

Abnormal Lipoprotein Lipase in Familial Exogenous Hypertriglyceridemia

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ABSTRACT A 5-yr old male proband and his sister have had hypertriglyceridemia and hepatosplenomegaly since birth. When studied on a metabolic ward, they demonstrated rapid decreases in serum triglycerides on 3 g fat/day diets. Oral glucose tolerance tests were normal. Postheparin lipolytic activity (PHLA) against chylomicrons was virtually absent in both children whereas the mother and a normolipemic sister had levels approximately 50% normal. However, all four had a normal PHLA against commercial triglyceride emulsion (Intralipid). Two unrelated children from different kindreds of typical type I hyperlipoproteinemia and two patients with acquired type V hyperlipoproteinemia had deficient PHLA against both substrates. No inhibitors of PHLA could be demonstrated in the proband's plasma, and his own PHLA could not be enhanced by either normal concentrated plasma or pooled $d > 1.063$ lipoprotein fraction. The proband's postheparin plasma required almost 20 times the normal chylomicron-triglyceride concentration to reach one-half maximal lipase velocity.

Both affected siblings showed heavy pre-beta lipoprotein electrophoretic bands plus chylomicrons in their fasting plasmas while ingesting a 33% carbohydrate, 30% fat diet. Incubation of their postheparin plasma with $S_r > 400$ chylomicrons in vitro produced a smaller S_r 20-400 "remnant" with pre-beta electrophoretic mobility that was not seen under the same conditions when normal postheparin plasma was used. Postheparin monoglyceridase and phospholipase activities were either normal or only moderately decreased when determined

with appropriate artificial substrates. These data are consistent with either (a) a mutant gene producing a lipoprotein lipase with unusual substrate specificities or (b) an absolute deficiency of normal lipoprotein lipase with a compensatory increase in some other postheparin triglyceridase.

INTRODUCTION

In the rare, familial type I (exogenous, fat-induced, Burger-Grütz) hyperlipoproteinemia, the postabsorptive plasma appears creamy due to light-scattering chylomicrons. The plasma triglycerides are markedly elevated whereas cholesterol is normal or only slightly increased. The disorder is usually present from childhood and lipemia retinalis, eruptive xanthomata, hepatosplenomegaly, pancreatitis, or obscure bouts of abdominal pain accompany this entity (1). When dietary fat is restricted to 5 g/day, serum triglycerides and clinical signs both improve.

The defect in this disease appears to be a diminished removal of fat particles secondary to a deficiency of lipoprotein lipase, an enzyme facilitating the uptake of chylomicrons by extrahepatic tissues. Adipose tissue extracts from homozygous type I patients show low lipoprotein lipase activity whereas heterozygous carriers may have intermediate tissue levels (2). Our studies deal with a family demonstrating the usual clinical history and fat-induction characteristics of type I hyperlipoproteinemia who have an abnormal lipoprotein lipase with unusual substrate specificity and kinetics.

METHODS

All subjects avoided alcohol, excessive dietary fat or carbohydrate, tobacco, and medications (except where indicated in Table I) for 48 h preceding study. Venous blood was collected the morning after an overnight fast into heparinized or EDTA tubes and immediately centrifuged at 4°C,

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5,000 rpm. Plasma was either refrigerated at 4°C or frozen at -20°C before analysis. Lipoprotein electrophoresis was performed within 24 h of collection by the method of Lees and Hatch (3). Plasma triglycerides (4) and cholesterol (5) were determined on the AutoAnalyzer (Technicon Instruments Corp., Tarrytown, N. Y.).

Postheparin enzyme assays were performed on plasma collected 6, 8, 10, and 12 min after intravenous injection of 0.1 mg/kg aqueous heparin (Eli Lilly and Co., Indianapolis, Ind., 100 U/mg). Lactescent plasmas were centrifuged at 106,000 *g* for 1 h at 4°C to remove chylomicrons that might serve as endogenous substrate or create pipetting errors. This 1 h centrifugation cleared all plasmas of any turbidity. For simplification, equal volumes of each sample were pooled for a given patient and enzyme assays were performed in duplicate on the single pooled specimen. Results obtained in this manner did not differ significantly from those derived from the mean activity of individual specimens taken 6–12 min after the injection of heparin.

Postheparin lipolytic activity (PHLA).¹ Both Intralipid (Vitrum-Stockholm) and human chylomicrons were used as substrates for the lipoprotein lipase released by heparin. The Intralipid represents an artificial triglyceride emulsion (containing small amounts of phospholipid and monoglyceride) commonly used for the assay of PHLA. The chylomicrons represent the $S_r > 400$ fraction obtained by ultracentrifugation at plasma density, 40,000 rpm for 60 min in a Spinco model L using a no. 40 fixed angle rotor (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.). The source of this substrate was a chylous ascites effusion occurring in a patient with lymphatic obstruction secondary to retroperitoneal carcinoma. The uncentrifuged effusion was stored under sterile conditions at 4°C and served as a reproducible source of chylomicron substrate for periods up to 4 mo. The actual chylomicron concentrate, decanted after ultracentrifugation, showed gross aggregation with rising FFA concentrations after 1 wk of storage under similar conditions and was therefore prepared fresh each day experiments were performed. There was no difference in enzyme activity against these chylomicrons as compared with those obtained from hyperlipemic plasmas, including the proband's own postheparin chylomicrons. The final assay mixtures were as follows: (a) *Chylomicrons*. 0.3 ml chylomicron suspension (135 mg triglyceride/ml) in 0.9% saline (pH 8.7), 0.3 ml 0.33 M $(\text{NH}_4)_2\text{SO}_4$ in 20% fatty acid-poor albumin (Pentex-Bovine fraction V) (pH 8.7), 0.4 ml plasma. Final pH after incubation ranged from 8.50–8.65. (b) *Intralipid*. 0.25 ml of 10% Intralipid (pH 8.7), 0.25 ml 0.4 M $(\text{NH}_4)_2\text{SO}_4$ in 20% fatty acid-poor albumin (pH 8.7), and 0.5 ml plasma. The reaction in each assay was linear for 45 min and substrate concentrations were not rate-limiting. The entire incubation mixture was extracted for FFA determination (6) at 0 and 45 min in duplicate.

To test for circulating plasma inhibitors in the patient's plasma or for a missing component that might be supplied by normal plasma, the following experiments were performed (Table III). Identical PHLA assays with chylomicron substrates were done with the exception of the plasma component. Normal postheparin plasma was mixed with equal volumes of either preheparin plasma, plasma concentrated 10-fold with polyacrylamide gel (Lyphogel, Gelman Instrument Co., Ann Arbor, Mich.), or the $d >$

1.063 fraction from either normal or patient's preheparin plasma to test for possible missing components necessary for full lipoprotein lipase activity.

Postheparin monoglyceridase activity (PHMA). A chromatographically pure mixture of 1-, 2-monoolein (3 g/100 ml) was added to Krebs-Ringer's phosphate buffer, pH 7.40, containing 10 g/100 ml FFA-poor albumin and 4 g/100 ml taurodeoxycholate and sonicated until clear. The resultant micellar suspension (0.6 ml) served as a substrate for 0.4 ml postheparin plasma. Samples were extracted at 0 and 90 min for FFA determinations. Results are expressed as microequivalents FFA released/milliliter plasma per hour. Preheparin plasma contains from 0.5 to 1.0 μeq FFA/ml per h monoglyceridase activity but was not assayed in all experiments and therefore the PHMA actually represents the sum of pre- and postheparin monoglyceridase activities.

Postheparin phospholipase. Inosthin, (Associated Concentrates, Woodside, N. Y.) a mixture of soybean phosphatides purified with acetone and ethanol, contains primarily phosphatidyl ethanolamine with trace sterol glycosides but no mono- or triglycerides. This was used as a substrate for phospholipase activity in postheparin plasma by a modification of the method described by Vogel and Zieve (7).

10 ml of 0.1 M glycine buffer, pH 9.6, containing 90 mg Inosthin and 47.5 mg sodium deoxycholate, was sonicated until clear. This suspension (0.7 ml) was then mixed with 0.3 ml plasma and incubated at 37°C for 1 h in duplicate. Samples for FFA determination were taken at 0 and 60 min. The reaction was linear for 2 h. Results are expressed as microequivalents FFA/milliliter plasma per hour.

Lipoprotein lipase: reaction products. To examine changes in size and composition of chylomicrons ($S_r > 400$ lipoproteins) after treatment with heparin-released plasma lipoprotein lipase, 6 ml of either normal or patient's postheparin plasma was incubated with 6 ml of chylomicrons suspended in 0.2 M $(\text{NH}_4)_2\text{SO}_4$, 20% FFA-poor albumin, pH 8.7, at 37°C for 4 h. At intervals, portions were removed from each flask and either applied to Whatman 3M chromatographic paper strips for electrophoresis or added to cellulose nitrate ultracentrifuge tubes containing 0.9 g/100 ml saline plus 3 mg/10 ml diethyl *p*-nitrosophenylphosphate (Paroxon-Calbiochem, San Diego, Calif), an inhibitor of lipoprotein lipase. The latter were then centrifuged at 106,000 *g* for 60 min to obtain the $S_r > 400$ fraction. The remaining infranate was resuspended in saline and centrifuged at the same speed for 24 h. The upper 2 ml was considered as containing only the very low density S_r 20–400 lipoprotein fraction. These migrated primarily in the pre-beta position with minor trailing from the origin on standard paper electrophoresis. Both $S_r > 400$ and 20–400 fractions were extracted for triglyceride and cholesterol determinations. The incubations were performed once each on two separate days using different chylomicron preparations as substrates. The results were in good qualitative agreement and those of one such experiment are illustrated in Fig. 4.

Lipoprotein lipase: Michaelis-Menten kinetics. Postheparin plasma from four normal young males and the proband was incubated for 1 h with the following final chylomicron-triglyceride concentrations; 16.0, 8.0, 5.3, 4.0, 3.3, and 1.6 mg/ml. The assay was otherwise identical with that described above for PHLA.

Buffer and plasma concentrations were constant at all substrate concentrations. One normal subject (no. 4) and the patient (Da. G.) received 0.5 mg/kg heparin IV in-

¹ *Abbreviations used in this paper:* PHLA, postheparin lipolytic activity; PHMA, postheparin monoglyceridase activity.

TABLE I
Clinical and Biochemical Data for the G. Kinship

Patient Age-Sex	Pedigree position	Triglycerides	Cholesterol	Lipoprotein electrophoresis	PHLA*
		mg/100 ml			μeq FFA/ml plasma per h
Normals n=12	—	75–150	75–250	—	6.94±1.96
Da. G. 5, M	III-1	935	125	Chylomicrons + ↑ pre-beta	0.14
C. G. 4, F	III-2	4200	365	Chylomicrons + ↑ pre-beta	0.22
Do. G. 7, F	III-3	83	158	Normal	2.46
P. B. G. 26, F	II-3	223	157	↑ pre-beta (+trace chylomicrons)	2.68
S. G., Jr. 29, M	II-2	360	212	↑ pre-beta + trace chylomicrons	5.45
S. G., Sr.† 60, M	I-1	310	276	↑ pre-beta	9.42
G. G. 58, F	I-2	200	274	↑ pre-beta	7.09
D. G. 32, M	II-1	235	247	↑ pre-beta	7.78
A. B. § 54, M	I-3	342	295	↑ pre-beta	5.75
M. B. 30, F	II-4	60	223	Normal	4.42
P. B. 27, M	II-5	42	160	Normal	5.37

* Postheparin lipolytic activity against chylomicrons: mean ±SD.

† Obesity and congestive heart failure controlled on digitalis and chlorothiazide.

§ Subject ingested approximately 120 cm³ of 87 proof whiskey, 8–10 h preceding study.

|| Subject was in the 4th mo of pregnancy.

stead of the usual 0.1 mg/kg dose. This was done in the latter to obtain sufficient PHLA at lower substrate levels. The one normal subject served as a control to eliminate possible heparin effects on the substrate-enzyme complex at this relatively low plasma substrate concentration. The results in Fig. 5 are illustrated as reciprocal plots of velocity and substrate (Lineweaver-Burk) with the latter expressed in millimolar concentrations. The average molecular weight of chylomicron triglyceride was estimated at 900.

RESULTS

Table I and Fig. 1 provide the data establishing a genetic basis for the disorder in this kinship. There was a virtual absence of PHLA against chylomicrons in the two affected siblings with approximately 50% normal PHLA in the unaffected mother and sister. However, the proband's father has a low normal PHLA with trace chylomicrons in the fasting state and, as with the remaining family, cannot be definitely established as a carrier. It should be especially noted that the paternal grandparents are well within the normal range for PHLA. Elevated pre-beta lipoproteins were found in

several asymptomatic family members and the mother (II-3) also contained trace chylomicrons by electrophoresis.

The lipoprotein electrophoretograms (Fig. 2) demonstrate the presence of chylomicrons in the proband's serum while on a regular diet with a distinct decrease

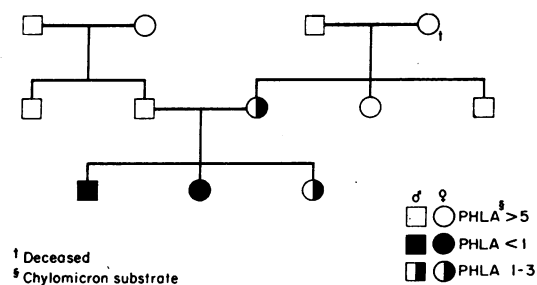


FIGURE 1 Pedigree for the G. family. There is no consanguinity known within the last four generations. Members are numbered (Table I) from left to right in each generation.

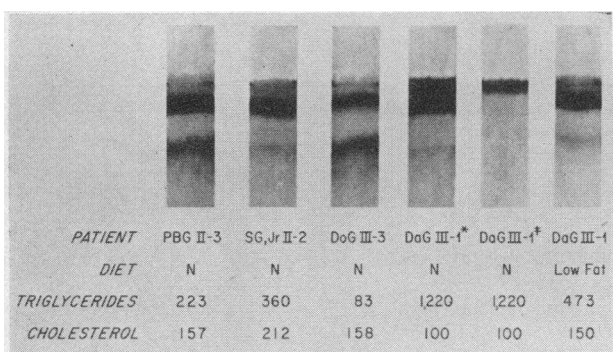


FIGURE 2 Lipoprotein electrophoretograms for the G. family. Serum triglycerides and cholesterol are in mg/100 ml. * Undiluted plasma; † Same plasma diluted to 10%; N = normal diet.

after a period of fat restriction. In undiluted specimens there is a heavy accumulation of pre-beta species and a pronounced trail from the origin. When the plasma is diluted to 10% the electrophoretic pattern more clearly represents the typical finding in type I disorders.

Fig. 3 illustrates the sharp fall in serum triglycerides on a fat-restricted diet in the proband's younger sister after 10 days. She was then given a "normal" diet for 12 days to demonstrate the persistence of pre-beta, very low density lipoproteins. The carbohydrate content of this diet comprised 37% of the total calories. Not shown in the illustration was the continuing absence of PHLA against chylomicrons on this same diet that contained 30% of its total calories as fat. The PHLA measured against an artificial triglyceride emulsion and true chylomicrons are compared in Table II. All family members had a normal or low normal PHLA when measured against Intralipid. However, the two affected siblings had marked decreases in PHLA against chylomicrons

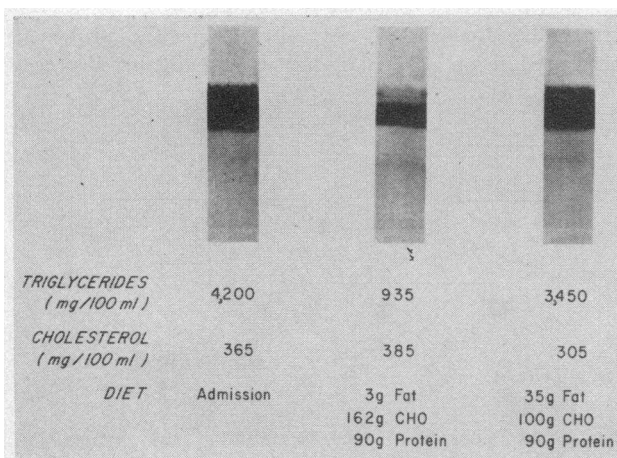


FIGURE 3 The effect of diet on serum triglycerides and cholesterol in the proband's sister, III-2.

and the healthy sister and mother had approximately one-half the activity of normal subjects. The two unrelated type I patients from different kinships and a type V patient with diabetes and pancreatitis all had very low PHLA with either substrate. Their post-heparin plasmas were treated identically with the proband's including removal of plasma chylomicrons before assay.

The in vitro conversion of large $S_r > 400$ chylomicrons to smaller, very low density species with pre-beta electrophoretic mobility is shown in Fig. 4. The patient's plasma, which had low PHLA, catalyzed the change of triglyceride-rich, cholesterol-poor $S_r > 400$ to a relatively cholesterol-rich chylomicron "remnant" (8) and a triglyceride-rich pre-beta particle. In effect, a purely exogenous type I phenotype was artificially converted to a mixed exogenous, endogenous type V (9) pattern as illustrated in the upper right panel.

The reaction kinetics of plasma lipoprotein lipase were examined in four normal subjects and the proband. The Lineweaver-Burk plots in Fig. 5 show close agreement in 'apparent' K_m for the normals (8.3–10.3 mM). However the patient's enzyme required almost 20 times

TABLE II
Postheparin Lipolytic Activity with Intralipid and True Chylomicron Substrates

Subject (pedigree position)	PHLA	
	Intralipid	Chylomicrons*
	$\mu\text{eq FFA/ml per h}$	
Normals (n = 12)	$6.22 \pm \text{SD } 2.42$	6.94 ± 1.96
Da. G., III-1	3.90	0.14
		1.20†
C. G., III-2	4.40	0.22
		1.25†
Do. G., III-3	6.30	2.46
		3.27
P. B. G., II-3	4.10	2.68
		2.94†
M. B., II-4	5.60	4.42
P. B., II-5	6.92	5.37
A. B., I-3	10.47	5.75
A. I.‡	0.66	1.80
D. E.‡	0.93	1.25
G. K.¶	0.27	1.80

* Normolipemic donor of chylous ascites chylomicrons.

† Plasma chylomicrons from patient C. G. were used as substrate.

‡ Type I—unrelated; patient of Dr. Gerald Salen, The Rockefeller University, New York.

¶ Type I = unrelated; patient of Dr. Allen Crocker, Children's Hospital, Boston, Mass.

¶ Type V patient with diabetes mellitus and pancreatitis.

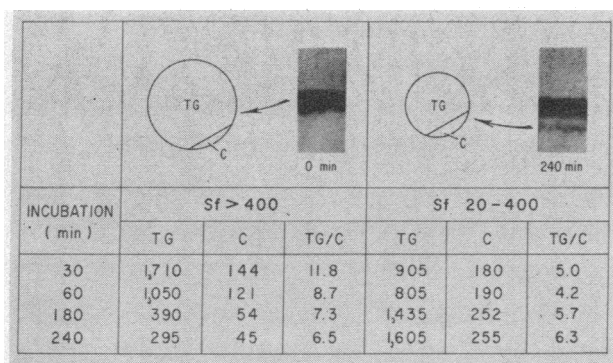


FIGURE 4 The reaction products of $S_f > 400$ chylomicrons after incubation with proband's postheparin plasma. The minutes listed at far left of the table indicate actual incubation time at 37°C but the substrate was exposed to enzyme throughout the approximately 3 h required for preparative ultracentrifugation and subsequent triglyceride extraction of the $S_f > 400$ fraction. Although Paroxon was added to these samples, continuing transformation may have occurred to account for the measurement of $S_f 20-400$ triglyceride at 30 and 60 min which did not appear on electrophoresis until 3 and 4 h. Results for triglyceride (TG) and cholesterol (C) are in mg/100 ml incubation medium. No attempt was made to analyze $S_f 0-20$ lipoproteins to possibly account for the "loss" of 24 mg/100 ml cholesterol. Not shown are the following data obtained with normal postheparin plasma. The $S_f > 400$ for 30 min was 545 and at 60 min, 50 mg/100 ml. The $S_f 20-400$ was 15 at 30 min and 10 mg/100 ml at 60 min.

that chylomicron concentration to reach half-maximal velocity. If the patient's enzyme were merely deficient, then only the y intercept ($1/V_{\max}$) would differ from normal since K_m is independent of enzyme concentration.

Table III eliminates an alternate explanation for the marked difference in 'apparent' K_m shown in Fig. 5. The presence of plasma inhibitors may alter the enzyme-

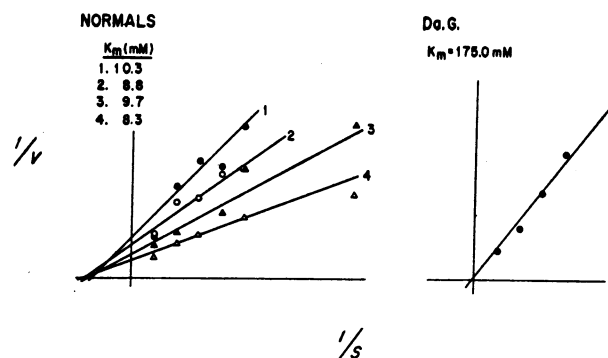


FIGURE 5 Lipoprotein lipase kinetics. Reciprocal plots of velocity and substrate concentration are shown for four normal subjects and the proband. The "apparent K_m " is expressed as millimolar chylomicron-triglyceride concentration. The patient Da. G. and normal no. 4 received 0.5 mg/kg heparin instead of usual dose of 0.1 mg/kg.

TABLE III
Absence of PHLA-Inhibitors in Patient's Plasma or Enhancement of Patient's PHLA by Normal Plasma

Plasma	PHLA*
	$\mu\text{eq FFA/ml per h}$
Normal postheparin	
+normal preheparin	10.76
+Da. G.'s. preheparin	9.76
+normal preheparin (concentrated $\times 10$)	9.94
+Da. G.'s. preheparin (concentrated $\times 10$)	9.32
+normal $d > 1.063$	10.59
+Da. G. $d > 1.063$	10.38
Da. G.'s postheparin	
+normal preheparin	1.33
+Da. G.'s preheparin	1.13
+normal $d > 1.063$	0.77
+Da. G.'s $d > 1.063$	1.05

* Chylomicron substrate.

substrate complex in such a way as to account for the above results. However there was not significant inhibition of normal PHLA detected by the $d > 1.063$ plasma fraction. In Table III, data fail to show any missing components necessary for lipoprotein lipase activation that might have been supplied by addition of normal plasma or normal plasma high density lipoprotein.

Table IV lists the postheparin monoglyceridase and phospholipase activities in selected patients. Monoglyceridase remained in the normal or low normal range in all family members examined as well as in the two un-

TABLE IV
Postheparin Monoglyceridase and Phospholipase Activities

Patient	Postheparin plasma activity	
	Monoglyceridase	Phospholipase
	$\mu\text{eq FFA/ml per h}$	
Normals (n = 10)	$7.93 \pm 1.65^*$	(n = 4) $4.01 \pm 0.49^*$
Da. G.	5.23	2.74
C. G.	6.47	2.80
Da. G.	5.70	
P. B. G.	4.90	
P. B.	8.00	
A. B.	5.70	
A. I.†	4.57	2.20
D. E.†	5.10	2.86
G. K.§	4.73	2.03
R. S.§		1.67

* Mean \pm SD.

† Familial type I patients from unrelated kindreds.

§ Acquired type V patients with diabetes mellitus and acute pancreatitis (see Table II for PHLA).

related type I patients and in a single patient with acquired PHLA deficiency (type V) secondary to poorly-controlled diabetes mellitus. The postheparin phospholipase was moderately decreased in all PHLA-deficient patients studied as compared with a small number of unmatched normal subjects.

DISCUSSION

The inheritance of type I hyperlipoproteinemia is established as autosomal recessive (1). Harlan, Winesett, and Wasserman (2) have phenotyped a family with exogenous hypertriglyceridemia on the basis of adipose tissue levels of lipoprotein lipase and were able to detect intermediate enzyme levels in the parents and one unaffected sibling. They further suggested an oral fat tolerance test as a useful tool for identifying carriers of this trait. However, this procedure has proven highly variable with normal volunteers in our laboratory and may be influenced by factors other than plasma PHLA (10) such as the fasting level of plasma triglyceride. In the family reported here, measurement of plasma PHLA was intermediate for the mother and healthy sister but was essentially normal in the father who would also be expected to be heterozygous for an abnormal gene. The most likely interpretation of these data is that of autosomal recessive transmission, however, PHLA determinations are not always a sensitive method for detection of heterozygotes. The significance of elevated pre-beta lipoproteins in other family members is unclear from our studies. Little, Whayne, Bhagwat, Buckley, and Kallos (11) have reported an adult female with type I whose father had type IV but whose five other relatives were normal.

The finding of diminished PHLA against chylomicrons in this kindred is in agreement with others. However normal lipolytic activity against a triglyceride emulsion has generally not been found in other type I families (2, 12-15) and may represent a variant of this disorder. The deficient PHLA against both substrates in our type I patients of unrelated kindreds (A. I. and D. E., Table IV) further confirms this differentiating feature. A single patient in reference 13 with typical type I features repeatedly gave normal PHLA against Ediol. Further investigations of this case have been reported (16) and will be discussed below. Steiner (17) has studied a 20-yr old alcoholic male, with no clear family history of hyperlipemia, who had features of fat-induced hypertriglyceridemia, normal PHLA with coconut oil emulsion (Ediol), but a deficient activity against natural chylomicrons similar to our subjects. Studies of five diabetic patients with acquired fat-induced hyperlipemia failed to show this substrate specificity (18) and these observations have been extended by patient

G. K. (Table II) who had diabetes and acute pancreatitis.

Another abnormality is the markedly increased 'apparent K_m ' against chylomicrons for the proband's PHLA. These K_m values are referred to as apparent because they reflect the activities of nonpurified enzyme(s) on substrate particles that also vary in size. However, even with these limitations, the comparison between normals' and proband's plasma remains valid and meaningful. The lack of circulating inhibitors in his plasma confirms the reports of others (12-14) and eliminates this as an explanation for the altered substrate kinetics. This apparent requirement for very high chylomicron concentration (15,000 mg triglyceride [TG] 100 ml) before half-maximal clearing occurs, might explain the metabolic abnormality as well as the clinical condition. The elevated apparent K_m indicates a different or mutant enzyme rather than simply a deficient normal one. Similar kinetics and very low PHLA were observed in postheparin plasma taken after a period of low-fat intake. Ultracentrifugation was not necessary because of the low chylomicron concentration and the possibility of artifact, introduced by removal of chylomicron-bound enzyme, is therefore eliminated. Our data confirm those of Bradford, Furman, and Bass (14) who first demonstrated these kinetic differences with the coconut oil substrate (Ediol) in three siblings with familial hyperchylomicronemia. Their PHLA however, were markedly deficient against coconut oil triglycerides and there were also differences from normal seen with ionic and detergent inhibitor properties. This latter phenomenon may reflect an action on the enzyme-substrate complex formation rather than on the enzyme itself (19-20). Further studies on the kinetics of PHLA and substrate specificities must be performed on more cases of this disorder to clarify whether these two kindreds with unusual lipoprotein lipase properties are representative of familial hyperchylomicronemia.

An alternate explanation for the above data might be the compensatory increase of another postheparin triglyceridase that is absent or present in only small amounts in normal postheparin plasma. Others (21-23) have characterized a rat liver lipase showing differing pH and inhibitor properties from adipose tissue lipoprotein lipase. Herbert et al. (16) have recently reported that postheparin plasma from four type I patients hydrolyzed artificial glyceride emulsions but had different inhibition and activation characteristics from normal postheparin plasmas. They speculate that this triglyceridase originates from the liver as suggested from rat experiments and may also be released by heparin into the circulation of normal subjects. These data, would offer an attractive explanation for our studies.

Still another possible explanation for the high apparent K_m in an assay system that expresses activity as FFA production from triglyceride, is that enzyme activity is influenced by the size of the lipoprotein-triglyceride particles. In vitro conversion of chylomicrons to "remnants" (9) was observed with our patients' postheparin plasma, but not with normal controls. The possibility exists that normally there are two postheparin triglyceridases (24), one having greater affinity for $S_r > 400$ and the other for the smaller S_r 20-400 lipoproteins. If our patients had a deficiency only in the latter case then this would be reflected in an abnormally low PHLA as measured against heterogeneous particle size. However, this explanation is less tenable because of our experiments in which the disappearance of substrate was followed (Fig. 4). The hydrolysis by the patients' postheparin plasma of both particles, $S_r > 400$ and S_r 20-400 "remnants" was reduced as compared with normal postheparin plasma. This would suggest that the patients' postheparin plasma had a deficiency of both lipases.

The exogenous pre-beta species produced in vitro may represent the same lipoprotein seen on electrophoresis of all undiluted plasmas from these patients (Fig. 2). The failure to observe this spectrum of lipoprotein density shifts with normal postheparin plasma may merely reflect the increased affinity of normal lipoprotein lipase for chylomicron triglyceride with completion of hydrolysis at a much faster rate. In this regard, Hazzard, Porte, and Bierman (25) have recently demonstrated in vitro production of triglyceride-poor chylomicrons after 16 h incubation of lipemic, postprandial plasma obtained from type II, III, and V patients. In view of these considerations, it seems prudent not to ascribe the pre-beta lipoproteins observed in our patient's fasting plasma as necessarily being of "endogenous" origin.

Heparin releases at least three other enzyme activities into the circulation: monoglyceridase (26, 27), phospholipase (7), and a nonspecific esterase (26, 27). We have measured the first two enzymes and found monoglyceridase activity to be normal or low normal in all patients with familial or acquired hyperchylomicronemia. The phospholipase was low but definitely present. Our findings suggest that these are distinct enzymes but do not exclude a mutant lipoprotein lipase with normal affinity for monoolein or phosphatidyl ethanolamine micelles but decreased activity for chylomicron triglyceride. Other workers have previously reported the varying characteristics of the postheparin lipases (7, 27-29) but a highly purified lipoprotein lipase preparation (30) still retains monoglyceridase activity. Another recent report documents the failure to separate phospholipase from lipoprotein lipase by a wide variety of fractionation tech-

niques (31). Perhaps the simplest explanation for these data is that lipoprotein lipase has activity against each of these glycerides but other separate heparin-released lipases also exist.

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