Int J Diabetes & Metabolism (2004) 11: 14-18

Effect of zinc deficiency and experimental diabetes on glutamate oxaloacetate, glutamate pyruvate aminotransferases and alkaline phosphatase activities in rats

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Abstract

The aim of this study was to investigate the effect of low dietary zinc intake and experimental diabetes on transaminases and alkaline phosphatase activities in rats. 8 week old male normal albino (Wistar) rats were fed diets containing either adequate (54 mg/kg) or deficient (1 mg/kg) quantities of zinc for one week. Ten rats from each group (n = 20) were injected with alloxan to induce diabetes. Control and alloxan rats were fed for three weeks and food intake and body weight gain were recorded daily and twice weekly, respectively. On day 28 the animals were killed and blood glucose, serum zinc, pancreatic zinc, serum glutamate oxalate transaminase (GOT), serum glutamate pyruvate transaminase (GPT) and serum alkaline phosphatase were determined after an overnight fast. Body weight gain of diabetic animals at the end of four weeks of dietary manipulation was significantly lower than those of the non-diabetic animals. Diabetic rats had higher food intake and lower serum and pancreatic zinc concentrations compared with non-diabetic rats. Dietary zinc intake significantly altered the body weight gain, food intake and serum zinc concentration of diabetic or non-diabetic rats. Serum GOT and GPT were significantly higher in alloxan-induced diabetic rats than in normal rats while the level of serum alkaline phosphatase was lower. The consumption of low-Zn diet led to increase in GOT and GPT, and decrease in serum alkaline phosphatase. In conclusion, the combination of zinc deficiency and diabetes affects the activities of GOT, GPT and alkaline phosphatase, and it appears that zinc deficiency may lead to the development of severe diabetes.

Key words: *Diabetes Mellitus, rats, alloxan, GOT, GPT, alkaline phosphatase.*

Introduction

It is well known that zinc forms an integral part of crystalline insulin.[1] Pancreatic zinc content of diabetic animals has been found to be strikingly reduced.[2] In addition, hyperzincuria has been demonstrated in many diabetic subjects.[3] Diabetic children have been shown to have low hair zinc levels, which return to normal after insulin administration.[4] Experimental diabetes can be produced by intravenous administration of dithisone[5] or by intraperitoneal injection of alloxan.[6] Experimental animals show a triphasic change in blood sugar levels, initial hyperglycaemia, hypoglycaemia, and finally hyperglycaemia, after the administration of alloxan. The development of such phases in alloxan diabetes is mainly due to insulin deficiency, insulin surplus and then the absence of insulin secretion, respectively.[7]

Many research workers reported a significant elevation in glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) activities in diabetic human[8] and animals.[7] Raised and decreased levels of alkaline phosphatase were also recorded in patients with diabetes.[9, 10]

In view of the relationship between zinc and diabetes, and the alteration of GOT, GPT and alkaline phosphatase associated with the diabetic state, this work was carried out to determine the effect of reduced dietary zinc on metabolic changes in alloxan-induced diabetes.

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Material and Methods

Animals and diet

Male normal Albino (Wistar) rats of 8 weeks of age with a body weight of 250 – 300 g were randomly divided into two groups of 20 each. 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 split food. Humidity was around 70 % and temperature was 22 ± 2° C. Food and distilled water were provided *ad libitum*. Animals were given access to either low zinc diet, 1mg Zn/kg or control diet, 54 mg Zn/kg. Zinc levels were checked in the diet as follows: Duplicates samples of 2 g were taken from each batch of diet for zinc analysis using Pye Unicam SP 9000 atomic absorption spectrophotometer. Modification of the American Institute of Nutrition[11] purified diet for rats and mice, were prepared. The dietary carbohydrate source was provided by equal amounts of corn starch 326 g/kg (ONAB EL Harrouch, Algeria) and sucrose 326 g/kg, protein 168 g/kg (egg white solids), lipids 80 (corn oil), fibre 40 g/kg (cellulose), vitamin mix 20 g/kg (Sigma), and mineral mix 40 g/kg. The latter was formulated to contain either

Table 1. Mean body weight gain (g/day), food intake (g/day), feed efficiency (Body weight gain/food intake×100) and liver fresh weight (g) of diabetic and non-diabetic rats given a low-Zn (1mg Zn/kg) or control (54 mg/kg) semi-synthetic diet for 26 days.

Animals	Diabetic				Non-diabetic			
Diet	Control		Low-Zn		Control		Low-Zn	
	(n= 10)		(n= 9)		(n= 10)		(n= 10)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Body wt gain	2.9[a]	0.07	1.2[b]	0.2	5.0[c]	0.06	3.4[d]	0.2
Food intake	17.0[a]	0.6	17.5[a]	0.2	12.6[b]	0.3	10.9	0.2
Feed efficiency	17.6[a]	2.0	7.1[b]	2.2	40.0[c]	3.7	32.3[d]	1.9
Liver fresh wt	12.4[a]	0.8	13.5[a]	0.9	12.9[a]	0.9	13.2[a]	0.6

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

Table 2. Mean blood glucose (mmol/l), serum zinc (µg/100 ml), pancreas dry wt (g), pancreatic zinc content (µg), pancreatic zinc concentration (µg/g dry wt) and serum GOT, GPT and alkaline phosphatase activities (UI/l) of diabetic and non-diabetic rats given a low-Zn (1mg Zn/kg) or control (54 mg Zn/kg) semi-synthetic diet for 26 days.

Animals	Diabetic				Non-diabetic			
Diet	Control		Low-Zn		Control		Low-Zn	
	(n= 10)		(n= 9)		(n= 10)		(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 zinc	80.6[a]	2.7	72.2[b]	3.9	114.8[c]	1.5	105.3[d]	2.7
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
Serum GOT	112[a]	10	137[b]	12	70[c]	1.6	80[d]	1.4
Serum GPT	86[a]	9.3	114[b]	7.3	19[c]	0.5	20[c]	1.0
Alkaline phosphatase	239[a]	21	178[b]	8.4	348[c]	27	272[d]	19

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

adequate (54 mg/kg) or deficient (1mg/kg) quantities of zinc, as determined by atomic absorption spectroscopy. Mineral mix supplied: calcium hydrogen orthophosphate 13 g/kg; disodium hydrogen orthophosphate 7.4 g/kg; calcium carbonate 8.2 g/kg; potassium chloride 7.03 g/kg; magnesium sulphate 4 g/kg; ferrous sulphate 0.144 g/kg; copper sulphate 0.023 g/kg; potassium iodate 0.001 g/kg; manganous sulphate 0.180 g/kg; zinc carbonate 0.1 g/kg. The low zinc diet contained no additional zinc carbonate. The diet was prepared as described by Fairweather-Tait et *al* [12] and Southon et *al*.[13] After one week of experiment, ten rats from each group were intraperitoneally injected with freshly prepared alloxan monohydrate solution (Alloxan; Sigma) in a dose of 150 mg/kg body weight to induce diabetes.[7] Diabetic rats were then pair-fed against non-diabetic rats in the same dietary group. Rats were maintained on the appropriate experimental diet for 26 days. Food intake and body weights were recorded regularly. Animals were fasted over-night on day 27, but allowed to feed for two periods of one hour each, between 11.00 -12.00 am and 17.00 -18.00 pm. Rats were then killed between 11.00 and 12.30 pm on day 28. One animal from each group was killed at approximately the same time by exsanguination from the heart, under diethyl ether anaesthesia. The blood was transferred into ice-cold centrifuge tubes and a portion taken for whole-blood glucose analysis, which was performed promptly after exsanguination. The remaining blood was centrifuged for 10 min at 3000 rpm and the serum was utilized for serum zinc, GOT, GPT and alkaline phosphatase assays. Livers were also rapidly excised and weighed. The pancreas was washed with isotonic saline (9 g NaCl/l distilled water) and blotted to dry. The pancreases were then weighed, dried at 80° C for 16 hours and zinc concentrations were determined.

Analytical methods

Glucose was measured in 10 µl samples of whole blood by the glucose oxidase method, using a YSI model 27 glucose analyzer. The dried pancreas 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). Standard reference materials, bovine liver and wheat flour were assayed using the same methods for zinc determination to assess zinc recovery. The recovery of zinc in the standard reference material exceeded 96 %. Zinc in serum was also measured in duplicate, after a twenty-fold dilution of serum in double distilled water by flame Atomic Absorption Spectrophotometer (Pye Unicam SP 9000). Zinc standards were prepared from a 1mg/ml zinc nitrate standard solution (BDH) using 5% glycerol to approximate the viscosity characteristics, and to avoid zinc contamination from exogenous sources. All tubes were soaked in HCl (10 % v/v) for 16 h and rinsed with double distilled water. GOT (EC 2.6.1.1), GPT (EC 2.6.1.2) and alkaline phosphatase (EC 3.1.3.1) activity were determined using commercial test kits, which were purchased from Biocom Company Ltd (Germany) for GOT/ and GPT and Biolabo Company Ltd (France) for alkaline phosphatase measurements, following the enzyme listing GOT, GPT[14] and alkaline phosphatase.[15] The results were compared using student's unpaired t-test for the effect of the zinc deficiency diet, and for the effect of diabetes. The results were also analyzed by one-way analysis of variance (ANOVA) where appropriate. A $p < 0.05$ was considered the limit for the statistical significance.

Results

The daily body weight gain of diabetic animals was significantly ($p < 0.05$) lower than those of the non-diabetic rats. The two diabetic groups had higher daily food intake compared with non-diabetic rats. Dietary zinc intake significantly ($p < 0.05$) altered the daily body weight gain of diabetic and non-diabetic animals and food intake of non-diabetic rats (Table 1). Serum and pancreatic zinc concentrations of diabetic rats were lower ($p < 0.05$) than those of non-diabetic rats. There was also low serum zinc level in both diabetic and non-diabetic rats fed on low zinc diet as compared to their control counterparts. However, there was no effect of dietary zinc on pancreatic zinc concentrations (Table 2). Blood glucose of diabetic rats either fed on low zinc or control diet was higher than that of non-diabetic rats and blood glucose of the two groups fed on low zinc diet was also higher than that of controls. As expected serum GOT and GPT of diabetic animals were significantly higher than those of non-diabetic animals, while alkaline phosphatase level of diabetic rats was lower than that of non-diabetic rats. The GOT level of diabetic and non-diabetic rats fed on low-Zn diet was significantly ($p < 0.05$) higher than in their control counterparts, and the GPT levels of low-Zn fed rats were also higher than those for controls but the differences were not always significant. Serum alkaline phosphates of low zinc fed animals were lower than those of control animals (Table 2).

Discussion

The present study shows that animals given alloxan to induce type I diabetes show elevated blood glucose levels and a decrease in body weight gain, although they consumed a larger quantity of food (hyperphagia) than did the non-diabetic animals. The mean daily value of consumed diet by rat is 14 g. This is comparable to that reported in the literature.[16] The mobilization of protein and fat stores may be responsible for the body weight loss noted in diabetic rats,[17] since food conversion efficiency of diabetic animals was lower than that of the non-diabetic animals. The low dietary zinc intake resulted in reduced food intake (non-diabetic animals), body weight gain, and serum zinc concentration in diabetic and non-diabetic rats. These results are in agreement with previously published reports.[18,19] Blood glucose is also affected by low-Zn diet. The higher blood glucose level observed in low-Zn fed animals may relate to altered glucose utilization by tissues or to the increased rate of endogenous glucose production.[20,21] Diabetic and non-diabetic rats fed on low zinc diet showed no differences in pancreatic zinc concentration, despite the fact that this tissue is generally regarded to be one of the most sensitive to variations in dietary zinc intake.[22] It appears, 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 may result in the maintenance of tissue zinc levels in the low zinc groups, despite the dietary concentration of zinc being fifty 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 results from this study clearly demonstrated the ability of both diabetic and non-diabetic animals to reduce zinc loss when dietary zinc intake was restricted. This may have been achieved by the decreased endogenous zinc secretion into the gastrointestinal tract [24,25] or by the high efficiency the animals to retain zinc.[26] On the other hand, pancreatic and serum zinc concentrations of diabetic groups were lower than that of the non-diabetic groups. This finding may be due to the ability of diabetic animals to excrete higher amounts of zinc in urine than normal ones,[3, 27] or to the degranulation, cytolysis and to other pathological changes in the pancreatic tissue, associated with progression of the condition.[28] It appears, therefore that diabetic rats were less able to adapt to conserving zinc. In this experiment there was a significant rise in serum GOT and GPT levels in diabetic rats, which could relate to excessive accumulation of amino acids (glutamate and alanine) in the serum of diabetic animals as a result of amino acids mobilization from protein stores.[17] These excessive amino acids are then converted to ketone bodies (α keto-glutaric and pyruvate) for which the enzyme GOT and GPT are needed, leading to increased enzyme activity. The higher levels of GOT and GPT in the low-Zn fed animals, may give rise to a high concentration of glucose. In other words, the gluconeogenetic action of GOT and GPT plays the role of providing new supplies of glucose from other sources such as amino acids. It is interesting to note that Greeley and Sandstead[29] found evidence of decreased oxidation of the carbon chain of alanine when zinc was restricted and led to alanine accumulation in the blood. The decrease in serum alkaline phosphatase activity in rats fed on low zinc diet is probably related to the decreased serum zinc concentration. Moreover, the observed variation in alkaline phosphatase

levels may result from the increased need of energy through glycolytic and oxidative pathways of glucose 6 phosphate, rather than alkaline phosphatase activity. Since these animals had higher blood glucose level,[30] coupled with low serum alkaline phosphatase activity in the diabetic compared to non-diabetic rats. This phenomenon could be attributed to the decrease in serum zinc level. However, Prasad *et al*[31] showed that zinc is present in several metalloenzymes such as alkaline phosphatase, and hence it is needed for their activities.

In conclusion, the combination of zinc deficiency and diabetes affected the activities of GOT, GPT and alkaline phosphatase. Zinc deficiency also appeared to contribute to the development of severe diabetes.

Acknowledgements
This work is supported by the research project N° F 2301/02/2002 funded by the Ministry of Higher Education, Algeria. We should thank members of Algiers Pasteur Institute for providing rats.

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