Short-term Effect of Insulin and 5(3-Piridyl) Tetrazole on Serum Triglycerides in Insulin-dependent Diabetic Patients

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SUMMARY

A group of insulin-dependent diabetic patients was studied in order to determine the possible short-term regulatory effect of insulin and that of an antilipolytic drug on serum triglycerides.

Subcutaneous crystalline insulin (one third of the usual morning dose of intermediate long-acting insulin, NPH) and an oral dose of 5(3-piridyl) tetrazole, 800 mg., were administered.

During the three-hour period of the study, the amount of both substances was sufficient to suppress free fatty acids and to produce a progressive lowering of serum triglycerides without significant changes in serum cholesterol. However, although the level of blood sugar was normalized with insulin, it did not change with the administration of 5(3-piridyl) tetrazole. The rate of fall of plasma free fatty acids and serum triglycerides was similar in both substances and positively correlated with its basal concentration.
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Hypertriglyceridemia secondary to diabetes mellitus is a late metabolic event.[1] It develops following a prolonged and severe insulin deficit[2] and reverses to normal after a period of at least 24 to 48 hours of insulin therapy.[3,4]

On the other hand, evidence suggests that insulin may also have a short-term regulatory effect on the serum triglycerides' (TG) concentration. Thus, a glucose load in normal subjects reduces alimentary chylomicronemia[5] and decreases the level of endogenous serum TG in one to two hours. If this immediate effect of glucose is due to insulin release under the influence of hyperglycemia, there should be observed first an absence of the TG-lowering effect of glucose in insulin-dependent diabetic subjects, and second the production of this effect with the administration of insulin. The first phenomenon has been reported[8,9] and the second was investigated in normal subjects with results that are contradictory and inconsistent.[10,11] It appeared that a more adequate subject to test this immediate effect of insulin would be one with low levels of endogenous hormone and consequently elevated blood glucose, thus overcoming severe hypoglycemia after insulin injection.

In the present work, a dose of subcutaneous crystalline insulin sufficient to suppress lipolysis and normalize average blood glucose in three hours also produced a fall in serum TG, which was significant at one hour and reached its maximal effect at the end of the three-hour period of the study. Oral administration of a potent antilipolytic drug, 5(3-piridyl) tetrazole (P3T), 800 mg.,[12] produced a decrease in serum TG, similar to that observed with insulin, even though the level of blood sugar did not change.

MATERIAL AND METHODS

Sixteen insulin-dependent diabetic patients (six women and 10 men) aged 22 to 65 were studied in the morning about 24 hours after the last injection of their usual morning dose (36 to 80 I.U.) of intermediate long-acting insulin (NPH) and 12 to 14 hours after the last meal.

The studies were performed while the patients remained seated in a quiet room. After a rest period, 12 to 20 U. of crystalline insulin was injected subcutaneously or 800 mg. of P3T was given by mouth. Blood samples were obtained immediately before and one, two, and three hours after insulin or P3T administra-

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tion. In control studies, only two blood samples with a three-hour interval were obtained, without administration of any drug. Twelve patients were subjected to control and insulin studies, five of them were also studied with P3T, and another four patients were studied only with P3T.

No patient had ketonuria at the time of the study, and only two showed moderately turbid serum, which did not demonstrate a "cream" layer after 12 hours at 5° C. Venous blood was analyzed for glucose,[13] cholesterol,[14] TG,[15] and free fatty acids (FFA)[16] content.

RESULTS

Table 1 summarizes the results obtained. We can see a decrease of serum TG caused by insulin, associated to that expected on blood glucose and plasma FFA. A steady decline was observed, starting from a moderately elevated fasting level, which was significant at one hour and persisted during the three-hour period of the study. P3T also produced a significant fall in plasma FFA and serum TG, even though blood sugar did not change significantly. Neither insulin nor P3T caused significant changes in serum cholesterol.

Figure 1 depicts the rate of fall (estimated from 0 to 180 minutes by the least-square method) of plasma FFA and serum TG after administering insulin and P3T. It can be observed that the antilipolytic and serum-TG-lowering effect of both substances were positively correlated with their fasting concentrations. The regression lines between fasting levels of FFA and its net fall rate were y: $-0.099(0.0030x)$, r: 0.70 for insulin, and y: $0.097(0.0028x)$, r: 0.85 for P3T; and between fasting level of TG and its net fall was y: $-0.057(0.0016x)$, r: 0.88 for insulin, and y:

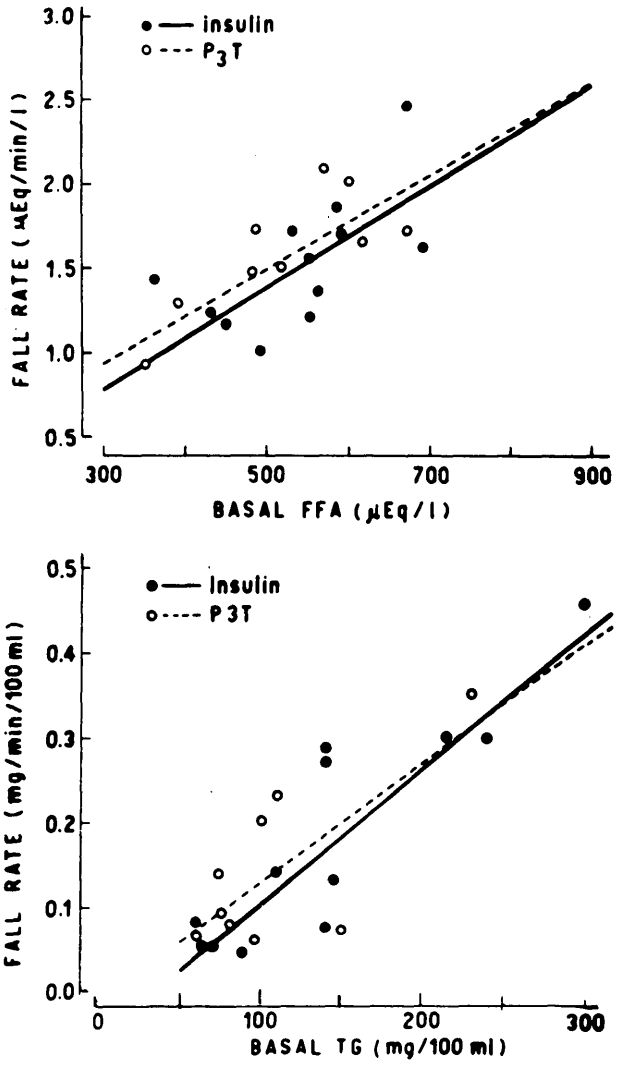

FIG. 1. Influence of basal levels of plasma FFA and serum TG on their rate of fall produced by insulin and P3T in a three-hour period.

TABLE 1

Effect of subcutaneous crystalline insulin (12 to 20 U.) or oral P3T (800 mg.) on blood glucose and fats in insulin-dependent diabetic patients

		no.	basal	60 min.	120 min.	180 min.
	Insulin	12	233±23	186±21*	131±18*	93±21*
Glucose	P3T	9	245±26	235±24	217±22	215±24
mg./100 ml.	Control	12	219±26	—	—	207±24
	Insulin	12	557±25	436±25*	326±30*	285±17*
Free fatty acids	P3T	9	560±51	366±30*	285±30*	230±28*
μEq./L.	Control	12	568±32	—	—	534±35
	Insulin	12	145±21	133±20*	120±17*	101±13†
Triglycerides	P3T	9	108±18	98±16†	91±16†	83±14*
mg./100 ml.	Control	12	144±25	—	—	138±24
	Insulin	12	254±16	252±15	250±16	249±16
Cholesterol	P3T	9	222±11	210±10	207±16	214±11
mg./100 ml.	Control	12	252±15	—	—	254±15

Mean ± S. E.; *P*: * < 0.01, † < 0.001, in respect to basal level by paired comparison *t*-test..

$-0.0053(0.0014x)$, r: 0.75 for P3T. The regression lines between both substances were not statistically significant.

DISCUSSION

The present work shows that a dose of subcutaneous crystalline insulin sufficient to suppress lipolysis and normalize average blood glucose in a three-hour period, as a function of its basal concentration, reduces the level of serum TG in diabetic patients.

Previous studies performed in normal subjects showed an immediate effect of insulin on serum TG different among females and males. In pregnant, puerperal, and normal females,[10] intravenous injection of 0.1 U. of insulin per kilogram of body weight results in a significant fall of serum TG in a three-hour period. In this study, the magnitude of the decrement was also positively associated to the basal level of serum TG. On the other hand, in normal males[11] the intravenous injection of 0.12 U. of insulin per kilogram of body weight did not change the level of serum TG. Furthermore, when a single pulse of insulin was replaced by a five-to-eight-hour constant infusion of 0.01 to 0.02 U./kg./hour, only three of the seven males reacted with a decline in serum TG during the infusion period. The reason for the variation in response is not clear. Severe decrement in blood sugar was induced by these doses of insulin in all subjects except pregnant females.[17] This group responded with a more pronounced TG-lowering effect. It is possible that hormonal response to hypoglycemia may block some of the mechanism by which insulin decreases serum TG levels. Epinephrine inhibits the activation of adipose tissue lipoprotein lipase produced by insulin in two to three hours in rats[18] and increases serum TG in 60 minutes in normal subjects.[19] In our study, in order to avoid hypoglycemia, subcutaneous rather than intravenous insulin was used. Under these conditions, net lipolysis was persistently suppressed during the period of observation, and both diabetic females and males responded with a decline in serum TG levels (females, from a basal level of 127 ± 32 mg./100 ml. to 102 ± 25 mg./100 ml. at 180 minutes, $P < 0.05$, and males, from a basal level of 158 ± 30 mg./100 ml. to 107 ± 14 mg./100 ml. at 180 minutes, $P < 0.01$).

Our study in diabetic humans shows a similar effect on plasma lipids of insulin and P3T, although the effect on blood glucose differed. This effect could be the consequence of an inhibition of lipolysis[20] and therefore TG production,[21] that is, a decrease in splanchnic, mainly hepatic, output, due to a diminished availability of free fatty acids, glycerol, and glucose, produced by insulin. Similar decreasing effect on plasma lipids was induced by nicotinic acid (of which P3T is a derivative) in the diabetic rat[22] and dog.[23]

The other possible mechanism of this effect on plasma lipids could be an acceleration on removal, induction, or activation of adipose tissue lipoprotein lipase, described for insulin[18] in the three species above mentioned and clearly seen in rats with nicotinic acid[22] but not in man[24] or dog.[23]

Another possibility is the interaction of both mechanisms described. Some possible causes of action are hepatic TG synthesis, incorporation of the TG into the lipoprotein, and finally the output of lipoprotein-bound TG from the liver to the circulation, as suggested by experiments on hepatic lipogenesis with nicotinic acid in normal rats.[25]

We cannot establish at the present which is the mechanism responsible for the short-term effect on serum TG described, but the effect of P3T suggests its possible clinical importance. It must still be determined if it acts with equal effectiveness during the whole day and when administered chronically. Our results indicate that the release of insulin normally observed during the absorptive stage has a short-term regulatory effect on endogenous serum TG concentration. Its absence may be of importance over the entire metabolic regulation in diabetic patients.[26]

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