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Effect of Low Dietary Zinc Intake and Experimental Diabetes on the Zinc and Carbohydrate Metabolism in Rats

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Abstract: To investigate the effect of low dietary zinc intake and experimental diabetes (IDDM) on the zinc and carbohydrate metabolism, 8-week-old male weaning normal albino (Wistar) rats were fed diets containing either adequate (54mg/kg) or low zinc (1mg/kg) quantities for one week. Ten rats from each group (n=20) were then intraperitoneally injected with alloxan to induce diabetes. The rats were sacrificed after a further three weeks. Body weight gain and food intake were recorded regularly. On day 28, after an overnight fast, the animals were sacrificed and blood glucose, serum insulin, serum cholesterol concentrations, liver glycogen contents, and femur and pancreatic zinc concentrations were determined. Diabetic rats fed a low zinc or control diet had a low body weight gain, high total food intake (hyperphagia), low serum insulin, low liver glycogen contents and high serum cholesterol concentrations compared to normal rats. The

consumption of the low zinc diet had only a minimal effect on the zinc status of rats as indicated by the growth rate, food intake and femur and pancreatic zinc concentrations. However, both diabetic and non-diabetic rats fed a low zinc diet had higher blood glucose than their control counterparts. Liver glycogen was also found to be higher in the low zinc non-diabetic rats than in their controls. Serum insulin and serum cholesterol concentrations were unaffected by dietary regimen. To conclude, the present study demonstrates that a reduced zinc intake had an effect on glucose utilization in both diabetic and non-diabetic rats and on glycogen deposition in non-diabetic rats. However, there were negligible changes in zinc status. Therefore, it appears that abnormalities in the carbohydrate metabolism may occur before tissue zinc depletion becomes apparent.

Key Words: Diabetic rats, non-diabetic rats, Alloxan, Low zinc levels

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Introduction

Zinc is required for normal insulin metabolism. It seems reasonable, therefore, that changes in body zinc status could affect the production, storage and secretion of insulin (1,2). A relationship between zinc and insulin storage is also suggested by the finding that acute stimulation of insulin secretion in rats also reduces the zinc content in the B-cells of the pancreas (3). There are several reasons for suspecting that an abnormal zinc metabolism could play a role in the pathogenesis of diabetes mellitus and in some of its complications. Cytological studies have demonstrated the presence of zinc in the crystal of insulin (4). In addition, microscopic studies indicated that the B cells of zinc deficient animals have decreased granulation and histochemically detectable insulin (5). In addition, some investigations have shown that rats given low-zinc diets had impaired glucose tolerance following intravenous or intraperitoneal glucose administration (6,7). Moreover, tissue zinc concentrations and zinc balances have both been reported to be altered in the diabetic state (8-10).

In view of the alteration of the carbohydrate and zinc status in a zinc deficient diet, the present study was undertaken to investigate the effects of dietary zinc deficiency and experimental diabetes (Insulin Dependent Diabetes) on the zinc and carbohydrate metabolism in diabetic rats fed a semi-synthetic diet containing either 1 or 54 mg Zn/kg, while non-diabetic rats were used as a comparison. Growth rate, food intake, zinc status, blood glucose, serum insulin and serum cholesterol concentrations, as well as liver glycogen content, were estimated.

Materials and Methods

Animals and diets

Male weaning normal albino (Wistar) rats of 8 weeks of age with a body weight ranging from 250-300 g were randomly divided into two groups of 20 each. The animals were housed individually in polypropylene cages with stainless-steel gridded tops and bottoms and stainless-steel food hoppers. Trays were placed under

each food hopper to collect spilt food. Humidity was around 70% and temperature was $22 \pm 2^\circ\text{C}$. Food and distilled water were provided *ad libitum*. The animals were given access to either a low-zinc diet, 1 mg Zn/kg, or the control diet, 54 mg Zn/kg. A modified version of the American Institute of Nutrition's (11) purified diet for rats and mice was prepared containing g/kg diet. The dietary carbohydrate source was provided by equal amounts of corn starch 326 (ONAB EL Harrouch, Algeria) and sucrose 326, protein 168 (egg white solids), lipids 80 (corn oil), fiber 40 (cellulose), Vitamin mix 20 (Sigma), and mineral mix 40. The latter was formulated to contain either adequate (54 mg/kg) or deficient (1 mg/kg) quantities of zinc, as determined by atomic absorption spectroscopy.

Mineral mix supplied (g/kg diet): calcium hydrogen orthophosphate 13; disodium hydrogen orthophosphate 7.4; calcium carbonate 8.2; potassium chloride 7.03; magnesium sulfate 4; ferrous sulfate 0.144; copper sulfate 0.023; potassium iodate 0.001; manganous sulfate 0.180; and zinc carbonate 0.1. The low zinc diet contained no additional zinc carbonate. The diet was prepared similar to that in the literature (12, 13).

After one week of the experiment, 10 rats from each group were intraperitoneally injected with freshly prepared alloxan monohydrate solution (Alloxan; Sigma) at a dose of 150 mg/kg of body weight to induce diabetes (14). Diabetic rats were then pair fed against non-diabetic rats in the same dietary group. The rats were maintained on the appropriate experimental diet for 26 days. Fasted overnight on day 27, but allowed to feed for two periods of 1 h each, between 11.00 and 12.00 a.m, and 5.00 and 6.00 p.m. The rats were then sacrificed between 11.00 and 12.30 p.m on day 28. One animal from each group was sacrificed at approximately the same time by exsanguination from the heart, under diethyl ether anesthesia. The blood was transferred into ice cold centrifuged tubes and a portion was taken for whole-blood glucose analysis, which was performed promptly after exsanguination. The remaining blood was centrifuged for 15 minutes at 3000 g and the serum stored at -20°C until insulin and cholesterol assays were performed. Livers were rapidly excised, weighed, freeze-clamped at -196°C , ground under liquid nitrogen and stored at -20°C for glycogen analysis. The pancreas was washed with isotonic saline (9 g sodium chloride/l distilled water) and blotted to dry. The right femur was taken and

the connective tissues and muscle were removed. After that, the pancreas and femurs were weighed, dried at 80°C for 16 hours and zinc concentrations were determined.

Analytical methods

Serum insulin was determined by a radio-immunoassay (15), using a rat insulin standard (NOVO, Research Institute, 4 ng/ml, Bagsvrend, Denmark), ^{125}I -insulin (Amercham International, Bucks, England) and guinea pig anti-porcine insulin serum (Wellcome Reagents Ltd., Beckenham, England). The blood glucose was measured in 10 ml samples of fresh whole blood by the glucose oxidase (EC 1.1. 3. 4) method, using a YSI Model 27 glucose analyzer and the kit constitute of phosphate buffer containing the enzymes (GOD, POD) and D-glucose (Sigma). The determination of total liver glycogen was as that of glucose following an enzymatic hydrolysis with amyloglucosidase (EC 3. 2. 1. 3) obtained from *Asperigillus niger*; (Sigma) (16). Both the dried pancreas and femurs were heated in silica crucibles at 480°C for 48 h and the ash taken up in hot hydrochloric acid (11.7 M) for Zn analysis by atomic absorption spectrophotometer (Pye Unicam SP 9000) (17). Using standard reference materials, bovine liver and wheat flour checked the accuracy of zinc recovery using this method. These standards were prepared and analyzed identically to assess recovery. The recovery of zinc from the standard reference material exceeded 96%. Cholesterol measurements were carried out in the hospital by the colorimetric enzymatic method (18) using commercial kits. Comparisons between the effect of diet and diabetes were made using Student's t-test.

Results

Induction of a type I diabetic state caused a decrease ($p < 0.05$) in body weight gain and higher food intake in the diabetic rats compared to the non-diabetic rats. However, dietary zinc deficiency did not significantly alter either body weight or total food intake (Table 1). Femur and pancreatic zinc concentrations, taken as an index of Zn status, indicated that both the low-zinc diabetic and non-diabetic rats were able to maintain a similar status to their control groups (Table 1). Pancreatic Zn concentrations were significantly lower in all diabetic individuals compared to the non-diabetic animals. In this experiment, mean femur zinc concentrations for diabetic

Table 1. Mean body weight gain (g/day), total food intake (g/day), femur zinc concentration (mg/dry wt), pancreas dry wt (g) and pancreatic zinc content (μg), and pancreatic zinc concentration ($\mu\text{g/g}$ dry wt) of diabetic and non-diabetic rats given a low zinc (1 mg Zn/kg) or control (54 mg Zn/kg) semi-synthetic diet for 28 days.

Animals Diet	Diabetic				Non-diabetic			
	Control (n= 10)		Low zinc (n=10)		Control (n= 10)		Low zinc (n= 10)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Body wt gain	3.0 ^a	0.4	2.5 ^a	0.4	5.2 ^b	0.5	5.9 ^b	0.4
Food intake	17.0 ^a	0.6	17.5 ^a	0.2	12.6 ^b	.03	11.7 ^b	0.5
*Feed efficiency	17 ^a	2.0	14 ^a	2.2	40 ^b	3.7	49 ^b	2.2
Femur Zn	117.8 ^a	5.7	118.4 ^a	5.6	150.7 ^b	3.7	118.1 ^a	4.1
Pancreas dry wt	0.85 ^a	0.08	0.96 ^a	0.05	0.88 ^a	0.2	0.76 ^a	0.06
Pancreatic Zn content	43.5 ^a	6.7	45.3 ^a	4.2	83.3 ^b	8.7	80.2 ^b	11.2
Pancreatic Zn concentration	50.4 ^a	3.9	44.7 ^a	1.4	104.0 ^b	6.1	86.5 ^b	16.5

^{a, b} values within a horizontal line with different superscript letters were significantly different ($p < 0.05$).

* body wt gain/food intake $\times 100$.

rats were relatively lower than those for non-diabetic rats, but the difference was not always significant. Analysis of blood glucose, serum insulin and cholesterol concentrations and liver glycogen content indicated that low-zinc diabetic rats had similar serum, insulin and cholesterol concentrations as well as liver glycogen content, but a higher fasting blood glucose level compared to the control diabetic animals (Table 2). Serum insulin and cholesterol concentrations were similar in both non-diabetic groups, but the low-zinc non-diabetic rats had higher blood glucose and liver glycogen contents than did the controls. The diabetic animals fed either a low-zinc or control diet had a higher ($p < 0.05$) blood glucose and serum cholesterol concentration, a decreased serum insulin concentration and lower liver glycogen content compared with the non-diabetic rats (Table 2).

Discussion

In this experiment, diabetic rats, with or without zinc deficiency, weighed less than the non-diabetic rats. However, the body weight gain in diabetic and non-diabetic rats was not affected by dietary zinc concentrations. This is not in good agreement with some previously published reports (19, 20). It is suggested, thus, that these animals may have an efficient homeostatic control mechanism for zinc, enabling them

to conserve its metabolically active pool. The diabetic rats fed either on the low-zinc or control diet had a lower feeding efficiency than the non-diabetic rats, although the food intake of these animals was higher (hyperphagia) than that of the non-diabetic rats. The daily mean of consumed diet by a rat is 14 g (21). This raises the possibility of a metabolic state disturbance of the animal, suggesting that the diabetic condition had exacerbated the reduced ability of the diabetic rats to utilize food intake as normal subjects. Diabetic and non-diabetic rats fed on low zinc or control diet had no differences in pancreatic and femur zinc concentrations, despite the fact that this tissue is generally regarded to be one of the most sensitive to variations in dietary zinc intake (22). It appeared, therefore, that these rats have an efficient mechanism for retaining body zinc, which results from a homeostatic response to the increased needs caused by the low dietary zinc intake. Such a mechanism results in the maintenance of tissue zinc levels in the low zinc groups, despite the dietary concentration of zinc being 50 times lower than the control groups. It is well known that animals and humans subjected to dietary mineral depletion are often able to conserve the mineral within certain tissues even in the face of a severe deficiency (23). The reduced total pancreatic zinc in diabetic rats fed at both levels of zinc compared with the non-diabetic rats is probably due to the degranulation, cytolysis and to other pathological changes in the pancreatic tissue, associated

Table 2. Mean fasting blood glucose (m mole/l), serum insulin (μ units/ml), serum cholesterol concentration (mg/100 ml), liver wet weight (g), liver glycogen content (mg) and liver glycogen concentration (mg/g fresh wt) of diabetic and non-diabetic rats given a low zinc (1 mg Zn/kg) or control (54 mg Zn/kg) semi-synthetic diet for 28 days.

Animals Diet	Diabetic				Non-diabetic			
	Control (n= 10)		Low zinc (n=10)		Control (n= 10)		Low zinc (n= 10)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Blood glucose	15.4 ^a	0.8	19.8 ^b	1.3	5.0 ^c	0.1	7.2 ^d	0.4
Serum insulin	19.0 ^a	3	16.1 ^a	4	35.5 ^b	2.8	31.2 ^b	4
Serum cholesterol	106 ^a	45	116 ^a	14	39 ^b	15	41 ^b	16
Liver wet wt	12.4 ^a	0.8	13.5 ^a	0.9	12.9 ^a	0.9	13.2 ^a	0.6
Liver glycogen content	66.4 ^a	12.7	53.9 ^a	14.5	150.9 ^b	33	307 ^c	26
Glycogen concentration	5.2 ^a	0.9	4.0 ^a	1.0	11.7 ^b	3.1	24.3 ^c	2.5

^{a, b, c, d} values within a horizontal line with different superscript letters were significantly different ($p < 0.05$).

with progression of the condition (24), or to the high excretion of zinc in the urine (25-27). However, the marked lack of reduction in the femur zinc levels of diabetic rats compared with the non-diabetic rats supports the work of Levine et al. (8) after using streptozotocin diabetic rats. In the current study, when the time of feeding was strictly controlled and the amount of food eaten by each animal before an overnight fast was known to be similar, the mean fasting blood glucose concentration in the low-zinc diabetic or non-diabetic rats was found to be higher than that of the controls. This suggests that the lower zinc intake had exacerbated the ability of diabetic and non-diabetic rats fed on a low-zinc diet to utilize glucose. Results from previous studies have also shown an increased blood glucose concentration, after intravenous injection with glucose in rats fed on a zinc deficiency diet (28), suggesting a relation between carbohydrate utilization and dietary zinc supply. The fasting glycogen content of both low-zinc and control diabetic rats was lower compared to that of non-diabetic groups, although liver weight was similar in different groups. This might be related to liver dysfunction (29,

30). Liver glycogen content in the low-zinc non-diabetic rats was higher than that of the control non-diabetic rats. These observations indicate that the carbohydrate metabolism of these animals is sensitive to reduced zinc intake and it is interesting to note that Reeves and O'Dell (31) have also observed an increased glycogen synthesis in zinc-deficient rats.

In conclusion, the findings presented in the present paper indicated that despite the apparently negligible effects of consuming the low-zinc diet on growth or the zinc status of the rats used in this experiment, significant differences in glucose metabolism were observed in both diabetic and non-diabetic rats and glycogen deposition in non-diabetic rats.

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