Plasma Lipolytic Activity

Relationship to Postheparin Lipolytic Activity and Evidence for Metabolic Regulation

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Lipolytic activity was measured in human plasma without prior administration of intravenous heparin. Eluted from heparin-Sepharose in a barbital buffer containing 6 mg/ml heparin, plasma lipolytic activities in 20 subjects were distributed between hepatic triglyceride lipase (HTGL, mean ± SE 60.6 ± 4.6%) and extrahepatic lipoprotein lipase (LPL, 39.4 ± 4.6%). Confirmation of the identities of HTGL and LPL was provided by inhibitory antisera. Preheparin LPL activity was absent in plasma from a patient with type I hyperlipoproteinemia. Both preheparin HTGL and LPL activities correlated with the respective activities measured in plasma obtained 15 min after intravenous injection of heparin (r_s = +.774 and +.685, respectively; n = 12). Evidence for the metabolic regulation of preheparin lipases was provided by measurement of significant increases in LPL and HTGL activities after oral glucose ingestion. Overall, preheparin plasma HTGL and LPL activities may reflect ongoing lipoprotein lipolytic activity in tissue beds, and because these measurements do not require the administration of intravenous heparin, they should prove useful for additional studies of short-term regulation of the lipases. *Diabetes* 37:610–615, 1988

Human plasma triglyceride lipolytic activity has been effectively determined only after the release of tissue lipases from peripheral capillary beds with intravenous heparin (1). This postheparin lipolytic activity (PHLA) includes predominantly hepatic triglyceride lipase (HTGL) and lipoprotein lipase (LPL) as well as other lipases such as monoglyceride hydrolase (2) and phospholipase (3), which have lesser hydrolytic activities against a triglyceride substrate.

Even when the activities of the separate enzymes can be measured with great specificity, the use of PHLA is not ideal for assessing the metabolic regulation of these triglyceride lipases. The predominant extrahepatic component, LPL, is released from various tissues and may not always correlate with enzyme activity measured directly in the tissue beds (4–6). In addition, heparin-induced anticoagulation both precludes the use of serial tissue biopsies to evaluate the relationship between plasma and tissue lipase activities and is associated with some risk. Finally, short-term metabolic studies with serial measurements of plasma activity are uninterpretable. This is because heparin-mediated release of tissue-bound lipases is probably associated with some depletion of tissue activities that require time for restoration. In addition, the release of lipolytic activities into plasma results in substantial intravascular lipolysis (7), a pharmacologic effect that perturbs lipid levels and may, in turn, alter lipase activities (8).

Studies were therefore undertaken to determine if triglyceride lipolytic activity could be measured in human plasma without prior intravenous injection of heparin. After a method for plasma lipolytic activity (PLA) was established, the relationship of PLA to PHLA was determined. Finally, experiments were carried out to examine the possibility that PLA could be altered by metabolic perturbation.

MATERIALS AND METHODS

Plasma samples. Blood was obtained from informed and consenting normal-weight human volunteers, all <45 yr of age. Blood for PLA was collected into EDTA (1 mg/ml) or heparinized tubes (Vacutainer, 143 USP U or 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. Fasting triglyceride (9) and cholesterol (10) were determined enzymatically. High-density lipoprotein (HDL) cholesterol was measured in plasma from which very-low- and low-density lipoproteins were precipitated by 4% sodium phosphotungstate (11). The HDL cholesterol was

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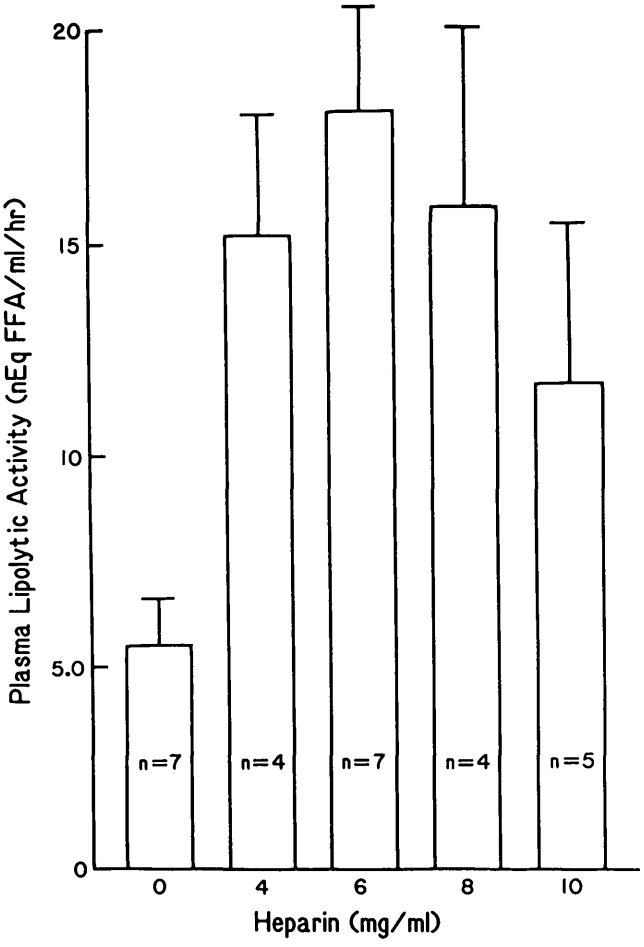

FIG. 1. Effect of heparin concentration of eluting buffer on recovery of plasma lipolytic activity (PLA) from heparin-Sepharose. One-half milliliter of heparinized plasma was placed onto 0.5 ml heparin-Sepharose 6B, which had been equilibrated and washed 3 times in 0.02 M barbital sodium buffer–0.3 M NaCl (pH 7.4, 4°C). Enzyme activity was eluted with 1.5 ml barbital sodium buffer containing 4–10 mg/ml heparin sodium. Data shown for 0 mg/ml heparin were obtained from measurements made in heparinized plasma before heparin-Sepharose chromatography. PLA was measured as described in MATERIALS AND METHODS. Data are means ± SE. *n* = number of samples of each heparin concentration. FFA, free fatty acids.

measured in the supernatant after centrifugation at 1500 × *g* for 30 min at 4°C.

In 15 subjects (8 men, 7 women), 75 g of oral glucose was administered, and samples for PLA, triglycerides, glucose, and insulin were obtained 30, 60, 120, 180, 240, 300, and 360 min later. Similar measurements were made in control subjects (2 men, 3 women), who did not ingest glucose. Glucose was measured by a glucose oxidase technique with the Beckman glucose analyzer (Fullerton, CA); insulin was measured by double-antibody radioimmunoassay (12). After fasting measurements, 12 additional subjects (4 men, 8 women) were injected with 100 IU heparin sodium/kg body wt i.v. (porcine intestine, Elkins-Sinn, Dallas, TX), and 15 min later, blood for PHLA was collected and placed on ice. Plasma was separated by centrifugation for 30 min at 480 × *g*. All pre- and postheparin plasmas were assayed the day they were obtained.

Assay of lipolytic activities. Initial experiments were performed by addition of 0.05 ml of plasma to 0.15 ml of substrate as outlined below. Additional assays of PLA 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 buffer–0.3 M NaCl, pH 7.4 at 4°C, a modification of the method of Boberg et al. (13) for human postheparin plasma. After washing 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 4–10 mg/ml heparin sodium (Fisher, Fair Lawn, NJ). Postheparin plasma was diluted 1:25 and also assayed after column elution.

Two substrates were used for the specific measurement of LPL and HTGL activities. For total 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 H_2O (0.8:1.3:1.0:0.9) by 100 s of sonication (10 s on followed by 10 s off for 10 cycles) with a sonicator (model W-220F, Heat Systems-Ultrasonics, Plainview, NY) at 4°C. For hepatic triglyceride lipase 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 H_2O.

Enzyme substrate incubations. After heparin elution from heparin-Sepharose 6B, 0.15-ml aliquots of enzyme effluent 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 Vaughn (14). 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 Plaines, IL).

Lipolytic activity after inhibition with goat anti-human HTGL and LPL serum. The preparation and characteristics of the antisera against HTGL (15) and LPL (16) have been described previously. For these studies, HTGL and LPL activities were measured in preheparin plasma from six normal-weight subjects after an overnight fast. Blood was collected in heparinized tubes and placed on ice. Plasma was separated by centrifugation and passed over heparin-Sepharose as outlined above. Subsequent to elution with 0.02 M barbital sodium, 0.3 M NaCl buffer containing heparin sodium (6 mg/ml), immunoinhibition was accomplished by dilution of postcolumn effluent with anti-LPL (1:1; 5 μg IgG) or anti-HTGL (1:5) and a subsequent 60-min incubation at 4°C. Nonimmune goat serum served as a control for HTGL and mouse IgG for LPL. The enzyme effluent was then incubated with lecithin-stabilized triolein substrate for 90 min at 37°C. Assays for PLA were then performed as described above.

Recoveries and expression of results. For both total lipolytic activity and HTGL (pre- and postheparin), upper-phase fatty acid recoveries were determined with a standard solution of [1-^{14}C]oleic acid in the presence of substrate. Mean ± SE for recoveries was 68.4 ± 1.2%. After correction for recovery, results were expressed in nanoequivalents of

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TABLE 1
Subject profile and fasting data

Subject	Sex	Glucose (mg/dl)	TG (mg/dl)	Chol (mg/dl)	HDL-chol (mg/dl)	Insulin (μU/ml)	PLA (neg · ml^{-1} · h^{-1})	HTGL (neg · ml^{-1} · h^{-1})	LPL (neg · ml^{-1} · h^{-1})
Oral glucose									
1	M	93	61	161	34	10	9.9	4.6	5.3
2	F	73	59	172	57	10	10.4	3.5	6.9
3	M	82	63	172	47	45	35.8	28.8	7.0
4	F	80	48	208	64	21	42.9	25.5	17.4
5	F	90	183	247	75	15	8.2	7.0	1.2
6	F	60	89	165	64	28	15.2	7.7	7.5
7	M	90	56	202	84	24	10.2	8.0	2.2
8	M	88	50	119	32	21	11.2	7.4	3.8
9	M	89	104	225	46	31	7.6	5.5	2.1
10	M	89	61	207	61	8	22.5	14.5	8.0
11	F	101	77	163	35	41	29.0	30.0	0.0
12	F	84	95	183	76	21	17.7	11.2	6.5
13	F	77	80	172	66	10	21.0	18.1	2.9
14	M	81	118	204	65	65	24.5	8.1	16.4
15	M	85	115	238	33	26	19.7	6.3	13.4
Mean ± SE		84 ± 2	84 ± 9	189 ± 9	56 ± 4	25 ± 4	19.1 ± 2.7	12.4 ± 2.3	6.7 ± 1.4
Saline control									
1	M	87	69	170	49	9	12.0	8.2	3.8
2	F	89	78	183	47	19	16.2	8.5	7.7
3	F	64	80	166	48	15	18.9	7.7	11.2
4	F	75	123	181	48	6	22.0	6.7	15.3
5	M	77	87	154	46	33	13.2	8.6	4.6
Mean ± SE		78 ± 5	87 ± 9	171 ± 5	48 ± 1	16 ± 5	16.5 ± 1.8	7.9 ± 0.4	8.5 ± 2.1
Mean ± SE (n = 20)		83 ± 2	85 ± 7	185 ± 7	54 ± 3	23 ± 3	18.4 ± 2.1	11.3 ± 1.0	7.7 ± 1.0

TG, triglycerides; HDL-chol, high-density lipoprotein cholesterol; PLA, plasma lipolytic activity; HTGL, hepatic triglyceride lipase; LPL, lipoprotein lipase.

fatty acid liberated over 1 h per milliliter of plasma. LPL activity was calculated as the difference between PLA and HTGL. Nonparametric statistics including the Mann-Whitney U and Spearman rank-correlation coefficient were used for statistical analyses.

RESULTS

Establishment of conditions for PLA. The substrate used for PLA determination contained [1-^{14}C]triolein at 0.8 μCi/mg triolein and provided a final triolein concentration of 625 μg/ml. When cold triolein concentrations were reduced to 500 and 312.5 μg/ml, total lipolytic activities were minimally affected (98 ± 7 and 88 ± 5%, respectively; n = 6). A further decrease in cold triolein concentration (125 μg/ml) resulted in a total loss of measurable lipolytic activity. The hydrolytic rate was maximized by preincubation of the sonicated substrate for 90 min at 37°C. Product formation was linear for 120 min but was routinely terminated at 90 min.

Initial experiments demonstrated the presence of lipolytic activity in fasting human plasma obtained from blood drawn into either EDTA- or heparin-coated collection tubes. However, much greater activity was present in heparinized (7.5 ± 1.1 neq · ml^{-1} · h^{-1}, n = 45) than EDTA plasma (1.5 ± 0.4 neq · ml^{-1} · h^{-1}, n = 14). The amount of heparin present in Vacutainer tubes (1000 μg) proved maximal, and these tubes were in turn used for further studies.

Although the addition of Ca^{2+} (3.3–73.6 μm) increased EDTA PLA, the activity remained <40% of that present in heparinized plasma. Moreover, when heparin was added to EDTA plasma, no increase in PLA occurred. Thus, immediate exposure of blood to heparin was important for preservation of maximum lipase activity.

To maximize PLA, 0.5 ml of heparinized plasma was placed over heparin-Sepharose 6B and eluted with 0.02 M barbital sodium–0.3 M NaCl buffer containing heparin (4–10 mg/ml) after the column was washed with three volumes of barbital buffer. Before loading onto heparin-Sepharose, PLA was 5.6 ± 1.1 neq · ml^{-1} · h^{-1}. The presence of heparin in eluting buffers increased PLA (Fig. 1), with 6 mg/ml of heparin producing the greatest effect (18.3 ± 2.4 neq · ml^{-1} · h^{-1}, n = 7).

Identification of lipase activities in PLA. Under enzyme substrate conditions where LPL activity is inhibited (absence of serum in the substrate, 1 M NaCl), HTGL activity was

TABLE 2
Preheparin plasma lipolytic activity: effect of anti-LPL and anti-HTGL serum

Patient	Preheparin lipolytic activity (neq · ml^{-1} · h^{-1})			
	Nonimmune serum or IgG	+Anti-LPL	+Anti-HTGL	+Anti-LPL +Anti-HTGL
1	23.2	21.0	4.3	0.1
2	13.5	10.3	5.5	0.4
3	14.6	8.0	8.1	0.2
4	11.0	8.1	1.4	0.2
5	11.8	9.9	4.0	0.4
6	18.8	18.1	3.2	0.1
Mean ± SE	15.5 ± 1.9	12.6 ± 2.3	4.4 ± 0.9	0.2 ± 0.1

LPL, lipoprotein lipase; HTGL, hepatic triglyceride lipase.

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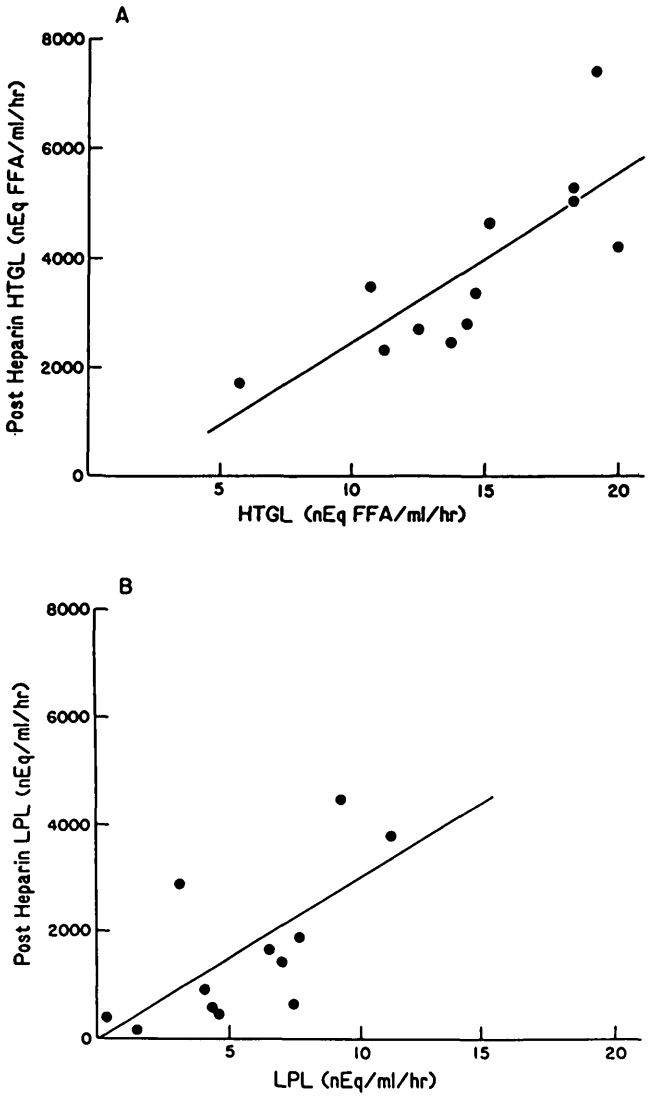

FIG. 2. Correlation of plasma lipolytic activity with postheparin lipoprotein lipase (LPL) and hepatic triglyceride lipase (HTGL) activities. Comparisons of postheparin lipolytic HTGL (*A*) and LPL (*B*) with preheparin HTGL and LPL were carried out in 12 subjects after overnight fast. After heparinized plasma was obtained, subjects were injected with 100 IU heparin sodium/kg i.v., and 15 min later postheparin plasma was obtained. Both plasma lipolytic and postheparin lipolytic activities were eluted from heparin-Sepharose 6B and measured as described in MATERIALS AND METHODS. FFA, free fatty acids. Spearman rank-correlation coefficients were significant for both: *A*, $r_s = .774$, $P < .02$; *B*, $r_s = .685$, $P < .05$.

determined in plasma samples after elution from heparin-Sepharose. A distribution of PLA, HTGL, and LPL data for 20 subjects (10 men, 10 women) is listed in Table 1. Fasting PLA, HTGL, and LPL were similar in men and women. HTGL represented 60.7 and 60.6% of PLA in men and women, respectively.

For both PLA and HTGL, established assay conditions resulted in 25 counts/min above blank to approximate 10 neq · ml^{-1} · h^{-1} of enzymatic activity. Intra-assay coefficients of variation for PLA and HTGL were 7.2 and 15.4%, respectively. One subject with impaired glucose tolerance (no. 11) had no measurable fasting LPL.

The biochemical determinations of HTGL and LPL were validated with inhibitory antibodies (15,16). In Table 2, data are presented from six subjects in whom PLA was measured after exposure to anti-HTGL, anti-LPL, anti-HTGL plus anti-LPL, or nonimmune serum. After addition of anti-LPL, the residual lipolytic activity was 81% of that measured in the presence of mouse IgG. Anti-HTGL lowered PLA more extensively (to 28% of control PLA). When both anti-LPL and anti-HTGL were added, only 1.3% of the original PLA remained. Moreover, a patient with known LPL deficiency (type I hyperlipoproteinemia) but normal HTGL activity in postheparin plasma was also studied. As in postheparin plasma, PLA was composed entirely of HTGL; LPL activity was absent. Thus, quantification of LPL in preheparin plasma as the difference between PLA and HTGL was well supported.

Relationship of PLA to PHLA. To determine if preheparin lipolytic activities mirrored those in postheparin plasma, 12 subjects were injected with 100 IU heparin sodium/kg after fasting blood was obtained for PLA (HTGL and LPL). When lipolytic activities in samples obtained 15 min after heparin injection were compared with those carried out in preheparin plasma, both postheparin HTGL and LPL correlated with preheparin HTGL and LPL ($r_s = .744$, $P < .02$ and $r_s = .685$, $P < .05$, respectively; Fig. 2).

PLA regulation. Studies were undertaken to determine the potential for regulation of preheparin plasma activities by metabolic perturbation. Data for fasting PLA, HTGL, LPL, insulin, triglyceride, and glucose are shown in Table 1. After ingestion of 75 g glucose ($n = 15$), PLA was higher at 180, 240, 300, and 360 min than in noningesting control subjects ($n = 5$) (Fig. 3). HTGL activity rose, but significant differences between the two groups (with or without oral glucose ingestion) were seen only at 240 min (Fig. 4). By contrast, the effect of oral glucose on LPL activity was significant at 180, 240, and 360 min after ingestion (Fig. 5). Both HTGL and LPL activities decreased slightly over 6 h in the control subjects (Fig. 5).

No relationship was found between fasting triglycerides or changes in triglycerides after oral glucose and fasting or postglucose PLA, HTGL, or LPL activities. There were also no relationships between fasting triglycerides, HDL cholesterol, or insulin and pre- or postheparin HTGL or LPL.

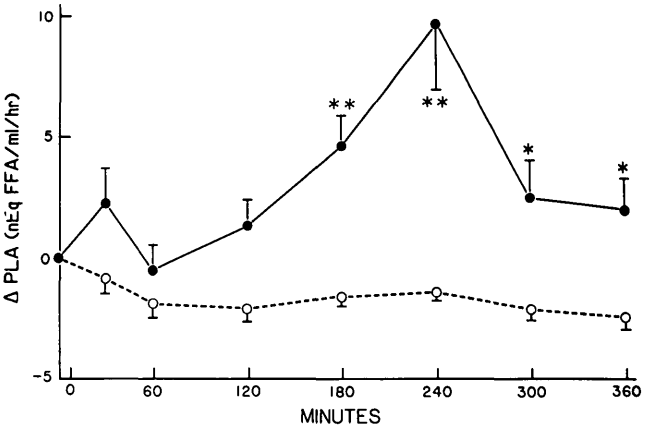

FIG. 3. Response of plasma lipolytic activity (PLA) to oral glucose tolerance test (OGTT). Twenty normal volunteers fasted for 12 h. Fifteen then received 75 g oral glucose. Blood samples were withdrawn as indicated, and PLA was measured in all subjects as described in MATERIALS AND METHODS. Data are means ± SE. FFA, free fatty acids. ●, OGTT; ○, control. *$P < .005$; **$P \leq .001$.

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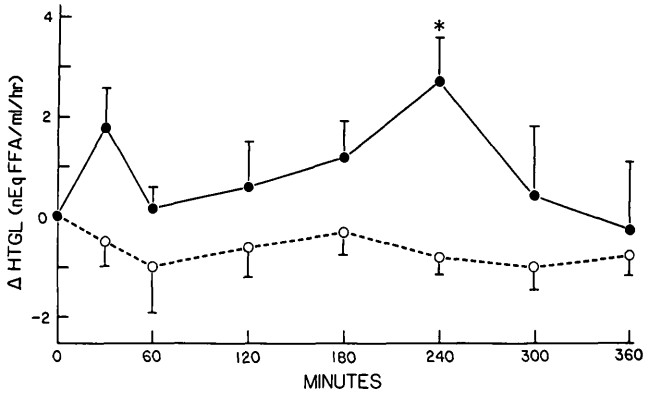

FIG. 4. Response of hepatic triglyceride lipase (HTGL) to oral glucose tolerance test (OGTT). Conditions are described in Fig. 3. HTGL activity was measured as described in MATERIALS AND METHODS. Data are means ± SE. FFA, free fatty acids. ●, OGTT; ○, control. *$P < .01$.

DISCUSSION

Measurements of preheparin lipolytic activity have been reported in rats (17–19), dogs (20), and humans (1,21–23). However, other than in the reports of Chajek-Shaul et al. (19) in rats and Greten et al. (23) in humans, selective measurement of HTGL and LPL activities has not been accomplished. In rats (19), comparisons of preheparin LPL and HTGL with the respective lipolytic activities in postheparin plasma were not made. In the report of Greten et al. (23), despite greater amounts of postheparin HTGL than LPL activity in both normal and hypertriglyceridemic subjects, preheparin plasma HTGL was near 0 $\mu eq \cdot ml^{-1} \cdot h^{-1}$ and LPL was ~1.0 $\mu eq \cdot ml^{-1} \cdot h^{-1}$. In addition, although postheparin LPL activity, in both normal and hypertriglyceridemic subjects, increased during clofibrate administration, no alteration in preheparin LPL occurred.

In this report, the distribution of PLA between HTGL (60.6 ± 4.6%) and LPL (39.4 ± 4.6%) is consistent with published data for postheparin HTGL and LPL in normal subjects (1,17,23), in addition to PHLA activities measured herein (HTGL 71.0 ± 4.9%, LPL 29.0 ± 4.9%). Although preheparin LPL and HTGL activities were very low when compared with values measured in postheparin plasma, the pre- and postheparin values were highly correlated, supporting the relevance of PLA as a measure of triglyceride hydrolytic potential.

To maximize the measurement of preheparin lipolytic activities, modifications of a previously published method for PHLA were required (13). First, PLA in heparinized plasma was found to be fivefold greater than in EDTA plasma. Heparin is not only capable of releasing lipases from their endothelial binding sites into plasma in vivo (24) but also delays the catabolic clearance of ^{131}I-labeled LPL injected into rats (25). Increased PLA when blood is drawn into heparinized tubes suggests either activation of the lipases or impedance of plasma-dependent inhibition of the enzymes. Although lipase activity in crude enzyme preparations is higher after exposure to heparin, this effect does not occur with purified enzyme (26). The addition of heparin to EDTA plasma failed to increase PLA. Thus, an immediate effect of heparin to prevent inhibition and/or destruction seems most likely.

Additional enhancement of PLA occurred after heparinized plasma was eluted from heparin-Sepharose with barbital buffer containing heparin sodium. The ability of heparin-Sepharose to bind plasma lipases, specifically LPL, was originally described by Olivecrona et al. (27) and has been used subsequently for purification of the enzyme (28,29). Elution with buffer containing heparin resulted in an approximately threefold increase in PLA. The ability of heparin-Sepharose chromatography to increase PLA could be due to the removal of *1*) factors that promote destruction of enzymatic activity or *2*) inhibitors of lipolytic activity. Plasma factors that inhibit LPL have been described in patients with severe hypertriglyceridemia (30). Perhaps the variabilities in PHLA and tissue LPL in normal subjects could be partially explained by the relative concentrations of such factors in plasma.

Inhibition of both HTGL and LPL activities by specific antisera provided strong evidence that the biochemical assays were valid determinations for the two lipases. Subsequent to immunoinhibition, the residual and extremely small amount of lipase activity (0.2 ± 0.1 $neq \cdot ml^{-1} \cdot h^{-1}$) could result from either pancreatic lipase or monoglyceride lipase (31). The activity of pancreatic lipase would necessarily be small due to the absence of colipase and bile salts (32) and the presence of known inhibitory substances for pancreatic lipase in the substrate, such as lecithin and albumin (33). Monoglyceride hydrolase activity should be limited by the trace contamination of the labeled triolein preparation with monoglyceride. Moreover, as for postheparin LPL, preheparin LPL activity was maximally inhibited by 1 M NaCl and the absence of serum in the substrate mixture (1,34,35), whereas no reduction of HTGL activity occurred. Preheparin LPL activity was also absent from plasma obtained from an LPL-deficient patient.

Increased PLA after ingestion of oral glucose indicates that PLA may undergo metabolic regulation. This increase was mostly in LPL activity, but some increase in HTGL (at 4 h only) also occurred. In rats, administration of glucose to fasting animals produced a decrease in preheparin LPL (19). This discrepancy may relate to the relative contribution of adipose tissue to PLA in the two species. In rats (36–38) and humans (39), glucose administration has been shown

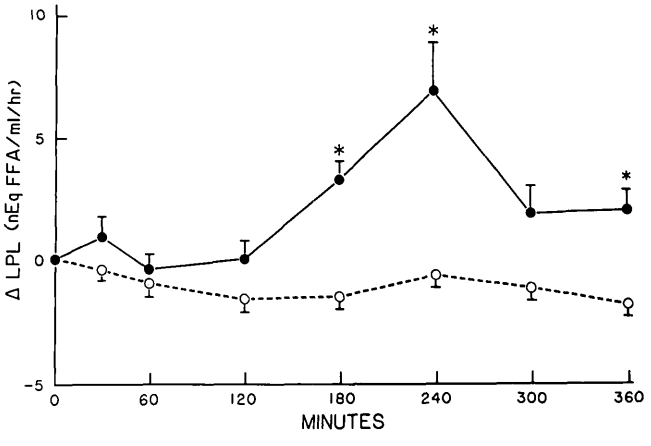

FIG. 5. Response of lipoprotein lipase (LPL) to oral glucose tolerance test (OGTT). Conditions are described in Fig. 3. Change in LPL activity (plasma lipolytic activity minus hepatic triglyceride lipase) was measured as described in MATERIALS AND METHODS. Data are means ± SE. FFA, free fatty acids. ●, OGTT; ○, control. *$P < .01$.

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to increase adipose tissue LPL. From data obtained during euglycemic clamp studies, this effect of glucose in humans is partly, if not entirely, mediated by insulin (40,41). Similar glucose- and insulin-dependent changes in LPL in other tissues, particularly muscle (38,42,43), have not been seen. In fact, inhibition of muscle LPL activity by glucose and insulin has been reported in some studies (38). Thus, a greater contribution of adipose tissue to PLA in humans could explain the postglucose results.

In summary, a method has been developed for the measurement of lipoprotein triglyceride lipolytic activities (LPL and HTGL) in preheparin plasma. The relationships of preheparin LPL and HTGL to respective activities in postheparin plasma predict the potential usefulness of this methodology in the assessment of local tissue or systemic lipolytic enzyme physiology and pathophysiology.

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