Preheparin lipoprotein lipolytic activities: relationship to plasma lipoproteins and postheparin lipolytic activities

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Abstract To determine the putative metabolic relevance of preheparin versus postheparin lipoprotein lipases, the relationships of both pre- and postheparin lipoprotein lipase (LPL) and hepatic triglyceride lipase (HTGL) to plasma triglycerides, low density lipoprotein (LDL) cholesterol, and high density lipoprotein (HDL) cholesterol were determined in 93 men. Relationships of preheparin lipases to their respective postheparin lipases were also examined. Although relationships between the preheparin lipases and plasma triglycerides and HDL cholesterol were not apparent, both preheparin LPL (r_s = 0.306, P = 0.0036) and HTGL (r_s = 0.348, P = 0.0008) correlated with LDL cholesterol, a relationship not seen with either postheparin lipase. Both postheparin LPL (r_s = 0.515, P = 0.0001) and postheparin HTGL (r_s = -0.228, P = 0.0028), however, correlated with HDL cholesterol. In addition, postheparin LPL was inversely correlated with postheparin HTGL (r_s = -0.363, P = 0.0003), whereas the relationship between preheparin LPL and preheparin HTGL was positive (r_s = 0.228, P = 0.0009). ■■ Overall, these data point to differences between pre- and postheparin lipases in their relationships to lipoproteins, and one to another. The relationships of LDL cholesterol to both preheparin LPL and HTGL suggest that displacement of active forms of both lipases from their endothelial binding sites may mark triglyceride-rich lipoproteins or their remnants for metabolic pathways that lead to LDL.—**Glaser, D. S., T. J. Yost, and R. H. Eckel.** Preheparin lipoprotein lipolytic activities: relationship to plasma lipoproteins and postheparin lipolytic activities. *J. Lipid Res.* 1992. **33:** 209–214.

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Measurements of lipoprotein lipases in plasma have been routinely carried out after the intravenous injection of heparin (1). Because of the high binding affinity of heparin for the lipases (2), the enzyme bound to endothelial surfaces is rapidly released by heparin from capillary beds into plasma. Peak activities can be measured 10–15 min after the intravenous injection of heparin (3). Our laboratory has developed a method to measure both lipases in plasma without the need of intravenous heparin (4). In a limited number of subjects (n = 12), the amount of preheparin LPL and HTGL correlated with the respective activities measured after the injection of intravenous heparin. However, comparisons between preheparin and postheparin lipolytic activities have not been carried out in large numbers of subjects. Moreover, the relationships of the preheparin enzymes to plasma lipids and lipoproteins have not been established.

The purpose of this study was to determine whether the relationships of LPL and HTGL in preheparin plasma with plasma triglycerides, low density lipoprotein cholesterol (LDL-C), and high density lipoprotein cholesterol (HDL-C) were similar to those of postheparin LPL and HTGL. To further consider the metabolic relevance of the lipases, relationships between the preheparin and postheparin lipases were also examined. The data to follow reveal interesting differences between the preheparin and postheparin lipases that may have important implications in lipoprotein metabolism.

MATERIALS AND METHODS

Blood was obtained on 12 different days from 93 informed and consenting male volunteers between the ages of 20 and 54. Subjects on medications known to affect carbohydrate or lipid metabolism and patients with diabetes mellitus, renal, hepatic, and/or oncologic diseases were excluded. Serum creatinine, BUN, glucose, electrolytes, SGOT, SGPT, bilirubin, and alkaline phosphatase were normal in all subjects.

Abbreviations: LDL, low density lipoproteins; HDL, high density lipoproteins; LPL, lipoprotein lipase; HTGL, hepatic triglyceride lipase; PLA, preheparin lipolytic activity; LRP, LDL receptor-related protein.

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Only males were studied in order to avoid potential but unproven variation in the lipases associated with the menstrual cycle. Blood for preheparin lipase was collected into heparinized tubes (Vacutainer, 1000 µg heparin sodium/10-ml tube) and immediately placed on ice. All preheparin samples were collected after an overnight fast of at least 12 h. Blood for postheparin plasma was collected in the same manner, but 15 min after an intravenous bolus injection of 100 IU heparin sodium/kg body weight (porcine intestine, Elkins-Sinn, Dallas, TX). Fasting plasma triglycerides (5) and cholesterol (6) were determined enzymatically. Separation of HDL was accomplished by use of the dextran sulfate–magnesium chloride precipitation method of Warnick, Benderson, and Albers (7). LDL-C was calculated according to the equation of Friedewald, Levy, and Fredrickson (8).

Assays of total plasma lipolytic activity and post-heparin lipolytic activity were performed after 0.5 ml of plasma was placed over a 0.5 ml column of heparin-Sepharose 6B (Pharmacia, Piscataway, NJ) that had been previously equilibrated in 0.02 M barbital sodium 0.3 M NaCl buffer, pH 7.4, at 4°C (4), a modification of the method of Boberg et al. (9) for human post-heparin plasma. After washing the column three times with 0.5 ml of the buffer, enzyme activity was eluted with 1.5 ml of barbital sodium 0.3 M NaCl buffer containing 6 mg/ml heparin sodium (Fisher, Fair Lawn, NJ). Postheparin plasma was diluted 1:25 and also assayed.

For lipase assays, two different substrates were used for the specific measurement of total lipase and HTGL activities. New substrates were prepared each assay day. For total preheparin lipolytic activity (PLA), the substrate was prepared with 10 mg triolein (Sigma, St. Louis, MO), 8 µCi [1-^{14}C]triolein (Amersham, Arlington Heights, IL), and 0.48 mg egg phosphatidylcholine (Calbiochem-Behring, La Jolla, CA). After drying under nitrogen, lipid components were emulsified in a 4-ml mixture of 10% fatty acid-poor bovine serum albumin (Miles, West Haven, CT), pooled normal human serum, 2 M Tris buffer (pH 8.2), and distilled water (0.8:1.3:1.0:0.9) by 100 sec of sonication (10 sec on followed by 10 sec off for 10 cycles) with a sonicator (model W-220F, Heat Systems-Ultrasonics, Plainview, NY) at 4°C. For HTGL the substrate was altered by the addition of NaCl to a final concentration of 3.89 M. The final pH was adjusted to 8.6 with 2 M Tris-HCl. Serum was omitted, and substrate volume was maintained with water. Using a standard post-heparin plasma, the interassay coefficient of variation for total lipase activity was 10.5% (n = 12); for HTGL, 11.4% (n = 12). Intra-assay variation was 3.3 ± 0.3% for total lipase activity, and 4.8 ± 0.5% for HTGL.

After heparin elution from heparin-Sepharose 6B, 0.15-ml aliquots of enzyme eluate were incubated with 0.05 ml of each substrate. Before addition of the enzyme, the substrates were preincubated for 90 min at 37°C. The reaction was carried out at 37°C and terminated after 90 min with the fatty acid extraction mixture of Belfrage and Vaughan (10). Reaction vessels were shaken for 5 min on a shaker (Eberbach, Ann Arbor, MI) and centrifuged at 600 g for 20 min. A 0.5-ml aliquot of the upper phase was removed and counted in a scintillation counter (Searle Mark III, Des Plains, IL).

After correction for recovery, results were expressed as nEq FFA liberated over 1 h per ml of plasma. LPL activity was calculated as the difference between the amount of total lipase activity measured using the serum-containing substrate and that measured as preheparin HTGL. Postheparin LPL was calculated as the difference between total postheparin lipolytic activity and postheparin HTGL.

Spearman rank correlations (r_s) were used for statistical analysis.

RESULTS

Fasting lipid data are provided in **Table 1**. LDL cholesterols were calculated for all but 4 of the 93 subjects. These four had triglycerides > 400 mg/dl (4.52 mmol/l). Preheparin and postheparin lipase results are in **Table 2**. The contribution of HTGL and LPL to the total lipolytic pool was similar for pre- and postheparin plasma.

No relationships were seen between the preheparin lipases (LPL, HTGL) and triglycerides or HDL-C. We did, however, find correlations between both postheparin lipases (LPL, HTGL) and HDL-C. Postheparin-LPL was positively correlated with HDL-C (r_s = 0.515, P = 0.0001) (**Fig. 1A**), whereas a weak inverse relationship was seen between postheparin-HTGL and HDL-C (r_s = –0.228, P = 0.028) (Fig. 1B).

TABLE 1. Lipid data

	n		SI Units
			mmol/l
Age	93	30.3 ± 1.0 yr.	
		mg/dl	
Total cholesterol	93	181 ± 4	4.66 ± 0.11
Triglyceride	93	132 ± 10	1.49 ± 0.12
HDL-cholesterol	93	43.1 ± 1.2	1.11 ± 0.03
LDL-cholesterol	89	110 ± 2.9	2.83 ± 0.08

Values are given as mean ± SEM.

TABLE 2. Lipase data

Activities	n	nEq FFA/ml per h
Preheparin plasma		
Total lipase	93	34.6 ± 1.4
Hepatic triglyceride lipase	93	20.9 ± 0.3
Lipoprotein lipase	93	13.7 ± 1.0
Postheparin plasma		
Total lipase	93	$17{,}092 \pm 246$
Hepatic triglyceride lipase	93	$12{,}184 \pm 239$
Lipoprotein lipase	93	$4{,}907 \pm 245$

Values are given as mean ± SEM.

Preheparin lipases, however, did correlate with plasma LDL-C. As portrayed in **Fig. 2**, LDL-C correlated with both preheparin LPL ($r_s = 0.306$, $P = 0.0036$) (Fig. 2A) and preheparin HTGL ($r_s = 0.348$, $P = 0.0008$) (Fig. 2B). No relationships between LDL-C and the postheparin lipases were found.
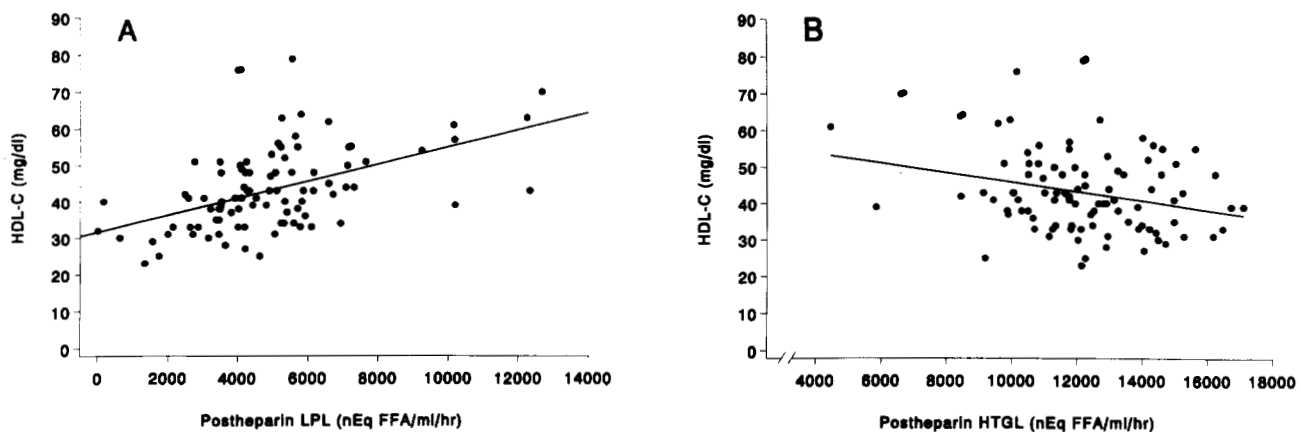
When the preheparin lipases and postheparin lipases were compared, interesting differences occurred. Although the relationship between preheparin LPL and HTGL was positive ($r_s = 0.338$, $P = 0.0009$) (**Fig. 3A**), an inverse correlation was found between the two lipases in postheparin plasma ($r_s = -0.363$, $P = 0.0003$). There was no relationship between postheparin LPL and preheparin LPL in these subjects ($r_s = 0.101$, $P = NS$) (**Fig. 4A**), whereas postheparin and preheparin HTGL were related ($r_s = 0.400$, $P = 0.0001$) (Fig. 4B).

DISCUSSION

As reported previously, relationships between postheparin LPL and HDL (11–14), and HTGL and HDL (13–17), were present in the current report. These relationships likely reflect the putative roles of each of the two lipases in HDL metabolism. LPL is a hydrolytic

enzyme that is rate-limiting for the removal of lipoprotein triglycerides from the circulation (18). During the LPL-dependent hydrolysis of chylomicrons and VLDL, some HDL molecules appear to acquire lipid and protein to become a more buoyant form of HDL, HDL_2 (12, 19). Like LPL, HTGL binds to the glycocalyx on the endothelium where it hydrolyzes triglycerides and phospholipids transported in plasma lipoproteins (20). Unlike LPL, the levels of HTGL in postheparin plasma are inversely related to HDL. Presumably, this reflects the removal of HDL (HDL_2) from the circulation and its uptake and further processing by the liver. The failure of preheparin LPL and HTGL to predict HDL cholesterol, however, provides less support for a physiologic contribution of this smaller pool of lipases to HDL metabolism.

In this study, the relationships of LDL-C to preheparin LPL and HTGL, however, were meaningful and may have physiologic implications. As previously shown, LPL can bind to circulating lipoproteins (21–23). Although this interaction may have little to do with the in vivo hydrolytic function of the lipases, it may direct lipoproteins for further processing, i.e., to LDL. As demonstrated by Felts, Itakura, and Crane (22), triglyceride-rich lipoprotein remnants that contain LPL are better recognized by hepatic receptors and are preferentially removed. This is followed by uptake of the particle and generation of LDL with the lipase being inactivated and the remaining lipids further metabolized by the liver. Triglyceride-rich lipoproteins do not readily interact with the LDL receptor or the LDL receptor-related protein (LRP) unless the lipoproteins are enriched with exogenous apoE3 (24, 25). However, as recently observed by Sehayek et al. (26), lipolysis of human and rat VLDL exposes unreactive endogenous apolipoprotein E-3, and possibly apolipoprotein B-100, which permits the more effi-

Fig. 1. HDL cholesterol and postheparin lipases. Plasma HDL cholesterol concentrations in mg/dl are plotted against LPL (nEq FFA/ml/h) (A) and HTGL (nEq FFA/ml/h) (B) obtained 15 min after the injection of 100 IU/kg heparin sodium into 93 men. For LPL, $r_s = 0.515$, $P = 0.0001$; for HTGL, $r_s = -0.228$, $P = 0.028$.
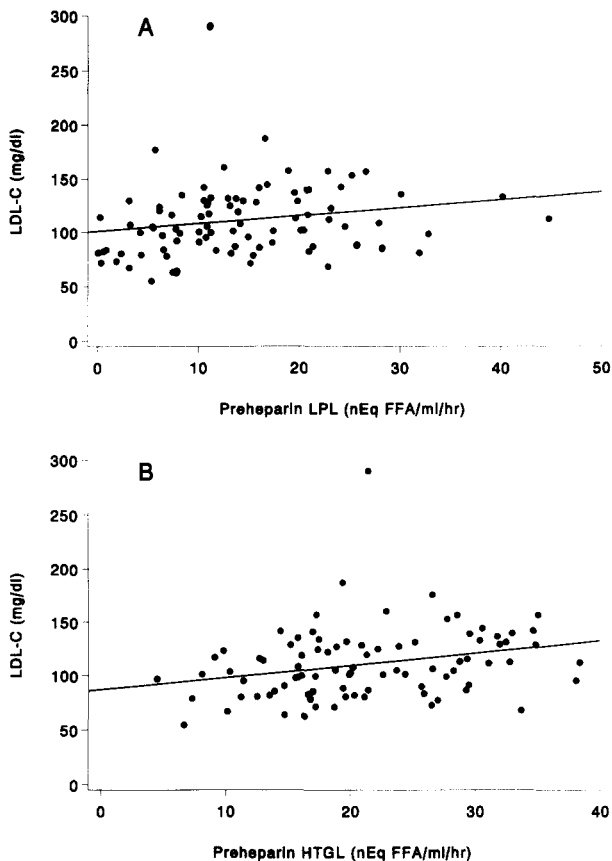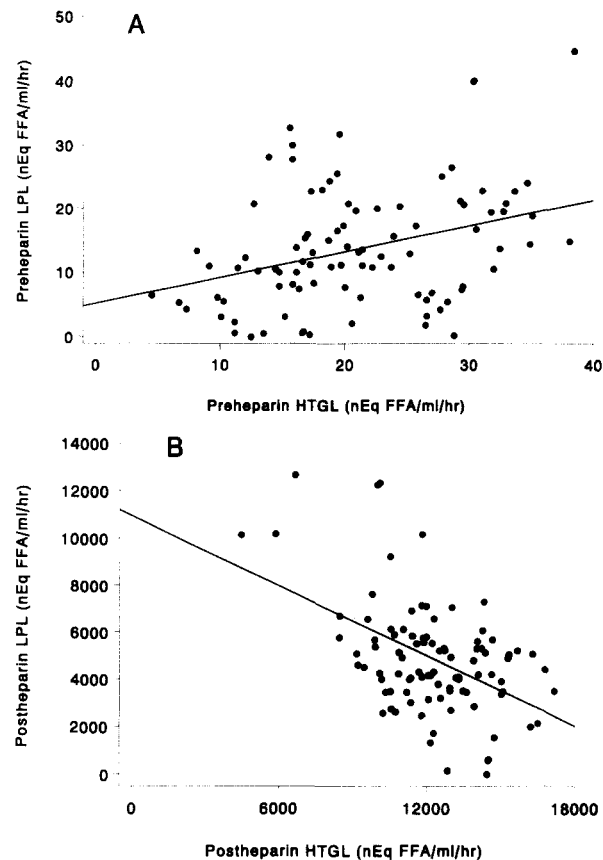
Fig. 2. LDL cholesterol and preheparin lipases. Plasma LDL cholesterol concentrations in mg/dl are plotted against LPL (nEq FFA/ml/h) (A) and HTGL (nEq FFA/ml/h) (B) obtained in plasma from 89 men after a 12-h fast. For LPL, $r_s = 0.306$, $P = 0.0036$; for HTGL, $r_s = 0.348$, $P = 0.0008$.

Fig. 3. Relationships between LPL and HTGL. In A, preheparin LPL is plotted against preheparin HTGL; in B, postheparin LPL is plotted against postheparin HTGL (see Fig. 1). For preheparin plasma $r_s = 0.338$, $P = 0.0009$; for postheparin plasma, $r_s = 0.363$, $P = 0.0003$.

cient and rapid removal of these particles. The possibility that the presence of LPL on lipoprotein surfaces further directs the hydrolyzed lipoproteins to LDL or LRP receptors, however, was not assessed in this group of subjects.

The factors which control the amount of LPL and HTGL attached to circulating lipoproteins are not known. As shown by Saxena, Witte, and Goldberg (27) and Peterson et al. (28), LPL can be displaced from endothelial binding sites by lipolysis products. Yet, most of this lipase is inactive. Berr, Eckel, and Kern (29) have previously shown that the injection of heparin increases LPL activity up to 1000-fold. Yet, the intravenous injection of heparin increases LPL mass by only 10-fold (30). Although the relationships between the preheparin lipases and LDL-C shown in this study could also exist for the inactive lipases, we have no such data at present.

Another mechanism by which active lipases could be released and sustained in preheparin plasma could be the action of circulating glycosaminoglycans. As previously shown by Staprans and Felts (31), glycosa-

minoglycans circulate bound noncovalently to plasma proteins. Although little is known about the physiologic role of glycosaminoglycans in plasma, heparan sulfate isolated from human plasma stimulated LPL in vitro several fold. Presumably, this effect of heparan sulfate represents, at least in part, the ability of glycosaminoglycans to stabilize the lipase (32). The displacement of LPL (and HTGL) from endothelial binding sites could thus serve to maintain the active form of the lipase(s) in circulating plasma. The relationship of LDL-C to preheparin LPL and HTGL could be attributed to levels of heparan sulfate in plasma. The relative rate of conversion of remnants to LDL could therefore be determined by the concentration of heparan sulfate:LPL:lipoprotein complexes, a hypothesis yet to be tested.

The potential for such a role of heparan sulfate is further suggested by the positive relationships between preheparin LPL and preheparin HTGL. Such a relationship was not found for the postheparin enzymes. In fact, the inverse relationship between the postheparin lipases could reflect the nonphysiologic set-
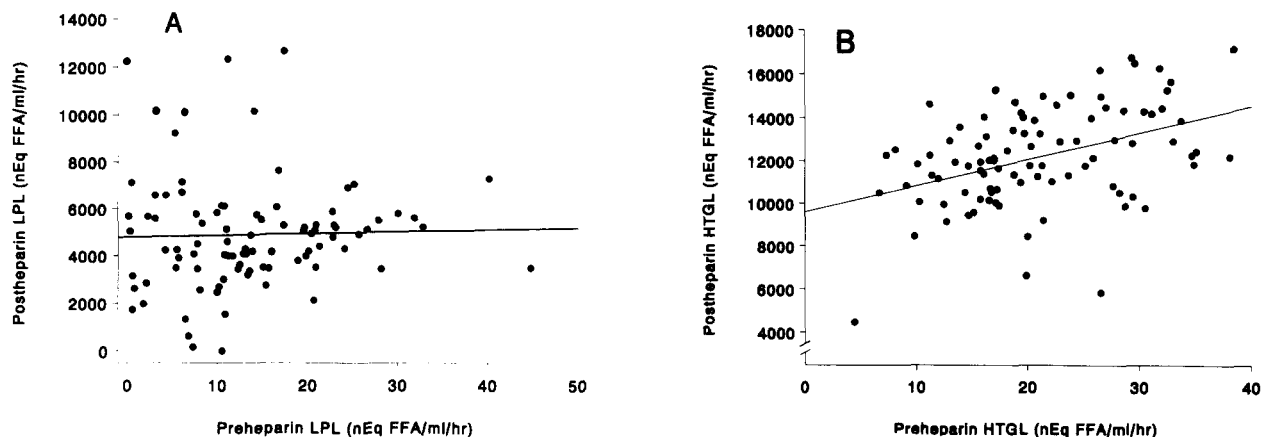
Fig. 4. Relationships between preheparin and postheparin lipases. In A, postheparin LPL is plotted against preheparin LPL. In B, postheparin HTGL is plotted against preheparin HTGL. For LPL, $r_s = 0.101$, $P = NS$; for HTGL, $r_s = 0.400$, $P = 0.0001$.

ting in which the postheparin lipases are quantified. In a previous study involving 12 subjects (4) we observed statistically significant correlations between postheparin LPL and preheparin LPL ($r_s = 0.685$, $P < 0.05$) and between postheparin HTGL and preheparin HTGL ($r_s = 0.774$, $P < 0.02$). In the current study only the relationship between postheparin and preheparin HTGL remained. An explanation for this discrepancy is not apparent.

In summary, evidence is provided that preheparin and postheparin lipases differ in their relationships to circulating lipoproteins and one to another. Whereas the relationships of postheparin lipases to HDL are only seen in the nonphysiologic setting after intravenous heparin administration, the relationships of preheparin LPL and HTGL to LDL-C and to one another are seen in unperturbed plasma and may have relevance to the physiology of lipoprotein metabolism.

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