Modified Heparin–Sepharose Procedure for Determination of Plasma Lipolytic Activities of Normolipidemic and Hyperlipidemic Subjects after Injection of Heparin

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A modified heparin–Sepharose affinity chromatography procedure (Boberg et al., J. Lipid Res. 18: 544–547, 1977) was developed to determine two different triglyceride lipase activities in human post-heparin plasma: hepatic triglyceride lipase (I) and lipoprotein lipase (II). With this procedure, lipoproteins were separated from the eluted lipases. The total lipolytic activity of II was eluted from heparin–Sepharose by heparin. The use of heparin as eluting agent prevents the partial inhibition of II, in contrast to the procedure based on elution of II with a high concentration of NaCl. In a comparative study with the modified heparin–Sepharose affinity column chromatography, the immunochemical and protamine sulfate inhibition procedures, the results indicated that these three procedures are equally suitable for the determination of I and II from normolipidemic subjects. However, because of possible interference by plasma, the column-chromatographic procedure is the preferred method for measuring lipase concentrations in post-heparin plasma of hyperlipidemic patients. The II activity of post-heparin plasma from normolipidemic subjects was not significantly age (20–39 and 40–60 years) or sex-related. I activity was also not significantly different with respect to age, but was significantly greater in men than in women.

Additional Keyphrases: triacylglycerol lipase · lipoprotein lipase · inhibition with protamine sulfate · immunochemical inhibition · sex- and age-related effects · hyperlipidemia · chromatography, affinity column · reference intervals · inhibition by apolipoprotein E

Plasma lipolytic activity after injection of heparin (“post-heparin”) consists of two lipolytic activities, hepatic triglyceride lipase (H-TGL; triacylglycerol lipase, EC 3.1.1.3) and lipoprotein lipase (LPL; EC 3.1.1.34), which originate from liver and extrahepatic tissues, respectively (1–4). These two lipases can be differentiated by procedures such as protamine inhibition (5), chromatography on heparin–Sepharose (6), and immunochemical inhibition assays (7, 8). Heparin–Sepharose affinity chromatography has been used to purify LPL from cow's milk (9). Ehnhoml et al. (10) demonstrated the NaCl concentration required for elution of the H-TGL in post-heparin plasma to be 0.6–0.74 mol/L and of the LPL to be 1.2–1.5 mol/L. This finding led to the development by Boberg et al. (6) of an affinity-chromatography procedure that enables selective measurement of these two enzymes. The decisive advantage of this method is that the eluted enzymes are free from their plasma substrates, such as chyloplacrons or very-low-density lipoproteins (3), obviating the varying influence of these substrate on the activities. However, because sodium chloride is an inhibitor of LPL activity, it is not possible to elute LPL activity quantitatively from heparin–Sepharose with NaCl solution.

We modified the procedure, using heparin as the eluting agent; this led to better analytical recovery (97 ± 4%) of both lipases from the heparin–Sepharose column. We also report LPL and H-TGL activities for both normolipidemic and hyperlipidemic subjects. These studies show that affinity chromatography is the method of choice for circumventing interference by plasma constituents in the assay of LPL and H-TGL activities, particularly in plasma from hyperlipidemic subjects.

Materials and Methods

Chemicals

Unless otherwise stated, all chemicals were purchased from Sigma Chemical Co., St. Louis, MO 63178. Pre-activated cyanogen bromide Sepharose 4B was obtained from Pharmacia, Piscataway, NJ 08854. Beef-lung heparin solution for use in human subjects was obtained from The Upjohn Co., Kalamazoo, MI 49001. The powder form of beef-lung heparin was a gift from The Upjohn Co. Protamine sulfate (lot no. 910123) was from Calbiochem, San Diego, CA 92112. Glycerol tri[1-14C]oleate with a specific activity of 55 Ci/mol was obtained from Amersham Corp., Arlington Heights, IL 60005. The disposable column for heparin–Sepharose affinity chromatography was obtained from Cooke Laboratory Products, Alexandria, VA 22314.

Preparation of Heparin–Sepharose

The pre-activated cyanogen bromide Sepharose 4B was used for coupling with heparin (beef-lung origin). Fifteen grams of gel was swollen and washed for 15 min on a sintered-glass filter with 1 L of 1 mmol/L HCl. Heparin (1 g) was dissolved in 75 mL of a 0.1 mol/L NaHCO3 buffer solution containing 0.5 mol of NaCl per liter and mixed with the gel in four 50-mL test tubes; the mixture was rotated end-over-end for 24 h at 4 °C. Unbound heparin was washed away with 200 mL of coupling buffer, which was quantitatively assayed for uronic acid (11). The remaining active groups of the gel were then reacted with ethanolamine (1 mol/L, pH 8, 2 h). The heparin–Sepharose was further washed with 1 L of sodium acetate buffer (0.1 mol/L, pH 4.0) containing 1 mol of NaCl per liter and then with 1 L of 0.1 mol/L sodium borate buffer, pH 8.0, containing 1 mol of NaCl per liter. The gel was washed with 3 L of distilled water and stored in NH4OH · HCl buffer (50 mmol/L, pH 8.5) at 4 °C. The amount of heparin covalently linked was 6 to 10 g per liter of Sepharose.

Subjects

Donors were classified as normolipidemic or hyperlipidemic according to the procedures of the Lipid Research Clinics (12). All hyperlipidemic subjects had a type V lipoprotein electrophoretic pattern. The samples were obtained before any medication or treatment. Blood samples were drawn by venipuncture from subjects who had fasted for 12–14 h. Fully

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informed written consent was obtained from all subjects after approval of the project by the Human Investigation Committee of the Oklahoma Medical Research Foundation. Heparin administration and blood drawing were performed in the Outpatient Clinic of the Oklahoma Medical Research Foundation.

Post-Heparin Plasma
Subjects were injected with 100 int. units of heparin per kilogram of body weight up to the limit of 10 000 int. units of heparin as maximum total dose. Blood was sampled 30 min after this injection, and the tubes were immediately placed in ice. Plasma, separated by centrifugation at 4 °C, was stored at −20 °C.

Preparation of Antiserum to Human Milk Lipoprotein Lipase
Human milk cream acetone–ether powder was prepared as described by Hernell and Olivecrona (13), solubilized with Triton X-100 surfactant, and fractionated on a column of heparin–Sepharose as described previously (14). The LPL, now concentrated 316-fold, was used for raising an antiserum to LPL. For this, 1 mL of the enzyme solution (0.2–0.5 g/L) in deoxycholate solution (9–10 g/L) was mixed with an equal volume of complete Freund's adjuvant (Difco Laboratories, Detroit, MI 48232) and injected intraperitoneally into goats at weekly intervals for four weeks. Precipitating antibodies were produced after the fourth week of injection. The antiserum showed a single precipitin line against human milk by immunodiffusion. For studies on the inhibition of LPL activity, the immunoglobulin fraction of antiserum was prepared according to a standard ammonium sulfate (33% saturation) precipitation technique. The immunoglobulin fraction was dialyzed against the NH₄OH - HCl buffer (with no added NaCl) and adjusted to a protein concentration of 15 g/L.

Enzyme Assay
The lipolytic activity of the enzyme preparations was measured with [14C]triolein emulsified in Triton X-100 (15). All assays were carried out in the NH₄OH - HCl buffer containing 60 g of bovine serum albumin per liter as fatty acid acceptor. We used 0.1 mL of serum from a normolipidemic subject as activator. The substrate concentration was 10 mmol (0.1 mC) of triolein per liter. The final volume of the assay mixture was 1.0 mL. After incubation at 37 °C for 1 h, the reaction was stopped by adding 4 mL of a mixture of isopropanol and 1.5 mmol/L H₂SO₄ (40/1 by vol). After mixing, the lipids were extracted by the further addition of 2 mL of water and 5 mL of n-hexane, and shaking. Fatty acid was extracted from 3 mL of the separated hexane layer with 1 mL of 0.1 mol/L KOH, and 0.5 mL of the aqueous KOH solution was taken for measurement of the radioactivity (15).

The unit of activity was defined as the amount of enzyme catalyzing the formation of 1 μmol of fatty acid per hour at 37 °C.

Selective Inhibition of LPL
LPL was inhibited by antibodies to human milk LPL by incubating 0.1-mL aliquots of post-heparin plasma with 0.1–0.9 mL of the immunoglobulin. The volume of the incubation mixture was adjusted to 1 mL with the NH₄OH - HCl buffer and incubated at room temperature (25 °C) for 1 h. We assayed 0.4 mL of each reaction mixture for the remaining activity.

We inhibited LPL with NaCl by including NaCl (1 mol/L) in the assay mixture.

We inhibited LPL with protamine sulfate according to Krauss et al. (5).

Chromatography on Heparin–Sepharose
For affinity chromatography we used two small disposable columns, 7 mm in diameter, packed with heparin–Sepharose to give a 2-cm column. Post-heparin plasma was mixed with an equal volume of the NH₄OH - HCl buffer containing 0.3 mol of NaCl per liter. A 4-mL aliquot of the diluted plasma

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**Table 1. Dependence of Post-Heparin Plasma Lipolytic Activity (PHLA) on the Procedure for Assay in Normolipidemic and Hyperlipidemic Plasma**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Sex</th>
<th>Triglyceride g/L</th>
<th>Cholesterol g/L</th>
<th>Plasma vol for assay, mL</th>
<th>PHLA, units/mL</th>
<th>PHLA after heparin–Sepharose chromat., units/mL</th>
<th>LPL*, units/mL</th>
<th>H-TGL, units/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Normal</td>
<td>♂️</td>
<td>1.01</td>
<td>1.89</td>
<td>0.1</td>
<td>19.8</td>
<td>20.3</td>
<td>14.2</td>
<td>6.1</td>
</tr>
<tr>
<td>2. Normal</td>
<td>♂️</td>
<td>0.58</td>
<td>1.56</td>
<td>0.05</td>
<td>23.8</td>
<td>23.6</td>
<td>16.5</td>
<td>7.1</td>
</tr>
<tr>
<td>3. Type V</td>
<td>♂️</td>
<td>1.052</td>
<td>1.92</td>
<td>0.05</td>
<td>20.9</td>
<td>46.5</td>
<td>12.7</td>
<td>33.8</td>
</tr>
<tr>
<td>4. Type V</td>
<td>♂️</td>
<td>4.069</td>
<td>6.30</td>
<td>0.05</td>
<td>5.1</td>
<td>29.7</td>
<td>7.4</td>
<td>22.3</td>
</tr>
<tr>
<td>5. Type V</td>
<td>♂️</td>
<td>9.050</td>
<td>8.40</td>
<td>0.05</td>
<td>0.8</td>
<td>13.3</td>
<td>7.0</td>
<td>6.3</td>
</tr>
</tbody>
</table>

* LPL and H-TGL were determined with column A procedure.
was applied to one column (A). After the sample was loaded, the column was washed with 4 mL of the NaCl-containing buffer, then LPL and H-TGL were eluted together with 4 mL of buffer containing 10 g of heparin per liter.

The other 4-mL aliquot of the diluted plasma was applied to the other column. After the loading and washing with 4 mL of the NaCl-containing buffer, H-TGL was eluted first with 4 mL of buffer containing NaCl (0.72 mol/L) and then LPL was eluted with 4 mL of buffer containing heparin (10 g/L).

The affinity chromatography was performed at 4 °C. The time required for performing the column chromatography with the column A procedure was 1–2 h and 2–3 h for normolipidemic and hyperlipidemic post-heparin plasma, respectively; an additional hour was required for the column B procedure.

Results

Enzyme Assay

With the usual assay procedure in which 10 mmol of emulsified triolein per liter is used as substrate, the lipolytic activities were directly proportional to the amount of post-heparin plasma. Because this relation was apparent for normolipidemic subjects (Figure 1 and Table 1), post-heparin plasma lipolytic activity could be reliably measured. This conclusion was confirmed by results obtained for more than 40 normolipidemic post-heparin plasma samples.

This relationship was not linear for post-heparin plasma (Table 1) from subjects 3, 4, and 5, in which the triglyceride concentrations were 10.52, 40.69, and 90.50 g/L, respectively. The estimation of lipase activity appeared to depend on the amount of post-heparin plasma in the assay mixture: in all three cases, the lower the concentration of post-heparin plasma in the assay, the higher was the lipolytic activity. Although it was unclear whether the abnormal behavior of these post-heparin plasma samples was due to the presence of endogenous lipoprotein triglyceride, to a lipase inhibitor, or to the lack of a lipase activator, it was evident that a preliminary fractionation step was needed prior to the lipase assay for the lipolytic activity to be fully expressed in these hyperlipidemic subjects.

Affinity Chromatography on Heparin-Sepharose

Under the present experimental conditions, plasma lipoprotein was not retained by heparin-Sepharose. As tested by immunodiffusion, with use of antisera to apolipoproteins B, C-II, C-III, and E (16), all of these apolipoproteins were detected in the loading fraction, with only trace amounts in the 0.3 mol/L NaCl fraction. Neither triglyceride nor these apolipoproteins were detected in the eluted fraction from column A or in the fractions eluted with 0.72 mol/L NaCl and heparin from column B.

The H-TGL and LPL mixture was quantitatively eluted from column A by the 10 g/L solution of heparin. A study in which we used various concentrations of heparin indicated that 7 to 15 g/L provided complete recovery (Figure 2). For six normolipidemic post-heparin plasma samples, the analytical recovery ranged from 92 to 105% of the activity applied to the column (mean, 97 ± 4%). A higher concentration of heparin (20 g/L) caused inhibition of the lipases and resulted in low recovery. Therefore, in our routine affinity chromatography procedure, 10 g of heparin per liter was chosen for the elution of lipases. The lipase activity measured without the activator from column A corresponded to the value for H-TGL as determined by column B in the fraction eluted with 0.72 mol/L NaCl. The lipolytic activity in this NaCl-eluted fraction was not increased by adding serum. Thus, a simplified assay of H-TGL can be done by use of column A alone. H-TGL can be measured directly from the heparin-eluted fraction of column A in the absence of the activator (serum), and the total activity can be measured in the presence of the activator. The difference between total activity and H-TGL activity would represent the LPL activity.

The sequential elution of H-TGL and LPL from column B resulted in unsatisfactory analytical recovery of LPL (50–70%), probably owing to partial irreversible inhibition of LPL by the high concentration of NaCl (0.72 mol/L) during elution of H-TGL. Therefore we used the column A procedure in routine analyses for LPL and H-TGL.

In our routine studies, we used Upjohn beef-lung heparin for the preparation of heparin-Sepharose and for the elution of the lipases (column A). However, the affinity chromatography procedure could also be performed with comparable results by using the same concentration of pig intestinal heparin (ICN Pharmaceuticals, Inc., Cleveland, OH 44128) to elute LPL and H-TGL. Pig intestinal heparin (10 g/L) could also be used to elute LPL and H-TGL from the Sepharose where it had been covalently linked with the beef-lung heparin.

Post-Heparin Lipolytic Activity in Plasma of Normal and Hyperlipidemic Subjects

The activities of H-TGL and LPL in men and women 20–39 and 40–60 years of age were determined by use of the heparin-Sepharose affinity chromatography procedure (Table 2). These two age groups showed no significant difference in the activities of either LPL or H-TGL. The LPL activity for men and women was similar, but H-TGL activity was higher in men than in women (p < 0.01). Neither LPL nor H-TGL was correlated with the serum triglyceride concentration measured during fasting.

Measurement of post-heparin lipolytic activities in patients with type V hyperlipidemia gave different values for the 0.1- and 0.05-mL plasma samples used for assay. This indicated that the LPL and H-TGL could not be differentially determined by using an LPL inhibitor because of the non-additive activities of the lipases. The heparin-Sepharose procedure with use of column A yielded lipolytic activities that were significantly higher than those assayed directly from unfractionated post-heparin plasma. This is consistent with the finding that the addition of pre-heparin plasma from hyperlipidemic patients 3, 4, and 5 (Table 1) exhibited an inhibitory effect (28, 83, and 90%, respectively) on the post-heparin.
Table 2. Lipoprotein Lipase (LPL) and Hepatic Triglyceride Lipase (H-TGL) Activities in Normolipidemic Subjects

<table>
<thead>
<tr>
<th>Age</th>
<th>n</th>
<th>LPL, a units/mL</th>
<th>PHLA, b %</th>
<th>H-TGL, b units/mL</th>
<th>PHLA, b %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20–39</td>
<td>11</td>
<td>15.7</td>
<td>59</td>
<td>10.9</td>
<td>41</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(3.9)</td>
<td>(4.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>40–60</td>
<td>9</td>
<td>12.5</td>
<td>52</td>
<td>11.7</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(3.3)</td>
<td>(5.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20–39</td>
<td>10</td>
<td>12.9</td>
<td>64</td>
<td>7.3</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(3.6)</td>
<td>(2.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>40–60</td>
<td>4</td>
<td>15.8</td>
<td>69</td>
<td>7.0</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(5.7)</td>
<td>(2.8)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a No significant difference between two age groups or with respect to sex.
b No significant difference between two age groups. H-TGL activity was higher in men than in women (p <0.01). c Standard deviation in parentheses.

The use of unfractionated normolipidemic post-heparin plasma provided results similar to those obtained by the heparin–Sepharose procedure (column A) when used directly for the assay of total post-heparin lipolytic activity. The values were independent of the volume of plasma used for assay, and these two results together suggest that normolipidemic plasma does not interfere in the lipase assay. On the other hand, the lack of a direct proportionality of lipase activity to plasma volume when type V hyperlipidemic post-heparin plasma was used as enzyme source indicated the presence of interference by such plasma. This finding indicates that fractionation of such hyperlipidemic post-heparin plasma is needed before the lipase assay. Removal of apolipoproteins B, C-II, C-III, and E, and of endogenous triglyceride during the loading and 0.3 mol/L NaCl washing step of heparin–Sepharose chromatography indicated that this fractionation procedure effectively removed possible interference by lipoproteins in the assay.

Discussion

The residual activity after addition of the inhibitor represents H-TGL. The inhibitors for LPL were: (a, top) antiserum to human milk LPL; (b, bottom) 4 mg/0.1 mL of protamine sulfate in the incubation mixture. (---) the least squares line fitted to the experimental data; (——) the line for identical values by both procedures.

plasma lipolytic activity of a normolipidemic subject. It is known that plasma apolipoproteins affect lipoprotein lipase activity variably (17–20). In view of the high content of apolipoprotein E in plasma from type V hyperlipidemic subjects (16), we examined the inhibitory effect of apolipoprotein E and found that 1 g of apolipoprotein E per liter of assay mixture caused 77% inhibition of post-heparin plasma lipolytic activity from a normolipidemic subject. The apolipoprotein E concentration in plasma of normolipidemic subjects was 0.1 (SD 0.04) g/L in serum (16); the concentrations in patients 3, 4, and 5 were 0.42, 0.72 and 1.01 g/L, respectively.

Differential Determination of LPL and H-TGL with LPL Inhibitors

In a study of 19 normolipidemic subjects, the mean values for H-TGL activity as estimated by the heparin–Sepharose or immunochemical procedure were essentially identical, representing 34 and 35% of post-heparin lipolytic activity (Figure 3b). The least-squares fitted line from the experimental data had a correlation coefficient of 0.92 and a slope of 0.96. The overall analytical error for the bulk lipase assay was 7.8%; the standard error of the estimate from linear regression of the data was 4.8%. The measured activity (H-TGL) remaining in the presence of 1 mol/L NaCl was greater than that determined by the heparin–Sepharose procedure; whereas 52% of post-heparin lipolytic activity was not inhibited by 1 mol/L NaCl, the H-TGL activity estimated by the heparin–Sepharose procedure represented 34% of post-heparin lipolytic activity. These results suggest that 1 mol/L NaCl incompletely inhibits LPL. We confirmed this by assaying human milk LPL in the presence of 1 mol of NaCl per liter, which indicated only 70% inhibition by the NaCl. A study in which protamine sulfate was used (5) also indicated incomplete inhibition of LPL, the mean value of H-TGL activity representing 43% of post-heparin lipolytic activity. However, an increase in protamine sulfate concentration from 3 mg/0.1 mL to 4 mg/0.01 mL of preincubation mixture resulted in more nearly complete inhibition of LPL (Figure 3b) and provided a better estimate of H-TGL. The mean value for H-TGL as determined by this inhibition procedure was 37% of post-heparin lipolytic activity, essentially the same as that determined by the heparin–Sepharose or immunochemical methods. The standard error of the estimate as determined by linear regression was 5.4% (Figure 3b).
Because we used heparin as the eluting agent for the lipases in the column chromatographic procedure, it was important to consider a possible modulating effect of heparin on the post-heparin lipolytic activity. From the excellent recovery of post-heparin lipolytic activity from normolipidemic subjects with the heparin-Sepharose column (97 ± 4%), we conclude that heparin had no effect on the lipase activities. Although heparin may play a role as a lipase inhibitor or activator (21), this was observed only when the assay conditions were suboptimal. From a kinetic study, Posner and Morrison (22) suggested that heparin behaves as a competitive inhibitor with respect to the lipoprotein cofactor. Thus it is possible that the inhibitory effect of heparin is overcome by the presence of a saturating concentration of the cofactor. The mixing of equal volumes of a hyperlipidemic fasting plasma (triglyceride concentration, 33.75 g/L) and a normolipidemic post-heparin plasma did not affect the essentially quantitative (98%) recovery of the lipases by elution with heparin. Therefore, we conclude that the higher lipid content of the post-heparin plasma had no effect on the recovery of lipases. However, the time required for elution of lipases may be increased because of the slower flow rate of the column.

The low lipolytic activities of unfractionated plasma from type V hyperlipidemic subjects (Table 1) cannot be accounted for solely in terms of isoform dilution by the endogenous triglyceride substrates. Other factors, such as the presence of inhibitor(s) and lack of an activator, should also be considered. In fact, plasma from subject 3 showed normal LPL activity and above-normal H-TGL activity when these determinations were carried out after heparin-Sepharose fractionation. The subject's post-heparin lipolytic activity was also shown to increase upon dilution of plasma as determined by the standard assay procedure. These results indicate that in this subject as well as in subjects 4 and 5 (Table 1), the presence of an inhibitor(s) may be responsible for the impaired triglyceride metabolism. Because the post-heparin plasma used in the assay mixture represented 10- and 20-fold dilutions, it is expected that the inhibition in vivo may be even more pronounced. Thus, the determination of plasma lipolytic activities by the modified heparin-Sepharose procedure and the standard assay for post-heparin lipolytic activity using various plasma dilutions may provide a new means for identifying hypertriglyceridemic states because of the presence of lipase inhibitor(s). Although we have confirmed the reported inhibitory effect of apolipoprotein E (19, 20), the possible presence of other lipase inhibitors remains to be established. Recently, Brunzell et al. (23) reported the first case of a familial serum LPL inhibitor and presented evidence that this heat-stable and nondialyzable inhibitor completely inhibited normal adipose tissue LPL, both in the presence and absence of the activator.

It seems that the inhibition of LPL by a specific antiserum is adequate for the differentiation of LPL and H-TGL activities in normolipidemic plasma. This also applies to protamine sulfate when preincubated with post-heparin plasma at a concentration of 4 mg/0.1 mL. However, because the total post-heparin lipolytic activity must be determined in both of these methods, it seems that the modified heparin-Sepharose procedure using heparin for the elution of lipases is best suited for hypertriglyceridemic plasma. The higher protamine sulfate concentration required for the inhibition of LPL could be due to a higher amount of heparin used in this study for obtaining post-heparin plasma. After comparing different methods for selective measurement of LPL and H-TGL, Greten et al. (24) found good correlation between the procedures for determining the activity of the lipases by heparin-Sepharose column chromatography and immunochemical methods but not between these and the protamine sulfate assay procedures. The recovery of LPL was not indicated in the study.

It has been shown that the release of lipolytic activities into plasma is dependent upon time, heparin, and heparin dose. The dose of heparin needed for maximal release of activity is 100 to 200 units/kg body weight for LPL, but only about 50 int. units/kg for H-TGL (7). Use of a lower heparin dose is not desirable (7), because the response of LPL is in a dose-sensitive range. Therefore, Huttunen et al. (7) selected a dose of 100 int. units/kg for maximal release of both enzymes. Because this dose of heparin is within the recommended therapeutic range (25), we have also chosen this dose for obtaining the post-heparin plasma. Thus, the low levels of activities for post-heparin lipase reported by Greten et al. (8) and Krauss et al. (5) may in part be due to the low doses of heparin they used in these studies. On the other hand, Huttunen et al. (26), using the same dose of heparin (100 int. units/kg), found activities of both LPL and H-TGL to be higher than those presented in this study. Although the difference may be due in part to differences in assay conditions, it is equally possible that there is a genuine difference in the lipolytic activities between the population of Helsinki and Oklahoma City. The significance of this finding remains to be explored in further studies.

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References