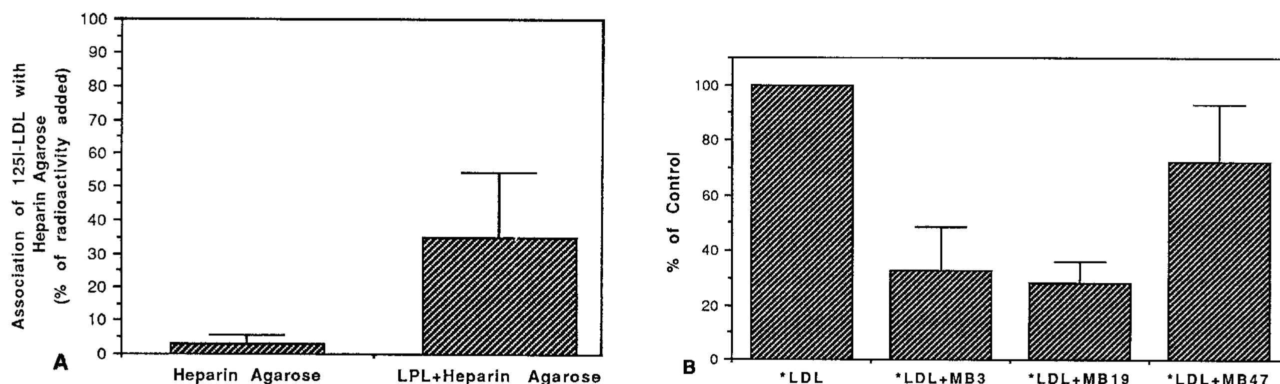

Fig. 3. Coprecipitation of ^{125}I-labeled LDL with LPL. The percentage of 1 μg of ^{125}I-labeled LDL that was precipitated with 10 μl of control or LPL-saturated heparin agarose gel as described under Methods is shown in panel A. In panel B precipitation was performed in the presence of monoclonal anti-apoB antibodies (MB3, MB19, and MB47, 50 μl of ascites) to inhibit the binding of ^{125}I-labeled LDL to LPL-saturated heparin agarose. Data are expressed as mean ± SD (n = 3).

(23) LPL association with other lipoproteins has also been noted. To determine whether very large numbers of triglyceride-containing lipoproteins compete for LPL binding to LDL size particles, we assessed whether LPL added to postheparin plasma of a type 1 subject would elute only with chylomicrons. When ^{125}I-labeled LPL was added to postheparin plasma from a type 1 subject, it eluted in two major peaks **(Fig. 5A).** We reported a similar pattern using postheparin plasma from subjects with type 5 hyperlipoproteinemia (12). The first peak of ^{125}I-labeled LPL found in the void volume probably represents LPL bound to chylomicrons or large chylomicron remnants formed by the addition of LPL to the type 1 plasma. The second peak of LPL eluted at a position similar to that found in normal subjects, overlapping the LDL peak. Although it may be that this second peak of LPL activity was due to hydrolysis of the larger triglyceride-rich lipoproteins into remnants, the LPL did not then transfer to the larger, more nascent lipoproteins. When the amount of recovered radioactivity in each peak was summed, 42% of the radioactivity eluted with the chylomicrons and 44% with the LDL (note that the LDL elution was broader). Thus, under these conditions the LDL did not have a markedly greater affinity for the triglyceride-rich lipoproteins.

The elution of HTGL activity after gel filtration of type 1 postheparin plasma is shown in Fig. 5B. HTGL activity was recovered between the peaks of LDL and HDL cholesterol and overlapped the elution of smaller LDL. This position was similar to that found in normal subjects.

Postheparin lipases elute with HDL in abetalipoproteinemia plasma

Although most of the LPL in postheparin plasma is found on apoB-containing lipoproteins, in occasional patients some LPL has been noted to co-elute with HDL (23). To determine whether in the absence of apoB LPL would associate with HDL-size particles, we assessed the elution of LPL activity in postheparin plasma from a subject with abetalipoproteinemia. As shown in **Fig. 6A** all LPL activity in postheparin plasma from the abetalipoproteinemic subject eluted at a later position and overlapped the elution volume of HDL. HTGL also eluted in the same position (Fig. 6B). This suggests that in the absence of apoB-containing lipoproteins, plasma LPL and HTGL activities are associated with HDL-sized lipoproteins.

DISCUSSION

LPL association with LDL appears to involve apoB–LPL interactions. This had been shown using ligand blots and microtiter plate assays (16). We now provide data from gel filtration and co-precipitation experiments that support the hypothesis that apoB on LDL interacts with LPL in solution. In both types of experiments, monoclonal antibodies to the amino-terminal region of apoB diminished the LPL–LDL interaction. MB47 did not have a similar effect. Thus, the amino-terminal region of apoB appears to mediate the association of LPL with LDL. Although the two antibodies against the amino-terminal region interact with epitopes that are not adjacent, the observation that they both block LPL–LDL interaction is not surprising. Rather than defining the exact region on apoB that interacts with LPL, it is much more likely that the IgG sterically blocks a large area of the globular amino-terminal region of apoB. For this reason, it is unlikely that use of additional monoclonal antibodies to this region

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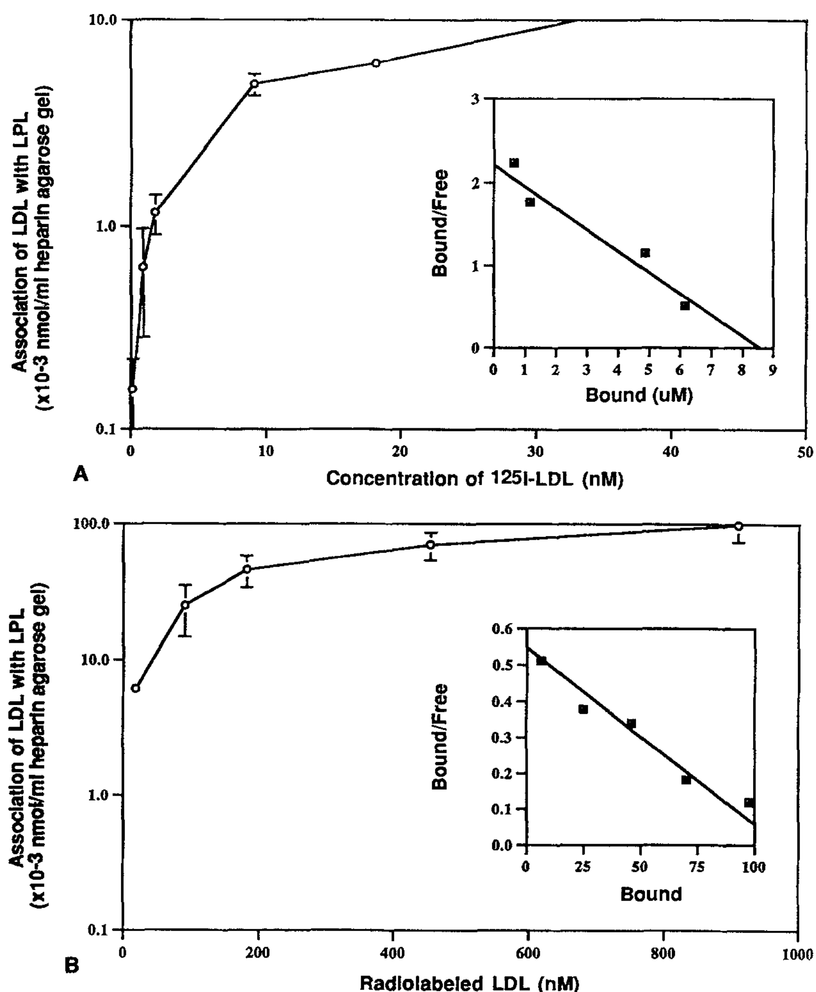

Fig. 4. ^{125}I-labeled LDL binding to LPL-saturated heparan agarose gel. The protocol used in this experiment was identical to the one described in the legend to Fig. 3 except that ^{125}I-labeled LDL at 0–900 μM was incubated with 50 μl of LPL-saturated heparan agarose gel. Binding to control gels was subtracted from the total binding. Panel A shows the ^{125}I-labeled LDL–LPL binding at lower concentrations of [125]-labeled LDL (0–500 nM). Panel B shows the binding at higher concentrations of ^{125}I-labeled LDL (50–900 nM). Data are expressed as mean + SD (n = 3). Insets: kinetic analysis of the data using Scatchard plot analysis.

will more exactly define the LPL binding motif on apoB. In contrast, the LDL receptor binding region of apoB must be sufficiently distant from the LPL binding region that even addition of a large immunoglobulin molecule does not block the LPL interaction with apoB.

The exposure of the amino-terminal region of apoB varies in different lipoprotein classes and is affected by the composition of the lipoproteins. Digestion of apoB with *Staphylococcus V8* protease leads to the production of a 120 kDa amino-terminal apoB fragment (24, 25). ApoB contained in LDL are more accessible to this protease than apoB in VLDL. In addition, smaller hypertriglyceridemic LDL are more susceptible to this proteolytic cleavage than are larger LDL (26). The amino terminal region of apoB has 6 disulfide bonds and is thought to extend away from the surface of the lipoprotein (26). The function of this apoB region, which is a component of both apoB-100 and apoB-48, is unknown. Several lines of data (15, 16) including those in this report support the hypothesis that this region is involved in interaction between LDL and LPL. However, the exact epitope responsible for this has not yet been determined.

Others have recently studied the interaction of LPL with lipoproteins. Connelly et al. (27) reported that LDL could act as a competitive inhibitor of LPL-mediated lipolysis. These data are consistent with LPL binding to LDL in solution. In a study similar to that reported from our laboratory, van Barlingen et al. (28) also found that LPL bound as well to LDL as VLDL when this interaction was studied using microtiter plate assays. However, in addition to lipoproteins, LPL will

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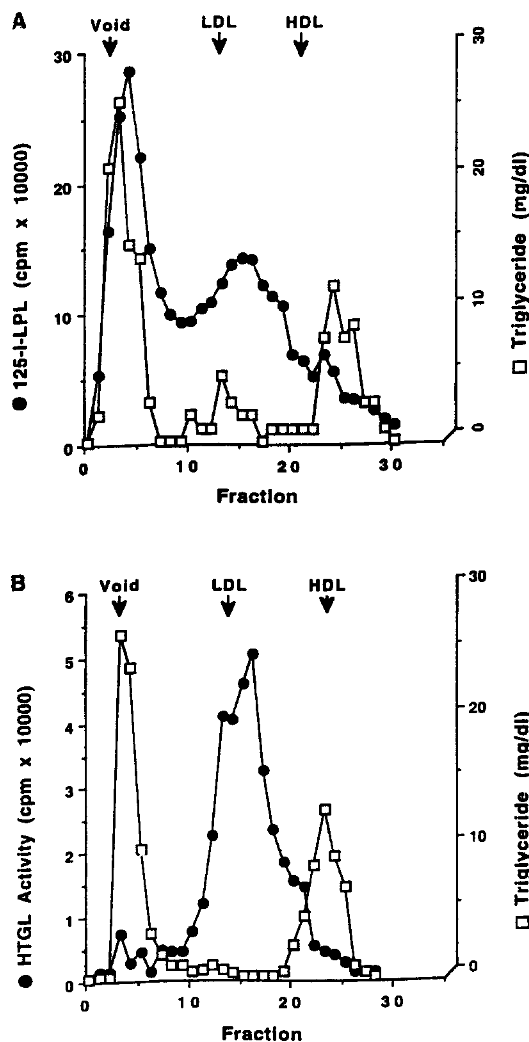

Fig. 5. LPL elution during gel filtration of postheparin plasma from a subject with LPL deficiency. A: LPL: ^{125}I-labeled bovine milk LPL (3.6 μg, 1 × 10^{6} cpm/μg) was added to 2 ml of plasma prior to gel filtration on 6% agarose as described in Methods and the ^{125}I radioactivity was measured in the column fractions. The void and elution volumes of LDL and HDL were determined for each individual column. B: HTGL: In panel B is shown the elution of HTGL after gel filtration of a postheparin plasma sample from the same subject. HTGL activity was measured in triplicate in each column.

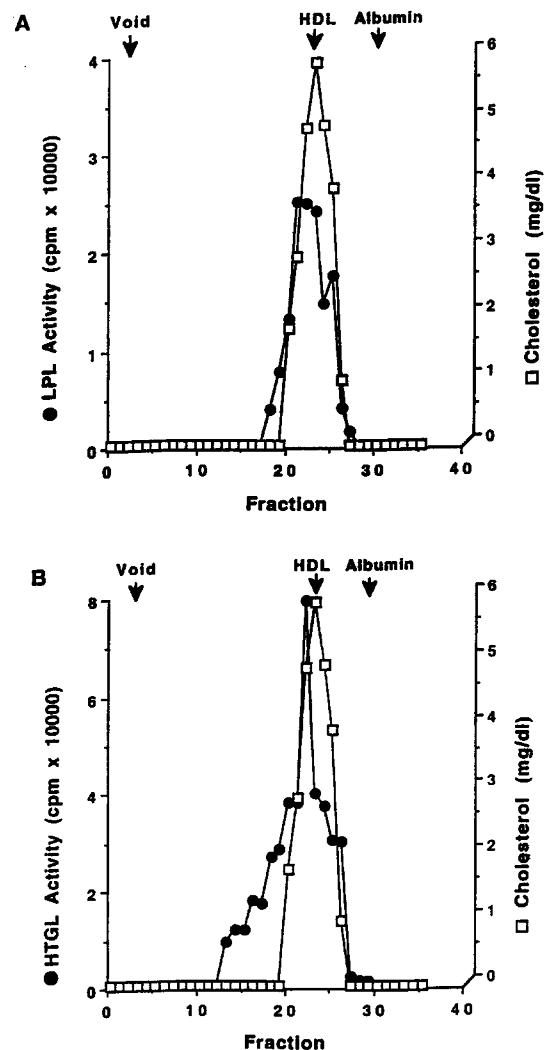

Fig. 6. LPL elution during gel filtration of postheparin plasma from a subject with abetalipoproteinemia. Two ml of postheparin plasma was gel-filtered on 6% agarose as described in Methods and LPL (panel A) and HTGL (panel B) activities were determined in 0.1-ml aliquots of each fraction (data shown are the average of triplicate determinations). The void and elution volumes of LDL and HDL were determined for each individual column.

also associate with lipid emulsion particles and this interaction likely is via association with cell surface phospholipids (29, 30).

In our studies and those recently reported by Carrero et al. (30), a method was required to gel-filter LPL without its aggregation. We used a small amount of heparin to do this. The other investigators mixed the LPL and lipoproteins with a Triton-containing buffer. Both of these methods could potentially affect the LPL–lipoprotein association in a nonphysiologic manner. While one might argue that LPL–heparin interaction reproduces the "normal" endothelial bound LPL-lipoprotein conditions, this is probably not entirely correct. Similarly, Triton alters LPL interaction with lipid emulsions and in low concentrations will enhance in vitro lipolysis while greater concentrations will block LPL actions in vitro and in vivo.

For these reasons, the best assessment of the specificity of LPL–lipoprotein interaction may be its association with lipoproteins in plasma. Unfortunately, this too has its possible artifacts stemming either from the introduction of heparin to form postheparin plasma or from the in vivo or in vitro (31) generation of lipolysis products. Nonetheless, the two studies of LPL elution in plasma

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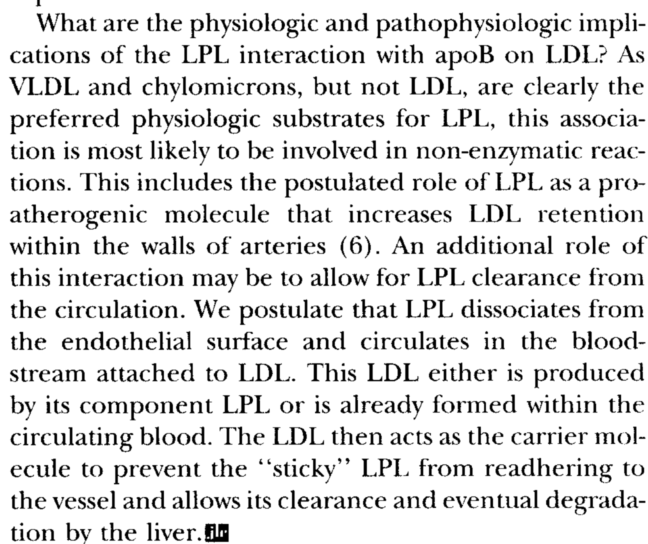
of dyslipoproteinemic subjects are instructive. In the case of the type 1 plasma, the elution of LPL with lipoproteins that are much smaller than chylomicrons demonstrates that the affinity of LPL for the larger particles is not so much greater than for smaller particles. If it were, the LPL were be expected, in vitro, to exchange from the smaller to the larger particles. The study in the abetalipoproteinemic plasma shows that when no apoB-containing lipoproteins are present, LPL will associate with the low affinity HDL. This, we presume, is similar to its association with phospholipid emulsions.

A number of investigators have reported that LPL significantly enhanced the uptake of LDL in cell culture experiments (reviewed in ref. 6). Mulder et al. (32) reported that both the number of binding sites and the affinity of the binding, based on binding curves and Scatchard analysis, in HepG2 cells are increased 20- to 30-fold by the addition of 3.4 μg/ml LPL. Further, the K_d of 3.76 nM obtained from our solution assay is in close agreement with the estimated K_d of approximately 2 nM (this calculation was based on molecular weight of 550,000 for apoB-100 of LDL). van Driel, Brown, and Goldstein (33) previously reported that in the solid phase assay ^{125}I-labeled LDL bound to LDL receptor with a K_d of 1.6 nM. Thus, together these data suggest that LDL appears to have a higher affinity for LDL receptors than for LPL.



What are the physiologic and pathophysiologic implications of the LPL interaction with apoB on LDL? As VLDL and chylomicrons, but not LDL, are clearly the preferred physiologic substrates for LPL, this association is most likely to be involved in non-enzymatic reactions. This includes the postulated role of LPL as a proatherogenic molecule that increases LDL retention within the walls of arteries (6). An additional role of this interaction may be to allow for LPL clearance from the circulation. We postulate that LPL dissociates from the endothelial surface and circulates in the bloodstream attached to LDL. This LDL either is produced by its component LPL or is already formed within the circulating blood. The LDL then acts as the carrier molecule to prevent the "sticky" LPL from readhering to the vessel and allows its clearance and eventual degradation by the liver.

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