A new method for the measurement of lipoprotein lipase in postheparin plasma using sodium dodecyl sulfate for the inactivation of hepatic triglyceride lipase

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Abstract Lipoprotein lipase (LPL) and hepatic triglyceride lipase (H-TGL) are lipolytic activities found in postheparin plasma. A simple and precise method for the direct determination of LPL in postheparin plasma is described. Preincubation of this plasma (45–60 min at 26°C) with sodium dodecyl sulfate (35–50 mM) in 0.2 M Tris-HCl buffer, pH 8.2, results in the inactivation of H-TGL, while leaving LPL fully active. Direct determination of H-TGL is done in a separate aliquot of the same postheparin plasma sample using previously reported assay conditions that do not measure LPL. The sodium dodecyl sulfate-resistant lipolytic activity has the characteristics of LPL as judged by a) its activation by serum and by apoE-apoproteinC-11; b) its inactivation (over 90%) by 0.75 M NaCl; and c) its inactivation by a specific antiserum. No sodium dodecyl sulfate-resistant activity was found in postheparin plasma from a patient with LPL deficiency (primary type I hyperlipoproteinemia). An excellent correlation of values was obtained (r = 0.99) for 30 samples assayed after sodium dodecyl sulfate treatment and after immuno-inactivation of H-TGL. The intra-assay coefficient of variation was ±11% and 4% before and after normalization of values, respectively.

Supplementary key words anti-lipoprotein lipase serum apoC-II

Lipoprotein lipase (LPL) (glycerol ester hydrolase, EC 3.1.1.3), an enzyme present in several extrahepatic tissues and released into the blood stream by heparin (1), is thought to play a key role in the removal of triglycerides from chylomicrons and very low density lipoproteins (2–5). A triglyceride hydrolase believed to originate in hepatic tissues is also released by heparin into the circulation (6–11), making a significant contribution to the total postheparin lipolytic activity. The function of this hepatic triglyceride lipase (H-TGL) in the clearance of plasma triglycerides has not yet been elucidated.

In 1955 Korn (12) observed that lipoprotein lipase from several tissues was inhibited by 1 M NaCl and by protamine sulfate. These inhibitory effects are not found with the triglyceride lipase released into plasma from liver by heparin (6, 13). These properties provide the basis of an assay (8, 14) in which the hepatic triglyceride lipase activity of postheparin plasma is determined after inhibition of most of the LPL activity by protamine sulfate. LPL is then estimated as the difference in activity determined in assays with or without protamine sulfate. Problems related to the indirect nature of this method for LPL determination make it less desirable than methods assaying LPL directly. Independent determinations of both LPL and H-TGL activities in postheparin plasma have been possible after their separation and partial purification by column chromatography on Sepharose gel containing covalently bound heparin (15). This permits the assay of each enzyme under its optimal assay conditions, but the method is laborious and involves significant losses during chromatography. Selective inhibition of H-TGL or LPL by antisera prepared against the purified enzymes has provided a rapid and precise method for the determination of each of these enzymes in human postheparin plasma (PHP) samples (16, 17). However, such specific antisera are not widely available.

The activities of LPL and H-TGL purified by affinity chromatography on heparin–Sepharose gel are affected quite differently by several detergents (18). It has recently been observed in this laboratory that sodium dodecyl sulfate (SDS) completely inactivates

Abbreviations: apoC-I, apolipoprotein C-I; apoC-II, apolipoprotein C-II; H-TGL, hepatic triglyceride lipase; LPL, lipoprotein lipase; PHP, postheparin plasma; SDS, sodium dodecyl sulfate; Tris, tris(hydroxymethyl)aminomethane.
partially purified H-TGL at concentrations that protect LPL activity. This laid the basis of a new method for the direct determination of LPL in PHP. Preliminary reports of these studies have been presented (19, 20).

MATERIALS AND METHODS

Pre- and postheparin plasma samples

The studies were carried out using plasma samples from normal subjects of both sexes and from patients with type I, III, IV, and V hyperlipoproteinemias. The system used for phenotyping was that proposed by Fredrickson and Levy (21) as adopted by the World Health Organization (22). All blood samples were drawn after an overnight fast of at least 12 hr. Before injection of heparin, a 15-ml blood sample was collected for the determination of total triglycerides, total cholesterol, and quantitation of lipoproteins (23). Sodium heparin derived from intestinal mucosa (Riker Labs, Inc.) was then injected at 60 IU per kg of body weight. Forty ml of blood were collected 15 min later, immediately cooled on ice, and centrifuged for 30 min at 480 g. The plasma was then either assayed immediately or after storage (up to 3 months) at −70°C.

Enzyme sources

Purified H-TGL and LPL were prepared from human PHP using affinity chromatography on heparin-Sepharose gel, as described previously (15, 24). All other studies used PHP as source of enzymes. Frozen PHP samples were thawed immediately prior to use. For determination of LPL, 0.5 ml of PHP was diluted with 0.5 ml of an SDS solution (at the indicated concentrations) in 0.2 M Tris-HCl, pH 8.2, and preincubated for the indicated times at 26°C before assay of hydrolytic activity as described below. For determination of H-TGL, 0.5 ml of PHP was diluted with 0.5 ml of 0.2 M Tris-HCl, pH 8.8 (room temperature), and assayed 40 sec later, as described below.

Source of activator peptides

Blood samples, used as a source of activator peptides for LPL determination, were obtained from several normal individuals after an overnight fast. The sera were pooled, heated for 60 min at 50°C (to inactivate lipolytic activity), and stored (−70°C) in aliquots until use. Purified apoC-I and apoC-II were prepared as previously described (18) and stored at −70°C in 0.05 M ammonium bicarbonate buffer.

Substrate

Routinely, the substrate was prepared by mixing sufficient unlabeled trioleoylglycerol (Nu Check Prep., Inc., Elysian, MN) with tri-[1-14C]oleoylglycerol (Dhom, North Hollywood, CA) to provide a specific activity in the range of 0.106–0.111 μCi/μmol. To remove free oleic acid the mixture, 4 g in 50 ml of benzene, was passed through a 30 × 1.6 cm column of Amberlite IRA-400 resin which had been equilibrated with 500 ml of benzene. The substrate emulsion was prepared by addition of 6.8 ml of a solution of gum arabic (15 g/100 ml) in 0.2 M Tris-HCl of pH 8.2 or pH 8.8 (see assays) to 200 mg of trioleoylglycerol. The latter had been deposited by evaporation from the appropriate volume of the benzene stock solution. Emulsions were then obtained by sonication, using a microtip of the Branson sonifier-cell disruptor (Model W185, Heat Systems, Ultrasonics, Inc., Plainview, NY). Sonication was done at room temperature, using 8–12 bursts lasting 10–15 sec (until no more fat globules could be noted on the walls of the tube when its content was swirled and the tube was examined against a strong light). The resulting emulsion contained 5 μmol of trioleoylglycerol per 0.15 ml.

Other materials

Amberlite IRA-400 (Mallinckrodt, Inc.), medium porosity, 20–50 mesh, was used both in the preparation of substrate (as above) and for quantitation of the oleic acid produced by the hydrolytic enzymes. After sequential washing with 2.5 M NaOH, isopropyl alcohol, and with a mixture of isopropyl alcohol–water 9:1, the resin was allowed to equilibrate with the latter for 2–3 hr with occasional shaking. After three such treatments, the resin was washed several times with n-heptane and then stored in heptane (at 4°C) in a dark container. More than 99% of added oleic acid standard was consistently absorbed on the resin; elution of oleic acid varied with the resin batch from 85 to 98%.

Fatty acid-free albumin was prepared by treating bovine serum albumin fraction V (Reheis Chemical Co., Chicago, IL) with charcoal, according to the method of Chen (25). Sodium dodecyl sulfate (BDH Laboratory Reagents, Lot #324810; Accurate Chemical and Scientific Corporation, Hicksville, NY) was recrystallized twice from hot water and twice from hot ethanol before use. Other preparations of SDS were tested either after recrystallization or directly as supplied by Eastman Kodak Chemical Co. (Lots #175X and C5A), BioRad (Lots #15836 and #15881), and BDH Laboratory Reagents (Lot #1889972). [1-14C]-Oleic acid was from ICN Pharmaceuticals Inc., Irvine, CA; unlabeled oleic acid, gum arabic, and Trizima Base from Sigma Chemical Co., St. Louis, MO; Triton X-100 (technical grade) was obtained from A. J. Linch & Co., Los Angeles, CA. Technical grade hep-
The preparation and characteristics of antisera specific for H-TGL and for LPL have been described previously (29). The protein contents in the two antisera and in a control (normal goat serum) were equalized (63 mg/ml) by the addition of fatty acid-free bovine serum albumin. Prior to use, the small trioleoylglycerol hydrolytic activities of these sera were inactivated by heating for 1 hr at 50°C in a water bath. Unless specified otherwise, the PHP samples were mixed with the anti-H-TGL (or anti-LPL) serum in the ratio 1:10 (v/v) and incubated for 60 min at 28°C. The incubated mixtures (in 1.5 ml polypropylene “Micro Test Tubes,” Brinkman Instruments, Inc., Westbury, NY) were cooled on ice and centrifuged at room temperature for 1 min at 12,000 g (Eppendorf Centrifuge 3200, Brinkman Instruments Inc.). Remaining triglyceride hydrolytic activity was measured by transferring (in triplicate) 0.06 ml of the supernatant solutions into tubes containing 0.44 ml of assay media appropriate for the determination of H-TGL or LPL as described above.

Recoveries and expression of results

In each experiment, recoveries under all assay conditions were determined using a standard solution of [1-14C]oleic acid bound to albumin (30). Routinely recoveries were 64–69%. An “apparent specific activity” which compensated for quenching due to the resin and the Triton-KOH solution in the counting vials, was determined for each new preparation of trioleoylglycerol and resin. For this, 10 μl of the stock tri-[1-14C]oleoylglycerol solution was added into vials containing resin, treated as above. This apparent specific activity was used in the calculations for fatty acid liberated in the enzymatic reactions. After correction for recovery, results were expressed in μmol of fatty acid liberated in 1 hr per ml of PHP.

RESULTS

Development of method

Concentrations of SDS and time of pre-incubation. As shown in Table 1, concentrations of SDS could be found that completely inhibit H-TGL activity (1000 μM), producing a modest increase in LPL activity. The latter activity rapidly declined after dilution with buffer alone or with concentrations of SDS below 200 μM. With sufficient SDS present the enzyme preparation was stabilized and, in fact, gave higher activity than in the initial concentrated solution as previously reported (18).

The possible use of SDS for the selective inhibition of H-TGL in PHP was tested in a model system in which...
which the purified enzymes mixed with normal serum were pre-incubated with SDS. In this model system, SDS also inhibited H-TGL activity, while not affecting that of LPL (19). However, a much higher concentration of SDS (greater than 25 mM) was required to obtain complete H-TGL inactivation. This was expected from the known binding of SDS by albumin and other proteins (31).

Preliminary experiments with PHP showed that the trioleoylglycerol hydrolase activity that remained after pre-incubation with SDS had the characteristics of LPL, i.e., required a serum activator and was markedly suppressed by assay in media with 0.75 M NaCl. On the other hand, all trioleoylglycerol hydrolase activity measured under the conditions optimal for H-TGL (10, 32) was negligible after pre-incubation with SDS. It was also noted in these studies that a) if the concentration of SDS or the time of pre-incubation of PHP with SDS greatly exceeded that required for H-TGL inactivation, inhibition of LPL activity also occurred; b) longer periods of pre-incubation with SDS were required with PHP samples from subjects with hyperlipoproteinemia than those from normals; and c) SDS concentrations above 1 mM in the final assay interfered with the determination of LPL activity. Therefore, conditions were sought under which complete inactivation of H-TGL was obtained without loss of LPL activity, using the minimum amount of SDS and a convenient pre-incubation time. Furthermore, these conditions should be applicable to PHP samples from both normal subjects and individuals with elevated lipid levels.

When assayed under conditions optimal for H-TGL, it was found that virtually all activity was suppressed after pre-incubation of PHP in SDS concentrations greater than 20 mM for 2 hr. This was true in normal subjects and in those with hypertriglyceridemia, (tri-glycerides greater than 2000 mg/dl). The activity measured under conditions optimal for LPL fell with addition of low levels of SDS but remained constant at the lower value when preincubated with SDS concentrations in the 20–35 mM range. Under the assay condition for LPL both H-TGL and LPL are measured, but with the addition of sufficient SDS H-TGL is suppressed and the only activity remaining is that of LPL. With some preparations of SDS, concentrations up to 50 mM could be used without further reduction in activity. In a medium containing activator peptide (serum), the trioleoylglycerol hydrolase activity remaining after pre-incubation with SDS was 80–90% suppressed by 0.75 M NaCl, indicating that the activity resistant to SDS was due to LPL.

The time course of H-TGL inactivation at 26°C was examined using PHP samples pre-incubated with 35–50 mM SDS. In some normal subjects the H-TGL activity was completely inactivated after 30 min of pre-incubation with 50 mM SDS (Eastman Kodak, Lot

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**TABLE 1. Dilution and pre-incubation of purified H-TGL and LPL with SDS in Tris-HCl buffer**

<table>
<thead>
<tr>
<th>SDS during Pre-incubation</th>
<th>H-TGL</th>
<th>LPL</th>
</tr>
</thead>
<tbody>
<tr>
<td>µM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Undiluted sample)</td>
<td>(100)</td>
<td>(100)</td>
</tr>
<tr>
<td>0</td>
<td>85.1</td>
<td>12.5</td>
</tr>
<tr>
<td>50</td>
<td>87.3</td>
<td>17.7</td>
</tr>
<tr>
<td>100</td>
<td>95.1</td>
<td>57.5</td>
</tr>
<tr>
<td>200</td>
<td>95.0</td>
<td>130.9</td>
</tr>
<tr>
<td>300</td>
<td>98.0</td>
<td>134.2</td>
</tr>
<tr>
<td>400</td>
<td>98.0</td>
<td>137.3</td>
</tr>
<tr>
<td>500</td>
<td>88.7</td>
<td>133.8</td>
</tr>
<tr>
<td>600</td>
<td>58.7</td>
<td>128.7</td>
</tr>
<tr>
<td>1000</td>
<td>1.4</td>
<td>121.9</td>
</tr>
</tbody>
</table>

H-TGL and LPL purified from PHP by heparin-Sepharose gel column chromatography (24) were diluted 6- and 3-fold, respectively, to a final enzyme protein concentration of 40 µg/ml. The diluant, 0.2 M Tris-HCl of pH 8.2, contained the appropriate quantities of SDS to give the final concentrations indicated. After 5 min of pre-incubation at 28°C, trioleoylglycerol hydrolase activity was measured as described previously (15) in an assay system containing in 0.5 ml: 1.15 µmol of trioleoylglycerol, 5 µg of albumin, 7.5 mg of gum arabic, and 375 µmol of NaCl for H-TGL or 80 µmol of NaCl plus 30 µl of serum as activator for LPL. The concentration of enzymes and detergents in the assay mixture was ½ that in the pre-incubation mixtures. The activities of the undiluted preparations were 225 and 185 nmol fatty acid/30 min per 5 µg enzyme protein for H-TGL and LPL, respectively.

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**Fig. 1. Effect of time of incubation of substrate with normal serum (activator) on LPL activity.** Assay mixtures for LPL determination containing either 0.08 ml (O---O) or 0.16 ml (●---●) of serum per assay were prepared as indicated in Methods. The tubes were then incubated at 37°C for the times indicated on the abscissa before the addition of enzyme. A PHP sample from a normal subject, pre-incubated for 45 min with SDS (3 mM, BDH, Lot #324810) was used as enzyme source.
Criteria for specificity

Serum and apolipoprotein activation of the SDS-resistant trioleoylglycerol hydrolase of PHP. These experiments used a PHP sample obtained from a normal female subject after injection of heparin (Upjohn, derived from lung) at a dose of 100 IU/kg body weight. The activity remaining after pre-incubation with SDS was measured in tubes containing increasing amounts of either normal serum, apoC-I, apoC-II, or mixtures of a constant amount of apoC-II (95 μg/tube) and increasing amounts of apoC-I. Results are summarized in Fig. 2. Essentially no activity was obtained without serum or activator apolipoproteins. The greatest activation was obtained with serum, followed by C-II, an apolipoprotein known to be the major serum component responsible for LPL activation (33, 34). A small activation was observed with increasing amounts of apoC-I, either when added alone or plus apoC-II. A LPL activated by apoC-I has been isolated by others in addition to that activated by apoC-II from PHP samples (35, 36). Activation by apoC-I of this magnitude has also been reported for purified milk LPL (37) and has been interpreted to be facilitatory and nonspecific. The preparation of apoC-I used in the experiment of Fig. 2 was prepared as described previously (18) and purified by means of a second DEAE-cellulose column chromatography step. Although this sample gave a single band when tested by polyacrylamide gel electrophoresis, the presence of trace amounts of other apolipoproteins cannot be ruled out.

Treatment with SDS of a PHP sample from a patient with type I hyperlipoproteinemia. S.G. is a 16-year-old female with a typical history for type I hyperlipoproteinemia. After experiencing recurrent episodes of abdominal pain from early childhood, she was found to have grossly lipemic plasma. The level of total trioleoylglycerol hydrolase activity in her PHP was low and no LPL activity could be detected after column chromatography on heparin–Sepharose gel.\(^2\) LPL deficiency is characteristic for type I hyperlipoproteinemia (14, 38). Studies using several PHP samples from this subject (freshly drawn or frozen) showed

that all trioleoylglycerol hydrolase activity was lost after 30 min of pre-incubation with SDS. This finding is in accord with the complete inactivation of H-TGL activity by treatment with PHP with SDS.

Effect of anti-LPL serum on SDS-resistant activity. Results presented in Fig. 3 show that the activity remaining after pre-incubation of PHP with SDS was decreased in proportion to the quantity of anti-LPL added and that over 90% of LPL activity was lost at the maximum ratio of PHP to anti-serum used (1:5). On the other hand, addition of anti-H-TGL serum produced a decrease in LPL activity of less than 15%, which is compatible with a known small reactivity of this anti-serum with LPL. In a similar experiment, lipolytic activity of six different PHP-SDS samples was measured. The activity of five samples was almost completely abolished by treatment with anti-LPL serum (1:10 ratio of PHP:anti-serum), while being unaffected by treatment with the control goat serum (Table 2). The sixth sample (no. 4) was from a type I patient and had essentially no activity before treatment with SDS.

Correlation of LPL activity as measured by the present assay by immunoassay. Thirty PHP samples from normal subjects and patients with hyperlipoproteinemia were used in this study. Their plasma triglyceride levels ranged from 40 to 1727 mg/dl. In each sample, LPL activity was determined after elimination of H-TGL by inactivation with SDS or by treatment with anti-H-TGL serum (see Methods). As shown in Fig. 4, there is an excellent correlation of results obtained by the two methods ($r^2 = 0.99$), using samples with a wide range of triglyceride levels and LPL activities. As a group, values for LPL activity in PHP pre-incubated with SDS were 29% higher than those obtained with the immunoassay. The data point showing no LPL activity by either method was obtained using a PHP sample from the patient with type I hyperlipoproteinemia.

Precision of the assays

Intra-assay error. A PHP sample from a normal donor (sample A) and one from a patient with type V hyperlipoproteinemia (sample B) were divided and each was

<p>| TABLE 2. Effect of anti-LPL serum on SDS-resistant trioleoylglycerol hydrolase activity |
|------------------------------------------|-----------------|--|---|</p>
<table>
<thead>
<tr>
<th>PHP Sample</th>
<th>Phenotype</th>
<th>No Serum Added</th>
<th>Control Serum</th>
<th>Anti-LPL Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal</td>
<td>7.42</td>
<td>8.14</td>
<td>0.50</td>
</tr>
<tr>
<td>2</td>
<td>Normal</td>
<td>11.95</td>
<td>12.29</td>
<td>0.57</td>
</tr>
<tr>
<td>3</td>
<td>Normal</td>
<td>16.20</td>
<td>15.65</td>
<td>0.68</td>
</tr>
<tr>
<td>4</td>
<td>Type I</td>
<td>0.03</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>Type IV</td>
<td>16.57</td>
<td>13.45</td>
<td>0.81</td>
</tr>
<tr>
<td>6</td>
<td>Type V</td>
<td>4.68</td>
<td>4.83</td>
<td>0.02</td>
</tr>
</tbody>
</table>

* $\mu$mol free fatty acid/hr per ml PHP.
PHP samples were incubated for 60 min at 26°C with 50 mM SDS (BioRad, Lot #15886, not recrystallized). Aliquots of these mixtures were assayed directly for LPL activity, as indicated in Methods. Other aliquots were mixed in a ratio of 1:10 (PHP-SDS:sera) with either normal goat serum or anti-LPL goat serum and pre-incubated for an additional 60 min at 28°C. Remaining LPL activity was then determined as described in Methods, using 0.1 ml of these mixtures per assay tube.

In Fig. 3, the effect of anti-H-TGL or anti-LPL serum on the SDS-resistant trioleoylglycerol hydrolase activity of PHP. A PHP sample from a normal subject was treated with crude SDS (50 mM, Eastman, Lot #175X) as indicated in Methods. Aliquots (120 $\mu$L) of the pre-incubated mixture were then combined with antiserum as indicated on the abscissa. In all cases, final volume was adjusted to 0.42 ml by addition of normal goat serum. After incubation at 28°C for 60 min the remaining activity was determined as indicated in Methods, using per assay 0.1 ml of these mixtures as source of enzyme.

In Fig. 4, the correlation of LPL activity as determined after inactivation of H-TGL by SDS (35 mM, BDH, Lot #324810) or by the use of anti-H-TGL serum. All assays were performed as described in Methods. The assay medium contained 0.08 ml of normal serum per tube, as source of activator apolipoprotein. Slope, 1.29; $r^2 = 0.99$.
residual activity was found under these assay conditions collected from subjects with a wide range of plasma fatty acid levels. The standard deviation of these samples was 90% inhibited by 0.75 M NaCl in the assay incubation with SDS is believed to be LPL, since it requires an apolipoprotein for full activity (Fig. 2). The much simpler method for fatty acid determination previously used (18), a liquid–liquid partition system (28), was found to be problematic due to high blanks. Glycerides were transferred into the aqueous phase along with the fatty acid. Possibly, this was related to the higher levels of albumin used in the assay mixtures. It seems virtually certain that a satisfactory partition system can be designed for this purpose if so desired.

The small number of subjects studied and the method of their selection does not allow description of the distribution of values for LPL and H-TGL activities in PHP. However, it is of interest that the levels of these enzymes in PHP samples for normal individuals, measured with the techniques here described, are well within the normal range as defined by the immunoassay method of Huttenen et al. (39). Also in agreement with this report is the observation that the values for LPL activity are higher in normal females than in normal males. The reverse was found for H-TGL activities in PHP. However, it is of interest that the SDS-resistant lipolytic activity of PHP was lost upon incubation with anti-LPL serum, but not with anti-H-TGL serum or control serum (Fig. 3, Table 2). Furthermore, the SDS-resistant lipolytic activity of PHP was lost upon incubation with anti-LPL serum, but not with anti-H-TGL serum or control serum (Fig. 3, Table 2). Finally, there was an excellent correlation between LPL and H-TGL in both sets of subjects, with a standard deviation of 1.2%.

The consistency of the inactivation of H-TGL by pre-incubation with SDS was determined by assay in 0.75 M NaCl, pH 8.8, without serum activator. Values below 0.4 µmol fatty acid/hr per ml PHP were obtained for all PHP samples from normal subjects and for most PHP samples from patients. However, some residual activity was found under these assay conditions with PHP from a few of the patients studied, varying up to 1.7 µmol fatty acid/hr per ml PHP.

### DISCUSSION

The lipolytic activity remaining in PHP after pre-incubation with SDS is believed to be LPL, since it requires an apolipoprotein for full activity (Fig. 2) and is over 90% inhibited by 0.75 M NaCl in the assay medium. Both of these characteristics are hallmarks of LPL (12, 33, 34). Also, a PHP sample from a type I hyperlipoproteinemic patient known not to have LPL as judged by heparin-Sepharose gel column chromatography (not shown) and by immunoprecipitation (Fig. 4), had no trioleylglycerol hydrolyase activity detectable after pre-incubation with SDS (Table 2). Furthermore, the SDS-resistant lipolytic activity of PHP was lost upon incubation with anti-LPL serum, but not with anti-H-TGL serum or control serum (Fig. 3, Table 2). Finally, there was an excellent correlation between LPL and H-TGL in both sets of subjects, with a standard deviation of 1.2%.

### TABLE 3. Intra-assay error

<table>
<thead>
<tr>
<th>Samples</th>
<th>Number of Assays</th>
<th>LPL Activity*</th>
<th>H-TGL Activity*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean ± SEM (Range)</td>
<td>Mean ± SEM (Range)</td>
</tr>
<tr>
<td>A*</td>
<td>40</td>
<td>15.5 ± 0.15 (14.8–17.0)</td>
<td>17.4 ± 0.17 (16.2–18.4)</td>
</tr>
<tr>
<td>B*</td>
<td>40</td>
<td>4.1 ± 0.3 (3.7–4.5)</td>
<td>54.0 ± 0.4 (52.2–55.9)</td>
</tr>
</tbody>
</table>

*The plasma triglyceride was 87 and 970 mg/dl for samples A and B, respectively.

*µmol fatty acid/hr per ml PHP.

Assays were performed as indicated in Methods, using PHP samples collected the same day and never frozen.
PHP samples from patients with hypertriglyceridemia. Thus, using commonly available equipment and materials, the method described herein will allow the precise and accurate determination of LPL in multiple PHP samples containing highly variable enzyme activities and triglyceride levels.

This work was made possible by the excellent assistance of Mrs. Florence E. Casanada. We are also greatly indebted to Dr. Jan Augustin for providing the purified enzymes and antiserum used in this work. The statistical analyses were kindly performed by Mr. Ngoc Anh Le. The collaboration of Dr. Henry Ginsberg, Dr. William Friedman, and Mr. Robert Troisi, as well as the valuable suggestions of Dr. Ray C. Pittman, are greatly appreciated. The assistance of Mrs. Mary Newman and Mrs. Simone Sauerheber is also acknowledged. This work was supported by NIH Grant #14197-05.

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