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The beneficial effect of vitamin E supplementation on zinc status, carbohydrate metabolism, transaminases and alkaline phosphatase activities in alloxan-diabetic rats fed on zinc deficiency diet

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Abstract
The aim of this study was to investigate the beneficial effect of vitamin E supplementation on zinc deficiency in experimental diabetes. Male alloxan-diabetic Wistar, albino rats of 10 weeks of age were divided into three groups. The first group received a diet containing 54 mg zinc/kg (adequate zinc group, AZ), the second group received a diet containing 1mg zinc/kg (zinc deficient group, ZD), and the third group received a diet containing 1mg zinc/kg supplemented with vitamin E (500mg/kg diet) (ZD+VE). Body weight gain and food intake of all rats were recorded regularly over a period of four weeks. On day 28, after overnight fasting, animals were killed and blood glucose, serum cholesterol, serum triglycerides, serum protein, serum urea, serum zinc, femur zinc, pancreatic zinc, testis zinc, liver glutathione concentrations and serum glutamic oxalic transaminase (GOT), serum glutamic pyruvic transaminase (GPT) and serum alkaline phosphatase activities were determined on blood and tissue samples. Body weight gain of zinc deficient diabetic animals at the end of four weeks of dietary manipulation was significantly lower than that of zinc adequate diabetic animals. Dietary zinc intake significantly increased blood glucose, serum cholesterol, serum triglycerides, and serum urea of zinc deficient diabetic rats. In contrast, serum zinc, femur zinc, pancreatic zinc, serum protein and liver glutathione levels were lower. The consumption of zinc deficient diet led also to an increase in serum GOT, GPT coupled with a decrease in serum alkaline phosphatase. Vitamin E ameliorated all the previous parameters. In conclusion, the present study demonstrates that vitamin E supplementation significantly reduced the severity of zinc deficiency in diabetes mellitus. (Int J Diabetes Metab 15: 46-50, 2007)

Key words: *Diabetic rats, alloxan, zinc deficiency, vitamin E, transaminases*

Introduction

Many of the features of zinc deficiency and essential fatty acid (EFA) deficiency are similar in humans and animals. These similarities include dermal lesion, poor growth, and bone and joint disorders. Several ways in which EFA and zinc may interact have been proposed. O'Dell[1] suggested that one point of interaction may be related to EFA oxidation, with zinc deficiency producing a high oxidation rate of unsaturated fatty acids in cell membranes leading to decreased membrane stability. Zinc plays a key role in the regulation of insulin production in pancreatic tissue. It seems reasonable therefore, that any changes in body zinc status could affect production, storage and secretion of insulin. There are several reasons for suspecting that an abnormal zinc metabolism could play a role in the pathogenesis of diabetes mellitus, which is accompanied by severe oxidative stress (especially lipid peroxidation) as a result of an increased oxygen free radical production.[2] Oxidative stress, induced by hyperglycemia, leads to an accelerated development of cellular and vascular damage. Bettger and co-workers[3] have reported that supplementation of zinc-deficient chicks with vitamin E significantly reduced the severity of skin and joint pathology. Dietary supplementation with the antioxidant vitamin E has been suggested as a possible means of controlling diabetic complications.[4,5] The administration of antioxidant, such as vitamin E may be needed to prevent damage to lipids by oxygen free radicals and thus signs of zinc deficiency in diabetic patients.[6] Therefore, the aim of this study was to examine the effects of dietary vitamin E supplementation and its beneficial effect on diabetic pathology observed in zinc deficient rats by evaluating body weight gain, food intake, zinc status, carbohydrate metabolism and some enzymes activities in alloxan diabetic rats.

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Materials and Methods

Animals and diet

Male albino (Wistar) rats of 10 weeks of age were housed in standards cages. Humidity and temperature were controlled with a 12-h light/dark cycle. Food and water were provided *ad-libitum*. After one week rats were injected intraperitoneally with a freshly prepared alloxan monohydrate solution (Alloxan; Sigma, UK) at a dose of

150 mg/kg of body weight[7] to induce diabetes. Alloxan diabetic rats manifested clinical and biochemical signs of diabetes. The animals were divided into three groups (12 rats per each) after the stabilization of diabetes. The first group received a diet containing a 54 mg zinc/kg diet (adequate zinc, AZ), the second group received a diet containing 1mg zinc/kg diet (zinc deficient group, ZD), and the third group received a diet containing 1mg zinc/kg diet supplemented with vitamin E (500mg/kg diet) (ZD+VE).[8] The composition of the diet was similar to that described previously by Southon et *al.* [9] Rats were maintained on the appropriate experimental diet *ad libitum* for 26 days. Body weight gain was recorded regularly. Animals were fasted overnight 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 was killed approximately at the same time by exsanguination from the heart whilst under diethyl-ether anaesthesia. Blood was transferred into ice cold centrifuge tubes and a portion was taken for whole-blood glucose analysis which was performed immediately after exsanguination. The remaining blood was centrifuged for 10 min at 3000 revolutions/min and the serum was utilized for serum zinc, cholesterol, triglycerides, urea, protein, GOT, GPT and alkaline phosphatase assays. Pieces of liver were rapidly excised, weighed, freeze-clamped at -196 C°, ground under liquid nitrogen and stored at -20 C° for glutathione analysis. The pancreas and testis were 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 pancreases, testes and femurs were weighed, dried at 80°C for 16 hours and zinc concentrations determined.

Analytical methods

Glucose was measured in 10 µl samples of whole blood by the glucose oxidase method, using an YSI model 27glucose analyser and the kit constitute of phosphate buffer containing the enzymes (GOD, POD) and D-glucose (Sigma). GOT, GPT and alkaline phosphatase activity were determined using commercial test kits for GOT, GPT [10] and alkaline phosphatise.[11] Cholesterol, triglycerides and urea concentrations were also determined using commercial test kits for cholesterol,[12] triglycerides,[13] and urea.[14] Serum total protein level was analyzed by the method of Lowry.[15] Liver glutathione (GSH) concentration was measured utilizing the method described by Weckbercker and Cory.[16] Dried pancreas, testis and femur were heated in silica crucibles at 480°C for 48 hours and the ash taken up in hot hydrochloric acid (11.7 M) for Zn analysis by atomic absorption spectrophotometer (Pye Unicam SP 9000).[17] The accuracy of zinc recovery was checked using standard reference materials; bovine liver and wheat flour. These standards were prepared and analysed in similar conditions to the test items to assess recovery. The recovery of zinc in the standard reference material exceeded 96 %. Zinc in serum was analysed after a twenty-fold dilution of the serum by Flame Atomic Absorption Spectrophotometer (Pye Unicam SP 9000), USA. 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. Statistical analysis was performed using Students' *t-test* and $p < 0.05$ was considered the limit for the statistical significance.

Results

Table 1 summarizes the body weight gain, serum zinc, femur zinc, pancreatic zinc and testis zinc concentrations of the animal groups studied. Body weight gain of diabetic zinc deficient animals (ZD) at the end of four weeks of dietary manipulation was significantly lower than those of adequate zinc diabetic rats (AZ). Compared with adequate zinc diabetic group, zinc deficient diabetic group had low serum zinc, femur zinc, pancreatic zinc and testis zinc levels. Vitamin E significantly increased the body weight gain, serum zinc, femur zinc, pancreatic zinc and testis zinc levels of zinc deficient diabetic rats. Blood glucose, serum cholesterol, triglycerides and urea concentrations of diabetic zinc deficient diabetic group (ZD) were higher than those of zinc adequate diabetic group (AZ). In contrast serum protein and liver glutathione concentrations of zinc deficient diabetic animals were lower than those of adequate zinc diabetic rats. Supplementation of the diet of zinc deficient alloxan diabetic rats with vitamin E restored most of the above parameters to values of diabetic rats fed on adequate zinc diet (Table 2). The GOT and GPT levels of alloxan diabetic rats fed on low zinc diet were significantly higher than of adequate zinc diet rats. However, the serum alkaline phosphatase of zinc deficient animals was lower than those of adequate zinc animals. Addition of vitamin E significantly reduced GOT, GPT and elevated alkaline phosphatase activities (Table 2).

Discussion

In this study, we clearly demonstrated the role of vitamin E supplementation in diabetic pathology associated with zinc deficiency. Rats fed on zinc deficient diet (DZ) had lower body weight gain compared with rats fed with adequate zinc diet (AZ). This is in agreement with some previously published reports.[18] This growth retardation was due in part to a decrease in appetite and impaired protein synthesis.[19] The mean fasting blood glucose concentration in animals fed with low zinc diet was higher than that of the adequate zinc group (AZ). This may relate to altered glucose utilization by tissues or to an increased rate of endogenous glucose production.[20] Serum zinc, femur, pancreatic and testis zinc concentrations in rats fed zinc deficient diet were lower than that of adequate zinc group. These findings, indicating the effect of low zinc diet on body zinc status, is in agreement with previous investigations.[21] The cholesterol, triglycerides and urea levels of animals that received zinc deficient diet were significantly higher than that of adequate zinc group. This might be due to the catabolism of lipid and proteins stores to produce energy. [22]

Table 1: Animals characteristics, serum zinc and tissues zinc concentrations of diabetic rats fed adequate zinc diet (AZ), zinc deficient diet (ZD) and zinc deficient diet supplemented with vitamin E (ZD+VE) at the end of experimental period.

Parameters	Experimental Group					
	AZ (n = 10)		ZD (n = 9)		ZD+VE (n =10)	
	Mean	SEM	Mean	SEM	Mean	SEM
Body weight gain (g/day)	3.6[a]	0.2	1.7[b]	0.1	3.3[a]	0.1
Serum zinc (µg/100ml)	117.8[a]	2.30	79.4[b]	5.31	102.8[c]	4.85
Femur Zn Concentration (µg/g dry wt)	151.9[a]	6.63	114.9[b]	3.41	139.8[a]	8.35
Pancreatic Zn Concentration (µg/g dry wt)	81.9[a]	5.36	44.4[b]	6.4	75.0[a]	5.74
Testis Zn concentration (µg/g dry wt)	152.7[a]	4	83.3[b]	5.9	134.2[a]	9.64

a, b, c: values within a horizontal line with different superscript letters were significantly different ($p < 0.05$). Values are means with their standard errors for n observation.

Table 2: Mean blood glucose, cholesterol, triglycerides, protein concentration, urea, GOT, GPT, alkaline phosphatase and liver glutathione levels of diabetic rats fed adequate zinc diet (AZ), zinc deficient diet (ZD) and zinc deficient diet supplemented with vitamin E (ZD+VE) at the end of experimental period.

Parameters	Experimental Group					
	AZ (n = 10)		ZD (n = 9)		ZD+VE (n =10)	
	Mean	SEM	Mean	SEM	Mean	SEM
Blood glucose (mg/100ml)	208.5[a]	11.5	291.6[b]	5.6	243.0[a]	15
Serum Cholesterol (mg/100m)	l 71.1[a]	2.63	109.4[b]	8.16	88.3[c]	2.4
Serum triglycerides (mg/100ml)	114[a]	4	351.7[b]	9.2	107.4[a]	8.16
Serum Protein (g/100ml)	6.2[a]	0.33	4.9[b]	0.12	5.77[a]	0.09
Serum urea (mg/100ml	73.3[a]	16.6	153.2[b]	15.7	110.3[c]	11
Serum GOT (U/l	74.7[a]	5.46	129.0[b]	6.03	98.9[c]	11
Serum GPT (U/l)	69.9[a]	11	129.2[b]	10	82.7[a]	6.8
Serum alkaline Phosphatase (U/l)	170.2[a]	14.1	76.1[b]	5	145.5[a]	12
Liver glutathione (nM/mg prot)	32.65[a]	3.06	23.05[b]	0.59	28.0[a]	1.66

a, b, c: values within a horizontal line with different superscript letters were significantly different ($p < 0.05$). Values are means with their standard errors for n observation.

We observed a significant rise in serum GOT, GPT activities in rats fed a low zinc diet compared to controls (AZ). This is in agreement with previous reports.[23] This finding confirms once again the result of high concentration of blood glucose found in these animals. In other words, the gluconeogenetic action of GOT and GPT plays a role in providing a new supply of glucose from other sources such as amino acids. It is interesting to note that Grefley and Sandstead [24] found evidence of decreased oxidation of the carbon chain of alanine when zinc was restricted resulting in alanine accumulation in blood. The decrease in serum alkaline phosphatase in rats given low zinc diet may be attributed to the decrease in serum zinc.

Prasad *et al*[25] showed that zinc is present in several metalloenzymes such as alkaline phosphatase, and hence it is needed for their activities. In general the present study indicated that some symptoms and signs associated with zinc deficiency in diabetic rats can be prevented by supplementation with vitamin E. The body weight gain of diabetic rats fed on low zinc diet supplemented with vitamin E (ZD+VE) was higher than that of zinc deficient diet (ZD)

group; this is in agreement with the results obtained by Kott *et al*[26] who noted an increase in body weight gain by older lambs supplemented with vitamin E. Serum zinc, and femur, pancreatic and testis zinc concentrations in diabetic rats fed zinc deficient diet supplemented with vitamin E (ZD+VE) are higher than those of the zinc deficient group. This result confirms the report of Hurley *et al*[27] who observed high plasma zinc concentration in rats supplemented with vitamin E. Zinc and vitamin E appear to interact during transport across biological membranes in a fashion dependent on their concentration in the membrane. In this study blood glucose was reduced in animals that received zinc deficient diet supplemented with vitamin E compared to zinc deficient rats. There are several possible explanations for the reduction of plasma glucose while on vitamin E supplementation. Supplementation of vitamin E might alter insulin receptors in muscle or adipose tissue by increasing membrane motility. In addition, vitamin E may enhance glucose uptake by the diaphragm.[28] There are also significant reductions in cholesterol, triglycerides and urea concentrations in animals fed ZD+VE compared with zinc deficient group. This finding corroborates that of Jain et al,[29] who reported that vitamin E supplementation can lower cholesterol and triglycerides levels in diabetic patients.

Transaminase activities were lower in ZD+VE compared to ZD group, normalising GOT and GPT levels after vitamin E supplementation. It could be concluded that this vitamin is capable of ameliorating the impaired hepatocellular function.[30] However, the increased activity of alkaline phosphatase in serum is probably a result of increased zinc concentration. The diminution in total protein might be due to microproteinuria, which is an important clinical marker of diabetic nephropathy,[31] and it might also be due to a reduction in protein synthesis in those on low zinc diet. The elevation of serum protein after vitamin E supplementation was probably due to decreased hepatic insulin resistance allowing insulin to stimulate the incorporation of amino acids into protein.[30] The decreased GSH concentration in low zinc diabetic rats, may be due to oxidative stress in animals with low zinc. In other words, the reduced antioxidant production was due to the increased oxygen metabolites, which caused a decrease in the activity of the antioxidant defense system.[32] The elevated glutathione level in diabetic animals that received vitamin E suggest that vitamin E decreased the level of free radicals thereby increasing the levels of antioxidants of diabetic rats to normal.[33, 34]

In conclusion, the combination of zinc deficiency and diabetes affected some biochemical parameters and liver function but dietary supplementation of vitamin E proved to be a most potent agent in protecting against the clinical disease associated with increased free radical activity, and had therapeutic benefits by reducing diabetic complications caused by zinc deficiency.

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