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Lipolytic activity of post-heparin plasma in hyperglyceridemia*

DONALD S. FREDRICKSON, KATSUTO ONO, and LOUIS L. DAVIS

Section on Molecular Disease, Laboratory of Metabolism, National Heart Institute, Bethesda, Maryland

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SUMMARY

A standard assay has been developed for the lipolytic activity of post-heparin plasma permitting rate measurements with zero order kinetics over wide ranges of enzymatic activity and concentrations of plasma glycerides. Measurements were made in 60 subjects with hyperglyceridemia, 18 of whom had been further subclassified by dietary studies into either fat-induced or carbohydrate-induced types. Activity in the majority of subjects with hyperglyceridemia, including all of the carbohydrate-induced type, was equal to or greater than that found in normal subjects. Subjects with fat-induced hyperglyceridemia, with one exception, and some of their relatives had abnormally low values. The level of activity in both normal and hyperglyceridemic subjects was uniformly dependent upon prior dietary fat intake.

In 1955, Havel and Gordon (1, 2) described three siblings with hyperglyceridemia directly related to dietary intake of fat and with decreased activity of "lipoprotein lipase" appearing in plasma after intravenous injection of heparin. They postulated a defect in tissue lipoprotein lipase as the basis of a defect in chylomicron metabolism. The lipolytic activity of post-heparin plasma has been determined in other subjects with elevated plasma glycerides with varying interpretations (3–6). Comparisons have been based on very small samples and by use of a variety of methods in which lipolysis might not have been measured under optimal conditions.

The purposes of this study were the development of a method designed to measure maximal rates of lipolysis *in vitro*, particularly in plasma samples containing very large amounts of endogenous lipid and its application to sufficient subjects with either normal or elevated glycerides to determine the usefulness of the test in possible segregation of traits leading to hyperglyceridemia.

* Portions of this work were reported at the April, 1962 meeting of the Federation of American Societies for Experimental Biology.

EXPERIMENTAL METHODS

The following stock solutions were prepared for use in the assay system: (1) albumin (bovine fraction V, Armour), 20 g/100 ml of 0.1 M $(NH_4)_2SO_4$ at pH 8.4; (2) coconut oil emulsion (Ediol, Schenlabs Pharmaceuticals, Inc., New York, N.Y.), containing 50 mg of glyceride/ml of isotonic saline; (3) extraction mixture for fatty acids containing isopropyl alcohol–isooctane–1 N H_2SO_4 40:10:1 (v/v) (7), to which was added 1.5% w/v polyoxyethylene lauryl alcohol ("Brij 35", Atlas Powder Corp., Wilmington, Del.); (4) sodium heparin (Upjohn Co., Kalamazoo, Mich.), 100 units (1 mg)/ml in sterile isotonic saline.

Glyceride Composition of the Ediol Substrate. An aliquot of the Ediol was extracted in chloroform–methanol 2:1, and the tri-, di-, and monoglycerides in the chloroform phase were eluted from a silicic acid column (8) with 60% benzene in hexane, 10% benzene in chloroform, and 10% methanol in chloroform, respectively. Thin-layer chromatography (9) was used to monitor column eluates for purity, and amounts of glyceride were determined by ester analysis (10). Fatty acids were determined as methyl esters by gas-

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TABLE 1. THE GLYCERIDE COMPOSITION OF THE COCONUT OIL EMULSION* USED AS SUBSTRATE†

Glyceride Content (%)		Fatty Acid Composition (%)							
		C:8	C:10	C:12	C:14	C:16	C:18	C:18:1	C:18:2
Triglyceride	47.0	10	6	62	14	7	<1	<1	<1
Diglyceride	1.9	<1	<1	29	15	22	21	12	<1
Monoglyceride	1.7	<1	<1	4	6	36	53	<1	<1

* Ediol, Schenlabs Pharmaceuticals, Inc.

† Tri-, di-, and monoglycerides were determined as the hydroxamates and calculated as trilaurin, dilaurin, and monostearin, respectively. Fatty acids were determined as methyl esters by gas chromatography employing 20% diethylene glycol adipate polyester–phosphoric acid at 192°. Linearity of the ionization detector response was determined through use of NIH Metabolism Study Section standards A-C. The stock emulsion was diluted 1:9 with isotonic saline for use in the assay system.

liquid chromatography. The composition is shown in Table 1. Approximately 7% were partial glycerides, the composition of the monoglycerides reflecting monostearin added as stabilizer by the manufacturer. Over 90% of the triglyceride fatty acids were between C_8 and C_{14} in chain length.

Triglycerides, cholesterol, and phospholipids were determined by previously described methods (11–13) using chloroform–methanol extracts of plasma or chylomicrons (14). Chylomicrons were prepared by layering plasma under saline at density 1.006 and centrifuging for 30 min at 100,000 × g at 15°. The packed layer at the top was re-suspended and washed twice before use as substrate. The partial glyceride content and fatty acid composition of chylomicrons were not determined.

Test. All subjects were tested after an overnight fast. Ten milliliters of blood was withdrawn, and heparin solution 0.1 mg/kg body weight was rapidly injected intravenously. A second sample was drawn exactly 10 min later. Four drops of the heparin solution, not exceeding 0.15 ml/10 ml blood, was added to the tube as anticoagulant. Bloods were kept at 2° during centrifugation and the plasma was either used immediately or frozen for subsequent assay.

Assay of Lipolytic Activity. Glass-stoppered tubes containing 100 mg albumin, 15 mg of the glyceride substrate, and 0.2 ml of plasma in a total volume of 1.0 ml were incubated in a Dubnoff shaker at 37°. Duplicate tubes were removed at 5 min, as a "zero-time" blank, and then 15, 30, and 45 min thereafter. The reaction was stopped immediately by adding 5.0 ml of the fatty acid extraction mixture. The tube was tipped once to allow mixing, allowed to stand 5 min, and then shaken vigorously. This procedure, combined with the presence of the detergent polyoxyethylene lauryl alcohol in the extraction mixture, was found useful in eliminating clumping and erratic extraction of free fatty acids (FFA) otherwise obtained with albumin in the presence of ammonium sulfate. Free fatty acids were then further extracted and titrated according to the method of Dole (7).

The FFA concentrations, minus the zero-time value, were plotted against time (Fig. 1) and the slope of the line connecting the points was used to calculate the reaction velocity (in μEq FFA/min/ml plasma).

Subjects. These are analyzed in detail in Table 2. *Normals* were selected only on the bases of plasma triglyceride concentrations less than 200 mg/100 ml

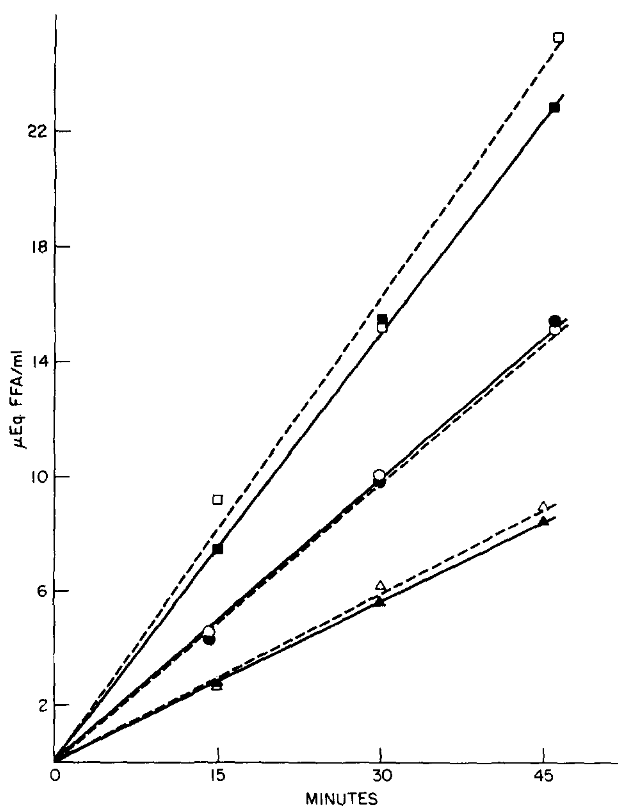

FIG. 1. A post-heparin sample from each of three different subjects was assayed (closed symbols), then diluted with an equal volume of the subject's pre-heparin plasma and assayed again, these second results being plotted × 2 for comparison (open symbols). Incubations were carried out with 0.2 ml plasma in 1.0 ml total volume as described in the text. Subjects and their plasma glyceride concentrations (in mg/100 ml) were: ■, OJ, 70; ● FW, 89; ▲ JP, 1080.

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TABLE 2. Summary of Age, Plasma Glyceride Concentrations, and Post-Heparin Lipolytic Activities of Normoglyceridemic and Hyperglyceridemic Subjects*

	Age	Plasma Glycerides	Post-heparin Lipolytic Activity
	years	*mg/100 ml*	*μEq FFA/ min/ml*
Normoglyceridemic (<200 mg/ml)			
Group (41)	36.0 ± 17	94.3 ± 41	0.37 ± 0.08
Males (17)	36.8 ± 15	89.8 ± 44	0.38 ± 0.08
	(18–68)	(30–152)	(0.27–0.57)
Females (24)	35.3 ± 16	97.7 ± 44	0.37 ± 0.07
	(18–75)	(32–193)	(0.24–0.53)
Hyperglyceridemic (>200 mg/100 ml)			
Group (60)	40.4	1,132	0.34 ± 0.14
	(4–70)	(211–5741)	(0.06–0.71)
Fat-Induced (9)			
	19.0 ± 8.8	2,028	0.16 ± 0.10
	(4–36)	(570–4200)	(0.16–0.41)
Carbohydrate-Induced (9)			
	51.9 ± 11.5	930	0.37 ± 0.09
	(34–66)	(211–2,908)	(0.26–0.50)

* Values are given as mean ± standard deviation with range in parentheses.

and the absence of any known relatives with hyperglyceridemia. The majority were "normal" volunteer subjects hospitalized at the Clinical Center, National Institutes of Health.

Hyperglyceridemic Subjects as a group were defined by plasma triglyceride concentrations in the post-absorptive state greater than 200 mg/100 ml. Although some had abnormal carbohydrate tolerance, none were considered diabetic in the sense of requiring treatment for this condition. All were ambulatory. They were further subdivided into three groups.

(1) *Fat-Induced Hyperglyceridemia (FIH)* (15). These nine subjects were characterized by the development of marked hyperglyceridemia within a few days of fat intake in the normal range of 30–40% of calories and a corresponding rapid decline to normal or nearly normal plasma glyceride levels upon reduction of fat intake to extremely low levels (5–10 g/day). The dietary responses of a single case representative of the nine subjects in this group are detailed in Fig. 2.

(2) *Carbohydrate-Induced Hyperglyceridemia (CiH)*. These nine subjects demonstrated a relationshp of plasma glycerides to diet opposite to that of the first group. All were considered to have carbohydrate-induced hyperglyceridemia as defined by dietary tests performed by Dr. E. H. Ahrens at The Rockefeller Institute and previously described by him and his colleagues (15).

(3) *Unclassified Hyperglyceridemia (UH)*. The other 42 subjects included only five who had also been rigorously studied for dietary response at the Clinical Center. The plasma glyceride levels of these five were not decisively affected by either high-fat or high-carbohydrate intake. Three of these were obese males with abnormal carbohydrate tolerance. When the body weight of two of these subjects was decreased by 10 kg, their plasma triglycerides were maintained at lower levels of 250–400 mg/100 ml. Another was myxedematous and her plasma glycerides fell only after thyroid replacement. The fifth had familial hypercholesterolemia with tendon xanthomas. These heterogeneous subjects were placed with the remaining 37 subjects and all considered unclassified for purposes of this study.

Relatives of Hyperglyceridemic Subjects. Members of several kindreds were tested. The results with normoglyceridemic members of such a family were excluded from the tabulation of normals and are described separately.

RESULTS

Selection of Assay Conditions. Certain of the assay conditions, including use of albumin at the pH optimum of 8.4 and the selection of Ediol as substrate, were adopted directly from extensive previous work with lipoprotein lipase by Korn and others (16). Ammonium

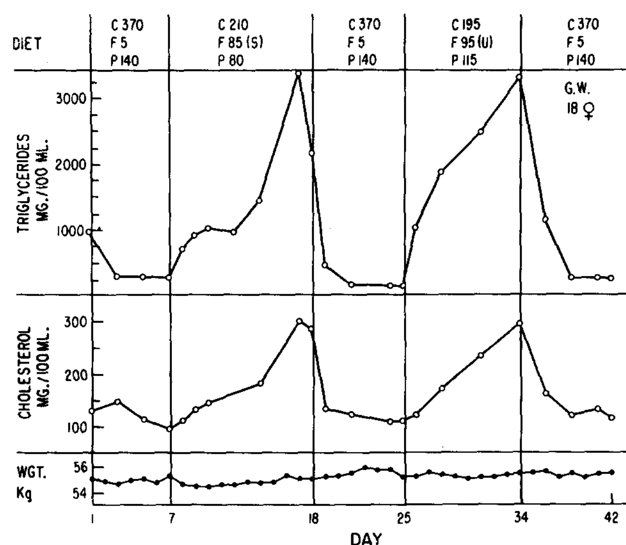

Fig. 2. The response of plasma lipids with changes in diet in a subject with fat-induced hyperglyceridemia (GW, Table 5). Small adjustments in caloric intake were made to maintain constant weight. C, F, and P refer respectively to the approximate grams of carbohydrate, fat, and protein in the daily diet. F(S) refers to provision of 95% of fat as butterfat and F(U) to 95% of fat as corn oil.

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cation, 0.05 M, previously shown to activate lipoprotein lipase from heart (17) or adipose tissue (18), was arbitrarily added. A final volume of 1.0 ml was chosen to permit cessation of the reaction and extraction of FFA in a 12-ml centrifuge tube with a minimum of time loss and handling.

The concentrations of Ediol and post-heparin plasma were selected to provide a high ratio of Ediol to "endogenous" plasma glyceride in the incubation mixture, thus minimizing variations in total glyceride concentration and composition anticipated in the assay of subjects whose plasma glyceride concentrations might differ by as much as 200-fold. Experimental support for this decision lay in the great differences in activities observed with Ediol compared to human chylomicrons such as are illustrated in Table 4. The concentration of 0.2 ml plasma/ml incubation mixture was found to provide sufficient activity for reproducible assay even in subjects with a very low response to heparin. The concentration of 15 mg Ediol/ml was adopted after it was observed that larger concentrations tended to form two layers during incubation and did not appear to provide any greater activity (see JL, Table 3).

The data in Table 3 illustrate that, in three representative subjects with widely differing plasma glyceride concentrations, the activities obtained with 10–15 mg Ediol were consistently higher than with 5 mg or less. From the data in the last two columns of Table 3, however, it can be seen that the higher activity obtained with the maximum feasible amount of Ediol was not correlated with maintenance of any specific ratio of coconut oil glyceride to "endogenous" glyceride. In several experiments similar to that shown for RB in Table 3, it was found that a significant decrease in activity often accompanied addition of pre-heparin plasma unless the Ediol concentration exceeded 5 mg/ml. It was assumed that the higher Ediol concentrations offset some substrate-sensitive inhibitory effect normally present in plasma. This "inhibition" was not obviously related to the glyceride concentration of either the pre- or post-heparin plasma used. Its relation to inhibitors previously reported in plasma (19–21) was not further explored. The provision of sufficient substrate to overcome this effect is quite important, however, since screening of plasma from hyperglyceridemic subjects for inhibitory or stimulatory factors involves use of an increased concentration of plasma in the incubation mixture.

In Fig. 1 are plotted results obtained when plasma from three different subjects was assayed before and after dilution with an equal volume of pre-heparin plasma. Constant rates of lipolysis for at least 45 min are illustrated over a six-fold range of activities from 0.09 to 0.53 μEq/min/ml post-heparin plasma and in the presence of a 15-fold difference in plasma glyceride concentrations. Linearity for this time period was

TABLE 3. RELATIONSHIP OF LIPOLYTIC ACTIVITY TO ADDED EDIOL

Subject	Content of 1.0 ml Incubation Mixture*				Lipolytic Activity
	Plasma	"Endogenous" Glyceride	Ediol Glyceride	Ediol/ Total Glyceride	
	ml	*mg*	*mg*		*μEq FFA/ min/ml*
JL	0.2	5	50	0.91	0.43§
	"	5	25	0.83	0.43§
	"	5	10	0.67	0.43
	"	5	5	0.50	0.36
	"	5	2.5	0.33	0.29
JP	0.2	2	15	0.88	0.19
	"	2	5	0.71	0.17
	"	2	2.5	0.56	0.15
RB	0.2	0.2	15	0.99	0.57
	0.4†	0.4	15	0.97	0.59
	0.4‡	9.2	15	0.62	0.50
	0.2	0.2	5	0.96	0.40
	0.4†	0.4	5	0.93	0.27
	0.4‡	9.2	5	0.35	0.25

* 0.015 M saline added, when necessary, to reach final volume; incubations otherwise as described in text.

† Includes 0.2 ml of pre-heparin plasma from subject RB.

‡ Includes 0.2 ml of pre-heparin plasma from LW, subject with fat-induced hyperglyceridemia.

§ Formed two layers during incubation.

TABLE 4. INHIBITION OF LIPOLYTIC ACTIVITY BY NaCl*

Source of Post-Heparin Plasma	Status	Substrate	Lipolytic Activity Control	1 M + NaCl
			μEq FFA/min/ml	
LW	FIH	Coconut oil	0.41	0.29
		Chylomicrons LW	0.12	0.03
WS	N	Coconut oil	0.36	0.11
		Chylomicrons LW	0.11	0.04
JP	FIH	Coconut oil	0.18	0.06
		Chylomicrons JP	0.04	
OJ	N†	Coconut oil	0.17	0.08
		Chylomicrons JP	0.10	0.05
PP	FIH	Coconut oil	0.12	0.12
		Chylomicrons PP	0.06	0.05
RM	N	Coconut oil	0.48	0.25
		Chylomicrons PP	0.25	0.09

* Compared as to source of substrate (coconut oil [Ediol] or chylomicrons, providing equal amounts of glyceride), and source of post-heparin plasma (N = normoglyceridemia; FIH = fat-induced hyperglyceridemia).

† On 5-g fat diet.

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subsequently observed over the entire range of activities and plasma glyceride concentrations reported in Table 2.

Pre-Heparin Lipolytic Activity. Pre-heparin plasma from over 30 subjects, 15 of them with hyperglyceridemia, was also assayed for lipolytic activity. Occasionally, activity of the order of 0.01–0.03 μEq FFA/min/ml appeared to be present, but it was never sufficient for accurate measurement. Such measurements were therefore not made routinely and none of the post-heparin activities reported is corrected for any pre-heparin activity.

Selection of Test Conditions. The optimum sampling time was tested in one normal subject and one with FIH in samples drawn 5, 7.5, 10, 12.5, 15, and 20 min after heparin. In both, maximum activity was obtained in 10 min. The differences in activity obtained with plasma removed at 7.5 and 12.5 min were not significant, although there was a steady decline after 12.5 min. The most important feature in preparation of the subject proved to be the antecedent diet as discussed below.

Reproducibility. The combined biological and analytical variability in the test was determined to be reasonably small. Tests were repeated on four normal and four hyperglyceridemic subjects after intervals of 2 to 42 days. The mean difference between the two tests was 8.4 ± 2.5%. As has been previously reported (16, 22), lipolysis can be determined on samples frozen for several weeks. Samples from 10 subjects were also assayed immediately and again after freezing for periods of 2 to 54 days. These repeated values differed by ±2–21%, but the mean values for the groups before and after freezing were identical.

Assays were therefore made on plasma shipped frozen from other institutions. These were obtained under standard conditions, including use of identical heparin preparations.

Possible Heterogeneity of Post-Heparin Lipolytic Systems. It has been repeatedly suggested that heparin causes more than one form of lipolytic activity to appear in plasma. The evidence has been reviewed and extended most recently by Skořepa and Páv and their co-workers (23, 24). The susceptibility of plasma (25) and tissue lipoprotein lipase (17, 18) to inhibition by 1 M NaCl has frequently been used to differentiate this enzyme from other esterases, and the inhibitory effect of salt was therefore examined in this system.

Adipose tissue lipoprotein lipase,[1] incubated with pre-heparin plasma under the conditions described above, was 95% inhibited by added 1 M NaCl. However, lipolytic activity in post-heparin plasma was inhibited to a considerably lesser and highly variable extent. In samples from 24 subjects, the mean inhibition by NaCl was 49.5 ± 22.5% (range 0–87%).

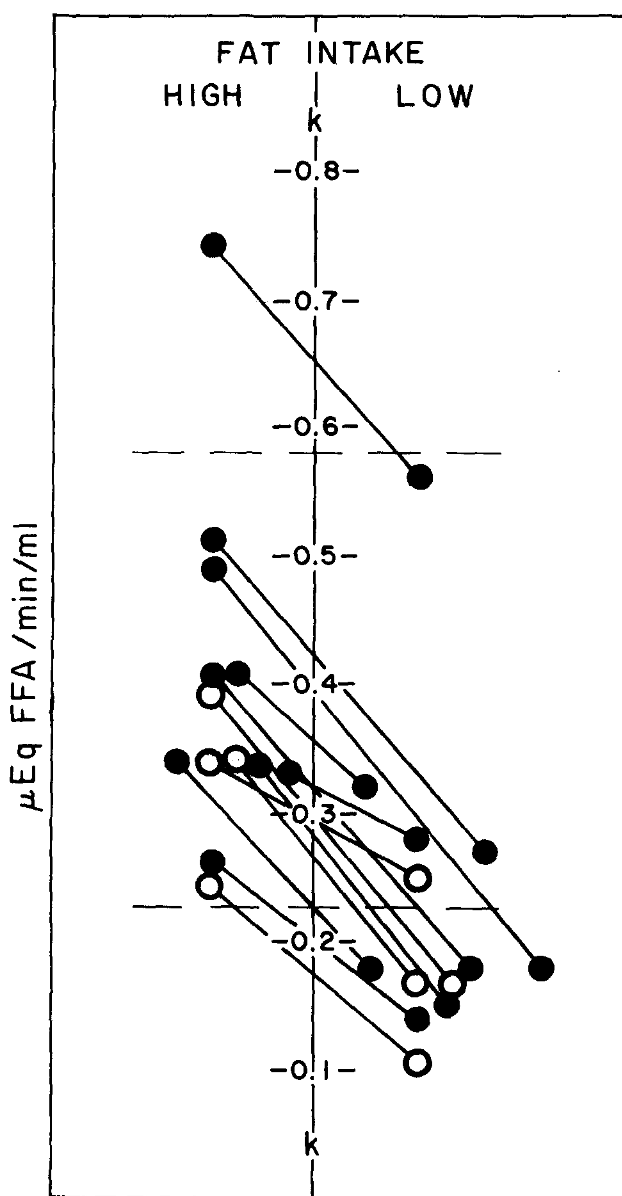

FIG. 3. Differences in lipolytic activity of post-heparin plasma obtained in 13 different normal (O) or hyperglyceridemic subjects (●) on high- and low-fat intake.

Table 4 illustrates, for specific subjects, our general failure to find consistent correlations between the degree of inhibition by NaCl and either the initial lipolytic activity of plasma, the glyceride concentration of plasma, or the nutritional state of the subject. Inhibition of activity from normals and subjects with fat-

[1] Rat adipose tissue lipoprotein lipase supplied by Dr. E. D. Korn, National Institute of Health.

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induced hyperglyceridemia was similar. The effect of NaCl was also independent of the choice of either coconut oil emulsion or human chylomicrons as substrate.

Effect of Diet. In preliminary studies, it was found that reduction in dietary fat intake to 5–20 g/day invariably lowered the lipolytic activity of post-heparin plasma to about 50% of that obtained on diets containing 50–150 g of fat (Fig. 3). This degree of reduction usually occurred with 7–10 days of fat restriction and activity returned to normal after restoration of adequate fat to the diet for a similar period. This phenomenon was independent of the plasma glyceride concentration or its variation with the diet. Interestingly, one patient with "a-β-lipoproteinemia" with a severe impairment of fat absorption, presumably of many years' standing, had post-heparin lipolytic activity of 0.17 μEq FFA/min/ml, a value comparable to that seen in normal subjects after a very low fat intake for 2–7 weeks. Subjects with FIH, some having activity at about this low level when on high-fat intake, showed still a further decrease to extremely low activity after a few days of fat restriction.

Unless specifically indicated, all values reported here for lipolytic activity were obtained after at least 1 week of normal fat intake.

Activity in Normoglyceridemic Subjects. The post-heparin lipolytic activity in 41 subjects with normal plasma glycerides followed a normal distribution over a relatively small range, having a mean and median value of 0.37 μEq FFA/min/ml. The results are summarized in Table 2 and Fig. 4. Variability was much less than the 10-fold differences observed in normals by Baker, who used a method of enzymatic assay based on optical density measurements (22). There was no correlation between activity and either age or sex.

Activity in Hyperglyceridemic Subjects. As indicated in Fig. 4, the lipolytic activity in the abnormal subjects extended over a much wider range, differing by as much as 10-fold. Of the 60 hyperglyceridemic subjects, 44 had normal activity and three exceeded the upper limit obtained in normals. The pre-heparin plasma of these three subjects did not contain significant activity nor did it enhance activity when incubated with post-heparin plasma from normal subjects. All nine of those demonstrated to have carbohydrate-induced hyperglyceridemia had normal activity (Table 2). Although their plasma glycerides rose with fat restriction, they had the same degree of fall in post-heparin lipolytic activity on this diet as did normals. Among the subjects with unclassified hyperglyceridemia, the five mentioned earlier as appearing to be neither fat- nor carbohydrate-induced also had normal activity.

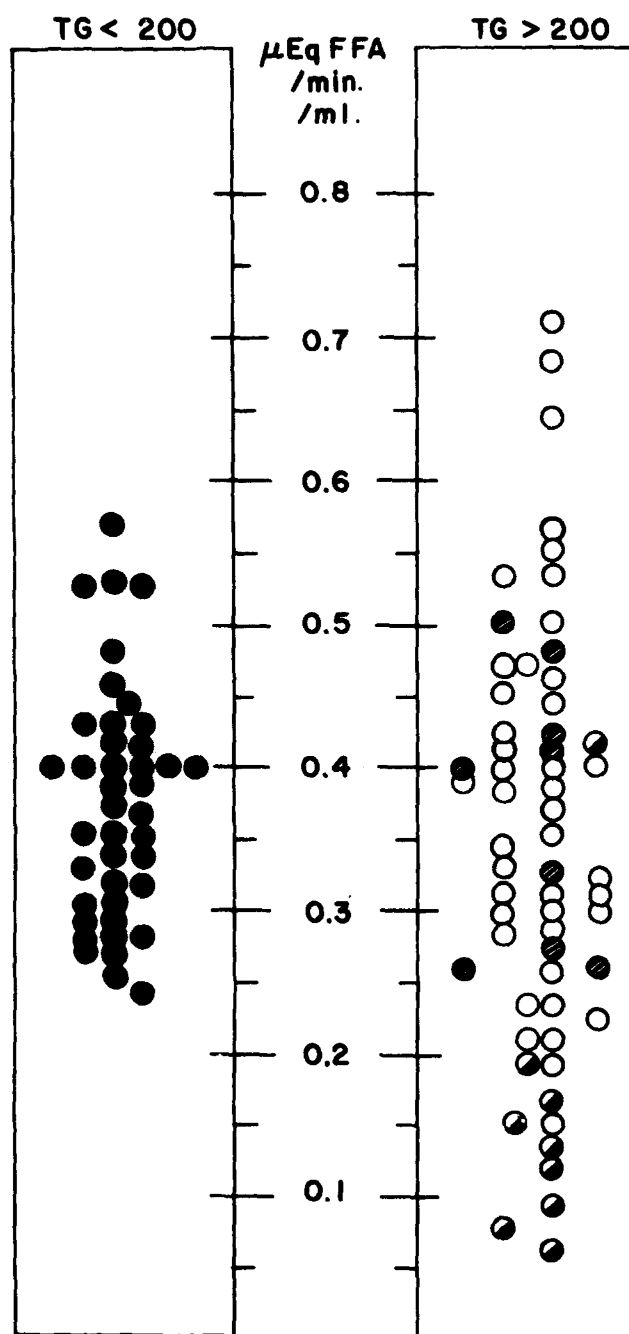

FIG. 4. Lipolytic activity of post-heparin plasma in subjects with normal (TG < 200) or elevated (TG > 200) plasma glyceride concentrations. Subjects in the latter group are further designated as fat-induced (◕), carbohydrate-induced (●), or unclassified (○).

With one important exception, the lipolytic activities of the subjects with fat-induced hyperglyceridemia were definitely subnormal (Table 2 and Fig. 4). Six of these were tested on multiple occasions. Some in this group were younger than the age range of normals tested,

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TABLE 5. Summary of Three Kindreds with Fat-Induced Hyperglyceridemia*

Subject		Sex	Age	Plasma TG	Plasma TC	Plasma PL	Lipolytic Activity
			years				μEq FFA/min/ml
Family G†							
RG	father	M	42	102	205	245	0.36
IG	mother	F	28	49	148	224	0.16
LG	propositus	M	4	932	73	162	0.06
RG	sib	M	7	42	147	205	0.30
MG	sib	F	2	72	189	224	0.15
Family P‡							
WP	father	M	66	183	220	242	0.21
DP	mother	F	55	155	271	234	0.28
JP	propositus	M	28	865	151	233	0.16
LP	propositus	M	22	2,166	252	389	0.12
PP	propositus	M	14	3,600	245	280	0.19
FP	sib	F	35	82	179	208	0.28
AP	sib	M	30	84			
CP	sib	M	21	75			
Family W							
LW	father	M	48	310	191	253	0.27
MW	mother	F	46	108	250	300	0.26
LW	propositus	M	19	2,290	256	352	0.41
GW	propositus	F	17	1,682	234	331	0.15
AW	sib	F	15	74	170	221	0.20
JW	sib	F	14	54	129	214	
TW	sib	M	12	62	157	253	
MW	sib	F	10	62	149	208	
JW	sib	M	21				

* M = male, F = female, TG = triglycerides, TC = total cholesterol, PL = phospholipids.
† Courtesy Dr. E. H. Ahrens, The Rockefeller Institute, N.Y.
‡ Also previously studied by Havel and Gordon (2).

but there was no intragroup correlation with age. The lowest value was obtained in a 36-year-old adult. Such low activities in FIH were often associated with plasma glyceride concentrations less than those in other subjects with normal activity. Pre-heparin plasma from these subjects did not inhibit normal activity in any instance when added to the incubation mixture.

The one exception in the group of nine subjects with FIH was a 19-year-old male, LW, whose dietary responses were identical to those shown for his sister in Fig. 2. His post-heparin response was normal on five separate tests performed over a 6-month period varying from 0.34–0.55 μEq FFA/min/ml (mean 0.41). This activity was reduced by fat restriction in the manner typical of all other subjects, and addition of his pre-heparin plasma to the incubation mixture had no stimulatory effect on normal lipolytic activity. His chylomicrons served equally well as substrate for his post-heparin activity as for that from a normal subject (Table 4).

Activity in Relatives of Subjects with FIH. Of the 60 hyperglyceridemic subjects, 12 had at least one sibling or parent known to have hyperglyceridemia. On the other hand, the families of only two of the remaining 48 had been screened sufficiently to exclude abnormal plasma lipids in both parents and all siblings.

Of the 12 cases known to be familial, six had not been classified by dietary responses. Only one of these unclassified patients had subnormal lipolytic activity, and he has several relatives with slightly elevated triglycerides without hypercholesterolemia. The other five had normal activity, and their relatives had varying plasma lipid abnormalities, most of them consistent with "essential familial hypercholesterolemia" with or without hyperglyceridemia. Three of the normoglyceridemic and nine of the hyperglyceridemic subjects shown in Fig. 4 were also considered to represent the syndrome of essential familial hypercholesterolemia, and all had a normal post-heparin response.

The six familial cases classified by dietary response all proved to be fat-induced and represented three different kindreds. Two of these kindreds and that of another fat-induced case, whose parents and siblings did not have hyperlipidemia, were examined further as to lipolytic response to heparin.

The results are summarized in Table 5. In family G, the mother and a sister had very low activity despite normal plasma glycerides. In the other two families, all of the four parents were well below the mean, but at least three were within the lower limits of normal. The value of 0.20 in the normoglyceridemic sibling, AW, is probably significantly low. The sampling of all families could not be complete and normal control data for very young subjects were not available. These data are therefore presented only for the strong suggestion they offer that the test may help to detect incompletely expressed forms of FIH.

Effect of Sonication. Robinson and Furman have reported (6) that sonication of post-heparin plasma from hyperglyceridemic subjects increased lipolytic activity. Their experiments were repeated using plasma samples from several different subjects and the results are summarized in Table 6. Activity in four cases of hyperglyceridemia, two of which were fat-induced, and in four normoglyceridemic subjects was unaffected by sonication. In one case of fat-induced hyperglyceridemia (CM, Table 6) the sonication of previously frozen plasma had a decided effect on total lipolytic activity. No explanation for this effect was

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TABLE 6. EFFECT OF SONICATION ON PLASMA LIPOLYTIC ACTIVITY*

Enzyme Donor	Status	Enzyme Source	Added Substrate	Lipolytic Activity	
				Control	Sonicated
				μEq FFA/min/ml	
CM	FIH	PHP	Coconut oil	0.11	0.30†
			"	0.10	0.35
			"	0.11	0.43
		AHP	"	0.00	0.00
JP	FIH	PHP	"	0.10	0.10
		PHP(CF)	"	0.10	0.10
		PHP(C)	"	0.00	0.00
LG	FIH	PHP	"	0.13	0.08
HS	CIH	PHP	"	0.29	0.30
CK	UH	PHP	"	0.22	0.25
CF	N(R)	PHP	"	0.57	0.61
GJ	N	PHP	"	0.46	0.36
JW	N	PHP	"	0.35	0.37
WH	N	PHP	"	0.30	0.34
JW + WH	N	PHP	"	0.33	0.37
		PHP	Chylomicrons JP	0.12	0.09

* Mixtures of substrate, plasma, and albumin in concentrations described in text for routine assays were exposed to sonication, using a Raytheon sonicator rated to deliver approximately 9.6 kc, for 60 min unless otherwise noted. The control mixture was kept at the same temperature, approximately 7°, for the same time period. Aliquots were then removed from the mixture and incubated at 37°, and lipolytic activity was determined. Abbreviations: FIH = fat-induced hyperglyceridemia, CIH = carbohydrate-induced hyperglyceridemia, UH = unclassified hyperglyceridemia, N = normoglyceridemia, N(R) = normoglyceridemic relative of hyperglyceridemic subject, PHP = post-heparin plasma, AHP = ante-heparin plasma, PHP(CF) = chylomicron-free post-heparin plasma, PHP(C) = chylomicrons from post-heparin plasma. Plasma from JP was used fresh, others had been frozen 1–34 days.

† Sonication for only 30 min.

offered by these experiments. It is possible that this is related to a nonspecific enhancement of the activity of other enzymes by sonication as described by Lawrence and Melnick (26); however, no lipase activity appeared in the pre-heparin plasma of CM after sonication.

Comparison with Previous Systems. The present system was compared with two published methods. During the course of this study, Kern, Steinmann, and Sanders (27) reported what is probably the first method designed to measure lipolytic activity of post-heparin plasma by combining a chemical, rather than turbidometric, end point with conditions permitting zero-order kinetics. Using 6 mg of Ediol and 0.4 ml of plasma/ml incubation mixture, they obtained an average rate of glycerol production equivalent to about 0.3 μEq FFA/min/ml, assuming complete hydrolysis of triglyceride. In our system, their heparin dose of 50 mg produced about double the activity obtained with our lower dose. Thus, the activities they obtained in normal subjects are lower than our values but of the same order of magnitude.

The second system, compared experimentally with the present system in Table 7, was that used by Havel and Gordon (2) in describing the P family (Table 5). Their method did not permit measurement of initial rates, and certainly contained suboptimal amounts of added substrate, and possibly of albumin. It consequently provided much lower calculated rates of hydrolysis, but differences between the subjects with FIH and others were nevertheless effectively detected by this method.

DISCUSSION

The present study has demonstrated that assay *in vitro* under standardized conditions of heparin-induced lipolytic activity in plasma has some clinical usefulness in differentiating types of hyperglyceridemia. At the same time, it may also fail to provide a completely reliable measurement of the ability to remove triglyceride from the blood.

The shortcomings of the test were apparent in the discrepancies between the lipolytic activities in two siblings who had hyperglyceridemia, which obviously depended upon the intake of dietary fat, and in the association of subnormal activity with normal plasma triglycerides in other members of this and similar kindreds.

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TABLE 7. COMPARISON OF TWO METHODS FOR MEASUREMENT OF LIPOLYTIC ACTIVITY*

Subject	Status	Plasma Triglyceride	Rate of Hydrolysis: Present Method	Single Tube: EDTA	Single Tube: Heparin
		mg/100 ml	*μEq FFA/min/ml plasma*		
RL	N	26	0.42	0.07	0.05
RZ	N	95	0.39	0.08	0.08
OJ	N	70	0.36	0.08	0.07
LL	N	135	0.35	0.09	0.07
HF	N	127	0.29		0.07
LW	FIH	650	0.30	0.02	0.02
"		1,966	0.41	0.07	
PP	FIH	5,038	0.29	0.04	0.08
JP	FIH	1,697	0.15	0.01	0.02
LP	FIH	2,166	0.12	0.02	0.03
ET	UH	472	0.58	0.12	0.12
AS	UH	1,882	0.56	0.09	0.06
GG	UH	1,229	0.38	0.06	0.10
HG	UH	1,409	0.33	0.11	0.13
SH	UH	283	0.23	0.10	0.09
LC	UH	907	0.13	0.08	0.08

* The single tube method refers to assay conditions as employed by Havel and Gordon (2). Two milliliters of plasma were mixed with 0.2 ml of 0.5% coconut oil emulsion and incubated at 37° for 15 min. The FFA concentration minus a zero-time control divided by 15 was used to estimate the rate of lipolysis. EDTA = 1 mg disodium ethylene diamine tetraacetate/ml blood used as anticoagulant in place of heparin (last column). Other abbreviations as in Table 6.

These discrepancies probably arise because the amount of enzyme activity appearing in plasma after heparin might not always be proportional to the amount in those tissues concerned with removal and hydrolysis of triglycerides. The total lipolytic activity so determined also may not reflect the "effective" concentration of lipase, particularly in view of the indication that the present system might be measuring more than one enzyme.

Standardized comparison of the lipolytic activity of post-heparin plasma with tissue lipolytic activity, particularly that of adipose tissue, will doubtless help to determine the validity of any plasma assay. The combination of a tissue or plasma assay and some type of fat tolerance test also may provide more accurate detection of lesser degrees of abnormality in triglyceride removal.

Despite the inadequacies of the plasma test, it does provide uniquely low values in one type of patient, where dietary responses suggest a severe impairment of the mechanism for removing chylomicron triglyceride from the blood. Extending the correlations between enzyme activity and dietary response observed in 23 of 60 subjects to the entire sample, it would be concluded that the large majority of cases of post-absorptive hyperglyceridemia do not represent a primary defect in a mechanism of clearing of dietary fat associated with decreased release of lipolytic activity by heparin. Since the full-blown syndrome of fat-induced hyperglyceridemia tends to have prominent familial expression, which makes it easier to collect such cases, no doubt this syndrome represents an even smaller percentage of all subjects with hyperglyceridemia than this sample indicates.

One of the most significant findings of this study is the absolute dependence of the response to heparin on the preceding diet. This appears to be an adaptive phenomenon making responsiveness to heparin a function of the degree of fat loading during the preceding several days. Caloric intake was maintained constant in the periods of fat deprivation during which heparin-released activity uniformly fell. These human experiments are not analogous, therefore, to experiments in animals, in which lipoprotein lipase has been found to decrease in adipose tissue (28–31) or, conversely, to increase in arterial tissue (32) after starvation. The effect of caloric deprivation on the heparin response has not been adequately studied in man.

At least one study has appeared showing decreased heparin responsiveness in cases of cystic fibrosis (33), and it may be predicted that all cases of malnutrition in which fat intake is severely limited will have low lipolytic activity of plasma after heparin.

It is concluded that measurement of post-heparin lipolytic activity under optimal *in vitro* conditions does not always provide a highly accurate view of the capacity for triglyceride removal. Nevertheless, a properly standardized test is sufficiently simple and useful to merit wider application in the study of hyperglyceridemic subjects and their relatives.

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REFERENCES

1. Havel, R. J., and R. S. Gordon, Jr. *Circulation* **12**: 485, 1955.
2. Havel, R. J., and R. S. Gordon, Jr. *J. Clin. Invest.* **39**: 1777, 1960.
3. Lever, W. F., P. A. J. Smith, and N. A. Hurley. *J. Invest. Dermatol.* **22**: 71, 1954.
4. Crofford, O. B., J. G. Coniglio, H. C. Meng, and E. V. Newman. *J. Clin. Invest.* **37**: 886, 1958.
5. Carlson, L. A., and B. Olhagen. *J. Clin. Invest.* **38**: 854, 1959.
6. Robinson, C. W., and R. H. Furman. *Clin. Research* **9**: 145, 1961.
7. Dole, V. P. *J. Clin. Invest.* **35**: 150, 1956.
8. Horning, M. G., E. A. Williams, and E. C. Horning. *J. Lipid Research* **1**: 482, 1960.
9. Malins, D. C., and H. K. Mangold. *J. Am. Oil Chemists' Soc.* **37**: 576, 1960.
10. Snyder, F., and N. Stephens. *Biochim. Biophys. Acta* **34**: 244, 1959.
11. Sperry, W. M., and M. Webb. *J. Biol. Chem.* **187**: 97, 1950.
12. Stewart, C. P., and E. B. Hendry. *Biochem. J.* **29**: 1683, 1935.
13. Bragdon, J. H. *J. Biol. Chem.* **190**: 513, 1951.
14. Bragdon, J. H. In *Lipids and the Steroid Hormones in Clinical Medicine*, edited by F. W. Sunderman and F. W. Sunderman, Jr., Philadelphia, Lippincott, 1960, p. 6.
15. Ahrens, E. H., Jr., J. Hirsch, K. Oette, J. W. Farquhar, and Y. Stein. *Trans. Assoc. Am. Physicians* **74**: 134, 1961.
16. Korn, E. D. In *Methods of Biochemical Analysis*, edited by David Glick, New York, Interscience Publishers Inc., 1959, vol. **7**, p. 145.
17. Korn, E. D. *J. Biol. Chem.* **215**: 1, 1955.
18. Korn, E. D., and T. W. Quigley, Jr. *Biochim. Biophys. Acta* **18**: 143, 1955.
19. Hollett, C., and H. C. Meng. In *The Blood Lipids and the Clearing Factor*. Koninkl. Vlaam. Acad. Wetenschap. Letter. en Schone Kunsten België Jaarboek, 1956, p. 190.
20. Lever, W. F., and E. Klein. *J. Invest. Dermatol.* **29**: 465, 1957.
21. Angervall, G., and B. Hood. *Acta Med. Scand.* **157**: 407, 1957.
22. Baker, S. P. *Circulation* **15**: 889, 1957.
23. Skořepa, J., B. Procházka, and H. Todorovičová. *Clin. Chim. Acta* **5**: 296, 1960.
24. Páv, J., and J. Wenkeová. *Clin. Chim. Acta* **5**: 90, 1960.
25. Brown, R. K., E. Boyle, and C. B. Anfinsen. *J. Biol. Chem.* **204**: 423, 1953.
26. Lawrence, S. H., and P. J. Melnick. *Proc. Soc. Exptl. Biol. Med.* **107**: 998, 1961.
27. Kern, F., Jr., L. Steinmann, and B. B. Sanders. *J. Lipid Research* **2**: 51, 1961.
28. Hollenberg, C. H. *Am. J. Physiol.* **197**: 667, 1959.
29. Cherkes, A., and R. S. Gordon, Jr. *J. Lipid Research* **1**: 97, 1959.
30. Robinson, D. S. *Biochem. J.* **74**: 25P, 1960.
31. Páv, J., and J. Wenkeová. *Nature* **185**: 926, 1960.
32. Hollenberg, C. H. *J. Clin. Invest.* **39**: 1282, 1960.
33. Slack, J., S. Nair, H. Traisman, G. Becker, S. Mahler, and D. Y. Hsia. *J. Lab. Clin. Med.* **59**: 302, 1962.

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