Serum zinc levels in diabetic patients and effect of zinc supplementation on glycemic control of type 2 diabetics

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ABSTRACT

Objectives: The present study is an attempt to assess serum zinc level in a sample of diabetic patients (both type 1 and type 2 diabetics) in comparison with those of apparently healthy controls, and to ascertain the relationship between the levels of serum zinc with some epidemiological variables. Furthermore, a trial of zinc supplementation for 3 months conducted to assess the effect of zinc supplementation on glycemic control of the studied type 2 diabetic patients, and the factors that affect the response to this supplementation.

Methods: Collection of data was carried out during the period between November 2002 to February 2003 at the Diabetic Center of Merjan Teaching Hospital in Babil Governorate, Iraq. In the first part of the study (a case-control study), the diabetic group included 133 diabetic patients (type 1 and 2) who were chosen from patients attending the Diabetic Center during the period of the study. The control group included 133 apparently healthy subjects who were selected from the workers of the same hospital. Selection of cases and controls was carried out by using systematic random sampling procedure. In the second part of the study (single blind were intervention study), type 2 diabetic patients (101 patients) divided into 2 groups; the first group included 50 patients supplemented with oral zinc sulfate (30 mg of elemental zinc/cap/day) for 3 months and second group included 51 patients given placebo and designed as control group.

Results: The first part of the study shows that the mean value for serum zinc level was significantly lower in diabetic patients than healthy controls (64.2 ± 12.6 μg/dl for type 1 diabetics, and 68.9 ± 11.9 μg/dl for type 2 diabetics versus 83.4 ± 12.5 μg/dl for healthy controls). Using simple linear regression, significant positive correlation was found between serum zinc level and years of education and significant negative correlation was found between serum zinc level and baseline HbA1c% value, in the diabetic group. While significant positive correlation found between serum zinc level and estimated zinc intake in the control group. Using multiple regression analysis, serum zinc level showed significant positive correlation with gender (being a male compared with female), and estimated zinc intake and significant negative correlation with diabetes state (diabetic compared with non-diabetic), residence (urban compared with rural residents), and plant protein intake. The second part of the study shows that the mean value for HbA1c% concentration of the supplemented group decreased significantly at the end of the 3 months of follow up, while no significant changes were found in the mean value for HbA1c% of the control group. The present study showed that the change in HbA1c% after supplementation had significant negative correlation with baseline HbA1c% value.

Conclusion: Diabetic patients have significantly lower mean serum zinc levels compared with healthy controls. Zinc supplementation for type-2 diabetics has beneficial effects in elevating their serum zinc level, and in improving their glycemic control that is shown by decreasing their HbA1c% concentration.

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Zinc is one of the essential trace elements that is required to maintain the normal physiological function of all forms of life.[1] Zinc occurs within a great variety of foods of both animal and plant origin. Zinc within animal products is more readily available than that within plant products.[2] Zinc has close interrelationships with the endocrine system and it is essential for normal growth, reproductive function, immune function, and glucose metabolism. Zinc deficiency has now been recognized to be associated with many chronic illnesses.[3] Diabetes mellitus (DM) is one of the diseases, which affect zinc homeostasis in different ways. The relationship between diabetes, insulin, and zinc is complex with no clear cause and effect relationship. The predominant effect of diabetes on zinc homeostasis is hypozincemia, which may be the result of hyperzincuria or decreased intestinal absorption of zinc or both.[4] Zinc has an important role in the glucose utilization by muscle and fat cells.[5] It is required as a co-factor for the function of intracellular enzymes that may be involved in protein, lipid and glucose metabolism.[6] Zinc may be involved in the regulation of insulin receptor-initiated signal transudation mechanism and insulin receptor synthesis.[7] Zinc also plays a key role in the synthesis, storage, and secretions of insulin by pancreatic tissue, and it accounts for the conformation integrity of insulin in its hexameric crystalline form.[8] Zinc may participate as an integral component of several antioxidant enzymes. Many of the complications of diabetes may relate to an increase in intracellular oxidant and free radicals associated with decrease in intracellular zinc and zinc dependent antioxidant enzymes.[9]

Laboratory tests for assessing zinc status are classified into 2 groups: those involving the analysis of zinc in body tissues or fluids and those testing zinc-dependent enzyme function.[10] The most often used approach to assess zinc status, particularly in large population studies, is the measurement of serum zinc levels.[11] The accepted reference range for serum zinc level is 70-120 μg/dl (10.7-18.4 mmol/L), and the level of 70 μg/dl (10.7 mmol/L) was used as cut-off value as an indicator of zinc deficiency.[10] There is no available information regarding the effect of zinc supplementation on glycemic control of Iraqi diabetic patients. This study is an attempt to assess serum zinc level in patients with DM in comparison with normal subjects, and to assess the effect of zinc supplementation on glycemic control of patient with type 2 DM.

Methods. The study was carried out during the period of November 2002 - February 2003. The first part of the study was a case-control study that included 133 diabetic patients (32 type 1 and 101 type 2 diabetics) who were selected from adult diabetic patients attending the Diabetic Center of Merjan Teaching Hospital, Babil Governorate, Iraq during the period of the study. The control group included 133 healthy subjects selected from the apparently healthy workers in the same hospital. Selection of patients and healthy controls was carried out by systematic random sampling technique. Serum zinc levels were assessed in diabetic patients and healthy controls.

The second part of the study was a single-blind, intervention study that includes only the selected, eligible type 2 diabetic patients who were on oral hypoglycemic drugs (101 patients). Patients in this part of the study were alternatively assigned to either the first group (who were supplemented with 30 mg/day of elemental zinc for 3 months) or the second group (who were given placebo, followed up for the same period, and designed as a control group). The HbA1c % levels were measured at the beginning of the study and after 3 months of follow up for both groups, and used as an indicator for glycemic control and a tool for evaluation of intervention during the follow up. The compound used in preparing the zinc supplement was zinc sulfate heptahydrate ($ZnSo4.7H_2O$), both zinc sulfate supplement and placebos were prepared as capsules by Ibin Sina Drug Development Center.

A structured questionnaire was used and completed by direct interview. Dietary intake was assessed for each subject using the 24-hour recall method. Dietary intake of zinc, calories and proteins, were calculated using food composition tables.

Height and weight were measured for subjects in both groups and used to calculate their body mass index that was used as an indicator of overweight or obesity. Waist and hip circumference ratio were measured to assess the fat distribution. Blood pressure was also measured and recorded for each subject. A sample of venous blood was drawn from each subject in the study following overnight fasting for 12 hours. Serum zinc level determination was carried out in Babylon Teaching Hospital laboratories using the atomic absorption spectrophotometer (the Buck model 210 GVP AAS). Fasting blood sugar (FBS) measurement was carried out using the calorimetric method, HbA1c% was assayed a few days after blood- sampling using improved colorimetric assay and both tests were carried out in Merjan Teaching Hospital laboratories.

Statistical analysis was carried out using SPSS version 10 computer software. The statistical significance of the difference in the mean of a continuous variable, which is known or assumed to

be normally distributed between 2 groups, was carried out by the use of sample t–test, while between more than 2 groups ANOVA test was used. Paired t-test was used to evaluate the statistical significance of mean change in a continuous variable after intervention (after-before comparison). The strength and direction of linear correlation between 2 quantitative continuous variables was tested with Pearson correlation coefficient. The *p*-value of less than the 0.05 level of significance was used as the criterion for determining statistical significance. Multiple regression models were used to study the independent and net effect of a set of independent (explanatory) variables on the response (dependent or outcome) variable.

Results. **Table 1** shows the mean and SD values for some epidemiological variables, estimated dietary intake and biochemical measurement of the studied groups with their significance. There was no significant difference between mean values for dietary zinc intake of the studied groups. Significant differences were noticed between mean ± SD values for total protein and animal protein intakes of diabetic patients and healthy controls. In addition, statistically significant difference was found between mean serum zinc level of diabetic patients and healthy controls. Mean serum zinc level was lower in type 1 diabetics compared with type 2 diabetics. In the current study, the mean ± SD value for dietary zinc intake of females versus males were equal to 10.8 ± 3.8 versus 13 ± 4.8 mg/day in the diabetics and 11.4 ± 3.8 versus 13.2 ± 4.7 mg/day in the control group.

The mean ± SD values for serum zinc level of males in the control group was 84.8 ± 13.2 μg/dl, (p=0.24), and in females was 81.8 ± 11.6 μg/dl, (p=0.24). In the mean ± SD values for serum zinc level of males in the diabetic group was 69.5 ± 14 μg/dl, (p=0.12) and in females was 66.2 ± 10 μg/dl, (p=0.12). Also, the mean ± SD values for serum zinc level of rural resident controls was 88.6 ± 13.2 μg/dl, (p=0.007), and in the urban resident controls was 80.9 ± 11.5 μg/dl, (p=0.007). In the mean ± SD values for serum zinc level of rural resident controls in the diabetic group was 68.7 ± 12.3 μg/dl, (p=0.45) and in the urban resident controls was 67.1 ± 12.2 μg/dl, (p=0.45). The present data showed that 82 diabetic patients (61%) and 20 healthy controls (15%) have serum zinc levels of <70 μg/dl (<10.7 mmol/L), and they were identified to have deficient serum zinc levels. Correlations between baseline serum zinc level and the studied variables are shown in **Table 2**. From these variables, only estimated zinc intake has significant positive correlation with serum zinc level in the control group. While in the diabetic patients, the table shows that serum zinc level has significant positive correlation with years of education. Base-line HbA1c% in type 2 DM patients was found to have a highly significant negative correlation with the level of serum zinc. To show the net and independent effect of a set of explanatory variables on baseline serum zinc level, multiple linear regression models were used. In the first step, all the possible explanatory variables entered in the model, and after serial infiltration, only 7 of these independent variables proved to have an effect on predictive power of the model. As shown in **Table 3**, the final regression model contained 7 independent variables listed according to decreasing magnitude of importance in their effect on serum zinc level.After controlling for the confounding effect of other independent variables included in the model, **Table 3** shows that having type 1 DM was associated with highest reduction in serum zinc level by a mean of 21.11 μg/dl, compared with healthy control. Next variable is having type 2 DM that is associated with reduction in serum zinc level by a mean of 12.56 μg/dl. All the 7 variables included in the last step of the multiple regression models had a statistically significant regression coefficient, and were able to explain 36% of variation in response variable.

The second part of this study, which is an intervention trial, involves type 2 DM cases of whom; 50 were supplemented with oral zinc, and 51 were supplemented with placebo and followed as a control group for 3 months. The number of patients who completed the 3 months of follow up was 86 (85%); of them 43 constitute the supplemented group and 43 constitute the control group. The remaining 15% of the patients were lost to follow up or showed non-compliance to the study regimen so they were excluded from the analysis.

Table 4 shows the mean ± SD values for serum zinc level, FBS concentration and HbA1c% changes after 3 months of follow up in the supplemented group compared with control group. As shown in the table, the mean serum zinc level for the supplemented group increased significantly by 4.3 ± 7.4 μg/dl (p<0.001). For the control group, the mean serum zinc level also significantly increased (p=0.004). The table also shows that the changes in the mean FBS for the supplemented group and that for the control group were found to be non-significant in both groups. However, the table shows that the mean HbA1c% of the supplemented group was decreased by 0.3%, and this change was statistically significant (p=0.01). While there was no significant changes in mean HbA1c% of the control group after 3 months of follow up.

Multiple regression analysis with backward elimination model was used to assess the effect of certain explanatory variables on change in the

Table 1 - The mean ± SD values for some epidemiological variables of the studied groups with their significance.

Variables	Healthy controls (mean ± SD) N=133	DM cases (mean ± SD) N=133		Significance
		Type 1 DM (N=32)	Type 2 DM (N=101)	
Age (in years)	39 ± 9.3	39.7 ± 18.1	54.6 ± 9.2	$p<0.001^{\dagger}$
Number of years of education	11 ± 4.8	5.1 ± 4.3	4.1 ± 4.7	$p<0.001^{\dagger}$
Crowding index	2.4 ± 0.8	2.6 ± 0.8	2.9 ± 0.9	$p<0.001^{\dagger}$
Cigarette smoking index	2.9 ± 7.5	1.4 ± 2.8	4.2 ± 9.8	$p=0.19^{*\dagger}$
Duration of DM in years	-	10.3 ± 12.8	5.1 ± 6	$p<0.002$
Body mass index (Kg/m^2)	26.9 ± 4.1	22.9 ± 5.6	28.6 ± 4.2	$p<0.001^{\dagger}$
Waist hip ratio	0.9 ± 0.1	0.9 ± 0.1	1 ± 0.1	$p<0.001^{\dagger}$
Estimated zinc intake (mg /day)	12.4 ± 4.4	10.7 ± 3.3	12.2 ± 4.6	$p=0.13^{*}$
Total protein intake (gm/ day)	78.2 ± 27.3	68.2 ± 22.4	61.9 ± 22.1	$p<0.001^{\dagger}$
Animal protein intake (gm/ day)	34.8 ± 23.4	31.6 ± 20.2	23.2 ±15.4	$p<0.001^{\dagger}$
Plant protein intake (gm/ day)	43.4 ± 15.3	36.6 ± 16.2	38.7 ± 15	$p=0.05^{*}$
Serum zinc level (μg/dl)	83.4 ± 12.5	64.2 ± 12.6	68.9 ± 11.9	$p<0.001^{\dagger}$
(μmol/L)	(12.8 ± 1.9)	(9.9 ± 1.9)	(10.6 ± 1.8)	

*not significant, †ANOVA analysis of variance, DM - diabetes mellitus

Table 2 - Correlation between baseline serum zinc level (μg/dl) and the studied variables with their significance in healthy controls and diabetic group.

Variables	Control group (r)	Cases with DM (r)
Years of education	0.004	0.23**
Estimated zinc intake (mg/day)	0.22*	0.02
Animal protein intake (gm/day)	0.11	0.08
Crowding index	-0.12	0.06
Waist hip ratio	0.07	0.05
Total protein intake (gm/day)	0.02	0.03
Calorie intake (kcal/day)	0.01	0.02
Age (in years)	-0.08	-0.03
Body mass index	0.04	-0.04
Plant protein intake (gm/day)	-0.14	-0.04
Cigarette smoking index	0.17	-0.05
Serum fasting blood sugar (mmol/L)	0.07	-0.05
Duration of disease (in years)	-------	-0.11
HbA1c% level - baseline (for type 2 DM)	-------	-0.33**

*Significant, $p<0.05$, **Highly significant, $p<0.001$, r- value is correlation coefficient, DM - diabetes mellitus

Table 3 - Multiple linear regression with baseline serum zinc level (in μg/dl) as the dependent variable and selected independent variables that had significant impact on improving the predictive power of the study.

Independent variables	ß	Standardized ß	*P*-value
Type 1 DM (compared with healthy controls)	-21.11	- 0.50	<0.001
Type 2 DM (compared with healthy controls)	-12.56	- 0.43	<0.001
Male gender (compared with female)	4.53	0.16	0.007
Plant protein intake (gm) (compared with animal protein)	-0.13	-0.14	0.02
Urban residence (compared with rural)	- 3.87	-0.13	0.02
Age (in years)	-0.13	- 0.12	0.03
Estimated zinc intake (mg)	0.012	0.11	0.04

DM - diabetes mellitus

Table 4 – Mean ± SD, change in serum zinc level (μg/dl), FBS (mmol/L) and HbA1c% value after 3 months of follow up in the supplemented group compared with control group.

Studied groups	Baseline levels mean ± SD	Levels after 3 months mean ± SD	Change in levels mean ± SD	Percent change after 3 months	*P* (paired t-test)
Serum zinc					
Supplemented group	64.1 ± 10.1	68.4 ± 8.7	4.3 ± 7.4	7.8 ± 12.6	$p<0.001$
Control group	73.3 ± 12.6	76.7 ± 12	3.4 ± 7.4	5.4 ± 10.6	$p=0.004$
FBS level					
Supplement group	10.6 ± 5.9	10.2 ± 4.5	-0.4 ± 6.3	15 ± 67.5	$p=0.72^{*}$
Control group	8.8 ± 3.8	9.8 ± 4.4	1 ± 4.4	24 ± 66.8	$p=0.15^{*}$
HbA1c% value					
Supplement group	8.1 ± 1.5	7.8 ± 1.3	-0.3 ± 0.8	2.9 ± 9.3	$p=0.01$
Control group	7.2 ± 1.3	7.2 ± 1.3	0 ± 0.9	0.8 ± 13.7	$p=0.94^{*}$

*not significant, FBS - fasting blood sugar

Table 5 - Multiple regression equation with change in serum zinc level and HbA1c% value as the dependent variables and selected independent variables among type 2 diabetic cases after 3 months of zinc supplementation.

Independent variables	Serum zinc level change		HbA1c% change	
	ß	*P*-value	ß	*P*-value
Age (in years)	0.22	0.07	-0.003	0.83
Gender (male compared with female)	1.47	0.53	-0.50	0.05
Cigarette smoking index	0.057	0.55	0.008	0.45
Body mass index	0.66	0.05	-0.026	0.43
Waist / hip ratio	-38.14	0.03	0.12	0.95
Estimated zinc intake	-0.022	0.933	0.012	0.65
Zinc serum level baseline (μg/dl)	-0.47	<0.001	0.017	0.13
HbA1c% value baseline	0.82	0.28	-0.28	0.001
Serum FBS level baseline	0.23	0.20	-0.010	0.61
P (model)	<0.01		0.009	
R^2	0.44		0.45	

FBS - fasting blood sugar

response variables (serum zinc level change and HbA1c% change), among the supplemented group of type 2 diabetics after 3 months of follow up (**Table 5**). After controlling for other independent variables included, only waist/hip ratio and base-line zinc level were found to have statistically significant correlation with the mean change in serum zinc level after 3 months of follow up. The resulting equation was statistically significant (p=0.01), and explains 44% of the observed variation in the response variable (serum zinc level change). Those with higher baseline zinc level at the start of the supplementation are expected to show a lower magnitude of change (response). For each unit (μg/dl) reduction in baseline zinc level, there is an increase in serum zinc level by a mean of 0.47 μg/dl. On the other hand, only baseline HbA1c% value was found to have a statistically significant negative correlation with the change in the HbA1c% after 3 months of follow up. The resulting equation was statistically significant, and explains 45% of the observed change in the response variable (HbA1c% change).

Discussion. A large body of experimental and clinical proof, especially in developed countries, supported alteration of zinc metabolism in patients with DM.[3,4] However, few studies examine the relationship between zinc and diabetes in developing countries.[12] The mean value for serum zinc levels in healthy individuals studied was within the normal range for healthy adults (11.4-17.8 μmol/L) reported by another study of zinc homeostasis in humans.[13] However, the minimum serum zinc value found in the present study (9.4 μmol/L) was lower than the lowest value reported by a number of studies carried out in Western countries.[14-16] Moreover, the mean serum zinc level of type 1 DM and type 2 DM patients found in the present study are much lower than the mean level reported in another study on zinc level in 18 subjects with type 1 DM and 22 subjects with type 2 DM that was equal to 18 ± 0.9 μmol/L.[17] Diabetic patient studied have also lower mean serum zinc levels compared with that reported in a study of 110 Tunisian adult diabetic subjects (both type 1 and type 2),[18] which showed that the mean ± SD value of plasma zinc level was 11.3 ± 0.18 μmol/L, and that 30% of the diabetic patients had serum zinc level below the cutoff level identified to show zinc deficiency (namely; 10.7 μmol/L), while in the present study 61% of the studied diabetic patients had serum zinc level below this cutoff level. The lower mean serum zinc level of diabetic patients in comparison to healthy controls noticed in this study is in agreement with many studies.[19-22] Furthermore, negative correlation was observed in this study between duration of diabetes (since diagnosis) and serum zinc level, and since type 1 diabetic patients studied have higher mean duration compared with type 2 diabetic patients, so the observed lower mean serum zinc level in type 1 diabetic group could be partly due to the longer duration of the disease they have in comparison to the type 2 group. A similar finding was reported by another study, which showed negative correlation between serum zinc level and duration of type-1 DM.[23]

In the current study, the mean dietary zinc intake of diabetic patients was found to be slightly lower in comparison to that of healthy subjects. This finding may partly explain the lower mean serum zinc level found in the studied diabetics, since it has been reported by many studies that circulatory zinc level (either plasma or serum) is a sensitive indicator of alteration

in dietary zinc intake.[16,24] Correlation analysis used in this study showed significant positive correlation between dietary zinc intake and serum zinc level in the healthy group only, which suggests that there could be other mechanisms apart from dietary zinc intake operating in diabetic patients that lowers their serum zinc level. In both type 1 and type 2 diabetes, impaired intestinal re-absorption of endogenous zinc and the increase in excretion of zinc into the intestine during the digestive process may lead to this low serum zinc level.[6] When all variables studied had been controlled in multiple regression models, serum zinc level still had significant correlation with estimated zinc intake in the studied subjects, which emphasizes the importance of consuming adequate zinc intake to maintain normal zinc level. This is in agreement with other studies.[12,25] Regarding protein intake, a positive correlation was found between serum zinc level and animal protein intake, while negative correlation was noticed between serum zinc level and plant protein intake in both healthy and diabetic individuals. Animal protein is considered as the main source for highly bioavailable zinc in the diet[26-28] thus, it can positively affect serum zinc level. This finding was also reported by several other studies that showed positive correlation between animal protein intake and estimated zinc intake.[29,30]

The multiple regression model used in this study showed a negative correlation between age and serum zinc level, when all other studied variables had been controlled, therefore, special attention should be paid to the diet of older diabetics to increase their dietary zinc consumption. This is consistent with results obtained by many studies, which showed that the elderly are at particular risk of zinc deficiency due to their low energy intake and poor dietary zinc consumption.[16,31,32]

In this study, a lower mean serum zinc value was noticed in females compared with males both in healthy and diabetic groups, which could be explained by their lower mean zinc intake compared with males. This finding is further confirmed in this study by the significant positive correlation obtained between gender (being male compared with female), and baseline serum zinc level, after controlling for other variables studied. This is in agreement with findings of several national food surveys suggesting that high a percentage of women worldwide were at particular risk of zinc deficiency as they consume less than the recommended zinc intake.[32,33]

In the present study, a positive correlation was found between serum zinc level and years of education in both diabetic and control groups, and it was significant in the diabetic group. This may reflect the positive effect of improvement in socioeconomic determinants on zinc status that may be related to the effect of socioeconomic factors on dietary zinc intake.[26] It has been shown that individuals with lower educational levels have chronically lower dietary zinc intake, that may be attributed mainly to selection of food with poor bioavailability.[34]

In the present study, mean serum zinc level was found to be higher in rural residents compared with urban residents in all studied groups, and residence in an urban area is expected to decrease the serum zinc level by a mean of 0.13 μg/dl. This is agreement with studies conducted in India reporting that serum zinc level and dietary zinc intake was significantly higher in rural Indian subjects compared with urban subjects.[35]

In the second part of this study, the increase in mean serum zinc level of the supplemented group after 3 months of follow up was found to be significant. In the control group, the significant change in mean serum zinc level observed after the trial can be explained by the possible change in pattern of dietary intake of these patients that occur due to enrolling them in the intervention study.

The mean value for HbA1c% of the supplemented group was reduced significantly by 0.3 ± 0.8% at the end of 3 months of follow up, while no significant change was found in the mean value for HbA1c% of the control group. The significant change in the supplemented group refers to the effective improvement in their glycemic control in response to zinc supplementation. This result is consistent with the results of other trials that were carried out on patients with type 2 DM to study the effect of zinc supplementation on their metabolic control.[5,18] The significant negative correlation noticed in the current study between serum zinc level change after supplementation and baseline serum zinc level suggested that the change in the mean value for serum zinc level after intervention is expected to be higher within groups of patients with lower mean value for baseline serum zinc level compared with other groups. This finding is consistent with results of other studies suggested that zinc treatment will have a high chance of success in changing the zinc status in the zinc-deficient subjects.[11]

In the present study, the mean HbA1c% change after supplementation was found to have negative correlation with baseline HbA1c% concentration, that is to say diabetic patients with higher baseline HbA1c% showed lower response to zinc supplementation in terms of change in mean value for HbA1c% concentration when the other variables had been controlled. It seems likely that there are

other factors affecting the level of HbA1c%, which perhaps ameliorate the beneficial effect of zinc supplementation in those patients.

References

1. Saradesai VM. Inorganic elements (minerals), Chapter 6: Introduction to clinical nutrition. 1st ed. **USA:** Marcel Dekker, Inc; 1998. p. 98-100.
2. Zheng JJ, Mason JB, Rosenberg IH, Wood RJ. Measurement of zinc bioavailability from beef and a ready-to-eat high-fiber breakfast cereal in humans: application of a whole-gut lavage technique. ***Am J Clin Nutr*** 1993; 58: 902-907.
3. Prasad AS. Zinc deficiency has been known of for 40 years but ignored by global health organizations. ***BMJ*** 2003; 326: 409-410.
4. Chausmer AB. Zinc, insulin and diabetes. ***J Am Coll Nutr*** 1998; 17: 109-115.
5. Song MK, Rosenthal MJ, Naliboff BD, Phanumas L, Kang KW. Effects of bovine prostate power on zinc, glucose and insulin metabolism in old patients with non-insulin dependent diabetes mellitus. ***Metabolism*** 1998; 47: 39-43.
6. Salgueiro MJ, Krebs N, Zubillaga MB, Weill R, Postaire E, Lysionek AE, et al. Zinc and diabetes. ***Biol Trace Elem Res*** 2001; 81: 215-228.
7. Tang X, Shay NF. Zinc has an insulin-like effect on glucose transport mediated by phosphoinositol-3-kinase and Akt in 3T3-L1 fibroblasts and adipocytes. ***J Nutr*** 2001; 131: 1414-1420.
8. Jindal RM, Taylor RP, Gray DW, Esmeraldo R, Morri PJ. A new method for quantification's of islets by measurement of zinc content. ***Diabetes*** 1992; 41: 1056-1062.
9. Szaleczky E, Prechl J, Pusztai P, Rosta A, Feher J, Somogyi A. Antioxidant status of patients with well controlled type 1 diabetes. ***Med Sci Monit*** 1997; 3: 163-166.
10. Milne DB. Essential trace elements, Section V, Chapter 30. In: Burtis CA, Ashwood ER. Tietz text book of clinical chemistry. 3rd ed. **Philadelphia (USA):** WB Saunders Company; 1999. p. 1029-1051.
11. Wood RJ. Assessing marginal zinc deficiency: where are we now and where are we going in the future? ***J Nutr*** 2000; 130: 1350S-1354.
12. Singh RB, Mohammad AN, Rastogi SS, Bajaj S, Gaoli Z, Shoumin Z. Current zinc intake and risk of diabetes and coronary artery disease and factors associated with insulin resistance in rural and urban populations in North India. ***J Am Coll Nutr*** 1998; 17; 564-570.
13. King JC, Shames DM, Woodhouse LR. Zinc homeostasis in human. ***J Nutr*** 2000; 130: 1360-1366.
14. Gibson RS, Martinez OB, McDonald AC. The zinc, copper and selenium status of selected sample of Cauadian elderly women. ***J Gerontol*** 1985; 40: 296-302.
15. Pilch SM, Senti FR. Analysis of zinc data from the Second National Health and Nutrition Survey. ***J Nutr*** 1985; 115: 1393-1397.
16. Bales CW, DiSilvestro RA, Currie KL, Plaisted CS, Joung H, Galanos AN, et al. Marginal zinc deficiency in older adults responsiveness of zinc status indicators. ***J Am Coll Nutr*** 1994; 13: 455-462.
17. Honnorat J, Accominotti M, Broussolle C, Fleuret A, Vallon J, Orgiazzi J. Effects of diabetes type and treatment on zinc status in diabetes mellitus. ***Biol Trace Elem Res*** 1992; 32: 311-316.
18. Anderson RA, Roussel AM, Zouari N, Mahjoub S, Matheau JM, Kerkeni A. Potential antioxidant effects of zinc and chromium supplementation in people with type 2 diabetes mellitus. ***J Am Coll Nutr*** 2001; 20: 212-218.
19. Walter RM, Uriu-Hare JY, Olin KL, Oster MH, Anawalt BD, Critchfield JW, et al. Copper, zinc, manganese, and magnesium status and complications of diabetes mellitus. ***Diabetes Care*** 1991; 14: 1050-1056.
20. Garg V, Gupta R, Goal R. Hypozincemia in diabetes mellitus. ***J Assoc Physicians India*** 1994; 42: 720-721.
21. Williams NR, Rajput WJ, West J, Nigdikar S, Foote J, Howard A. Plasma, granulocyte and mononuclear cell copper and zinc in patients with diabetes mellitus. ***Analyst*** 1995; 120: 887-890.
22. Car N, Car A, Granic M, Skrabalo Z, Momcilovic B. Zinc and copper in the serum of diabetic patients. ***Biol Trace Elem Res*** 1992; 32: 325-329.
23. Pedrosa LF, Ferreira SR, Cesarini PR, Cozzolino SM. Influence of glycemic control on zinc urinary excretion in patients with type 1 diabetes. ***Diabetes Care*** 1999; 22: 362-363.
24. Walsh CT, Sandstead HH, Prasad AS, Newberne PM, Fraker PJ. Zinc in health effects and research priorities for the 1990s. ***Environ Health Perspect*** 1994; 102: 5-46.
25. Lee S, Xiang M, Miller LV, Krebs NF, Lei T, Hambidge KM. Zinc absorption and intestinal losses of endogenous zinc in young Chinese women with marginal zinc intake. ***Am J Clin Nutr*** 1996; 63: 348-353.
26. De Jong N, Gibson RS, Thomson CD, Ferguson EL, McKenzie JE, Green TJ, et al. Selnium and zinc status are suboptimal in a sample of older new Zealand women in a community-based study. ***J Nutr*** 2001; 131: 2677-2684.
27. Johnson JM, Walker PM. Zinc and iron utilization in young women consuming a beef-based diet. ***J Am Diet Assoc*** 1992; 2: 1474-1478.
28. Lonnerdal B. Dietary factors influencing zinc absorption. ***J Nutr*** 2000; 130: 1378-1383.
29. Gerrior S, Bente L. Nutrient content of the US Food Supply, 1990-1997. Home Economics Research Report No 54. Washington, DC: US Department of Agriculture, Center for Nutrition Policy and Promotion; 2001.
30. Sandstrom B, Cederblad A. Zinc absorption from composite meals. II. Influence of the main protein source. ***Am J Clin Nutr*** 1980; 33: 1778-1783.
31. Kramer L, Spencer H, Osis D. Zinc and mineral content of weight reducing diets. ***Am J Clin Nutr*** 1981; 34: 1372-1378.
32. Wood RJ, Suter PM, Russell RM. Mineral requirements of elderly people. ***Am J Clin Nutr*** 1995; 62: 493-505.
33. Sandstead HH. Requirements and toxicity of essential trace elements, illustrated by zinc and copper. ***Am J Clin Nutr*** 1995; 61(suppl): 621-624.
34. Subar AF, Krebs SM, Cook A, Kahle LL. Dietary sources of nutrient among US adult 1989 to 1991. ***J Am Diet Assoc*** 1998; 98: 537-547.
35. Chadda SL, Radhakrishnan S, Ramachandran K, Kaul U, Gopinath N. Epidemiological study of coronary heart disease in urban population of Delhi. ***Indian J Med Res*** 1990; 92: 424-430.