A new method for the measurement of lipoprotein lipase in postheparin plasma using sodium dodecyl sulfate for the inactivation of hepatic triglyceride lipase[1]

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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 apolipoproteinC-II; *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.— **Baginsky, M. L., and W. V. Brown.** A new method for the measurement of lipoprotein lipase in postheparin plasma using sodium dodecyl sulfate for the inactivation of hepatic triglyceride lipase. *J. Lipid Res.* 1979. **20:** 548–556.

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 both 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

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[1] No reprints will be available for distribution.

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 hydrolase 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-^{14}C]oleoylglycerol (Dhom, North Hollywood, CA) to provide a specific activity in the range of 0.106–0.111 $\mu Ci/\mu mol$. 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 consistantly 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-^{14}C]-Oleic acid was from ICN Pharmaceuticals Inc., Irvine, CA; unlabeled oleic acid, gum arabic, and Trizma 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-

tane used for washing the resin after adsorption of the fatty acid (26) was from Eastman Kodak Chemical Co. All other reagents and solvents were of analytical grade and were obtained from Mallinckrodt, J. T. Baker Chemical Co., or similar suppliers.

LPL determination

The assay mixture for LPL contained in 0.49 ml the following: 5 μmol of tri-[1-^{14}C]oleoylglycerol, 22.5 mg of gum arabic, 100 μmol of Tris-HCl (pH 8.2), 50 μmol of NaCl, 25 mg of bovine serum albumin, and serum in the amount found optimal for activation (0.05–0.125 ml). After incubation for 80 min at 37°C in 16 × 125 mm culture tubes (screw cap), the contents were mixed (Vortex mixer) and then equilibrated for 15 min at 28°C in a second water bath. The reaction was started by addition of 0.01 ml of the PHP–SDS mixture. The reaction was terminated 60 min later and the liberated [1-^{14}C]oleic acid was determined, using the resin method of Kelley (26) as described by Huttenen et al. (27), with some modifications. These modifications were: a) after stopping the reaction by addition of the 5 ml of isopropyl alcohol–heptane–sulfuric acid mixture (27), 0.5 ml of 0.2 M Tris buffer (pH 8.2) was added to the tubes to compensate for the smaller assay volume used in these experiments; b) fatty acid was eluted from the resin (1.3–1.4 g, wet weight) by incubation for 90 min at 70°C with 1 ml of an equal mixture of Triton X-100 and 2 M KOH in methyl alcohol; and c) 0.1 ml of glacial acetic acid was added to each vial before counting (Liquid Scintillation Counter, Mark II, Nuclear, Chicago, IL). The counting solution was prepared as described by Pittman, Khoo, and Steinberg (28). All PHP–SDS samples were also assayed under conditions optimal for H-TGL determination (see next section) to document inactivation of H-TGL by SDS.

H-TGL determination

The assay mixture for H-TGL determination contained in 0.49 ml the following: 5 μmol of tri-[1-^{14}C]-oleoylglycerol, 22.5 mg of gum arabic, 98 μmol of Tris-HCl (pH 8.8), 375 μmol of NaCl, and 25 mg of bovine serum albumin. The assay tubes were then equilibrated at 28°C for 15 min and the reaction was started by addition of 0.01 ml of the PHP–Tris-HCl mixtures described above. After 60 min at 28°C, the reaction was terminated and fatty acid was measured as described for LPL determination.

Lipase assay after inhibition with antisera

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 hydrolase 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 hydrolase 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-^{14}C]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,[2] was determined for each new preparation of trioleoylglycerol and resin. For this, 10 μl of the stock tri-[1-^{14}C]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

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[2] Efficiency of counting of [^{14}C]toluene, added to vials containing resin which had been treated as in an assay, was 76–79%.

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
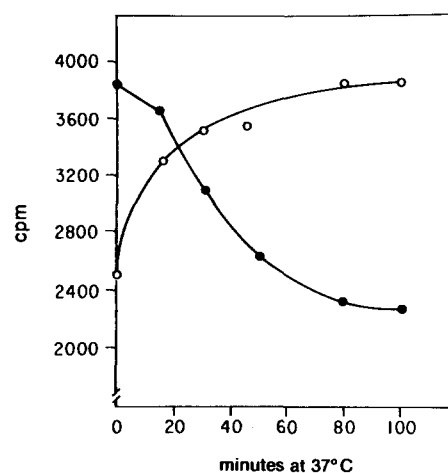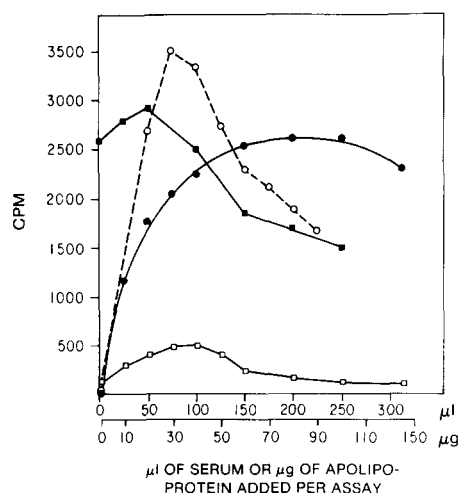

Fig. 1. Effect of time of incubation of substrate with normal serum (activator) on LPL activity. Assay mixtures for LPL determination containing either 0.08 ml (O – – – O) or 0.16 ml (● – – – ●) of serum per assay were prepared as indicated in Methods. The tubes were then incubated at 37°C for the times 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.

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, (triglycerides 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

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

SDS during Pre-incubation	Percent of Initial Activity	
	H-TGL	LPL
μM		
(Undiluted sample)	(100)	(100)
0	85.1	12.5
50	87.3	17.7
100	93.1	57.5
200	95.0	130.9
300	98.0	134.2
400	98.0	137.3
500	88.7	133.8
600	58.7	128.7
1000	1.4	121.9

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 26°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 mg 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 ⅛ 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.

Fig. 2. Activation of SDS-resistant trioleoylglycerol hydrolase activity in PHP by serum and apolipoproteins. Aliquots of apoC-I and apoC-II that had been kept frozen in 0.05 M ammonium bicarbonate solution were lyophilized. The protein residue was then dissolved in a solution of bovine serum albumin (70 mg/ml) in 0.15 M NaCl–0.25 M $CaCl_2$. The amounts of apolipoproteins indicated on the abscissa were added to the assay tubes (in 0.2 ml). Then the tubes received 0.3 ml of a solution of Tris-HCl (pH 8.2) and NaCl with the substrate emulsion to provide the assay mixture described in the text. Tubes with serum as activator received the volume of serum as indicated in the abscissa plus the appropriate volume of albumin solution as above, to give a volume of 200 μl. The final volume was 0.525 ml in these assay mixtures. The buffer added with substrate contained less albumin to accommodate that added with apolipoproteins or serum. The tubes were incubated for 15 min at 37°C before assay for LPL activity. O – – – O, Normal serum; ● —— ●, apoC-II; □ —— □, apoC-I; ■ —— ■, apoC-II (95 μg) added to each tube plus quantity of apoC-I indicated on abscissa.

#175X) as supplied by the manufacturer. At this time, activity measured under conditions for LPL reached a plateau which was sustained for an additional 90 min or more. Using PHP samples from subjects with type IV and type V hyperlipoproteinemia, 45–60 min of pre-incubation with the SDS solution were required to inactivate over 98% of the H-TGL activity. using SDS from BDH (Lot #324810) at 35 mM, the time chosen for H-TGL inactivation was 45 min. Unless otherwise stated, these conditions were routinely applied.

No differences in the time of pre-incubation with SDS or the concentration of SDS required for inactivation of H-TGL activity were noted when comparing PHP samples assayed immediately after phlebotomy (never frozen) and samples that had been stored for 1–3 months at −70°C.

Other important factors in the assay. It was found that amounts of serum that proved to be optimal for activation of the substrate when the assay mixtures were used immediately after preparation were highly inhibitory if these assay mixtures were used 100–120

min later (**Fig. 1**). Therefore, both the amounts of serum required for maximal activation and the time of equilibration of the serum with the substrate had to be defined to obtain reproducible results. For the serum batch used in this study, these values were 0.08 ml/assay and 80 min of equilibration. Since SDS binds to both proteins and lipids, the assay mixture should contain sufficient albumin and substrate to minimize SDS interference in the assay.

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.
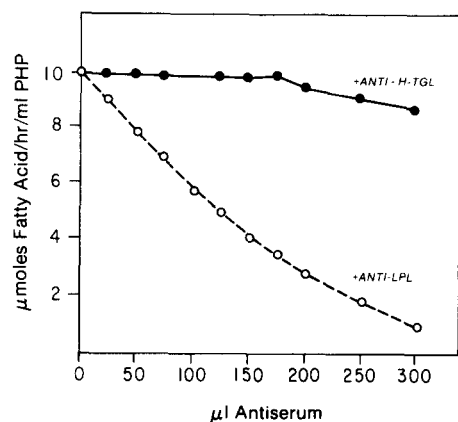
Treatment with SDS of a PHP sample from a patient with type I hyperlipoproteinemia. S.G. is a 16-year-old female with a typical history for type I hyperlipoproteinemia. After experiencing recurrent episodes of abdominal pain from early childhood, she was found to have grossly lipemic plasma. The level of total trioleoylglycerol hydrolase activity in her PHP was low and no LPL activity could be detected after column chromatography on heparin–Sepharose gel.[3] LPL deficiency is characteristic for type I hyperlipoproteinemia (14, 38). Studies using several PHP samples from this subject (freshly drawn or frozen) showed

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[3] Baginsky, M. L., and W. V. Brown. Unpublished data.

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 of 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 1 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

TABLE 2. Effect of anti-LPL serum on SDS-resistant trioleoylglycerol hydrolase activity

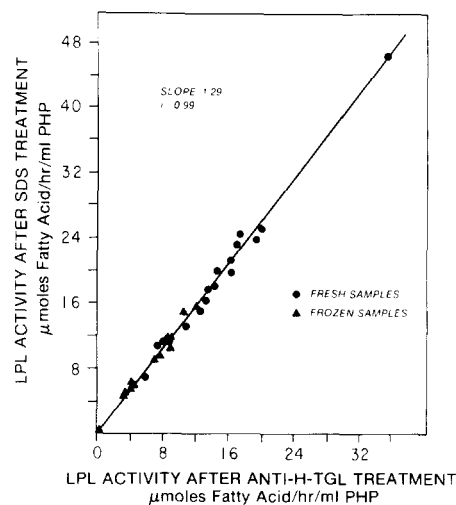| PHP Sample	Phenotype	No Serum Added	After Subsequent Treatment with	
			Control Serum	Anti-LPL Serum
		activity[a]	*activity*[a]	*activity*[a]
1	Normal	7.42	8.14	0.50
2	Normal	11.95	12.29	0.57
3	Normal	16.20	15.65	0.68
4	Type I	0.03	0	0
5	Type IV	16.57	13.45	0.81
6	Type V	4.68	4.83	0.02

[a] μmol free fatty acid/hr per ml PHP.

PHP samples were incubated for 60 min at 26°C with 50 mM SDS (BioRad, Lot #15836, 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.

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

Fig. 3. 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 μl) 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.

Fig. 4. 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; $r^2 = 0.99$.

TABLE 3. Intra-assay error

Samples	Number of Assays	LPL Activity[b] Mean ± SEM (Range)	H-TGL Activity Mean ± SEM (Range)
A[a]	10	15.5 ± 0.15 (14.8–17.0)	17.4 ± 0.17 (16.2–18.4)
B[a]	10	4.1 ± 0.3 (3.7–4.5)	54.0 ± 0.4 (52.2–55.9)

[a] The plasma triglyceride was 87 and 970 mg/dl for samples A and B, respectively.
[b] μmol fatty acid/hr per ml PHP.
Assays were performed as indicated in Methods, using PHP samples collected the same day and never frozen.

treated as 10 individual samples in all steps of the assays. The results obtained for LPL and H-TGL activities are summarized in **Table 3.** The coefficient of variation was approximately ±2–5% in both sets of assays and for both enzymes.

Inter-assay variations. Thirty-one PHP samples were collected from subjects with a wide range of plasma triglyceride levels (40–2109 mg/dl) and aliquots of 30 of these samples were assayed 3–6 times for LPL and H-TGL activities over a period of 2 months. The remaining PHP sample, from a normal male donor, was used as a control and was included in each of the assays in this study. The mean value for eight LPL determinations of this control sample was 15.2 μmol fatty acid/hr per ml PHP, with a standard deviation of 1.8, giving a coefficient of variation of 12%. The average coefficient of variation for the other 30 PHP samples was 11%. When all values were normalized to the mean value obtained for LPL with the control sample, the average coefficient of variation was reduced to 4%. For H-TGL, the average coefficient of variation was 9% and this value was not changed significantly by normalizing to the control sample.

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 trioleoylglycerol hydrolase 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 of the LPL values in 30 PHP samples (normal subjects and patients with hyperlipoproteinemias) as determined after inactivation of H-TGL with SDS or by addition of anti-H-TGL serum (Fig. 4).

The assay using SDS inhibition of H-TGL has the advantage of permitting the *direct* determination of LPL activity and it is simple, involving the addition of a solution of inexpensive and easily available chemicals followed by a short pre-incubation near room temperature. Critical factors for reproducible results are adequate sonication of the substrate to obtain homogeneous trioleoylglycerol emulsions and proper equilibration of this substrate with the activator apolipoprotein(s). The measurement of the hydrolytic products of LPL action on triglyceride or some other substrate may be done by a variety of methods. Although laborious, the method using the ion exchange resin for binding the released fatty acid has proven most precise and reliable over time in our laboratory and for that reason was used in the initial documentation studies with this procedure. 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 with the values for H-TGL activity. In addition, high values for H-TGL activity were obtained with some

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.█

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