

ORIGINAL ARTICLE

Zinc Status of and its Association to Cardiovascular Risk Biomarkers

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Abstract

Background: The antioxidant action of some nutrients is important in vascular protection. Zinc, particularly, has been associated with reduced risk of atherosclerosis, stroke and thrombosis.

Objective: The study evaluated zinc status and its association to cardiovascular risk biomarkers in healthy adults.

Methods: Cross-sectional study with 186 university students of both genders, aged between 20 and 30 years, selected using the convenience sampling method. The cardiovascular risk biomarker measurements included the lipid profile, Castelli index I and II, and waist circumference. Zinc analysis was performed by a three-days food record using NutWin program version 1.6.0.7. Plasma and erythrocyte mineral concentrations were determined by flame atomic absorption spectrophotometry. The lipid profile was determined by enzymatic colorimetric methods.

Results: The mean values of zinc intake were higher than the EAR in both genders. Participants had mean plasma and erythrocyte zinc concentrations lower than the cutoff points. The mean values of the lipid profile, Castelli index I and II, and waist circumference were adequate. There was a negative correlation between dietary zinc and total cholesterol and triglycerides.

Conclusions: The participants have a high dietary zinc intake and reduced plasma and erythrocyte concentrations of this mineral. Additionally, this study showed a negative association between zinc dietary intake and total cholesterol and triglycerides, biomarkers of cardiovascular risk, suggesting the importance of zinc in protecting against cardiovascular disease. (Int J Cardiovasc Sci. 2016;29(5):355-361)

Keywords: Cardiovascular Diseases / mortality; Biomarkers; Antioxidants; Adult.

Introduction

Cardiovascular disease is a major cause of morbidity and mortality worldwide. Interactions between environmental and genetic factors result in increased risk for this disease. Clinical and observational studies have shown the association between diet quantitative and qualitative characteristics and the occurrence of cardiovascular diseases, since certain dietary components may be involved in lipoprotein metabolism.^{1,2}

On the other hand, the antioxidant action of some nutrients is important in vascular protection. Zinc, particularly, participates in the antioxidant

defense system, contributing to the action of several enzymes involved in this process and, therefore, has been associated with reduced risk of atherosclerosis, stroke and thrombosis.^{3,4}

Zinc can play an important role in enzymes involved in lipid metabolism. It has been suggested that zinc supplementation can be effective in the marked reduction in triglycerides (TG), total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) levels.⁵⁻⁷

Therefore, changes in zinc concentrations may be associated with reduction of antioxidant activity and, consequently, with a higher risk to develop cardiovascular diseases.^{8,9} Thus, this study aims to assess

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zinc status and its association with cardiovascular risk biomarkers in healthy adults.

Methods

A cross-sectional study was conducted with 186 healthy subjects of both genders, aged between 20 and 30 years who were undergraduate students from a University, using the convenience sampling method. Volunteers were recruited from a public call and the participants were screened using a registration form.

Students taking part in the study met the following eligibility criteria: no chronic non-transmissible diseases (cardiovascular diseases, diabetes mellitus, obesity and cancer), liver disease and thyroid disorder; were not using vitamin-mineral supplementation and/or other drugs that could interfere with the nutritional zinc status.

This research was approved by the Research Ethics Committee the Federal University of Piauí.

Evaluation of the Nutritional Status

The body mass index was calculated from the participant's body weight divided by height square. The classification of the nutritional status, based on the distribution of the body mass index, was performed according to the recommendation of the World Health Organization.¹⁰

Determination of Dietary Zinc

For the assessment of zinc dietary intake, the three-day food record was used and the amounts of zinc in the diet were calculated using the "Nutwin" program, version 1.5.¹¹ After analyzing the diet composition, zinc was adjusted for energy, using the residual method.¹² To verify the adequacy of the dietary zinc intake, the Estimated Average Requirement (EAR) was used as reference, with 6.8 mg Zn/day for females and 9.4 mg Zn/day for males, aged between 20-30 years.¹³

Biological Material Collection

A 20-mL blood sample was collected in the morning, after at least a 12-hour fasting. The collected blood was distributed into separate tubes: (1) glass tube containing 30% sodium citrate as anticoagulant (10 μ L/mL of blood) for zinc analysis (10 mL); (2) and to determine the activity of SOD enzyme (5 mL); (3) tube not containing anticoagulant to determine the lipid profile (5 mL).

Determination of Zinc Biochemical Parameters

Zinc analysis in plasma and erythrocytes was performed using flame atomic absorption spectrophotometry, according to the method described by Rodriguez et al.¹⁴. All materials used for collection and mineral analysis were previously demineralized and the method precision and accuracy were checked using certified standards and secondary standards for plasma and erythrocytes. The reference values were the following: plasma: 70 - 110 μ g/dL¹⁵ and erythrocytes: 40 - 44 μ g/gHb.¹⁶

Cardiovascular Risk Biomarkers

Determination of Serum Lipid Concentrations

The enzymatic colorimetric method was used to assess serum concentrations of triglycerides, total cholesterol, HDL-cholesterol, LDL-cholesterol and VLDL-cholesterol. HDL was determined after precipitation of LDL and VLDL fractions. LDL and VLDL fractions were calculated from the equation: $LDL - c = TC - (VLDL - c + HDL - c)$; $VLDL - c = \text{triacylglycerol} / 5$, using reference values according to the recommendations of the V Brazilian Guidelines for Dyslipidemia and Atherosclerosis, Brazilian Society of Cardiology.¹⁷

Determination of Castelli Risk Indexes I and II

To determine the risk of cardiovascular disease, Castelli risk indexes I and II were used, which correspond to the ratio of total cholesterol and HDL-cholesterol and the ratio of LDL-cholesterol and HDL-cholesterol, respectively. Reference values are: Castelli Index I ≤ 4.3 mg/dL; Castelli Index II ≤ 2.9 mg/dL.¹⁸

Determination of Waist Circumference

The measurement of waist circumference was performed using a flexible, inelastic tape, considering the reference values of the World Health Organization.¹⁹

Statistical Analysis

Data were analyzed using the statistical software SPSS 18.0 for Windows. Kolmogorov-Smirnov's test was applied to check data normality and Levene's test for homogeneity of variances. To compare the groups, Student's *t*-test was used for parametric values and Mann-Whitney test for non-parametric values, with a significance level of $p < 0.05$. The variables were expressed as mean \pm standard deviation and the comparisons

between the groups was performed using the unpaired *t* test. Pearson's correlation coefficient was used to check the potential interrelationship between variables.

Results

Mean and standard deviation for age and anthropometric parameters used to assess participants' nutritional status are provided in Table 1.

Dietary zinc intake was assessed based on the mean intake of the study sample. Mean values of adjusted zinc were higher than EAR ($11,21 \pm 4,01$ mg/day) with statistical difference between genders ($p < 0.05$), with 10.65 ± 3.99 mg/day for females and 11.91 ± 3.97 mg/day for males.

Mean and standard deviation of participants' zinc parameters are provided in Table 2. Plasma zinc

values were adequate only for male individuals, with statistically significant difference in relation to women. Both groups had zinc deficient values in the erythrocyte compartment.

Mean and standard deviation of lipid profile in the participants' serum are provided in Table 3. Statistically significant difference was found between female and male individuals in relation to HDL- cholesterol, Castelli index I and II, and waist circumference, also being adequate according to the reference values for low cardiovascular risk.

The results of the correlation analysis between zinc parameters and cardiovascular risk biomarkers of participants are described in Table 4. There was a negative correlation between dietary zinc and total cholesterol and triglycerides ($p < 0.05$)

Table 1
Mean and standard deviation of participants' age and anthropometric indicators

Parameter	Total (n=186) Mean \pm SD	Female (n=99) Mean \pm SD	Male (n=87) Mean \pm SD	<i>p</i> -value
Age (years)	22.46 \pm 2.24	22.29 \pm 2.20	22.64 \pm 2.27	0.271
Weight (kg)	61.18 \pm 11.07	55.92 \pm 8.64	67.18 \pm 10.49	< 0.001*
Height (m)	1.67 \pm 0.083	1.62 \pm 0.065	1.72 \pm 0.067	< 0.001*
BMI (kg/m ²)	21.95 \pm 3.03	21.33 \pm 2.88	22.65 \pm 3.07	< 0.003*

SD: Standard Deviation; BMI: Body Mass Index; (*) Values significantly different between genders, Mann-Whitney's test ($p < 0.05$) or Student's *t*-test ($p < 0.05$).

Table 2
Mean and standard deviation of participants' plasma and erythrocyte zinc

Parameter	Total (n=186) Mean \pm SD	Female (n=99) Mean \pm SD	Male (n=87) Mean \pm SD	<i>p</i> -value
Plasma Zinc (μ g/dL)	68.64 \pm 14.68	65.97 \pm 14.40	71.69 \pm 14.47	0.008*
Erythrocyte Zinc (μ g/g Hb)	32.96 \pm 9.72	32.20 \pm 10.27	33.83 \pm 9.03	0.255

SD: Standard Deviation; (*) Value significantly different between genders, Student's *t*-test ($p < 0.05$); Reference values: plasma zinc 70-110 μ g/dL¹⁵; erythrocyte zinc 40-44 μ gZn/gHb¹⁶.

Table 3
Mean and standard deviation of participants' cardiovascular risk biomarkers

CR Biomarkers	Total (n=186) Mean ± SD	Female (n=99) Mean ± SD	Male (n=87) Mean ± SD	p-value
TC (mg/dL)	170.05 ± 35.35	172.62 ± 33.44	167.12 ± 37.38	0.294
LDL-c (mg/dL)	99.77 ± 29.66	97.86 ± 28.50	101.94 ± 30.96	0.535
HDL-c (mg/dL)	50.48 ± 13.70	54.99 ± 14.82	45.34 ± 10.16	< 0.001*
TG (mg/dL)	96.00 ± 65.13	92.75 ± 74.84	99.71 ± 52.12	0.116
Castelli I (mg/dL)	3.56 ± 1.11	3.34 ± 1.14	3.82 ± 1.02	< 0.001*
Castelli II (mg/dL)	2.12 ± 0.87	1.93 ± 0.85	2.34 ± 0.84	< 0.001*
WC (cm)	74.27 ± 8.74	70.41 ± 7.34	78.66 ± 8.16	< 0.001*

CR: cardiovascular risk; SD: standard deviation; TC: total cholesterol; LDL-c: low-density lipoprotein cholesterol; HDL-c: high-density lipoprotein cholesterol; TG: triglycerides; WC: waist circumference; (*) Values significantly different between genders, Student's t-test ($p < 0.05$) or Mann-Whitney's test ($p < 0.05$).

Table 4
Simple linear correlation analysis between zinc parameters and cardiovascular risk biomarkers

CVR Biomarker	Dietary Zinc (mg)		Erythrocyte Zinc (µg/g Hb)		Plasma Zinc (µg/dL)	
	r	p	r	p	r	p
TC	-0.163	0.026*	-0.015	0.842	0.056	0.449
LDL-c	-0.077	0.299	0.006	0.938	0.063	0.390
HDL-c	-0.065	0.380	0.080	0.280	-0.070	0.341
TG	-0.160	0.029*	-0.100	0.174	0.059	0.422
Castelli Index I	-0.092	0.212	-0.070	0.345	0.054	0.463
Castelli Index II	-0.050	0.495	-0.032	0.661	0.041	0.576
WC (cm)	0.061	0.411	-0.017	0.820	0.113	0.125

CVR: cardiovascular risk; TC: total cholesterol; LDL-c: low-density lipoprotein cholesterol; HDL-c: high-density lipoprotein cholesterol; TG: triglycerides; WC: waist circumference; (*) Pearson's linear correlation ($p < 0.05$).

Discussion

In this study, biochemical zinc parameters and cardiovascular risk biomarkers in healthy adults were

determined and a correlation analysis between these variables was performed.

The mean zinc concentrations in the assessed individuals' plasma were below the normal range;

however, when sorted by gender, the male group had adequate plasma levels, which were statistically and significantly higher than in women. These results are in agreement with those obtained by Ghasemi et al.²⁰ and Hussain et al.²¹ who also found higher zinc plasma concentrations in this group.

Higher plasma zinc levels in males may be the result of protein turnover, which is proportional to the muscle mass found in males, as well as serum albumin concentrations, which are higher in this group.²² Thus, it is emphasized that protein metabolism seems to contribute to zinc mobilization from the muscle tissue into plasma. On the other hand, reduced zinc concentrations in women may be associated with the action of the hormones estrogen and progesterone during ovulation and the luteal phase of the menstrual cycle, which directs this mineral from plasma to the endometrium.²³

Erythrocyte zinc levels were below the reference values in the study subjects. Similarly, Girish et al.²⁴ also found reduced erythrocyte concentrations of this mineral in healthy subjects. In this regard, it is worth mentioning that mean zinc concentrations found in the diet consumed by the participants were higher than the recommended values, which seem not to affect plasma and erythrocyte concentrations. In this regard, we highlight some factors that seem to interfere with zinc concentrations in blood components, such as reduced absorption and increased demand or losses of this mineral.

There is no universally accepted method to assess zinc nutritional status. Plasma is considered a sensitive biomarker to identify zinc deficiency in the population.²⁵ Additionally, red blood cells are also a reliable biomarker for zinc status analysis, as their concentrations of this mineral are nearly ten times higher than those in plasma, not reflecting recent changes in body zinc concentrations, since it has a longer half-life.^{15,27}

Regarding the lipid profile parameters, it was observed that the findings were in line with the recommended values. Some important aspects can be highlighted in this study, such as the reduced size of waist circumference and the adequacy of Castelli Risk Index I and II of the participants, factors that seem to have contributed to the lipid fractions found in the study.

It should be noted that other studies regarding this topic also had similar results. Freitas et al.²⁷ and Zemdeg

et al.²⁸ also conducted studies in University students and found normal lipid profile markers and waist circumference values in the study subjects.

It is noteworthy that the fat distribution, particularly presence of abdominal fat, is considered a determining factor for metabolic profile alterations and, hence, for the development of cardiovascular diseases, since it is a very lipolytic tissue that contributes to dyslipidemia.^{29,30} Thus, the appropriate values for these markers found in this study suggest reduced risk for such chronic diseases.

As for the correlation analysis between zinc parameters and biomarkers of cardiovascular risk, a negative correlation was found between dietary zinc and total cholesterol and triglycerides. It is important to emphasize that, in situations of low zinc concentrations in plasma and erythrocytes, as seen in this study, the body makes metabolic adjustments for the mobilization of this mineral from a tissue pool through tissue catabolism, such as in the muscle, to maintain its physiological functions³¹. Thus, under these conditions, the mobilized zinc acts on the activity of lipoprotein lipase, which in turn, hydrolyzes the lipid fractions, improving the profile of these markers³².

Based on data obtained in this study and according to the assessed biomarkers, the study participants are not at risk of developing cardiovascular diseases. Adequate zinc intake seems to have not changed its plasma and erythrocyte concentrations. Additionally, this study shows a negative association between zinc dietary intake and total cholesterol and triglycerides, cardiovascular risk biomarkers.

One limitation must be taken into consideration. The assessment of dietary intake is susceptible to random and systematic errors and can be affected by the number of days. To minimize these errors, the data obtained on zinc were subsequently adjusted based on energy intake and intra-individual variation.

Conclusion

The participants have a high dietary zinc intake and reduced plasma and erythrocyte concentrations of this mineral. Additionally, this study showed a negative association between dietary zinc intake and total cholesterol and triglycerides, cardiovascular risk biomarkers, suggesting the importance of zinc in protecting against cardiovascular disease.

Author contributions

Conception and design of the research: Revoredo CMS, Almondes KGS, Nogueira NN, Marreiro DN. Acquisition of data: Revoredo CMS, Aguiar HDSP, Lima SMT, Saffnauer ES, Almondes KGS. Analysis and interpretation of the data: Revoredo CMS, Holanda AON, Araújo CGB. Statistical analysis: Aguiar HDSP. Writing of the manuscript: Revoredo CMS. Critical revision of the manuscript for intellectual content: Revoredo CMS, Lima SMT, Holanda AON, Araújo CGB, Nogueira NN, Marreiro DN.

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Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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Study Association

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