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Mechanism of Excessive Postprandial Lipemia in the Diabetic Rat* (33505)

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The dog (1), rabbit (2), and rat (3) made diabetic by the administration of alloxan, exhibit a rise in concentration not only of plasma glucose but also of plasma triglyceride (PTG). The reasons for the rise of PTG were not completely ascertained, although it was discovered that the diabetic rat lacks lipoprotein lipase both in its blood (4), and in its adipose tissue (5), a defect remedied or markedly ameliorated by the administration of insulin (6). Recently Bierman *et al.* (7) reported that the alloxanized rat appeared to become hyperlipemic *only* if fed excess fat but these authors did not attempt to measure either the intestinal absorption, synthesis, and discharge of triglyceride (TG) or the rate of disappearance of intravenously administered TG from the plasma of their diabetic rats.

For the past several years we studied the disposition of exogenously derived TG in the normal rat (8–10), and we thought it of interest also to study the lipid dynamics of the diabetic rat. The results of these studies are described herein. They indicate that the diabetic rat exhibits a mildly elevated fasting PTG and a markedly elevated postprandial PTG, both of which appear due to the relative inability of this type of animal to rid its blood of fat.

Methods. A. Determination of pre- and postprandial PTG values of treated and untreated diabetic rats. Forty mature male rats, weighing an average of 290 g, (Long Evans strain) were given 40 mg of alloxan monohydrate/kg of weight by intravenous injection after being starved for 72 hr. One week later, 18 of the 35 surviving rats, after prior starvation for 15 hr, were given 3 ml of cottonseed oil by oral intubation. The remaining 17 rats received 0.5 units of regular insulin (Iletin, Lilly) by subcutaneous injection prior to the administration of the cottonseed oil. Twenty normal rats (controls) also were given the oil. Blood samples obtained before, and 6 hr after the oil feeding were analyzed for their PTG content according to the method of Van Handel and Zilversmit (11) and their glucose content according to the method of Marks (12) using uranyl acetate[1] precipitation.

B. Determination of intestinal absorption of TG in treated and untreated diabetic rats. Five rats made diabetic by the administration of alloxan 1 week previously, were starved for 15 hr and then given by oral intubation, 3 ml of cottonseed oil to which was added tripalmitin-^{14}C sufficient to supply 500,000 cpm/3 ml dose. The animals were bled before and 6 hr after the feeding. The initial sample was analyzed for its PTG and glucose content and the 6 hr sample was

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[1] The reagents were supplied as a "Biochemica-test" by C. F. Boehringer & Soehne.

analyzed for PTG and also for its content of labeled dietary-derived TG. All animals were killed after the 6 hr bleeding and the entire gastrointestinal tract, including its contents, and also the total liver of each animal were removed and assayed for their content of labeled dietary-derived TG. The methods employed for this assay of labeled TG in the plasma, gastrointestinal tract and liver have been previously described (9).

Eight similarly diabetic rats were treated in an identical manner except that prior to administration of the oil, they received a 0.5 unit of regular insulin by subcutaneous injection.

For control purposes, 9 normal rats were subjected to the same treatment.

C. Determination of hepatic discharge of TG in treated and untreated rats. We employed the Triton technique (13, 14) to determine the comparative rates of hepatic discharge of TG in normal and diabetic rats. Twenty rats made diabetic by the administration of alloxan monohydrate 1 week previously were starved for 15 hr and then given 200 mg of Triton WR-1339[2] (tyloxapol) by intravenous injection. Blood samples obtained before and 6, 12, 18, 24, 72, and 96 hr after this injection were obtained and analyzed for PTG. Nineteen of the 20 rats survived the experiment. Ten similarly diabetic rats were treated in an identical manner except that immediately prior and again 24 hr after the injection, they were given 0.5 unit of insulin and also blood samples were not obtained from them after the 48-hr period. Ten normal rats (controls) also were treated and studied exactly as was done on the untreated diabetic rats.

D. Determination of the rate of disappearance of intravenously administered TG from plasma of treated and untreated diabetic rats. Twenty rats made diabetic by the administration of alloxan monohydrate 1 week previously were starved for 15 hr and then given 2.0 ml of pooled hyperlipemic plasma (av PTG content = 2800 mg/100 ml) obtained from donor rats given 200 mg of Triton WR-1339, 24 hr previously. Bood samples were obtained immediately, 1 and 2 hr after the administration of the hyperlipemic plasma. Ten additional diabetic rats were similarly treated except they were given 0.5 unit of insulin immediately prior to the administration of the hyperlipemic plasma. Twenty normal rats also were given the hyperlipemic plasma and subsequently treated exactly as the untreated diabetic rats.

E. Determination of intravenously administered tripalmitin-^{14}C in diabetic rats. Ten rats made diabetic by the administration of alloxan monohydrate and 5 normal rats were given tripalmitin-^{14}C by intravenous injection. The tripalmitin was prepared as follows. One-half ml of a 5% solution of bovine albumin (Armour Laboratories, Fraction V) in water was swirled in a 25-ml Erlenmeyer flask until the interior was coated. A gentle stream of air was directed into the flask until the interior was barely damp. At this point tripalmitin-1-^{14}C in benzene was pipetted into the bottom of the flask in the proportion of 50 μl containing 1 μCi for each ml of hyperlipemic plasma to be added later. The air stream was reintroduced until the benzene had evaporated. Purified unsaturated phospholipid (Lipostabil[3]) was weighed onto Teflon[4] tape and the loaded tape dropped into the flask. One hundred mg of phospholipid was used for each ml of plasma added later. The flask was securely corked and stored at 4° until needed.

On the day of use, sufficient hyperlipemic plasma, obtained from rabbits fed standard chow with 2% cottonseed oil added, was added to allow withdrawal of 0.2 ml for i.v. injection into each rat. The flask was recorked and immersed in water at 37° and shaken with horizontal motion, to avoid foaming, for 1 hr. Tripalmitin-1-^{14}C prepared in this way continued to circulate in rat plasma for several hours, whereas simpler preparations disappeared within minutes of injection. Three hr after the i.v. injection of this preparation, the rats were killed and

[2] Rohm and Haas Co., polyoxyethylated tertiary octylphenol formaldehyde polymer.

[3] Natterman Co. in Koln, through the kindness of Dr. Hans Eikerman.

[4] E. I. Dupont de Nemours, Inc.

TABLE I. The Pre- and Postprandial PTG of Untreated and Treated Diabetic Rats.

No. of rats	Av wt. (g)	Plasma glucose (mg/100 ml)		Plasma triglyceride (mg/100 ml)	
		Before feeding	After feeding (6 hr)	Before feeding	After feeding (6 hr)
Normal rats					
20	302	176	164	27	69
Range:		(146–212)	(135–200)	(10–44)	(32–144)
SEM:		± 3.4	± 3.8	± 2.2	± 5.5
Diabetic rats					
18	290	331	267	38	632
Range:		(113–522)	(138–559)	(19–77)	(98–1665)
SEM:		± 34	± 29	± 3.7	± 127
Diabetic rats given insulin					
17	280	319	143	36	189
Range:		(159–485)	(77–401)	(15–93)	(22–697)
SEM:		± 27	± 17	± 3.9	± 46

their livers were removed and analyzed for labeled fatty acid.

Results. A. The pre- and postprandial PTG values of treated and untreated diabetic rats. As Table I indicates, the average preprandial PTG (38 mg/100 ml) of the diabetic rats was moderately but significantly greater ($p<0.02$) than that (27 mg/100 ml) of the normal rats. A far more dramatic difference was observed in their respective 6-hr PTG values in that the average PTG content of the untreated diabetic rats was more than nine times greater than that of the normal rats. The diabetic rats which were given insulin exhibited far less hyperlipemia 6 hr after their receipt of the oil. As Table I also shows, the fasting and postprandial plasma glucose values of the untreated diabetic rats were significantly greater ($p<0.001$) than these respective values of the normal rats. The values in both groups are elevated by heavy ether anesthesia (15). As might be expected, the diabetic rats given insulin exhibited a considerable decline in their 6-hr plasma glucose values.

B. Intestinal absorption of TG in treated and untreated diabetic rats. The diabetic rats given oil by mouth (see Table II) were as capable as the normal rats of absorbing TG. Moreover, the absorption of TG in the diabetic rats was not altered by the administration of insulin. Despite this observed equivalence of absorption of TG in normal and diabetic rats, a very marked difference was observed in their respective plasma and liver contents of dietary derived TG as determined by the assay of labeled fatty acids. Thus (see Table II) there was approximately three times more fatty acid of dietary origin in the total plasma volume of the diabetic rats than in the total plasma of the normal rats. As might be expected (given the equivalence of absorption but the disparity in PTG values of the two types of rats), significantly less ($p<0.001$) dietary derived fatty acid was found in the livers of diabetic than in normal rats. However, the diabetic rats given insulin with their oil not only exhibited a rate of intestinal absorption similar to that of normal rats but also (see Table II) a similar plasma and liver content of fatty acid of dietary origin.

C. Hepatic discharge of TG in treated and untreated diabetic rats. The response of untreated and insulin-treated diabetic rats was essentially the same as that of normal rats during the first 48 hr following a single injection of Triton (see Fig. 1). These results suggested that the rate of hepatic discharge of TG in the plasma of both the untreated and insulin-treated rats was essentially the same as that occurring in normal rats. At the

TABLE II. The Absorption and Disposition of Exogenous TG in Untreated and Treated Diabetic Rats.

No. of rats	Av wt. (g)	Av plasma glucose (mg/100 ml)	Plasma triglyceride (mg/100 ml) Before feeding	Plasma triglyceride (mg/100 ml) After feeding (6 hr)	Intestinal absorption of triglyceride (mg in 6 hr)	Dietary triglyceride fatty acid (in 6 hr) In total plasma vol (mg)	Dietary triglyceride fatty acid (in 6 hr) In total liver (mg)
Normal rats							
9	298	168	17	87	638	5.4	56
Range:	(263–310)	(138–198)	(5–27)	(62–144)	(410–950)	(2.5–10.2)	(34–74)
SEM:		± 4.0	± 2.3	± 7.7	± 50	± 0.8	± 3.7
Diabetic rats							
5	280	322	25	222	678	18.3	33.5
Range:	(260–304)	(150–620)	(21–30)	(112–348)	(400–1180)	(8.8–28.2)	(28–40)
SEM:		± 31	± 1.4	± 37.6	± 78	± 4.1	± 2.4
Diabetic rats given insulin							
8	278	340	23	104	614	5.9	56
Range:	(258–298)	(170–480)	(18–26)	(86–170)	(520–674)	(3.9–6.9)	(44–87)
SEM:		± 33	± 1.8	± 8.7	± 48	± 0.7	± 3.2

end of 72 hr (at which time most of the Triton had disappeared from plasma (16)), the average PTG of the diabetic rats was somewhat lower than that of the normal rats but this difference was not statistically significant. However, at the end of 96 hr there was a relative and statistically significant ($p<0.05$) lag in the fall of PTG in the diabetic rats compared to the normal rats.

D. Rate of disappearance of intravenously administered TG in the treated and untreated diabetic rats. As Fig. 2 illustrates, when the PTG content of the untreated diabetic rat is elevated by the administration of hypertriglyceridemic rat plasma, the rate of disappearance of this excess TG from the plasma, is significantly slower than that observed in the normal rat as measured 1 and 3 hr after the administration of the hyperlipemic plasma. As might be expected (see Fig. 2), the administration of insulin to diabetic

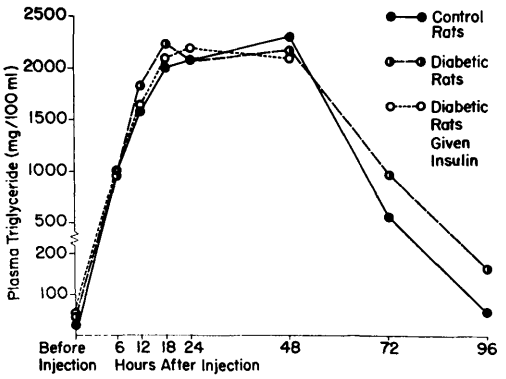

FIG. 1. The PTG response of the normal, diabetic, and diabetic rat given insulin to a single injection of Triton. The delay in the return of the PTG to preinjection level of the diabetic rat is seen 48–96 hr after injection.

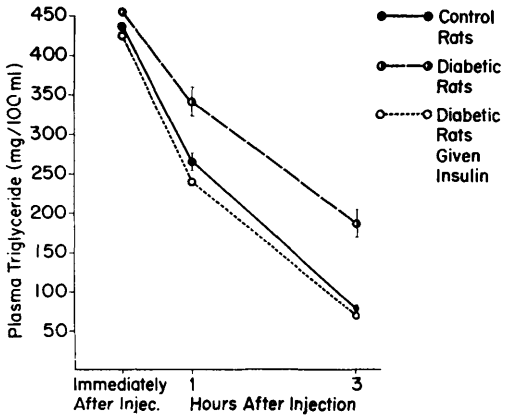

FIG. 2. The decline in PTG of the normal, diabetic, and diabetic rat given insulin after intravenous administration of hypertriglyceridemic serum. The lag in decline of the PTG in the untreated diabetic rat is clearly shown.

rats enabled them to rid their plasma of the excess TG as rapidly as the normal rats.

These results appeared to be confirmed when the livers of the normal and diabetic rats given tripalmitin-^{14}C intravenously were assayed for their labeled fatty acid. Thus the livers of 10 diabetic rats had an average content of labeled fatty acid equal to 15.8% (SEM: ± 0.8) of the amount of labeled TG originally injected and the five livers of the 5 control rats had an average content of labeled fatty acids equal to 45.5% (SEM: ± 0.9) of the amount of labeled TG previously injected.

Discussion. The results show that rats made diabetic by administration of alloxan not only exhibit a moderately elevated fasting PTG but also, when administered fat orally, become far more lipemic than the normal rat. This excess postprandial lipemia was not found to be due to any change in the intestinal absorption of TG in the diabetic rat nor was any change found in the hepatic rate of discharge of TG into plasma.

Rather the evidence obtained indicated that the lipid defect of the diabetic animal was primarily due to its relative inefficiency in removing TG from its plasma; this defect clearly became manifest when additional TG was brought into the animal's plasma by either the ingestion or parenteral administration of excess TG. In view of these facts, it would appear that the observed slightly higher fasting PTG of the diabetic rat was due primarily to a lag in its capacity to rid itself of the fat ingested 15 hr before blood sampling. The ability of insulin to correct or palliate either orally or intravenously induced hypertriglyceridemia suggests that the lipid defect present in the rat given alloxan is specifically due to its insulin deficit.

As already known, the plasma and the adipose tissue of the diabetic animal is deficient in lipoprotein lipase (4–6) and this abnormality is correctible by insulin administration (5, 7). The results obtained in this present study, while not directly demonstrating such a deficiency of lipoprotein lipase, nevertheless are compatible with this concept.

Summary. Rats made diabetic by administration of alloxan were found to exhibit a marked impairment of their ability to rid their blood either of exogenous or endogenously derived triglyceride. No defect in the absorption of triglyceride or secretion of endogenous triglyceride into plasma was observed. The defect in triglyceride clearing was largely correctible by administration of insulin.

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