EFFECT OF REDUCED DIETARY ZINC AND EXPERIMENTAL DIABETES ON GOT, GPT AND ALKALINE PHOSPHATASE ACTIVITIES IN RATS

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Abstract

To investigate the effect of low dietary zinc intake and experimental diabetes (IDDM) on transaminases and alkaline phosphatase activities in rats, 8 weeks old male wealing normal albino (Wistar) rats were fed on semi-synthetic diet 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 then intraperitoneally injected with alloxan to induce diabetes. The rats were sacrificed after further three weeks. Body weight gain and food intake were recorded regularly. On day 28 after an overnight fast, animals were killed and blood glucose, serum zinc, serum glutamic oxalic transaminase (GOT), serum glutamic pyruvic transaminase (GPT) and serum alkaline phosphatase were determined. 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. Compared with non-diabetic rats both diabetic groups had higher food intake and lower serum zinc. Dietary zinc intake did not significantly alter the body weight gain, total food intake and serum zinc of diabetic or non-diabetic rats. In alloxan diabetes serum (GOT), (GPT) were significantly increased compared to normal rats, while the level of serum alkaline phosphatase was decreased. The consumption of low-Zn diet led to increasing of GOT, GPT. However, serum alkaline phosphatase was decreased.

***Key words**: Alloxan, Diabetic rats, Non-diabetic rats, GOT, GPT, Alkaline phosphatase.*

الملخص

مجموعتين من الفئران البيضاء من نوع Albino Rats (Wistar) ، كل واحدة عددها 20 المجموعة الأولى غذيت على غذاء عادي يحتوي على 54 ملغ زنك/كلغ من الغذاء والمجموعة الثانية غذيت على غذاء منقوص لعنصر الزنك 1ملغ/كلغ من الغذاء وهذا لمدة أسبوع. بعد ذلك لقد تم حقن من كل مجموعة 10 فئران بواسطة مادة الألوكسان لإحداث مرض السكر مع ترك الحيوانات لتتناول غذاءها و شرابها بحرية وهذا لمدة ثلاثة أسابيع أخرى بحيث تم في هذه المدة وزن الحيوانات وكذلك كمية الغذاء المستهلكة دوريا. ولقد تم ترك الحيوانات في حالة صيام خلال الليل ثم تم قتلها في اليوم الثامن والعشرين أي بعد أربع أسابيع. و من ثم تم تقدير ذلك سكر الجلوكوز في الدم الكلي وعنصر الزنك والإنزيمات الناقلة (GOT, GPT) وإنزيم الفوسفتاز القلوي في المصل. من النتائج المتحصل عليها نرى أن الوزن بالنسبة للحيوانات المصابة بمرض السكر منخفضا معنويا مقارنة مع الحيوانات الغير مصابة وهذا عند نهاية الدراسة. كذلك مقارنة مع الفئران الغير مصابة أن الفئران المصابة إستهلكت كمية عالية من الغذاء بالإضافة إلى ذلك نرى أن هناك نقص في تركيز عنصر الزنك في المصل بالنسبة للحيوانات المصابة. أما فيما يخص تأثير نقص عنصر الزنك في الغذاء على الوزن أو كمية الغذاء المستهلكة أو نسبة عنصر الزنك في المصل فلا يوجد هناك أي إختلاف سوى عند الحيوانات المصابة أو الغير مصابة. أما فيما يخص نشاط إنزيمات ناقلات الآمين فنلاحظ إرتفاع في نشاط هذه الإنزيمات لدى الحيوانات المصابة أو الغير مصابة بينما هناك إنخفاض في نشاط إنزيم الفوسفتاز القلوي، وان إستهلاك الغذاء المنقوص لعنصر الزنك أدى أيضا إلى زيادة إضافية في نشاط إنزيمات ناقلات الآمين في حين نلاحظ هناك إنخفاض في نشاط إنزيم الفوسفتاز القلوي.

مفتاح الكلمات: الألوكسان، فئران مصابة بمرض السكر، فئران غير مصابة بمرض السكر، الإنزيمات الناقلة(GOT, GPT) ، إنزيم الفوسفتاز القلوي.

Introduction

It is well known that zinc forms an integral part of crystalline insulin (1). The zinc content of the pancreas of diabetic animals has been found to be strikingly reduced (2). In addition hyperzincuria has been demonstrated in many diabetic subjects (3, 4). Diabetic children have been shown to have low hair zinc levels, which return to normal after insulin administration (5). Experimental diabetes was produced by intravenous administration of dithizone to animals (6) or intraperitoneal injection of alloxan (7). Experimental animals show a triphasic changes in blood sugar levels, initial hyperglycemia, hypoglycaemia, and finally hyperglycaemia. The developments of these phases of alloxan diabetes are mainly due to insulin deficiency, insulin surplus and then insulin lack, respectively (8).

Many workers reported a significant elevation in glutamic oxalic transaminase (GOT) and glutamic pyruvic transaminase (GPT) activities in diabetes (9). Raised and decreased levels of alkaline phosphatase were also recorded in diabetes (10, 11). In view of the relationship between zinc and diabetes, and the alteration of enzyme activities of GOT, GPT, alkaline phosphatase associated with the diabetic state. Therefore this work was carried out to determine the effect of reduced dietary zinc on metabolic changes of these enzymes in alloxan diabetes.

Material and methods

Male wealing normal albino (Wistar) rats of two months and initial body weight was 262 ± 8 ranging from 250-300 g, were randomly divided into two groups of 20. 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 and temperature were controlled with a 12 hours light/dark cycle. Food and water were provided *ad-lib*. The first group received a diet containing 1 mg Zn/kg (low zinc group), and the second group received a diet containing a 54 mg Zn/kg (control group). The recommended dietary zinc concentration for both mice and rats is 12 - 30 mg / kg, depending on the protein source (12). The composition of diet was similar to that described previously by Southon *et al* (13), but with egg albumin as the protein source. The low zinc diet was prepared by omitting zinc carbonate from the mineral mix. After one week ten rats from each group were injected intrapretonealy of freshly prepared alloxan monohydrate solution (Alloxan; Sigma) in a dose of 150 mg/kg of body weight (8) to induce diabetes. Diabetic rats were then pair fed against non-diabetic rats in the same dietary group. Rats were maintained on the appropriate experimental diet *ad-lib* for 26 days. Fasted over night and on day 27 given access to food for two periods of 1 hour between 11.00 - 12.00 hours and 17.00 - 18.00 hours so that time of feeding on day before death was similar for all groups. Rats were then killed between 11.00 and 12.30 hours on day 28. One animal from each group being killed approximately the same time by exsanguination from the heart whilst under diethyl-ether anesthesia. Blood was transferred into an ice cold centrifuged tubes and a portion taken for whole-blood glucose analysis which was performed promptly after exsanguination. The remaining blood was centrifuged for 10 minutes at 3000 rpm and the serum was utilized for serum zinc, GOT, GPT and alkaline phosphatase assays. Livers were also rapidly excised and weighed. Glucose was measured in 10 µl samples of whole blood by the glucose oxidase method, using a YSI model 27 glucose analyzer. Zinc in serum was analyzed, after a twenty fold dilution of the serum by flame Atomic Absorption Spectrophotometer (Py Unicam SP 9000). GOT, GPT and alkaline phosphatase activity were determined using commercial test kits following the enzyme listing GOT, GPT (14) and alkaline phosphatase (15). Comparison between the effect of diet and diabetes were made using Student' s unpaired *t. test*.

Results

Table I summarizes the body weight gain, food intake, feed efficiency and the liver weight of the animal groups studied. The body weight of diabetic animals at the end of four weeks of dietary manipulation were significantly lower than those of the non-diabetic rats. Compared with non-diabetic rats, both diabetic groups had higher food intake.

Dietary zinc intake did not significantly alter the body weight gain, liver weight and total food intake of diabetic or non-diabetic rats. Serum zinc concentration of diabetic rats was lower ($p < 0.05$) than those of non-diabetic rats. However, it was no effect of dietary zinc on serum zinc concentration (Table II). Blood glucose of diabetic rats either fed on also higher than those for controls but differences were not always significant. Serum alkaline phosphatase of low zinc animals were lower than those of control animals (Table II).

Table I. 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 28 days.

Animals	Diabetic				Non-diabetic			
Diet	control (n = 10)		Low-Zn (n = 9)		control (n = 10)		Low-Zn (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]	0.3	11.7[b]	0.5
Feed efficiency	17[a]	2.0	14[a]	2.2	40[b]	3.7	49[b]	2.2
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 values within a horizontal line with different superscript were significantly different ($p < 0.05$)

Table II. Mean blood glucose (m mole/l), serum zinc (µg/100 ml), 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 28 days.

Animal	Diabetic				Non-diabetic			
Diet	Control (n = 10)		Low-Zn (n = 9)		Control (n = 10)		Low-Zn (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[a]	2.7	75[a]	4.1	110[b]	2.5	106[b]	4.0
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 ($p < 0.05$

low zinc or control diet was higher than of non-diabetic rats and blood glucose of the two groups fed on low- Zn diet was also higher than their controls. As expected serum GOT and GPT of diabetic animals were significantly higher than of non-diabctic animals. Whilc alkalinc phosphatasc level of diabetic rats was lower than in non-diabetic rats. The GOT level of diabetic and non-diabetic fed on low-Zn diet was significantly higher than in their control counterparts, and the GPT levels of low-Zn rats were

Discussion

The present study shows that animals given alloxan to induce a type I diabetic state show elevated blood glucose levels and a decrease in body weight gain, although they consumed high food (hyperphagia) than did the non-diabetic animals. The daily mean of consumed diet by rat is 14 g (16), therefore the mobilization of protein and fat stores may be responsible for the body weight loss noted in the diabetic rats (17), as much as the food conversion efficiency of diabetic animals was lower than the non-diabetic animals. Body weight gain and food intake of diabetic and non-diabetic rats was unaffected by reduced dietary zinc (18, 19). However, blood glucose has been affected by low-Zn diet. The observed higher blood glucose in the present study of low-Zn animals may relate to altered glucose utilization by tissues or to the increased rate of endogenous glucose production (20, 21). Serum zinc concentration clearly demonstrated the ability of both diabetic and non-diabetic animals to reduce zinc loss when dietary zinc intake restricted. This may have been achieved by the increased efficiency of zinc absorption from diet (22), or decreased endogenous zinc secretion into the gastrointestinal tract (23, 24) or the animals was have high efficiency to retain zinc in their bodies (25). On the other hand serum zinc concentration of both diabetic groups was lower than of the non-diabetic groups. This finding may be due to the diabetic animals, which excrete higher amount of zinc in urine than normal ones (3, 26). It appears, therefore that the diabetic rats were less able to adapt to diabetic condition. In this experiment it was also found that there is significant rise in serum GOT and GPT levels in diabetic rats, which could relate to

excessive accumulation of amino acids (glutamic and alanine) in serum of diabetic animals as a result of amino acids mobilization from protein stores and it was not to any degranulation, cytolysis or other pathological changes in the liver tissue. This excessive amino acids is then converted to ketonic bodies (α keto glutaric and pyruvate) for which the enzyme GOT and GPT are needed, therefore activities of these enzymes are increased. Serum GOT and GPT levels were higher in the low-Zn animals than their controls. This finding confirms the result of high concentration of blood glucose found in these animals. In other words, the gluconeogenetic action of GOT and GPT plays role to provide new supply of glucose from other sources such as amino acids. It is interesting to note that Grefley and Sandstead (27) found evidence of decreased oxidation of the carbon chain of alanine when zinc restricted and this result leaded to alanine accumulation in blood. The decrease of serum alkaline phosphatase in rats fed on low zinc diet is unlikely related to the decreased serum zinc concentration, because the latter was unchanged. The observed variation in alkaline phosphatase could result from the increased call of energy through glycolytic and oxidative pathway of glucose 6 phosphate, rather than alkaline phosphatase activity. Since these animals had higher blood glucose than their controls (28). Serum alkaline phosphatase activity was also found low in the diabetic rats compared to non-diabetic rats. This result could be attributed to the decrease of serum zinc. Prasad *et al* (29) showed that zinc is present in several metalloenzymes such as alkaline phosphatase, and hence it is needed for their activities and it was also found that carbonic anhydrase activity was deceased in streptozotocin diabetic rats (30).

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

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