Lipoprotein Lipase Activity in Patients with Diabetes Mellitus, with and without Hyperlipemia

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SUMMARY

No detectable abnormalities or changes in the post heparin plasma lipoprotein lipase (LPL) activity were found in fifteen "insulin independent" (stable) or sixteen "insulin dependent" (labile) diabetics, with or without hyperlipemia, in four patients with ketoacidosis and hyperlipemia both during the acute phase and after recovery, in two subjects with insulin induced hypoglycemia, and in two patients with gross hyperlipemia and diabetes during marked fluctuations in lipid levels in one, and during correction of the hyperlipemia in the other.

Abnormalities in the LPL system, as reflected in the post heparin plasma LPL activity, appear to play a minor role, if any, in the pathogenesis of diabetic hyperlipemia in man. DIABETES *15*:565-70, August, 1966.

Alterations in lipoprotein lipase* (LPL) activity have been described in various conditions associated with hyperlipemia in man.[1-3] Studies of LPL in diabetes mellitus have mentioned either decreased or unchanged activity.[4,5] Investigations in untreated diabetic rats have documented considerable increases of LPL activity in heart muscle and decreases in adipose tissue which were correctable by insulin.[6,7] In vitro studies have suggested the need for glucose and insulin in maintenance of normal LPL activity in adipose tissue.[8] These considerations have led to a reevaluation of the activity of LPL in human patients with diabetes. Various types of diabetes, both controlled and decompensated, with and without hyperlipemia, were studied in an effort to detect alterations in LPL activity which might be implicated in the pathogenesis of diabetic hyperlipemia in man.

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*The term lipoprotein lipase is used synonymously with "clearing factor" and "clearing factor lipase." In this study, unless otherwise specified, it is meant to denote "post heparin plasma clearing activity" as distinguished from similar enzymatic activity in tissues.

METHODS

Two groups of patients were selected from the Boston City Hospital Diabetic Clinic. Group 1 consisted of fifteen patients classified as having "insulin independent" (stable) diabetes. Their ages ranged from thirty-six to seventy-five years (median age, fifty-two). Eight of these patients took 1 to 2 gm. of tolbutamide and seven took 15 to 30 U. of insulin daily. Fasting glucose concentrations ranged from 88 to 160 mg. per 100 ml. (median, 115 mg. per 100 ml.) (table 1). Two patients had fasting glycosuria. Group 2 contained sixteen patients with "insulin dependent" (labile) diabetes, ten to forty-two years of age (median age, twenty-two). All took 30 to 80 U. of insulin daily. Fasting glucose concentrations were 88 to 320 mg. per 100 ml. (median, 140 mg. per 100 ml.) (table 1). Five had fasting glycosuria. Both groups were evaluated before taking their daily insulin or tolbutamide.

Four patients were studied while in acute diabetic ketoacidosis. Studies were repeated in three of these after their recovery. The clinical data of these patients are presented in table 2. After recovery, all patients were fasted for twelve hours or had saline running intravenously for six hours prior to evaluations.

In one normal subject and in one subject with diabetic hyperlipemia (Patient B, below), LPL activity was studied during varying times after heparin injection on five separate days (figure 1).

In two other normal subjects, LPL activity was determined on a control day and subsequently at the nadir of the hypoglycemic response following the the intravenous injection of 0.12 U. per kg. of glucagon-free insulin.*

Two patients were selected for prolonged observation on the ward of the Thorndike Clinical Research Center because of gross hyperlipemia and diabetes. Patient A (table 3, A) was a thirty-six-year-old white female who had diabetes discovered seven years prior to admission, had fasting serum lactescence noticed two years

*Kindly supplied by Eli Lilly and Co., Indianapolis, Indiana, through the courtesy of Dr. W. R. Kirtley.

TABLE 1

Patient No.	Plasma triglyceride (mg. per 100 ml.)	LPL activity (μEq./min./ml.)	Plasma glucose (mg. per 100 ml.)
Group 1 "insulin independent" (stable)			
1	155	0.458	110
2	111	0.468	110
3	375	0.400	136
4	127	0.254	112
5	171	0.321	115
6	175	0.336	132
7	183	0.420	155
8	176	0.303	88
9	179	0.331	105
10	129	0.360	92
11	160	0.309	106
12	162	0.384	118
13	100	0.412	140
14	243	0.310	158
15	650	0.376	160
Mean ± S.E.	206.4 ± 35.9	0.364 ± 0.016	122.5 ± 6.0
Group 2 "insulin dependent" (labile)			
1	137	0.420	140
2	54	0.382	132
3	76	0.374	110
4	200	0.347	115
5	236	0.302	146
6	74	0.334	115
7	117	0.214	182
8	86	0.395	88
9	93	0.222	170
10	178	0.311	110
11	212	0.254	142
12	220	0.337	122
13	69	0.336	162
14	56	0.628	215
15	120	0.368	320
16	117	0.254	135
Mean ± S.E.	127.8 ± 15	0.342 ± 0.025	150.2 ± 13.8
Normals (15) Mean ± S.E.	109.5 ± 8.9	0.415 ± 0.027	

prior to admission (whereas it had been documented to be absent on prior occasions) and had, during the year before admission, poor control of her carbohydrate metabolism, persistent hyperlipemia and several episodes of abdominal pain consistent with pancreatitis. Hyperlipemia had preceded any attacks of abdominal pain by at least one year. Patient B (table 3, B) was a fifty-year-old white male who had sustained a myocardial infarction six months prior to admission at which time a diagnosis of diabetes was established. Within three months, insulin treatment was begun, but in spite of increasing dosage, poor control persisted and marked hyperlipemia developed. On the metabolic ward, these patients were placed on reducing diets. Insulin was carefully withdrawn. After several weeks of observation, both were given tolbutamide for control of fasting blood sugar.

The activity of LPL in all patients was compared with that of a group of fifteen normal subjects, twenty-two to eighty-six years of age (median age, twenty-four) who had no known disease or abnormality of lipid metabolism. The upper limit of triglyceride concentration considered normal was established by the upper value of concentration found in twenty-five normal subjects (which included the above fifteen).

Lipoprotein lipase activity was determined by measuring the rate of free fatty acid (FFA) release from a buffered artificial coconut oil emulsion,* when incubated with citrated plasma obtained fifteen minutes (except

TABLE 2

Patient No.	Sex	Age	Cerebral function	Time after insulin Rx	Plasma glucose (mg. per 100 ml.)	Plasma ketones	Plasma carbon dioxide content (mEq./L.)	Plasma free fatty acid concentration (μEq./L.)	Plasma triglyceride concentration (mg. per 100 ml.)	LPL activity (μEq./min./ml.)
1	Male	48								
Acute			Light coma	½ hour	650	4+ (1-4 dil.)	10	1,500	420	0.364
Follow-up			Alert	2 days	140	0	26	320	82	0.417
2	Female	81								
Acute			Deep coma	Prior to Rx	815	4+ (1-4 dil.)	8	1,005	432	0.386
Follow-up			Drowsy	2 days	180	±(undil.)	22	426	169	0.372
3	Male	22								
Acute			Light coma	½ hour	650	4+ (1-4 dil.)	12	960	364	0.288
Follow-up			Alert	24 hours	145	0	28	425	150	0.287
4	Male	36								
Acute			Confused	1 hour	380	4+ (1-4 dil.)	12	1,135	235	0.305
No follow-up										

as noted) after the intravenous injection of 0.5 mg. per kg. of heparin. Details of this method are given in a previous communication.[3]†

All blood samples were drawn after twelve hours of fasting and were transferred to iced tubes immediately. The plasma was separated and deep frozen at —18° C. within two hours. Glucose[9] and triglyceride[10] concentrations were measured in heparinized plasma, and glucose was measured in urine. FFA were determined in heparinized plasma.[11,12]

RESULTS

The LPL activity in the normal subjects was 0.415 μEq./min./ml. ± 0.027 S.E. (range—0.212-0.566 μEq./min./ml.) (table 1). The highest value of triglyceride concentration found in normal subjects was 177 mg. per 100 ml.

Three of the patients in Group 1 had triglyceride concentrations of 375, 243, and 650 mg. per 100 ml. The latter two patients had the highest fasting glucose concentrations (158 and 160 mg. per 100 ml., respec-

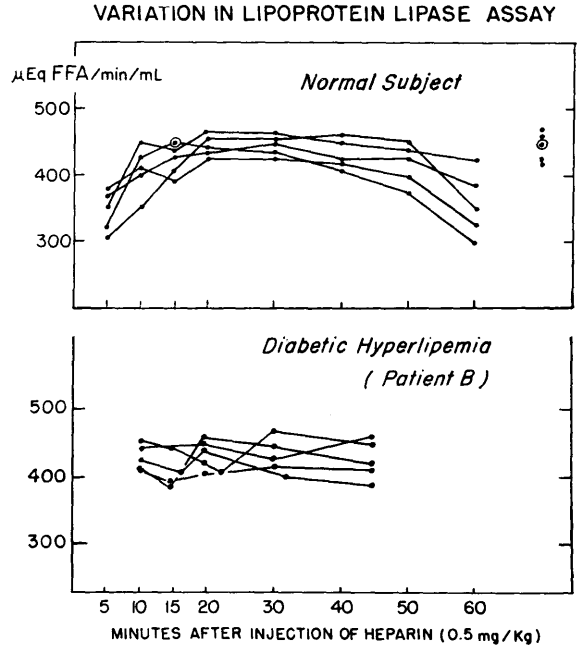

FIG. 1. The activities of lipoprotein lipase for varying times after the injection of 0.5 mg. per kg. heparin intravenously in a normal subject and in Patient B with diabetic hyperlipemia. Each line represents a different day; three on consecutive days and two, one and two weeks later. The five dots on the right (upper graph) represent repeated determinations of LPL activity on the same single sample indicated by the circled dot.

*Ediol, Schenlabs, Pharmaceuticals, New York, N.Y.

† One modification consisted of buffering the albumin to *p*H 8.5 with dilute NaOH prior to adding it to the incubation mixture.

TABLE 3

Patient	Treatment	Time after admission	Diet	Weight (pounds)	Fasting blood glucose concentration (mg. per 100 ml.)	24-hour urine glucose (grams)	Triglyceride concentration (mg. per 100 ml.)	LPL activity (μEq./min./ml.)
A	60 U.							
	insulin/day	on admission	Ward*	165	220	20	2,050	0.355
	no insulin	1 week	Ward*	158	158	35	1,150	0.372
	no insulin	2 weeks	Ward*	153	210	35	820	0.320
	no insulin	4 weeks	Ward*	148	190	42	360	0.342
	tolbutamide	5 weeks	Ward*	144	196	38	220	0.365
	tolbutamide	6 weeks	Ward*	142	130	20	170	0.348
B	45 U.							
	insulin/day	on admission	Ward†	210	155		2,600	0.402
	no insulin	10 days	Ward†	202	203	not	1,050	0.422
	no insulin	17 days	Ward†	200	222	done	820	0.415
	no insulin	on pass (10 days)	Uncontrolled	—	—		—	—
	no insulin	27 days	Ward	208	180		3,450	0.398
	no insulin	30 days	Ward	206	192		3,600	0.440
	no insulin	33 days	Ward	204	188		2,200	0.452
	no insulin	on pass (10 days)	Uncontrolled	—	—		—	—
	tolbutamide	38 days	Ward	206	192		3,400	0.441
	tolbutamide	42 days	Ward	203	140		1,600	0.422
	tolbutamide	56 days	Ward	196	130		720	0.418

*Calories —1823, Protein —70 gm., Carbohydrate —190 gm., Fat —87 gm.
†Calories —2386, Protein —100 gm., Carbohydrate —240 gm., Fat —114 gm.

tively). All patients in Group 1 had normal activities of LPL (Mean = 0.364 μEq./min./ml. ± 0.016 S.E.). The patients with hyperlipemia had LPL values of 0.400, 0.310, and 0.376 μEq./min./ml., respectively, not at either end of the distribution of values (table 1).

Four patients in Group 2 had triglyceride concentrations of 200, 236, 212, and 220 mg. per 100 ml. Their fasting plasma glucose concentrations were, respectively, 115, 146, 142, and 122 mg. per 100 ml. All patients in Group 2 had normal LPL activity (Mean = 0.342 μEq./min./ml. ± 0.025 S.E.). The patients with hyperlipemia had activities of 0.347, 0.302, 0.254, and 0.337 μEq./min./ml., respectively, values not different from others of the group (table 1).

The variation encountered in the assay of LPL is demonstrated in figure 1. The activity was reproducible to within approximately 0.060 μEq./min./ml. whether determinations were repeated on a single sample, on different days, or at times after heparin injection varying from fifteen to forty minutes. Similar variations in the LPL assay were noted in one normal subject and in Patient B with diabetic hyperlipemia.

Results in the four patients with diabetic ketoacidosis are presented in table 2. When first seen, all were clearly ketotic and acidotic, had markedly elevated FFA, and had elevated plasma triglyceride concentrations. Initially, as well as on follow up when the triglyceride and FFA concentrations had returned to normal, the activity of LPL was within the normal range. There were no detectable changes in activity in any individual patient after the ketoacidosis and hyperlipemia were corrected.

The two subjects in whom LPL activity was measured before and during insulin hypoglycemia had, respectively, before insulin, glucose concentrations of 86 and 92 mg. per 100 ml., and activities of LPL of 0.412 and 0.320 μEq./min./ml. After insulin and during hypoglycemia, the values were, respectively, glucose, 36 and 42 mg. per 100 ml. and LPL, 0.420 and 0.336 μEq./min./ml.

Both patients with gross hyperlipemia had decreasing triglyceride concentrations when their dietary intake was restricted and they lost weight (table 3). Patient B had increases in triglyceride concentration which correlated on two occasions with unrestricted eating (and drinking) while he was on pass. Both patients showed considerable fluctuation in the state of their glucose metabolism, reflected in changes in their fasting glucose concentrations and especially in the urinary glucose excretion in Patient A. During these changes in lipid and carbohydrate metabolism, repeated LPL determinations in both showed no detectable alterations in activity.

DISCUSSION

The present study was designed to evaluate LPL activity in various types of diabetes, compensated and decompensated, with and without hyperlipemia. The data indicate no detectable alteration from normal LPL activity in juvenile or adult forms, whether or not hyperlipemia is present. Neither hypoglycemia, hyperglycemia, ketoacidosis, nor hyperlipemia were associated with any abnormalities in LPL. In particular, it is evident in the two patients with gross diabetic hyperlipemia, that marked fluctuations in plasma lipid concentrations, including a return to normal, can occur with no changes in lipoprotein lipase in plasma.

An increased activity of LPL in heart muscle combined with decreased activity in adipose tissue has been demonstrated in alloxan diabetic rats.[7] In addition, increases in blood lipids, occurring upon withdrawal of insulin in alloxan diabetic rats, were correlated with decreases in adipose LPL activity.[6] All these alterations were correctable by insulin. These studies strongly imply a relation between the LPL system and hyperlipemia in diabetes. On the other hand, because of species variation in the behavior of LPL systems, caution is warranted in relating these animal observations to the human diabetic. The finding of normal plasma LPL in human diabetics may not reflect changes in tissue LPL. In addition the association of tissue LPL activity with a physiological role in fat transport is still indefinite.

Although low plasma LPL activity as described in some hyperlipemic states had focused attention on defects in the clearance of fat in these conditions, the present data indicate that the LPL system is not associated with the pathogenesis of diabetic hyperlipemia in the same manner as it may be in these other hyperlipemic states.

One is inclined at present, therefore, to attribute the changes in lipid metabolism seen in diabetes to control mechanisms other than defective clearance through a deranged LPL system. Primary defects in adipose tissue have been considered whereby continuous mobilization of abnormally large amounts of FFA causes the liver to deflect increased amounts of fatty acids to triglycerides, which are secreted into the plasma, resulting in hyperlipemia.[13] Although this concept is appealing and is often quoted, there is little experimental evidence for it. On the contrary, two recent studies have demonstrated reciprocal changes in FFA mobilization and the concentration of plasma triglycerides.[14,15]

A relationship between the diabetic state and "carbohydrate induced" lipemia has been reported.[16,17] Normal postheparin plasma LPL activity has been found in "carbohydrate induced" lipemia.[18] Diets high in carbohydrate result in greater incorporation of the C-14 from labeled glucose into plasma triglycerides.[19] These results suggest that the excess lipid in "carbohydrate induced" lipemia (and perhaps in some types of diabetic hyperlipemia) may be formed from dietary carbohydrate. However, no dominant mechanism has yet been identified in the pathogenesis of diabetic hyperlipemia in man.

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Relationships Between Alcohol, Heart Disease, and Liver Disease

Alcohol metabolism has recently come under close scrutiny. N. Tygstrup, K. Winkler, and F. Lundquist (*J. Clin. Invest.* *44*:817, 1965) reviewed the current knowledge of ethanol utilization. Normally alcohol is metabolized at a rather constant rate by the liver of both animals and man. This rate can be altered to a slight extent by various substances including glucose, pyruvate, amino acids, dinitrophenol, insulin, or thyroid hormone. The authors cited the work of two groups, K. Stuhlfauth and H. Neumair (*Med. Klin.* *46*:591, 1951) and A. Pletscher, A. Bernstein, and H. Staub (*Helv. Physiol. Pharmacol. Acta* *10*:74, 1952), indicating that large increases in the rate of ethanol metabolism accompanied administration of fructose. They evaluated this fructose effect in sixteen subjects and offered a possible explanation. Elaborate studies were performed employing a double lumen catheter introduced through the antecubital vein, the distal opening being placed in one of the right hepatic veins and the proximal opening in the upper part of the right atrium. This permitted ethanol, fructose, or both to be infused into the right atrium at a precise rate and samples of blood to be withdrawn from the hepatic vein. Simultaneous samples of blood were drawn from a peripheral artery. This permitted an evaluation of "clearance" of various metabolites by the liver.

Infusion of fructose alone resulted in a continuous rise in lactate. With ethanol and fructose the hepatic venous concentration of ethanol fell while that of lactate rose. Total oxygen consumption and carbon dioxide formation both increased when fructose was infused.

Hepatic elimination of alcohol is accomplished by oxidation to acetaldehyde by alcohol dehydrogenase, which is dependent upon nicotinamide adenine dinucleotide (NAD). Further dehydrogenation to free acetate is accomplished by aldehyde dehydrogenase, which is also dependent on NAD. The authors reasoned that, if the limiting step in alcohol metabolism is dissociation of the ADH-NADH complex, then the limit of the liver's capacity to metabolize alcohol may depend on its ability to reoxidize the NADH thus formed.

Although this concept has been questioned by some, the authors suggested that an estimate of the NADH/NAD ratio can be made by measuring certain metabolites which form parts of redox systems involving enzymes dependent on NADH, e.g., the beta-hydroxybutyrate/acetoacetate ratio. Furthermore the ratio of lactate/pyruvate is assumed to reflect the ratio of free NADH/NAD in hepatic cells under conditions of high pyruvate concentration.

The authors found that the NADH/NAD ratio was ten times higher during ethanol-fructose infusion than during infusion of fructose alone. Addition of fructose

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