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Effect of Long Term Excessive Zn Supplementation on Blood Lipid Profile and Tissue Minerals Status in Wistar Rat

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Abstract

During the last two decades the amount of Zn consumed from Zn-fortified foods and Zn rich dietary supplements have doubled for all age groups and the amount of this will further increase over time. The effect of long term increasing Zn load in the blood lipid profile and tissue minerals status in the body has not been worked out so far. In this study, three groups of rats were fed on semi-synthetic diet containing 20 mg Zn/kg (control, group-I), 40mg Zn/kg (group-II) and 80mg Zn /kg (group-III) diet respectively for a period of 180 days. The results revealed that the gained in body weight increased in rats in Zn concentration dependent manner. The blood lipid profile displayed a significant rise in serum total lipids, cholesterol, triglycerides, LDL-cholesterol, VLDL- cholesterol whereas HDL-cholesterol showed a reduction in their levels in group-II and III than their control counter parts. The tissue metal status showed an increase of Zn, Cu, Mg and Mn in the serum, a rise in Zn in liver and kidney and fall in Cu, Mg and Mn in kidney and liver. This data suggest that excess Zn in diet when fed for longer periods of time alter blood lipid metabolisms and tissue minerals status.

Key words: Zn, Cu, Mg, Mn, Zn- supplementation, Blood lipid profile, Minerals status

Introduction

It is well documented that zinc (Zn) is an essential trace mineral which is necessary for health and growth and is particularly important for the function and activity of over 200 metalloenzymes (Chen et al. 1991, NRC, 1989). Zinc also acts as indirect antioxidants via stabilization of cell membranes and the inhibition of free radicals production (Bray and Bettger, 1990). Inspite of the fact that Zn is an essential micronutrient, there are studies which reported that both deficiency and excess of Zn can cause dyslipidemia and impair hepatic cholesterol and enhance oxidative stress (Subramanyam and Vijaya, 1997). Contradictory evidences and results were reported from different studies regarding the effect of Zn supplementation on plasma lipids levels both in animals (Allen and Klevay, 1978, Koo and Lee, 1989) and humans (Hopper et al.1980, Chandra, 1984, Freeland Graves et al. 1982). The majority of human trials studies have shown either increase/decreased or unaffected by the supplementation of excess Zn in blood cholesterol (AREDS Report, 2002, Bogden et al. 1988), triglycerides (Gato and Samman , 1995), LDL-cholesterol (Chandra, 1984) and HDL- cholesterol level (Black et al. 1988).

Moreover, the association of altered metal status with change in blood lipid profiles has been reported by different investigator differently. Sugawara, in 1984, found a positive correlation between serum cholesterol and serum Cu and Zn concentration. A positive relationship between serum Zn and Zn to Cu ratio with plasma total cholesterol and LDL-cholesterol has also been reported (Tully et al. 1995). The ratios of Zn to Cu appear to affect the level of lipoprotein in the blood. High levels of Zn intake have been shown to lower HDL - cholesterol levels in blood, raise total and LDL- cholesterol, induce platelet aggregation and lead to atherosclerosis in animals (Klevay and Hyge 1973). Cu deficiency is associated with atherogenic changes in lipid profiles i.e increased LDL – cholesterol and decreased HDL- cholesterol which are risk factor for cardiovascular diseases (Klevay, 1983). Moreover, evidence is available to suggest that there is association between dietary Mg and Mn deficiencies, plasma lipid disorders and cardiovascular diseases (Seelig and Heggtveit, 1974, Seelig, 1980, Dorothy et al. 2003).

Even though Zn is an essential micronutrient, the use of Zn supplements has been discouraged by some health professional because excessive intake of Zn has been reported to induce Cu, Mg, and Mn deficiencies due to their antagonistic interactions (Aaseth et al. 1998, Couzy et al. 1993, Yadrik et al. 1989). This antagonistic interaction is of practical concern because experimental data suggested that deficiency these trace minerals impairs enzymes of antioxidant system and can produce variety of biochemical and physiologic changes and has been implicated in the etiology of chronic diseases (Rayssiguier et al. 1993, Thompson et al. 1992). During the past two decades, there has been a rise in the consumption of higher Zn either from Zn fortified foods as in the USA (Arsenault and Brown, 2003), or from vegetables (40mg/kg or more in above ground vegetables and 120mg/kg above in the underground vegetables) and meat foodstuffs (120 mg Zn/kg) as in some States of India (Ram et al, 2005; Singh and Taneja, 2010) and this will increase over time. This guided us to investigate the real situation of the long term effect of increasing Zn load in the blood lipid profile and tissue minerals status of the body. Therefore, the supplementation of pharmacological dosage of Zn in otherwise Zn adequate diet was investigated with the aim if excess Zn in the diet triggers alteration in blood lipid metabolism and minerals status in rats which are not genetically predisposed to any diseases. The results of the present study are given in this communication.

Material and Methods

For the present investigations, a semi-synthetic basal diet rich in fat and refined sucrose was preferred over the standard pellet rat diet consisting of the natural ingredients to keep the consistency of the composition of diet particularly of fat, sucrose and micronutrients through out of the experiment and to rule out the possibility of Zn-interaction with fibers and phytates (Oberleas and Harland, 1981, Turnland et al. 1984) which are known to reduced bioavailability of Zn by binding it in digestive tract and may take longer time duration of feeding to manifest the impact excessive Zn in diet. Accordingly, isocaloric semi-synthetic basal diet for the rats was prepared following modified Orgebin – crist et al. (1971). It contained (g/100g of diet).

The basal diet was further divided into 2 parts: control diet consisting of basal diet containing 20 mg Zn/kg diet (per-se) for Group- I and Zn-supplement-diet-I containing 40 mg Zn/kg diet for Group – II and Zn supplemented-diet-II containing 80mg Zn/kg diet for Group-III by accordingly increasing $ZnSO_4.7H_2O$ in basal diet. For each diet, the mineral & water-soluble vitamins were ground in sucrose and fat-soluble vitamins were dissolved in corn oil. Agar, which served as a binder and was dissolved in 25 ml of triple distilled, deionised warm water (60°C). On cooling to 40°C, the contents of each diet were thoroughly mixed in agar solution 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°C.

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Table 1. Composition of Basal Diet

Diet Components	g/100g	*Vitamin Mixture (mg/ kg)	**Mineral Mixture (g/kg)
Casein	30	Ascorbic acid – 500	CaH_2PO_4 - 25.3
Agar	2.0	Biotin – 4	$CoCl_3$ – 0.04
Corn oil	5	Calcium- D- pentothenate – 320	$CuCl_2$ – 0.10
Cellulose	8	Choline chloride – 2500	$FeSO_4 7H_2O$ - 0.60
Sucrose	51.0	Folic acid – 10	Mn $SO_4.5H_2O$ - 0.31
*Vitamin mixture	0.50	Inositol – 1000	Mg $SO_4.H_2O$ - 4.05
**Mineral mixture	3.50	Retinol - 0.31	NaF - 0.088
Total diet	100	Pyridoxine HCl – 80	KI - 0.004
		Riboflavin -120	Na_2CO_3 -1.15
		Ergacalciferol - 0.0031	KCL – 3.430
		Thiamin HCl – 200	Zn $SO_4.7H_2O$ - 0.088
		α-Tocopherol acetate (E) – 60	
		Cyanocobalamin - 0.40	
		Nicotinic acid – 300	

Experimental Design

Male wistar rats (30), aged 6 weeks, weighing 60-70g were procured from Central Animal House, Panjab University, Chandigarh. They were maintained in plastic cages with stainless steel top grill at room temperature (25° - 28°C) with 10:14 hr L: D cycles of 70-80% RH as per guide lines of Institutional Animals Ethics Committee and in accordance with the internationally accepted principles for laboratory animal use and care.

They were fed on standard pellet rat feed for one week to acclimatize. Thereafter, the rats were divided into 3 groups – I, II and III and in such a way that their mean initial body weights remained almost similar in each group. The animals were fed on their respective diets ad libitium and triple distilled deionised water was made freely available to them for 180 days. The body weights were recorded at the beginning of the dietary treatment and thereafter every week. After the end of the dietary treatment of 180 days, the male rats of each group were sacrificed using diethyl ether as anaesthesia.

The blood samples were collected by puncturing the heart and blood serum was prepared by centrifuging blood at 2500 rpm for 15 minutes. The freshly prepared serum was analyzed for cholesterol (Roeschlau et al, 1974, Allain et al. 1974), triglycerides (McGowan et al. 1983, Fossati and Prencipe, 1982), HDL-cholesterol (Burstein et al. 1970) (all by using commercially available kits- Reckon Diagnostic Pvt, Ltd, Baroda, India and ERBA diagnostic Mannheim GmbH, Mannheim, Germany, supplied through Transasia Bio- Medicals LTD, Daman) and total lipids (Frings and Dunn, 1971). The LDL and VLDL-cholesterol was calculated by Friedewald's equation (Friedewald et al. 1972).

Zn, Cu, Mg and Mn were estimated on atomic absorption spectrophotometer (Electronic Corporation of India Limited, Hyderabad- AAS 4139) using hollow cathode lamps (213.9nm, 324.8nm, 285.2nm and 279.5nm for Zn, Cu, Mg and Mn respectively). Samples of serum, liver and kidney are 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 10mM HNO_3 and filtered through ash free filter paper before analysis. Standards of Zn, Cu, Mg and Mn from Sigma Chemical Co., USA were prepared by dilution in triple distilled deionised water (TDW). The results were subjected to statistical analysis applying one way ANOVA.

Results and Discussion

The data of the study revealed that the body weight of the rats increased with increase in Zn concentration in diet during the first 150 days of dietary treatment in the three groups of rats and thereafter it fell in group-II and III during the next 30 days with respect to their weight at 150 days than their control counterparts at day 180 of the experiment. The data revealed that Zn is highly potent nutrient which initially promotes gain in body weight in concentration dependent manner and its prolong supplementation results in reduction of body weight (Table 2).

Table 2: Month wise body weight 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) during 180 days of dietary treatment.[Values are mean ± SE of 10 observation each]

Time duration(in days)	Group-I (Control)	Group-II	Group-III
0	68.0 ± 0.68	67.0 ± 0.67	67.2 ± 0.79
30	162.15 ± 1.12	192.25 ± 1.13[a]	231.46 ± 1.67[a]
60	199.17 ± 1.53	271.67 ± 1.67[a]	297.25 ± 1.14[a]
90	261.36 ± 1.97	318.33 ± 1.70[a]	363.43 ± 0.860[a]
120	280.50 ± 1.70	346.14 ± 1.60[a]	384.55 ± 1.39[a]
150	320.67 ± 1.13	359.57 ± 1.78[a]	411.65 ± 1.67[a]
180	371.35 ± 0.82	331.83 ± 1.10[a]	371.67 ± 1.67[a]

Units: gram; *P* values: [a] < 0.001(values of group- II and group-III were compared with group-I).

This is in conformity with the reports of the previous studies (Pomp et al. 1996, Chen et al. 1996, Taneja and Mandal, 2006) wherein Zn more than 20mg/kg diet (control) and not exceeding 100mg/kg diet fed to rats for a period of extending 4 to 6 weeks resulting in a significant gain in body weight than the control has been recorded. Investigatory studies on laboratory animals also provides evidences that the anabolic effects of Zn results from the higher absorption of nutrients i.e amino acids (Moran and Lyerly, 1985), fatty acids (Koo and Turk, 1977) and glucose (Taneja and Arya, 1992) in addition to activation protein and nucleic acid syntheses. A positive correlation between hair Zn and body weight or BMI has been seen to exist (Taneja et al.1996, Taneja and Mahajan. 1999).

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) during 180 days of dietary treatment.[Values are mean ± SE of 10 observation each]

Parameters	Group-I(Control)	Group-II	Group-III
Total Lipids*	202.50 ±2.14	248.17 ± 1.78 [a]	310.33 ± 2.86 [a]
Cholesterol*	61.67 ± 2.17	82.0 ± 1.02 [a]	115.0 ± 1.86 [a]
Triglycerides*	63.67 ± 1.56	104.83 ± 1.76 [a]	121.67 ± 2.67 [a]
VLDL- Cholesterol*	12.7 ± 0.320	20.97 ± 0.360 [a]	24.3 ± 0.340 [a]
HDL-Cholesterol*	20.67 ± 1.12	12.67 ± 0.710 [a]	8.83 ± 0.410 [a]
LDL- Cholesterol*	28.27 ± 1.35	48.37 ± 0.760 [a]	81.83 ± 1.94 [a]

Units: *: mg/dl; *P* values: [a] <0.001(values of group- II and group-III were compared with group-I).

The blood profile of treated rats revealed significant rise of total lipids, cholesterol, triglycerides, VLDL – cholesterol and LDL-cholesterol and decreased HDL-cholesterol after 180 days of Zn supplementation in group-II and group-III as compared to the control group-I (Table-3). These observations are consistent with previous reports from both in human and animal studies where supplementation of Zn increased blood cholesterol (both LDL and VLDL-c) and decreased in HDL- cholesterol level (Subramanyam and Vijaya, 1997, Chandra, 1984, Black et al, 1988, Hopper et al. 1980). This alteration in serum lipoproteins profiles in the Zn treated group-II and III in the present investigations may be resulted from deficiency of Cu, Mg and Mn in the tissues as a consequence of high Zn supplementation. The assessment of the metal status in the present study showed that Cu, Mg and Mn were approximately up to 30% less in group-II and up to 50% less in group- III rats both in liver and kidney indicating the induction of the Cu, Mg and Mn deficiencies or rise in ratio of Zn to Cu/ Mg/Mn in the tissues. This rise of serum total lipids, cholesterol, triglycerides, VLDL and LDL-cholesterol an fall in HDL- cholesterol after 180 days of treatment in group-II and III rats coincide very well with degree of fall of Cu concentration in these tissue, inspite of the fact that blood serum has higher Cu concentration than the control rat. Cu deficiency induced hypercholesterolemia has been demonstrated in animals and humans (Bureau et al. 1998, Nielsen and Milne, 1992). The plasma cholesterol increase by increasing dietary Zn at all levels Cu suggests that dietary Zn seems to suppress the plasma cholesterol lowering effect of dietary Cu (Klevay, 1975, Lata and Mehta, 1989). Cholesterol appeared to clear from the liver to blood plasma faster in the copper deficient rats than the control (Allen et al. 1982, Shao and Lei, 1980), as a result of the increased in the activity of liver enzyme, 3-hydroxy-3 methylglutamate coenzyme A reductase (Yount et al. 1990, Koo et al. 1993), the rate limiting enzyme in cholesterol biosynthesis and decrease in the lipoprotein lipase, hepatic lipase and lecithin cholesterol acyltransferase (ICAT) activity (Lau and Klevay, 1981).

Experimental Mg deficiency has also been shown to increase triglycerides, cholesterol, VLDL, LDL rich lipoproteins and reduced HDL-cholesterol (Rayssiguier and Gueux, 1986, Gueux et al. 1991), almost similar to Cu deficiency**.** In Mg deficient animals, hypertriglyceridemia could have arisen as a consequence of increased synthesis of triglycerides in the liver, decreased removal of lipid from the blood or combination of both. An increased in the HDL–cholesterol concentration is closely associated with triglyceride metabolism and defective triglyceride removal from the plasma compartment might explain low HDL-cholesterol level as found in the present investigations. Kawano et al. 1987 also observed reduced HDL- cholesterol, HDL protein and HDL apo-E levels with Mn deficiency. Because HDL particles carry a major portion of plasma cholesterol in the rat, these changes could affect cholesterol metabolism in Zn treated groups. One more possible mechanism for the alteration of lipid levels in Mn-deficient animals is that Mn deficiency resulted in increased lipid peroxidation of the endoplasmic reticulum, which is the site of lipoprotein synthesis. Bell and Hurley, 1973, observed swollen and irregular endoplasmic reticulum in tissues of Mn- deficient mice. This suggests that the observed rise of in level of cholesterol, triglycerides, VLDL- cholesterol and LDL-cholesterol and reduction in HDL- cholesterol in the group-II and III rats in the present investigations resulted due to Cu deficiency couple with Mg and Mn deficiency in tissues caused by excessive Zn in diet. Such lipid metabolism disorders have become common and important problems in clinical medicine. Interest in lipid disorder arises from their close relationship to vascular diseases, a major problem of western and currently in developing countries. Prospective data have been shown that the risk of coronary heart diseases is related to the serum cholesterol and triglyceride levels (Morrison and John, 2009, Larsa and Böttiger, 2003). The contribution of the serum total cholesterol to risk has also been found to be determined by its partitioned in the various lipoprotein fractions. A relatively large amount of cholesterol in the LDL-lipoprotein faction is atherogenic whereas that in the HDL fractions appears protective (Gordon et al. 1977).

The results of the blood lipid profile in the present study were consistent with deterioration of the metal status in the group –II and III rats which in turn led to an increase of the cholesterol, triglycerides, VLDL-c, LDL-c and decrease in HDL-c in their respective groups. The element analysis in the three groups of rats revealed that serum Zn, Cu, Mg and Mn increased significantly in group-II and III compared to those control rats in group-I. Moreover, Zn in liver and kidney was found to be significantly higher whereas Cu, Mg and Mn were significantly lowered in group-II and III compared to those control counterpart (Table 4). The increase of these elements were more in serum of group-III than in group-II inspite of the fact that only the amount of Zn on the diet has been increased while Cu, Mg and Mn were adequate and were in equal amount to the diet of group-I. The metal status in the blood plasma at a given time represents a total of bound, exchangeable and catabolic components of metals and therefore could lead to confusing results and predictions. The concentration of metal in tissues are better indicator of their status in the body than the blood plasma in assessing metal deficiencies or excess since their level are maintained by a dynamic equilibrium between tissue metals and exchangeable metal components of blood plasma. The present data of micro and macro elements indicated that Zn when in excess, even in pharmacological doses in diet, over a period of time replaces Cu,

Mg and Mn leading to their leaching in the blood and excretion in the urine even if these metals are adequate in diet. This leaching of Cu, Mg and Mn from the soft tissues in to the blood resulted in their deficiencies in the tissue as observed in liver and kidney of group-II and III rats.

Table-4: Mean Zinc(Zn), Copper(Cu), Magnesium(Mg) and Manganese (Mn) concentration in the serum, liver and kidney 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) during 180 days of dietary treatment.[Values are mean ± SE of 10 observation each]

Parameters	Group-I(Control)	Group- II	Group-III
Serum Zn*	0.57 ± 0.05	1.70 ± 0.02 [a]	1.90 ± 0.05 [a]
Serum Cu*	0.95 ± 0.04	2.35 ± 0.05 [a]	3.23 ± 0.14 [a]
Serum Mg*	1.56 ± 0,08	1.70 ± 0.06 [a]	1.96 ± 0.04 [a]
Serum Mn*	0.21 ± 0.05	0.67 ± 0.05 [a]	1.01 ± 0.06 [a]
Liver Zn[@]	32.7 ± 0.88	44.1 ± 0.84 [a]	57.0 ± 0.65 [a]
Liver Cu[@]	60.3 ± 0.68	43.9 ± 1.13 [a]	30.3 ± 0.77 [a]
Liver Mg[@]	61.0 ± 1.03	48.0 ± 0.49 [a]	34.0 ± 0.90 [a]
Liver Mn[@]	47.80 ± 0.59	36.40 ± 0.65 [a]	30.90 ± 0.66 [a]
Kidney Zn[@]	35.4 ± 0.54	42.7 ± 0.69 [a]	55.5 ± 1.52 [a]
Kidney Cu[@]	53.5 ± 0.74	46.50 ± 0.68 [a]	38.10 ± 0.63 [a]
Kidney Mg[@]	54.50 ± 0.84	45.90 ± 0.54 [a]	35.20 ± 0.76 [a]
Kidney Mn[@]	42.80 ± 1.87	37.90 ± 0.94 [a]	28.20 ± 0.62 [a]

Units: *: mg/dl; [@]: µg/g tissue weight; *P* values: [a] < 0.001(values of group- II and group-III were compared with group-I).

However, in the present study urinary Zn, Cu, Mg and Mn level was not assessed because only trace amounts are found in the urine and urinary loss of metal is only small fraction of their turnover, thereby limiting its use as a sensitive indicator of metal status. The observed increase of Zn level in liver and kidney in group –II and III rats than their control group-I may be attributed to the overexpression of Zn metallotheionein gene during treatment period due to high and prolong Zn intake. As a result of this they absorbed and retained greater amount of Zn than their control counterpart leading to deficiencies of Cu, Mg and Mn due to their interactions (Irato et al. 1996; Nielsen and Milne, 2004).

The results of the present study thus provide strong evidence that excessive Zn in diet even in pharmacological doses alter blood lipid metabolism and deteriorated mineral status of the body. These findings are particularly significant for the people of some States of India where the foodstuffs are overloaded with Zn (Singh and Taneja, 2010, Ram et al. 2005). The rising prevalence of cardiovascular related diseases even in the young age population during the past two decades may not be essentially due to change in lifestyle but also may be associated with excessive intake of Zn from Zn rich foods which in turn may alter trace minerals and lipoproteins metabolism of the body up to a level enough to manifest as diseases. Thus, there appear to be several potential adverse consequences of such pharmacological doses of Zn particularly when such intakes are continued for a prolonged period.

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