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I J Goldberg, ... , C B Blum, H N Ginsberg

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Research Article

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Association of Plasma Lipoproteins with Postheparin Lipase Activities

Ira J. Goldberg, Jessica J. Kandel, Conrad B. Blum, and Henry N. Ginsberg

Department of Medicine, Columbia University College of Physicians & Surgeons, New York, New York 10032

Abstract

Studies were designed to explore the association of lipoprotein lipase (LPL) and hepatic triglyceride lipase (HTGL) activities with lipoproteins in human postheparin plasma (PHP). The major peak of LPL activity after gel filtration of PHP eluted after the triglyceride-rich lipoproteins and just before the peak of low density lipoprotein (LDL) cholesterol. When PHP contained chylomicrons, an additional peak of LPL activity eluted in the void volume of the column. Most HTGL activity eluted after the LDL and preceded the elution of high density lipoprotein cholesterol. LPL activity in preheparin plasma eluted in the same position, relative to lipoproteins, as did LPL in PHP. Gel filtration of purified human milk LPL mixed with plasma or isolated LDL produced a peak of activity eluting before LDL. During gel filtration of PHP in high salt buffer (1 M NaCl) or after isolation of lipoproteins by ultracentrifugation in high salt density solutions, most of the lipase activity was not associated with lipoproteins. LPL activity was removed from PHP by elution through immunoaffinity columns containing antibodies to apolipoprotein (apo) B and apo E. Since lipoproteins in PHP have undergone prior *in vivo* lipolysis, LPL activity in PHP may be bound to remnants of chylomicrons and very low density lipoproteins.

Introduction

Two enzymes, lipoprotein lipase (LPL)¹ and hepatic triglyceride lipase (HTGL), constitute the majority of triglyceride hydrolytic activity in human postheparin plasma (PHP). LPL is synthesized in many cells and tissues, including adipose tissue, muscle (1), brain (2), and macrophages (3, 4). After secretion from these sites, it is thought to bind to the luminal surface of endothelium by interacting with heparin-like molecules (5). LPL, in the presence of apolipoprotein (apo) C-II (6), hydrolyzes triglycerides contained in lipoproteins, initiating catabolism of chylomicrons and conversion of very low density lipoproteins (VLDL) to low density lipoprotein (LDL) (7). Accompanying this hydrolysis of triglycerides, apo C and apo A, phospholipids, and free cholesterol are transferred to high density lipoproteins (HDL) (8). Thus,

Please address all correspondence to Ira J. Goldberg, Dept. of Medicine, Columbia University College of Physicians and Surgeons, 630 West 168th St., New York, NY 10032.

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1. *Abbreviations used in this paper:* apo, apolipoprotein; FFA, free fatty acid; HDL, high-density lipoprotein; HTGL, hepatic triglyceride lipase; LDL, low density lipoprotein; LPL, lipoprotein lipase; PHP, postheparin plasma; VLDL, very low density lipoprotein.

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LPL influences the circulating levels of both HDL and triglyceride. This latter relationship is substantiated by the positive correlation of LPL activity and HDL cholesterol levels in humans (9) and the low levels of HDL found in LPL-deficient subjects (10).

The physiological role of HTGL has been less clearly defined. Immunological inhibition of this enzyme in both rats (11, 12) and monkeys (13) resulted in an increased level of plasma triglycerides. A similar increase in plasma triglyceride levels was reported in a family with a genetic deficiency of HTGL (14). These studies support *in vitro* data that demonstrate VLDL triglyceride hydrolysis by HTGL (15). Since, unlike LPL, HTGL lipolytic activity is not dependent on the presence of apo C-II, a subclass of triglyceride-rich lipoproteins deficient in this apoprotein may require HTGL for their catabolism. Inhibition of HTGL activity in rats has also produced an increase in the phospholipid content of HDL (12, 16), suggesting an important role for HTGL in HDL metabolism. The inverse correlation between HTGL activity and HDL levels in humans (17) supports this hypothesis.

In circulation, both LPL and HTGL probably interact with triglycerides transported in lipoproteins. A number of observations suggest that LPL and HTGL are associated with lipoproteins in PHP. Fielding found that after PHP was mixed with Intralipid (Vitrum Company, Stockholm, Sweden), LPL activity was isolated with the lipid layer obtained by centrifugation (18). Shirai et al. reported that LPL bound *in vitro* to phospholipid vesicles in the presence or absence of apo C-II (19). Bengtsson and Olivecrona mixed purified HTGL with HDL and demonstrated that lipase activity and lipoproteins co-eluted during gel filtration (20). Accordingly, in the present studies, we undertook to explore further the possible physical association of LPL and HTGL with lipoproteins in plasma. Experiments were designed in particular to investigate which lipoproteins, if any, were associated with LPL and HTGL in PHP. We now report the results of these studies.

Methods

Sources of plasma. Plasma was obtained from normal volunteers (24-35 yr old) and from hyperlipoproteinemic patients followed at the Arteriosclerosis Research Center at Columbia Presbyterian Medical Center. Informed consent was obtained from all subjects. Individuals with a history of anemia or gastrointestinal or bleeding disorders were excluded from these studies. Plasma was obtained from subjects who had fasted for at least 12 h and from a normal subject who had eaten 500 ml of ice cream containing 96 g of butter fat 5 h previously. PHP (20-50 ml) was obtained 15 min after intravenous administration of 60 U/kg body wt of heparin (Upjohn Co., Kalamazoo, MI). Blood samples were placed on ice immediately, and plasma and cells were separated in a refrigerated centrifuge within 1 h. The PHP was chromatographed immediately, and duplicate studies were performed using samples frozen at -20°C for up to 4 wk. Samples for the study of lipases in preheparin plasma were obtained by withdrawing 10-20 ml of blood directly into 7-ml vacuum tubes, each containing 100 USP units of heparin. The plasma triglyceride and cholesterol were measured by enzymatic methods with an ABA 100

auto analyzer (Abbott Laboratories, North Chicago, IL). HDL cholesterol was obtained after precipitation of the lower density lipoproteins by heparin-manganese. Lipid levels for each subject studied are shown in Table I.

Gel filtration chromatography. 2 ml PHP and 4 ml pre-heparin plasma were applied to 1.0×120 cm columns containing 6% agarose (A-5 M, Bio-Rad Laboratories, Richmond, CA). The samples were chromatographed in a buffer containing 150 mM NaCl, 10 mM NaPO_4 , 0.01% EDTA, pH 7.4 (phosphate-buffered saline, PBS) or in 1.0 M NaCl, 10 mM sodium phosphate, pH 7.4 (high salt buffer), at 4°C. Column fractions (~1 ml) were collected at a rate of four per hour, and the eluate was monitored for absorbance at 280 nm. Column fractions were assayed for protein (21) and cholesterol (22). Elution volumes of LDL and HDL were determined for each study from the elution of the major peaks of cholesterol. These volumes were compared with the elution volumes of LDL and HDL cholesterol obtained by gel filtration of lipoproteins, collected by ultracentrifugation of normal human plasma at a density of 1.21 g/ml. Elution of chylomicrons was used to determine the void volume of each column. All gel filtration studies were performed with columns and buffers maintained at 4°C.

Ultracentrifugation of PHP. 1 ml PHP was mixed with KBr or sucrose solutions in ultracentrifuge tubes. The tubes were centrifuged in a 50.3 Ti rotor (Beckman Instruments, Inc., Palo Alto, CA) at 40,000 rpm at 4°C for 24 h, resulting in final densities of 1.006 and 1.019 g/ml, or for 48 h, resulting in densities of 1.063 and 1.22 g/ml. (23). The top 1 ml, containing those lipoproteins less dense than the buffer, was aspirated, and 20- μ l aliquots of this supernatant and of the infranatant were assayed for LPL and HTGL activities as described below.

Preparation of human milk LPL. Human milk was obtained frozen from the Human Milk Bank at Columbia-Presbyterian Medical Center. Acetone-ether powders of the milk cream were prepared by the method of Hernell and Olivecrona (24). 5 g of this powder was resuspended in 100 ml of buffer (0.1% Triton X-100, 40 mM NH_4OH , pH 8.5). The resulting solution was clarified by centrifugation at 5,000 rpm for 30 min in a RC-5B centrifuge (E. I. DuPont de Nemours & Co., Inc., Sorvall Instruments Div., Newtown, CT). The supernatant was gently mixed overnight at 4°C on a platform rocker with 10 ml of heparin-Sepharose gel (Pharmacia, Inc., Piscataway, NJ). The gel was then eluted using a linear salt gradient (0.4–1.5 M NaCl) in buffers containing 10 mM sodium phosphate, pH 7.4. The fractions obtained were assayed for LPL activity and those containing the greatest lipase activity were pooled. This preparation was concentrated by dialysis against 3.6 M ammonium sulfate followed by centrifugation at 10,000 rpm for 30 min. The pellet was resuspended in 2 ml of either human preheparin plasma, PBS with 1% bovine serum albumin (BSA), human plasma dialyzed against high salt buffer, or 1 ml of human LDL (2.8 mg protein/ml). These preparations were then dialyzed against either PBS or high salt buffer to remove residual ammonium sulfate before chromatography.

Measurement of lipase activities. Triglyceride hydrolytic activities in partially purified LPL preparations, PHP samples, and column fractions after gel filtration of PHP were measured with 100 μ l of the substrate emulsion described by Nilsson-Ehle and Schotz (25), containing 1.5 μ mol of triolein (Nu-Chek Prep, Inc., Elysian, MN) and 1.6 μ Ci of glycerol tri(9,10- ^3H)oleate (Amersham Corp., Arlington Heights, IL). The enzymatically released free fatty acids (FFA; ~280 counts/min per nmol) were extracted as described by Belfrage and Vaughan (26). Each column fraction was assayed in duplicate (50- μ l aliquots). For LPL determinations, HTGL activity was inhibited with a previously described anti-HTGL antiserum (13). HTGL activity in PHP and column fractions obtained from chromatography of PHP was measured in a manner similar to LPL, but with an emulsion containing 1 M NaCl for inactivation of LPL and without serum and anti-HTGL antiserum.

Activity of LPL in fractions obtained after gel filtration of preheparin plasma was measured using 150- μ l aliquots of each fraction mixed with 10 μ l of anti-HTGL antiserum and 50 μ l of a high specific activity emulsion. This emulsion containing 0.21 μ mol of triolein, 0.46 μ Ci of glycerol tri(9,10- ^3H)oleate, and 9.0 μ g of egg yolk phosphatidylcholine (Sigma Chemical Co., St. Louis, MO) sonicated in 33.5 μ l of buffer (0.3 M Tris-

HCl, 3% BSA, pH 8.6), and 16.5 μ l of human serum. The human serum had been previously incubated at 57°C for 60 min to inactivate any endogenous lipase activity. The emulsion was incubated at 37°C for 60 min before use. The assay was carried out in a 37°C shaking water bath for 90 min, and FFA (4,025 counts/min per nmol) were extracted as described above. A similar emulsion that contained 50 μ l of 1 M NaCl buffer and no human serum was used for measurement of HTGL.

Immunoaffinity chromatography. Immunoaffinity columns to human apoproteins were used to selectively remove lipoproteins containing specific apoproteins from PHP. Antisera to apo E and apo A-I were produced using isolated apoproteins as previously reported (27, 28). Rabbit antiserum to apo B was produced using human LDL isolated at $d = 1.030$ – 1.050 g/ml. Specific antibodies to human apo A-I, apo E, and LDL were prepared by chromatography of $(\text{NH}_4)_2\text{SO}_4$ precipitates of antisera over columns containing purified apoproteins or LDL ($d = 1.030$ – 1.050 g/ml) immobilized on 6% agarose. The purified antibodies were eluted with 50 mM glycine, pH 2.5. pH of the eluates was immediately adjusted to 7.4 by addition of 1 M NaPO_4 . The purified antibodies were bound to cyanogen bromide-activated Sepharose beads. The gels were washed with glycine containing buffer to block any unreacted sites. 20 ml of each gel was packed into 2.5-cm-diameter columns. 1 ml PHP was applied to each immunoaffinity column and to a similar column containing Sepharose CL-4B, and elution was then carried out with PBS containing 1% BSA. 10 3-ml fractions were collected from each column, and each fraction was assayed in triplicate for LPL activity. The percentage of activity recovered was calculated by dividing the sum of activity recovered in the fractions from each immunoglobulin-containing column by the total activity recovered from the control Sepharose column. The recovered activity was 58–81% of that applied to the column. Apo A-I, apo B, and apo E were measured in the eluted fractions by a previously described radioimmunoassay (29). These assays confirmed that over 90% of each apoprotein was removed by the appropriate immunoaffinity gel. After each experiment the immunoaffinity gels were regenerated by rapid elution of each column with 30 ml of 0.2 M glycine buffer, pH 2.5, 30 ml of 0.5 M sodium phosphate, pH 8.0, and 50 ml of PBS.

Results

Gel filtration of PHP. The results of gel filtration chromatography of PHP from a representative normal subject are shown in Fig. 1. Elution volumes of LDL and HDL (arrows) were determined by the elution of two peaks of cholesterol measured in the fractions. The peaks of LPL and HTGL activities were well separated from the void volume. A peak of LPL activity consistently eluted just before the LDL cholesterol peak in all five normal subjects (Table I, subjects 1–5). In addition, a shoulder of LPL activity from that peak overlapped the elution of LDL. HTGL eluted after the LDL cholesterol peak (Fig. 1 B) and before the HDL peak. The major peak of plasma proteins (data not shown) co-eluted with and after HDL cholesterol and was not associated with measurable lipase activity. 25–55% of total PHP lipolytic activity was recovered in the column fractions when the total activity recovered in the fractions was compared with that of PHP from the same subjects stored at 4°C for the duration of the chromatography. This loss of activity may be due to inactivation of LPL that occurred when the PHP was diluted with PBS during the gel filtration. Alternatively, some activity may have bound to the agarose gel.

Gel filtration in high salt buffer. The effects of high salt on the interaction of LPL and HTGL with lipoproteins was assessed by chromatographing PHP in high salt buffer. PHP was obtained from a normal subject (subject 3) 15 min after intravenous injection of heparin and was immediately mixed with an equal volume of 2.0 M NaCl. 3 ml of this preparation was gel filtered (Fig. 2). While some LPL eluted before the LDL cholesterol

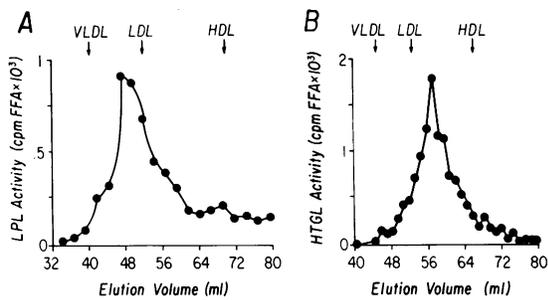


Figure 1. Gel filtration of human PHP on 6% agarose. 2 ml of PHP was gel filtered on a 1.0×120 -cm column containing 6% agarose. The void volume was determined by first gel filtering a preparation containing human chylomicrons. The elution volumes of LDL and HDL for each subject (arrows) were determined by assaying cholesterol in the column fractions obtained after gel filtration of PHP. Column fractions were assayed for LPL (A) and HTGL (B) with the emulsion described by Nilsson-Ehle and Schotz.

peak, most LPL activity now eluted with and after the HDL cholesterol. Since the elutions of plasma proteins and HDL were not clearly separated with 6% agarose, it is probable that much of the LPL in this experiment was not associated with lipoproteins (Fig. 2 A). HTGL eluted in a single peak which may have also contained some smaller HDL and the remainder of the plasma proteins. (Fig. 2 B). Thus, the elution patterns of both LPL and HTGL in PHP were strikingly altered by high salt. These findings suggest that the high salt concentration may have disrupted the association of the lipases with particular lipoproteins in PHP.

Gel filtration of partially purified human LPL. 2 ml of plasma was dialyzed against high salt buffer and mixed with purified human milk LPL. This mixture was gel filtered in high salt buffer. As shown in Fig. 3 A, a single peak of LPL activity eluted at a volume where HDL and the majority of plasma proteins eluted in previous studies. When the purified LPL preparation was resuspended in PBS with 1% BSA (i.e., with no lipoproteins) and gel filtered on 6% agarose, the peak of LPL activity eluted in a similar position (data not shown). In both experiments, this peak of LPL activity probably represented LPL—perhaps a dimer or tetramer of the 60,000 M_r molecule, not associated with lipoproteins. Purified human milk LPL was added to preheparin

Table I. Plasma Lipid Levels of Subjects Studied

Subject	Cholesterol mg/dl	Triglyceride mg/dl	HDL cholesterol mg/dl
1	169	80	42
2	209	30	52
3	191	88	45
4	195	134	62
5	193	89	105
6	295	33	97
7	230	425	43
8	213	257	35
9	399	2,597	20
10	211	684	25
11	516	1,234	18

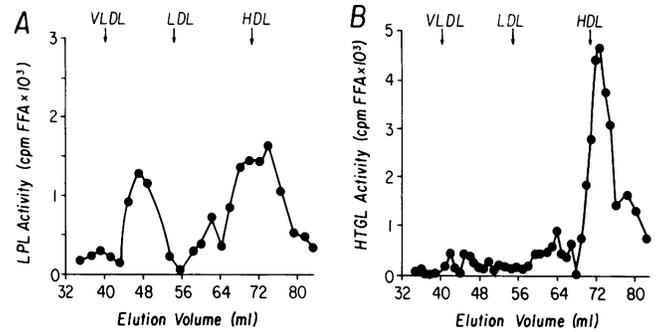


Figure 2. Gel filtration of human PHP in 1 M NaCl. PHP obtained from subject 3 was mixed with an equal volume of 2.0 M NaCl before gel filtration in high salt buffer on a 1.0×120 -cm column containing 6% agarose. Column fractions were assayed for cholesterol, LPL activity (A), and HTGL activity (B). The elution volumes of LDL and HDL are indicated.

plasma from a normal human volunteer (2 ml from subject 2) before gel filtration. In this study an additional peak of LPL activity eluted before LDL (Fig. 3 B). This peak of activity was at an elution position relative to lipoproteins that was similar to the position of LPL activity after gel filtration of PHP.

Purified human milk LPL was mixed with LDL isolated by ultracentrifugation, and this mixture was gel filtered to determine whether the peak of LPL activity in PHP might represent an enzyme-LDL complex. A single peak of LPL activity eluted just before the major cholesterol peak (Figure 3 C). This result suggested that the peak of LPL activity in PHP eluting before LDL represented an interaction between LPL and a subclass of lipoproteins of $d = 1.019$ – 1.063 g/ml.

Gel filtration of hyperlipoproteinemic plasma. PHP samples from subjects with either hypertriglyceridemia (subjects 7–9, 11) or who had ingested fat (subject 10) were gel filtered to assess the effect of increased levels of VLDL or chylomicrons on the elution of lipases (Fig. 4). A major peak of cholesterol eluted in the void volume using PHP from subjects with elevated VLDL or chylomicrons (subjects 7–11). As was found in studies using PHP from normal subjects, the major peak of LPL activity preceded or co-eluted with the LDL cholesterol. However, gel filtration of the hyperchylomicronemic plasmas (subjects 9–11) also resulted in an additional LPL peak eluting in the void volume of the column. HTGL activity in these subjects eluted in a position similar to that observed in PHP from normolipidemic subjects.

Gel filtration of preheparin plasma. Plasma from two hyperalipoproteinemic subjects (subjects 5 and 6) was chromatographed on 6% agarose to determine the elution volume of LPL activity in preheparin plasma. Eckel et al. have demonstrated a correlation between preheparin and PHP-lipase activities (30). Hence, use of plasma from a subject with a high level of PHP-LPL activity should increase the likelihood of detecting such activity after gel filtration of the preheparin sample. LPL activity was measured with high specific-activity emulsion. As shown in Fig. 5, LPL activity eluted just before LDL. This peak of activity was in a position similar to that observed in PHP from the same subject. The LPL activity in preheparin plasma was fully inhibited by a monoclonal antibody produced against human milk LPL (31). HTGL activity eluted in two peaks, one of which was in a similar position to that found after gel filtration of PHP. The second peak of HTGL activity eluted

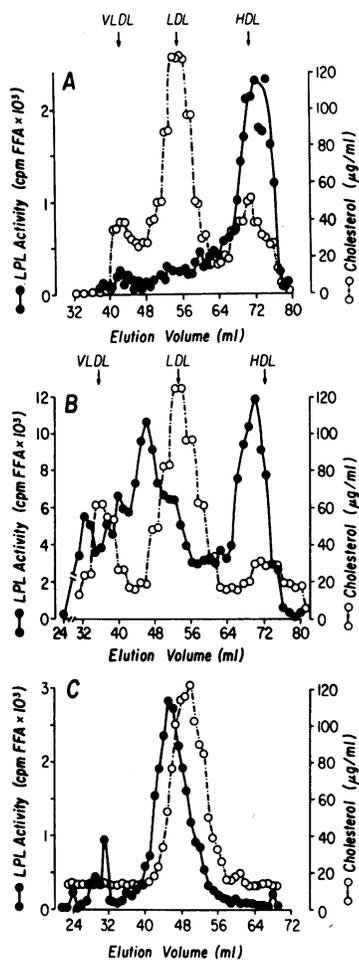


Figure 3. Gel filtration of purified human LPL. (A) Preheparin plasma was dialyzed against 1 M NaCl, and then mixed with purified human milk LPL. 2 ml of this preparation was then gel filtered in high salt buffer on a 1.0×120 -cm column containing 6% agarose, and column fractions were assayed for LPL activity and cholesterol. (B) Preheparin plasma was mixed with purified human milk LPL before chromatography. 2 ml of this preparation was gel filtered on a 1.0×120 -cm column containing 6% agarose, and column fractions were assayed for LPL activity and cholesterol. (C) LDL isolated by ultracentrifugation were mixed with purified human milk LPL. This preparation was gel filtered on a 1.0×120 -cm column containing 6% agarose, and column fractions were assayed for LPL activity and cholesterol. The elution volumes of VLDL, LDL, and HDL are indicated.

after the HDL cholesterol peak. This second peak was noted after gel filtration of preheparin plasma from subjects 2, 5, and 6. This late-eluting activity may be due to an effect of the high concentration of heparin in these samples, which were drawn into heparin-containing tubes.

Ultracentrifugation of PHP. PHP was also fractionated by ultracentrifugation at $d = 1.006, 1.019, 1.063,$ and 1.22 g/ml with both KBr- and sucrose-containing solutions. Most of the recovered LPL and HTGL activity (>80%) was found in the

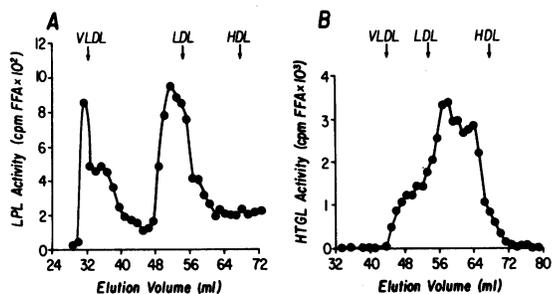


Figure 4. Gel filtration of hyperlipoproteinemic plasma. PHP was obtained from a patient with type V hyperlipoproteinemia (subject 9) and gel filtered on a 1.0×120 -cm column containing 6% agarose. (A) Column fractions were assayed for LPL and cholesterol. (B) Elution of HTGL activity after gel filtration of PHP from subject 7 with type IV hyperlipoproteinemia. The elution volumes of VLDL, LDL, and HDL are indicated.

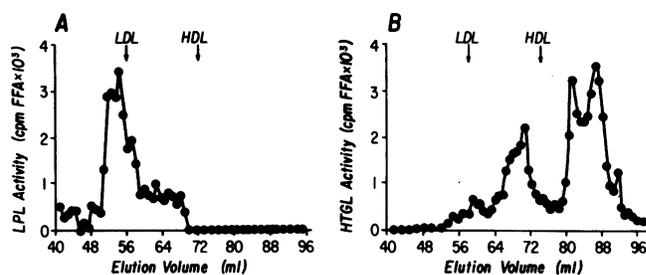


Figure 5. Gel filtration of preheparin plasma. Plasma obtained from subject 4 was gel filtered on a 1.0×120 -cm column containing 6% agarose. Column fractions were assayed for LPL and HTGL activities with a high specific-activity emulsion. The elution volumes of LDL and HDL are indicated.

infranant. <5% of total LPL activity in PHP was recovered after ultracentrifugation of PHP, adjusted to $d = 1.063$ and 1.21 g/ml with KBr solutions, perhaps due to irreversible inactivation of LPL by high salt concentration. Ultracentrifugation of PHP with sucrose solutions resulted in a higher percentage of LPL activity (35–64%) recovered with <20% of the lipase activity in the lipoprotein-containing supernatants. Therefore, an association of LPL and HTGL with lipoproteins was not found after ultracentrifugation of PHP.

Immunoaffinity chromatography of PHP. The percentages of LPL activity found in the eluate after immunoaffinity chromatography of PHP on anti-apo A-I, apo E anti-LDL Sepharose columns are shown in Table II. LPL activity was decreased by elution through columns containing antibodies to apo B and apo E to 40% (range 13–60%) and 60% (range 36–70%) of that found in the eluate from the control column, respectively. These results suggest that some LPL activity is associated with lipoproteins containing apo B and apo E. Hepatic lipase activity was also measured in the immunoaffinity experiments. While some HTGL activity (<50%) was removed by the anti-LDL column, we have been unable to obtain reproducible results in experiments ($n = 10$) using anti-apo A-I and anti-apo E immunoaffinity gels. The reasons for this lack of reproducibility are not apparent, but because variability occurred with different aliquots of PHP from a single subject, the effects of *in vitro* lipolysis before the defrosted PHP samples were applied to the immunoaffinity columns may have been important.

Discussion

Both LPL and HTGL activities co-eluted with particles the size of lipoproteins on gel filtration of PHP. LPL in PHP eluted with

Table II. Recovery of LPL Activity After Immunoaffinity Chromatography

Anti-apo A-I Sepharose	Anti-apo B Sepharose	Anti-apo E Sepharose	Sepharose CL-4b
111.7±22.4	40.3±18.3	60.0±12.8	100%

1 ml of PHP was applied to each column and eluted with PBS containing 1% BSA. 10 3-ml fractions were collected from each column and assayed in triplicate for LPL activity. The total recovered activity was divided by the activity recovered from the control (Sepharose CL-4B) column. Shown are means±SD for six experiments.

lipoproteins slightly larger than LDL. When purified human milk LPL was mixed with plasma or LDL, a peak of LPL activity eluted in a similar position. In addition, LPL activity was partially removed by immunoaffinity chromatography of PHP on anti-apo B and anti-apo E Sepharose, suggesting that LPL was attached to lipoproteins. Gel filtration of hyperchylomicronemic plasma produced an additional peak of LPL activity in the void volume of the column, where chylomicrons and chylomicron remnant particles elute.

The final metabolic product of LPL-mediated hydrolysis of VLDL may be an apo E-containing particle slightly larger than circulating LDL. Deckelbaum et al. incubated VLDL with bovine milk LPL and produced a particle slightly larger than native LDL (32). Blum et al. (27) and Gibson et al. (29) demonstrated the association of apo E with lipoprotein particles eluting with and just before the major peak of LDL cholesterol. Our studies showing elution of LPL activity in the same area suggested that LPL may be associated with this apo B- and apo E-containing lipoprotein species. Immunoaffinity experiments support this hypothesis. Rubinstein et al. (33) have postulated that this particle, which is increased in subjects deficient in HTGL, is the final metabolic product of LPL hydrolysis of triglyceride-rich lipoproteins. Since rapid intravascular hydrolysis of lipid occurs after intravenous heparin, it is possible that LPL in PHP is associated with lipoproteins that under normal physiological conditions are remodeled by interaction with HTGL, cellular receptors, or lipid transfer proteins. The second major lipoprotein substrate for LPL is chylomicrons. We hypothesize that the additional finding of a peak of LPL activity in the void volume of hyperchylomicronemic plasmas may represent LPL bound to chylomicron remnants.

HTGL activity eluted after the peak of LDL cholesterol and before the peak of HDL cholesterol. This enzyme has been postulated to be involved in the metabolism of HDL and some subclasses of LDL. An apo E-rich subclass of particles has been described that elutes before HDL during gel filtration (27, 29). HTGL in PHP may co-elute with these lipoproteins. The association of HTGL with small LDL during gel filtration may suggest physiological actions of this lipase. A role for HTGL in the production of denser, more atherogenic LDL from larger particles within the LDL size and density range has been postulated by other investigators (13, 34).

The nature of the association between lipase activities and lipoproteins was explored by examining the effects of ultracentrifugation and gel filtration in 1 M NaCl. These studies suggested that the interaction between lipases and lipoproteins may in part be ionic. Bengtsson and Olivecrona reported that the *in vitro* association of bovine LPL with some triglyceride-containing substrates was inhibited by addition of high salt (35). The later elution of LPL when PHP was gel filtered in the presence of high salt concentrations may be due to conformational changes in the enzyme molecules that altered their binding to lipoproteins.

The recovery of $\leq 50\%$ of LPL activity after gel filtration was not an unexpected finding. This enzyme rapidly loses its activity when it is stored in physiological buffers. In the absence of an assay for LPL protein, our studies were limited to measurements of enzyme activity. It is therefore conceivable that some of the activity in PHP is not associated with lipoproteins. This activity of free LPL may be less stable than the enzyme attached to lipoproteins. If this were the case, then non-lipoprotein-asso-

ciated LPL may have been rapidly inactivated and would not have been detected in the gel filtration experiments.

The association of LPL with lipoproteins in PHP and pre-heparin plasma has a number of physiological implications. LPL, as well as apo E, has been postulated to augment removal of circulating lipoproteins. Felts et al. demonstrated that remnant lipoproteins in the rat bound LPL, and that LPL and these lipoproteins were removed by the liver at the same rate, presumably as the enzyme-lipoprotein complex (36). These workers hypothesized that LPL functioned as the signal for removal of remnant lipoproteins by the liver. Alternatively, the binding of LPL to this class of lipoproteins may serve as the mechanism for removal of LPL by the liver. This in turn may play an important role in the regulation of the amount of LPL available on the endothelial surface to interact with circulating lipoproteins. Finally, LPL has been shown to function as a cholesterol-transfer protein (37). In this capacity, lipoprotein-associated LPL may be involved in the final remodeling of VLDL remnants to LDL.

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