Lipoprotein lipase in human plasma is mainly inactive and associated with cholesterol-rich lipoproteins

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Abstract This study was designed to further ascertain the presence in plasma of lipoprotein lipase (LPL) bound to circulating lipoproteins. Lipoprotein lipase mass and activity values in preheparin plasma from 20 volunteers were 69.8 ± 6.6 ng• ml^{-1} and 1.54 ± 0.15 mU• ml^{-1}, respectively, and no significant correlation between mass and activity was observed. Fifteen min after heparin injection, LPL mass had increased to 536 ± 60 ng• ml^{-1} and LPL activity to 261 ± 34 mU• ml^{-1} and a highly significant correlation between the increments in mass and activity was observed. The released material had a specific activity of 0.57 ± 0.03 mU• ng^{-1}. The LPL mass in preheparin plasma eluted early from heparin-Sepharose, in the position expected for inactive LPL monomers. Western blot analysis showed that the eluted material had the size expected for the LPL subunit (55 kDa). The increment of mass and activity after heparin eluted later from heparin-Sepharose, in the position expected for active LPL dimers. It is concluded that preheparin plasma contains substantial amounts of inactive LPL protein, and that heparin releases mainly active LPL into circulation. On gel filtration LPL activity and mass in postheparin plasma eluted mainly in the positions of LDL and HDL. Electron microscopy of immunostained fractions showed reaction for LPL and apolipoprotein B, or apolipoprotein A-I, on the same particles. LPL mass in preheparin plasma eluted in a similar pattern, associated with LDL and HDL. In postprandial plasma substantial amounts of LPL protein eluted with the triglyceride-rich lipoproteins. When ^{125}I-labeled bovine LPL was added to plasma or to ultracentrifugally isolated lipoproteins and then analyzed by gradient gel electrophoresis, the labeled lipase moved with the lipoproteins. The presence of substantial amounts of inactive LPL protein associated with lipoproteins in plasma may have important implications for the metabolism of the particles in view of recent reports on avid binding of LPL-lipoprotein complexes to cell surfaces and receptors.—**Vilella, E., J. Joven, M. Fernández, S. Vilaró, J. D. Brunzell, T. Olivecrona, and G. Bengtsson-Olivecrona.** Lipoprotein lipase in human plasma is mainly inactive and associated with cholesterol-rich lipoproteins. *J. Lipid Res.* 1993. **34:** 1555–1564.

Supplementary key words apolipoprotein • heparin-Sepharose • hepatic lipase • gel filtration • gradient gel electrophoresis • electron microscopy

Lipoprotein lipase (LPL) is an enzyme that hydrolyzes triglycerides in plasma lipoproteins at the endothelial "binding-lipolysis" sites where the enzyme is noncovalently attached to heparan sulfate chains (1, 2). The active enzyme is a noncovalent homodimer which, when converted to the monomeric form, loses its catalytic activity (3, 4). Some recent studies in animals indicate that there is a continuous dissociation of the enzyme from the endothelium to the blood (5, 6); the enzyme is carried to the liver where it is avidly taken up and degraded (7, 8). The presence of circulating LPL in human plasma has been already noted (9–13), measuring protein mass rather than activity, but the distribution in plasma has been scarcely studied. Goldberg et al. (14) reported that on gel filtration of postheparin plasma the major peak of LPL activity eluted after the triglyceride-rich lipoproteins and just before the peak of low density lipoproteins (LDL). With postprandial plasma an additional peak of LPL activity eluted in the void volume of the column. They concluded that LPL was probably bound to remnants of chylomicrons and very low density lipoproteins (VLDL). Conversely, some of us (EV and JJ) (9) used an ELISA which detects the active, dimeric form of the enzyme, and found the LPL mass probably bound to cholesterol-rich lipoproteins.

Abbreviations: apo, apolipoprotein; BSA, bovine serum albumin; ELISA, enzyme-linked immunosorbent assay; HDL, high density lipoprotein; IDL, intermediate density lipoprotein; LDL, low density lipoprotein; Lp[a], lipoprotein[a]; LPL, lipoprotein lipase; bLPL, bovine lipoprotein lipase, hLPL, human lipoprotein lipase; MAb, monoclonal antibody; PBS, phosphate-buffered saline; SDS, sodium dodecylsulfate; VLDL, very low density lipoprotein.

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The primary purpose of the present study was to confirm these data, to explore and quantitate the presence of active and inactive material in normal individuals, and to study its distribution in plasma.

MATERIALS AND METHODS

Collection of blood and plasma handling

Normolipemic volunteers were recruited to obtain citrated pre- and postheparin blood (60 IU heparin • kg^{-1} body weight, 15 min). Pre- and postheparin blood samples were also obtained in EDTA-containing tubes from normo- and hyperlipemic (Type IV) subjects after 10–14 h fasting or after a meal. Blood was kept at 4°C, centrifuged, and the plasma was immediately frozen (−80°C) when analyses were not performed on the same day.

Purification and labeling of lipases

Bovine LPL (bLPL) was purified from milk as described (15). Protein concentration of the preparation was calculated from the absorbance at 280 nm and the absorption coefficient ($A_{1\%}$) of 16.8 cm^{-1}. Human LPL (hLPL) was purified from postheparin plasma by adsorption to heparin-Sepharose. The preparation was treated with rabbit antibodies against human hepatic lipase (16) before gradient elution from a second column of heparin-Sepharose. The LPL content of the human preparation was calculated from activity data assuming a specific activity of 600 U • mg^{-1}. Bovine LPL was iodinated using the lactoperoxidase and glucose oxidase method and was repurified on heparin-Sepharose as described (7). The lipase was stored in 10 mM Tris-HCl (pH 7.4), 20% (vol/vol) glycerol, ~1 M NaCl, 2 mg • ml^{-1} of bovine serum albumin (BSA). The labeled lipase preparations contained ~1 ng of protein per μl and had a specific radioactivity of 10–30 × 10^3 cpm • ng^{-1}.

Antibodies

Female chickens were immunized with bLPL as previously described (17). Immunoglobulins were isolated from egg yolks by precipitation (18) followed by chromatography on DEAE-Affi Gel Blue (Pharmacia LKB Biotechnology, Sweden) (19). They were then affinity-purified using a bLPL-Sepharose column (20). The antibodies were eluted with 0.2 M glycine, pH 2.7, then immediately dialyzed against 10 mM Tris-HCl, pH 7.4, and stored frozen in this buffer at a concentration of about 0.5 mg • ml^{-1}. The polyclonal chicken antibodies used in this study were called P66. Features for the 5D2 monoclonal antibody (MAb) used were previously reported (13, 21). Conjugation of antibodies with peroxidase was done with the periodate method (22). Gold-coupled protein A and rabbit anti-mouse IgG were from Amersham (U.K.). Rabbit antisera against apolipoproteins were from Orion (Finland). Peroxidase-conjugated polyclonal antibodies against apolipoproteins A-I and B were from The Binding Site LTD. (U.K.), and MAb against apolipoprotein E was from Biogenesis LTD. (U.K.)

Quantitation of LPL mass

For this we used a solid phase sandwich ELISA with the polyclonal chicken antibodies for coating, and the 5D2 MAb for detection. Briefly, wells were coated with 100 μl affinity-purified antibody P66 (10 μg • ml^{-1} in phosphate-buffered saline (PBS, 10 mM phosphate, 150 mM NaCl, pH 7.4)) for 4 h at 37°C. After three washes with PBS/0.05% (v/v) Tween-20, 100 μl hLPL standard (0 to 300 ng • ml^{-1}) and samples diluted in PBS/0.05% (v/v) Tween-20/1 mg • ml^{-1} heparin/4 mg • ml^{-1} BSA were incubated for 12–16 h at 4°C. The wells were rinsed three times with PBS/0.05% (v/v) Tween-20, then the horseradish peroxidase-conjugated 5D2 antibody (diluted 1:5000 in PBS/0.2% (v/v) Tween-20) was added and the plates were incubated for 4 h at room temperature. After four rinses with PBS/0.05% (v/v) Tween-20, development was carried out with O-phenylene diamine substrate (0.4 mg • ml^{-1}, DAKO). Standards were linear to 300 ng • ml^{-1} with a correlation coefficient greater than 0.97. For measurement of plasma samples three dilutions of each sample were used, and the values that fell on the linear portion of the standard curve were used. The lower limit of detection was about 1 ng • ml^{-1}.

Lipase assays

Lipase activity measurements were performed as previously described (23). Hepatic lipase activity in plasma samples were inhibited by anti-hepatic lipase IgG (23). Fifteen μl of pre- and postheparin plasma samples (10 μl plasma + 5 μl IgG) or of fractions from the chromatographic separations were incubated with substrate in the presence of 10 μl of inactivated rat serum (as source of apoC-II) in a total volume of 200 μl. The assays were linear with time and amount of enzyme within the range used. The incubations were carried at 25°C for 15 min up to 100 min depending on the level of activity in the sample. One unit corresponds to 1 μmol of fatty acid released per min.

Quantitation of lipids and apolipoproteins

Cholesterol, triglycerides, and phospholipids were determined with enzymatic colorimetric assays from Boehringer Mannheim. Apolipoproteins A-I, B, and E in chromatography fractions were determined by direct ELISA using peroxidase-coupled polyclonal antibodies for qualitative detection. Turbidimetric assays using Orion kits for apoA-I and B were used for quantitation in plasma samples.

Heparin-Sepharose affinity chromatography

To assess the nature of hLPL from pre- and postheparin plasma the lipase was isolated by chromatography on a heparin-Sepharose column as described by Östlund-Lindqvist and Boberg (24). The chromatographies were performed at 4°C at a flow rate of 1 ml • min^{-1} and 1-ml fractions were collected. Fractions to be assessed for LPL mass were immediately diluted (1:5) with the ELISA sample-buffer. Fractions were also assessed for apoB and apoE. Lipoproteins eluted at NaCl concentration lower that 0.4 M where no LPL mass could be detected.

Gel filtration chromatography

A Superose 6 HR 10/30 column was used in an HPLC system (Pharmacia LKB, Sweden) and run with PBS to separate lipoproteins. Samples of 500 μl, 0.22 μm-filtered plasma were applied to the column and chromatographed at a flow rate of 0.3 ml per min under a pressure of 1.0–1.4 MPa. Fractions of 0.3 ml were collected. All chromatography steps were performed in a cold room (10°C). Fractions were immediately used to measure lipids or apolipoproteins or assayed for lipase mass or activity. The column was calibrated with rabbit IgG which eluted with a peak in fraction 29 and with BSA which eluted with a peak in fraction 33. ^{125}I-labeled bLPL run together with 130 μg unlabeled bLPL eluted with a peak of both activity and radioactivity in fraction 51. As LPL eluted after albumin it must have been retarded by interaction with the gel matrix. When ^{125}I-labeled LPL was run in the presence of plasma it distributed approximately as the endogenous LPL.

Ultracentrifugation of human plasma lipoproteins

Plasma from a blood donor was subjected to sequential, preparative ultracentrifugation to isolate different lipoprotein subclasses. Lipoprotein of hydrated densities $\leq$ 1.006 kg • l^{-1} (VLDL), 1.006–1.019 kg • l^{-1} (IDL), 1.019–1.063 kg • l^{-1} (LDL), 1.063–1.125 kg • l^{-1} (HDL_2), and 1.125–1.250 kg • l^{-1} (HDL_3) were separated according to Schumaker and Puppione (25). The remaining infranatant was considered as lipoprotein-deficient plasma. Lipoprotein-[a] (Lp[a]) was isolated from a subject with high plasma Lp[a] concentration (0.83 g • l^{-1}) by single vertical spin ultracentrifugation (26). The peak fractions were pooled and respun by the same procedure. All fractions were dialyzed against 50 mM Tris-HCl, 150 mM NaCl, pH 7.4. Ultracentrifugation in the salt solutions disrupted the binding of LPL to lipoproteins as was previously noted (9, 14). No immunoreactive LPL remained in the lipoprotein preparations after isolation, but was recovered in the infranatant.

Incubation experiments of ^{125}I-labeled bLPL with plasma and lipoproteins

Affinity of bLPL for lipoproteins was assayed in vitro as follows. Plasma, lipoprotein-depleted plasma, or isolated lipoproteins (VLDL, IDL, Lp[a], LDL, HDL_2, and HDL_3) were incubated with ^{125}I-labeled bLPL (~ 150 ng per ml of sample) for 1 h at 4°C with constant shaking. The samples were then immediately electrophoresed under nondenaturing conditions in gradient polyacrylamide gels (2–30%) (27). Gels were first autoradiographed and then stained for lipid (Oil red O) and protein (Coomassie G-250). For further identification, parallel gels were electroblotted onto nitrocellulose paper and apolipoproteins A-I, B, and E were localized by incubation with peroxidase-conjugated antibodies.

Western blot analysis of hLPL

To assess the relative molecular mass (M_r) of LPL in preheparin plasma, 20 ml was subjected to heparin-Sepharose chromatography and the peak fractions (LPL measured by ELISA, around 0.7 M NaCl in the gradient) were pooled. After removal of contaminating immunoglobulins by adsorption to protein A-Sepharose (Pharmacia LKB), the unbound material was dialyzed against 50 mM Tris-HCl, 150 mM NaCl, pH 7.4. To concentrate the samples by water absorption, the dialysis bag was covered during 12 h with carboxymethyl-cellulose. The samples were then electrophoresed in 12.5% SDS-polyacrylamide gels (28), and electroblotted to nitrocellulose membranes. After blocking with 4% BSA, the membranes were incubated overnight with the primary antibody (5D2 1:2000 in 25 mM Tris-HCl, 0.15 M NaCl, pH 7.4/0.05% (v/v) Tween-20/3% (w/v) BSA). The membranes were then washed with the same buffer but without BSA and with 0.1% (w/v) SDS and 1.0% (v/v) Nonidet P40 and incubated with a peroxidase-conjugated rabbit anti-mouse IgG antibody (Dako, Denmark). Development was done with the chemiluminescence kit (ECL) of Amersham (U.K.) following the manufacturer's instruction.

Electron microscopy immuno-staining

Selected samples from LPL mass peaks observed in the gel filtration chromatography profiles were applied on Formvar-carbon-coated grids. After removing excess sample, the grid was incubated for 30 min with antibodies against LPL (5D2) or apolipoproteins B or A-I diluted in PBS/0.5% (w/v) BSA. The first incubation was followed by three washes in PBS and a second incubation (20 min at room temperature) with an anti-mouse immunoglobulin antibody coupled to 10-nm gold particles to reveal 5D2 and 16 nm gold-coupled protein A to visualize the polyclonal antibodies against apolipoproteins. Finally, the grids were thoroughly washed, negatively stained with 2% sodium phosphotungstate, and immediately observed under the electron microscope. The efficiency of the labeling was calculated on LDL peak fractions, assuming that an LDL particle has only an apoB molecule, with the following equation:

Efficiency = (amount of 16-nm gold particles/amount of LDL particles) × 100.

This equation could not be applied on the LPL peak fraction used for the double immunostaining as it contained both LDL and HDL particles.

RESULTS

Levels of LPL in plasma

In this study we used a sandwich ELISA with polyclonal chicken antibodies to bovine LPL to capture the antigen. These antibodies inhibit the activity of both bovine and human LPL (17); hence they react with the native form of human LPL. The antibodies were used to immunoprecipitate human LPL which was unfolded by treatment with SDS, and they detected human LPL after SDS polyacrylamide gel electrophoresis and blotting onto nitrocellulose (G. Bengtsson-Olivecrona, unpublished results). Hence, they react also with unfolded forms of human LPL. Recovery experiments with ELISA plates and ^{125}I-labeled bovine LPL in catalytically active form, or after unfolding with SDS or with 1 M guanidinium chloride, showed similar binding, about 60%, when about 1 ng LPL was added per well. As shown in **Fig. 1**, this assay was able to recognize the inactive monomeric (4) and the active dimeric forms of LPL. We conclude that this coat will likely capture most or all forms of LPL protein in plasma. The 5D2 MAb has previously been shown to react with both native and unfolded forms of LPL (13). It does not react with human hepatic lipase (13).

The present ELISA, applied to samples from 20 normal male volunteers, gave a value of 69.8 ± 6.6 ng • ml^{-1} for LPL mass. The LPL activity was 1.54 ± 0.15 mU • ml^{-1}, yielding a specific activity of about 0.02 mU • ng^{-1}. There was no significant correlation between LPL activity and mass in plasma (r = 0.07, P = 0.78). The specific activity of purified human LPL has been reported to be 0.40–0.45 (24), 1.55 (29), and 0.51 mU • ng^{-1} (30). We used an assumed value of 0.6 mU • ng^{-1} for our standard. Hence, most of the LPL mass in basal plasma represents catalytically inactive protein.

Injection of 60 IU heparin • kg^{-1} body weight released LPL mass and activity into plasma in the volunteers. The values 15 min after injection were 536 ± 60 ng • ml^{-1} and

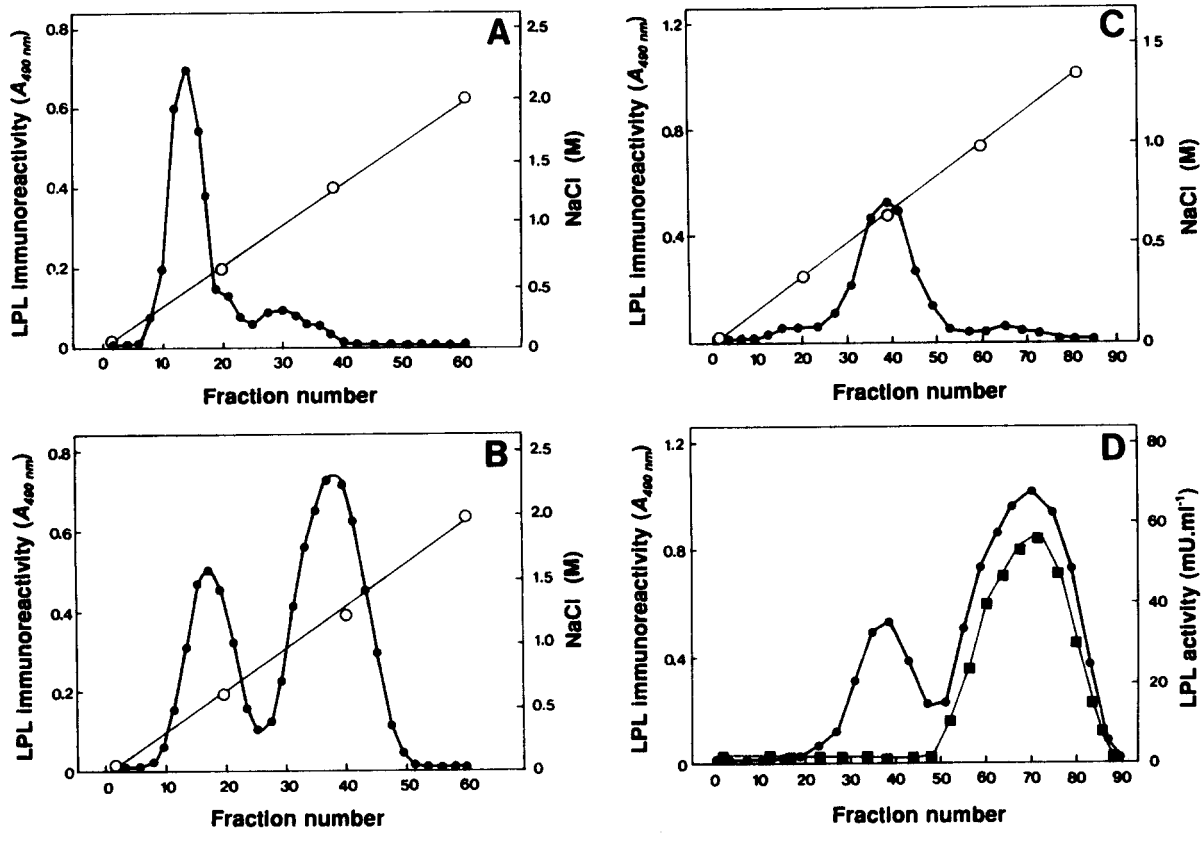

Fig. 1. Heparin-Sepharose chromatography of pre- and postheparin plasma. A and C show LPL mass profiles for preheparin plasma. B and D show LPL mass in postheparin plasma. C and D are from the same subject, and in D LPL activity (-■-) is also presented (the postheparin plasma LPL activity was 179 mU • ml^{-1}). In D the NaCl gradient, equivalent to that in panel C, is not shown for clarity. Note that the mass values are expressed in absorbance and are not corrected with respect to a human LPL standard.

261 ± 34 mU • ml^{-1}. There was no significant correlation between the LPL mass after heparin to that present in plasma before heparin (r = 0.27, P = 0.25). In contrast, there was a highly significant correlation between the LPL activity released by heparin (i.e., postheparin minus preheparin) to the LPL mass released (r = 0.92, $P \leq 0.0001$). The specific activity for the released material (i.e., the increase in activity divided by the increase in mass) was 0.57 ± 0.03 mU • ng^{-1}, i.e., close to the value of 0.6 for the LPL from human milk which was used as standard.

Forms of LPL in plasma

Earlier studies have shown that the monomeric and dimeric forms of LPL can be separated by chromatography on heparin-Sepharose (13, 31, 32). Four plasma samples (pre- and postheparin) from normolipemic volunteers were therefore subjected to heparin-Sepharose chromatography (Fig. 1). With preheparin plasma, essentially all LPL immunoreactivity eluted around 0.7 M NaCl, the position expected for monomeric LPL (Fig. 1, A and C). This LPL-immunoreactive material was checked by Western blot for protein size which was about 55 kDa, similar to the size of LPL from human milk (not shown). In accord with previous observations, the LPL activity in postheparin plasma eluted from heparin-Sepharose at an NaCl concentration of about 1.1 M, together with LPL mass (Fig. 1, B and D). No increase in material eluting at 0.7 M NaCl was noted (Fig. 1, C and D). These observations support the conclusion that virtually all the LPL mass in preheparin plasma represents inactive monomeric LPL, whereas the material released by heparin represented mainly the catalytically active dimeric form of LPL.

Association of LPL with lipoproteins

Some of us have previously reported co-elution of LPL-immunoreactive material with cholesterol-rich lipoproteins in fractions of gel filtered pre- and postheparin plasma (9). In that study we used the 5D2/5D2 ELISA (21), which recognizes the dimeric, active form of LPL (13). These results are now confirmed and extended with the P66/5D2 ELISA, which recognizes also monomeric, inactive forms of LPL. Plasma was separated by gel filtration. A typical profile for pre- and postheparin plasma is presented in **Fig. 2A,** showing co-elution of LPL with the lipoprotein peaks, from VLDL to HDL. The major peaks of LPL were associated with LDL and/or HDL, with some trailing into the late fractions. Recoveries for LPL mass in plasma fractions were usually higher than 100%, probably due to the known sample dilution effect. In postheparin plasma (Fig. 2B) LPL activity and mass eluted with the LDL and HDL peaks. Hepatic lipase (HL) activity eluted in a single peak located between the peaks of HDL and LDL, as reported by Goldberg et al. (14). Recoveries of lipase activities were between 67 and

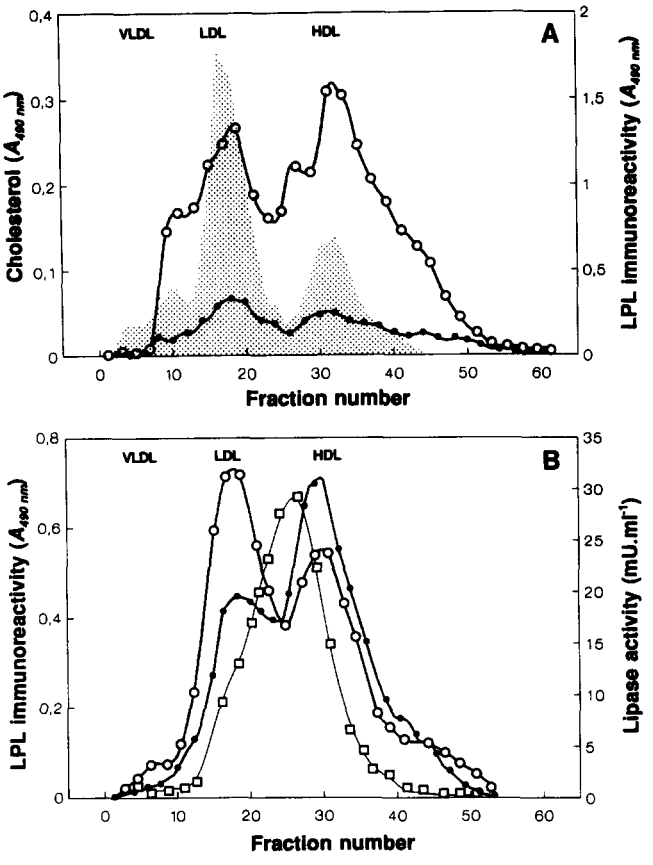

Fig. 2. Gel filtration of human plasma. (A) pre- (-●-) and postheparin (-○-) LPL mass profiles. The stippled area shows the cholesterol profile. (B) postheparin plasma from another subject; LPL mass (-●-), LPL activity (-○-), hepatic lipase activity (-□-). Note that the LPL mass cholesterol values are expressed in absorbance and are not corrected with respect to a standard.

75%. The profiles varied somewhat from plasma to plasma. **Fig. 3** shows two other examples with preheparin plasma. Panel A represents the profile for LPL mass in plasma of a fasting subject. Here most of the LPL mass was associated with the LDL and HDL peaks. Panel B in Fig. 3 shows the profile from another subject in the postprandial state. Here a distinct peak of LPL mass eluted where VLDL and larger lipoproteins were found.

Co-elution on gel filtration does not necessarily demonstrate binding of LPL to lipoproteins. Therefore, fractions rich in LPL were examined by electron microscopy after immunostaining with the 5D2 antibody plus gold-coupled rabbit anti-mouse IgG (**Fig. 4**). This experiment was done with postheparin plasma as the expected frequency of LPL-lipoprotein complexes in preheparin plasma was low. In the chromatographic fractions corresponding to LDL, every 900 to 1000 of the particles showed immunoreaction for LPL. This corresponds roughly to the frequency expected from the mass data.

To further study the association of LPL with lipoproteins, ^{125}I-labeled bovine LPL was incubated with

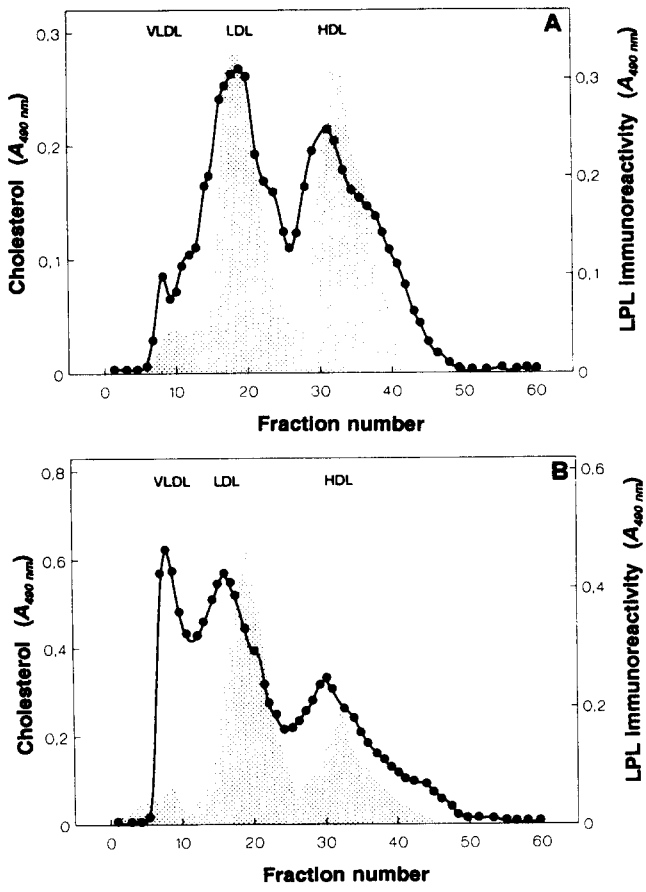

Fig. 3. Illustration of the variations of LPL mass profiles on gel filtration of preheparin plasma. A shows plasma from a fasted subject. B shows plasma from another, nonfasted subject. LPL mass (-●-). The stippled area shows the cholesterol profile. Note that the values are expressed in absorbance and therefore are not corrected with respect to a control value. In B the triglyceride profile with a major peak in the VLDL fraction is not shown for clarity.

plasma or lipoprotein-depleted plasma. The lipoproteins were then separated by gradient gel electrophoresis (**Fig. 5A**). Autoradiographies of the gels showed labeled LPL associated with several bands. As found on gel chromatography, the major bands were in the positions of LDL and large HDL. Little or no LPL was found associated with Lp[a] or with HDL_3. A band seen between LDL and HDL may represent LPL bound to $\alpha 2$ macroglobulin (E. Vilella, G. Bengtsson-Olivecrona, T. Stigbrand, and P.E.H. Jensen, unpublished observation). LPL added to lipoprotein-depleted plasma moved slightly further than the HDL_3 band. In another experiment, labeled bovine LPL was mixed with ultracentrifugally isolated lipoproteins. It should be noted that ultracentrifugation in the salt solutions used for isolation of the lipoproteins causes dissociation of LPL (9, 14). Therefore, the isolated lipoproteins used in the experiments were essentially free of endogenous LPL. Except with HDL_3 and Lp[a], LPL moved with the lipoprotein to which it had been added, in most cases with considerable streaking.

DISCUSSION

In this study we have used a solid phase sandwich ELISA to detect and quantitate LPL protein in plasma. A polyclonal antibody was used to coat the plates for capture of the antigen. A MAb 5D2 (13, 21) was used for detection, ensuring specificity. Both of these antibodies react with both native and unfolded forms of LPL. A previous ELISA that used the MAb 5D2 both for capture and for detection required that two copies of the epitope were present together, as would be the case in active dimeric LPL. Using this ELISA Babirak et al. (21) reported a value of 196 ± 59 ng • ml^{-1} for LPL mass in postheparin plasma of 34 normal subjects. Ikeda et al. (11) used an ELISA with two different MAbs and reported values of 33.4 ± 4.7 and 223 ± 66 ng • ml^{-1} for LPL mass in pre- and postheparin plasma, respectively, somewhat lower than the values found here. Kern et al. (12) reported 8–25 and 75–145 ng • ml^{-1} for LPL mass in pre- and postheparin plasma, respectively, of eight normal subjects. The different values may, at least in part, be due to the nature of the standards used for calibration. We used the peak fraction of LPL purified on heparin-Sepharose from human milk as standard, and based our estimate of LPL mass in this fraction on an assumed specific activity of 0.6 mU • ng^{-1}.

A major observation was that preheparin plasma consistently contains LPL mass with low activity, suggesting that the mass represents mainly catalytically inactive LPL and confirming previous results (11–13). In our subjects there was no correlation between LPL mass and activity in preheparin plasma, neither was there any correlation between LPL mass before and after heparin. The nature and metabolic significance of this LPL mass is not clear. On heparin-Sepharose it eluted at the NaCl concentration expected for monomeric LPL. Western blot analysis of LPL from preheparin plasma indicated that the material corresponds to the full-size protein.

There was a strong correlation between the increments of LPL mass and activity after heparin injection in our study. On heparin-Sepharose chromatography the increment appeared in the position for dimeric, active LPL ("peak two"). Hence, heparin appears to release mainly the active form of LPL. In accord with this, Peterson, Fujimoto, and Brunzell (13) detected an increase after heparin with a MAb that reacts with active LPL, but not with a MAb that only detects unfolded forms of the enzyme. Previous studies (11, 21) report a strong correlation between LPL mass and activity after heparin, further supporting the view that heparin releases the active form of the enzyme. The implication of these findings is that there are two parameters to measure with an ELISA, the

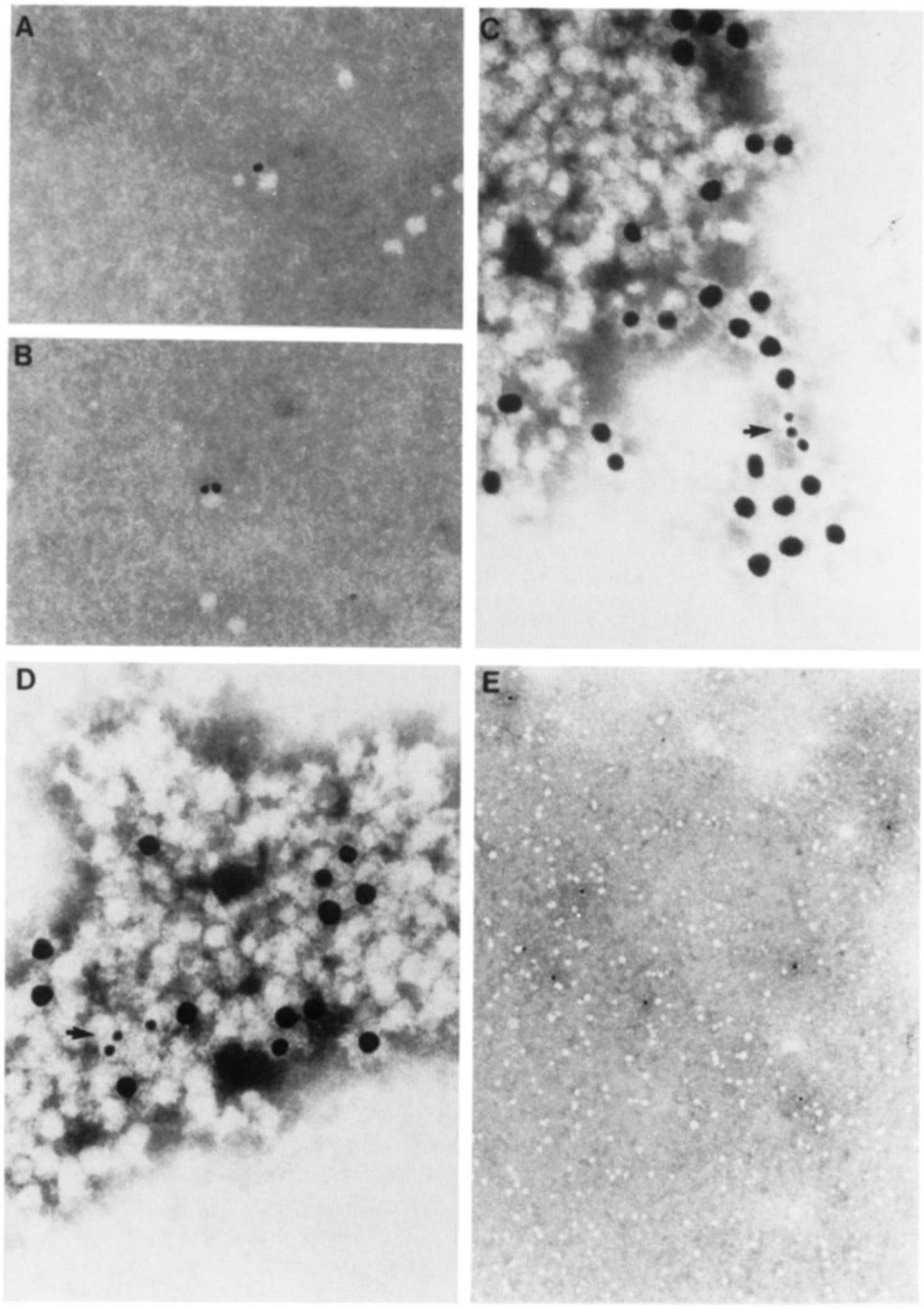

Fig. 4. Electron micrographs of lipoproteins reacted with antibodies against LPL and/or apolipoproteins A-I and B. Samples were obtained by gel filtration of postheparin plasma, and correspond to the LPL peak fractions where both small LDL and large HDL are present. Panels A and B show LPL bound to lipoproteins revealed by incubation with 5D2 MAb and subsequent incubation with anti-mouse-immunoglobulin antibody coupled to 10-nm gold particles. Panels C and D show double immunogold labeling to detect LPL (as in panel A, 10-nm gold particles, see arrows), and apoA-I (C) or apoB (D) with pig polyclonal antibodies visualized with protein A coupled to 16-nm colloidal gold. E is a low magnification micrograph from a control grid incubated with only protein A coupled to 16-nm colloidal gold used as a control of the binding of protein A to plasma immunoglobulins co-eluting in the same fractions and to calculate the labeling efficiency (40%).

amount of inactive LPL mass in preheparin plasma, and the increment of active LPL released by heparin.

Our results on the distribution of lipase activities in postheparin plasma agree well with those of Goldberg et al. (14). Most of the LPL activity eluted with particles the size of LDL or slightly larger than LDL. Goldberg et al. (14) further showed that LPL activity was removed from postheparin plasma on passing through immunoaffinity

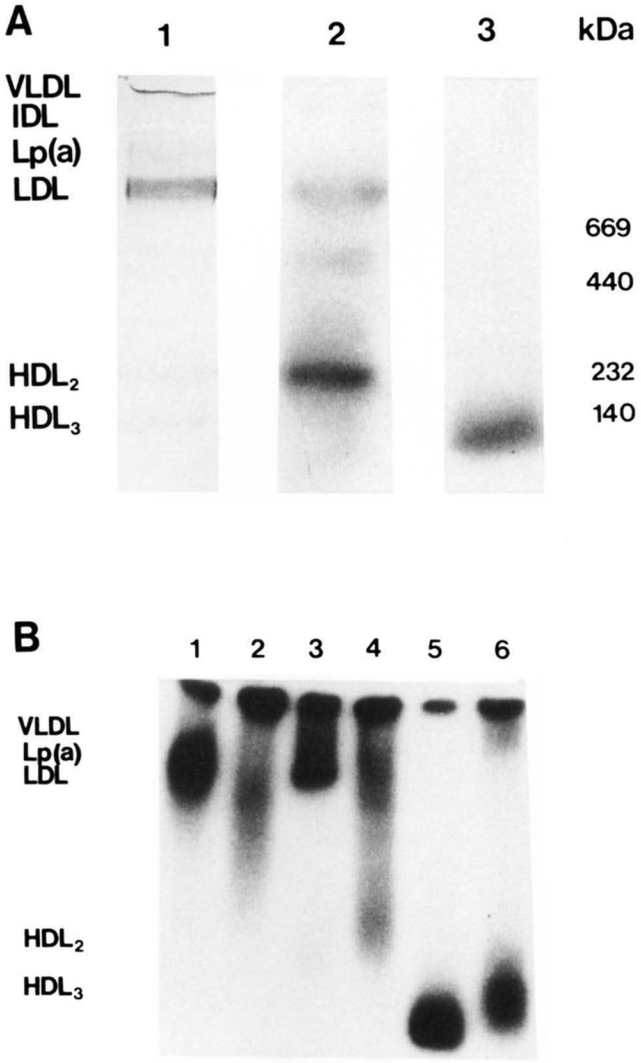

Fig. 5. In vitro binding of ^{125}I-labeled bLPL to lipoproteins. Samples of plasma or isolated lipoproteins were incubated with the enzyme and the lipoprotein particles were then separated by nondenaturing polyacrylamide gradient gel electrophoresis. A: For the experiment in lanes 1 and 2, ^{125}I-labeled bLPL was incubated in plasma; for the experiment in lane 3, ^{125}I-labeled bLPL was incubated in plasma depleted of lipoproteins. Lane 1 shows Oil red O staining. The lipoproteins were further identified by immunoreaction for apolipoproteins A-I and B (not shown). Lanes 2 and 3 show autoradiograms. B: ^{125}I-labeled bLPL was incubated with ultracentrifugally isolated lipoproteins. Lane 1: VLDL, lane 2: IDL, lane 3: LDL, lane 4: HDL_2, lane 5: HDL_3, and lane 6: Lp[a]. The plate is an autoradiogram. With our methods, HDL_2 and Lp[a] are presumably contaminated with LDL and HDL, respectively.

columns containing antibodies to apoB and apoE, substantiating that LPL is in fact bound to lipoproteins of the IDL-LDL classes. Further evidence for this is our demonstration by electron microscopy of LPL immunoreaction on particles that also reacted with antibodies to apoB. In samples from postheparin plasma, LPL was found on lipoproteins with a frequency of one LPL on every 900 to 1000 lipoprotein particles. For comparison, the proportion of LPL mass (around 0.5 $\mu g \cdot ml^{-1}$) to apoB mass (around 1000 $\mu g \cdot ml^{-1}$) would correspond to a frequency of one LPL per 800 LDL particles assuming that each LDL particle had one apoB (M_r 556 kDa), that LPL was present as homodimers (M_r 110 kDa), and that half of the LPL molecules were on LDL.

In the present study LPL with low specific activity was detected with HDL. Previously (14), less LPL activity was detected here. The authors described a shoulder of LPL from the LDL-associated peak. The difference may lay in part in the columns and conditions used for separation. On electron microscopy lipoprotein particles from these fractions reacted with both LPL and apoA-I. On gradient gel electrophoresis of plasma with added ^{125}I-labeled LPL, most of the enzyme moved in the position expected for HDL_2. That LPL can interact with HDL is evident also from earlier observations; it can hydrolyze lipids in HDL (33), enhance transfer of lipids from HDL to cells (34), and enhance binding of HDL to cells and extracellular matrix (35).

The largely inactive LPL mass in preheparin plasma distributed between lipoproteins similar to active LPL. The active LPL, however, accounts for only a small proportion of LPL mass in preheparin plasma. Model studies have shown that monomeric LPL retains ability to bind to lipid emulsion droplets and liposomes (G. Bengtsson-Olivecrona, unpublished results). Whether specific protein–protein interactions are also involved in binding to the lipoproteins is presently not known. However, using a ^{125}I-labeled crosslinker bound to bLPL and after incubation with lipoproteins and further delipidation, we observed more than 80% of the radioactivity associated to the apoprotein moiety (E. Vilella, unpublished results). A candidate would be apoC-II, but this seems unlikely as the most consistent peak of LPL protein was in the LDL range, lipoproteins which are relatively poor in apoC-II.

Triglyceride-rich lipoproteins are the preferred substrate for LPL. Yet most of the enzyme in plasma was found on cholesterol-rich particles. When postprandial plasma (Fig. 3B) or plasma from a type IV hyperlipidemic patient (data not shown) were used, some LPL mass eluted in the position of large triglyceride-rich particles. This is similar to the observations of Goldberg et al. (14) who suggested these were chylomicron remnants. The enzyme in postheparin plasma moves readily to added substrate emulsion particles during assay (2). Furthermore, the distribution of the enzyme shifts with the conditions, e.g., salt concentration, used during separation (14). All of this suggests that the LPL interacts in a reversible fashion with the lipoproteins and that the patterns observed reflect the distribution of freely movable LPL molecules, active as well as inactive, between the lipoproteins present in the plasma sample.

The association of LPL with lipoproteins, even when catalytically inactive, may have physiological implica-

tions. Friedman et al. (34) suggested that LPL may play a role in cellular uptake of cholesteryl esters from lipoproteins. Eisenberg et al. (35) and Mulder et al. (36) have shown that LPL enhances the binding of lipoproteins to cells and to extracellular matrix through heparan sulfate proteoglycans. Saxena et al. (37) proposed a mechanism for LPL-mediated retention of LDL in the endothelial extracellular matrix, and demonstrated enhancement of the cellular binding of VLDL and LDL particles by LPL. Felts, Itakura, and Crane (38), in 1975, already proposed that LPL attached to lipoprotein remnants might be the signal for liver uptake of these particles. Recently Beisiegel, Weber, and Bengtsson-Olivecrona (39) demonstrated that LPL enhances the binding of chylomicrons to a cell surface protein, and that LPL bound a protein that resembled the low density lipoprotein receptor-related protein, the putative receptor for chylomicron remnants. Presumably acting through a similar mechanism, LPL has been shown to modulate hepatic uptake of apolipoprotein B-containing lipoproteins (40). The LPL mass in plasma is such that there could be one LPL on each 500 to 1000 LDL particles. This is a low proportion, but if the presence of LPL influences the metabolism of the particles, the lipase could have significant impact, in view of the normally slow turnover of these particles. Moreover, LPL bound to remnant particles may be difficult to detect because the particles may be rapidly taken up by the liver.

The technical assistance of Ann-Sofie Jacobsson and Asa Lundsten is greatly appreciated. This work was supported by the CICYT grant PB 92-0843, the NIH grant DK02456, by the Swedish Medical Research Council, by the Swedish Margarine Fund for Research in Nutrition, and by the Spanish Ministry of Health (FIS). E. V. is in receipt of a postgraduate fellowship award from the Fundación Ferrer para la Investigación.

Manuscript received 18 December 1992 and in revised form 7 April 1993.

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