

Quantitative determination of hepatic and lipoprotein lipase activities from human postheparin plasma¹

Jonas Boberg,² Jan Augustin,³ Marietta L. Baginsky, Pablito Tejada, and W. Virgil Brown

Department of Medicine, School of Medicine,
University of California, San Diego, La Jolla,
CA 92093

Summary A method was developed to separate and quantitatively determine two different triglyceride lipase activities in human postheparin plasma: hepatic triglyceride lipase (H-TGL) and lipoprotein lipase (LPL). Affinity chromatography on heparin-Sepharose columns was used for the separation. Rechromatography of purified H-TGL on heparin-Sepharose resulted in recoveries of 74 and 97% of these enzyme activities, respectively. The analytical errors for the determinations of the two activities were 11.4 and 9.6%, respectively.

Supplementary key words triglyceride lipase activities

Triglyceride lipolytic activity in blood plasma after administration of heparin has been determined by several methods (1–4). Previous studies have reported low postheparin lipolytic activities (PHLA) in patients with hypertriglyceridemia (1, 5). However, there was a considerable overlapping of the values for PHLA activities between the patients with hypertriglyceridemia and the control subjects. One explanation for this might be that there are at least two triglyceride lipolytic activities in postheparin plasma that have been demonstrated (6, 7). Animal studies in the rat (8), in the dog (9), and in the pig (10) have demonstrated that one of the triglyceride lipase activities originates in the liver⁴ and the other activity originates in extrahepatic tissues.⁴ This latter enzyme activity has properties similar to adipose tissue lipoprotein lipase from the pig (11), from man (12), and from rat heart muscle (12) in requiring a specific apolipoprotein

activator, apolipoprotein C-II, and in its response to certain inhibitory substances.

The aim of the present investigation was to develop a rapid method by which the two triglyceride lipolytic activities of postheparin plasma in man (H-TGL and LPL) could be separately measured with precision and accuracy.

Materials and Methods

Subjects. Healthy non-obese laboratory personnel and students were recruited as volunteer subjects. They were under no dietary restrictions unless specifically indicated. All subjects fasted 12–14 hr before blood was taken. The tests were done between 8 and 10 AM. Subjects taking prescription medications were specifically excluded.

In all subjects a preheparin blood sample was taken for plasma lipid determinations and lipoprotein estimations (13). Routinely 60 IU of sodium heparin per kg body weight (Riker Laboratories) was injected intravenously and postheparin blood samples were taken 15 min after the injection.

Blood samples. The blood samples (10–30 ml) were taken into Vacutainer test tubes without any further addition of anticoagulant. The blood was separated from the cells immediately by centrifugation (4°C) at 2000 rpm for 15 min, and stored frozen at –70°C.

Heparin-Sepharose affinity chromatography. One volume of postheparin plasma (usually 2–4 ml) was mixed with one volume of 0.005 M Na-barbital buffer, pH 7.4, containing 0.45 M NaCl. A measured volume (4–8 ml) of this mixture was applied to Sepharose 4B columns (8 × 20 mm) containing covalently linked heparin (14). The column had previously been equilibrated with a 0.005 M Na-barbital buffer, pH 7.4, containing 0.15 M NaCl. A stepwise elution was carried out (using the barbital buffer with NaCl as indicated) and the following six fractions were collected. 1) The volume that went through during loading; 2) A wash buffer fraction of 8 ml containing 0.3 M NaCl; 3) 6 ml of buffer containing 0.72 M NaCl; 4) 6 ml of buffer containing 0.72 M NaCl; 5) 6 ml of buffer containing 1.5 M NaCl; and 6) 6 ml of buffer containing 1.5 M NaCl. The columns were reused for another separation after a new equilibration with 20 ml of 0.005 M Na-barbital containing 0.15 M NaCl. Each column was used no more than 10 times or for more than 2 weeks.

Assay of triglyceride lipase activity. Total assay volume was 0.1 ml containing 0.23 μmol of triolein (0.05 μCi ¹⁴C/μmol); 0.4 mg of gum arabic as emulsifier; and 0.5 mg of bovine serum albumin. LPL activity was assayed in 0.2 M Tris-HCl, pH 8.2, 0.13 M NaCl, added to the components above with 2 μg of apolipo-

¹ Preliminary report given at the American Heart Association 47th Scientific Sessions, November 18–21, 1974.

² Visiting postdoctoral NIH research fellow; supported by grants from the Swedish Medical Research Council (60F-4187 and 19R-4187). Present address: Department of Geriatrics, University of Uppsala, Box 641, S-751 27 Uppsala, Sweden.

³ Research training grant from Deutsche Forschungsgemeinschaft.

⁴ In this paper these two enzyme activities will be mentioned as hepatic triglyceride lipase (H-TGL) and lipoprotein lipase (LPL) in spite of the fact that they have not been completely characterized in man.

Abbreviations: PHLA, postheparin lipolytic activity; H-TGL, hepatic triglyceride lipase; LPL, lipoprotein lipase.

protein C-II purified as previously reported (15). For the assay of H-TGL, 0.2 M Tris (pH 8.8) containing 0.75 M NaCl was used (6). The assay was started by addition of 10 μ l (Unimetric automatic pipetter) of enzyme fraction and incubation was performed for 30 min at 28°C. The assay was terminated by addition of 1.6 ml of chloroform-heptane-methanol 5.0:4.0:5.6 (v/v/v) and 0.5 ml of 0.5 M NaOH, essentially according to the method of Belfrage and Vaughan (16). After mixing and centrifugation, 0.6 ml of the upper phase was transferred together with 10 ml of scintillation solution (4 g PPO and 0.5 g POPOP dissolved in 15 ml of acetic acid and 1 l of toluene) into liquid scintillation counting vials with a Micromedic automatic dispenser. Dilutions, losses, and quenching were measured using [¹⁴C]oleic acid standards and appropriate corrections were made.

The enzyme activity was always measured at substrate saturation concentration and release of fatty acids was linear for at least 60 min during incubation.

Results

Separation and quantification of H-TGL and LPL by heparin-Sepharose affinity chromatography. The series of fractions obtained by the stepwise elution procedure was assayed under the conditions optimal for H-TGL and LPL. The elution profiles of the triglyceride lipase activities are shown in Figs. 1 and 2. Loading of the sample at a NaCl concentration of 0.3 M resulted in the lowest loss of activity both during loading and in the first wash fraction. Accordingly this condition gave the highest recoveries. Partially puri-

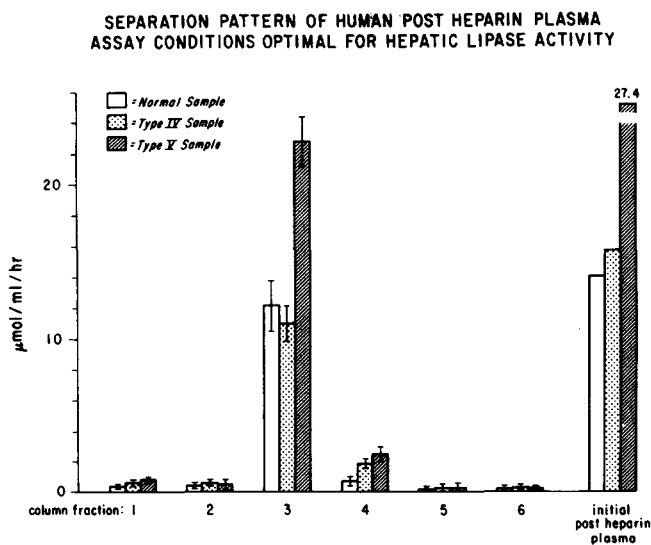


Fig. 1. Separation pattern of triglyceride lipase activities of human postheparin plasma. Assay conditions chosen were those optimum for H-TGL activity (pH 8.8, 0.75 M NaCl and without added apolipoprotein-C-II activator). All column fractions were 6 ml.

SEPARATION PATTERN OF HUMAN POST HEPARIN PLASMA
ASSAY CONDITIONS OPTIMAL FOR LIPOPROTEIN LIPASE ACTIVITY

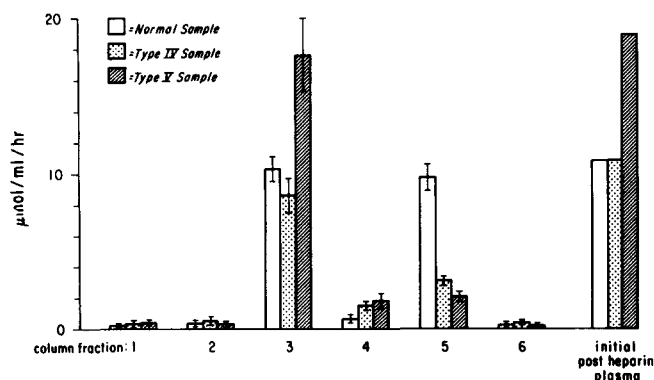


Fig. 2. Separation pattern of triglyceride lipase activities of human postheparin plasma. Assay conditions chosen were those optimum for LPL activity (pH 8.2, 0.13 M NaCl and with apolipoprotein-C-II as substrate activator). All column fractions were 6 ml.

fied enzyme fractions were rechromatographed, after dialysis to adjust the NaCl concentration back to 0.3 M; the recoveries of H-TGL in elution fraction 3 and of LPL in elution fraction 5 were 74 and 97%, respectively. Similar high recoveries were found for these enzymes from postheparin plasma of both normal subjects and patients with hypertriglyceridemia. The PHLA elution patterns from one normal subject and two patients with hyperlipoproteinemia (lipoprotein patterns in preheparin plasma samples were normal, Type IV and Type V respectively) are shown in Figs. 1 and 2. The recovery of H-TGL activity was similar for the three subjects, in spite of the fact that their serum triglyceride concentrations were quite different—83, 362, and 1840 mg/dl, respectively.

An additional check of the effect of lipemic plasma on the isolation of the two enzyme activities was made by mixing preheparin lipemic plasma with postheparin normal plasma. This did not change the values for either of the two lipolytic activities.

The triglyceride lipolytic activity measured in whole plasma in Fig. 2 represents the sum of LPL activity under optimal conditions and H-TGL activity under quite suboptimal conditions. During chromatography, the H-TGL is exposed to high ionic strength (0.72 M NaCl) and is thereby activated (17). This activation is thought to be due to disruption of a large molecular weight complex and is persistent even when the assay is conducted in a medium of much lower NaCl concentration (0.15 M NaCl). For these reasons very high values for recovery (150–200%) are obtained.

Reproducibility of the assay for the two enzyme activities over a period of time is shown in Fig. 3. One large sample of postheparin plasma was portioned and

stored frozen at -70°C for approximately 2 months. Each day a series of determinations was done. No significant systematic changes occurred during the 2 months of storage. Mean values and standard deviations for the H-TGL and LPL activities during the test period were 17.2 ± 2.3 and $3.9 \pm 0.7 \mu\text{mol}\cdot\text{ml}^{-1}\cdot\text{hr}^{-1}$, respectively.

The overall analytical errors of the method were 12.3 and 10.8% for H-TGL and LPL, respectively, when the whole procedure was done within one day. The corresponding proportion of the errors attributable to the affinity chromatography procedure was 11.4 and 9.6%, respectively, and the errors of the assay procedure were 4.6 and 4.3%, respectively, for the determination of the H-TGL and LPL activities.

The kinetics of the two enzyme activities after heparin injection were studied with the present method. The appearance of the LPL activity was slightly delayed compared to the appearance of the H-TGL activity. On the other hand LPL activity disappeared more rapidly from the blood than H-TGL, which is in agreement with earlier findings (18).

In six healthy subjects with normal serum lipid concentrations, the effect of a 48-hr fast on the H-TGL and LPL activities was studied. The H-TGL activity was unchanged, 11.6 ± 4.8 (SD) $\mu\text{mol}\cdot\text{ml}^{-1}\cdot\text{hr}^{-1}$, while the LPL activity significantly ($2P < 0.05$) increased from 3.8 ± 0.3 (SEM) to 5.4 ± 0.6 (SEM) $\mu\text{mole}\cdot\text{ml}^{-1}\cdot\text{hr}^{-1}$.

Discussion

The use of affinity chromatography with heparin-Sepharose appears to provide a useful and reproducible technique to determine quantitatively and separately the different triglyceride lipase activities in postheparin plasma. Previously described procedures (6) to separate H-TGL from LPL activities of postheparin plasma were associated with low recoveries of enzyme activities. One reason for the low recoveries was the delipidation of the plasma before affinity chromatography. The present procedure has thus been essentially modified in two ways. First, the postheparin plasma was not delipidated before the affinity chromatography. Secondly, application of the postheparin plasma in the heparin-Sepharose column was done at a higher ionic strength. These two changes increased the recovery considerably. The average recovery of H-TGL activity was 88 ± 11 ($\bar{x} \pm \text{SD}$) % in 18 normal subjects. This figure was calculated by comparing the H-TGL activity (isolated partially purified activity) with the original postheparin lipolytic activity in whole plasma assayed at high ionic strength (0.75 M NaCl). The recovery of the LPL activity was difficult to determine under ideal

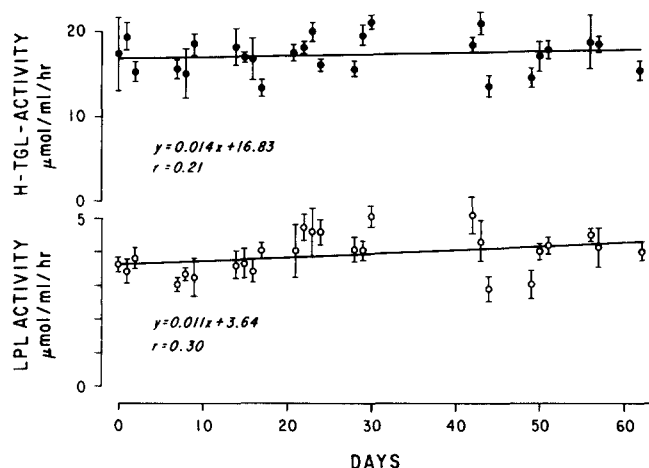


Fig. 3. Hepatic triglyceride lipase (H-TGL) and lipoprotein lipase (LPL) activities in a postheparin plasma. About 300 ml of postheparin plasma taken from a healthy female subject after intravenous injection of 60 IU heparin/kg body weight. The plasma was divided into 12-ml batches and stored frozen at -70°C . Each determination was done in triplicate and mean value \pm SD is indicated for each day of assay.

conditions. However, when the partially purified fraction of LPL activity was rechromatographed, 97% was recovered.

Two other methods have been described for determining H-TGL and LPL activities separately in human postheparin plasma (7, 18, 19). One of them (18, 19) is based on a technique by which an antibody to H-TGL is preincubated with the postheparin plasma. This procedure abolishes the H-TGL activity and the LPL activity can be determined exclusively. The other method (7) is based on the suggestion that protamine sulfate might be a selective inhibitor of LPL activity. Both of these earlier methods may have a disadvantage, since certain amounts of plasma containing lipoproteins and apolipoproteins are present in the incubation medium during the assays. Thus differences in recovered enzyme activities may not be correctly characterized. In the present method both the H-TGL and the LPL activity seem to be isolated free from major serum lipoprotein components. However, the importance of this aspect is not fully evaluated since in vitro addition of different concentrations of serum lipoproteins did not significantly change estimated activities of either H-TGL or LPL in the two methods described earlier (7, 18). The similar results obtained with the present method and the method described by Huttunen et al. (18) in applied clinical studies (20, 21) suggest that these two methods both adequately measure the two lipolytic activities in postheparin plasma.

During fasting the LPL activity increased while no change was found in H-TGL activity. Earlier studies by Robinson (22) in the rat showed that heparin-

releasable LPL activity in adipose tissue decreases during fasting while heparin-releasable LPL activity from heart muscle increases. In the rat, heparin-releasable LPL activity in skeletal muscle also increases during fasting (23). The present data in man thus suggest that the net increase of the postheparin LPL activity during fasting probably reflects an increase in muscle LPL activity. However, this remains to be proved by direct measurements. ■■

This work was supported by grants USPHS HL 14197 and HL 13304.

Manuscript received 20 July 1976 and in revised form 8 February 1977; accepted 23 February 1977.

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