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Glucose Tolerance, Plasma and Pancreatic Insulin Levels in Zinc Deficient Rats[1, 2] (39077)

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As early as 1937 Hove *et al.* (1) noted that glucose tolerance curves for zinc deficient rats differed from normal "in some cases." However, they concluded that there were no disorders in carbohydrate metabolism. Reexamination of the problem has led to contrasting results. Some investigators have reported reduced glucose clearances in zinc deficient rats given intravenous or intraperitoneal glucose (2–5), while others (6, 7) have found no difference in the tolerance to intraperitoneal glucose between zinc deficient and control rats fed *ad libitum*. One group of workers has reported that oral glucose tolerance was not impaired in zinc deficient rats (2). However, abnormal glucose tolerance curves were obtained in some human zinc deficient dwarfs after an oral dose of glucose (8).

In rats and hamsters, one group of workers (3) found differences in basal blood glucose levels while other groups (2, 4, 5, 7) did not.

Immunoreactive blood insulin concentrations do not appear to change in zinc deficient rats when compared with pair-fed controls (3). Huber and Gershoff (3) reported that pancreatic slices from zinc deficient rats released less insulin in vitro than slices obtained from pair-fed controls. However, there were no significant differences in the insulin concentration of pancreatic tissue.

Adequate chromium is necessary for normal glucose tolerance (9), yet several studies (2, 3, 7), used semipurified diets which contained no added chromium. Other differences which may account for the contrasting results include variations in the route of glucose administration, in the composition of the diet, in body weight between groups, in food intake before glucose loading, and in the type and degree of stress upon the animals during the experimental period. Therefore, we have reexamined glucose metabolism in zinc deficient rats under more closely controlled conditions, using a semipurified diet adequate in chromium and other trace elements.

Methods. Experiment 1. Two trials of this experiment were conducted on a total of 63 animals. In each trial, male weanling rats (CD Strain, Charles River Breeding Laboratories, Wilmington, Mass.), 45–50 g, were housed in individual stainless steel wire suspended cages and offered deionized water ad libitum. They were fed a basal diet (Table I) supplemented with $ZnCO_3$ to contain 20 ppm of elemental zinc until their average body weight was 150 g. At this time they were divided into two groups: a zinc deficient group which received the basal diet with no added zinc and a zinc sufficient group which continued to receive the supplemented diet. The food intake of the latter was restricted so as to maintain the average weight of the zinc deficient group. Thus the two groups were pair-weighted. The animals remained on these dietary regimens until the zinc deficient group displayed typical signs of zinc deficiency (55 days in Trial 1 and 39 days in Trial 2).

At the end of the feeding period, the rats were fasted overnight, anesthetized lightly (sodium pentobarbital, 5 mg/100 g body wt, Trial 1 and sodium amobarbital, 4 mg/100 g body wt, Trial 2) and kept under anesthesia

[1] Some of these data were presented at the Fifty-Eighth Annual Meeting of the Federation of American Societies for Experimental Biology, Atlantic City, N.J., April 7–12, 1974.

[2] The animals were maintained in animal care facilities accredited by the American Association for Accreditation of Laboratory Animal Care.

until sacrificed. Samples of blood were obtained from the abdominal aorta of each animal before gastric intubation and 30, 60, 120, or 180 min after a glucose load (500 mg/100 g body weight using a 50% glucose solution). A single blood sample was taken from each rat and five to eight animals were used for each time period.

The heparinized blood samples were cooled immediately in ice and aliquots were removed for whole blood glucose determination by the glucose oxidase method (Glucostat, Worthington Biochem Corp., Freehold, New Jersey). The remainder was centrifuged for the measurement of plasma insulin levels by radioimmunoassay (10).

A femur was removed from several animals and analyzed for zinc concentration by atomic absorption spectrophotometry (11).

The results of the two trials were combined and analyzed statistically using the Students' *t* test.

Experiment 2. Twenty-two male weanling rats were divided into three groups: Group I (six rats) received a zinc sufficient diet, *ad libitum*, Group II (eight rats) received a zinc deficient diet *ad libitum*, and Group III (eight rats) received a zinc sufficient diet pair-fed with Group II. Unlike Expt 1 where the zinc sufficient animals were maintained at the mean weight of the zinc deficient group, in this experiment Group III was pair-fed the average daily food intake of Group II.

The animals received the basal diet (Table I) with and without zinc supplementation. However, in this experiment vitamin A was given orally (0.258 g vitamin A acetate, two times per week) rather than being mixed with the diet.

After 21 days the animals were sacrificed and the pancreas was removed and weighed. Pancreatic insulin was determined by a method previously reported (10).

Results. Experiment 1. At the time of sacrifice, the zinc deficient animals displayed typical signs of zinc deficiency such as anorexia, rough coats, and scaly dermatitis about the feet and mouth. Table II shows that the zinc deficient group had a significantly lower femur zinc concentration than the zinc sufficient group.

TABLE I. COMPOSITION OF BASAL ZINC DEFICIENT DIET.[a]

Ingredient	g/100 g of diet
Dried egg white[b]	20.0
Glucose monohydrate	67.9
Corn oil	5.0
Salts (Fox–Briggs, zinc free)[c]	6.0
Trace element supplement[d]	0.1
Vitamins[e]	1.0

[a] <3 ppm zinc by analysis.

[b] Obtained from Henningson Foods, White Plains, New York.

[c] Provided in diet (mg/kg): $CaCo_3$, 10,000; $CaHPO_4$, 28,400; $CuSO_4$, 7; $FeC_4H_2O_4$, 98; $MgSO_4$, 3000; $MnSO_4H_2O$, 250; KCL, 7000; KIO_3, 10; NaCl, 4000; Na_2HPO_4, 7000.

[d] Provided in diet (mg/kg): $Co(C_2H_3O_2)_2 \cdot 4H_2O$, 21.2; $Na_2MoO_4 \cdot 2H_2O$, 5.0; Na_2SeO_3, 0.3; NaF, 44.2; $CrCl_3 \cdot 6H_2O$, 10.2; $NiCl_2 \cdot 6H_2O$, 4.0; $Na_2B_4O_7$, 23.3; $Na_2HAsO_4 \cdot 7H_2O$, 1.3.

[e] Provided in diet (mg/kg): thiamin·HCl, 50; riboflavin, 25; pyridoxine·HCl, 30; calcium pantothenate, 100; niacin, 200; menadione, 50; folic acid, 20; biotin, 5; vitamin B_{12}, 0.05; choline dihydrogen citrate, 5000; *i*-inositol, 1000; α-tocopherol acetate, 200; ascorbic acid, 1000; *p*-aminobenzoic acid, 100; and (in micrograms) vitamin A, 6,900; and vitamin D_2, 100.

TABLE II. FEMUR ZINC CONCENTRATIONS OF ZINC DEFICIENT AND ZINC SUFFICIENT CONTROL RATS, PAIR-WEIGHTED.

Diet	Number of animals	μg Zn/g dry wt
Zinc deficient, *ad libitum*	6	58 ± 13[a]
Zinc sufficient, pair-weighted	6	158 ± 38
		$P < 0.001$

[a] Mean ± SD.

Table III presents the results of the glucose tolerance tests. No significant differences in the blood glucose level were noted between the two groups, although the maximum value appeared 30 min after loading in the zinc deficient group and 60 min after loading in the sufficient group. There were no significant differences in fasting insulin levels or in the insulin response to the glucose load.

TABLE III. ORAL GLUCOSE TOLERANCE AND BLOOD INSULIN IN FASTED ZINC DEFICIENT RATS AND FASTED ZINC SUFFICIENT RATS, PAIR-WEIGHTED.

Minutes after oral glucose load[a]	Zinc deficient			Zinc sufficient (pair-weighted)		
	Number of rats	Blood glucose (mg/100 ml)	Plasma insulin (μU/ml)	Number of rats	Blood glucose (mg/100 ml)	Plasma insulin (μU/ml)
0	7	78 ± 13[b, c]	72 ± 20	7	79 ± 18	66 ± 12
30	9	133 ± 33	137 ± 30	6	114 ± 33	135 ± 39
60	6	122 ± 20	159 ± 27	6	121 ± 11	160 ± 30
120	6	108 ± 9	102 ± 23	5	109 ± 11	99 ± 25
180	5	103 ± 21	95 ± 29	6	97 ± 13	92 ± 33

[a] 500 mg glucose/100 g body weight.
[b] Mean ± SD.
[c] Blood glucose and plasma insulin values were not significantly different between zinc deficient and zinc sufficient, pair-weighted groups for each sampling period.

TABLE IV. PANCREATIC INSULIN LEVELS IN ZINC DEFICIENT AND CONTROL RATS.

Dietary treatment	Number of rats	Units insulin/g pancreas	Significance level[a]
Zinc deficient *ad lib.*	8	0.55 ± .12[b]	—
Zinc sufficient pair-fed	8	0.55 ± .11	NS
Zinc sufficient *ad lib.*	6	0.26 ± .05	$P < 0.001$

[a] Significance level in comparison with Zinc deficient, *ad lib.* group.
[b] Mean ± SD.

Experiment 2. The pancreatic insulin levels are listed in Table IV. The mean insulin concentration was significantly lower in the zinc sufficient group fed *ad libitum* than in the zinc deficient rats. However, no significant differences were noted between the zinc deficient and the pair-fed zinc sufficient groups.

Discussion. Particular care was taken throughout these investigations to maintain conditions that were as close as possible to the physiologic state. Adequate chromium and other trace elements were added to the basal diet. Also, a high quality protein was used at a level sufficient to promote normal growth.

Control animals were either pair-weighted or pair-fed to insure similar body weights or similar dietary intakes. Prior to the glucose tolerance tests all rats were fasted overnight to minimize differences in blood glucose levels due to feeding patterns.

All animals remained lightly anesthetized throughout the glucose tolerance tests to reduce struggling and epinephrine release. Glucose was administered orally since this is the normal feeding route, while intravenous or intraperitoneal injections circumvent the influence of the gastrointestinal hormones that stimulate insulin release (12–14). Furthermore, the stress of the intraperitoneal injection of a hypertonic glucose solution may cause the release of corticoids, and thus alter glucose tolerance.

The data from Expt 1 show that there is no significant difference in oral glucose tolerance or circulating insulin between pair-weighted zinc sufficient and deficient rats.

Experiment 2 demonstrated that insulin concentration in the pancreas of zinc deficient rats fed *ad libitum* was significantly decreased when compared to zinc sufficient rats fed *ad libitum*. However, no significant differences were noted when food intake was adjusted by pair-feeding. These data suggest that dietary restriction rather than zinc deficiency was the causative factor increasing the pancreatic insulin levels.

Thus, neither of these experiments indicates that zinc deficiency per se precipitates an alteration in glucose tolerance or in plasma and pancreatic insulin.

Summary. Two experiments were con-

ducted to examine the effect of zinc deficiency on glucose tolerance, and on blood and pancreatic insulin concentrations. In the first study, no significant differences in blood glucose or plasma insulin levels were noted between pair-weighted zinc deficient and zinc sufficient rats after an oral glucose load. In the second experiment, the concentration of pancreatic insulin in pair-fed zinc sufficient rats did not differ significantly from that of zinc deficient rats. However, a zinc sufficient group fed *ad libitum* had significantly lower pancreatic insulin levels, suggesting that food restriction may cause increased pancreatic insulin. Thus, zinc deficiency per se had no apparent effect on oral glucose tolerance or pancreatic insulin concentrations.

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