Long term excessive Zn-supplementation promotes metabolic syndrome-X in Wistar rats fed sucrose and fat rich semisynthetic diet

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During the last two decades Zinc (Zn) as a micronutrient is being used indiscriminately in agricultural and husbandry practices and also in baby foods and multivitamin supplements with a view that Zn is non-toxic and promotes linear growth and body weight in the consumers. The long-term effect of increasing Zn load in the body has not been worked out so far. In this study, three groups of rats were fed on a semi-synthetic diet containing 20 mg (control, group-I), 40 mg (group-II) and 80 mg Zn/kg (group-III) diet respectively for 6 months. The results revealed that the gain in body weight increased in rats in Zn-concentration dependent manner. The urine examined on weekly basis showed glucosuria in group-II on week 10 and in group-III on week 8 and thereafter. The arterial blood pressure was significantly higher in group-II and III than their control counterparts on monthly basis. Histochemical examination of skin revealed an increase in the number of adipocytes filled with triglycerides making a subcutaneous fatty tissue thicker in group-II and group-III than that of control group. The blood profile after 180 days of dietary treatment, displayed a significant rise in glucose, total lipids, cholesterol, triglycerides, LDL-cholesterol, VLDL-cholesterol, insulin, cortisol and aldosterone whereas HDL-cholesterol, T3, T4 and TSH showed a reduction in their levels in the blood serum. The tissue metal status showed an increase of Zn, Cu and Mg in the serum, a rise in Zn in liver, hair and abdominal muscles and fall in Cu and Mg concentrations in liver, hair and abdominal muscles. This data suggest that Zn in excess in diet when fed for longer periods of time induces metabolic syndrome-X.

Keywords: Dyslipidemia, Hypertension, Metabolic syndrome-X, NIDDM, Obesity, Zn-supplementation

Metabolic syndrome-X includes abdominal obesity, dyslipidemia (increased cholesterol, LDL-c and decreased HDL-c), insulin resistance, raised blood pressure, elevated C-reactive protein and prothrombotic state. The prevalence of metabolic syndrome-X is increasing world over especially in young individuals and scientists are unable to contain it due to their restricted knowledge about the factors that trigger it. High fats and high sugar rich diet coupled with sedentary life style have been implicated as the main causes of the onset of metabolic syndrome-X.

Zinc is an essential micronutrient for normal growth and development and its deficiency has been linked with diabetes. It has been reported that an increase of Zn in diet to double the amount of recommended dietary allowance (RDA) increases the portion of the body fat in healthy children and leads to obesity. The percentage of Zn consumed from Zinc-fortified foods doubled from 14% for children surveyed in 1994 to 28% for children surveyed in 1998 in the US preschool children and this is also true for all age groups. Only 1% of the children have inadequate intakes. The percentage of this zinc intake from Zinc-fortified food will be increased over the time.

In spite of the fact that zinc is an essential micronutrient the use of zinc supplements in free-living population has been discouraged by some health professionals because doses greater than the recommended daily allowance is associated with copper deficiency. A positive association between the amount of fat and ratio of zinc to copper was found in 71-food items studied. High ratio of zinc to copper is associated with high concentration of serum cholesterol and high risk of IHD. A positive correlation between the zinc/copper ratio of hair and serum cholesterol was found in human beings.

The genetically obese mice (ob/ob), diabetic mice (db/db), zucker diabetic fatty rats (zef/brt) and transgenic rodents were used as a model of research to understand the etiology of metabolic syndrome-X. One of the important features of these animals is that they absorb and retain greater amount of Zn in liver, adipose tissue and tricep muscles than their lean...
control counter parts. They have greater potential of absorbing orally administered Zn from the lumen of gastrointestinal tract to blood plasma and can absorb 60% or more of glucose than those of lean control counter parts\textsuperscript{14-17}.

There are some evidences that both deficiency and excess of zinc can cause dyslipidemia and impair hepatic cholesterol and enhance oxidative stress\textsuperscript{18}. Further, high Zn-supplementation results in decrease in serum HDL-cholesterol\textsuperscript{19-21} and a significant increase in LDL-cholesterol\textsuperscript{21}. Lower HDL-cholesterol is associated with insulin resistance through increase in aldosterone secretion\textsuperscript{22}. Zn-supplementation also results in increased leptin production\textsuperscript{23,24} and leptin is associated with diabetes and hypertension\textsuperscript{25-29}.

The increased Zn mass action increases HbA\textsubscript{1C} and urinary Zn in both diabetics and normal individuals. Accordingly, excess Zn-supplementation results in altered glycosylation\textsuperscript{30}, and haemoglobin A\textsubscript{1C} is a reliable quantitative indicator of long-term hyperglycemia. Secondly, high Zn-supplementation in individuals with diabetes results in high serum Zn concentrations, and blocking of peripheral insulin receptors\textsuperscript{31} leading to decreased glucose tolerance\textsuperscript{32}. Thirdly, the use of zinc supplements in free-living population has been discouraged because it results in copper deficiency\textsuperscript{8,9,33,34}. And experimental data suggest that impairment of glucose tolerance can be secondary to copper deficiency\textsuperscript{35}.

It is believed that human individuals susceptible to syndrome–X are genetically predisposed to absorb and retain more Zn than their control counterparts like those of db/db mice and Zucker rats and on spontaneous development of diabetes, they start losing Zn in their urine. This led us to investigate if excess Zn in pharmacological doses in diet triggers the metabolic syndrome–X in rats, which are not genetically predisposed to metabolic syndrome-X. The results of this study are reported in this paper.

**Materials and Methods**

For the present investigations, a semi-synthetic diet rich in fat and refined sucrose was preferred over the standard rat pellet diet consisting of natural ingredients to keep the consistency of the composition of diet particularly of fat, sucrose and micronutrients through out the experiment and to rule out the possibility of Zn-interactions with fibers and phytates\textsuperscript{36,37} which are known to reduce bioavailability of Zn by binding it in digestive tract and may take longer time duration of feeding to manifest the impact of excessive Zn in diet.

Accordingly isocaloric semi-synthetic basal diet for the rats was prepared following modified Orgebin crist et al.\textsuperscript{38} It contained (g/100g of diet):

<table>
<thead>
<tr>
<th>Composition of basal diet</th>
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</thead>
<tbody>
<tr>
<td>Total Diet (g/100g)</td>
</tr>
<tr>
<td>Casein 30</td>
</tr>
<tr>
<td>Agar 2.0</td>
</tr>
<tr>
<td>Cornoil 5</td>
</tr>
<tr>
<td>Cellulose 8</td>
</tr>
<tr>
<td>Sucrose 51</td>
</tr>
<tr>
<td>Vitamin* Mixture 0.5</td>
</tr>
<tr>
<td>Mineral** Mixture 3.5</td>
</tr>
<tr>
<td>Total Diet 100</td>
</tr>
<tr>
<td></td>
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</table>

The basal diet was further divided into 3 parts and modified as: control diet consisting of basal diet containing 20 mg Zn /kg diet (per se) for Group-I, Zn supplemented- diet-I containing 40 mg Zn /kg diet for Group-II and Zn supplemented-diet-II containing 80 mg Zn /kg diet for Group-III by accordingly increasing ZnSO\textsubscript{4},7H\textsubscript{2}O in basal diet. For each diet, the mineral and water-soluble vitamins were ground in sucrose and fat-soluble vitamins were dissolved in corn oil. Agar, which served as a binder was dissolved in 25 ml triple distilled deionized warm water (60\textdegree C). On cooling to 40\textdegree C, the contents of each diet were thoroughly mixed in separate containers. The dough so formed was put in petridishes and solidified in refrigerator. The solidified diet was cut into small pieces of 2 × 2 × 2 cm size and stored in the container at the temperature > -4\textdegree C.

Male wistar rats (30) aged 6 weeks; weighing 60-70 g were procured from Central Animal House of the Panjab University, Chandigarh. They were maintained in plastic cages with stainless steel top grills at room
temperature (25°-28°C) with 14:10 hr L: D cycles and 70-80 % RH. They were fed on standard pellet rat feed for one week to acclimatize. Thereafter, rats were equally divided into 3 groups – I, II and III in such a way that their mean body weights remained almost similar in each group.

The animals were fed on their respective diets ad libitum and triple distilled deionised water was made freely available to them for 180 days. The body weight and blood pressure of rats were recorded at the beginning of the dietary treatment and thereafter every week. Ugobasile blood pressure recording instrument installed at Pharmacology Division, University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh, India, was used for recording the blood pressure and heart rates after anaesthetising the animals by injecting thiopentone sodium (25 mg/kg body weight) intraperitoneally. The urine samples were tested by Benedict’s test for diabetes every week. After the end of the dietary treatment of 180 days, the male rats of each group were sacrificed using diethyl ether as an anaesthesia.

The blood samples were collected by puncturing the heart and blood serum was prepared by centrifuging blood at 2500 rpm for 15 min. The freshly prepared serum was analyzed for glucose, cholesterol, triglycerides, HDL-cholesterol (all by using commercially available kits- Reckon Diagnostics PVT, LTD, Baroda, India and ERBA diagnostics manheim GmbH, Mannheim, Germany, By Transasia Bio-Medicals LTD, Daman.) and total lipids. The LDL and VLDL cholesterol were calculated by using Friedelwald’s equation. Hormone insulin was determined by a solid phase two-site enzyme immunoassay kit (DRG Diagnostics, USA) and cortisol was determined by enzyme immunoassay (Microtiter strips kit supplied by Immuno-Biological Laboratories, Hamburg).

Zn, Cu and Mg were estimated on atomic absorption spectrophotometer (Electronic Corporation of India Limited, Hyderabad–AAS 4139) using hollow cathode lamps (213.9, 324.8 and 285.2 nm for Zn, Cu and Mg, respectively). Samples of blood serum, tissues including liver, hair and abdominal muscles were digested separately in 3:1 v/v nitric acid and perchloric acid on a sand bath until a white ash formed. The ash was dissolved in 6 ml of 10 mM HNO₃ and filtered through ash-free filter paper before analysis. Standards for Zn, Cu and Mg (sigma) were prepared by dilution in triple distilled deionised water (TDW).

The skin separated from the ventral side of the abdominal region of rats of group-I (control), II and III was employed for the localization of adipocytes by light microscopy. Skin was fixed in formalin calcium and processed for cold gelatin sections and stained in Sudan Black B (SBB).

### Table 1

<table>
<thead>
<tr>
<th>Time duration (in days)</th>
<th>Group–I (Control)</th>
<th>Group–II</th>
<th>Group–III</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Body weight</td>
<td>Blood pressure</td>
<td>Body weight</td>
</tr>
<tr>
<td>0</td>
<td>68 ± 0.84</td>
<td>-</td>
<td>68.29 ± 0.68</td>
</tr>
<tr>
<td>30</td>
<td>162.29 ± 1.90</td>
<td>93.57 ± 0.92</td>
<td>192.57 ± 1.67*</td>
</tr>
<tr>
<td>60</td>
<td>205.57 ± 2.19</td>
<td>97.14 ± 1.01</td>
<td>269.57 ± 1.59</td>
</tr>
<tr>
<td>90</td>
<td>262.29 ± 2.35</td>
<td>98.57 ± 0.92</td>
<td>*319.86 ± 1.18a</td>
</tr>
<tr>
<td>120</td>
<td>280.86 ± 1.47</td>
<td>102.86 ± 1.84</td>
<td>*346.57 ± 1.27a</td>
</tr>
<tr>
<td>150</td>
<td>325.43 ± 1.19</td>
<td>104.29 ± 2.77</td>
<td>*361.43 ± 0.92a</td>
</tr>
<tr>
<td>180</td>
<td>378.14 ± 0.86</td>
<td>111.43 ± 0.92</td>
<td>*329.57 ± 1.86a</td>
</tr>
</tbody>
</table>

* Onset of diabetes mellitus as revealed by positive Benedict test of urine.

P values: * < 0.001(values of group-II and group-III were compared with group-I)
(Table 2) in group-II and III during the 180 days of dietary treatment.

After 180 days of dietary treatment, the number of adipocytes in control rats (group-I), were of small size, consisting of two irregular layers between dermis and subcutaneous layers of skin from ventral abdominal part. Some of them were very close to each other and some were separated by a space between them (Fig. 1). In group-II, the number of adipocytes increased from 2 layers in group-I to irregular four layers and the spaces between the adipocytes decreased. Most of them were lying very close to each other. Few adipocytes were seen to extend in dermis (Fig. 2). In group-III, there was a further increase in number of layers of adipocytes, consisting of 8 irregular layers between dermis and subcutaneous layer and almost 4 times thicker as compared to group-I. The adipocytes in large number were also seen extending in the dermis (Fig. 3).

After six months of dietary treatment, a significant rise in serum glucose, total lipids, cholesterol, triglycerides, VLDL-c, LDL-c was observed with a significant decrease in serum HDL-c in group-II and III (Table 3). Serum insulin, cortisol and aldosterone were increased significantly in group-II and III. But an unusual increase in serum aldosterone was observed in group-II than the group-III (Table 3). A significant decrease in serum T₃, T₄ and TSH in them in group-II and group-III rats as compared to control group-I (Table 4).

**Discussion**

The increase in body weight in three groups of rats varied significantly with the increase in Zn concentration in diet during the first 150 days of dietary treatment and thereafter it fell in group-II and III during the next 30 days with respect to their weight at 150 days than that of their control counter parts at day 180 of the experiment. It suggests that Zn is highly potent nutrient, which initially promotes the gain in body weight in concentration dependent manner but its prolonged treatment results in the reduction in body weights (Table 1).

A study on relation of Zn absorption and deposition of fat in ob/ob mice and high fat diet induced ICR-mice fed a high fat diet containing Zn in greater amount has been shown to increase significantly higher gain in body weight and more fat deposition identical to ob/ob mice than did their lean control counter parts fed a diet containing high fat but low zinc⁵⁴ lends support to this observation.

**Table 2—Month wise heart rates (beats/min) in male rats of Group-I [fed on basal diet (control)], Group-II (fed on Zn-supplemented-diet-I) and Group-III (fed on Zn-supplemented-diet-II) during 180 days of dietary treatment.**

<table>
<thead>
<tr>
<th>Time duration (in days)</th>
<th>Group-I (Control)</th>
<th>Group-II</th>
<th>Group-III</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>182.8 ± 61.84</td>
<td>287.14 ± 3.60a</td>
<td>350.0 ± 3.09a</td>
</tr>
<tr>
<td>60</td>
<td>201.43 ± 0.92</td>
<td>308.57 ± 2.37a</td>
<td>385.71 ± 1.70a</td>
</tr>
<tr>
<td>90</td>
<td>215.29 ± 0.29</td>
<td>314.29 ± 3.69a</td>
<td>400.0 ± 3.09a</td>
</tr>
<tr>
<td>120</td>
<td>232.14 ± 3.25</td>
<td>355.71 ± 2.02a</td>
<td>417.14 ± 4.74a</td>
</tr>
<tr>
<td>150</td>
<td>241.43 ± 0.92</td>
<td>377.14 ± 7.14a</td>
<td>452.86 ± 3.60a</td>
</tr>
<tr>
<td>180</td>
<td>248.57 ± 0.92</td>
<td>410.0 ± 3.09a</td>
<td>457.14 ± 1.84a</td>
</tr>
</tbody>
</table>

*P values: * < 0.001(values of group-II and group-III were compared with group-I)

**Table 3—Blood profile of male rats of Group-I [fed on basal diet (control)], Group-II (fed on Zn-supplemented-diet-I) and Group-III (fed on Zn supplemented-diet-II) after 180 days of dietary treatment**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group-I (Control)</th>
<th>Group-II</th>
<th>Group-III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Lipids*</td>
<td>205.95 ± 1.54</td>
<td>249.28 ± 0.71a</td>
<td>312.86 ± 0.36a</td>
</tr>
<tr>
<td>Cholesterol*</td>
<td>61.65 ± 1.50</td>
<td>82.70 ± 1.50a</td>
<td>114.9 ± 0.83a</td>
</tr>
<tr>
<td>Triglycerides*</td>
<td>65.93 ± 1.55</td>
<td>105.49 ± 1.42a</td>
<td>122.64 ± 0.44a</td>
</tr>
<tr>
<td>VLDL-Cholesterol*</td>
<td>13.19 ± 0.31</td>
<td>21.09 ± 0.28a</td>
<td>24.53 ± 0.09a</td>
</tr>
<tr>
<td>HDL-Cholesterol*</td>
<td>18.71 ± 0.34</td>
<td>14.63 ± 0.34a</td>
<td>11.64 ± 0.27a</td>
</tr>
<tr>
<td>LDL-Cholesterol*</td>
<td>29.76 ± 1.31</td>
<td>46.98 ± 1.94a</td>
<td>78.80 ± 0.47a</td>
</tr>
<tr>
<td>Glucose*</td>
<td>75.27 ± 0.77</td>
<td>157.14 ± 1.5a</td>
<td>206.59 ± 1.09a</td>
</tr>
<tr>
<td>Insulin*</td>
<td>17.93 ± 1.29</td>
<td>72.05 ± 0.85a</td>
<td>107.62 ± 1.12a</td>
</tr>
<tr>
<td>Cortisol*</td>
<td>52.29 ± 4.29</td>
<td>102.14 ± 4.74a</td>
<td>108.29 ± 3.75a</td>
</tr>
<tr>
<td>Aldosterone*</td>
<td>135.36 ± 5.07</td>
<td>441.14 ± 19.36a</td>
<td>387.5 ± 23.15a</td>
</tr>
<tr>
<td>T3</td>
<td>246.57 ± 0.37</td>
<td>212.14 ± 1.01a</td>
<td>231.43 ± 0.09a</td>
</tr>
<tr>
<td>T4</td>
<td>9.76 ± 0.02</td>
<td>8.31 ± 0.03a</td>
<td>8.03 ± 0.02a</td>
</tr>
<tr>
<td>TSH</td>
<td>1.08 ± 0.01</td>
<td>0.91 ± 0.009a</td>
<td>0.34 ± 0.009a</td>
</tr>
</tbody>
</table>

Units: *mg/dl; + pmole/L; * ng/ml; $ pg/ml; ^ ng/dl; ^ ug/dl; $ μU/ml.

*P values: * < 0.001(values of group-II and group-III were compared with group-I)
Figs 1-3—Photomicrographs of V.S of skin of rats (Formalin Calcium/SBB) showing subcutaneous adipocytes in 1, Group-I (Control); 2, Group-II (Zn-supplemented diet-I); 3, Group-III (Zn-supplemented diet-II) [A = subcutaneous adipocytes containing sudanophilic granules; D = dermis; SCML = subcutaneous muscular layer]
The supplementation of Zn promotes food intake, linear growth and body weight increase in children suffering from marginal to severe Zn-deficiency. Investigatory studies on laboratory animals, however, provide evidences that the anabolic effects of Zn result from higher absorption of nutrients i.e. amino acids, fatty acid and glucose in addition to the activation of protein and nucleic acids syntheses. These anabolic effects of Zn are unlikely to diminish after cessation of growth. The continuous input of excess nutrients in tissues by excessive bioavailability of Zn after adolescence may create environment essential for obesity. It lends support to the report that an increase of Zn in diet to double the amount of recommended dietary allowance (RDA) increases the portion of the body fat during 25 weeks period of dietary treatment in healthy children. A positive linear correlation between Zn concentration in their hair than those with normal body weight for their height and BMI. Zn promotes deposition of fat was confirmed in the present investigations by employing Sudan Black B-test on a portion of skin from the ventral side of the abdomen after six months of dietary treatment. The histochemical studies of skin clearly revealed an increase in adiposity in Zn concentration dependent manner and support the observation of Pomp et al. that Zn increases the number of adipocytes leading to obesity. The deposition of fat in group-II and III supports the proposed potential mechanism for development of obesity involving Zn based on the observations on the activation of ovine metallothioneine ovine growth hormone transgene (OMT-la-OGH) by ZnSO₄ as it activates the genes of growth hormone during early period of growth and induces the relatively undifferentiated preadipocytes to be committed to become adipocytes. This may increase their population. The enhanced population of adipocytes responds by filling with triacylglycerol due to increased absorption of nutrients caused by Zn and eventually leads to the observed state of obesity. Further, the elevated growth hormone level may increase the insulin level which lead to increased glucose transport into adipocytes, thereby enabling them to increase lipogenesis. Therefore, the over supply of Zn during growth phase sets in the conditions for the onset of obesity.

However the obese human subjects possess either lower or normal plasma level of Zn compared to normal control. The lower plasma Zn level in obese individuals can return to normal following dietary therapy suggesting increased requirement of Zn in them.

Infact, plasma Zn status does not truly reflect body Zn status as it tends to maintain Zn-homeostasis either by increasing Zn-absorption or decreasing Zn excretion, depending upon Zn-intake and metabolic requirement. Therefore no significant difference can exist between BMI and serum zinc in healthy subjects. Tissue Zn status is thus more reliable to determine relationship between the two. It is evident that the hair Zn concentration increases with increase in BMI in Indian women. And hair Zn reflects chronic Zn status over the period of hair growth and is more stable indicator of body Zn reserves than is plasma Zn. Thus the present study clearly supports the fact that excessive zinc either in food chain or due to genetic disorder results in the obesity.

The estimation of insulin, T₃, T₄ and TSH in blood serum revealed an increase in insulin and fall of T₃, T₄ and TSH in group-II and III rats during present investigations (Table 3) estimated at the end of the experiment. This suggests that the lipogenesis in adipocytes is promoted by increase in insulin level accompanied by cutting down the basal metabolic rate or reduction in thermogenesis by depressing T₃, T₄ and TSH release from thyroid and pituitary glands. This is also supported by the fact that decreased T₃ concentration in diabetes mellitus is a result of inhibition of hepatic 5'-deiodinase enzyme activity, which is responsible for the conversion of T₄ to T₃. The reduction in T₄ response may have resulted from impaired T₄ synthesis or release by the thyroid gland.

Obesity poses a formidable challenge to the growing population as it is etiologically linked to insulin resistance; an accompanying insulin dependent diabetes mellitus, hypertension, and coronary artery disease. Insulin resistance results in hyperglycemia, resulting in glucosuria and hyperzincuria. McNair et al. confirmed hyperzincuria relationship to the degree of hyperglycemia but not glucosuria. Chausmen has postulated that hyperglycemia interferes with the active transport of Zn back into the renal tubular cell and results in hyperzincuria.

High dose Zn-supplementation in diabetes and normal individuals resulted in more hyperzincuria and increase in haemoglobin A₁C in both diabetes and normal individuals. Epidemiological studies have
shown that hyperzincuria is a consistent feature in NIDDM and has been associated with hyperglycemia and haemoglobin A1c in patients with type-II diabetes. In the present study, the glucose in urine was detected by Benedict test at week 8 in group-III and week 10 in group-II of dietary treatment suggesting the onset of obesity linked NIDDM in them. The loss of glucose in urine and hyperzincuria increased with increase in time duration and amount of Zn present in diet (data not shown). Urinary Zn-excretion has also been correlated with the degree of either glucosuria or albuminuria.

The animals in group-II and III displayed arterial hypertension wherein their arterial blood pressure was significantly higher than that of control group (Table 1). At the end of dietary treatment of 180 days, the arterial blood pressure was recorded more than 100% higher in both the dietary treatment groups than those of the control group rats. Leptin, a product of ob gene, is expressed mainly in adipose tissue and participates in diabetes and hypertension in obese individuals. Lower and higher leptin levels in the Zn-deficient and Zn-supplemented dietary groups were observed which couldn’t be explained solely with changes in fatty tissues. Moreover it is possible that Zn interacts with more important and complex systems involved in leptin secretion regulation. These reports indicate that leptin may cause an increase in blood pressure by affecting sympathetic nervous system by excessive Zn-supplementation in the present investigations.

Two factors, hormonal and trace metals have also been implicated in the onset of hypertension, when it is associated with metabolic syndrome-X. Hormonal factors include hyperinsulinemia, insulin resistance, high cortisol and aldosterone levels. All the three hormones in blood increased significantly in group-II and III (Table 3) after Zn-supplementation. The maximum increase in these hormones in blood serum was observed in group-III compared to group-II (except aldosterone which was more in group-II than group-III) and group-I and so was the degree of systolic arterial blood pressure. Hyperinsulinemia has been reported to induce resetting in cardiac autonomic control mainly secondarily to an increase in sympathetic activity. This lends support to the hypothesis that hyperinsulinemia/insulin resistance is directly implicated in the pathogenesis of vascular mortality associated with NIDDM, to sustain over activation of the cardiac sympathetic nervous system. This is evident during present study if the heart rate was considered. The heart rate was more than 80% higher in group-II and III than that of the control individuals, which coincided well with insulin resistance (Table 2). Infact insulin resistance occurs in respect to increase glucocorticoids, as revealed by the increase in cortisol levels in diabetes mellitus in the present study.

The analysis of trace element status in three groups of rats revealed that blood serum Zn, Cu and Mg increased significantly than their control counter parts in group-II and III compared to those of control rats in group-I. The increase of micro and macro elements were more in group-III than in group-II inspite of the fact that only the amount of Zn in the diet has been increased while Cu and Mg were adequate and were equal in amount to the diet of group-I (Table 4). Higher Cu concentration in blood plasma has been seen to occur in hypertensive humans and SHR rats than their control counter parts. The degree of increase of serum Cu and Mg in diabetes mellitus has been associated with its complications of hypertension or macrovascular diseases. The plasma Mg levels has been reported to be less when diabetes mellitus associated with retinopathy but the greatest mortality rate occurred in hypermagnecemic patients leading to congestive heart failure.

There are contradictory reports regarding the serum Zn concentrations in diabetes; while some show a reduction in serum Zn in patients with diabetes mellitus, the recent investigations observed an increased serum Zn in these patients. The concentrations of metals in blood plasma at a given time represent a total of bound, exchangeable and catabolic components of metals. They, therefore, lead to confusing results and predictions, more so in case of chronic diseases where urinary losses are high. The metal concentrations in tissues are more dependable indicator of assessing metal deficiencies/excesses, since their concentrations are maintained through dynamic equilibrium between tissue metals and exchangeable metal components of the blood plasma. The metals concentrations in tissues therefore are better indicators of their status in the body than that of blood plasma.

Zn in liver, hair and abdominal muscles was found to be significantly higher whereas Cu and Mg were significantly lower in group-II and III compared to those of control animals in group-I (Table 4). In rats, experimentally induced diabetes mellitus causes elevated Zn concentrations in liver, kidney, plasma...
and bone\textsuperscript{102-104}. This results from a combination of diabetes-induced hyperphagia and an altered absorption and retention of Zn\textsuperscript{105}. Diabetic and control rats appear to absorb the same amount of Zn from diet but excreted significantly less endogenous Zn from their intestine. This reduction in intestinal Zn excretion may be an important factor contributing to the elevated Zn concentration observed in some organs of diabetic rats\textsuperscript{102,103,105-108}. It is further supported by epidemiological studies where instead of hyperzincuria, the diabetic patients have not appeared to be Zn deficient\textsuperscript{70}. This is further evident by elevated Zn concentration in serum, urine and mononuclear cell in diabetic patients after high dose Zn-supplementation which has a greater ramification in diabetes\textsuperscript{30}. Mineral Zn is found in the tissues bound to metallothionein, and the synthesis of this protein is stimulated by Zn itself and also by specific hormones such as glucocorticoids. In obese mice, the concentration of glucocorticoid is normally high, which keeps the levels of metallothionein bound to Zn high by a “sequestration” of Zn in some specific tissues. In present studies Zn concentration in tissues was significantly higher and coincides well with the levels of cortisols in the blood in group-II and III consistent with the previous report\textsuperscript{15}.

The present data of macro and micro elements also suggest that Zn when in excess even in pharmacological doses in diet over a period of time replaces Cu and Mg in tissues leading to their leaching into the blood resulting their rise in blood plasma and increased excretion in urine even if these metals are adequate in diet. This is also evident in other studies wherein Cu in hair and nails in patients of diabetes mellitus, hypertension and coronary artery disease have been found to be low\textsuperscript{109,110}. Similarly, low Cu concentration has been reported in muscle biopsies of diabetics\textsuperscript{111}.

The blood profile of treated rats showed significant rise of glucose, total lipids, cholesterol, triglycerides, VLDL and LDL-cholesterol, decreased HDL-cholesterol indicating dyslipidemia and hyperglycemia (Table 3). The association of altered metal status with changes in blood profile has been reported by different investigators differently. Hyperglycemia has been reported to occur under Zn-deficiency conditions as it adversely affects the glucose-disposal due to its stimulating effect of glucose transport in muscles and adipocytes. Accordingly, the therapeutics of Zn-supplementation has been reported in subjects with diabetes mellitus and obesity which both have insulin resistance\textsuperscript{112}. If this is so, then the hyperglycemia should not have appeared in rats of group-II and III which exhibit hyperzincemia. The present data thus clearly contradict the protective role of zinc reported \textit{per se}, rather its excess supplement could aggravate hyperglycemia, as it would further reduce Cu-bioavailability to the cells. Infact the reality of Zn–deficiency in diabetes has been criticized as its supplementation has been seen to increase serum haemoglobin A\textsubscript{1C} values in diabetes mellitus\textsuperscript{30}.

\begin{table}[h]
\centering
\caption{Mean Zinc (Zn), Copper (Cu) and Magnesium (Mg) concentrations in the serum, liver, hair and abdominal muscles of male rats of Group-I [fed on basal diet (control)], Group-II (fed on Zn-supplemented-diet-I) and Group-III (fed on Zn supplemented-diet-II) after 180 days of dietary treatment}

\begin{tabular}{lccc}
\hline
Parameters & Group-I (Control) & Group-II & Group-III \\
\hline
Serum Zn\textsuperscript{*} & 0.68 ± 0.04 & 1.65 ± 0.01\textsuperscript{a} & 1.96 ± 0.053\textsuperscript{a} \\
Serum Cu\textsuperscript{*} & 0.98 ± 0.02 & 2.67 ± 0.03\textsuperscript{a} & 3.27 ± 0.17\textsuperscript{a} \\
Serum Mg\textsuperscript{*} & 1.41 ± 0.01 & 1.75 ± 0.008\textsuperscript{a} & 1.84 ± 0.01\textsuperscript{a} \\
Liver Zn\textsuperscript{\&} & 34.97 ± 0.66 & 42.17 ± 0.48\textsuperscript{a} & 56.03 ± 0.63\textsuperscript{a} \\
Hair Zn\textsuperscript{\&} & 23.14 ± 0.97 & 32.74 ± 1.16\textsuperscript{a} & 47.31 ± 0.24\textsuperscript{a} \\
Abdominal muscles Zn\textsuperscript{\&} & 26.06 ± 0.43 & 35.83 ± 0.31\textsuperscript{a} & 49.11 ± 0.30\textsuperscript{a} \\
Liver Cu\textsuperscript{\&} & 63.94 ± 0.90 & 40.11 ± 0.24\textsuperscript{a} & 37.89 ±0.24\textsuperscript{a} \\
Hair Cu\textsuperscript{\&} & 45.26 ± 0.73 & 28.11 ± 0.24\textsuperscript{a} & 27.08 ± 0.44\textsuperscript{a} \\
Abdominal muscles Cu\textsuperscript{\&} & 52.97 ± 0.48 & 33.43 ± 0.41\textsuperscript{a} & 30.17 ± 0.48\textsuperscript{a} \\
Liver Mg\textsuperscript{\&} & 35.14 ± 0.34 & 30.86 ± 0.33\textsuperscript{a} & 29.83 ± 1.71\textsuperscript{a} \\
Hair Mg\textsuperscript{\&} & 32.91 ± 0.24 & 27.77 ± 0.48\textsuperscript{a} & 26.91 ± 0.24\textsuperscript{a} \\
Abdominal muscles Mg\textsuperscript{\&} & 34.97 ± 0.17 & 30.34 ± 0.22\textsuperscript{a} & 28.11 ± 0.24\textsuperscript{a} \\
\hline
\end{tabular}

Units: * mg/dl; \& µg/g of tissue wt.

\textit{P} values: \textit{a} < 0.001(values of group-II and group-III were compared with group-I)
\end{table}
There are evidences that both Zn deficiency and its excess can cause dyslipidemia and impair hepatic cholesterol synthesis and enhance oxidative stress. Chandra observed a significant increase in serum LDL-cholesterol after high Zn-supplementation.

Serum HDL-cholesterol level was also found to decrease significantly in healthy adults as in present study after high-Zn supplementation. Low levels of serum HDL-c is one component of a cluster of coronary disease risk factors linked to abdominal obesity, hypertension, hyperinsulinemia and insulin resistance. Its inverse correlation was observed with aldosterone levels in subjects with the coronary artery disease risk factor cluster. HDL-c is reported to inhibit adrenal aldosterone secretion by altering calcium channels in some cells since intracellular calcium is involved in regulating aldosterone secretion. Fall in HDL-cholesterol in blood depresses the calcium dependent regulating system, which results in increased secretion of aldosterone. Further high insulin levels are correlated with low HDL. Elevated aldosterone further aggravates coronary disease risk factor cluster such as low HDL-c, insulin resistance and abdominal obesity.

The assessment of metal status in present study showed that Cu in liver, hair and abdominal muscles was approximately 40% less in severely diabetic animals in group-III and 35% less in less severe diabetic rats in group-II indicating the induction of Cu-deficiency or rise of Zn-Cu ratio in the tissues. The rise of serum glucose, total lipids, cholesterol, triglycerides, VLDL and LDL-cholesterol and fall of HDL-cholesterol observed after 180 days of treatment in group-II and III rats coincide very well with the degree of fall of Cu-concentration in these tissues, in spite of the fact that blood serum has higher Cu-concentration than that of control rat.

Sugawara found a positive correlation between serum cholesterol and serum Cu and Zn concentrations. Salonen et al. have investigated an interaction among serum Cu and LDL-cholesterol concentration in progression of carotid atherosclerosis, a positive relationship between serum Zn and plasma total cholesterol and LDL-cholesterol has also been reported. Increased plasma Cu levels were also correlated with increased leptin levels in hypertensive patients but not with serum-Zn levels. High dietary ratio of Zn to Cu is associated with high concentration of serum cholesterol and high risk of IHD. It has been hypothesized that an imbalance of Zn and Cu status may be involved in the etiology of human hypertension because they are involved in the regulation of blood pressures by participating in the stimulation of several enzymes.

The plasma cholesterol increase by increasing dietary Zn at all levels of Cu suggests that dietary Zn seems to suppress the plasma cholesterol lowering effect of dietary Cu. Excessive Zn-supplementation has been seen to result in Cu deficiency. Cholesterol appeared to clear the liver to blood plasma faster in Cu deficient rats than in control due to reduction in new lecithin cholesterol acyltransferase (ICAT) activity, lipoprotein lipase activity and thyroid function and increased activity of 3-hydroxy-3-methylglutaryl coenzyme A reductase, the rate-limiting enzyme in cholesterol biosynthesis. Induced Cu deficiency increased absolute amount of cholesterol and triglycerol in the VLDL and LDL in present investigations.

Experimental Mg deficiency has also been shown to increase triglycerides, cholesterol, VLDL, LDL-rich lipoproteins and a reduced HDL-c almost similar to Cu deficiency. During the present investigations, high-Zn supplementation resulted in fall of Cu and Mg concentrations in the tissues of the treated group. The effect of Zn on Mg has not been studied except by El-Yazigi et al. where a positive correlation between heamoglobin A1C and urinary loss of Mg and Zn in patients with type-II diabetes has been observed. This suggests that the observed rise in absolute amount of cholesterol, triglyceride, LDL-c and VLDL-c in the present investigations resulted due to Cu deficiency coupled with Mg deficiency in tissues caused by excessive Zn in diet. These results are consistent with the report per se.

High serum Cu levels in patients with diabetes mellitus may be attributing to hyperglycemia that may stimulate glycation and release of more copper ions that accelerates the oxidative stress. And HbA1c levels further contributes to the changes in the profile of other blood trace elements and as a result of this, contributes to the degree of oxidative stress in patients of diabetes mellitus. Secondly, the fall in tissue Cu/Zn ratio adversely affects cytosolic superoxide dismutase resulting in alteration of antioxidant defense system. Also the reduction of selenogluthathione peroxidase and increased synthesis of cholesterol promotes lipoprotein oxidation under
Cu-deficiency in particular\textsuperscript{138,139}. These effects are important given the etiological role of low-density lipoprotein in initiation of atherosclerosis leading to high risk of coronary artery disease in NIDDM patients\textsuperscript{140}. Arrhythmias, hypertension and sudden cardiac death reported under Mg deficiency\textsuperscript{144} may not be the effect of Mg deficiency alone but due to the combined effect of Cu and Mg deficiencies, which appear to be secondary to excessive Zinc influx in the body.

The results of the present study thus provide strong evidence that excessive Zn in diet even in pharmacological doses stimulates the conditions that favor the onset of metabolic syndrome-X. The rising prevalence of metabolic syndrome-X during past two decades therefore may not essentially be due to change in the lifestyle but also may be associated with genetic predisposition for excessive Zn absorption or excessive Zn intake through food chain due to its indiscriminate use in agricultural and animal husbandry practices\textsuperscript{142}, Zn fortified baby foods and other Zn rich dietary supplements\textsuperscript{7}. The recent survey revealed that the percentage of Zn consumed from Zn fortified food doubled from 14% for children surveyed in 1994 to 28% for children surveyed in 1998 in the US preschool children and this is also true for all age groups. The percentage of this intake form Zn-fortified food will increase over the time\textsuperscript{7}. Intake of Zn fortified food for a longer period of time may make the growing children more venerable to these diseases. The manifestation of metabolic syndrome-X at relatively younger age may be outcome of Zn rich food, which in turn may induce the conditions favoring the cluster of diseases included in metabolic syndrome-X.

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