Post-Heparin Lipolytic Activity in Diabetic Patients with a History of Mixed Hyperlipemia

Relative Rates Against Artificial Substrates and Human Chylomicrons

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SUMMARY

The mechanism responsible for the accumulation of chylomicrons in mixed hyperlipoproteinemia (Type 5) is not known. Assays of the plasma-clearing factor, lipoprotein lipase, with artificially prepared substrates have been normal or only mildly depressed in this condition. The recent report of a patient in whom there was marked depression of activity against native chylomicrons despite normal activity against an artificial substrate suggested, however, that there might exist a qualitative defect in the function of lipoprotein lipase undisclosed by assays with the artificial substrate in general use. Accordingly, the lipolytic activity of post-heparin plasma from five diabetic patients with mixed hyperlipoproteinemia was measured against two artificial substrates and two preparations of human chylomicrons. There was good correlation between activity against coconut oil and each of the three other substrates over a wide range of plasma glyceride concentrations and post-heparin lipolytic activities. The data support the conclusion that plasma post-heparin lipolytic activity in diabetic patients with mixed hyperlipoproteinemia is qualitatively normal. DIABETES *18*:562-66, August, 1969.

The mixed hyperlipemia characterized by the presence of chylomicrons and increased amounts of pre-beta lipoprotein on electrophoresis of fasting plasma (Type 5) is an etiologically heterogenous group.[1] The majority of patients with this disorder have been found to have coexisting diabetes mellitus and normal or mildly depressed plasma post-heparin lipolytic activity (PHLA) against artificially prepared substrates. Recently, a nondiabetic patient with Type 5 hyperlipoproteinemia has been described in whom plasma PHLA against chylomicrons was nearly absent although there was normal activity against emulsified coconut oil, the substrate in general use for the assay.[2] The purpose of the present study was to reexamine the assumption that PHLA against artificially prepared substrates is an adequate description of activity against human chylomicrons in Type 5 hyperlipoproteinemia associated with diabetes mellitus.

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MATERIALS AND METHODS

Patients

Healthy volunteers served as donors of normal post-heparin plasma and were recruited from the medical staff or were convalescing male patients free of diabetes and lipid disorders. The five diabetic patients in the study had gross plasma lactescence with chylomicronemia and pre-beta lipoproteinemia initially in the course of their illness (table 1). Four of the five patients had maturity onset diabetes, rarely displayed more than trace ketonuria, and ultimately had good control of diabetes with diet alone (patients No. 2, 3, 4) or oral agents (patient No. 5). Patient No. 1 had typical juvenile onset diabetes and suffered two episodes of ketoacidosis related to acute infections in the year prior to study (table 1). At the time of this study, two patients (No. 3 and 4) were in relapse with chylomicronemia and increased pre-beta lipoprotein (Type 5) and three (No. 1, 2, and 5) were in remission with increased pre-beta lipoprotein alone (Type 4). Thus, the extremes in the natural history of Type 5 hyperlipoproteinemia are represented. The initial absolute values for PHLA were obtained with several assay methods,[3-5] and tended to be low when compared with a series of values for normals obtained in this laboratory. No systematic attempt was made to examine the effect of treatment on PHLA.

Laboratory methods

Electrophoresis of plasma was performed in agarose gel by a modification of the method of Noble.[6] Serum glyceride concentration was measured by the method of Kessler and Lederer.[7] PHLA was determined on sam-

TABLE 1 (*continued on page 564*)

Clinical and laboratory findings in the diabetic patients in this study

Patient	Age/sex	Diabetes: onset/duration	P.I.*	Complications	Treatment
No. 1	22 F	Juvenile 19 yr.	13.11	Retinopathy Albuminuria Infections	Diet Insulin
No. 2	56 M	Maturity 6 yr.	10.62	Myocardial infarction (at age 39)	Diet
No. 3	52 F	Maturity 1 yr.	11.46	Vascular insufficiency Eruptive xanthomatosis	Diet Clofibrate
No. 4	53 M	Maturity 3 mo.	12.28	Alcoholism Lipemia retinalis	Diet
No. 5	53 F	Maturity 1 yr.	12.97	Eruptive xanthomatosis	Diet Tolbutamide

*P.I. = Ponderal index: $\frac{\text{height (inches)}}{\sqrt[3]{\text{weight (pounds)}}}$.

†Sample obtained at time of maximum hyperglyceridemia.

‡Values obtained by the method of D. V. Datta (mean normal 28.3 ± 11.8 μEq./ml./hr.).[3] The remaining PHLA values in this table were determined by the method of Muir (mean normal 6.50 ± 1.75 μEq./ml./hr.).[4]

ples obtained ten minutes after the intravenous injection of sodium heparin, 10 units/kg. Four substrate preparations were used. Emulsified soybean oil* and coconut oil† were diluted to a glyceride concentration of approximately 50 mg./ml. with 0.85 per cent sodium chloride. Chylomicrons were obtained from thoracic fluid of a normolipemic subject with traumatic chylothorax and from the plasma of a hyperlipemic patient (No. 4 in this study) with fasting glycerides of 5,480 mg./100 ml. The chylomicrons were separated by ultracentrifugation at 0.8×10^6 g × min. and were washed in 1.1 per cent sodium chloride. Assays were performed by a modification of the method of Frederickson, Ono, and Davis.[5] Activity was expressed as μEq. of free fatty acid liberated/ml. of plasma/hr. Since several of the plasmas were assayed on more than one occasion after refreezing and rethawing, maximal lipolytic rates cannot be derived from the data presented here. Hyperlipemic plasmas were cleared by ultracentrifugation prior to assay with use of a #40 angle head motor and at 20° C. temperature since the contained particulate glyceride was a potential competing substrate. Tests of significance (Student's *t* test), regression lines, and correlation coefficients were calculated with the Mathatron 4280 computer.

*Intralipid. Vitrum, Stockholm, Sweden

†Lipostrate-CB ("Ediol"). Calbiochem, Los Angeles, California

RESULTS

The ratios of lipolytic activity against soybean oil and chylomicrons to the activity against coconut oil emulsion are shown in table 2. There were no significant differences in the relative lipolytic activities against soybean oil emulsion or the two chylomicron preparations. There was a high degree of correlation between activity against coconut oil and each of the three other substrates over a wide range of plasma glyceride concentrations and PHLA (figure 1). Fasting plasma from the hyperlipemic patients obtained before heparin was given did not inhibit plasma PHLA from a normolipemic donor (figure 2). The modest increase in activity observed in the latter experiment resulted from

TABLE 2

Relative post-heparin lipolytic activity against different substrates in normals and in diabetics with mixed hyperlipemia

Substrate*	$\frac{\text{Activity against substrate}}{\text{Activity against coconut oil}}$ ± S.D.M.	
	Normal subjects (five)	Hyperlipemic patients (five)
Soybean oil	0.70±0.04	0.66±0.24
Chylomicrons normolipemic donor	0.55±0.15	0.60±0.06
Chylomicrons hyperlipemic donor	0.70±0.20	0.74±0.16

*Final substrate concentrations (mg./ml.): coconut oil, 26.6; soybean oil, 24.6; chylomicrons, normolipemic donor, 6.5; chylomicrons, hyperlipemic donor, 19.1.

TABLE 1 (*continued from page 563*)

Clinical and laboratory findings in the diabetic patients in this study

Patient	Serum cholesterol mg./100 ml. maximum	minimum	Serum glycerides mg./100 ml. maximum	minimum	at study	Post-heparin μEq./ml./hr.	Lipolytic activity per cent normal
No. 1	400	—	4,502	456	456	1.71	26.3
No. 2	1,170	255	Creamy plasma	296	296	12.2	188
No. 3	530	196	7,050	330	4,750	6.70†‡ 6.54	23.7†‡ 100.6
No. 4	600	176	6,040	91.5	5,480	2.86† 10.4	44.0† 160
No. 5	910	201	3,371	174	233	7.08†‡ 5.56	25.0†‡ 85.5

*P.I. = Ponderal index: $\frac{\text{height (inches)}}{\sqrt[3]{\text{weight (pounds)}}}$.

†Sample obtained at time of maximum hyperglyceridemia.

‡Values obtained by the method of D. V. Datta (mean normal 28.3 ± 11.8 μEq./ml./hr.).[3] The remaining PHLA values in this table were determined by the method of Muir (mean normal 6.50 ± 1.75 μEq./ml./hr.).[4]

stimulation by the two fasting plasmas with the highest glyceride concentrations and was attributed to partial correction of suboptimal substrate concentrations in the assay. There was no increase in activity of post-heparin plasma from the hyperlipemic patients when normal donor plasma was added (99.9 ± 11.7 per cent of control).

DISCUSSION

In early studies with lipoprotein lipase, investigators found similar rates of hydrolysis for chylomicrons and for artificially prepared fat emulsions.[8,9] In many subsequent studies of PHLA in disease states, workers have assumed that normal rates of hydrolysis measured with artificial substrate preparations implied normal rates for native chylomicrons. The recent report of a qualitative defect in PHLA in which normal rates were found with a coconut oil emulsion but in which chylomicrons were resistant to hydrolysis[2] prompted our re-examination of this assumption in Type 5 hyperlipoproteinemia, a disease in which PHLA measured with coconut oil emulsions has been normal or only mildy depressed.[1] A selective inability to hydrolyze chylomicrons should be readily apparent when the ratios of rates of hydrolysis of chylomicrons to artificially prepared substrate are compared with normal. The data presented in this study, however, are strong evidence against the hypothesis that there is a selective defect in the hydrolysis of chylomicrons by PHLA from patients with Type 5 hyperlipoproteinemia and diabetes since the relative rates did not differ significantly from normal.

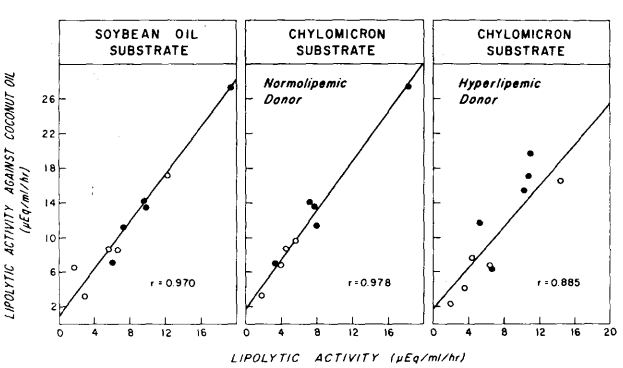

FIG. 1. Plasma PHLA against soybean oil and human chylomicrons versus activity against coconut oil in normolipemic (•) and hyperlipemic (o) individuals.

The mechanisms responsible for the accumulation and persistence of chylomicrons in fasting plasma from certain diabetic patients are only partly understood. Insulin lack appears to lead to a decrease in PHLA in man and laboratory animals[10-12] and may result in the accumulation of particulate lipid in the plasma. Insulin treatment has been followed by prompt increases in PHLA and decreases in plasma glyceride concentration. This study was neither designed nor intended to test that point although PHLA in Patients 3, 4, and 5 was initially low and increased after treatment.

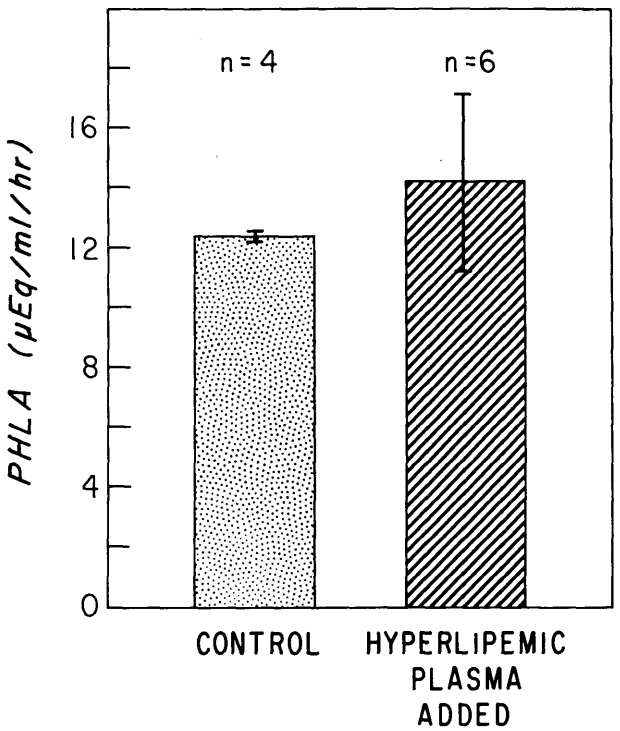

FIG. 2. The effect of addition of cleared pre-heparin plasma from hyperlipemic individuals on the lipolytic activity (± S.D.M.) of normal post-heparin plasma using human chylomicrons as substrate.

A second mechanism for the accumulation of chylomicrons has been suggested for Type 5 hyperlipoproteinemia. Chylomicronemia has been attributed to delayed removal on the basis of competition for common removal sites between endogenous and alimentary particles.[1] The delay in disappearance of intravenously administered chylomicrons demonstrated in the presence of endogenous hyperlipemia is indirect evidence favoring that proposal.[13]

Inhibitors of PHLA have been reported in acute pancreatitis.[14] In this study, there was no inhibition of normal PHLA by plasma from the hyperlipemic patients nor was there stimulation of plasma PHLA from the hyperlipemic patients by the addition of normal plasma. Thus, in Type 5 hyperlipoproteinemia associated with diabetes, both chylomicrons and PHLA appear qualitatively normal and there are neither missing cofactors supplied by normal plasma nor circulating inhibitors of PHLA against human chylomicrons.

Several potential limitations are implicit in the method for lipoprotein lipase assay by PHLA. First, commercially available triglyceride emulsions are mixtures and may contain phospholipid, monoglyceride, preservatives, and detergents. The close correlation of PHLA values in this study for the two artificially prepared substrates which are quite dissimilar in monoglyceride and phospholipid content suggests that the activity being measured is primarily lipoprotein lipase and not monoglyceridase or heparin-released phospholipase.[15,16]

Also, there is evidence that some tissue lipoprotein lipase is unavailable for release by heparin,[17] that metabolic changes may have diametrically opposite effects on lipoprotein lipases in different tissues,[18] and indirect evidence that apparent PHLA deficiency may be a phenomenon related to the dose of heparin administered.[11,19]

In view of the potential limitations, conclusions regarding the integrity of the lipoprotein lipase system based on plasma PHLA may be misleading. Nevertheless, tissue lipoprotein lipase and plasma PHLA have appeared to undergo parallel changes in states of insulin lack,[11,12] and in familial deficiency of lipoprotein lipase.[9,20] Indeed, experiments with the isolated perfused rat heart[17] have suggested that the heparin-released enzyme may be functionally more important than the lipoprotein lipase which is fixed to tissues in the uptake and utilization of triglyceride fatty acids. Although no qualitative defect in plasma PHLA was present in the five diabetic patients in this study, these data do not exclude such a defect in tissue lipoprotein lipase nor in patients with Type 5 hyperlipoproteinemia associated with other diseases.

ACKNOWLEDGMENT

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Manganese and Glucose Tolerance

(*Continued from page 561*)

19:348, 1961). Trivalent chromium is necessary for optimal utilization of glucose and the action of insulin at cellular level. Chromium III is, by itself, not a hypoglycemic agent and is active only in the presence of insulin (W. H. Glinsmann, F. J. Feldman, and W. Mertz, Science *152*:1243, 1966). In adult diabetics, occasional improvements in glucose tolerance have been reported following oral supplements of trivalent chromium. There has been much speculation over the role played by zinc in the action of insulin and glucagon, and this trace element has also been implicated in the regulation of blood glucose levels. Low blood levels of other trace elements such as silicon, aluminum, titanium, and copper have been found in diabetic patients by Kosenko...

Although adequate manganese in the diet may correct the impaired glucose tolerance of manganese deficient guinea pigs and blood levels of manganese may be relatively low in human diabetes, there is no evidence, apart from one isolated instance, that administration of manganese improves glucose tolerance in man. Since manganese is omnipresent in food and water, there is no reason at this time to believe that a dietary deficiency of this trace element occurs in man.

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