EXPERIMENTAL / LABORATORY STUDIES

Effect of Zinc Deficiency on Zinc and Carbohydrate Metabolism in Genetically Diabetic (C57BL/Ksj Db+/Db+) and Non-Diabetic Original Strain (C57BL/Ksj) Mice

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Abstract: Our aim was to investigate the effect of low dietary zinc intake on zinc and carbohydrate metabolism in type II diabetes. Male, 4–5-week-old, genetically diabetic (C57B/LKsJdb+/db+) and nondiabetic original strain (C57BL/KsJ) mice were fed a diet containing 1 mg of Zn/kg (low zinc groups) or 54 mg of Zn/kg (control groups) for 27 days. Food intake and body weight gain were recorded regularly. On day 28, after an overnight fast, the animals were sacrificed and blood glucose, serum insulin concentrations, liver glycogen contents, and zinc levels in the femur and pancreas were determined. The consumption of the low zinc diet had only a minimal effect on the zinc status of the diabetic mice as indicated by growth rate, food intake and femur and pancreatic zinc concentrations. In fact, diabetic mice fed on the low zinc diet had a higher total food intake than those fed on the control diet. The low zinc diabetic mice also had higher fasting blood glucose and liver glycogen levels than their control counterparts. However the growth rate and femur zinc concentration of the original strain mice were affected by the low zinc diet. To conclude, the present study demonstrates an adverse effect of reduced dietary zinc intake on glucose utilization in the genetically diabetic mice, which occurred before any significant tissue zinc depletion became apparent and also showed that the original strain of mice was more susceptible to the low zinc diet than were the diabetic mice.

Key Words: Strain, zinc depletion, diabetic mice (C57BL/KsJdb+/db+), nondiabetic mice (C57BL/KsJ)

Introduction

The effect of zinc deficiency on insulin and carbohydrate metabolism has been studied in some detail in laboratory animals (1-6) but in many instances the findings are difficult to interpret because of the effect of dietary zinc depletion on food intake (7). Consequently, there is no clear evidence demonstrating that inadequate dietary zinc is associated with changes in insulin secretion and action, although microscopic studies indicate that the *B-cells* of zinc deficient animals have decreased granulations and histochemically detectable insulin (8). The effect of low zinc status on hormonal action may, however, become apparent in conditions where insulin secretion and function are already abnormal. For example, it has been suggested that the altered zinc metabolism and reduced zinc status reported to occur in type II (maturity onset) diabetic subjects may aggravate insulin resistance, which is characteristic of this condition

(9). It has been speculated that where there is evidence of poor zinc status a program of zinc repletion may improve insulin sensitivity and reduce the severity of some of the complications of this disease (10,11). There appears to be little information, however, on the effect of varying dietary zinc intake on the progression and severity of this form of diabetes.

A semi-synthetic diet containing either 1 or 54 mg of Zn/kg was fed to 4- and 5-week-old male, genetically diabetic mice (C57BL/KsJ db+/db+). Diabetes (db) is inherited as a unit autosomal recessive for the diabetic gene (db/db) and displays symptoms similar to non-insulin dependent diabetes in humans, namely hyperglycemia, hyperinsulinemia and polyuria (12). Original strain mice (C57BL/KsJ) cannot be distinguished morphologically or physiologically from normal mice and were used for comparison purposes in the present study. Growth rate, food intake, zinc status, blood glucose and

insulin concentration and glycogen content in the liver were measured in the 2 dietary groups of each mouse genotype.

Materials and Methods

Animals and diets

The experiment was performed on 4- and 5-weeks-old male diabetic mice C57BL/KsJdb+/db+ and original strain mice C57BL/KsJ. Mice were randomly allocated in 2 groups. Approximately half of each genotype (20 diabetic and 8 nondiabetic) received a diet containing 1 mg of Zn/kg (low zinc groups) and the remaining animals (20 diabetic and 8 nondiabetic) received a diet containing 54 mg of Zn/kg (control groups). Modifications of the American Institute of Nutrition (13) purified diet for rats and mice were prepared containing a 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; zinc carbonate 0.1. The low zinc diet contained no additional zinc carbonate. The diet was prepared similarly to that of the reference (14,15). The recommended dietary zinc concentration for both mice and rats is 12-30 mg/kg (13). In this experiment mice were caged singly in polypropylene cages with stainless steel gridded tops and bottoms and stainless steel food hoppers, in a room kept at 21 °C, with humidity at around 70%, and a 12-h light/12 h dark cycle. Trays were placed under each food hopper to collect spilt food. Food and distilled water were provided ad libitum. Food intake was measured daily and body weight was recorded twice weekly for 28 days. Mice were maintained on the appropriate experimental diet ad libitum for 26 days. They were fasted overnight, and on day 27 they given access to food for 2 periods of 1 between 11:00 and 12:00 and 17:00 and 18:00 so that the time of feeding on the day before death was similar for all groups. Mice were then sacrificed between 11:00 and 12:30 on day 28. One animal from each group was killed at approximately the same time by cardiac puncture under ethyl-ether anesthesia. Blood was transferred into an ice cold heparinized vial and a portion was taken for whole blood glucose analysis which was performed promptly after exsanguination. The remaining blood was centrifuged and the plasma stored at -20 ºC before insulin assay. Livers were rapidly excised, weighed, freeze-clamped at -196 ºC, ground under liquid nitrogen and stored at -20 ºC before glycogen analysis. The pancreas was washed with isotonic saline (9 g of sodium chloride/l distilled water) and blotted to dry. The right femur was taken and connective tissues and muscle were removed. The right femur and pancreas were then weighed and dried at 80 ºC for 16 h and zinc concentrations were determined.

Analytical methods

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 a kit of phosphate buffer containing the enzymes (GOD, POD) and D-glucose (Sigma). Serum insulin was determined by a radio-immunoassay (16), using a rat insulin standard (NOVO, Research Institute, 4 ng/ml, Bagsvrend, Denmark),

[125]I-insulin (Amersham International, Bucks, England) and guinea pig anti-porcine insulin serum (Wellcome Reagents Ltd., Beckenham, England). The determination of total liver glycogen followed that of glucose following enzymatic hydrolysis with amyloglucosidase (EC 3. 2. 1. 3) obtained from *Asperiguillus niger*, (Sigma) (17). The dried pancreas and femur were heated in silica crucibles at 480 ºC for 48 h and the ash was taken up in hot hydrochloric acid (11.7 M) for zinc analysis by atomic absorption (Pye Unicam PU 9000) (5). The accuracy of zinc recovery using this method was checked by utilizing standard reference materials (bovine liver and wheat flour). The recovery of zinc in the standard reference material exceeded 96%. Comparisons between the effect of diet and the genotype were made using Student's t test.

Results

Although the total food intake of the low zinc diabetic animals was 7% higher (P < 0.01) than that of the

control diabetic mice, the body weight gain of diabetic mice fed on a low zinc diet for 27 days was not significantly different from that of their food control counterparts (Figure 1). The body weight gain of original strain mice fed a low zinc diet was lower (P < 0.01) than that of the controls (Figure 2). However, the amount of food consumed by the low zinc and control original strain mice was not significantly different. Values for growth rate and food intake were significantly higher for diabetic animals compared with the original strain (Table 1). Femur and pancreatic zinc levels taken as an index of zinc status indicated that both the low zinc diabetic and original strain mice were able to maintain a similar status to that of the control group, except for a significant reduction in femur zinc concentration in the original strain (C57BL/KsJ) mice fed a low zinc diet compared with the controls (Table 2). On day 27 of the study, time of feeding was controlled to allow a more accurate comparison of blood glucose and insulin concentrations between groups on day 28 when the mice were sacrificed following an overnight fast. Food intake measurements showed that there was no difference between genotypes or dietary groups in the amount of food consumed at either of the 2 meals on the day before sampling. Weights of food consumed at the last meal (Mean ± SEM) were (g) control groups: diabetic (0.9 ± 0.05), original strain (1 ± 0.1), low zinc groups: diabetic (0.8 ± 0.07), original strain (0.8 ± 0.02). Analysis of fasting blood glucose and plasma insulin concentration and liver glycogen content, indicated that low zinc diabetic mice had similar plasma insulin concentrations, higher fasting blood glucose levels (P < 0.05) and increased glycogen contents (P < 0.05)

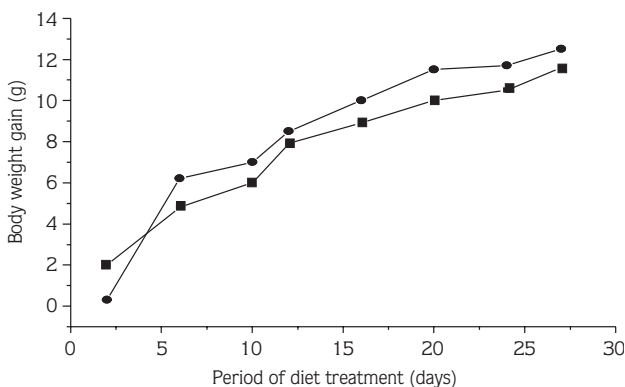compared with the control diabetic animals (Table 3). The higher liver glycogen values in the low zinc diabetic group were associated with a significant increase in liver weight (P < 0.05) together with a trend towards a higher glycogen concentration. Liver weight and liver glycogen content were similar in both the low zinc and control groups of the nondiabetic (original strain) mice (Table 3).

Discussion

In this experiment the body weight gain of diabetic mice was not significantly affected by low dietary zinc concentration (1 Zn/kg), although the diabetic mice fed on the low zinc diet had a higher food intake than those fed on the control diet. This is surprising in view of the many studies demonstrating reduced appetite in animals fed on low zinc diets (18,19) and raises the possibility that the degree and direction of response to dietary zinc depletion may be influenced by the metabolic state of the animals. The body weight gain of the original strain (C57BL/KsJ) mice fed a low zinc diet was lower than that of the control group, and this is in agreement with the results obtained with other animal species given inadequate dietary zinc (20,21). There was, however, no significant difference in the amount of food consumed by these 2 groups of mice; consequently, the food conversion ratio over 27 days was significantly higher in the control group compared with the low zinc group. This supports the work of Chesters and Quarterman (22), who found in force-feeding experiments that the low growth rate of zinc deficient rats was not entirely due to reduced food consumption, but also to other metabolic

Figure 1. Body weight gain (g) over 27 days for the diabetic mice (C57BL/KsJ db+/db+) fed a control (●) or low zinc diet (■).
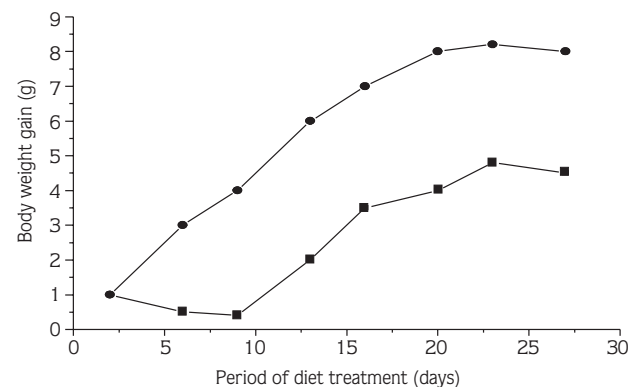

Figure 1. Body weight gain (g) over 27 days for the original strain (C57BL/KsJ) mice fed a control (●) or low zinc diet (■).

Table 1. Mean body weight gain (g), total food intake (g) and food conversion (body weight gain/food intake x 100) of diabetic and nondiabetic (original strain) mice given a a low zinc (1 mg of Zn/kg) or control (54 mg of of Zn/kg) semi-synthetic diet for 28 days.

Genotype	Diabetic				Nondiabetic (Original strain)			
Diet	Control (n = 20)		Low zinc (n = 19)		Control (n = 8)		Low zinc (n = 8)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Initial body-wt	19.6[a]	0.3	19.5[a]	0.1	15.2[b]	1.1	17.9[b]	0.3
Body-wt gain	11.9[a]	0.8	12.7[a]	0.7	7.8[b]	0.8	4.3[c]	0.4
Total food intake	121[a]	2	130[b]	2	93.8[c]	1.6	87.0[c]	3.1
Food conversion %	9.9[a]	0.6	9.8[a]	0.7	8.2[a]	0.7	4.9[b]	0.5

a, b, c values within a horizontal line with different superscript letters were significantly different
(P < 0.05), n: number of animals.

Table 2. Femur zinc concentration (µg/g dry wt), pancreas dry weight (mg), pancreatic zinc content (µg), and pancreatic zinc concentration (µg/g dry wt) of diabetic and nondiabetic (original strain) mice given a a low zinc (1 mg of of Zn/kg) or control (54 mg of of Zn/kg) semi-synthetic diet for 28 days.

Genotype	Diabetic				Nondiabetic (Original strain)			
Diet	Control (n = 20)		Low zinc (n = 19)		Control (n = 8)		Low zinc (n = 8)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Femur Zn Concentration	120[a]	3	121[a]	6	149.6[b]	2.9	116.7[a]	3.8
Pancreas dry wt	59[a]	3	57[a]	3	34[b]	1	33[b]	2
Pancreatic Zn content	3.2[a]	0.1	2.7[b]	0.1	3.6[ac]	0.2	3.4[ac]	0.1
Pancreatic Zn Concentration	56.5[a]	4.1	49.0[a]	3.8	108.1[b]	6.1	106.0[b]	5.9

a, b, c values within a horizontal line with different superscript letters were significantly different
(P < 0.05), n: number of animals.

Table 3. Mean fasting blood glucose (m mol), plasma insulin (µ units/ml), liver fresh wt (g), total liver glycogen (mg), and liver glycogen concentration (mg/g fresh wt) of diabetic and non-diabetic (original strain) mice given a low zinc (1mg Zn/Kg) or control (54 mg Zn/kg) semi-synthetic diet for 28 days.

Genotype	Diabetic				Nondiabetic (Original strain)			
Diet	Control (n = 20)		Low zinc (n = 19)		Control (n = 8)		Low zinc (n = 8)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Blood glucose	12.4[a]	0.4	15.3[b]	0.9	6.97[c]	0.5	6.9c	0.3
Plasma insulin	52.1[a]	5.6	46.0[a]	5.4	15.2[b]	3.3	9.4[b]	1.4
Liver fresh wt	1.86[a]	0.08	2.1[b]	0.5	0.84[c]	0.04	0.81[c]	0.02
Total liver glycogen	22.1[a]	3.5	32.7[b]	3.9	5.64[c]	1.3	3.77[c]	1.4
Liver glycogen Concentration	11.4[a]	1.5	15.7[a]	1.9	6.57[b]	1.5	4.54[b]	1.7

a, b, c values within a horizontal line with different superscript letters were significantly different
(P < 0.05), n: number of animals.

processes for which zinc is essential. The rate of growth of the diabetic mice, whether fed a low zinc or control diet, was markedly higher than for any of the nondiabetic groups of animals. This is likely to be related to the high food intake of these mice compared to the nondiabetic animals, or to the possibility that insulin-dependent enzyme activities (glucokinase, citrate lyase, glucose 6 phosphate dehydrogenase) are probably much higher in the adipose tissues of the diabetic (C57BL/KsJdb+/db+) mice because of their hyperinsulinemia. They would therefore be capable of converting glucose to carbon dioxide much more efficiently than the nondiabetic mice, thus producing the energy required for free fatty acids and fat synthesis, leading to obesity (12,23,24). Pancreatic zinc concentration was similar in dietary groups of the same genotype, despite the fact that tissue is generally regarded to be one of the most sensitive factors to variation in dietary zinc intake (25). 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 levels in the low zinc groups, despite the dietary concentration of zinc being 50 times lower than that 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 (26). The results from this study clearly demonstrated the ability of both diabetic and nondiabetic animals to reduce zinc loss when dietary zinc intake was restricted. This may have been achieved by decreased endogenous zinc secretion into the gastrointestinal tract (27). The lower pancreatic zinc concentration observed in the diabetic mice fed at both levels of zinc compared with that of the original strain is probably related to their hyperinsulinemia, the early onset of *B-cell* degranulation and other pathological changes in this tissue associated with the progression of the condition (12), and is consistent with human studies showing that the pancreatic zinc concentration of diabetics is depressed compared with that of healthy individuals (28-30). The reduction in femur zinc concentration in the original strain low zinc group, compared with the controls, is interesting in view of the finding that the growth rate of these animals was also significantly reduced. These mice appear, therefore, to be more susceptible to low dietary zinc intake than the diabetic animals studied. There are 2 possibilities, that

may explain the loss of femur zinc in these C57BL/KsJ mice. Less of the absorbed zinc may have been transferred to the relatively non-mobilisable pool of zinc in bones, thus preserving zinc in the metabolically active pool, or else bone zinc stores may have been mobilized to maintain tissue zinc levels. Despite the apparently minimal effects of consuming the low zinc diet on growth and zinc status of mice used in the present study, significant differences in glucose metabolism were observed. In this experiment, when the time of feeding was strictly controlled and the amounts of food eaten by each animal before an overnight fast were known to be similar, the mean blood glucose concentration in the low zinc diabetic mice was found to be 23% higher than that in the control diabetic group. This suggests that the lower zinc intake had exacerbated the reduced ability of the diabetic mice to utilize glucose. Results from the present study, and those from a previous study showing increased blood glucose concentrations after oral dosing in rats fed on a marginal zinc diet in late pregnancy (4), suggest a relation between carbohydrate utilization and dietary zinc supply, particularly in conditions associated with hyperinsulinemia and tissue in insulin resistance. Since the circulating insulin level in this study was unaffected by the reduced dietary zinc intake, the possibility of increased insulin resistance or reduced physiological potency of the hormone should be considered. Aside from the well known large accumulation of subcutaneous fat in the genetically diabetic mice, the most striking anatomical deviation is the size of the liver, which is in part due to metabolic defects resulting in increased fat and glycogen deposition (12). In the present study, the livers of diabetic mice were 2 times heavier than those in the original strain and it was noted that livers from low zinc diabetic mice were significantly heavier than those from their control counterparts. In addition, the fasting glycogen content of the low zinc diabetic mice was approximately 48% higher than that in the control diabetic animals, although food intake on the day before death was similar for these 2 groups. This again indicates that the carbohydrate metabolism of these animals is sensitive to reduced zinc intake. It is interesting to note that Reeves and O'Dell also found evidence of increased glycogen synthesis in zinc deficient rats (3). The fresh weights of liver from both groups of diabetic mice were higher than for any of the nondiabetic groups of animals. This is likely to be related to the higher glycogen content of the livers of these mice and possibly to other

compositional changes not considered in this study. The increased glycogen deposition in these mice may be related to the higher food intake of the diabetic mice, the high blood glucose concentration, and possibly to an increased activity of hepatic enzymes such as pyruvate carboxylase and phosphoenol pyruvate carboxylase, which increase the rate of gluconeogenesis in the liver.

In conclusion, the findings in the present paper demonstrate that reduced zinc intake had an adverse effect on glucose utilization in the genetically diabetic mice, although changes in zinc status appeared to be minimal. It appears therefore that abnormalities in carbohydrate metabolism may occur before tissue zinc depletion becomes apparent. However, the original strain mice were more susceptible to low dietary zinc than were the diabetic mice, in that the original strain mice given a low zinc diet had lower body weight gain and femur zinc concentration than the controls.

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References

1. Huber AM, Gershoff SN. Effect of dietary zinc deficiency in rats on insulin release from the pancreas. Nutr 103: 1739-1744, 1973.

2. Brown ED, Penhos JC, Recant L et al. Glucose tolerance, plasma and pancreatic insulin levels in zinc deficient rats. Exper Biol Med 150: 557-560, 1975.

3. Reeves PG, O'Dell BL. The effect of zinc deficiency on glucose metabolism in meal-fed rats. Brit Nutr 49: 441-452, 1983.

4. Fairweather-Tait SJ, Wright AJA, Cooke J et al. Studies of zinc metabolism in pregnant and lactating rats. Brit J Nutr 54: 401-413, 1985.

5. Kechrid Z, Nazan D, Abdenour C et al. Effect of low dietary zinc intake and experimental diabetes on the zinc and carbohydrate metabolism in rats. Turk J Med Sci 32: 101-105, 2002.

6. Quarterman J, Mills CF, Humphries WR. The reduced secretion of and sensitivity to insulin in zinc-deficient rats. Biochem Biophys 25: 354-361, 1966.

7. Boquist I, Lernmark A. Effect of the endocrine pancreas in Chinese hamsters fed zinc-deficient diets. Acta Pathol Microbol Scand 76: 215-228, 1969.

8. Kinlaw WB, Levine AS, Morley JE et al. Abnormal zinc metabolism in type II diabetes mellitus. Am Med 75: 273-277, 1983.

9. Mooradian AD, Morley JE. The role of zinc status in altered cardiac adenylate cyclase activity in diabetic rats. Am Nutr 45: 877-881, 1987.

10. Levine A, McClain C, Handwerger B et al. Tissue zinc status of genetically diabetic and streptozotocin-induced diabetic mice. Am Clin Nutr 37: 382-386, 1983.

11. Chausmer AB. Zinc, insulin and diabetes. Am Coll Nutr 1998, 17: 109-115, 1998.

12. Coleman DL, Hummel KP. Studies with the mutation diabetes in the mouse Diabetologia 3: 238-248, 1967.

13. American Institute of Nutrition. Report of the American Institute of Nutrition ad hoc Committee on Standards for Nutritional studies. Nut 107: 1340-1348, 1977.

14. Kechrid Z, Bouzerna N, Zio MS. Effect of low zinc diet on ^{65}Zn turnover non-insulin dependent diabetic mice. Diabetes Metab 27: 580–583, 2001.

15. Scott AM, Atwater I, Rojas EA. Method for the simultaneous measurement of insulin release and B-cell membrane potential in single mouse islets of langerhans. Diabetologia 21: 470–475, 1935.

16. Bergmeyer HU: Methods of enzymatic analysis, Academic Press. New York, San Francisco, London, 1963. vol. 3. p 434.

17. Mills CF, Quarterman J, Chesters JK et al. Metabolic role of zinc. Am Clin Nutr 22: 1240-1249, 1969.

18. McNall AD, Etherton TD, Fosmire GJ. The impaired growth induced by zinc deficiency in rats is associated with decreased expression of the hepatic insulin like growth factor I and growth hormone receptor genes. Nutr 125: 874-879, 1995.

19. Zhao C, Yang H, Jiang H et al. Effect of zinc deficiency on the distribution of elements in the tissue of pregnant rays and their fetuses. Chinese Med Sci 30: 277-279, 2001.

20. Kelleher SL, Lonnerdal B. Marginal maternal zinc intake in rat alters mammary gland Cu transporter levels and milk Cu concentration and affects neonatal Cu metabolism. Nutr 133: 2141-2148, 2003.

21. Chesters JK, Quarterman J. Effects of zinc deficiency on food intake and feeding patterns of rats. Brit Nutr 24: 1061-1069, 1970.

22. Ming-Derchen PL, Lin VC, Wen-Han L. Zinc supplementation aggravates body fat accumulation in genetically obese mice and dietary-obese mice. Biol Trace Element Res 52: 125-132, 1996.

23. John YJ, Scott K, Reaves YI et al. Zinc deficiency decreases plasma level and hepatic mRNA abundance of apolipoprotein A-I in rats and hamsters. Am Physiol 275: 1516-1525, 1998.

24. Williams RB, Mills CF. The experimental production of zinc deficiency in the rat. Brit Nutr 24: 989-1003, 1970.

25. Huber AM, Gershoff SN. Effect of dietary zinc and calcium on the retention and distribution of zinc in rats fed semi-purified diets. Nutr 100: 949-954, 1970.

26. Johnson WT, Canfield K. Intestinal absorption and excretion of zinc in streptozotocin-diabetic rats as affected by dietary zinc and protein. Nutr 115: 1217-1227, 1985.

27. Scott DA, Fisher AM. The insulin and zinc content of normal and diabetic pancreas. Clin Invest 17: 725-728, 1938.

28. Melinkeri RR, Sontake AN, Kulkarni AM. Serum zinc and zinc containing enzymes in diabetes mellitus. Ind Med Sci 44: 173-177, 1995.

29. Dura-Trave T, Villa EI. Actividad biologica del zinc. Acta Pediatr Esp 42: 27-33, 1984.