Lipoprotein lipase in plasma after an oral fat load: relation to free fatty acids

Fredrik Karpe,[1,*] Thomas Olivecrona,[†] Göran Walldius,[*] and Anders Hamsten[*]

King Gustav V Research Institute and Department of Medicine,[*] Karolinska Hospital, Karolinska Institute, S-104 01 Stockholm, and Department of Medical Biochemistry and Biophysics,[†] University of Umeå, S-901 87 Umeå, Sweden

Abstract Lipoprotein lipase (LPL) releases fatty acids from triglyceride-rich lipoproteins for use in cellular metabolic reactions. How this hydrolysis, which occurs at the vascular endothelium, is regulated is poorly understood. A fatty acid feedback system has been proposed by which accumulation of fatty acids impedes LPL-catalyzed hydrolysis and dissociates the enzyme from its endothelial binding sites. We examined this hypothesis in humans who were subjected to an oral fat tolerance test of a mixed-meal type. Plasma triglycerides, free fatty acids, and LPL activity were measured before and repeatedly during a 12-h period after intake of the fat load. Since soybean oil with a high content of linoleic fatty acid was the source of triglycerides, a distinction could be made between endogenous free fatty acids (FFA) and FFA derived directly from lipolysis of postprandial triglyceride-rich lipoproteins. Mean LPL activity was almost doubled ($P < 0.01$) 6 h after intake of the oral fat load. The rise in LPL activity was accompanied by an increase of plasma triglycerides and linoleic free fatty acids (18:2 FFA), but not of total plasma FFA, which instead displayed a heterogeneous pattern with essentially unchanged mean levels. The postprandial response of LPL activity largely paralleled the postprandial responses of 18:2 FFA and triglycerides. The highest degree of parallelism was seen between postprandial 18:2 FFA and LPL activity levels. Furthermore, the integrated response (area under the curve, AUC) for plasma measurements of LPL correlated with the AUC for 18:2 FFA ($r = 0.40$, $P < 0.05$), but not with the AUC for plasma triglycerides ($r = 0.21$, ns). The high degree of parallelism and significant correlation between postprandial plasma LPL activity and 18:2 FFA support the hypothesis of fatty acid control of endothelial LPL during physiological conditions in humans.—**Karpe, F., T. Olivecrona, G. Walldius, and A. Hamsten.** Lipoprotein lipase in plasma after an oral fat load: relation to free fatty acids. *J. Lipid Res.* 1992. **33:** 975–984.

Supplementary key words hepatic lipase • triglycerides • alimentary lipemia

Metabolism of triglyceride-rich lipoproteins in human plasma provides energy-rich substrate for immediate use in tissues or for storage in adipose tissue. However, in this process potentially atherogenic lipoprotein particles are also generated. Lipolysis of triglycerides from chylomicrons and very low density lipoproteins (VLDL) is catalyzed mainly by lipoprotein lipase (LPL). This enzyme is bound to the vascular endothelium by affinity for heparan sulfate, and only low levels of LPL are found in the circulation (1, 2). The binding of LPL to the endothelium is thought to be weakened by local fatty acid accumulation that may result from lipolysis of plasma triglycerides. This has been indicated in individuals in whom the lipolytic system has been overloaded by infusion of triglyceride emulsions (2, 3) and by cell culture experiments (4). Excess of free fatty acids (FFA) released from triglyceride-rich lipoproteins have therefore been proposed to exert feed-back control of lipolysis.

To explore factors that govern the level of LPL in the circulation and whether evidence of the proposed fatty acid feed-back control system could be obtained under physiological conditions in humans, we have studied postprandial LPL activity and lipid concentrations in individuals subjected to an oral fat load of a mixed-meal type. The study group was selected to provide a wide range of basal plasma triglyceride concentrations, which gave a good opportunity to search for associations between plasma LPL activity and markers of the metabolism of triglyceride-rich lipoproteins.

MATERIALS AND METHODS

Materials

Constituents of the oral fat load were mixed the day before the test. Soybean oil was a generous gift from Mr. Staffan Sennfält, Karlshamns Oils & Fats AB, Karlshamn, Sweden. The fatty acid composition of the soybean oil was as follows: palmitic acid (16:0) 10.0%, stearic acid (18:0) 4.2%, oleic acid (18:1) 23.1%, linoleic acid (18:2)

Abbreviations: AUC, area under the curve; BMI, body mass index; FFA, free fatty acids; LPL, lipoprotein lipase; HL, hepatic lipase; VLDL, very low density lipoprotein.

[1]To whom correspondence and reprint requests should be addressed.

53.9%, linolenic acid (18:3) 7.6%, and the sum of remaining minor fatty acid species 1.2%. Dried egg whites and yolks were bought from Sigma Chemical Co., St. Louis, MO, and glucose was purchased from the hospital pharmacy at Karolinska Hospital. Antiserum to human hepatic lipase (HL) was raised in a goat (5). Immunoglobulins were isolated and dialyzed against phosphate-buffered saline. Apolipoprotein C-II was prepared from human serum as previously described (6). Intralipid® is a product of Kabi-Pharmacia Parenterals, Stockholm, Sweden. The 10% emulsion contained 10 g soybean triglycerides and 1.2 g egg yolk phospholipids per 100 ml. Bovine serum albumin (fraction V) and gum arabic were obtained from Sigma. Heparin-agarose was prepared as previously described (7). [^{3}H]oleic acid-labeled triolein was synthesized by Dr. Lennart Krabisch, Dept. of Physiological Chemistry, University of Lund, Sweden. Heparin (5000 IE/ml) used for intravenous injection was from Kabi, Stockholm, Sweden and heparin used in the assay mixture was from AB Lövens, Malmö, Sweden. Bovine LPL was prepared from milk as described (7). A plasma sample used as standard for lipase assays was obtained from a healthy, normolipidemic 30-year-old man. It was prepared as the study samples, aliquoted into small tubes and stored together with the study samples at −80°C.

Study protocol

Forty-four male subjects (age, 48.9 ± 3.2 years; body mass index, body mass/height^2, 26.3 ± 3.5 kg/m^2) were investigated in the study. Of these, 34 had suffered a myocardial infarction and 10 were apparently healthy and normolipidemic men who had been recruited from participants in a population survey. The patient group was selected to provide a wide range of fasting plasma triglyceride levels. Subjects with polygenic or monogenic hypercholesterolemias (based on repeated lipoprotein classifications showing type IIa pattern and studies of first degree relatives) were not considered. More than 5 years had elapsed between the infarction and the present study in all postinfarction patients. Obese (≥130% ideal weight) subjects and subjects with manifest diabetes mellitus, ongoing lipid-lowering medication, or alcohol abuse were not included. A total of 25 of the 34 patients were on treatment with a cardioselective β-blocker at the time of the study.

Participants were admitted to the Clinical Research Unit in the early morning after an overnight fast. They had been asked to refrain from smoking during the fasting period and from alcohol intake during the preceding 3 days. A mixed meal consisting of soybean oil (50 g/m^2 body surface area), glucose (50 g/m^2), egg white protein (25 g/m^2), dried egg yolk (6.3 g/m^2), and lemon flavor was homogenized in 200 ml water and ingested within 10 min between 07.00 and 07.30 AM. The test meal was well tolerated by all subjects. A fasting blood sample was taken before intake of the test meal. Subsequent blood samples were drawn hourly for the first 9 h, and the last sample was taken 12 h after ingestion of the emulsion. Participants were allowed to be ambulant throughout the test. Smoking was prohibited. Water or tea, but no food, was allowed during the test.

Blood sampling

Venous blood samples were drawn into pre-cooled sterile tubes (Vacutainer, Becton Dickinson, Meylan Cedex, France) containing Na_2EDTA (1.4 mg/ml) for the lipid and Na-heparin (29 IE/ml) for the lipase analyses. The tubes were immediately placed in ice-water and plasma was recovered for lipid analyses within 30 min by low-speed centrifugation (1750 *g*, 20 min, +1°C). Analyses of plasma triglycerides and FFA determinations were started within 1 h after blood sampling. Plasma samples for lipase activity determinations were frozen at −80°C within 1 h of blood sampling.

Analyses of free fatty acids and triglycerides

Total plasma FFA were determined according to Ho (8) after extraction according to Dole (9). The fatty acid composition of the FFA was determined by gas–liquid chromatography after preparative thin-layer chromatography of FFA from a total plasma lipid extract. One ml plasma was extracted with 5 ml methanol, 10 ml chloroform, and 15 ml 0.1 mmol/1 disodium phosphate, pH 6.8. The lower chloroform phase was evaporated under a gentle stream of N_2 and applied to a silicic acid thin-layer chromatography plate containing 1.0% dimethyl-POPOP, Packard Instruments, Downers Grove, IL. The thin-layer chromatogram was developed in petroleum ether–diethylether–acetic acid 80:19:1. The FFA bands were identified in UV light and scraped into glass tubes for methylation which was performed in 5% H_2SO_4 in methanol for 4 h at 60°C. The fatty acids were then extracted with heptane, and an aliquot was injected into a Hewlett-Packard GC 5720A gas chromatograph equipped with a 1.8-m 10% DEGS-PS column of 2 mm inner diameter (10). The eight predominant fatty acid peaks were recorded and taken to 100%. The percentage of linoleic acid was multiplied with the total amount of FFA. Fractionation of FFA was only made in 29 individuals, 5 controls and 24 patients. Analysis of FFA composition was prevented by lack of manpower in 15 consecutive experiments.

Total triglycerides and cholesterol were determined in triplicate in plasma. Lipids were first extracted with chloroform–methanol (11). Cholesterol (12) and triglycerides (13) were then determined on an Ultrolab (LKB).

Lipase assays

Plasma samples were shipped on dry ice from Stockholm to Umeå where the lipase assays were done. The assay systems were as described elsewhere (14), using total

assay volumes of 200 μl and sample volumes of 15 μl or less. Assays for both LPL and HL were at 25°C, for 15 to 100 min, depending on the expected activity. Control experiments with postheparin plasma showed that both assays were linear with amount of plasma sample and time over the ranges used. All determinations were run in triplicate. The coefficients of variation of samples from the same pool measured on the same day were 3.7% and 5.3% for LPL and HL, respectively. Lipase activities were expressed in milliunits (mU), which correspond to 1 nmol of fatty acid released/min. The contribution of triglyceride from the sample to total triglycerides in the assays was always less than 15%.

The low LPL activity in plasma samples generates only small amounts of labeled product fatty acids, even with the high specific activity substrate used here. To keep the blank at minimum, the labeled triolein was repurified by thin-layer chromatography about once every month to remove traces of fatty acids and partial glycerides. In most of our previous work we have used the Belfrage and Vaughan (15) procedure to extract fatty acids for counting. This method was used here for assay of LPL and HL in samples of postheparin plasma. For assay of LPL activity in plasma without previous heparin injection we extracted the fatty acids by another procedure developed by Spooner, Garrison, and Scow (16) which reduced the background.

For assay of LPL the substrate was prepared by sonication of [^{3}H]triolein into Intralipid®. This emulsion was then mixed with a medium containing albumin and apolipoprotein C-II, and stood at least 30 min at room temperature before the sample was added. The plasma samples were preincubated 2 h on ice with 0.5 volume of a solution of goat antibodies to HL. This reduced the HL activity by more than 98% in samples of purified HL, or as estimated in postheparin plasma. The final composition of the medium was (excluding contribution from the sample itself): NaCl, 0.1 M; Tris, 0.1 M; triglycerides, 4 mg/ml; phospholipids, 0.24 mg/ml; heparin, 0.1 mg/ml; albumin, 60 mg/ml; and apolipoprotein C-II, 5 μg/ml. The pH was 8.2.

Measurement of low lipase activities in plasma (without prior heparin injection) requires a sensitive assay system, meriting a close look at some technical aspects and reproducibility. Thirteen subjects in the laboratory staff were sampled in order to test the influence of freezing on plasma LPL and HL activities. Aliquots of fresh plasma samples were stored in an ice-bath and frozen at −80°C. Fresh and thawed plasma samples were analyzed and the mean activity levels of fresh and frozen samples were calculated along with the mean difference between the respective measurements. For LPL the activity in fresh plasma was 1.41 ± 0.58 mU/ml and after freezing 1.51 ± 0.51 mU/ml, with a mean difference of +0.10 ± 0.24. Corresponding HL activities were 0.92 ± 0.48 and 1.00 ± 0.44 mU/ml with a mean difference of +0.08 ± 0.15. Thus, no deterioration of lipase activities was seen after freezing. A methodological study was also run in connection with an earlier study of lipases in fasting and postprandial plasma, which has not been reported before. In that study a frozen standard plasma was used to assess the inter-assay variability. One tube of that standard plasma was thawed and assayed on each of 21 days when study samples were analyzed over a period of 108 days. The LPL value for this standard was 0.92 ± 0.22 mU/ml (mean ± SD). Regression analysis of the registered activities versus time elapsed since first assay did not reveal any significant change. The slope for activity values versus log-normalized time was 0.07. We conclude that there is no substantial loss of LPL activity when plasma samples are stored frozen at −80°C for a few months.

As a further quality control in the earlier study we assayed 19 of 57 series of basal and postprandial plasma samples a second time. At least one series of samples from each assay day was reanalyzed. The mean ratio for values-1 divided by values-2 was 1.01 ± 0.26 (mean ± SD). These data indicate that the assay gives stable and reproducible values, but the high standard deviation indicates that there is a substantial variation in individual values, with a variation between measurements months apart being around 25%. This is because the activities are close to background values, and because there is day-to-day variation of the substrate emulsion and other assay parameters.

When results of basal and postprandial HL measurements were available from the first 18 experiments (5 controls and 13 patients), this analysis was stopped, since no significant changes in HL activity were found in postprandial plasmas.

Postheparin plasma lipase measurements

All subjects returned 1 week after the oral fat tolerance test for an intravenous injection of heparin (100 U/kg body weight) after an overnight fast. Venous blood for determination of postheparin plasma LPL and HL activities was taken before and 15 min after the heparin injection into pre-cooled Vacutainer tubes containing heparin. Plasma was prepared, handled, and analyzed as described above.

Statistics

Results are shown as mean ± SD (tables) or mean ± SEM (Figures) after testing for skewness. Calculation of statistical significance for differences between group means was done by Student's unpaired *t*-test and for differences between various time points within groups by paired *t*-test.

To investigate whether the responses of LPL activity on the one hand and total FFA, 18:2 FFA, and triglycerides

TABLE 1. Basic characteristics and responses to the test meal for all subjects

	Controls (n = 10)	Patients (n = 34)
Age, years	49.2 ± 3.6	48.8 ± 3.2
BMI, kg/m²	24.5 ± 2.8	26.8 ± 3.5
Tg (fasting), mmol/l	1.09 ± 0.40 (96 ± 35)[a]	2.39 ± 1.66[b] (212 ± 147)
Tg (peak postprandial), mmol/l	2.83 ± 0.97 (250 ± 86)	5.45 ± 3.45[b] (482 ± 305)
Cholesterol (fasting), mmol/l	5.58 ± 0.56 (215 ± 22)	5.94 ± 0.92 (229 ± 36)
Cholesterol (peak postprandial), mmol/l	5.69 ± 0.74 (220 ± 29)	6.07 ± 1.07 (234 ± 41)
FFA (fasting), μmol/l	591 ± 207	647 ± 242
FFA[c] (peak postprandial), μmol/l	497 ± 130	639 ± 208[b]
18:2 FFA[d] (fasting), μmol/l	86 ± 16	93 ± 40
18:2 FFA (peak postprandial), μmol/l	206 ± 44	236 ± 60
LPL (fasting), mU/ml	0.66 ± 0.22	1.16 ± 0.77[b]
LPL (peak postprandial), mU/ml	1.27 ± 0.43	1.82 ± 0.76[b]
HL[e] (fasting), mU/ml	1.13 ± 0.48	0.89 ± 0.34
HL[c] (peak postprandial), mU/ml	1.12 ± 0.42	0.87 ± 0.34
PHPL-LPL, mU/ml	483 ± 180	361 ± 133[b]
PHPL-HL, mU/ml	338 ± 128	345 ± 156

Results are mean ± SD. Group differences were tested by Student's unpaired *t*-test. BMI, body mass index; Tg, plasma triglycerides; FFA, free fatty acids; 18:2, linoleic acid; LPL, lipoprotein lipase; HL, hepatic lipase; PHPL, postheparin plasma.

[a] Levels in mg/dl are shown in parentheses.

[b] $P < 0.05$.

[c] The peak postprandial value was defined as the value at 6 h after intake of the oral fat load.

[d] Measurement of 18:2 FFA was made in 29 subjects, 5 controls, and 24 patients.

[e] Measurement of HL activity was made in 18 subjects, 5 controls and 13 patients.

on the other hand were parallel, correlations were made for each individual between LPL and FFA, LPL and 18:2 FFA, and LPL and triglycerides measured at 3, 6, and 9 h after intake of the test meal. These time points, and not the fasting (0 h) and 12 h time points, were chosen to make possible the study of dynamic events induced by the oral fat intake. Confidence intervals for the mean values of all individual correlation coefficients were calculated. For comparison, the relationship between LPL and HL responses, two variables for which no association was indicated, was also tested. The mean of individual correlation coefficients between LPL and HL was found to be close to zero.

To quantify the total increases of LPL, 18:2 FFA, and triglycerides in plasma during the entire 12-h postprandial period, areas between the curves for plasma measurements plotted against time after the test meal and the fasting plasma concentration were calculated. This integrated value is designated area under the curve (AUC).

Ethical considerations

The experimental protocol was approved by the Ethical Committee of the Karolinska Hospital. All subjects gave their informed consent to participate in the study.

RESULTS

Basic metabolic characteristics of the study group and responses to the test meal are shown in **Table 1** and Figs. 1-5. The average fasting plasma triglyceride level of the healthy controls was 1.09 ± 0.40 mmol/l (range; 0.53-1.99 mmol/l) and the peak level was reached 3 h after intake of the test meal at 2.83 ± 0.97 mmol/l (range; 1.34-4.62 mmol/l) (**Fig. 1**). The corresponding figures for the patients were 2.39 ± 1.66 mmol/l (range; 0.74-8.17 mmol/l) and 5.45 ± 3.45 mmol/l (range; 0.97-15.41 mmol/l), and the average time to the postprandial triglyceride peak was

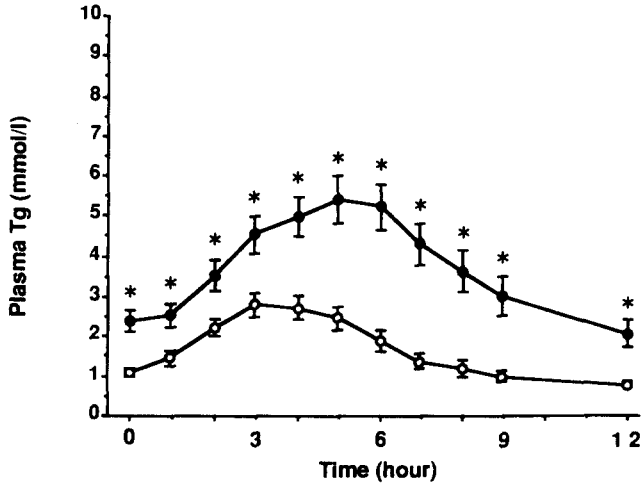

Fig. 1. Response of plasma triglycerides (Tg) to the oral fat load in the control and patient group. Controls (n = 10) are shown with open symbols and patients (n = 34) with filled symbols. Levels are given as mean ± SE. *, $P < 0.001$, controls vs. patients.

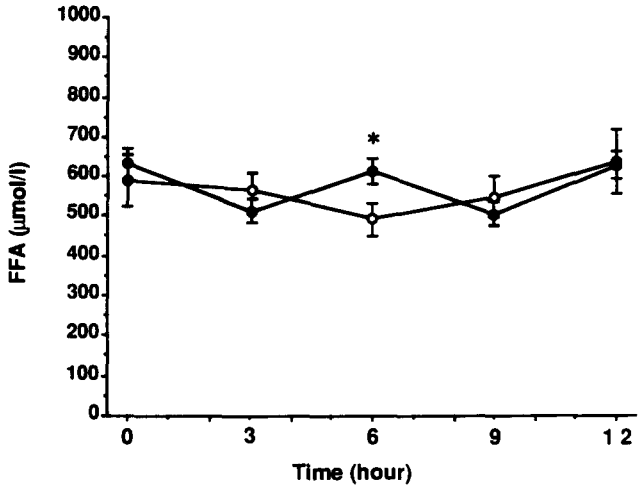

Fig. 2. Response of plasma total free fatty acids (FFA) to the oral fat load. Controls (n = 10) are shown with open symbols and patients (n = 34) with filled symbols. Levels are given as mean ± SE. Group means were significantly different ($P < 0.05$) at 6 h.

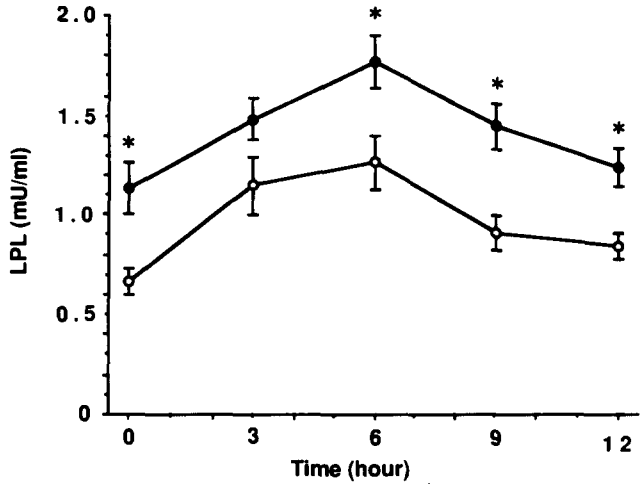

Fig. 4. Plasma levels of lipoprotein lipase (LPL) activity during the oral fat tolerance test. Controls (n = 10) are shown with open symbols and patients (n = 34) with filled symbols. Levels are given as mean ± SE. Significant group differences were found at 0, 6, 9, and 12 h. LPL activity increased in both groups after intake of the oral fat load (by 92 and 59%, respectively), $P < 0.01$ for both groups at 6 h.

5 h in this group. The return to baseline of plasma triglycerides was also delayed among the patients as compared to the controls. The group differences in fasting and postprandial plasma triglycerides were highly significant as a consequence of patient and control subject selection. Fasting plasma cholesterol levels were slightly, but statistically insignificantly elevated in the patient group, and no significant changes were recorded after intake of the fat meal in either group (data not shown).

Compared to the change in plasma triglycerides, FFA responses were small and heterogeneous (**Fig. 2**). The mean fasting levels in patients and controls were similar (647 ± 242 vs 591 ± 207 μmol/l, not significant). No distinct pattern was recorded in either patients or controls. The FFA concentration at 6 h after intake of the fat load was, however, higher in the patients compared with the controls (639 ± 208 vs. 497 ± 208, $p < 0.05$).

Linoleic FFA of total plasma FFA (18:2 FFA) was determined by gas–liquid chromatography in order to obtain a measure of FFA derived directly from postprandial triglyceride-rich lipoproteins. The fasting levels of 18:2 FFA did not differ between patients and controls (93 ± 40 vs. 86 ± 16 μmol/l, ns). Peak levels were attained at 6 h in the patients (236 ± 60 μmol/l) and at 9 h in the con-

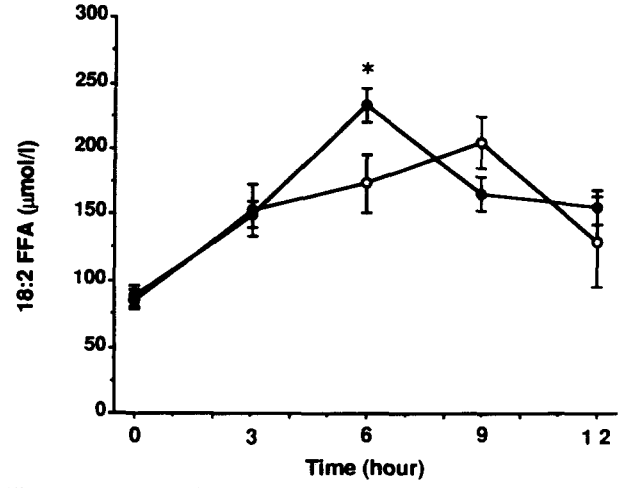

Fig. 3. Response of plasma linoleic free fatty acids (18:2 FFA) to the oral fat load. Controls (n = 5) are shown with open symbols and patients (n = 24) with filled symbols. Levels are given as mean ± SE. A significant group difference was found at 6 h ($P < 0.05$).

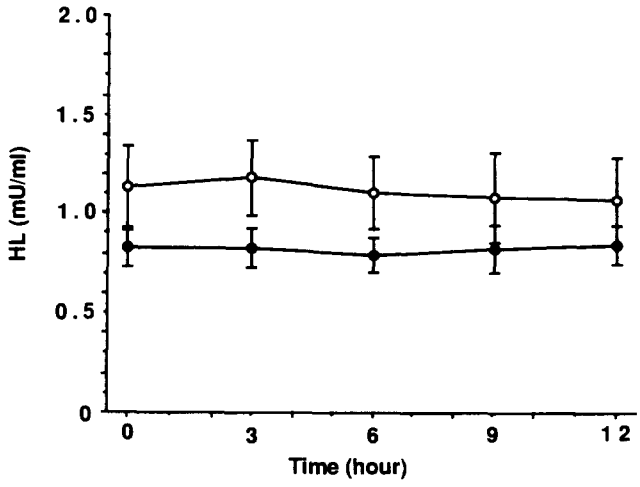

Fig. 5. Plasma levels of hepatic lipase (HL) activity during the oral fat tolerance test. Controls (n = 5) are shown with open symbols and patients (n = 13) with filled symbols. Levels are given as mean ± SE. Plasma levels were not influenced by the oral fat load.

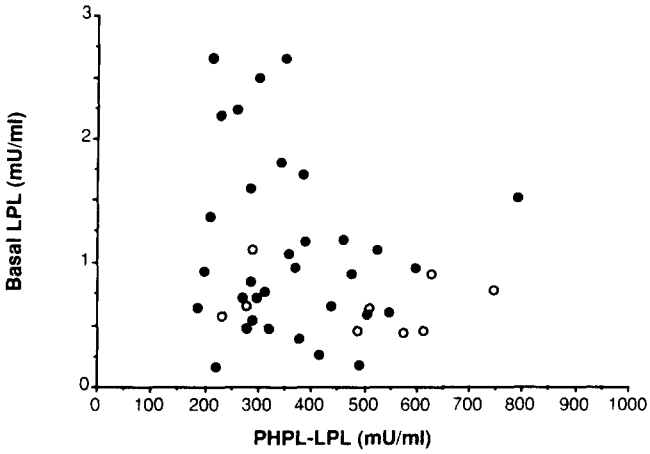

Fig. 6. Scattergram showing the relationship between basal and postheparin lipoprotein lipase (LPL) activity levels in controls (n = 9, open symbols) and patients (n = 34, filled symbols) (r = 0.21, ns).

trols (206 ± 44 μmol/l) (**Fig. 3**). In both groups increases from fasting to peak levels were highly significant ($P < 0.01$). A difference in 18:2 FFA concentration between the groups was found only at 6 h, at which time the 18:2 FFA level was slightly higher in the patient group (236 ± 60 vs. 174 ± 51, $P < 0.05$).

LPL activity in fasting plasma was almost twice as high in the patient group as compared to the control group (1.16 ± 0.77 vs. 0.66 ± 0.22 mU/ml, $P < 0.05$). LPL activity increased in both groups after intake of the fat load (by 59 and 92%, respectively, $P < 0.01$) and reached a maximum at 6 h at which time the group difference in LPL activity remained significant (1.82 ± 0.76 vs. 1.27 ± 0.43 mU/ml, $P < 0.05$) (**Fig. 4**). In contrast, the fasting HL activity tended to be lower in the patients (0.89 ± 0.34 vs. 1.13 ± 0.48 mU/ml, ns). Plasma levels of HL were not influenced by the oral fat load as shown in **Fig. 5.**

Postheparin plasma determinations of LPL and HL were made 1 week after the oral fat load. The patient group had significantly lower levels of postheparin LPL activity, whereas the level of HL was similar in the two groups (Table 1). No correlation was found between basal and postheparin LPL activity (r = 0.21, ns, **Fig. 6**), whereas a positive linear correlation was found between the two HL activities (r = 0.86, $P < 0.001$, **Fig. 7**).

To investigate whether the responses of LPL, on the one hand, and the responses of FFA, 18:2 FFA and triglycerides, on the other hand, were parallel, correlations were made for each individual subject between LPL and FFA, LPL and 18:2 FFA, and LPL and triglycerides measured at 3, 6, and 9 h after intake of the test meal. Confidence intervals for the mean values of all individual correlation coefficients were calculated and found to be positive (**Fig. 8**). This suggests that the postprandial response of LPL paralleled the postprandial responses of 18:2 FFA, total FFA, and triglycerides. The responses of LPL, 18:2 FFA, and plasma triglycerides to the oral fat load in five subjects with parallel response between LPL and 18:2 FFA are illustrated in **Fig. 9**. The triglyceride peak generally preceded the LPL and 18:2 FFA peaks.

The AUC for LPL was also analyzed in relation to the AUC values for 18:2 FFA and plasma triglycerides. An association was found with the AUC for 18:2 FFA (r = 0.40, $P < 0.05$, **Fig. 10**), but not with the AUC for triglycerides (r = 0.12, ns). There was no correlation between the AUC values for 18:2 FFA and triglycerides (r = 0.21, ns), nor between fasting or postprandial triglyceride and 18:2 FFA or LPL activity measurements. Thus, within the patient group, the normotriglyceridemic subjects did not

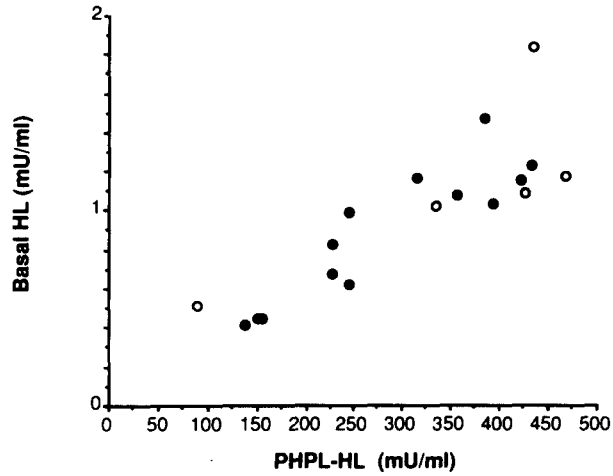

Fig. 7. Scattergram showing the relationship between basal and postheparin hepatic lipase (HL) activity levels in controls (n = 5, open symbols) and patients (n = 13, filled symbols) (r = 0.86, $P < 0.001$).

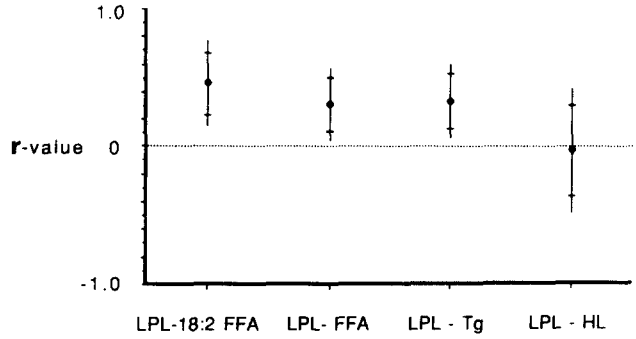

Fig. 8. Confidence intervals (95th and 99th) for the mean values of individual Pearson correlation coefficients (r-value) between lipoprotein lipase (LPL) activity and linoleic free fatty acids (18:2 FFA) (n = 29), LPL activity and total plasma free fatty acids (FFA) (n = 44), and LPL activity and triglycerides (n = 44) at 3, 6, and 9 h after intake of the oral fat load. The confidence interval for the mean value of the individual correlation coefficient between LPL and HL activities at the three time points is also shown.

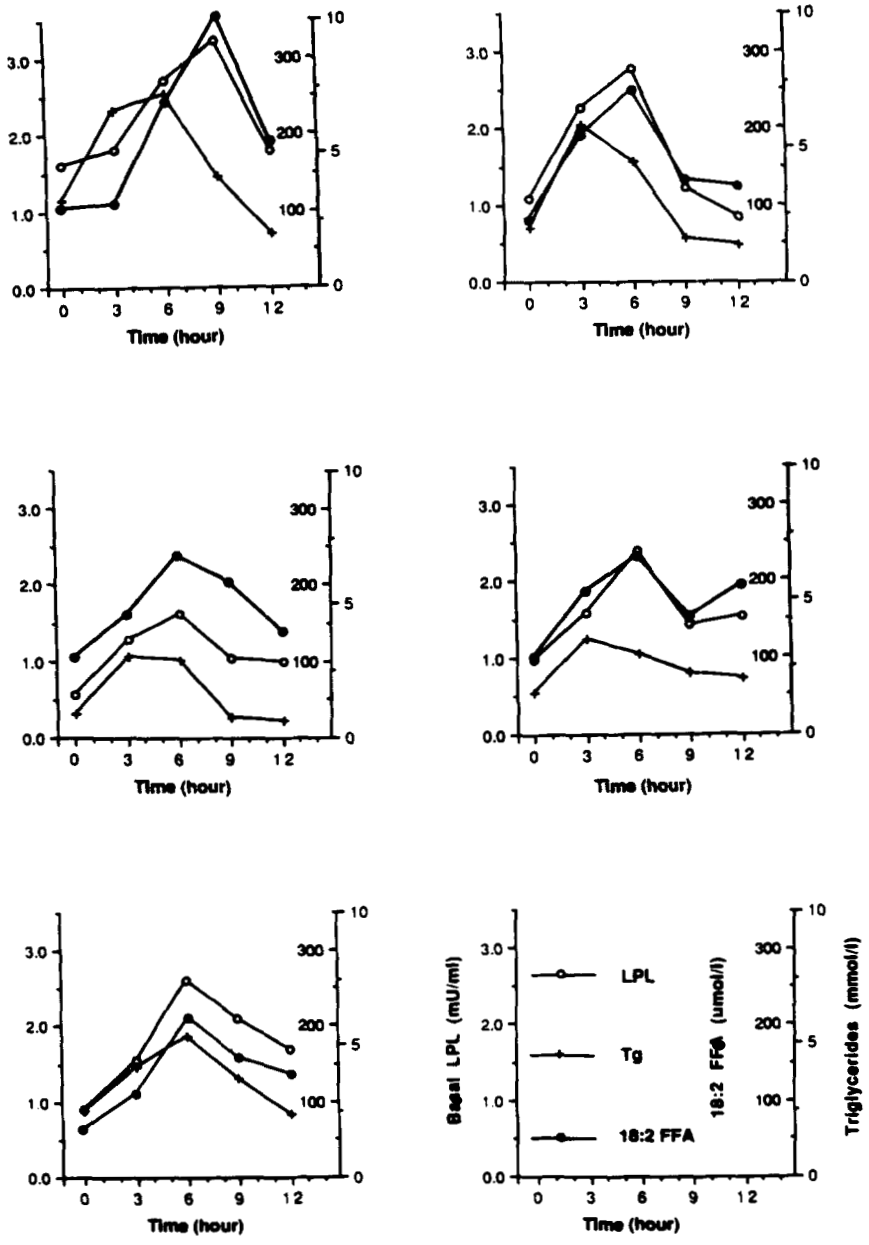

Fig. 9. Line plots of the time courses for plasma lipoprotein lipase (LPL) activity, linoleic free fatty acids (18:2 FFA) and triglycerides after intake of the oral fat load in five subjects chosen to illustrate the congruent responses of LPL and 18:2 FFA.

have lower LPL levels than the hypertriglyceridemic subjects.

There were no differences between patients with and without β-blocker medication with respect to fasting and postprandial plasma levels of LPL, FFA, 18:2 FFA, and triglycerides.

DISCUSSION

The present study dealt with the regulation of plasma LPL activity during alimentary lipemia. The experimental design resulted in a substantial increase of plasma triglycerides after fat intake. LPL activity was almost doubled 6 h after intake of the oral fat load and the increase of LPL was parallel above all with the postprandial response of FFA derived directly from chylomicrons. Why this happens is not obvious. Two parameters that might be causally related are the rise in plasma triglycerides (1), since LPL is known to bind to triglyceride-rich lipoproteins (17, 18), and recirculation in plasma of unesterified fatty acids derived from hydrolysis of lipoprotein triglycerides (2), since there is evidence that fatty acids can dissociate LPL from its binding sites (3, 4). The fact

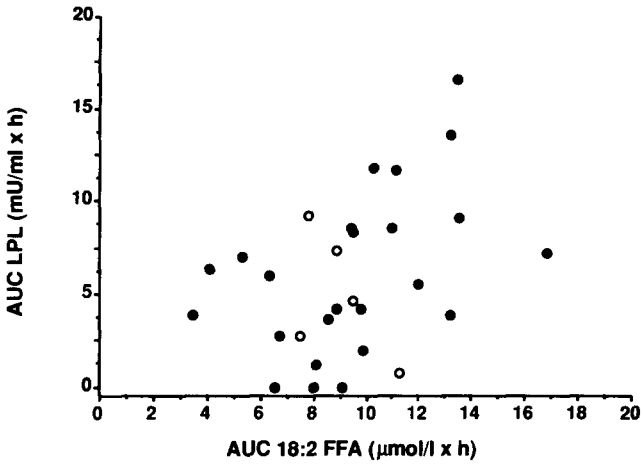

Fig. 10. Scattergram showing the relation between the area under the curve (AUC) for plasma linoleic free fatty acids (18:2 FFA) and postprandial LPL activity during the oral fat tolerance test in controls (n = 5, open symbols) and patients (n = 24, filled symbols) (r = 0.40, $P < 0.03$). AUC was calculated as the area between the curve for plasma measurements plotted against time after intake of the test meal (0–12 h) and the fasting plasma concentration.

that linoleic acid made up 54% of the fatty acids in the test meal was used to evaluate the origin of the FFA. A variable but massive increase of 18:2 FFA was seen in all subjects. As illustrated in Figs. 8 and 9 there were parallel changes in plasma triglycerides, total FFA, 18:2 FFA, and plasma LPL activity, but 18:2 FFA was the parameter that showed the strongest association to plasma LPL. Furthermore, the AUC for 18:2 FFA correlated to the AUC for LPL (Fig. 10), whereas there was no significant correlation between AUC values for plasma triglycerides and LPL. This indicates that the magnitude in response was similar between LPL and 18:2 FFA, but not between LPL and plasma triglycerides. The high degree of parallelism between the fluctuations of chylomicron-derived FFA and plasma LPL activity is evidence for a simultaneous and possibly coordinated metabolism and supports the hypothesis of fatty acid feed-back control of LPL at the vascular endothelium under physiological conditions in humans. However, it should be emphasized that actual release of LPL from the vascular endothelial binding site for obvious reasons could not be determined in this study.

The present findings are in accordance with those of Peterson et al. (3). In that study healthy young men were subjected to an intravenous infusion of a triglyceride emulsion (Intralipid®) at a rate exceeding the removal capacity for triglycerides. In this overload situation a substantial increase of plasma FFA occurred in some, but not in all subjects, and correlated closely to a rise in plasma LPL. The studies by Saxena, Witte, and Goldberg (4) in which oleic acid was found to dissociate LPL from cultured endothelial cells are also in accord with our findings and interpretations. The same group more recently studied the effect of different FA species on the release of LPL from endothelial cells (19). High concentrations of either short-chain or saturated FA, or both, showed only weak capacity to dissociate LPL from the endothelial cells in vitro, whereas specifically *cis*-monounsaturated but also polyunsaturated FA both were highly active in this respect.

It should be emphasized that the mixed meal used in the present study contained a substantial amount of glucose which may lead to postprandial hyperinsulinemia. Although, insulin is strongly antilipolytic (20, 21), total FFA was not diminished which presupposes input of FFA to the plasma compartment from other sources than adipose tissue. The increase of 18:2 FFA in conjunction with the stable total FFA thus most likely indicates that 18:2 FFA to a major extent is derived from chylomicrons in the postprandial state. However, insulin may also have a direct effect on plasma LPL activity. Eckel et al. (22) demonstrated that plasma LPL activity increased after an oral glucose load. Thus it cannot be entirely ruled out that some of the increase in plasma LPL due to the oral fat load was mediated by insulin.

Some degree of parallelism was also seen between plasma triglycerides and LPL activity. This could imply lipoprotein binding of LPL. Recently Beisiegel, Weber, and Bengtsson-Olivecrona (23) presented data suggesting that LPL may serve as a ligand for recognition of chylomicron remnants by hepatic receptors. Hence, it could be speculated that LPL is released from its endothelial binding by a high local FFA concentration induced by active lipolysis and subsequently binds to lipoprotein remnants, serving as a signal that the particle is ready to be taken up by the liver. Plasma LPL may thus reflect the presence of remnant lipoprotein particles.

There was no correlation between basal and postheparin LPL activities. Basal LPL was almost twice as high in patients compared to controls, whereas postheparin LPL was lower in patients than in controls. These findings support the previous conclusion that plasma LPL is not regulated by simple equilibrium with LPL at endothelial binding sites (24). This is not surprising, since there is much evidence for net transport of LPL in plasma from extrahepatic tissues (25, 26), where the enzyme is produced, to the liver, where the enzyme is degraded (27–29).

In sharp contrast to the findings for LPL, there was no change in HL activity after intake of the oral fat load. Peterson et al. (3) found no change of HL activity after infusion of lipid emulsions. These findings indicate that HL in plasma is in a fairly static equilibrium with HL at vascular binding sites, and that shifts in metabolic conditions do not rapidly change HL activity or its interaction with the vascular binding sites. To the best of our knowledge the influence of FFA on binding of HL to vascular binding sites has not been investigated. The present experimental situation most probably did not result in high local FFA concentrations near the HL binding site,

due to the lipoprotein specificity of HL which essentially does not include large triglyceride-rich lipoproteins (30). There was a strong, positive correlation between basal and postheparin HL activity in this study, as in a previous study (24). The heparin challenge was made 1 week after the oral fat load, hence the correlation implies that HL activity remained stable in each individual, despite large variations between individuals. This is in accord with a study (24) in which postheparin HL was measured on two occasions months apart and a high degree of correlation was found between the two measurements in each individual. Thus, HL seems to be stable both in amount and in terms of lipid and lipoprotein influence.

LPL activity in plasma is low, posing questions on the specificity and reproducibility of the assay used. In a previous study Peterson et al. (3) found that only a very low lipase activity remained, less than 0.2 mU/ml, when HL and LPL were eliminated with antisera. This residual lipase activity did not change during the course of a 6-h infusion of Intralipid® which raised the plasma triglyceride concentration to more than 1000 mg/dl and caused large increases in plasma LPL activity. Hence, other lipases present in plasma, e.g., pancreatic lipase, contribute only little to the activity registered in the present assay, and do not contribute to the rise of LPL activity during experimental hyperlipidemia. The contribution of HL is, however, substantial. Therefore this lipase was eliminated from the samples using specific antibodies. The present results show that good reproducibility for determinations of lipase activities can be maintained over several months, but that the values are associated with a considerable variation; the SD for duplicate measurements of the same sample on separate occasions (months apart) was 20–25%. This needs to be taken in consideration when evaluating the data.

In summary, the present study examined the response of plasma LPL activity to an oral fat load in subjects covering a wide range of fasting plasma triglyceride levels. A rise in LPL activity was found that paralleled the accumulation of chylomicron-derived FFA. From previous studies a fatty acid feed-back control system has been proposed for regulating LPL at the vascular endothelium. The present findings support the hypothesis that the rate of lipolysis of triglyceride-rich lipoproteins can be partly regulated by accumulating FFA that dissociate LPL from its endothelial binding sites. Further studies on the nature of these regulatory mechanisms are now under way in our laboratories.

This study has been supported by grants from the Swedish Medical Research Council (204, 727, and 8691), the Swedish Heart-Lung foundation, the foundation for Old Servants, King Gustaf V 80th birthday fund, Professor Nanna Svartz' fund, the Swedish Society for Medical Research (Karolinska Institute), the Swedish Medical Society, and the Swedish Margarine Industry Fund for Research on Nutrition. Dr. Karpe has research fellowships from the Swedish Heart-Lung foundation and Förenade Liv Insurance Company. Dr. Hamsten is career investigator of the Swedish Heart-Lung Foundation.

Manuscript received 5 December 1991 and in revised form 5 March 1992.

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