Acute Insulin Withdrawal and the Regulation of Plasma Triglyceride Removal in Diabetic Subjects

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SUMMARY

The effects of acute insulin deprivation on the enzyme lipoprotein lipase (LPL) and on triglyceride (TG) removal from plasma in man are unknown. To assess the role of insulin availability on TG removal, plasma TG concentration and postheparin lipolytic activity (PHLA)—an indirect means of quantitating LPL—were measured in seven insulin-dependent diabetics before and forty-eight hours after insulin withdrawal. The TG elevation ($p < .01$) and diminished PHLA levels ($p < .01$) observed in all subjects were consistent with impaired TG removal. The delayed disappearance of C-14-labeled TG after insulin deprivation ($p < .01$) demonstrated in two subjects further suggests that insulin is required for maintaining normal LPL and TG removal. DIABETES *17*:127-32, March, 1968.

Lipoprotein lipase (LPL) is a tissue enzyme believed to play an important role in the removal of triglyceride from plasma.[1] This enzyme may be estimated indirectly by the measurement of lipolytic activity in plasma after the intravenous administration of heparin (PHLA). Decreased PHLA levels associated with markedly elevated plasma triglyceride concentration (TG) have recently been demonstrated in symptomatic diabetics with chronic insulin insufficiency.[2] To determine whether acute insulin deprivation also affected LPL and caused impaired triglyceride removal, PHLA was measured before and after a brief period of insulin withdrawal in seven insulin-dependent diabetics under carefully controlled metabolic conditions.

METHODS

Seven volunteers, aged nineteen to twenty-seven years, with stable, uncomplicated diabetes mellitus but otherwise apparently healthy, were hospitalized and studied on a metabolic ward. The duration of required insulin treatment ranged from three to twenty-one years. Blood specimens were obtained the morning after an overnight fast, both before and after a forty-eight-hour period of insulin withdrawal. Blood glucose, electrolytes, free fatty acids, triglyceride, and total protein concentration were determined by previously described methods.[2] Before insulin withdrawal, each patient was given subcutaneously one half his normal daily maintenance insulin dose as crystalline insulin. Thirty minutes later PHLA was measured by the method of Fredrickson,[3] after the injection of 380 U. of heparin per square meter of body surface area. Since several patients showed more than one activity peak at different times after heparin (table 1), the entire thirty-five-minute lipolytic response (the area circumscribed by the time-response curve) was quantitated by planimetry. Insulin was then withheld for the next forty-eight hours from all subjects except F.L., who required insulin treatment after thirty-two hours. PHLA and TG levels again were measured after insulin withdrawal. During this interval, all patients were carefully observed, and blood glucose, electrolytes, and serum acetone (Denco, acetone test powder) were determined every twelve hours. All patients were allowed free access to food and liquid and encouraged to eat and drink liberally. Urinary electrolyte and water losses were replaced by mouth and intravenously.

To test whether TG removal was influenced by acute insulin deprivation, plasma obtained from two subjects (L.S., F.L.) before and twenty-four hours after insulin withdrawal was labeled in vitro with C-14 tripalmitin (Nuclear-Chicago) by previously described methods.[4] This labeled plasma was layered under isotonic saline (1:1), and centrifuged at 3×10^6 G minutes in a Spinco SW-39 swinging bucket rotor under sterile conditions. The layer of fat particles present at the top of the tube after centrifugation corresponding in flotation rate to $Sf > 400$ was carefully aspirated and discarded. The lipoproteins harvested from the infranatant

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TABLE 1

Clinical data and metabolic changes before and after insulin withdrawal

Subject	Age (yrs.)	Duration insulin treatment (yrs.)		Fasting blood sugar (mg./100 ml.)	Fasting triglyceride (mg./100 ml.)	Fasting free fatty acid (μEq./L.)	Duration insulin withdrawal (hrs.)	Total protein (gm./100 ml.)	Carbon dioxide (mEq./L.)	Postheparin lipolytic activity (μEq. fatty acid/ml./min.) minutes 4	6	8	10	15	25	35	PHLA response (area units)
			Before*	124	40	476		7.1	22	.335	.351	.388	.475	.371	.413	.200	550
S.M	19	2	After	215	72	874	48	7.1	19	.219	.253	.369	.233	.227	.168	.126	309
J.W.	19	3		68	38	832		6.5	25	.411	.452	.476	.490	.438	.318	.265	577
				238	49	1,129	48	6.4	27	.328	.353	.309	.303	.253	.223	.142	372
D.H.	23	4		68	41	448		6.6	23	.125	.218	.387	.403	.210	.199	.043	308
				311	77	1,309	48	7.2	20	.208	.290	.263	.210	.196	.185	.118	272
C.S.†	27	6		49	99	934		6.0	28	.252	.225	.252	.210	.128	.059	.099	195
				320	373	1,013	48	6.5	17	.220	.222	.190	.175	.159	.087	.032	205
L.S.	22	8		113	45	754		5.7	22	.292	.327	.311	.338	.251	.140	.101	327
				478	60	2,913	48	7.1	7	.000	.013	.014	.023	.000	.010	.025	28
M.W.†	25	18		283	59	844		5.9	24	.654	.692	.497	.644	.491	.430	.271	714
				510	86	2,888	48	7.8	8	.263	.206	.199	.158	.166	.166	.110	257
F.L.	25	21		165	37	668		5.6	21	.346	.297	.441	.404	.349	.196	.155	410
				461	41	1,982	32	6.0	8	.150	.125	.154	.134	.108	.088	.039	139
								Mean: Before		.345	.366	.393	.424	.320	.251	.162	440
								After		.198	.209	.214	.177	.158	.132	.085	226

*Data before and after the indicated duration of insulin withdrawal.
†Patients treated only with oral fluids and electrolytes.

fraction (Sf < 400), which correspond in size predominantly to endogenous very low-density lipoprotein, were administered to each patient by rapid intravenous injection. The study was repeated in subject F.L. thirty-two hours, and subject L.S. forty-eight hours following insulin withdrawal. It was assumed that the labeled lipoproteins injected in the untreated state were similar to those injected in the treated state. Blood specimens were obtained at frequent intervals from three to sixty minutes after injection, and plasma radioactivity determined by liquid-scintillation counting. The slope of the decline of radioactivity, and half-times (T ½) were calculated from the linear portion of the log C-14 disappearance curve by regression analysis with use of a digital computer.

RESULTS

Measurements

Fasting plasma triglyceride concentrations (TG) in these subjects were within the range reported for both diabetic and nondiabetic young subjects.[5,6] TG increased in all subjects following insulin withdrawal and in one subject (C.S.) reached markedly elevated levels (figure 1, table 1). This uniform increase in TG levels was not significant by parametric statistical methods owing to the high variance but was significant ($p < .01$) by the Wilcoxon matched pairs test.[7] Thus TG levels increased in this group of insulin-dependent diabetics following a brief period of insulin deprivation.

This TG elevation following insulin withdrawal was associated with a significant decrease in PHLA. The means of the individual PHLA values after insulin deprivation were significantly reduced at 8, 10 ($p < .01$) and 15 min. ($p < .02$; figure 2). The total PHLA response determined by planimetry of the activity-time curve was also significantly reduced ($p < .01$; paired comparisons) (figure 3). However, PHLA was unchanged in the subject (C.S.) with the highest TG elevation (table 1). She was later found to have had a brief febrile illness before hospitalization during which her dietary intake and diabetic control were poor. Although her PHLA was unchanged after insulin withdrawal, her PHLA levels were significantly higher when she was later tested at a time when her diabetic control was optimal. In patient L.S., PHLA fell to almost undetectable levels during the development of ketoacidosis, but insulin restored PHLA to control levels twenty-four hours after treatment was instituted.

Delayed clearance of C-14 labeled plasma triglyceride in the two subjects so studied suggested that triglyceride

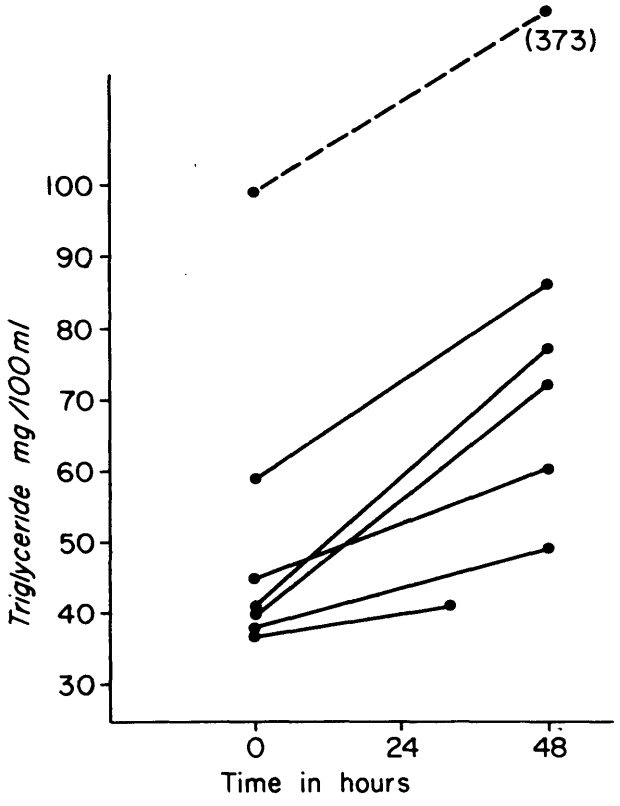

FIG. 1. Plasma triglyceride concentrations before and after insulin withdrawal in seven insulin-dependent diabetics.

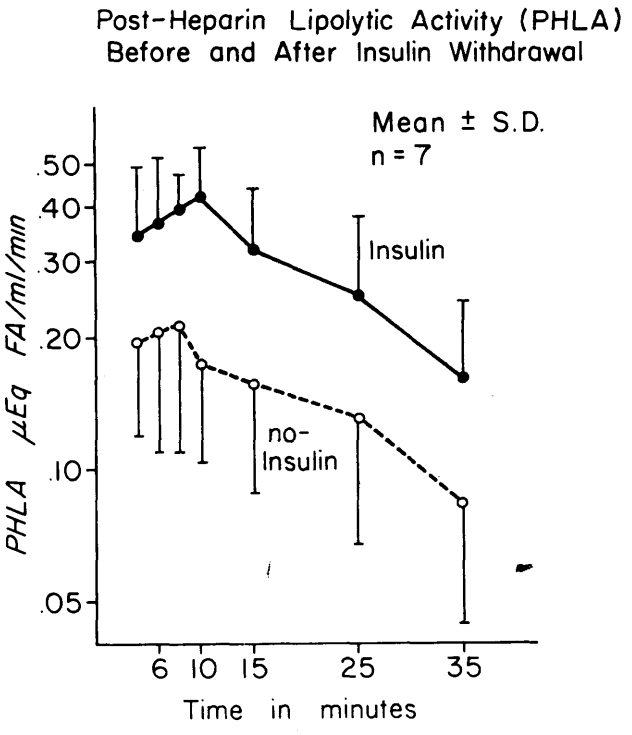

FIG. 2. Mean postheparin lipolytic activity (PHLA) before and after insulin withdrawal in seven insulin-dependent diabetics.

removal was impaired (figure 4). When one subject (F.L.) was studied in ketoacidosis after thirty-two hours without insulin, the T ½ had increased from 7.8 to 11.3 min. ($p < .01$). Similarly, in a second subject (L.S.) in ketoacidosis forty-eight hours after insulin withdrawal, the T ½ had increased from 6.4 to 18.8 min. ($p < .001$).

The fasting blood sugar and FFA levels rose in all subjects (table 1). The highest FFA levels were observed in the subjects who developed the most severe ketoacidosis.

Clinical observations

All subjects became symptomatic following insulin withdrawal. Thirst was a poor indicator of body hydration since the two subjects who received only oral fluid and electrolyte replacement developed hemoconcentration and ketosis (C.S. and M.W.) The three subjects who best tolerated the period of insulin withdrawal and showed no signs of volume depletion and ketosis had received vigorous parenteral fluid replacement. By this means, volume depletion in several patients was minimized, though not prevented, in spite of twenty-four-hour urine volumes ranging from 12-18 L. These sub-

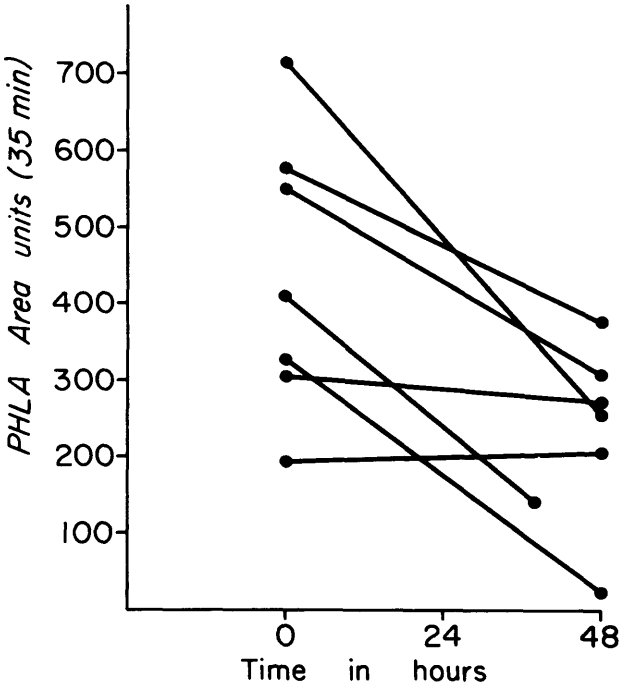

FIG. 3. Individual changes in postheparin lipolytic activity (PHLA) before and after insulin withdrawal in seven insulin-dependent diabetics.

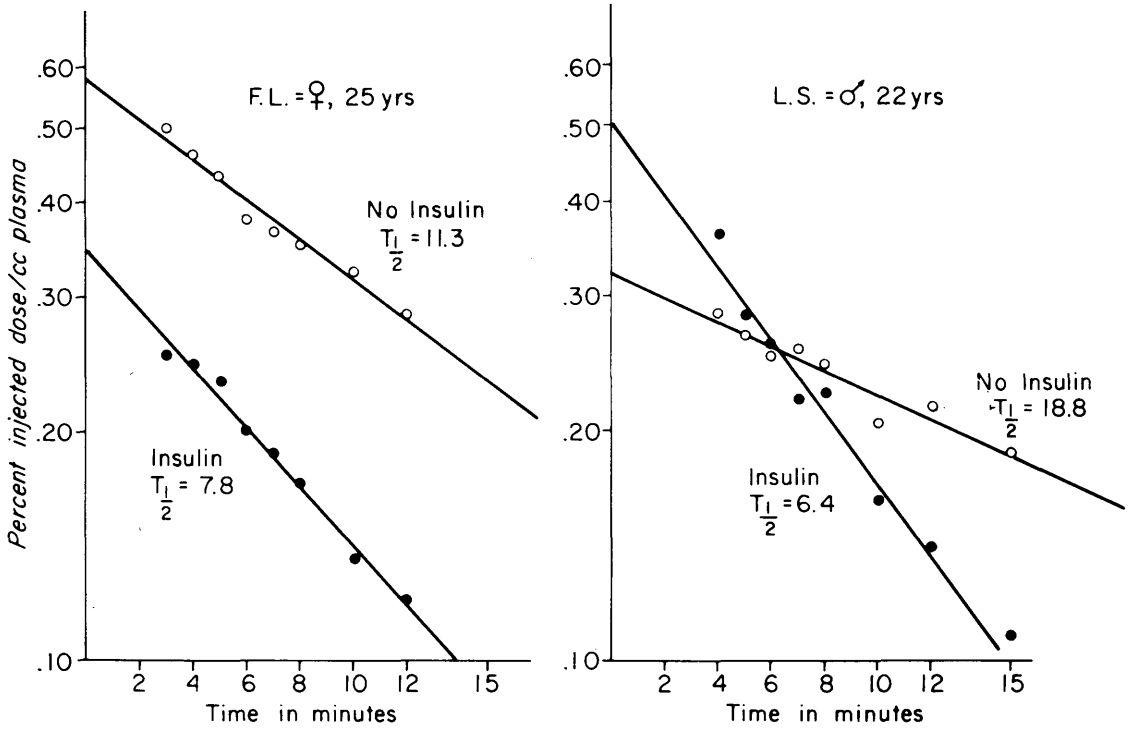

FIG. 4. Disappearance of C-14-labeled triglyceride before and after insulin withdrawal in two insulin-dependent diabetics.

jects also had a shorter duration of prior insulin treatment. Despite aggressive parenteral fluid repletion, two subjects, L.S., F.L., who had received insulin treatment for eight and twenty-one years, respectively, became severely ketoacidotic. Thus, development of ketoacidosis in this group of diabetic subjects related both to the duration of prior insulin treatment and to the adequacy of fluid and electrolyte replacement.

DISCUSSION

Results in this study illustrate that a brief period of insulin deprivation does not uniformly result in the development of ketoacidosis in insulin-dependent diabetics. In the patients in whom a normal plasma volume was maintained, residual pancreatic insulin secretion was apparently sufficient to suppress lipolysis in adipose tissue and prevent ketoacidosis. Since two subjects who developed ketoacidosis were severely dehydrated, reflex stimulation of the sympathetic nervous system secondary to reduced plasma volume may have been an important factor in the development of ketoacidosis, perhaps due to both stimulation of lipolysis[8] and inhibition of insulin release.[9]

In each subject, fasting plasma TG concentration was initially normal but rose after insulin withdrawal. This TG elevation could result from either increased endogenous TG synthesis exceeding the normal removal capacity, a primary removal defect causing the accumulation of both dietary and endogenous triglyceride, or a combination of these factors. Increased hepatic triglyceride production may not explain the TG elevation in these patients, since recent evidence in dogs[10] and rats[11] suggests that accelerated fatty acid mobilization which occurs in uncontrolled diabetes does not increase endogenous triglyceride synthesis and release but rather preferentially contributes to ketoacidosis. In this study, increased TG levels, associated with decreased PHLA and delayed disappearance of isotopically labeled triglyceride, are consistent with impaired removal.

The decline in PHLA observed after insulin withdrawal suggests that acute reduction in insulin availability promptly affects tissue LPL. A quantitative reduction in available enzyme is indicated by low activity at all times after heparin. However, the virtually parallel PHLA time curves before and after insulin suggest that there is no alteration in enzyme release or removal from plasma. The lowest PHLA responses after insulin withdrawal were observed in the three severely ketoacidotic subjects who had the longest duration of clinical diabetes and were likely to have had the least available

pancreatic insulin. The more recently treated diabetics also became symptomatic and developed decreased PHLA. Although the insulin levels in these subjects were sufficient to prevent the development of ketoacidosis, they were nonetheless inadequate to maintain normal carbohydrate and protein metabolism, including presumably LPL synthesis. Thus, insulin appears to closely regulate LPL.

This association between insulin and LPL is described in a recent report of decreased PHLA and hypertriglyceridemia in symptomatic diabetics with chronic insulin insufficiency. Insulin treatment in these diabetics promptly restored PHLA, triglyceride levels and presumably TG removal to normal.[2] Further experimental evidence that insulin influences the enzyme system important in TG removal are the observations that insulin administration restores to normal the decreased adipose tissue LPL[12] and PHLA[13] in alloxan-diabetic animals.

The results of this study appear to conflict with those of a recent report of normal PHLA in a few ketoacidotic diabetics.[14] On this basis, it was concluded that LPL did not significantly contribute to triglyceride elevation in diabetes. However, these studies may not be comparable for several reasons. In that report, some of the ketoacidotic patients tested already had received some insulin treatment prior to study, which may have obscured the effects of insulin lack on PHLA levels. PHLA in such severely dehydrated patients also could be elevated spuriously as a result of plasma volume contraction. Furthermore, these investigators used a dose of heparin to release LPL which was five times greater than the dose employed in this study. This larger dose may have obscured differences in PHLA.

The significantly delayed disappearance of C-14-labeled plasma TG after insulin withdrawal in two subjects in this study is further evidence of impaired TG removal. From earlier observations[15] it appeared that a shorter period of insulin deprivation in insulin-dependent diabetics did not elevate plasma TG levels or alter the removal rate of similarly prepared radioactive TG. However, in that study only the C-14 labeled plasma TG in the particulate fat fraction ($Sf > 400$) was administered. The nature and size of the labeled TG injected may be important, since in this study impaired removal was demonstrated with the use of a nonparticulate plasma lipoprotein fraction ($Sf < 400$). Since impaired clearance of labeled exogenous fat particles in alloxan-diabetic rats was not apparent until the TG level markedly increased,[16] impaired triglyceride removal demonstrated after insulin withdrawal from diabetic patients similarly may depend on the degree of TG elevation, as well as on the duration of insulin deprivation and the nature of the administered lipoprotein fraction. Thus, acute insulin deprivation appears to result in low PHLA, impaired TG removal, and increased TG levels.

Despite a fall in PHLA to levels comparable to those found in symptomatic diabetics with gross lipemia and prolonged periods of poor control, the increase in TG concentration noted in six of the patients in this study was small. This small change in TG may reflect the short duration of low PHLA levels, since it would be expected that the magnitude of the rise in TG is influenced by the duration, as well as the severity of impaired removal.

Thus the development of lipemia (lactescent plasma) in diabetes appears to depend upon the presence of a low insulin level for an extended period of time. The available insulin must be sufficient to prevent uncontrolled lipolysis and ketoacidosis but inadequate to maintain PHLA and normal TG removal. It seems reasonable to suggest that the patients in this study also would have developed lipemia had they continued to eat and remained free of acidosis. Since PHLA appears to be exquisitely sensitive to changes in effective levels of circulating insulin, TG elevation resulting from impaired removal of both exogenous (dietary) and endogenous triglyceride may be a common occurrence in the clinical course of diabetes mellitus.

ACKNOWLEDGMENT

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Gastrectomy in Children

Symptoms of the malabsorption syndrome, weight loss, the dumping syndrome, macrocytic anemia, and subacute combined degeneration of the spinal cord, are fairly common after subtotal and total gastrectomy in adult patients. This experience has been generally extrapolated into childhood, so that there has been considerable apprehension among surgeons concerning gastrectomy in the growing child. The indications for gastrectomy in childhood are infrequent enough so that no one surgeon or even a large medical center has accumulated enough such cases to develop any significant statistics. The recent report of an international survey of such cases by T. C. Moore (Ann. Surg. *162*:91, 1965) tends to allay these apprehensions.

The only experimental study which would seem to be pertinent to this report was that of J. A. Thompson, J. A. Bonta, and H. W. Clatworthy, Jr. (Surg. Forum *6*: 297, 1956). These authors reported that weight gain was unimpaired in healthy mongrel puppies subjected to 50 per cent gastrectomy, vagotomy, and gastroduodenostomy at three weeks of age.

A questionnaire on gastric resection was mailed by Dr. Moore to all members of the surgical section of the American Academy of Pediatrics and to all members of the British Association of Pediatric Surgeons. The percentage of members responding to this mailing was not mentioned, but information was obtained regarding 113 gastric resections during the first twelve years of life. A marked difference in results was reported, depending upon whether or not the gastrectomy included the cardioesophageal junction and esophagogastric anastomosis.

There were ninety-eight subtotal and total gastrectomies in which the cardioesophageal junction remained intact. Forty-three of these were done in the first two years of life. Operative mortality was 10 per cent and limited to those under the age of six. There were four late deaths, three due to cancer. Indications for surgical intervention included peptic ulcer (forty-two), gastric scarring or perforation due to the ingestion of corrosive agents (thirteen), duplication of the stomach (eleven), benign neoplasm (seven), malignant neoplasm (seven), and miscellaneous other conditions of the stomach (eighteen). Available follow-up data in eighty-four survivors revealed a uniformly *normal growth and development* (author's italics), even after total gastrectomy. The major difficulty after total gastrectomy was said to be the development of anemia, although this was not further documented or defined.

(*Continued on page* 146)