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The Effect of Zinc Deficiency on Zinc Status, Carbohydrate Metabolism and Progesterone Level in Pregnant Rats

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Aim: Our aim was to investigate the effect of low dietary zinc intake in late gestation on zinc status, transaminases and alkaline phosphatase activities, carbohydrate metabolism and progesterone level in albino (Wistar) rats.

Materials and Methods: Eight-week-old female pregnant and non-pregnant albino (Wistar) rats were fed with diets containing either adequate (54 mg/kg) or low zinc (1 mg/kg) quantities for 18 days. Food intakes and body weight gain were recorded regularly. On day 19, after an overnight fast, blood samples were collected and animals dissected. Femur, pancreas, placenta and fetuses were removed. Concentrations of glucose, cholesterol, and urea; transaminases and alkaline phosphatase activities; and progesterone level in serum and zinc amount in femur, pancreas, placenta and fetus were determined, respectively.

Results: The consumption of the low-zinc diet had an effect on animal characteristics and zinc status as indicated by the growth rate, food intake and femur, pancreas, placenta and fetus zinc concentrations. Dietary zinc intake also significantly altered glucose, cholesterol, urea, and progesterone concentrations and GOT, GPT and alkaline phosphatase activities of pregnant rats compared to their control counterparts.

Conclusions: These data revealed that reduction of zinc in the diet during pregnancy affected growth rate, food intake, fetus development, zinc status, carbohydrate metabolism and progesterone concentration, and caused disturbance in transaminases and alkaline phosphatase enzyme activities.

Key Words: Zinc, pregnancy, transaminases, carbohydrate, progesterone, alkaline phosphatase

Gebe Ratlarda Çinko Eksikliğinin Karbonhidrat Metabolizması, Çinko ve Progesteron Düzeylerine Etkisi

Amaç: Gebeliğin son döneminde düşük çinko içeren diyetin Wistar albino ratlarda, karbonhidrat metabolizması, transaminaz ve alkalen fosfataz aktiviteleri, çinko ve progesteron düzeylerine etkisinin araştırılması amaçlandı.

Yöntem ve Gereç: Sekiz haftalık gebe ve gebe olmayan Wistar albino ratlar ya normal (54mg/kg) ya da düşük(1mg/kg)düzeyde çinko içeren diyetle 18 gün süreyle beslendi.Gıda alımı ve ağırlık düzenli olarak kaydedildi. 19. gün, gece boyunca açlığı takiben, kan örnekleri alındı ve hayvanlar sakrifiye edildi. Femur, pankreas, plasenta ve fötus çıkarıldı. Serum glükoz, kolesterol, üre, transaminaz, alkalen fosfataz ve progesteron düzeyleri ile plasenta, pankreas, femur ve fötusun çinko düzeyleri ölçüldü.

Bulgular: Düşük çinko içeren diyetin, büyüme oranı, gıda alımı, femur, plasenta, pankreas ve fötusun çinko düzeylerinden de anlaşılacağı gibi ratların karekteristiğine ve çinko düzeylerine etkili olduğu görüldü. Kontrol grupları ile karşılaştırıldığında diyetle alınan çinkonun serum glükoz, kolesterol, üre ve progesteron düzeyleri yanında GOT, GPT ve alkalen fosfataz aktivitelerini de değiştirdiği saptandı.

Sonuç: Gebelikte düşük çinko içeren diyetle beslenme, büyüme oranını, gıda alımını, fötus gelişimini, karbonhidrat metabolizması, çinko ve progesteron düzeylerini olumsuz etkilerken, transaminaz ve alkalen fosfataz enzim aktivitelerinde de oynamalara yol açmaktadır.

Anahtar Sözcükler: Çinko, gebelik, transaminaz, karbonhidrat, progesteron, alkalen fosfataz

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Introduction

A dietary deficiency of zinc has been demonstrated in several species of animals and human. In the early studies with rats, growth retardation and skin lesion were found to be among the most obvious overt symptoms of the deficiency (1,2). Other symptoms include diminution of food intake leading to anorexia, thinning and depigmentation of hair, skin lesions around the neck and inside the hind legs and impairment of reproductive function in both male and female animals (3,4). Reproductive dysfunction includes reduced testicle size, lower spermatogenesis and retarded sexual maturity in the male, increased malformation in young and difficult parturition (5,6). In birds fed on inadequate zinc diet, egg reproduction and hatch ability are reduced, and often those hatched are weak with abnormal skeletal development and feathering (7). Numerous biochemical changes have been identified to occur as a result of dietary zinc deficiency. Zinc deficiency generally causes decreased tissue zinc content, but some tissues such as testes, bones, liver, kidney and muscles are more susceptible to zinc loss than the others (8). Biochemical changes within susceptible tissues include reduction of RNA and DNA synthesis (9), decreased protein synthesis (10), impairment of glucose tolerance (11), and a reduction in the activity of several enzymes including alkaline phosphatase and carbonic anhydrase (12). Therefore, in view of the alteration in zinc status associated with reproduction and in light of the above observations, this work was carried out to determine the effect of reduced dietary zinc on growth rate, food intake, zinc concentration in femur, pancreas, fetus and placenta, metabolic changes (glucose, cholesterol, urea), enzyme activities (glutamic-oxaloacetic transaminase-GOT, glutamic-pyruvic transaminase-GPT, alkaline phosphatase) and reproductive parameters such as progesterone hormone, in late gestation in albino (Wistar) rats.

Materials and Methods

Animals and diets

Female normal albino (Wistar) rats of two months 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 spilled food. Humidity and temperature were controlled with a 12-hour light/dark cycle. The animals were acclimated for seven days prior to mating, during which time they were fed a complete purified spray-dried egg white control diet and water ad-lib. After the seven-day period, half of each group (10 rats) was mated overnight with males of the same strain. Mating (day 0 of gestation) was confirmed by the presence of vagina plugs and sperm-positive smears. Then pregnant and non-pregnant animals received a diet containing either 1 mg zinc (Zn)/kg or containing 54 mg Zn/kg. The recommended dietary zinc concentration for both mice and rats is 12 - 30 mg/kg, depending on the protein source (13). The composition of the diet was similar to that described previously by Southon et al. (14), but with egg albumin as the protein source. The low-zinc diet was prepared by omitting zinc carbonate from the mineral mix. Rats were maintained on the appropriate experimental diet ad-lib for 17 days. They were then fasted overnight and on day 18 given access to food for two periods of 1 hour each between 11.00 - 12.00 hours and 17.00 - 18.00 hours so that time of feeding on the day before death was similar for all groups. Rats were then decapitated after being slightly anesthetized with diethyl ether between 11.00 and 12.30 hours on day 19. Animals were sacrificed by exsanguination from the heart whilst under diethyl-ether anesthesia. Blood was transferred into ice-cold centrifuged tubes and a portion taken for whole-blood glucose analysis, which was performed promptly after decapitation. The remaining blood was centrifuged for 10 minutes at 3000 round/minute and the serum was utilized for serum cholesterol, serum urea, serum zinc, GOT, GPT, alkaline phosphatase and serum progesterone assays. The uterus was examined intact, and the number of implantation sites was recorded. The uterus was then cut open, fetuses were removed, cleaned and weighed, the number of normal and resorbed fetuses was recorded, and fetuses from each litter were used for zinc determination. Maternal pancreas and placenta were also rapidly excised, washed with isotonic saline (9 g sodium chloride/l distilled water) and blotted to dry. The right femur was taken and connective tissues and muscle were removed. The pancreas, femur, fetus and placenta were then weighed and dried at 80°C for 16 hours, and then zinc concentrations determined.

Analytical methods

Glucose was measured in 10 µl samples of whole blood by the glucose oxidase method, using an YSI model 27 glucose analyzer. Zinc in serum was analyzed, after a 20-fold dilution of the serum by flame atomic absorption spectrophotometer (AAS) (Pye Unicam SP 9000). GOT, GPT and alkaline phosphatase activities and cholesterol and urea measurements were determined using commercial test kits following the enzyme and the biochemical listing for GOT and GPT (15), alkaline phosphatase (16), cholesterol (17) and urea (18). Progesterone concentration was estimated by enzyme-linked immunosorbent assay (ELISA), as described by Siekmann and Breuer (19). Dried pancreas, femur, fetus and placenta were heated in silica crucibles at 480°C for 48 hours and the ash taken up in hot hydrochloric acid (11.7 M) for zinc analysis by AAS (Pye Unicam SP 9000) (15). Bovine liver and wheat flour were used as standard reference materials to check the accuracy of zinc recovery using this method). These standards were prepared and analyzed identically to assess recovery. The recovery of zinc in the standard reference material exceeded 96%. The results were analyzed by one-way analysis of variance (ANOVA), multiple comparison test. A p value < 0.05 was considered the limit for statistical significance.

Results

Table 1 summarizes the body weight gain, food intake, and weight of fetuses of the animal groups studied. There were no significant differences in body weight gain and food consumption of pregnant animals at the end of 19 days of dietary manipulation as compared to non-pregnant rats (Table 1). In general, dietary zinc intake significantly alters maternal weight gain, fetus weight, and total food intake. Serum and femur zinc concentration in non-pregnant and pregnant rats fed on low-zinc diet were lower ($P < 0.05$) than in their control counterparts. However, pancreas zinc concentration was higher in low-zinc groups than control groups (Table 3). In addition, placenta and fetus zinc concentrations were significantly decreased in low-zinc pregnant rats compared to the control pregnant rats. Indexes of both resorbed and malformed fetuses are given in Table 2. There was a high percentage of resorbed fetuses and malformed fetuses in low-zinc pregnant rats. Blood glucose of pregnant rats fed on either low zinc or control

Table 2. Fetus indexes of pregnant rats fed on low-Zn (1 mg Zn/kg) vs control (54 mg/kg) diet.

Animals	Pregnant rats	
Diet	Control (n = 10)	Low-Zn (n = 10)
Total implant sites	75	73
Normal fetuses	73	58
Resorbed fetuses	2	15
Malformed fetuses	0	38
Resorbed (%)	2.6	20.5
Malformed (%)	0	65.5

Table 1. Body weight gain, food intake, and weight of fetuses of the animal groups studied [low-Zn (1 mg Zn/kg) vs control (54 mg/kg) diet].

Animals	Non-pregnant				Pregnant			
	Control (n = 10)		Low-Zn (n = 10)		Control (n = 10)		Low-Zn (n = 10)	
Diet	Mean ± SE		Mean ± SE		Mean ± SE		Mean ± SE	
Body weight gain	15.7[a]	1.5	10.1[b]	1	5.1[a]	0.7	9.4[b]	0.6
Food intake	21.8[a]	0.9	18.4[b]	0.8	21.5[a]	1	17.9[b]	0.7
Fetal body weight					3.4[a]	0.4	2[b]	0.3

[a, b]: Values within a horizontal line with different superscripts were significantly different ($P < 0.05$). Values are means with their standard errors for n observations.

Table 3. Investigated parameters of zinc status of non-pregnant and pregnant rats fed on low-Zn (1 mg Zn/kg) vs control (54 mg Zn/kg) diet.

Animals	Non-pregnant				Pregnant			
Diet	Control (n = 10)		Low-Zn (n = 10)		Control (n = 10)		Low-Zn (n = 10)	
	Mean ± SE		Mean ± SE		Mean ± SE		Mean ± SE	
Blood glucose	79.3[a]	7.9	139.4[b]	16	155c	12	201.1[d]	8.9
Cholesterol	186.4[a]	9.7	225.3[b]	10.3	265.7[b]	13.2	313.9[c]	16.4
Urea	41.2[a]	2.6	60.5[b]	3.5	74.4[c]	3.5	86.3[d]	3.4
Serum GOT	45.8[a]	3.4	65[b]	4.3	117.7[c]	2.7	160.9[d]	3.6
Serum GPT	37.7[a]	4.2	67.1[b]	2.2	117[c]	5.2	155.7[d]	6
Alkaline phosphatase	170.3[a]	8	165.7[b]	5.8	228.7[c]	14.5	180.7[db]	12.6
Progesterone	4.5[a]	0.2	13.9[b]	0.4	48.9[c]	2.6	75.3[d]	1.2
Serum zinc	20.3[a]	1.1	17.5[b]	0.07	17.5[b]	0.04	15.1[c]	0.8
Femur zinc	196.4[a]	27.3	119.2[b]	21.8	155.8[ab]	20.7	88.5[c]	5.2
Pancreas Zn	62.5[a]	16.1	161.1[b]	28.6	50.1[a]	10.2	143.2[c]	16.6
Fetus Zn					138.1[a]	2.6	87.8[b]	3
Placenta Zn					189.8[a]	7.3	95.8[b]	11.7

[a, b, c, d]: Values within a horizontal line with different superscripts were significantly different ($P < 0.05$). Values are means with their standard errors for n observations.

diet was higher than of non-pregnant rats. In addition, blood glucose of the two groups fed on low-zinc diet was higher than their controls. Both pregnant animal groups fed either on low-zinc or control diet had higher ($P < 0.05$) serum GOT and GPT activities than non-pregnant rats. Serum cholesterol and urea concentrations in low-zinc groups were also found to be higher than in normal zinc groups (Table 3). The GOT and GPT levels of pregnant and non-pregnant rats fed on low-zinc diet were significantly higher than in their control counterparts, whereas serum alkaline phosphatase activity of low-zinc diet animals were lower than those of control animals. As expected in pregnant state, serum progesterone concentration was significantly higher in pregnant rats than in non-pregnant rats and the consumption of the low-zinc diet led to a further increase in the concentration of progesterone (Table 3).

Discussion

In this experiment, body weight gain and food intake in pregnant and non-pregnant rats was affected by dietary zinc concentrations. This is in good agreement with some previously published reports (20). This raises the possibility of the metabolic state disturbance in animals, suggesting that the low-zinc condition reduced the ability of rats to utilize food intake as in normal subjects, and led to the decrease in fetal body weight and to the high incidence of resorbed fetuses, of total affected implantation sites and of malformed fetuses. These results are likely a result of the negative effect of low dietary zinc on formation and development of fetuses. The reduction in serum zinc and femur zinc concentrations in low-zinc groups compared with their controls is interesting in view of the finding that the growth rate of these animals was also significantly reduced. However, an increase was observed in pancreatic zinc concentration despite the fact that this tissue is generally regarded as being one of the most sensitive to variations in dietary zinc intake (21). 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 results in

the maintenance of tissue zinc level in the low-zinc groups, despite the dietary concentration of zinc being 50 times lower than in 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 (22). The zinc concentration in the analyzed fetuses and placentas was lower in the zinc-deficient than in the zinc-adequate group, and this finding was due to the less-absorbed maternal zinc being transferred to the fetus and placenta. In the current study, in which the time of feeding was strictly controlled and the amount of food eaten by each animal before an overnight fast was known to be comparable, the mean fasting blood glucose concentration in the low-zinc pregnant or non-pregnant rats was found to be higher than that of controls. This suggests that the lower zinc intake had exacerbated the ability of pregnant and non-pregnant 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-deficient diet

(23,24), suggesting a relation between carbohydrate utilization and the dietary zinc supply. A significant rise in serum GOT and GPT levels in pregnant rats was also observed, which could relate to excessive accumulation of amino acids (glutamic and alanine) in the serum of pregnant animals as a result of amino acid mobilization from maternal protein stores (25). These excessive amino acids are then converted to ketonic bodies (a keto glutaric and pyruvate) for which the enzymes GOT and GPT are needed; therefore, the activities of these enzymes are increased. Serum GOT and GPT levels were also found higher in the low-zinc animals than their controls. This finding confirms the result of a 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 Greeley & Sandstead (26) found evidence of decreased oxidation of the carbon chain of alanine when zinc was restricted, and this result led to alanine accumulation in the blood. Serum alkaline phosphatase decreased in rats fed on a low-zinc diet, and this result could be attributed to the decrease in serum zinc. Prasad et al. (27) showed that zinc is present in several metalloenzymes such as alkaline phosphatase, and hence it is needed for their activities. The elevation in cholesterol and urea concentrations in pregnant and non-pregnant rats fed on a low-zinc diet is probably due also to the mobilization of fat and protein stores, which are assumed to provide an alternate energy source. Serum progesterone was significantly increased in the low-zinc group. One possibility is that this increase might be due to the high percentage of cholesterol converted to progesterone.

In conclusion, zinc deficiency in pregnancy state affected the growth rate, food intake, and fetus development, zinc status, carbohydrate metabolism and progesterone concentration, and caused disturbances in the activities of some enzymes.

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