Evidence for separate monoglyceride hydrolase and triglyceride lipase in post-heparin human plasma

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ABSTRACT Post-heparin plasma contains an enzyme or enzymes with both triglyceride lipase (TGL) and monoglyceride hydrolase (MGH) activities. A simple and reproducible radioactive assay for measurement of MGH activity was developed and used, with a previously reported assay for TGL, to study lipolysis in plasma.

After the injection of heparin, enzymatic activity against both tri- and monoglycerides appeared and disappeared from plasma at approximately the same rates. However, in contrast to TGL activity, MGH activity was: (*a*) much greater, (*b*) considerably less heat-sensitive, (*c*) unaffected by three inhibitors (NaCl, protamine, and pyrophosphate), (*d*) not influenced by radical changes in fat and carbohydrate content of the diet, and (*e*) normal in familial Type I hyperlipoproteinemia. The dichotomy between MGH and TGL activities in patients with genetic deficiency of TGL constitutes strong evidence that these are two different enzymes.

The findings further indicate that when post-heparin lipolytic activity is measured for the purpose of detecting TGL deficiency, it may be necessary to perform the assay with a substrate free from partial glycerides.

SUPPLEMENTARY KEY WORDS radioactive assay · lipoprotein lipase · inhibitors · hyperlipoproteinemia

POST-HEPARIN plasma contains both triglyceride lipase (TGL) (1), which is considered to be synonymous with lipoprotein lipase (EC 3.1.1.3), and monoglyceride hydrolase (MGH) activities (2). Hydrolysis of both substrates is markedly enhanced after the administration of heparin, and both of these enzymes form a major part of what is often called post-heparin lipolytic activity or PHLA. This report describes the measurement of MGH activity by a simple radioactive assay and its comparison with TGL activity. Normal subjects and patients with a genetic deficiency of PHLA that is accompanied by severe hyperchylomicronemia (Type I hyperlipoproteinemia) (3) were studied. In the latter individuals a marked dichotomy between MGH and TGL activities was found. This and other evidence suggests that these separate hydrolytic activities are probably catalyzed by different enzymes

MATERIALS AND METHODS

Materials

Glycerol-^{3}H monooleate was synthesized by acylation of randomly labeled glycerol-^{3}H with oleoyl chloride. The resulting monoglyceride was separated from small amounts of di- and triglyceride by column chromatography (4). It was found to be chemically and radiochemically pure by thin-layer chromatography. Its specific activity was 29.3 mc/mmole. Glycerol trioleate-1′-^{14}C, with specific activity of 36.4 mc/mmole, was purchased from Nuclear-Chicago Corp.; according to thin-layer chromatography, 99.7% of the radioactivity was found to be in triglyceride. Both glycerides were stored in dry redistilled benzene at −20°C. Unlabeled monoolein and triolein were obtained from The Hormel Institute, Austin, Minn., and sodium taurodeoxycholate from Maybridge Chemical Company, Tintagel, Corn-

Part of this work was presented at the 22nd Annual Meeting of the American Heart Association, Council on Arteriosclerosis (Bal Harbour, 19–20 November 1968).

Abbreviations: TGL, triglyceride lipase; MGH, monoglyceride hydrolase; PHLA, post-heparin lipolytic activity.

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wall, England; these were stored at −20°C. All organic solvents were redistilled before use.

Methods

Post-heparin plasma samples were obtained as described by Fredrickson, Ono, and Davis (5), 10 min after the i.v. injection of 10 units of heparin/kg of body weight unless otherwise indicated.

Diets

All normal subjects and patients were hospitalized on a metabolic ward and their weights kept constant. Blood was drawn after a minimum of 7 days on each of three diets. The starting diet was a basal one that provided approximately 20, 40, and 40% of calories from protein, fat, and carbohydrates, respectively. Other diets were a high-fat diet (>200 g of fat and <50 g of carbohydrates per day) containing approximately 20% of calories from protein, 75% from fat, and 5% from carbohydrates; and a high-carbohydrate diet (>7 g of carbohydrates/kg per day) containing approximately 20% of calories from protein, 80% from carbohydrates, and less than 2% from fat.

Enzyme Assays

MGH was assayed in tubes fitted with screw caps. In a total volume of 0.70 ml each vial contained: 35 μg of glycerol-^{3}H monooleate (6.6×10^6 dpm); 1.0 mg of unlabeled monoolein; 5.0 mg (0.9 mmoles) of sodium taurodeoxycholate; and 0.5 ml of 0.1 M Tris buffer (pH 8.6). The incubation mixture was thoroughly shaken on a Vortex mixer until a water-clear solution was obtained, and an amount (1–200 μl) of post-heparin plasma was added. The samples were placed in a Dubnoff shaker at 27°C and the reaction was stopped by the addition of 10 ml of chloroform–methanol 2:1. 2 ml of 0.5 N H_2SO_4 was then added and the phases were separated by centrifugation. Radioactivity was determined in duplicate on 0.1-ml aliquots of the upper aqueous methanol phase with a Tri-Carb liquid scintillation spectrometer (Packard, model 3375) provided with an external standard for quenching correction. Samples were counted in 10 ml of scintillation mixture (6). Less than 1% of the radioactive substrate was present in the upper phase when the incubations were run in the absence of post-heparin plasma, this blank being between 1 and 2% of the usual counting rate obtained with enzyme present. Incubations were run in duplicate and paired samples agreed to within 3%.

TGL assays were carried out as described by Greten, Levy, and Fredrickson (7). Each vial contained, in a total volume of 3.14 ml, 7.06 mmoles of glycerol trioleate-1′-^{14}C, 3.0 ml of 1.35 M Tris buffer (pH 8.6), 0.1 ml of 1% albumin solution, and 0.04 ml of a 1% aqueous Triton X-100 [a nonionic detergent, mixture of *p*,*t*-octylpoly(phenoxyethoxy) ethanols] solution. Each incubation mixture was sonicated for exactly 30 sec at 6.6 amp and 4°C (Branson sonifier). Post-heparin plasma, 150 μl, was added just before incubation. The post-heparin plasma from the patients with Type I hyperlipoproteinemia was delipidated (7) before use. Total lipids were extracted and ^{14}C-labeled free fatty acids, diglycerides, and triglycerides were separated by thin-layer chromatography. The bands containing these compounds were located by means of iodine vapor and quantitatively transferred to counting vials.

For both assays, incubations were carried out for 60 min at 27°C unless otherwise indicated. Under these conditions both triglyceride [see previous report (7)] and monoglyceride were hydrolyzed at linear rates. MGH activity was linearly proportional to the volume of added post-heparin plasma between 25 and 150 μl (Fig. 1). With 60 min of incubation, the amount of hydrolysis was found to vary linearly over a 200-fold range of concentration of post-heparin plasma (i.e., from 1 to 200 μl of post-heparin plasma added in the assay).

RESULTS

The addition of from 10 to 5000 μg of pure triolein or of plasma containing 250–2400 μg of triglyceride to the incubation mixture had no effect on the total amount of glycerol-^{3}H monooleate hydrolyzed. Thus triglyceride, added either as an emulsion or present in lipoproteins,

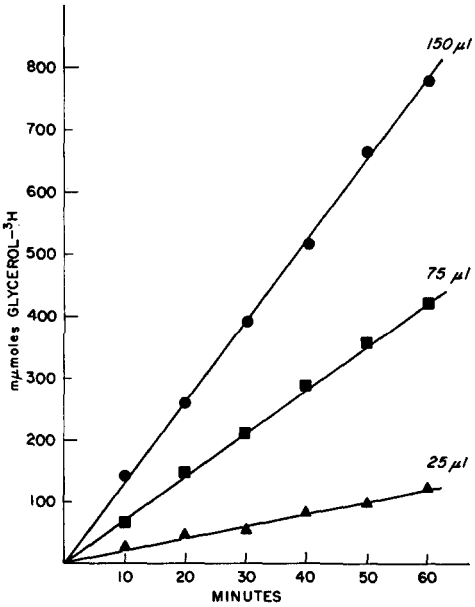

Fig. 1. Hydrolysis of glycerol-^{3}H monooleate with various amounts of the same sample of post-heparin plasma from one normal subject. Temperature, 27°C.

did not compete with monoglyceride as substrate for MGH.

Pre-heparin plasma has been shown to be essentially free from TGL activity, as measured with the radioactive assay (7). Pre-heparin plasma (100 μl) from six of the fasting normal subjects released only 560 (range 240–900) mμmoles of glycerol-^{3}H/ml in 60 min. This was significantly higher than the blank and about 10% of the post-heparin level. In three patients with Type I hyperlipoproteinemia the pre-heparin MGH activity was approximately the same, 390 mμmoles of glycerol-^{3}H/ml per hr (range 300–500). All subsequent studies of enzyme activity were carried out in post-heparin plasma.

TABLE 1 INFLUENCE OF INHIBITORS ON MGH AND TGL IN POST-HEPARIN PLASMA

Added Inhibitors	MGH Activity* Level	MGH Activity* Inhibition	TGL Activity* Level	TGL Activity* Inhibition
	mμmoles glycerol-^{3}H/ml per hr	%	*mμmoles FFA-^{14}C/ml per hr*†	%
None	4600	—	21.7	—
NaCl, 1 M	4310	0	7.0	68
Protamine sulfate, 150 μg/ml	4300	0	9.1	58
$Na_4P_2O_7 \cdot 10\ H_2O$, 10 μM	4700	0	10.8	50

All enzyme determinations were made on the same sample of post-heparin plasma. Final concentrations of inhibitors in the incubation mixture are shown.

* Mean of three experiments.

† FFA, free fatty acids.

TABLE 2 INFLUENCE OF TEMPERATURE ON MGH AND TGL IN POST-HEPARIN PLASMA

	MGH Activity* Level	MGH Activity* Expected Activity	TGL Activity* Level	TGL Activity* Expected Activity
	mμmoles glycerol-^{3}H/ml per hr	%	*mμmoles FFA-^{14}C/ml per hr*†	%
No preincubation	3900	100	19.6	100
60 min preincubation at 37°C	3930	100	7.2	37
15 min preincubation at 54°C	1950	60	2.0	10
15 min preincubation at 60°C	0	0	0	0

All determinations were performed simultaneously and made on the same sample of post-heparin plasma. Post-heparin plasma was heated as described prior to its addition to the incubation mixture. Incubations were then carried out at 27°C.

* Mean of three experiments.

† FFA, free fatty acids.

Post-heparin TGL was distinctly inhibited by NaCl (1 M), protamine sulfate (150 μg/ml), and sodium pyrophosphate (1×10^{-5} M), whereas MGH activity was unaffected (Table 1). The two activities also differed in temperature stability (Table 2). At 37°C, TGL was partially inactivated within 1 hr (7), while MGH activity remained stable at this temperature. Both activities were decreased at 54°C, but to different extents, as previously reported by Shore and Shore (2), and both were completely inactivated at 60°C.

The influence of different diets on post-heparin TGL and MGH were compared. Five normal subjects were tested after 1 wk on each of the basal, high-fat, and high-carbohydrate diets. The latter two diets varied in sequence, but were separated by at least 1 wk of basal diet. In each of five normal subjects TGL activity was the same during the basal or high-carbohydrate diet and an average of 38% higher (range 25–57%) on the high-fat diet (Fig. 2). MGH activity determined in these subjects under the same dietary conditions was not affected by either the high-fat or high-carbohydrate diet (Fig. 2).

In the five normal subjects on the basal diet, the mean plasma TGL activity 10 min after heparin was 12.1 mμeq of free fatty acids-^{14}C per ml of post-heparin plasma per hr (range 9.1–15.1). In two siblings with Type I hyperlipoproteinemia, who had the typical defect in ability to remove dietary glyceride from plasma, the corresponding values for TGL activity were 3.7 and 3.1 mμeq of free fatty acids-^{14}C/ml per hr. In contrast, MGH activity in four patients with Type I hyperlipoproteinemia (5010 mμmoles of glycerol-^{3}H/ml per hr, range 3480–6300) was not different from normal [5600 ± 2200 (SD, n = 20); range, 3300–9500]. There

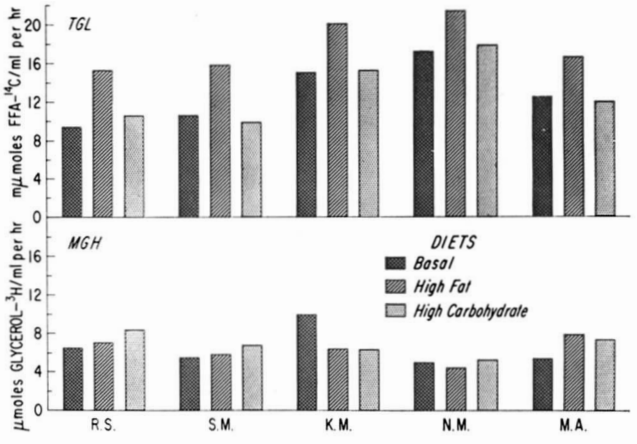

FIG. 2. Effect of different diets on post-heparin TGL and MGH activity. Enzyme determinations were made on plasma from five normal subjects who had been on a basal, high-fat, or high-carbohydrate diet for at least 1 wk, respectively, as described in the text. TGL, triglyceride lipase; MGH, monoglyceride hydrolase.

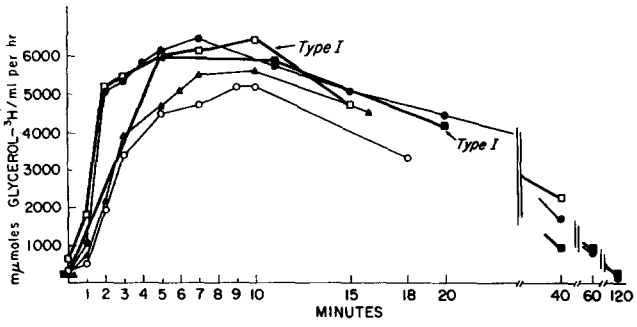

FIG. 3. MGH activity in plasma after i.v. injection of heparin into three normal subjects (○, ▲, ●) and two patients with familial Type I hyperlipoproteinemia (□, ■). Temperature, 27°C. MGH, monoglyceride hydrolase.

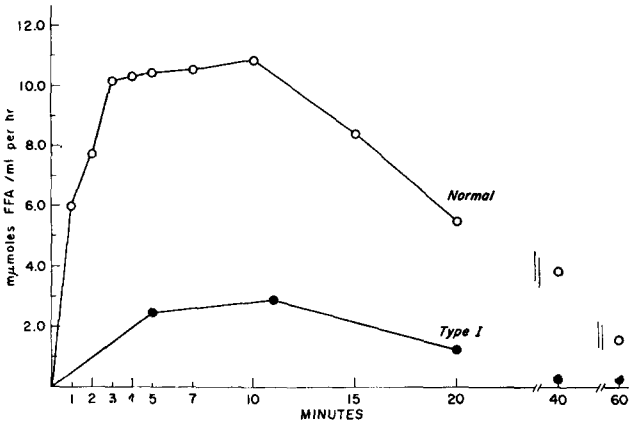

FIG. 4. TGL activity in plasma after i.v. injection of heparin into one normal subject and one patient with familial Type I hyperlipoproteinemia. Substrate, glycerol trioleate-1′-^{14}C; temperature, 27°C. TGL, triglyceride lipase.

was thus a marked discrepancy between TGL and MGH activities in this disorder.

Plasma MGH and TGL activities were also measured at different time intervals after heparin injection in three normal subjects and two patients with Type I hyperlipoproteinemia. The time course of TGL activity in plasma coincided with that for MGH (Figs. 3 and 4). The rates of release and decline of MGH in the Type I patients were not significantly different from those in the normals (Fig. 3), while TGL activity was at all time intervals less than a third of that obtained from a normal subject (Fig. 4).

The assay systems employed for the determination of post-heparin TGL and MGH activities differed in the nature of the detergent used and in the ionic strength of the buffer. Several experiments were therefore performed in which MGH activity was determined in the presence of 13 μl of 1% Triton X-100 per ml and with 1.35 M Tris buffer in the medium. In these experiments 33 μl of 1% aqueous albumin per ml was also present, as employed for TGL assay. Under these conditions, MGH activity in a patient with Type I hyperlipoproteinemia was not decreased and neither 1 M NaCl nor preincubation at 37°C produced any decrease in activity, all of these results being compatible with those obtained with the assay conditions employed here for all other studies of MGH.

DISCUSSION

These experiments were designed to provide an accurate method of measuring MGH activity in post-heparin plasma and to examine whether this activity is identical with triglyceride lipase. The assay used was linear over a 200-fold range of activity. The determinations of MGH activity were unrelated to plasma triglyceride concentrations of up to 5000 mg/100 ml.

It is difficult to compare hydrolytic activities, as obobtained with water-soluble substrates (esterases), on the one hand, and water-insoluble substrates (lipases) on the other. For example, different effects of salts and other inhibitors may be due to their influence on the physical state of the substrate, rather than specific effects on the enzyme or enzymes present. The differences obtained here and elsewhere (2) between effects of salt on TGL and MGH activities thus do not provide helpful evidence of heterogeneity. On the other hand, it seems much more likely that temperature effects are related to direct effects on the enzyme, and the difference in stability of MGH and TGL activities obtained at 37°C argues for the separateness of these enzymes. The dichotomy observed between MGH and TGL activities in the patients with genetic deficiency of TGL offers the strongest evidence yet obtained that these are two different enzymes.

Rat and rabbit adipose tissues have also been found to contain a monoglyceride hydrolase activity that appears to be distinct from hormone-sensitive lipase activity and probably from lipoprotein lipase (8). The adipose tissue monoglyceride-splitting activity was much greater for 2-monoglycerides than for 1-monoglycerides. We have not made such a comparison of positional specificity for the plasma MGH. At present there is no basis for determining the possible identity of the plasma and adipose tissue monoglyceridase activities.

In clinical studies TGL activity is usually assayed by measuring the release of free fatty acids or glycerol from artificial oil emulsions (Ediol, Calbiochem, Los Angeles, Calif., or Intralipid, Vitrum, Stockholm, Sweden). These also contain significant amounts of mono- and diglycerides. One of our patients (L.W.), with familial Type I hyperlipoproteinemia (9), repeatedly showed normal values of post-heparin lipolytic activity, as determined by an assay employing Ediol (5), in contrast with those in his affected sibling and other patients with

this disorder. This gave cause for speculation that the assay employing Ediol was less than ideal for the detection of Type I hyperlipoproteinemia (3). L.W. was found here to have low values of TGL, like other Type I hyperlipoproteinemic patients, when a pure ^{14}C-labeled triglyceride was used as substrate. The normal values for post-heparin lipolytic activity in L.W., as determined with a mixed emulsion, might have been due to hydrolysis of partial glycerides that compensated for a specific deficiency in TGL in this patient; however, his post-heparin MGH activity was not significantly greater than that obtained in three other Type I patients who had low PHLA values, as obtained with Ediol, and the anomalous results in L.W. cannot be explained at present. In the light of present experience, however, the determinations of PHLA should include measurements made with a pure triglyceride substrate. The suggestion that the rate-limiting defect in Type I hyperlipoproteinemia concerns the initial hydrolysis of one ester linkage is being further investigated.

We would like to thank Miss Nanci Briggs for her valuable technical assistance.

Manuscript received 28 October 1968; accepted 27 January 1969.

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