

# Selective Measurement of Two Lipase Activities in Postheparin Plasma from Normal Subjects and Patients with Hyperlipoproteinemia

RONALD M. KRAUSS, ROBERT I. LEVY, and DONALD S. FREDRICKSON

*From the Molecular Disease Branch, National Heart and Lung Institute,  
National Institutes of Health, Bethesda, Maryland 20014*

**ABSTRACT** An assay has been developed for specific measurement of two different lipase activities in postheparin plasma. Lipoprotein lipase, derived from extrahepatic sources, is measured as protamine-inactivated lipase activity; hepatic lipase activity is protamine-resistant under the conditions of this assay. In 100 normal subjects, both enzyme activities were noted to be related to age and sex. Protamine-resistant lipase, which comprised 46–95% of the total activity, was highest in men over 18. Protamine-inactivated lipase activity was greatest in younger males and was age-correlated in women, doubling between the second and sixth decades.

In 12 patients with hyperchylomicronemia, including five previously shown to have familial type I hyperlipoproteinemia, protamine-inactivated lipase activity was markedly reduced, whereas protamine-resistant lipase was below normal in only 1. The results were not due to lack of plasma activator, presence of plasma inhibitor, or diet, and the deficiency was not overcome by increasing the provoking dose of heparin from 10 U to 75 U/kg. Mean values for both lipase activities were not reduced in 32 other patients with hyperchylomicronemia, nine with “floating beta” lipoproteins (type III hyperlipoproteinemia), and 23 with hyperprebeta-lipoproteinemia (type IV). Mean protamine-resistant lipase activity was below normal in a group of four women with hypothyroidism, in whom protamine-inactivated lipase was not reduced. Both of the lipase activities were capable of hydrolyzing lipid in very low-density lipoproteins, but the relative rate of hydrolysis of chylomicrons by protamine-resistant lipase was markedly limited. These results indicate the importance of distinguishing between lipases of hepatic and extra-

hepatic origin in the measurement of postheparin lipolytic activity.

## INTRODUCTION

The clearance of circulating lipoprotein triglyceride is thought to be mediated primarily by lipoprotein lipase (glycerol-ester hydrolase, EC 3.1.1.3) (1, 2). This enzyme activity is present in a number of extrahepatic tissues and is released into plasma after heparin administration. Total postheparin lipolytic activity (PHLA)<sup>1</sup> has been used to assess the activity of lipoprotein lipase, but PHLA is heterogeneous. It includes monoglyceride hydrolase (3, 4) and phospholipase (5, 6) activities, as well as triglyceride lipase activity of hepatic origin, which differs from lipoprotein lipase in substrate specificity and certain other properties (7–12).

Clinical interest in PHLA derives from its potential use in assessing lipoprotein clearing capacity. It has been inferred that low PHLA in a number of clinical states associated with hyperglyceridemia reflects a deficiency of lipoprotein lipase and that this in turn is related to a defect in lipoprotein catabolism. These inferences, however, have not been tested heretofore with an assay that distinguishes between lipoprotein lipase and the hepatic triglyceride lipase in postheparin plasma.

We have recently described separate measurement of these enzyme activities in postheparin plasma in the rat (9). This assay is based on selective inactivation of extrahepatic lipase by protamine sulfate. In the current studies, we have evaluated the applicability of this assay

<sup>1</sup> Abbreviations used in this paper: FFA, free fatty acids; PHLA, postheparin lipolytic activity; VLDL, very low-density lipoprotein.

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to measurement of human plasma lipases, and examined possible differences in lipase activities among normal subjects and patients with hyperglyceridemia.<sup>2</sup> Of special interest were patients with familial type I hyperlipoproteinemia (14, 15) in whom clearance of chylomicrons is severely impaired (14–16). The primary defect in this disorder is generally thought to be a deficiency of lipoprotein lipase (14–18), but PHLA measurements are not consistently reduced in all patients with this disorder (14, 15, 17, 19). The selective lipase assay was also performed in patients with hypothyroidism, a condition in which low PHLA has been reported (20), but other features of the type I syndrome have not been observed.

## METHODS

*Human tissue sources.* Samples of human omentum, mesentery, and liver were obtained from patients with stage I lymphoproliferative disorders in the course of diagnostic laparotomy under Fluothane anesthesia. The patients had previously been eating a regular hospital diet and fasted 12–16 h before the operation. Pathologic examination revealed no evidence of neoplasm in any of the tissues. The specimens were immediately frozen and stored at  $-20^{\circ}\text{C}$ . They were thawed just before use, and homogenized in cold acetone (20:1, vol/wt). The homogenates were filtered over Whatman No. 1 filter paper in a Buchner funnel and washed six times with 200 ml of acetone, and three times with 200 ml of diethyl ether. The resulting powders were air-dried and homogenized with a Potter-Elvehjem homogenizer in 0.025 M  $\text{NH}_4\text{OH}$ . Aliquots of these extracts, containing approximately 30–60  $\mu\text{g}$  of protein, were employed in enzyme assays.

In one experiment, fresh, unfrozen slices (2 g wet wt) of mesenteric adipose tissue and liver from a patient with chronic lymphocytic leukemia were incubated at  $37^{\circ}\text{C}$  with 0.194 M Tris buffer, pH 8.6, containing 0.15 M NaCl (Tris-NaCl buffer, ionic strength 0.2) in the presence and absence of heparin. For the adipose tissue, the heparin concentration was 3 U/ml and for the liver, 1000 U/ml. After 45 min, the reaction vessels were iced and the contents centrifuged at 450 *g* for 20 min in an IEC refrigerated centrifuge (International Equipment Company, Needham Heights, Mass.). 0.1-ml aliquots of the supernate were immediately used for enzyme assay as described below. The higher concentration of heparin was chosen for the liver because of a previous report that the activity of a liver heparinase may increase the requirement of rat hepatic lipase for heparin (21). This heparin concentration did not alter the effect of protamine on the enzyme extracted from acetone-ether powders of liver.

*Plasma lipids and lipoproteins.* Plasma triglyceride was determined by the method of Kessler and Lederer (22) and cholesterol by the  $\text{N}_2\text{A}$  method (23). Paper electrophoresis of lipoproteins and lipoprotein quantitation were performed as described previously (24).

*Subjects.* Healthy male and female adult laboratory workers, spouses of patients with familial hyperlipoproteinemia, and N.I.H. normal volunteers, all of whom had normal cholesterol and triglyceride concentrations (15),

<sup>2</sup>A preliminary report of these data has been previously presented in abstract form (13).

served as controls. The subjects' ages ranged from 5 to 64 yr. Some were sampled as outpatients, while consuming regular American diets. Others were inpatients, eating a regular hospital diet or special diets as described in results. All subjects fasted 14–16 h before blood samples were obtained for lipid analyses and lipase assays.

Clinical data pertaining to the patients with primary hyperglyceridemia are listed in Tables V–VII, and data on patients with hypothyroidism are listed in Table XI. All patients had been referred for evaluation of hyperlipidemia; in some instances affected members of the same kindred were studied. For the purpose of comparing their lipolytic activities, patients with elevated plasma triglyceride were grouped into one of three categories according to their plasma lipoprotein patterns at the time postheparin plasma was obtained for enzyme assay: (a) patients with hyperchylomicronemia as defined by the presence of a distinct fat layer floating on top of the plasma after the latter had been stored at  $4^{\circ}\text{C}$  for 18 h (15, 25), (b) patients with floating beta lipoprotein, i.e., having lipoproteins of density  $<1.006$  that had beta mobility on electrophoresis (26); these patients also had faint chylomicronemia; (c) patients with hyperprebeta lipoproteinemia, without chylomicrons. None of the patients had low density lipoprotein concentrations above limits previously defined as normal (15). The first of the above categories includes patients with types I and V hyperlipoproteinemia in the classification of Fredrickson, Lees, and Levy (14, 15), the second is equivalent to type III, and the third to type IV.

All subjects with hyperlipoproteinemia were sampled while hospitalized on a metabolic ward. Patients with hyperchylomicronemia were routinely fed diets containing at least 30 g of fat/24 h; the fat content was reduced to less than 10 g/24 h for some studies. Other patients were fed diets providing approximately 20% of calories from protein, 40% from fat, and 40% from carbohydrate, the total intake being adjusted daily to maintain a constant body wt.

Plasma glucose values were obtained for all patients in the fasting state and in most patients 2 h after 100 g of oral glucose. The percentage of ideal body weight was calculated from the Metropolitan Life Insurance Tables (27). Routine measurements of renal, hepatic, and thyroid function were normal in all patients except where indicated (Table XI). None of the patients or normal subjects had received any medication known to affect lipid metabolism (including insulin, oral anti-diabetic agents, steroid hormones, or hypolipidemic drugs) for at least 4 wk before study. Hyperglyceridemia was also documented in one or more first-degree relatives of all but 14 of the patients with "primary" hyperglyceridemia (Tables V–VII). Ascertainment was not complete in all the families, and in some cases no relatives were available for sampling (Tables V–VII).

*Postheparin plasma.* All blood samples were collected in tubes containing 2 U heparin/ml whole blood, and kept at  $4^{\circ}\text{C}$ . Samples were obtained before and 10 min after rapid intravenous administration of sodium heparin. For the standard test, 10 U/kg heparin was employed; in special studies the dose was increased up to 100 U/kg body weight. In some experiments, blood samples were also obtained at intervals from 5 to 120 min after heparin injection. These samples were withdrawn from an indwelling intravenous cannula in an antecubital vein washed with physiological saline between samplings. Plasma was separated in a refrigerated IEC centrifuge, and frozen in dry ice in 0.5-ml

portions. Samples were stored at  $-20^{\circ}\text{C}$ , and were shown to retain full enzyme activity after 6 mo.

**Assay of postheparin lipase activity.** Assay of triglyceride lipase activities was performed as described previously (9). Plasma or tissue extract (0.01–0.10 ml) was incubated in duplicate in a total volume of 0.1 ml of the Tris-NaCl buffer containing 0.1 M sodium chloride and heparin 1.5 U (ionic strength 0.15) for 10 min at  $27^{\circ}\text{C}$ . Duplicate samples were also incubated in the same buffer, containing 3.0 mg/0.1 ml protamine sulfate (Calbiochem, A grade, San Diego, Calif.). After this incubation, 0.9 ml of substrate medium was added. The substrate was prepared within 30 min of use by sonification of the following components with a Branson Sonifier-Cell Disruptor (Branson Instruments Co., Danbury, Conn.): glyceryl-tri-[1- $^{14}\text{C}$ ]oleate (Amersham/Searle Corp., Arlington Heights, Ill., 39–41 mCi/mmol) 5  $\mu\text{Ci}$ ; triolein (Applied Science Labs Inc., State College, Pa.) 113  $\mu\text{mol}$  (100 mg); fatty acid-free albumin (Pentex Biochemical, Kankakee, Ill.) 200 mg, and 1% (vol/vol) aqueous Triton X-100, 0.6 ml, in a total volume of 12 ml of the Tris-NaCl buffer. When tissue extracts were used as enzyme source, 20 U of heparin and 0.8 ml of plasma from a fasted normal subject were included/12 ml of the substrate mixture.

To maximize the reproducibility of the assay results, the sonification procedure was performed in a standardized manner. The flat tip of the sonifier was centered and inserted  $\frac{1}{2}$  in below the surface of the assay mixture, contained in a standard 25-ml liquid scintillation vial suspended in a beaker of ice. Sonification was carried out for 1 min at setting 5 (60 W), and, after a 15-s pause, for an additional 30 s. During the last 5 s, the output was increased (setting 7) to achieve foaming. A creamy translucent emission was obtained, there being only a few small lipid droplets visible at the surface.

Incubations were routinely carried out for 60 min at  $27^{\circ}\text{C}$ . Reactions were terminated and free fatty acids (FFA) extracted into 0.1 M KOH by the method of Schotz, Garfinkel, Huebotter, and Stewart (28). The [ $^{14}\text{C}$ ]FFA were counted in Instagel (Packard) in a Packard Tri-Carb Liquid Scintillation Spectrometer (Packard Instrument Co., Inc., Downers Grove, Ill.). Total release of FFA was calculated after correction for efficiency of extraction and quenching, and activity expressed in units of micromoles FFA/milliliter plasma/hour. When the same substrate emulsion was used, duplicate values differed by less than 5%. They differed by less than 10% when different triolein emulsions were employed for assay of the same plasma. No differences were obtained with different lots of grade A protamine sulfate.

For experiments employing lipoprotein substrates, very low-density lipoproteins (VLDL), and chylomicrons were prepared as described below, and were dialyzed 14 h at  $6^{\circ}\text{C}$  against the Tris-NaCl buffer just before use. A single pool of each lipoprotein was employed for all assays. Incubation mixtures were prepared to contain the same concentrations of triglyceride, albumin, Tris, and NaCl as in the triolein substrate. Enzyme assay was performed as described above, except that incubations were carried out for 90 min and FFA were re-extracted from the KOH phase and titrated by the method of Dole and Meinertz (29).

**Other methods.** Chylomicrons were prepared by layering postprandial plasma from patients with hyperchylomicronemia under 0.85% saline and centrifuging in a 60 Ti rotor in a Beckman L265B ultracentrifuge (Beckman Instruments,

Inc., Fullerton, Calif.) at 100,000 *g* for 10 min. The packed lipid layer at the top of the tube was washed once and stored under  $\text{N}_2$  in 1 mM EDTA at  $4^{\circ}\text{C}$  for no longer than two weeks. VLDL were prepared by previously described techniques (30).

Protein was assayed by the method of Lowry, Rosebrough, Farr, and Randall (31). Plasma glucose was determined by the glucose oxidase method (32) and thyroxine by a semiautomated technique (33).

**Statistical methods.** Mean lipase values were compared by the two tailed *t*-test; the Aspin-Welch modification was employed when the variances were not equal (34). Non-parametric tolerance limits were calculated from the tables of Somerville (35). Regression was estimated by the method of least squares. A family of simultaneous tolerance intervals for the regression line was calculated with the Bonferroni inequality (36).

## RESULTS

### Assessment of the validity of the assay using human enzymes

**Enzymes in tissues.** Enzyme contained in either acetone-ether powders of adipose tissue or liver or extracted by heparin from wet tissues was preincubated with protamine for 10 min at  $27^{\circ}\text{C}$ . The adipose tissue lipase was inhibited completely, or nearly so (Table I), while the liver lipase activity was not significantly affected. The absolute activities in these tissues were low and the increase in activity produced by protamine with two of the livers was within the experimental error ( $\pm 10\%$ ). These results were in accord with those obtained with liver and adipose tissue enzymes prepared in the rat (9).

TABLE I  
Inactivation of Human Adipose Tissue and Liver Lipase Activities by Protamine Sulfate

Enzyme source	Triglyceride lipase activity		Change with protamine
	–protamine	+protamine 3 mg/ml	
	nmol FFA/mg · protein/h		%
Adipose tissue			
Patient 1	76	15	–80
2	114	10	–91
3	19	0	–100
		Av	–90
Liver			
Patient 1	170	163	–4
2	114	119	+4
3	70	82	+17
		Av	+6

Acetone-ether powders were prepared from tissues of patients 1 and 2 and were extracted with 0.025 M  $\text{NH}_4\text{OH}$ ; tissues from patient 3 were extracted with heparin and the assays carried out as described in Methods.

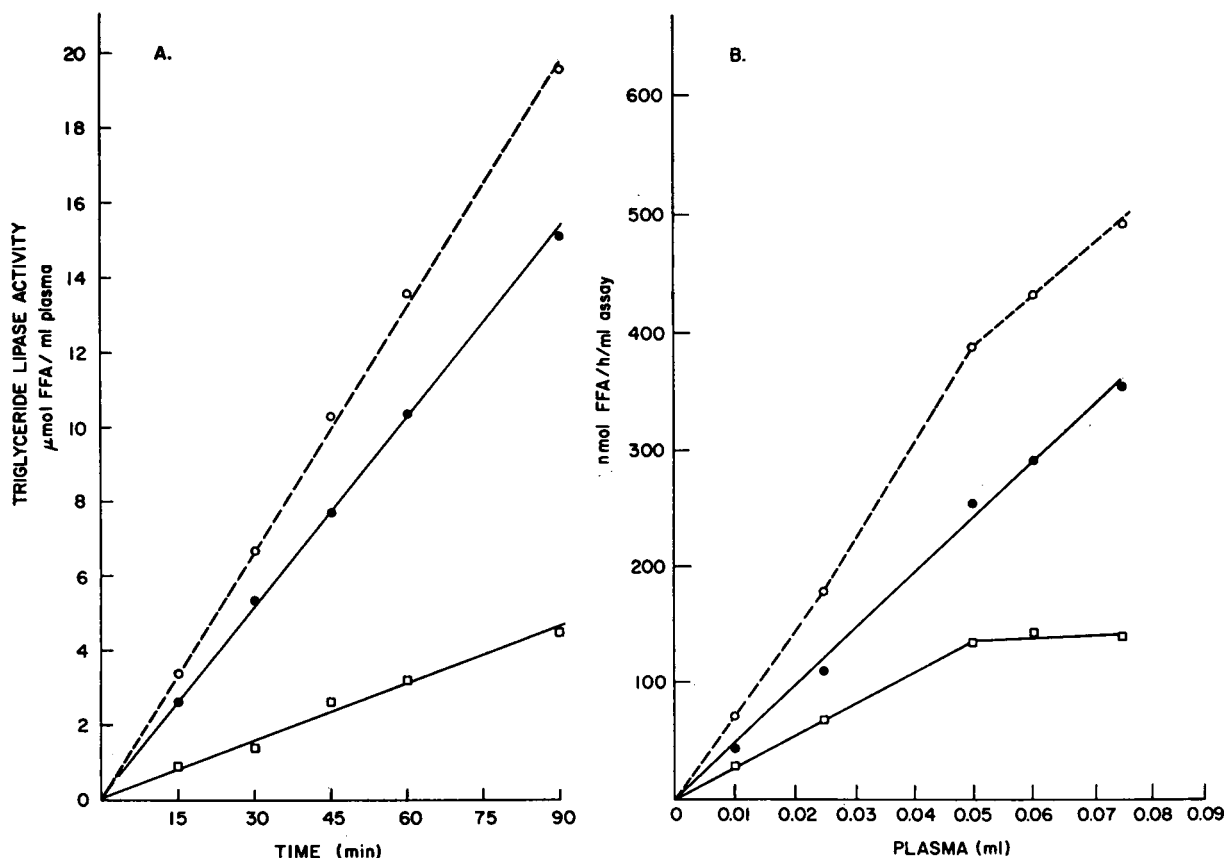


FIGURE 1 The relationship of heparin-released triglyceride lipase activities to incubation time and amount of postheparin plasma. Incubations were carried out in a total volume of 1 ml as described in the text.  $\circ$ - $---$ - $\circ$ , Total activity (protamine absent);  $\bullet$ - $---$ - $\bullet$ , protamine-resistant activity (protamine present);  $\square$ - $---$ - $\square$ , protamine-inactivated activity (total - protamine-resistant).

*Enzymes in postheparin plasma.* By preincubation of plasma in the presence or absence of protamine, the "protamine-resistant" and the "total" lipase activities were measured in postheparin plasma from normal subjects. The "protamine-inactivated" activity was determined as the difference between the total and the protamine-resistant activity (Fig. 1A and 1B). The reactions proceeded linearly for at least 90 min (Fig. 1A). Since partial glycerides were present in only trace quantities in the substrate, it was assumed that the initial rate of FFA release primarily reflects hydrolysis of the triglyceride ester bonds, although subsequent hydrolysis of partial glyceride reaction products also occurs (3, 4, 8). Activities were not enhanced by addition of preheparin plasma and were proportional to the amount of added postheparin plasma, up to 0.05-0.06 ml/ml of assay mixture (Fig. 1B). Above this concentration, protamine-inactivated lipase activity did not increase. The limited range of proportional response

was not obviously substrate- or temperature-dependent and the maximum was reproducibly obtained in numerous assays of plasma from both normal subjects and patients. In subsequent experiments, therefore, no more than 0.05 ml of plasma/ml of assay was employed for enzyme assays.

The time course of the two lipolytic activities in plasma after two doses of heparin was studied (Figs. 2 and 3). Preheparin plasma contained relatively little total triglyceride lipase activity ( $<0.6$  U). After administration of 10 U/kg of heparin, both lipase activities were maximal between 5 and 30 min, and then declined rapidly, returning to the base line by 90-120 min (Fig. 2). Similar results were obtained in two female and two male normal volunteers.

Two of these patients then received 75 U/kg heparin (Fig. 3). After this dose, protamine-resistant activity was maximal within 5 min and declined exponentially with an estimated  $t_{1/2}$  of 4-9 h. The protamine-inacti-

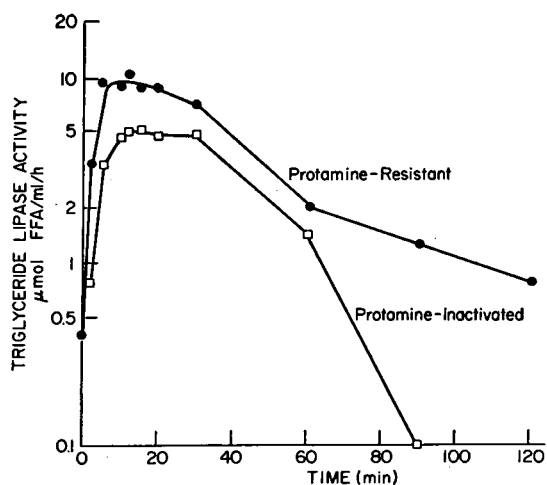


FIGURE 2 Time-course of changes in plasma triglyceride lipase activities after intravenous administration of 10 U/kg heparin in a normal subject.

vated lipase activity reached a peak after 40–60 min, and decayed more rapidly, the  $t_{1/2}$  being roughly 60 min in one patient and 75 min in the other.

In four subjects, an increase in the heparin dose from 10 U/kg to 75 U/kg caused a rise of 30–100% in protamine-resistant activity and a 40–190% increase in protamine-inactivated lipase activity measured 10 min after heparin. The effects of varying doses of heparin on the plasma lipase activities in one normal volunteer are shown in Fig. 4.

#### Enzyme activities in normal subjects

The results obtained in a group of normal subjects on their regular diets are summarized in Table II. Significant age and sex variations were observed for both protamine-resistant and protamine-inactivated lipase activities. The protamine-resistant activity, which comprised 46–95% of the total in individual subjects, was highest in adult men. A significant correlation with age was observed for protamine-inactivated lipase in women (Fig. 5), the mean lipase activity doubling between the second and sixth decades. Protamine-inactivated lipase activity in young males ( $\leq 16$  yr) was comparable to that in the older women (Table II). In male subjects older than 18 yr, the activity was significantly lower. This reduction in subjects over 18 was abrupt; there was no apparent continuous regression with age.

Nonparametric tolerance limits were established for 90% of the normal population with 85% confidence (Table III and Fig. 5). The number of subjects in the group of males 18 and under was inadequate for estimation of the tolerance limits with a high degree of confidence.

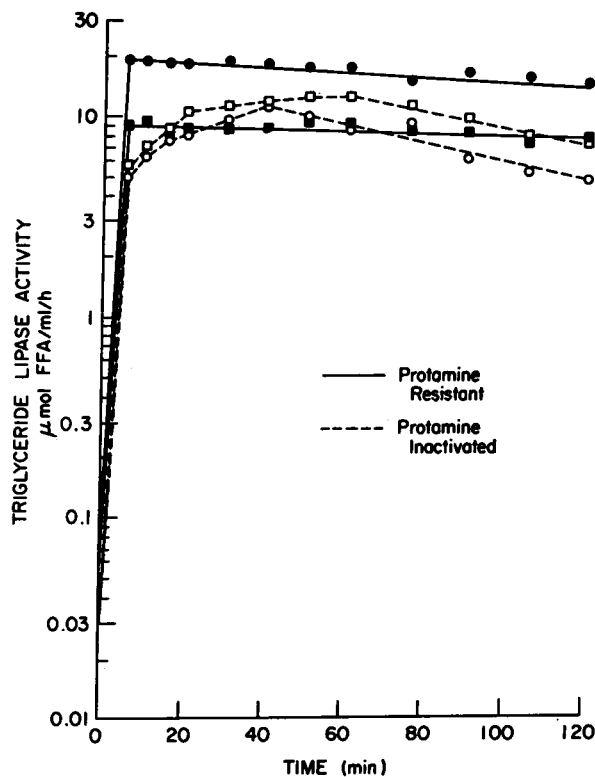


FIGURE 3 Time-course of changes in plasma triglyceride lipase activities after a 75 U/kg dose of heparin given to two different normal subjects.

Neither of the lipase activities was correlated with plasma triglyceride or cholesterol concentration or with body weight. Although there was a tendency for protamine-inactivated lipase activity to be higher in menopausal women than in age-matched controls (Fig. 5), this difference was not statistically significant. The two women with the highest activities of protamine-inactivated lipase (Fig. 5) were, in fact, premenopausal, and no common clinical or biochemical parameter could be identified to distinguish them from the other normal women.

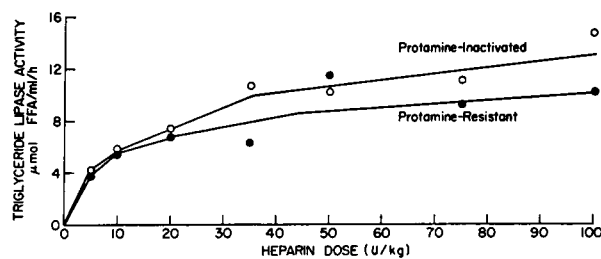


FIGURE 4 Plasma triglyceride lipase activities at 10 min after varying doses of heparin in a normal volunteer. The heparin dose was sequentially increased, a 2–3 day interval separating each administration.

TABLE II  
Heparin-Released Plasma Lipase Activities  
in Normal Subjects

Sex	Age yr	No.	Triglyceride lipase activity	
			Protamine- inactivated	Protamine- resistant
			$\mu\text{mol FFA/ml/h} \pm \text{SD}$	
Male	5-16	10	6.7 $\pm$ 1.5*†	11.0 $\pm$ 3.1*
	19-65	40	4.2 $\pm$ 1.5*	15.0 $\pm$ 5.4*†
Female	7-16	11	3.8 $\pm$ 1.1†§	11.1 $\pm$ 3.8
	19-62	39	4.9 $\pm$ 2.3§	10.5 $\pm$ 3.3‡

\* Difference between younger and older men was significant,  $P < 0.01$ .

† Difference between men and women was significant,  $P < 0.001$ .

§ Significant correlation with age,  $P < 0.001$ .

#### Diet-induced changes in triglyceride lipase activity

Eight normal volunteers were fed a basal diet containing approximately 20% of calories from protein, 40% from fat, and 40% from carbohydrate, which maintained their body weights. The diet was also low in cholesterol (< 300 mg) and high in its content of polyunsaturated fat (a polyunsaturated:saturated ratio of > 2:1). After a period of 1-3 wk, during which plasma triglyceride and cholesterol concentrations stabilized, the fat content was reduced to less than 10 g by isocaloric substitution of carbohydrate. This low-fat diet was continued for 10-14 days. The resultant changes in enzyme activities and plasma lipids are shown in Table IV. Protamine-inactivated lipase activity was markedly reduced in all subjects (mean

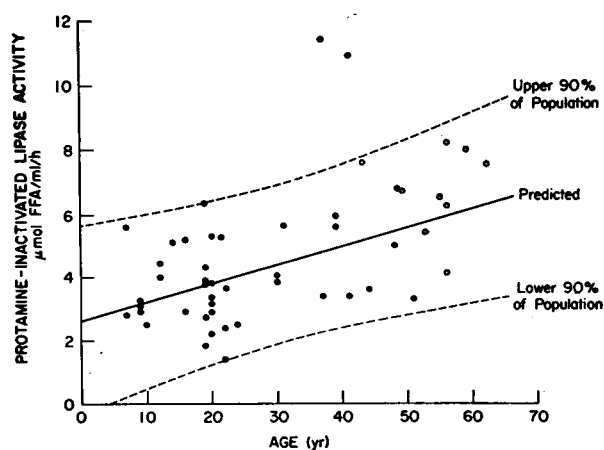


FIGURE 5 Regression of protamine-inactivated lipase activity upon age in women. Open circles indicate postmenopausal females.

TABLE III  
Heparin-Released Plasma Lipase Activities: Tolerance  
Limits for 90% of Normal Population\*

Sex	Age	Triglyceride lipase activity	
		Protamine- inactivated	Protamine- resistant
		$\mu\text{mol FFA/ml/h}$	
Male	19-65	1.6-7.1	3.4-28.7
Female	7-62	Age-dependent	5.1-19.0

\* Estimated with 85% confidence (see Methods).

reduction 56%,  $P < 0.001$ ). Protamine-resistant lipase activity increased in some of the subjects, but the mean increase for the group was not significant. There was no significant change in either enzyme activity in normal subjects fed diets containing 30-150 g of fat/day.

The studies summarized in Table IV also afforded the opportunity to estimate the variation in the lipase activities measured repeatedly in individual subjects on the basal diet. The percent standard deviation (biological variation) for five or six determinations over a 2-wk period ranged from 6 to 18% (av = 12%) for protamine-resistant lipase, and 16-32% (av = 23%) for protamine-inactivated lipase.

#### Plasma lipase measurements in hyperlipoproteinemia

Effects of lipoprotein concentrations on the lipase assay. A possible effect of high lipoprotein concentrations on enzyme measurements was assessed by adding both chylomicrons and VLDL to 0.05-ml aliquots of postheparin plasma from a normal volunteer. In Fig. 6,

TABLE IV  
Effect of Diet Composition on Lipase Activities in Normals

Diet	Sex	N	Lipase activity	
			Protamine- inactivated	Protamine- resistant
			$\mu\text{mol FFA/ml/h} \pm \text{SD}$	
Basal	Male	4	4.1 $\pm$ 1.8	12.5 $\pm$ 3.4
	Female	4	3.8 $\pm$ 1.0	12.8 $\pm$ 3.0
	All	8	3.9 $\pm$ 1.3*	12.6 $\pm$ 3.0
Low fat	Male	4	2.0 $\pm$ 1.0	13.4 $\pm$ 1.6
	Female	4	1.6 $\pm$ 0.3	14.5 $\pm$ 4.8
	All	8	1.7 $\pm$ 0.7*	13.9 $\pm$ 3.8

\* Difference between basal and low fat, significant,  $P < 0.001$ . The basal diet contained approximately 20% of calories from protein, 40% from carbohydrate, and 40% from fat. The low-fat diet contained less than 10 g fat/day, with isocaloric substitution of carbohydrate.

the amount of added lipoprotein is expressed in terms of the plasma triglyceride concentration achieved. The lipase activity measured in the absence of protamine was the same at all triglyceride levels below 2,200 mg/100 ml, beyond which a slight reduction occurred. The inactivation produced by protamine was, however, reduced by added lipoproteins. This resulted in an apparent lowering (> 5%) of protamine-inactivated activity at triglyceride concentrations greater than 800 mg/100 ml for VLDL and 1,500 mg/100 ml for chylomicrons (Fig. 6).

The interference with protamine inactivation in lipoprotein-rich plasma could be reversed by dilution of the plasma, thus reducing the final lipoprotein triglyceride concentration in the assay. This was demonstrated in postheparin plasma from a normal volunteer in which the plasma triglyceride concentration was increased from 125 to 3,400 mg/100 ml by addition of VLDL. Protamine inactivation was reduced > 60% but was restored to base line by a fivefold dilution of the plasma with 0.15 M NaCl solution. Changes of similar magnitude were observed by diluting plasma from patients with comparable plasma triglyceride concentrations. It has been previously shown that high triglyceride concentrations interfere with protamine inactivation of triglyceride lipase from adipose tissue (37).

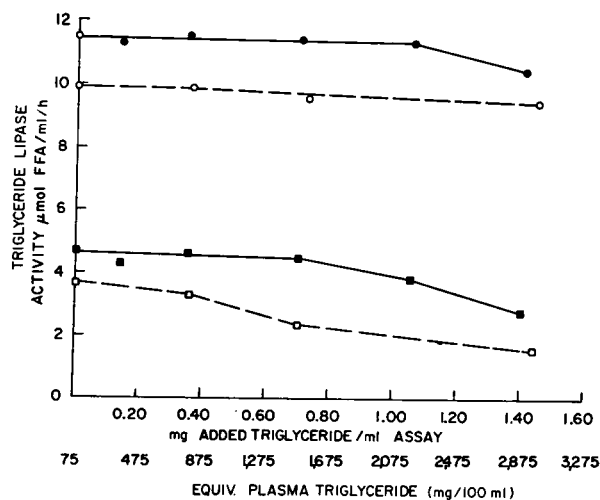


FIGURE 6 The effect on total (circles) and protamine-inactivated triglyceride lipase activity (squares) of adding chylomicrons (—) or VLDL (---) to postheparin plasma from a normal volunteer. The quantity of added lipoprotein is expressed in milligrams of triglyceride; the resultant triglyceride concentration in the plasma is also shown.

For the standard enzyme assay, plasma was diluted with physiologic saline, when necessary, to achieve a concentration of triglyceride of less than 500 mg/100 ml. At this concentration, the endogenous triglyceride

TABLE V  
Triglyceride Lipase Activities in Patients with Floating Beta Lipoproteins

Pt	Age	Wt	% Ideal body wt.	Plasma glucose fasting/2 h	Plasma triglyceride	Plasma cholesterol	Triglyceride lipase activity	
							Protamine-inactivated	Protamine-resistant
		kg	%		mg/100 ml	mg/100 ml	μmol FFA/ml/h	
<b>Men</b>								
J. B.	24	75.4	108	77	870	508	2.5	20.0
W. E.	42	71.4	96	106/296	1,584	616	3.4	21.6
J. A.	48	100.7	129	95/120	808	424	3.5	10.8
P. F.	29	83.3	115	86/122	234	216	3.6	15.3
D. W.*	19	71.5	100	94	804	752	6.5	11.7
Mean ± SD, n = 5							3.9 ± 1.5	15.9 ± 1.5
Normal							4.2 ± 1.5	15.0 ± 1.5
P value							NS	NS
<b>Women</b>								
E. Sh.	43	96.6	156	262	744	464	3.9	15.3
L. H.	46	90.8	139	80/104	193	246	4.2	13.8
E. C.	44	60.9	100	88/188	562	390	5.9	9.7
O. S.	40	98.4	158	104	402	261	6.0	17.4
Mean ± SD, n = 4							5.0 ± 1.2	14.1 ± 3.3
Normal							4.9 ± 2.3	10.5 ± 3.3
P value							NS	NS

\* Patient without documented hyperglyceridemia in a first-degree relative (no relatives available).

TABLE VI  
Triglyceride Lipase Activities in Patients with Hyperprebetalipoproteinemia

Patient	Age	Wt. kg	% ideal body wt. %	Plasma glucose fasting/2 h mg/100 ml	Plasma triglyceride mg/100 ml	Plasma cholesterol mg/100 ml	Triglyceride activity	
							Protamine- inactivated $\mu\text{mol FFA/ml/h}$	Protamine- resistant
<b>Men</b>								
J. Cal.	24	73.4	105	86/107	192	176	1.7	26.2
G. G.	58	68.2	86	120/95	362	167	2.2	14.8
Ri. B.	36	59	81	90/95	272	148	3.0	16.0
J. L.*	47	80.8	110	107/146	273	274	3.5	18.5
J. G.	37	95.2	130	98/240	442	247	3.7	16.3
J. S.	24	82.1	104	86/120	210	117	3.7	19.2
A. G.	35	99.4	135	95/114	284	212	3.8	9.3
Ro. B.	33	74.6	92	97/135	378	235	4.3	11.3
R. R.	36	130.1	161	95/101	432	258	5.0	18.9
P. B.	35	90.6	112	100/193	432	252	5.4	9.2
J. Cam.	49	62.2	80	71/155	233	273	5.6	12.2
E. W.	59	76.3	104	100/146	840	249	5.9	20.7
R. M.*	51	64.4	90	95	350	284	7.0	5.8
C. M.	58	89.2	112	120/213	244	261	7.3§	10.1
J. O.*	35	82.9	113	90	372	258	7.3§	13.6
Mean±SD, n = 15							4.9±1.7	14.5±5.6
Normal							4.2±1.5	15.0±5.4
P value							NS	NS
<b>Women</b>								
L. C.	31	62.7	107	93/150	161	170	2.0	7.3
G. G.	51	75.4	113	94/147	206	261	3.0	11.5
L. M.	48	76	119	92/176	448	237	3.0	12.7
I. P.	47	76.7	117	83/103	239	285	5.4	6.1
M. P.	66	79	129	107/205	582	292	5.7	2.1†
M. L.	41	67	105	90/128	362	270	6.7	5.7
S. G.	65	78.1	113	179/180	360	272	6.9	10.4
M. B.	48	67	103	100/167	342	221	7.6	11.7
Mean±SD, n = 8							5.0±2.1	8.4±3.7
Normals							4.9±2.3	10.5±3.3
P value							NS	NS

\* Patients without documented hyperglyceridemia in a first-degree relative (R. M. and J. O., no relatives available; J. L., two first-degree relatives with normal plasma lipid concentrations).

† Value below 90% normal tolerance limit.

§ Values above 90% normal tolerance limit.

constituted less than 4% of the total in the assay. When the dilution was more than fivefold, a substrate of higher specific activity was used to restore the sensitivity of the assay.

*Lipase activities in patients with hyperglyceridemia.* Lipase activities were measured in postheparin plasma obtained from individuals with primary hyperglyceridemia (Tables V–VII).

In the 23 patients with hyperprebetalipoproteinemia, and the 9 with floating beta lipoproteins, mean protamine-resistant and protamine-inactivated lipase activi-

ties were normal (Tables V and VI). Individual values were within the 90% normal tolerance limits, except for a low activity of protamine-resistant lipase in one woman (M. P.), and elevated protamine-inactivated lipase in two men (C. M., J. O.).

In the 44 patients with hyperchylomicronemia, the measurement of protamine-inactivated lipase activity appeared to identify two distinct populations of patients (Table VII). 12 patients (6 men, 6 women) had activities far below the 90% normal tolerance limits and 4–10-fold less than the lowest values in the remainder



TABLE VII  
Triglyceride Lipase Activities in Patients with Hyperchylomicronemia

Patient	Age	Wt kg	% ideal body wt %	Plasma glucose fasting/2 h mg/100 ml	Plasma triglyceride mg/100 ml	Plasma cholesterol mg/100 ml	Triglyceride activity	
							Protamine- inactivated $\mu\text{mol FFA/ml/h}$	Protamine- resistant
Men								
L. Wy.*	30	60.6	87	94	4,590	364	0.0§	12.0
P. P.*	22	69.8	93	98/100	785	171	0.3§	12.0
J. P.*	38	64.7	87	90/330	1,463	273	0.6§	6.1
I. S.†	36	69.2	94	118/163	2,057	171	0.6§	13.9
J. K.*†	21	61.2	96	83/104	3,528	260	0.7§	7.6
L. P.*	34	70.0	101	89/105	1,974	259	0.8§	6.6
W. P.	40	82.4	96	97/188	2,244	212	3.0	8.4
J. V.	31	72.6	87	75/65	770	226	3.1	8.3
C. S.	45	71.5	95	90/75	405	162	3.2	14.7
A. W.	41	96.4	116	82/73	530	285	3.3	11.6
P. G.	41	86.0	114	92/112	1,938	359	3.6	11.1
J. B.	39	101.7	127	114/180	6,386	680	4.0	13.7
G. S. (2)	68	77.6	98	89/200	720	295	4.0	18.1
J. F.	38	136.8	162	82/102	860	301	4.4	15.6
F. F.	34	92.8	129	83/77	1,239	359	4.8	12.6
R. P.	68	88.6	119	100/173	903	305	5.0	13.8
G. S. (1)	38	84.2	110	90/176	1,740	363	5.1	20.8
D. R.	50	81.8	109	88/160	8,525	988	5.6	20.9
D. M.†	36	84.7	118	147	2,673	281	5.7	18.9
Cl. D.	50	106.4	133	90/185	1,417	404	5.8	11.3
J. H.	30	89.6	113	91/125	323	142	5.9	21.0
R. B.	37	87.8	111	78/134	2,233	399	6.7	20.7
H. C.	52	75.1	105	102/75	1,716	247	6.8	5.5
G. M.†	53	103.1	145	182/410	2,025	560	7.6	24.6
Ch. D.	53	108.4	131	93/120	1,035	403	8.1	15.4
M. K.	49	85.4	106	90/70	1,233	296	9.4	24.9
L. A.†	28	82.1	118	87	4,179	416	11.4	10.7
Women								
G. Wy. J.*	29	50.1	92	84	267	96	0§	4.0§
S. K.*†	11	29.2	—	86	2,750	185	0§	11.1
M. W.	53	60.1	95	80/168	1,281	187	0§	11.2
Ga. S.*†	9	28.5	—	80	1,496	280	0.2§	9.7
P. W.*†	25	58.9	102	78/123	759	178	0.2§	11.3
D. T.*†	15	45.8	—	99	800	121	0.3§	15.8
F. J.	39	47.6	80	156	552	164	3.2	4.2§
A. L.	37	78.5	131	98/178	1,085	333	3.5	8.3
R. S.	39	78.3	127	194	3,043	250	3.6	7.8
C. B.	48	88.5	128	193/381	1,870	298	3.9	11.5
H. V.	60	78.5	115	91/129	540	285	4.2	14.3
H. M.	44	61.4	103	104/228	1,106	322	4.4	11.4
A. P.	33	81.1	133	90	1,290	435	4.7	7.3
J. G.	53	66.1	112	100/176	1,442	418	6.0	17.7
E. L.†	43	70.7	114	133/286	1,024	293	6.1	12.9
E. Sn.	55	72.0	110	243/426	5,460	884	10.6	14.8
R. J.	48	64.2	99	140/330	1,295	349	12.8	9.8

\* Patients with documented hyperchylomicronemia before age 20.

† Patients without documentation of hyperglyceridemia in first-degree relatives (I. S., D. M., L. A., P. W., and E. L., no relatives available; G. M., Ga. S., and D. T., one relative with normal plasma lipid concentrations; J. K. and S. K., three relatives with normal plasma lipid concentrations).

§ Value below 90% normal tolerance limit.

|| Value above 90% normal tolerance limit.

TABLE VIII  
Comparison of Hyperchylomicronemic Patients with and without Deficiency of Protamine-Inactivated Lipase Activity

	n	Mean plasma triglyceride	Mean plasma cholesterol	Mean triglyceride lipase activities	
				Protamine-inactivated	Protamine-resistant
		mg/100 ml ± SD		μmol FFA/ml/h ± SD	
Men					
Protamine-inactivated					
Lipase activity					
Reduced	6	2,400 ± 1,404	250 ± 72	0.5 ± 0.3*	9.7 ± 3.3‡
Not reduced	21	2,052 ± 2,035	366 ± 188	5.5 ± 2.2§	15.4 ± 5.5
Normal	40			4.2 ± 1.5	15.0 ± 5.4
Women					
Protamine-inactivated					
Lipase activity					
Reduced	6	1,234 ± 863	175 ± 65	0.1 ± 0.1*	10.5 ± 3.8
Not reduced	11	1,700 ± 1,421	366 ± 187	5.6 ± 3.2	10.9 ± 3.9
Normal (all women)	50			4.7 ± 2.0	10.6 ± 3.4

\* Significantly lower than normal,  $P < 0.001$ .

‡ Significantly lower than normal,  $P < 0.05$ .

§ Significantly higher than normal,  $P < 0.01$ .

of the group. The activities in these 12 patients were all at the limit of sensitivity of the assay. In several of them, total activity was higher in the presence than in the absence of protamine; this yielded slightly nega-

tive values for protamine-inactivated lipase ( $-0.1$ – $-0.6$  U), which are listed as zero in Table VII.

The protamine-inactivated lipase activities in the rest of the patients with chylomicronemia were distributed

TABLE IX  
Triglyceride Lipase Activities in Patients with Reduced Protamine-Inactivated Lipase Activity on Moderate and Severe Fat Restriction

Patient	Diet	Plasma triglyceride	Plasma cholesterol	Triglyceride lipase activity	
				Protamine-inactivated	Protamine-resistant
		mg/100 ml	mg/100 ml	μmol FFA/ml/h	
L. Wy.	A	4,590	364	0.0	12.0
	B	672	242	0.3	11.7
I. S.	A	2,057	171	0.6	13.9
	B	362	127	0.2	13.9
J. K.	A	3,528	360	0.7	7.6
	B	292	102	0.2	4.5
S. K.	A	2,750	185	0.0	11.1
	B	820	152	0.0	9.8
M. W.	A	1,281	187	0.0	11.2
	B	1,572	257	0.5	12.6
Ga. S.	A	1,496	128	0.0	10.9
	B	585	109	0.2	9.7

All patients received basal diet, containing  $>30$  g fat/day (A) or fat-restricted diet containing  $<10$  g fat/day (B) for 7–11 days before being sampled. Caloric contents of diets were adjusted daily to maintain stable body weights. No chylomicrons were detectable at the end of period B in any of the patients.

continuously within the normal range, except in 4 men and 2 women who had abnormally high activities. Protamine-resistant lipase activity was normal in all the patients except for two women with low values, one of whom (G. Wy. J.) had a coexistent reduction of protamine-inactivated lipase.

Because of the obvious discontinuity in the distribution of protamine-inactivated lipase activity, the mean enzyme activities were calculated separately for the first 12 patients with extremely low activities and for the remainder who had normal or high values (Table VIII). Both groups had comparable mean triglyceride concentrations. Mean protamine-inactivated lipase in the first or "deficient" group was less than one-eighth that of normals; mean activity was higher than normal in the second group (men,  $P < 0.01$ ; females, NS). The mean activity of protamine-resistant lipase was normal in each category, except for a one-third reduction in the males with low protamine-inactivated lipase activity (Table VIII).

Lipase activities were measured in 6 of the 12 patients with low protamine-inactivated lipase after isocaloric reduction of dietary fat to less than 10 g/day (Table IX). There were marked reductions in plasma triglyceride concentrations in all patients except M. W., who developed a high level of VLDL; the postheparin lipase activities were not significantly changed.

An increase in heparin dose from 10 U/kg to 75 U/kg (Table X) resulted in no significant increase of protamine-inactivated lipase activity in each of seven patients with low enzyme activities. Protamine-resistant activity was roughly doubled, however, similar to the response seen in normals. In two of the patients (L. Wy. and J. P.) the lipase activities were measured at 5, 10, and 20 min after this dose of heparin. Protamine-inactivated lipase activity was the same at each of the time points, while protamine-resistant lipase followed the same time course observed in normals.

Addition of normal plasma to postheparin plasma

TABLE X  
*Triglyceride Lipase Activities after 75 U/kg Heparin in Patients with Reduced Protamine-Inactivated Lipase Activity*

Patient	Triglyceride lipase activity	
	Protamine-inactivated	Protamine-resistant
	$\mu\text{mol FFA/ml/h}$	
L. Wy.	0.7	19.9
P. P.	1.1	24.1
J. P.	0.2	12.7
I. S.	0.7	24.2
L. P.	0.7	8.5
D. T.	0	26.3
P. W.	0.7	20.5
Mean $\pm$ SD, $n = 7$	$0.6 \pm 0.4$	$19.5 \pm 6.9$
Heparin 10 U/kg, mean $\pm$ SD, $n = 7$	$0.4 \pm 0.3$	$10.6 \pm 3.6$

from three patients with reduced protamine-inactivated lipase (P. W., G. S., and J. K.) produced no increase of either lipase activity. Plasma from the three patients produced no inhibition of the postheparin plasma lipase activities in normals, and activities in mixtures of postheparin plasma from patients and normals were additive.

Many of the patients with primary hyperlipoproteinemia were above ideal body weight and many also had abnormal glucose tolerance (Tables V-VII). Although these parameters were normal in most patients with lipase deficiency, there was no overall correlation of either lipase activity with these abnormalities or with plasma lipid concentrations.

*Lipase activities in patients with hypothyroidism.* Lipase measurements were carried out in four women with different types of hyperlipoproteinemia and with clinical and biochemical evidence of hypothyroidism

TABLE XI  
*Triglyceride Lipase Activities in Patients with Hypothyroidism*

Patient	Age	Sex	Serum thyroxine	Plasma triglyceride	Plasma cholesterol	Lipoprotein electrophoresis pattern (15)	Triglyceride lipase activity	
							Protamine-inactivated	Protamine-resistant
			$\mu\text{g}/100 \text{ ml}$	$\text{mg}/100 \text{ ml}$	$\text{mg}/100 \text{ ml}$		$\mu\text{mol FFA/ml/h}$	
G. G.	56	F	1.8	404	448	II-B	5.5	6.0
D. K.	54	F	0.9	251	277	IV	5.7	7.2
D. M.	35	F	0.6	141	381	II	3.0	5.3
G. C.	48	F	0.4	1,191	505	V	6.1	7.4
Mean $\pm$ SD, $n = 4$							$5.4 \pm 1.8$	$6.2 \pm 0.8$
Normal							$4.9 \pm 2.3$	$10.5 \pm 3.3$
$P$ value							NS	$< 0.001$

(Table XI). Protamine-inactivated lipase was normal in all four patients, while the mean protamine-resistant lipase activity was significantly reduced.

#### Hydrolysis of lipoproteins by postheparin lipase activities

The activities of protamine-resistant and protamine-inactivated lipases against human chylomicrons and VLDL were measured and compared with the respective activities against the triolein substrate (Tables XII and XIII). The results indicated a distinct difference in substrate preference of the two postheparin lipase activities. This was true for both normal subjects and patients with hyperglyceridemia. The protamine-resistant activity was much higher against the triolein emulsion than against chylomicrons. Activity against VLDL was intermediate. On the other hand, the protamine-inactivated activity was in the same range with all three substrates (Table XII).

The statistical significance of the differences just noted are better seen in Table XIII, where the ratio of activity against each lipoprotein substrate to that against triolein is displayed. For either substrate and enzyme, the mean ratios in patients and normals did not differ significantly, permitting the calculation of mean ratios for the entire group of subjects. The ratios

obtained for hydrolysis of VLDL (0.84) and chylomicrons (1.04) by the protamine-inactivated lipase were not significantly different. In contrast, the protamine-resistant activity hydrolyzed chylomicrons at a rate only about one-fourth of that obtained with VLDL (0.16 vs. 0.59;  $P < 0.001$ ).

Although the ratios varied considerably among individual subjects, the findings in the group as a whole suggested that measurement of protamine-inactivated lipase activity with the triolein emulsion was comparable to measurement with VLDL or chylomicrons. This was not true for the protamine-resistant activity, however. Thus, the portions of total activity determined to be protamine-inactivated and protamine-resistant varied with the different substrates. With the triolein emulsion, protamine-inactivated lipase activity was  $33 \pm 3\%$  of the total (mean  $\pm$  SEM for the patients in Table XII, excluding J. P. and G. Wy. J.); with VLDL,  $42 \pm 4\%$ , and with chylomicrons,  $78 \pm 5\%$ . The difference between the first and second substrates was not statistically significant; that between the second and third was ( $P < 0.001$ ).

#### DISCUSSION

Triglyceride lipase activities of hepatic and extra-hepatic origin are released into plasma by heparin.

TABLE XII  
Postheparin Plasma Lipase Activities with Lipoprotein Substrates

Subject	Sex	Age	Lipase activity					
			Protamine-inactivated			Protamine-resistant		
			Triolein	VLDL	Chylos.	Triolein	VLDL	Chylos.
$\mu\text{mol FFA/ml/h}$								
Normal								
G. H.	M	43	2.6	3.5	1.7	18.1	6.8	2.4
J. V. S.	M	22	4.6	4.0	5.2	9.9	4.3	1.6
J. J.	M	43	4.6	2.4	2.5	16.1	9.8	1.4
S. S.	F	39	2.8	2.9	2.5	4.3	2.6	1.3
A. T.	F	56	4.6	3.6	6.0	7.2	4.5	1.9
J. Li.	F	19	5.2	4.9	5.3	6.9	2.6	1.1
Hyperglyceridemic*								
J. P.	M	38	-0.1	-0.6	-0.3	3.7	2.1	0.7
G. Wy. J.	F	28	0.5	0.7	0.2	1.3	1.4	0.6
J. V.	M	31	4.3	2.6	3.4	8.5	6.7	0.3
A. P.	F	33	3.7	2.7	3.8	6.2	3.6	1.3
J. B.	M	24	3.3	4.5	5.2	12.7	6.3	0.7
E. C.	F	44	4.8	2.2	5.9	8.8	5.4	0.3
R. M.	M	51	5.7	4.2	5.5	5.0	1.9	0.4
S. G.	F	65	4.5	3.0	6.0	10.4	7.9	0.5

Release of FFA from all substrates was measured by titration (see Methods). Chylos, chylomicrons.

\* Subjects J. B. and E. C. are listed in Table V; subjects R. M. and S. G. in Table VI; and the remainder in Table VII. Samples were not the same as those used for Tables V-VII (obtained up to 1 yr later), but in all cases the lipid concentrations and the lipoprotein patterns were consistent with the initial ones.

TABLE XIII  
*Postheparin Plasma Lipase Activities: Relative Rates of Hydrolysis of Lipid in Lipoproteins and Triolein Emulsion*

Subject	Hydrolytic activity: lipoprotein/triolein			
	Chylomicrons		VLDL	
	Protamine-inactivated	Protamine-resistant	Protamine-inactivated	Protamine-resistant
Normal				
G. H.	0.65	0.13	1.35	0.38
J. V. S.	1.13	0.16	0.87	0.43
J. J.	0.54	0.09	0.52	0.61
S. S.	0.89	0.30	1.04	0.61
A. T.	1.30	0.26	0.78	0.63
J. Li.	1.02	0.16	0.94	0.38
Mean±SEM	0.92±0.12	0.18±0.03	0.92±0.11	0.51±0.05
Hyperglyceridemic				
J. P.	*	0.19	*	0.57
G. Wy. J.	*	0.46	*	1.08
J. V.	0.79	0.04	0.61	0.79
A. P.	1.03	0.21	0.73	0.58
J. B.	1.57	0.06	1.36	0.50
E. C.	1.23	0.03	0.46	0.62
R. M.	0.97	0.08	0.74	0.38
S. G.	1.33	0.05	0.67	0.76
Mean±SEM	1.15±0.11	0.14±0.05	0.76±0.13	0.66±0.08
All Mean±SEM	1.04±0.09	0.16±0.03	0.84±0.08	0.59±0.05
P value	<0.02		<0.001	

The ratios represent FFA release from lipoprotein/FFA release from triolein (data from Table XII).

\* Activities against triolein were less than 1  $\mu$ mol FFA/ml/h; ratios not calculated.

Hepatic lipase differs from extrahepatic (lipoprotein) lipase in several major ways. Lipoprotein lipase activity appears to be dependent on the presence of the apolipoprotein apoC-II (37-39). We have failed to demonstrate an activator requirement for highly purified hepatic lipase from rat (10), although activation of canine hepatic lipase by human apoC-I has been reported (40). Hepatic and extrahepatic lipases both hydrolyze primary ester bonds preferentially, but preparations of hepatic lipase cleave the secondary ester bond of triglyceride at a greater rate, possibly due to the higher activity of an associated monoglyceride hydrolase (8, 12).

Another distinction between those two enzyme activities is the relative resistance of hepatic lipase to lipoprotein lipase inactivators (7-9). This feature has served as the basis for a specific assay of the two enzyme activities in rat postheparin plasma that utilizes selective inactivation of extrahepatic lipase with protamine sulfate (9). The validity of this assay was established by showing that protamine-inactivated lipase was

the only component present in totally hepatectomized rats and that protamine-resistant lipase correlated with the amount of liver tissue remaining in partially hepatectomized rats (9).

The procedure developed for the selective assay of hepatic and extrahepatic lipase activities in rat postheparin plasma was applied to the study of hyperglycemia in humans after several criteria were satisfied. Firstly, under the conditions of this assay, lipase from human liver was shown to be protamine-resistant and lipase from adipose tissue was demonstrated to be protamine-inactivated like the rat enzymes. It remains to be demonstrated in man, as has been shown for the rat (7, 9), that lipase activities from other extrahepatic tissues exhibit the same sensitivity to protamine as enzyme from adipose tissue.

Further, the optimal conditions for assay of the two activities in human plasma were established. Using a standard heparin dose of 10 U/kg, we found that plasma obtained 10 min after heparin contained maximal levels of both enzyme activities and that these could be mea-

sured reproducibly with a triolein emulsion at pH 8.6. Both lipase activities remained constant (within the range of error of the assay) between 10 and 20 min, and then declined rapidly. At the higher dose of heparin, the decay was markedly slowed, particularly for the protamine-resistant lipase. Prolonged survival of total PHLA after higher heparin doses has been observed previously in man (41) and the rabbit (42).

A third requirement was the establishment of the normal range of lipase activities in man as measured by this assay. Protamine-inactivated lipase activity rose linearly with age in women, while the activity fell sharply in men after age 16–18. Protamine-resistant lipase activity was 40% higher in adult men than in any other category. While the biochemical bases for these age and sex variations are not understood, hormonal effects on lipase activities (43–46) may prove to be important, and there undoubtedly are environmental influences that have not been perceived. One such factor that definitely influences lipase activities is diet. The drop in PHLA reported previously with fat-restricted diets (17) was here shown to be due to a decrease in the protamine-inactivated lipase component of PHLA.

Finally, conditions were determined for eliminating any effect of the concentration of triglycerides in plasma upon the enzyme activities. High lipoprotein levels reduced the extent of protamine inactivation, but this was not apparent when plasma was diluted so that the endogenous triglyceride contributed less than 4% to the total in the assay.

The application of this assay procedure to the study of 76 patients with primary hyperglyceridemia revealed a marked deficiency of protamine-inactivated lipase activity in postheparin plasma from 12 patients with hyperchylomicronemia. These low activities could not be ascribed to dietary fat restriction or to the presence of a plasma inhibitor, and were not corrected by addition of normal plasma. Protamine-resistant lipase activity was within the normal range in all but 1 of the 12 patients: mean activity was normal in the women, and reduced by about one-third in the men. Persistence of the severe deficiency of protamine-inactivated lipase after a much higher dose of heparin (75 U/kg) appeared to rule out an antagonism to heparin, a suspected cause of decreased PHLA in patients receiving estrogens (46) and others with paraproteinemia (47).

Patients with primary hyperchylomicronemia have been further categorized as having type I or type V hyperlipoproteinemia in the classification of Fredrickson, Lees, and Levy (14, 15). Technically, the distinction between these two lipoprotein patterns is an associated increase in VLDL in type V (14, 15). An as-

essment of VLDL concentration is implied in this distinction, whether qualitatively from electrophoretograms or quantitatively through use of chromatography, nephelometry, or ultracentrifugation. A more practical and less exact alternative criterion has been the response of plasma triglyceride concentrations to substitution of all dietary fat by carbohydrate. There results a precipitous decline to much lower levels in type I, little decline or even a rise in type V (14, 15). It has also been observed that type I patients are usually detected in childhood, while type V patterns are most often seen in adults (14, 15).

10 of the 44 chylomicronemic patients in this study population were known to have had severe hyperglyceridemia before age 20 (Table VII); the remainder were detected at a later age. 5 of the 10 patients (representing two sibships) previously have been designated as having familial type I hyperlipoproteinemia on the basis of detailed studies, including response to dietary fat restriction (P. P., L. P., and J. P.; L. Wy. and G. Wy. J.) (14, 17). The other five, previously unpublished, had similar dietary responses. These are shown for three of the patients (J. K., S. K., and Ga. S.) in Table IX. On the basis of this criterion, these five patients were also considered to have type I hyperlipoproteinemia.

The group of 12 patients with deficiency of protamine-inactivated lipase activity (Tables VII, VIII) included all 10 of the type I subjects described above. Thus, the present results are consistent with earlier reports of reduced total PHLA or clearing factor in plasma from patients described as having type I (14–17, 48). The failure to detect a reduction in PHLA in one of the patients (L. Wy.) by an earlier assay (14, 17) appears to have been due to a relatively high activity of the protamine-resistant lipase at that time.

Two other patients with chylomicronemia, both detected in adulthood, were also in the group of patients with low protamine-inactivated lipase activity (Table VII). One, I. S., had a precipitous drop in plasma triglycerides on a fat-restricted diet (Table IX). He would have been classified as type I. The other, M. W., showed a rise in triglycerides on the fat-restricted diet and was considered to represent type V (Table IX).

The finding of low protamine-inactivated lipase activity in postheparin plasma from this group of patients is in accord with a report of reduced levels of lipoprotein lipase in adipose tissue from type I patients (18). However, other abnormalities may be responsible for an enzyme defect in postheparin plasma, and it is possible that reduced tissue lipoprotein lipase is not the primary defect in all of the patients reported here. In a recent study, heparin-released plasma histaminase activity was also found to be low in all but two of the

patients shown here to have reduced protamine-inactivated lipase (I. S. and J. K.) and normal in other hyperglyceridemic patients (49). The interrelationship of histaminase and lipoprotein lipase remains to be determined; possible explanations for the combined deficiency in these patients include abnormality of a common protein subunit, activating mechanism, or tissue binding site from which both enzymes are released into plasma by heparin. It has also not been excluded that accelerated removal of lipase activity from plasma is responsible for an apparent deficiency in some patients.

Mean plasma lipase activities were normal or elevated in the other group of patients with hyperchylomicronemia. Some individuals had abnormalities (low protamine-resistant lipase or elevated protamine-inactivated lipase) also found within the group of patients with hyperprebetalipoproteinemia alone. The hyperglyceridemia in most of these patients was familial (see Methods), and it is possible that the variations in lipase activities have a genetic basis.

While it is apparent that multiple abnormalities are represented in the group of patients with hyperchylomicronemia, the present results suggest utility in segregating a category of patients on the basis of reduced extrahepatic lipase activity in postheparin plasma. As suggested above, the discrimination between type I and type V lipoprotein patterns is not based on a precise biochemical test. A deficiency of lipoprotein lipase activity thus may define better a category of patients with either lipoprotein pattern who clearly represent a biochemical defect distinct from others within these same general groups. There are potentially many other abnormalities leading to chylomicronemia that are not associated with lipoprotein lipase deficiency. These include saturation of the normal clearance mechanisms (50) or a deficiency of another tissue factor (51) or lipoprotein binding site. Similar mechanisms might also be operative in patients with hyperprebeta- and floating beta lipoproteinemia, who may have abnormalities in VLDL metabolism (52-54), but whose heparin-released enzyme activities were found to be normal in the present study.

The results in the patients with hypothyroidism suggest that the deficiency of total PHLA previously described (20) represents a reduction in protamine-resistant lipase activity. While the lipase activity was within the normal 90% tolerance limits for each of the four patients, the mean activity for the group was significantly below normal. The finding of normal protamine-inactivated lipase in these patients appears to be consistent with the data of Persson (55) who found that lipoprotein lipase activity in adipose tissue was within the normal range in five patients with myxe-

dema, although the average activity was somewhat below the mean for normals.

Evaluation of possible differences in substrate specificity between hepatic and extrahepatic lipase activities was complicated by several factors. Although the effects of protamine were presumed to occur chiefly during the preincubation period (9), it was not possible to exclude influences of the different substrates on the protamine response. In addition, although assays contained a large excess of added substrate, interaction of enzymes with endogenous plasma lipoproteins may have affected the lipase measurements. Finally, the assay procedures did not discriminate among individual extrahepatic or hepatic enzymes, which may have hydrolyzed different lipoprotein species at different rates.

The data in Tables XII and XIII revealed considerable intersubject variation in the relative lipase activities against the lipoprotein substrates. This variance may have related to one or more of the above-mentioned factors influencing the enzyme measurements. Consistent patterns were apparent, however, in the results of the group as a whole. Protamine-inactivated lipase activity was generally comparable with triolein, VLDL, and chylomicron substrates; while protamine-resistant was highest with triolein, lowest with chylomicrons, and intermediate with VLDL. These results may help to explain the finding in postheparin plasma of an "abnormal" lipoprotein lipase with reduced activity against chylomicrons, but normal hydrolysis of triglyceride emulsions (19, 56). This has been described in patients considered to have type V (56) or type I (19) hyperlipoproteinemia, and may represent predominance of protamine-resistant (hepatic) lipase activity.

While the relative activities of hepatic and extrahepatic lipases in postheparin plasma do not necessarily reflect the activities of these enzymes in tissues, the present results suggest possible specificity of function of the enzymes in lipoprotein catabolism. The hydrolysis of chylomicron and VLDL lipids at similar rates by protamine-inactivated lipase is in accord with previous observations that both types of particles are catabolized (1, 2) and taken up (57-59) by extrahepatic tissues. The relatively low activity of protamine-resistant lipase against chylomicrons is in agreement with the findings in patients with type I hyperlipoproteinemia, many of whom had diminished chylomicron clearance in the presence of normal or only slightly reduced protamine-resistant lipase activity. These results are also consistent with previous observations that chylomicrons are cleared to a relatively small extent by liver *in vivo* (59-63) and *in vitro* (7, 11, 64-68), while they raise the possibility that hepatic lipase may have some function in the catabolism of VLDL.

The selective assay for heparin-released hepatic and extrahepatic lipase activities has been shown by these studies to be useful in defining categories of patients with primary hyperchylomicronemia and in evaluating clinical states such as hypothyroidism, where a reduction in total PHLA previously has been associated with hyperglyceridemia. Other conditions reported to have low PHLA include diabetes mellitus (69), hormonal steroid treatment (43, 46, 70), liver disease (65), alcoholism (71), renal failure (72), and pancreatitis (73). Specific changes also might be detected with hypolipidemic diets or drugs found to increase total PHLA (44, 45, 74). Further studies of individual lipase activities, both heparin-released and *in situ*, may better define their respective roles in lipoprotein catabolism.

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