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ERYTHROCYTE SOD ACTIVITY, COPPER AND ZINC DAILY INTAKES, THEIR PLASMA LEVELS, ERYTHROCYTE CONTENT AND PLASMA CERULOPLASMIN OXIDASE ACTIVITY IN FEMALES WITH LOW COPPER INTAKE

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Abstract

Introduction: Zinc and copper cooperate in the regulation of superoxide dismutase activity (SOD – EC 1.15.1.1) with copper at the active site and zinc maintaining a secondary enzyme function. Copper deficiency brings about depression of erythrocyte SOD activity. Numerous data indicated that females are at greater risk of copper deficiency than males.

Aim of the study: This study aimed to evaluate erythrocyte SOD activity, dietary copper and zinc intakes, their plasma levels and erythrocyte content and plasma ceruloplasmin activity in females with low dietary copper intake.

Methods: A total of 37 females volunteered to participate in the study. Dietary copper and zinc intakes were assessed from 24 h dietary records taken 4 times before blood withdrawal. Plasma and erythrocyte copper and zinc were determined using atomic absorption spectrometry. Erythrocyte SOD and plasma ceruloplasmin oxidase activities were assayed colorimetrically at 480 nm and 460 nm, respectively.

Results: In 70.3% of the women daily copper intakes were lower than 2/3 of the safe intake level. In 16.2% of the participants plasma copper concentrations were lower than the accepted physiological limit. The incidents of low plasma ceruloplasmin oxidase activity were observed in 56.6 % of the participants. It was found that the best model predicting erythrocyte SOD activity included zinc/copper ratio in the diet and plasma ceruloplasmin oxidase activity (model adjusted R^2=0.313, F= 9.2, p<0.0006).

Conclusions: Our study indicated that in females with low dietary copper intakes there was no association between erythrocyte SOD activity and plasma copper and zinc concentrations and the content of both trace metals in the erythrocytes. The best predictors of SOD activity were zinc to copper ratio in the diet and plasma ceruloplasmin oxidase activity.

Key words: females, superoxide dismutase, zinc/copper ratio, ceruloplasmin oxidase activity

Introduction

Copper, zinc and other essential trace metals have a vitamin-like effect on living systems. Practically all forms of life need copper and zinc to render essential metabolic processes functional. Zinc has been shown to be essential for the structure and function of a large number of macromolecules and for over 300 enzymatic reactions. It has both catalytic and structural roles in enzymes, while in zinc finger motifs, it provides a scaffold that organizes protein subdomains for the interaction with either DNA or other proteins. Zinc is critical for the function of a number of metalloproteins, including members of oxido-reductase, hydrolase, ligase and lyase family (1). Additionally, it has been demonstrated that in mammals zinc plays an important role in the maintenance of immune function and in the regulation of neural transmission and insulin secretion (2-5).

Similarly, copper plays an important role as an integral part of many enzymes including lysyl oxidase essential for collagen synthesis, ferroxidase contributing to iron metabolism and several monooxygenases and mitochondrial cytochrome c oxidase (6). Both zinc and copper cooperate in the regulation of superoxide dismutase activity (SOD – EC 1.15.1.1) with copper at the active site and zinc maintaining a secondary enzyme function (7,8).

There is a wealth of studies indicating that copper deficiency due to low dietary copper intake in the diet brings about depression of erythrocyte SOD activity and diminished antioxidant protection (9,10). On the other hand, inadequate copper intakes are not the only reason for copper deficiency. It is well established that zinc excess may contribute to copper deficiency due to zinc-induced inhibition of copper absorption from the intestine and zinc to copper ratio in the diet should not to exceed 6.0 to prevent adverse effects of zinc on copper homeostasis (11-17).

Low daily copper intakes and marginal zinc deficiency are common throughout the world (18-20). Our previous study has demonstrated that in young, physically active male and female physical education students copper daily intake covered 71.7% and 45% of the recommended intake level, respectively, and was accompanied by unfavorable zinc to copper ratio (8.7 and 10.2 in females and males, respectively) (21). In addition, in 47% of females participating in the study the plasma copper level was lower than the physiological limit of 12.6 μmol·l^{-1}. On the contrary, the copper level was lower than physiological limit of 11.0 μmol·l^{-1} in only 6.2% of male subjects. Similarly, Raczyńska et al. (22) have found high zinc to copper ratio in male and female athletes and inadequate copper dietary intake mostly in junior female subjects, covering only 59% of the recommended intake level.

Thus, it could be presumed that dietary habits of females make them more prone to copper deficiency in comparison with males and a high zinc to copper ratio may even strengthen unfavorable effects of low copper intake on erythrocyte SOD activity.

It should be stressed that erythrocyte SOD is of special importance for red cell protection against superoxide production due to hemoglobin autooxidation (23). On the other hand, erythrocyte SOD has been recognized as a potent antioxidant for other tissues, since anion channel allows the transport of superoxide and other radicals into red cells (24,25).

However, there is no data concerning the relationship between erythrocyte SOD activity, dietary copper and zinc intakes, their plasma levels, erythrocyte copper and zinc content and plasma ceruloplasmin activity in females with low dietary copper intake and the evaluation of these associations was the aim of the present study.

Materials and Methods

Subjects

A total of 37 healthy, regularly menstruating females volunteered to participate in the study. All the participants were physical education students not engaged in high-performance sport, but engaged in different forms of physical activity (swimming, track and field, games, gymnastics) as a part of the obligatory physical education program. All the subjects were non-smokers and none of them were taking oral contraceptives. The participants were informed about the procedures and gave their written consent prior to the participation in the study. The experimental protocol was approved by the Ethics Commission in the Academy of Physical Education in Warszawa.

Dietary records

Daily intake of energy, macro- and micronutrients was briefly assessed from 24 h food records collected over 4 days preceding blood collection and analyzed using a computer program. A set of pictures of meals and foods was shown to the participants by an experienced interviewer. The household measures of food intake were converted into gram weights. An interviewer assigned codes to the foods reported by the subjects and performed computer analysis using the Food 2 program purchased from the Institute of Food and Nutrition in Warsaw.

Blood samples

Fasting venous blood was drawn into lithium heparin tubes using disposable needles and syringes under aseptic conditions between 8:30 and 9:00 a.m. To avoid a possible female hormone effect on SOD activity and copper and zinc plasma levels blood was collected between days 5-8 of the menstrual cycle (26,27). Plasma and erythrocytes were separated by centrifugation (15 min/4000 rpm, 4^0 C). The erythrocytes for SOD, copper and zinc determination were washed 3 times with cold isotonic (0.9%) saline. Both plasma and erythrocytes were stored at – 70^0 C until analysis.

Biochemical analysis

SOD activity and zinc and copper levels were determined in the hemolysates of erythrocytes. SOD activity was measured colorimetrically at 480 nm according to Misra et al (28) and one unit of enzyme activity was defined as the amount of SOD required to inhibit adrenaline autooxidation by 50% at 25^{0}C and pH 10.2. Zinc and copper content in the erythrocytes and their plasma levels were assayed using atomic absorption spectrometry (29). Inter-and intra-assay coefficient of variations for zinc and copper determination were lower than 5%. Ceruloplasmin oxidase actitivy was assayed colorimetrically with o-dianisidine dichloride at 460 nm and 30^0 C (30,31).

Statistical analysis

Data distribution was evaluated using the Shapiro-Wilk test. The only variables which were not normally distributed was plasma ceruloplasmin oxidase activity and erythrocyte copper content. To evaluate associations among SOD activity and other variables (zinc to copper ratio in the diet, zinc and copper plasma levels and erythrocyte content and plasma ceruloplasmin oxidase activity) Pearson product-moment or non-parametric Spearman rank correlations were calculated. These variables were then used in multivariate analysis, by use of forward stepwise regression, to determine the model which best predicts erythrocyte SOD activity. Plasma ceruloplasmin oxidase activity was logarithmically transformed (natural logarithm) before it was included into the model. All calculations were made using Statistica 6.0 (Statsoft, USA). All data are presented as means ± SD.

Results

The subjects' physical characteristics are presented in Table 1. The females participating in the present study were characterized by inadequate daily energy intake (1751 kcal) which covered only 83.8% of the recommended intake level (Table 2). The mean copper intake covered 62.7 % of the safe intake level, the mean zinc daily intake covered 79.4% of the safe intake level. The mean zinc to copper ratio equaled 8.7.

Table 1. *Physical characteristics of the subjects*

	Means ± SD
Age (years)	20.5 ± 1.1
Body mass (kg)	62.8 ± 8.0
Body height (cm)	170.3 ± 7.5
BMI ($kg \cdot m^{-2}$)	21.6 ± 2.1
Activity ($h \cdot wk^{-1}$)	7.6 ± 2.7

Table 2. *Daily intake of energy, macronutrients, zinc and copper in female physical education students*

	Means ± SD
Energy (kcal)	1751 ± 629
Energy (kcal/kg m.c.)	28.8 ± 13.3 (83.8%)
Protein (g)	56.2 ± 16.2 (13.4 ± 2.6)*
Fat (g)	69.1 ± 34.2 (34.4 ± 7.5)*
Carbohydrates (g)	241.3 ± 85.9 (51.9 ± 7.3)*
Zinc (mg)	7.94 ± 2.33 (79.4)#
Copper (mg)	0.94 ± 0.33 (62,7 %)#
Zinc/copper ratio	8.7 ± 1.9

* - percent of energy derived from the respective macronutrient;
# - percent of the safe intake level
(safe intake levels: copper – 1.5 $mg \cdot day^{-1}$; zinc – 10 $mg \cdot day^{-1}$)

Erythrocyte SOD activity, zinc and copper contents, plasma zinc and copper levels and plasma ceruloplasmin oxidase activity are presented in Table 3. In Table 4 the analysis of individual data of trace metal intakes, zinc and copper plasma levels and ceruloplasmin oxidase activity are presented with respect to reference values. In 70.3% of the women daily copper intakes were lower than 2/3 of the safe intake level. Zinc intakes less than 2/3 of the safe intake level was noted in 32.4% of the participants. In 16.2% of the participants plasma copper concentrations were lower than the accepted physiological limit of 12.6

Table 3. *Erythrocyte SOD activity, zinc and copper content, plasma zinc and copper leves and ceruloplasmin oxidase activity in female subjects*

	Means ± SD
SOD ($U \cdot l^{-1}$)*	1765.8 ± 171.9
Zinc ($\mu g \cdot g^{-1}$)*	10.5 ± 1.2
Copper ($\mu g \cdot g^{-1}$)*	0.67 ± 0.13
Zinc ($\mu mol \cdot l^{-1}$)**	15.0 ± 0.2
Copper ($\mu mol \cdot l^{-1}$)**	15.1 ± 2.4
Ceruloplasmin ($U \cdot l^{-1}$)**	62.4 ± 23.1

* - in erythrocytes, ** - in plasma;

Table 4. *Percent of participants with zinc and copper daily intake less than 2/3 of the safe intake level, and lower than physiological limit copper and zinc plasma level and ceruloplasmin oxidase activty*

	%
Copper ($mg \cdot day^{-1}$)	70.3
Zinc ($mg \cdot day^{-1}$)	32.4
Copper ($\mu mol \cdot l^{-1}$)	16.2
Zinc ($\mu mol \cdot l^{-1}$)	0
Ceruloplasmin ($U \cdot l^{-1}$)	56.6

Table 5. *Pearson product-moment and Spearman rank correlations between erythrocyte SOD activity and other variables*

	r
Zinc intake ($mg \cdot day^{-1}$)	- 0.16
Copper intake ($mg \cdot day^{-1}$)	0.11
Zinc/copper ratio (diet)	- 0.43[a]
Zn (erythrocytes)	0.34[b]
Cu (erythrocytes)	0.031
Zinc/copper ratio (erythrocytes)	- 0.04
Plasma Zn	- 0.06
Plasma Cu	0.14
Zinc/copper ratio (plasma)	- 0.11
Ceruloplasmin	0.39[c]

a - $p < 0.008$; b – $p < 0.038$; c- $p < 0.02$

Table 6. *The model used to predict erythrocyte SOD activity in female physical education students (n=37)*

Independent variables	Model adjusted R^2	F	p
Zn/Cu (diet)	0.163	8.01	0.008
Ceruloplasmin ($U \cdot l^{-1}$)*	0.313	9.20	0.0006

Independent variables in order of introduction into the model
* Ceruloplasmin activity was logarithmically transformed (natural logarithm) before introduction into the model

Table 7. *Regression and partial correlation coefficients of the best model used to determine predictors of erythrocytes SOD activity in females*

Independent variables entered	Regression coefficient (B)	Standard error of B	Partial correlation coefficient	t	p
Zn/Cu (diet)	-38.005	12.609	-0.459	- 3.013	0.0048
Ceruloplasmin ($U \cdot l^{-1}$)*	193.938	66.008	0.450	2.938	0.0059

*Ceruloplasmin activity was logarithmically transformed (natural logarithm) before introduction into the model

$\mu mol \cdot l^{-1}$. However, the incidence of low plasma ceruloplasmin oxidase activity (lower than 62 $U \cdot l^{-1}$) were more frequent and was observed in 56.6 % of the women. Plasma zinc levels were within physiological limit in all of the participants.

Pearson product-moment and Spearman rank correlations between erythrocyte SOD activity and other variables are presented in Table 5. There were significant and positive correlations between erythrocyte SOD activity and erythrocyte zinc content and plasma ceruloplasmin oxidase activity ($p<0.04$ and $p<0.02$, respectively) and a negative correlation between SOD activity and zinc/copper ratio in the diet ($p<0.008$). However, multiple regression analysis has revealed that the best model predicting erythrocyte SOD activity included zinc/copper ratio in the diet and plasma ceruloplasmin oxidase activity (model adjusted $R^2=0.313$, $F= 9.2$, $p<0.0006$) (Table 6 and 7). It is worth noting that when copper and zinc intakes were separately included into the model instead of zinc to copper ratio, adjusted R^2 equaled 0.264 with $F=5.30$ ($p<0.004$).

Discussion

The participants of the present study were characterized by mean copper intakes much lower than the safe intake level of 1.5 mg/day (10). In addition, the majority of the participants were at risk of copper deficiency since their diet provided less than 1.0 mg copper daily i.e. less than 2/3 of the safe intake level. However, despite copper inadequacy in the diet plasma copper concentrations lower than physiological limit of 12.0 $\mu mol \cdot l^{-1}$ was observed only in minority of the participants (32). Zinc plasma concentrations were within physiological limits (10.1-16.8 $\mu mol \cdot l^{-1}$) (32) in all the women despite mean zinc intake lower than the safe intake level of 10 $mg \cdot day^{-1}$ in most of the participants and the risk of zinc deficiency in subjects with daily zinc intakes lower than 2/3 of the safe intake level of 6.7 mg (33). These findings confirm others' data concerning a minor importance of copper plasma and zinc levels as an index of their daily intakes since despite low (10,19).

However, we indicated that the incidence of low ceruloplasmin oxidase activity were more frequent (56.7% of all participants) than low plasma copper levels suggesting greater sensitivity of the former to low copper intakes and/or to high zinc to copper ratio in the diet. This assumption seems feasible since it has been demonstrated that copper absorbed from the diet is primarily taken up by the liver, the main source of ceruloplasmin synthesis and excretion into the plasma (34). Thus, the liver is the first target tissue which responds to copper supply in the diet. Incorporation of copper into ceruloplasmin is constant at higher intakes but reduced at intakes that results in copper deficiency and this may be the case in the current study (35).

In the present study copper and zinc contents in the erythrocytes was within limits reported by others in female subjects (36). However, both trace metal levels in the erythrocytes of our participants were lower than reported by Singh et al (37) in females characterized by higher (by 40%) zinc intake and higher (by 48%) copper intake in comparison with our subjects. This finding is in agreement with other data concerning the associations between both trace metal intakes and their erythrocyte contents (38,39).

Multiple regression analysis revealed that the best model predicting erythrocyte SOD activity included exclusively zinc to copper ratio in the diet and plasma ceruloplasmin oxidase activity with no effects of other tested variables.

These findings indicate that the zinc to copper ratio in the diet is more important for erythrocyte SOD activity than copper and zinc daily intakes per se. As was mentioned in the Introduction there is a powerful effect of zinc on copper intestinal absorption (13). On the other hand, it is well recognized that copper intestinal absorption increases with decreased copper content in the diet (17). However, our data indicate that zinc and copper interaction operates even at low dietary copper intakes.

In addition, our data suggest significant contribution of plasma ceruloplasmin in the regulation of erythrocyte SOD activity. Despite the well-recognized role of ceruloplasmin in iron intestinal absorption and antioxidant protection (40-43), its contribution to copper supply into the tissues is still under debate (44). However, it should be pointed out that ceruloplasmin receptors were identified in the hepatocyte and erythrocyte membranes (45-47). In addition, in vitro externally added ceruloplasmin increased both copper levels and Zn-Cu-SOD activity in K562 erytholeukemia cells (48). The mechanism of this ac-

tion is not fully understood, however, it is postulated that ceruloplasmin provides copper to intracellular GSH, which complexes copper ions and provides them to apo-SOD (49). It could not be excluded that the same mechanism operates in the erythrocytes.

In conclusion, our study indicated that in females with low dietary copper intakes there was no association between erythrocyte SOD activity and plasma copper and zinc concentrations and both trace metal content in the erythrocytes. The best predictors of SOD activity were zinc to copper ratio in the diet and plasma ceruloplasmin oxidase activity. Thus, the balance between zinc and copper in the diet seems to be of special importance for erythrocyte SOD activity and in consequence the whole-body protection against superoxide action.

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