Effect of dietary zinc deficiency on rat lipid concentrations

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ABSTRACT

Objectives: Evaluation of the lipid profile in serum, liver, and testis of rats fed marginal and severe zinc deficient diets.

Methods: Three groups of rats were treated for 8 weeks with normal diet, marginally zinc deficient diet and severely zinc deficient diet. Lipid concentrations were measured in serum, liver, and testis of these groups.

Results: The concentrations of serum lipids were not significantly altered between marginally zinc deficient diet treated and control rats. However, in rats treated with severely zinc deficient diet, the concentrations of serum total cholesterol, high density lipoprotein cholesterol and phospholipids were significantly increased ($P < 0.01$) and ($P < 0.001$), whereas the concentration of triacylglycerol was significantly decreased ($P < 0.01$). However, low-density lipoprotein cholesterol concentration was non-significantly different from controls. The concentrations of liver total cholesterol, triacylglycerol and phospholipids were significantly decreased ($P < 0.001$) in rats treated with severely zinc deficient diet. The testicular concentration of total cholesterol was increased but this increase was non-significantly different from controls, whereas the testicular concentrations of triacylglycerol and phospholipids were significantly decreased ($P < 0.001$) in rats treated with severely zinc deficient diet.

Conclusion: These results suggest that a marginally zinc deficient diet does not play a significant role in altering rat lipid concentrations. However, the changes in serum lipid concentrations could be related to those changes in tissue lipid concentrations.

Keywords: Lipids, serum, liver, testis, rat, zinc, deficiency.

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Zinc, an important nutritive factor, is a co-factor for many metalloenzymes. Over a hundred metalloenzymes require zinc for proper function, including red blood cell carbonic anhydrase, alkaline phosphatase, lactic and alcohol dehydrogenases and many enzymes involved in ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) synthesis (such as DNA and RNA-dependent, RNA Polymerizes).[1,2] Zinc deficiency has many causes, but malnutrition and malabsorption are the most common. Pregnant women are at risk for suboptimal levels of zinc, which can adversely affect fetal outcome.[3] The role of zinc in the development and maintenance of a normally functioning immune system has been well established.[2] The most important clinical syndrome of zinc deficiency is acrodermatitis enteropathica that is characterized with skin lesions, loss of hair, severe growth retardation, extreme irritability, and increased susceptibility to infection.[2,4] A very low serum zinc level (190 μg/L) was shown in a patient with acrodermatosis.[5] Symptoms seen in milder zinc deficiency states include mental lethargy, growth retardation, poor appetite, male hypogonadism, and skin changes.[6] Zinc deficiency can be associated with high rates of oxidative damage to testes lipids, proteins, and DNA in male rats.[7] A previous report stated that zinc deficiency could increase in vivo lipid peroxidation and decrease rat insulin sensitivity.[8] Previous studies have shown that zinc deficiency in rats affected the activities of the enzymes involved in the formation of triacylglycerol and phospholipids.[9] The increased synthesis of

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triacylglycerol and phospholipids in zinc deficiency may be due to the increased availability of substrates as well as to the increased activities of the enzymes involved in these processes.[10] As an essential micronutrient, zinc provides little, if any, antioxidant protection against low density lipoprotein (LDL) oxidation in humans.[11,12] It has been earlier reported that higher serum zinc levels were associated with higher levels of total serum cholesterol, LDL-cholesterol, and triacylglycerol.[13] No significant changes were noted for high density lipoprotein (HDL) cholesterol.[12] Recently, however, the effect of pregnancy and a chronic, marginal intake of zinc on zinc kinetics was studied in rats.[14] It was concluded that there was an increase in the turnover rate of the exchangeable plasma zinc pool when dietary zinc intake was marginal during pregnancy. It could help maintain a supply of zinc to the growing fetus when plasma zinc concentrations are reduced. The purpose of this study is to investigate the lipid profile in serum; liver and testis of rats fed marginal and severe zinc deficient diets.

Methods. All chemicals were purchased from BDH (Poole, United Kingdom). Kits for lipid determination were purchased from BioMerieux Laboratory Reagents and Instruments (Marcy-L'Etoile, France). Weanling male rats (140g–190g) were obtained from King Fahd Medical Research Center, College of Medicine and Allied Sciences, King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia. Animals were divided into 3 groups. Group one. Received marginally zinc deficient diet (MZD) which contained 2.8 mg/Kg. Group 2. Received severely zinc deficient diet (SZD) that contained 0.2 mg/Kg. Group 3. Received normal diet containing sufficient zinc (30.8 mg/Kg). All different types of diets were obtained from TEKLA DIETS (Aharan Sprague Dawley Inc., Co.). All diets were given for 8 weeks with free access to distilled water. Animals were kept in a controlled environment (constant temperature of 24ºC and a light cycle of 12 hours on / 12 hours off). At the end of the period, blood specimens were collected from rats by cardiac puncture under light ether anesthesia into plain tubes. Serum was then separated, divided into aliquots and were used either immediately or stored at -20ºC until analyzed. Testis and liver were exercised and stored in a deep freeze at -80ºC until use. Total cholesterol, HDL-cholesterol, LDL-cholesterol, triacylglycerol, and phospholipids were estimated in samples by standard enzymatic methods using BioMerieux kits.[15–17] Total lipids were extracted from frozen testis and liver using chloroform-methanol mixture (2:1 v/v), then extracts were filtered and filtrates were evaporated to dryness in a rotary evaporator under partial vacuum at -40ºC. Residues were then quickly redissolved in a known volume of petroleum ether-chloroform mixture (90:10, v/v) and stored at -20ºC until analyzed. Total lipid extracts prepared from testis and liver were analyzed for total cholesterol, triacylglycerol and phospholipids using the same method described above. Serum, testis and liver were digested with concentrated nitric acid on a hot plate at approximately 140ºC. The concentration of zinc was measured with a flame Atomic Absorption Spectrophotometer (AAS), Model 5000, Perkin-Elmer, Norwalk. The data presented in this investigation are expressed as means ± standard error of the mean (SEM) and comparisons between experimental and corresponding control rats was made by using student's t-tests.

Results. **Table 1** shows that only the control rats gained body weight significantly, whereas MZD and SZD rats significantly lost body weight ($P<0.001$). Significant differences were noted in the weights of liver and testis between control, MZD and SZD rats. Serum concentrations of zinc of MZD and SZD rats were significantly less than those of their matched controls ($P<0.001$). **Table 2** shows the changes in serum lipid concentrations among control, MZD and SZD rats. The concentrations of total cholesterol in both MZD and SZD rats were significantly increased ($P<0.05$ and $P<0.01$). High density lipoprotein-cholesterol concentrations in MZD rats were not significantly different from those in control rats, whereas they were significantly higher ($P<0.001$) in SZD rats. However, LDL-concentrations in both MZD and SZD rats were not significantly different from those in control rats (**Table 2**). The concentrations of triacylglycerol were only significantly decreased in SZD rats ($P<0.01$). The concentrations of phospholipids in both MZD and SZD rats were significantly increased ($P<0.01$). **Table 3** shows the concentrations of zinc, total cholesterol, triacylglycerol, and phospholipids in liver and testis among MZD and SZD rats. Liver concentrations of total cholesterol in MZD rats was not significantly different from those in control rats, whereas they were significantly lower ($P<0.001$) in SZD rats. However, testis concentrations of total cholesterol in both groups were not significantly different from those of control rats (**Table 3**). The concentrations of triacylglycerol in liver and testis of both MZD and SZD rats were significantly decreased ($P<0.02$ and $P<0.001$). The concentrations of phospholipids of liver and testis of MZD rats were not significantly different from those of control rats. On the other hand, the concentrations of phospholipids in both tissues of SZD rats were significantly reduced ($P<0.001$). The concentration of liver zinc of both MZD and SZD rats was not significantly different from that of their matched controls (**Table 3**). The concentration of testis zinc

Table 1 - General characteristics of control, marginally zinc deficient, and severely zinc deficient rats.

Characteristics	Control Rats	Marginally zinc deficient rats	Severely zinc deficient rats
Initial body wt. (g)	140 ± 2.3 (12)	168.8 ± 2.9 (12)	183.6 ± 4.2 (12)
Final body wt. (g)	380.8 ± 3.9 (12)	106.2 ± 2.7 (12)**	131.4 ± 2.9 (12)**
Liver wt. (% 100 g body wt.)	0.82 ± 0.03 (12)	5.90 ± 0.22 (12)**	3.93 ± 0.08 (12)**
Testis wt. (% 100 g body wt.)	0.22 ± 0.02 (12)	1.74 ± 0.15 (12)**	0.81 ± 0.10 (12)**
Serum zinc (μmol/L)	15.1 ± 0.048 (6)	10.5 ± 0.049 (6) **	8.3 ± 0.043 (7) **

Results are presented as means ±standard error of the mean (SEM), with number of rats given in parentheses. As compared with matched control rats, *P < 0.01; **P < 0.001 (Student's t-test), wt - weight.

Table 2 - Serum concentrations of total cholesterol, HDL-cholesterol, LDL-cholesterol, triacylglycerol, and phospholipids in control, marginally zinc deficient and severely zinc deficient rats.

Analysate	Control Rats	Marginally zinc deficient rats	Severely zinc deficient rats
Total cholesterol (mmol/L)	1.71 ± 0.08 (10)	2.00 ± 0.11 (10)*	2.04 ± 0.07 (10)**
HDL-cholesterol (mmol/L)	1.09 ± 0.04 (9)	1.17 ± 0.06 (10)	1.39 ± 0.04 (10)***
LDL-cholesterol (mmol/L)	0.023 ± 0.01 (8)	0.023 ± 0.02 (8)	0.022 ± 0.02 (8)
Triacylglycerol (mmol/L)	0.53 ± 0.03 (8)	0.49 ± 0.04 (8)**	0.42 ± 0.02 (8)**
Phospholipids (mmol/L)	2.04 ± 0.05 (8)	2.22 ± 0.004 (8)**	2.30 ± 0.06 (8)**

Results are presented as means ± standard error of the mean (SEM), with number of rats given in parentheses. As compared with matched control rat, *P < 0.05; **P < 0.01; ***P < 0.001 (Student's t-test); HDL - High density lipoprotein; LDL - Low density lipoprotein.

Table 3 - Liver and testis concentrations of total cholesterol, triacylglycerol, phospholipids, and zinc in control, marginally zinc deficient and severely zinc deficient rats.

Analysate	Control Rats	Marginally zinc deficient rats	Severely zinc deficient rats
Liver			
Total cholesterol (mg/g wet tissue)	1.00 ± 0.04 (8)	0.90 ± 0.04 (8)	0.73 ± 0.02 (8)**
Triacylglycerol (mg/g wet tissue)	4.81 ± 0.26 (8)	3.94 ± 0.23 (7)*	2.99 ± 0.08 (7)**
Phospholipids (mg/g wet tissue)	7.58 ± 0.23 (8)	7.90 ± 0.11 (7)	4.36 ± 0.25 (8)
Zinc (μg/g wet tissue)	24.74 ± 0.49 (7)	23.74 ± 0.50 (7)	23.57 ± 0.51 (6)
Testis			
Total Cholesterol (mg/g wet tissue)	1.08 ± 0.04 (8)	1.15 ± 0.04 (8)	1.27 ± 0.10 (8)
Triacylglycerol (mg/g wet tissue)	5.00 ± 0.29 (7)	2.80 ± 0.23 (7)**	2.78 ± 0.20 (7)**
Phospholipids (mg/g wet tissue)	5.49 ± 0.14 (10)	5.44 ± 0.10 (9)	4.33 ± 0.16 (8)**
Zinc (μg/g wet tissue)	29.12 ± 0.72 (6)	26.89 ± 0.35 (6)*	23.85 ± 0.54 (5)**

Results are presented as means ± standard error of the mean (SEM), with number of rats given in parentheses. As compared with matched control rat, *P < 0.02; **P < 0.001 (Student's t-test).

of both MZD and SZD rats was significantly less than that of control rats ($P < 0.02$ and ($P < 0.001$).

Discussion. Results of the present study show that the serum zinc concentrations of MZD and SZD rats were significantly less than that of control rats. These results are consistent with the dietary zinc given to rats and the concentrations are in agreement with the results reported by O'Dell et al.[18] The present results show that the serum concentrations of total cholesterol in both MZD and SZD rats were elevated by 17% and 19%, whereas HDL-cholesterol in SZD rats was also elevated by 27.5%. These findings coincide with the finding of Faure et al[19] and are superficially contradictory with many findings in humans and rats.[11,13,20-22] Recent studies in animals and in vitro support the hypothesis that zinc, an essential micronutrient, possesses antioxidant properties.[12,23] However, our results have shown that zinc did not provide a significant antioxidant protection against LDL oxidation since serum concentrations of LDL cholesterol in both MZD and SZD rats were not changed significantly from those of control rats. The significant increase in serum concentrations of phospholipids in both marginally and severely zinc deficient rats, and the decrease of serum concentration of triacylglycerol in SZD rats, may be due to the fact that zinc deficiency has distinct effects on fatty acid metabolism.[24-25] Liver concentrations of total cholesterol, triacylglycerols, and phospholipids in SZD rats were significantly decreased ($P<0.001$). These findings coincide with the findings reported that zinc deficiency stimulates lipid peroxidation that can be indexed by elevated malondialdehyde.[26] Testicular concentrations of triacylglycerol, phospholipids and zinc in severely deficient rats were significantly decreased ($P<0.001$), these changes may be due to a consequent increase of reactive oxygen species generation or reductions in zinc-dependent antioxidant processes, or both.[27]

The present study suggests that zinc deficiency has adverse effects on risk markers of cardiovascular diseases namely, serum total cholesterol and HDL-cholesterol. It is also suggested that the changes observed in serum lipid concentrations may be related to the changes in tissue lipid concentrations. From this view, the findings of this study are in agreement with other studies.[28-29] However, the higher serum HDL-cholesterol concentrations observed in zinc deficient rats support the hypothesis that zinc supplements may decrease serum HDL-cholesterol level.[30]

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