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Circulating zinc levels and cardiometabolic risk-related variables in adults

Determinants of serum zinc levels

Niveles de zinc circulante y variables relacionadas con el riesgo cardiometabólico en adultos

Milton Fabián Suárez-Ortegón [1], Alejandra Arbeláez [2], José Guillermo Ortega-Ávila [3], Mildrey Mosquera [2,4]

1. Departamento de Alimentación y Nutrición, Facultad de Ciencias de La Salud, Pontificia Universidad Javeriana Seccional Cali, Cali, Colombia
2. Grupo de Nutrición, Universidad del Valle, Cali, Colombia
3. Departamento de Ciencias Básicas, Facultad de Ciencias de La Salud, Pontificia Universidad Javeriana Seccional Cali, Cali, Colombia
4. Departamento de Ciencias Fisiológicas, Universidad del Valle, Cali, Colombia

Correspondencia:

Milton Fabián Suárez-Ortegón, Pontificia Universidad Javeriana seccional Cali, Calle 18 No. 118-250, Cali, Colombia.

Tel.: +57 2 3218200

milton.suarez@javerianacali.edu.co

Corresponding author:

Milton Fabián Suárez-Ortegón: conceived the study design, obtained funding, collected, researched and analysed data, and drafted the manuscript.

Alejandra Arbeláez: collected and researched data.

José Guillermo Ortega-Ávila: researched data.

Mildrey Mosquera: obtained funding, collected and researched data.

Introduction. Altered serum zinc levels, lower and higher in respect to healthy controls, have been observed in people affected by non-communicable chronic diseases. However, to date, studies describing potential determinants of zinc levels in general populations free of chronic diseases appear to be limited.

Objective. To evaluate whether nutrient intake, biochemical and clinical measures, lifestyle and family history of cardio-metabolic diseases were independently associated with zinc levels in apparently healthy individuals.

Materials and methods. Two-hundred-thirty-nine healthy subjects were evaluated. Serum zinc was measured by Flame Atomic Absorption Spectrometry and the rest of biochemical marker by enzymatic-colorimetric methods. Standard techniques were used to measure waist circumference, height and weight. Body fat was estimated by impedanciometry and blood pressure by digital sphygmomanometers. A survey was applied to record personal and familial history of non-communicable chronic disease (NCCD), and nutrient intake was estimated by using the 24-h recall method.

Results. Women had lower serum zinc levels than men. In multivariate analyzes total fat intake [Beta=-0.15, Standard error(SD) 0.03,P<0.001], plasma log-triglycerides[Beta=-10.18,SD 3.9,P=0.010], and female gender[Beta=-6.81,SD 3.3,P=0.043] were significant predictors for serum zinc levels. Zinc intake was not significantly related to serum zinc in both, univariate and multivariate analyses.

Conclusions. Variables related to cardiometabolic risk such as plasma triglycerides levels and total fat intake were associated with serum zinc levels in individuals without diagnostic of chronic or infectious/inflammatory diseases.

Further studies are required to confirm our findings as well as evaluation of possible mechanisms for these relationships.

Key words: Zinc; heart disease risk factors; triglycerides; micronutrients.

Introducción. Se han observado niveles séricos alterados de zinc, tanto más altos como más bajos, en personas afectadas por enfermedades crónicas no transmisibles. Sin embargo, información sobre determinantes de zinc sérico en poblaciones sin enfermedad crónica es muy limitada.

Objetivo. evaluar si la ingesta de nutrientes, las medidas bioquímicas y clínicas, el estilo de vida y los antecedentes familiares de enfermedades cardiometabólicas se asociaron de forma independiente con los niveles de zinc en individuos aparentemente sanos.

Materiales y métodos. Se evaluaron 239 sujetos sanos. El zinc sérico se midió por espectrometría de absorción atómica de llama y el resto de marcadores bioquímicos por métodos enzimático-colorimétricos. Se utilizaron técnicas estándar para medir antropometría. Se aplicó una encuesta para registrar antecedentes personales y familiares, y se estimó el consumo de nutrientes por recordatorio de 24 h.

Resultados. Las mujeres tenían niveles séricos de zinc más bajos que los hombres. En análisis multivariados, la ingesta total de grasas [Beta=-0.15,Standard error(SD) 0.03,P<0.001], los triglicéridos plasmáticos[Beta=-10.18,SD 3.9,P=0.010], y el sexo femenino[Beta=-6.81,SD 3.3,P=0.043] fueron predictores significativos de los niveles séricos de zinc. La ingesta de zinc no estuvo significativamente relacionada con el zinc sérico en los análisis univariados y multivariados.

Conclusiones. Variables relacionadas con el riesgo cardiometabólico como los niveles de triglicéridos y la ingesta total de grasas se asociaron con los niveles de zinc en individuos sin diagnóstico de enfermedades crónicas o infecciosas/inflamatorias. Se requieren más estudios para confirmar nuestros

hallazgos, así como la evaluación de posibles mecanismos para estas relaciones.
Palabras clave: Zinc; factores de riesgo de enfermedad cardiaca; triglicéridos; micronutrientes.

Zinc is the most abundant intracellular trace element, being both structural and functional part of many enzymes involved in processes of cell division and growth, immune response, gene expression, sexual reproduction, and antioxidant defense, among others (1). Although zinc status has been mainly studied in relation to nutritional deficiency, in recent years there has been a growing interest on zinc status in cardiometabolic diseases (CMD) such as type 2 diabetes and cardiovascular diseases (CVD), and results are still conflicting. A systematic review and meta-analysis reported a positive association between serum or blood zinc and type 2 diabetes, presumably because of disturbed body zinc distribution (2). In contrast, Hennigar et al. did not find a significant difference in serum zinc concentration between individuals with and without diabetes in populations from the NHANNES 2011-2014 (3). Meanwhile, according to in-vitro evidence zinc deficiency may lead to alterations of insulin secretion and predispose to T2D (4,5). However, the studies in humans on blood circulating zinc and T2D are few and most of them with cross-sectional design (2).Similarly, accumulated observations in cross-sectional studies has shown a trend for lower zinc status in people affected by CVD in comparison with controls (6). Additional reverse causation between CMD and zinc is highly plausible since metabolic alterations proper of CMD such as inflammation and increased oxidative stress could also affect zinc balance via up-regulation of metallothionein, a protein which catch zinc ions and blocks its body distribution (1).

To date, studies describing potential determinants of zinc levels in general populations free of chronic diseases appear to be limited. Thus, we conducted a study to assess whether metabolic, nutritional, clinic, and lifestyle variables and

also family history of cardio-metabolic diseases (CMD) could be independently associated with zinc levels in apparently healthy individuals.

Materials and methods

Subjects

This study was performed by using available collected data from a project on irons status, insulin resistance and type 2 diabetes. This project has a bank of the blood serum of the participants preserved at -70 Celsius grades. In 239 out of 245 samples, serum zinc measurement was achieved on the basis of feasible volume for the laboratory technique. The original project had a convenience sample and the participants were voluntarily recruited from the staff of a hospital, a university, a governmental health department and a supermarket chain in Cali-Colombia, after responding to advertisements describing the research. Apparently healthy subjects (120 men, 119 women) aged 25-64 years old were included in the final sample. To ensure healthy condition and avoid confounding in serum zinc, lipid profile variables, insulin, glucose or high sensitivity C Reactive Protein (hs-CRP) levels, we excluded individuals with the following conditions: clinically significant hepatic, neurological or endocrinologic disease; cardio-metabolic diseases (except class I obesity) or other major systemic disease; tobacco use; long-term multivitamin or vitamin supplements consumption (two or more days/week in the last six months); therapy with drugs to lower lipid or glycemia levels; evidence of acute or chronic inflammatory or infective diseases. The Universidad del Valle Research Ethics Committee approved the research (Permission number: 0016-07). All participants gave written informed consent.

Clinical measurements

Trained researchers measured waist circumference (WC), height and weight using standard techniques by Lohman (7,8), and body mass index (BMI) was calculated. In addition, percentage of body and blood pressure were estimated by using an impedanciometer (OMRON®) and digital sphygmomanometers (OMRON®), respectively.

Biochemical markers

The following biochemical markers were determined: glycemia, plasma triglycerides, total cholesterol and HDL cholesterol (HDL-C) (direct method) by colorimetric assay (Biosystems®, Spain); insulin levels by chemoluminescence test (kit IMMULITE 1000, San José, CA), and levels of high sensitivity C reactive protein (hs-CRP) by turbidimetry (Biosystems®, Spain). An autoanalyzer A-15 was used (Biosystems®, Spain). LDL-cholesterol (LDL-C) was calculated by Friedwald equation (total cholesterol – HDL-cholesterol-(tryglicerides /5)(9). HOMA-IR (Homeostatic Model Assessment Insulin Resistance) index was calculated as ([glucose mmol/L] x [insulin mU/mL]/22.5) (10).

The Flame Atomic Absorption Spectrometry method standardized by Smith et al. was used to measure serum zinc levels. (11). Test tubes intended for Zn analysis underwent thorough cleaning with 0.1% Nitric acid to avoid contamination of the samples. To analyze Zn, a plasma sample was diluted fivefold with deionized water, meaning 500 µL of plasma was mixed with 2 mL of deionized water. Zinc calibrators were sequentially aspirated by the spectrometer (Shimadzu AA-7000), starting from the least concentrated to the most concentrated, until stable readings were obtained. These values were

utilized to create calibration curves through least squares regression fitting to extrapolate samples concentrations. The variation coefficient was 5.4% and the recovery percentage was 106.6%.

Dietary intake and personal and family history

Food intake was estimated via the 24-h dietary recall method using the food composition table of the center of nutritional service elaborated by Quintero and Escobar (12). All of the nutrients available in the table were evaluated. The dietary recalls were conducted in one day, Thursdays or Fridays, which were the days scheduled for complete evaluation of the individuals recruited. Similarly, a survey to record personal data and cardio-metabolic diseases history in siblings, aunts/uncles, parents, and grandparents was applied by trained interviewers**.** Practice of physical activity peer week was also recorded according to having or not sessions of vigorous or moderate physical activity (3 sessions of at least 30 mins per week). None physical activity was defined as not having any session of physical activity peer week.

Data analysis

The analyses were carried out in the whole group without any kind of stratification. In case of not having a Gaussian distribution, the study variables were reported as median and interquartile range, while mean and standard deviation were used to describe normally-distributed variables. The relationship between potential determinants and serum zinc levels was tested via linear regression. Multiple linear regression models were built to explain serum zinc levels. First, we explored potential determinants via a preliminary univariate analysis, in which those variables with at p value <0.10 reached the multiple explanatory model (13). In this multiple model, a p value <0.05 was considered

significant. Variables non-normally distributed were log-transformed before their inclusion in the linear regression analyses (intakes of carbohydrate, ascorbic acid, vitamin A, zinc, and iron; calories/day; HOMA-IR index; hs-CRP; and plasma triglycerides).

Complementarily, we ran a linear regression analysis in which the built multivariable explanatory model of serum zinc levels was additionally adjusted for a set of covariates (potential confounders or variables of influence in the predictors-outcome´s relationship) regardless of the statistical significance of these covariates as predictors of zinc levels. The planned covariates were: age, sex, hs-CRP levels, total intake (calories/day), BMI (Kg/m^2), and none physical activity per week (yes/no). The inclusion of a covariate was obviated if this already was part of the multivariable explanatory model. All the analyses were carried out by using software STATA 10.0.

Results

Table 1 describes the study population. Individuals were adults (mean age: 45.5 years) and BMI was in the overweight range. A total of 110 individuals (46%) had overweight, and 48 (20%) had obesity class I. Average LDL–C and plasma triglycerides levels were in the upper reference range; and family history of hypertension was the most prevalent CMD antecedent.

The univariate analysis showed negative significant negative associations ($p < 0.1$) with serum zinc levels, except for log-plasma triglycerides levels and glycaemia, which showed positive relationships (table 2). Intakes of minerals, including zinc itself, were not found associated with serum zinc levels. Female sex was found inversely associated with serum zinc levels. Log-CRP levels and

self-reported familial history of obesity, and total fat intake were also related to serum levels ($p < 0.1$).

In the multivariate analysis (table 3) variables that emerged as significant predictors ($p < 0.05$) for serum zinc levels, were total fat intake and log-plasma triglycerides in the whole group, total fat intake and age (negative predictor with a marginal significance) among men, and log-plasma triglycerides levels, log-hs-CRP levels (negative predictor with a marginal significance) and total fat intake among women.

Table 4 shows the multiple model from table 3 additionally adjusted a preestablished set of covariates (age, calories/day, BMI, and physical activity practice). Findings were similar to those from the multiple model in table 3. Particularly, female gender improved its statistical significance as negative predictor of serum zinc levels ($p=0.076$ to $p=0.043$).

Discussion

The present study evaluated the independent relationship of a group of nutritional, sociodemographic, biochemical, anthropometrical and life style variables with serum zinc levels. Female gender and variables related to cardiometabolic risk such as plasma triglycerides levels and total fat intake were associated with serum zinc levels.

We found plasma triglycerides as the only lipid profile variable significant and independently related to serum zinc levels in the whole group and among women. To our knowledge this is the first report of a relationship between plasma triglycerides and serum zinc in a multivariate analysis. Previously, Tully et al. described positive correlation of serum zinc with total and LDL cholesterol in elderly women (77-99 years old) and Laitinen et al. with LDL-C, HDL-C and

total cholesterol in young people (3-18 years old)(14,15), while He et al. did not find relationship between serum zinc and lipid profile in men(16). Tully et al. supported their findings in relation to red meat consumption, a source of both zinc and saturated fat. On the other hand, in our study, we found that the higher the total fat intake, the lower the serum zinc levels. We did not find similar studies with evaluation of total fat intake as a potential predictor of serum zinc levels. Serum zinc levels could negatively response to fat load meals via consumption of the metal in peripheral tissues because there is evidence of zinc as a promoter factor of insulin signaling (17). Another potential explanation for the inverse association between fat intake and zinc levels might lay on pro-inflammatory effects of intracellular fat (18). Inflammation in turn, triggers sequestration of tissue zinc (19). Meanwhile, the positive association between plasma triglycerides levels and serum zinc could be related to lipolytic effects of serum zinc-alpha2-glycoprotein (20). Nevertheless, this hypothesis and other possible mechanisms have to be evaluated in further studies.

In this analysis, women presented lower zinc values than men, and female gender was a negative predictor of serum zinc levels. However, the relationship of serum zinc levels with gender is not conclusive. While our finding by gender agrees with those reported by Mariani et al. (21), other authors did not observe that relationship (22,23), and even Schumacher et al. had reported higher serum zinc levels in women (24). Although Andriollo-Sanchez et al. argued the lack of difference in serum zinc by gender based on a low hormonal status in their middle aged and old populations, the Mariani et al. study was carried out in healthy old subjects. Women in our analysis presented higher body fat percentage and hs-CRP levels than men, and these variables were inversely

associated with serum zinc levels in univariate analysis. In particular, hs-CRP levels were significant serum zinc predictors in the multiple model if gender and body fat percentage were not included, while body fat percentage was found associated only if gender was not in the multivariate analysis (data no shown). These findings are consistent with the idea of a possible subclinical inflammation derived from adipose tissue as modulator of serum zinc levels, as previously discussed.

Among the possible nutritional determinants of serum zinc levels, zinc intake was not a significant predictor. This finding is coherent with two explanations: one, is the fact that serum zinc levels is not an ideal marker for assessment of zinc status, with the exception of severe deficiency cases (25,26); and two, that serum zinc levels are influenced or modulated by non-nutritional factors, as we found. A not significant correlation between zinc intake and serum zinc was described by Hyun et al. ($r = 0.005$, $p = 0.929$), but it became significant when zinc supplements consumption was added to zinc intake, although this correlation was weak ($r = 0.114$, $p = 0.027$)(27). Andriollo-Sanchez et al. also reported a weak but significant correlation between zinc intake and serum zinc ($r = 0.129$), but zinc intake was not discriminated between food and supplements consumption (22). In agreement with our non-significant finding, Hennigar et al. did not find an association between zinc intake and serum levels of the same micronutrient in U.S general population (3). In contrast, some studies (25,28,29) have found significant increments in serum zinc levels after supplementation with zinc. It seems zinc supplement use would improve the relationship between intake and circulating levels, perhaps because a better bioavailability of commercial zinc salts and considerable increase in the amount

of consumed zinc. However, the bioavailability would depend on the kind of supplement (e.g. aqueous, tablets) and salt used (e.g. zinc sulfate, histidinate) (30,31). The use of mineral and/or vitamin supplements was an exclusion criterion in our study and therefore the zinc intake is clearly nutritious.

There are limitations of the present study that have to be mentioned. 24 hours recall survey was from one day and data average of two days could be more acute in terms of estimations of individual intakes. In addition, the cross-sectional nature of the study does not allow to determine cause-effect relationships. The group of individuals of the original project was a convenience sample and thus, the findings of this study should be contrasted with results from future population-based studies in free-NCCD individuals. Similarly, excluding individuals with overweight/obesity had been recommendable due to potential effects of excess adiposity on the relationship between cardiometabolic determinants and serum zinc. However, in Colombia one out of each two individuals is affected by overweight or obesity (32), and excluding individuals with this condition would drastically affect the sample size and the capability to detect associations. At any rate, when the relationships obtained were adjusted for BMI and other covariates, these did not substantially change. On the other hand, the kind of recruited sample of our study represents a strength in terms of apparently healthy condition, and absence of potential confounding factors (tobacco use, multivitamin supplements and medications consumption). Similarly, the evaluation of a wide range of cardiovascular risk markers and CMD family history (and not only nutritional and anthropometrical variables) as predictors of serum zinc levels is other favorable point of the present research.

In summary, variables related to cardiovascular risk such as plasma triglycerides levels, total fat intake and hs-CRP levels were predictors for serum zinc levels. Further studies are required to confirm our findings as well as evaluation of possible mechanisms for the relationship between serum zinc and predictor variables.

Conflict of interest

The author declare no conflicts of interest exist.

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Table 1. Description of study population

n	239
Age (years)[1]	45.5 ± 7.7
Clinical variables	
BMI (kg/m^2) [1]	26.1 ± 3.7
WC (cm)[1]	80.5 ± 10.4
Body Fat (%)[1]	31.7 ± 10.0
SBP (mmHg)[1]	117 ± 16.4
DBP (mmHg)[1]	74 ± 11.3
Menopausal Women n(%)	50 (20.9)
Biochemical variables	
Zinc (µg/dL)[1]	76.6 ± 13.5
Cholesterol (mg/dL)[1]	197 ± 34.1
HDL-C (mg/dL)[1]	48 ± 11.1
LDL-C (mg/dL)[1]	115 ± 29.1
Log- Plasma triglycerides (mg/dL)[2]	131 (94-196)
Log-Glucose	88 (83-93)
Insulin	8.4 (5.7-13.3)
Log- hs CRP (mg/L)[2]	1.5 (1.1-2.4)
Log-HOMA IR[2]	1.7 (1.1-2.8)
Dietary intake	
Total energy intake (Calories/day)	1859.3 (1555.5-2111.8)[2]
Total fat(g/day)[1]	89.5 ± 27.7
Carbohydrates (g/day)	197.6 (163.0-251.3) [2]
Protein (g/day)[1]	65.9 ± 25.7
Calcium (mg/day)[2]	366.5 (204.6-543.7)
Iron (mg/day)[2]	9.1 (6.8-12.4)
Zinc (mg/day)[2]	6.9 (4.3-10.1)
Vitamin A (mg/day)[2]	406.3 (256.6-712.6)
Vitamin C (mg/day)[2]	58.6 (37.5-115.4)
Copper (mg/day)[2]	0.5 (0.3-0.7)
Myocardial infarction n(%)	87 (36.4)
Stroke n(%)	42 (17.6)
Dyslipidemia n(%)	95 (39.7)
Diabetes n(%)	111 (46.4)
Hypertension n(%)	140 (58.6)
Obesity n(%)	81 (33.9)
Lifestyle variables	

None physical activity peer week n(%)	118 (49.4)

[1]Mean ± Standard Deviation
[2]Median (Interquartile range)
WC, Waist circumference. BMI, Body mass index. hs-CRP, high sensitivity C reactive protein. HDL-C, HDL cholesterol. LDL-C, LDL cholesterol. SBP, systolic blood pressure. DBP, diastolic blood pressure. IR-HOMA, Insulin resistance homeostatic Model Assessment.

Table 2. Univariate analysis with serum zinc levels as dependent variable in whole group and by gender

Variable	β Coefficient	Std. Error	p Value
Clinical variables			
BMI	-0.18036	0.239	0.452
WC	0.13669	0.083	0.104
% Body Fat	-0.27333	0.087	**0.002**
SBP	0.01147	0.053	0.831
DBP	-0.01801	0.077	0.817
Menopause	2.42550	2.422	0.318
Biochemical variables			
Cholesterol	0.02452	0.025	0.340
HDL-C	-0.09298	0.078	0.239
LDL-C	-0.03552	0.030	0.243
Log- Plasma triglycerides	14.3445	3.610	**<0.001**
Log-Glucose	41.2479	19.189	**0.003**
Insulin	0.91705	3.381	0.786
Log-HOMA	1.80126	3.176	0.571
Log-hs CRP	-0.50104	3.042	0.034
Dietary intake			
Calories	-12.07119	7.711	0.116
Total fat	-0.14774	0.030	**<0.001**
Carbohydrates	5.92232	5.496	0.282
Protein	0.02194	0.034	0.524
Calcium	3.49406	2.660	0.190
Iron	-1.03839	4.827	0.830
Zinc	0.06269	0.190	0.742
Log-Vitamin A	1.69669	1.900	0.393
Log-Vitamin C	1.66690	2.422	0.492
Copper	0.57591	1.518	0.705
Family history			
Myocardial infarction	0.53947	1.819	0.767
Stroke	1.77387	2.297	0.441
Dyslipidemia	2.43881	1.782	0.172
Diabetes	-1.07516	1.754	0.541
Hypertension	-1.74105	1.774	0.327
Obesity	-3.81356	1.833	**0.039**
Sociodemographic variables			
Gender (Female)	-7.21638	1.687	**<0.001**
Age	-0.07133	0.113	0.529
Lifestyles			
None physical activity peer week	-0.64469	1.750	0.714

WC, Waist circumference. BMI, Body mass index. hs- CRP, high sensitivity C reactive protein. HDL-C, HDL cholesterol. LDL-C, LDL cholesterol. SBP, systolic blood pressure. DBP, diastolic blood pressure. IR-HOMA, Insulin resistance homeostatic Model Assessment.

Table 3. Multiple linear regression model[1] with serum zinc levels as the outcome

	β Coefficient	Standard Error	p Value
% Body Fat	-0.07303	0.135	0.589
Log-Plasma plasma triglycerides	10.18376	3.901	**0.010**
Log-hs CRP	-3.19418	3.162	0.314
Log-Glucose	1.72965	19.498	0.929
Total fat Intake	-0.15065	0.031	**<0.001**
Familial history of obesity	-0.76590	1.805	0.672
Gender (Female)	-4.77600	2.683	0.076

[1] Multiple model was built with variables related to serum zinc ($p < 0.1$) in a preliminary univariate analysis.
hs- CRP, high sensitivity C reactive protein.

Table 4. Serum zinc´s explanatory multiple model[1] adjusted for a preestablished set of covariates[2]

	β Coefficient	Standard Error	p Value
% Body Fat	0.1237	0.188	0.512
Log-Plasma plasma triglycerides	10.7795	3.948	**0.007**
Log-hs CRP	-2.6393	3.190	0.409
Log-Glucose	12.5293	20.120	0.534
Total fat Intake	-0.1844	0.044	**<0.001**
Familial history of obesity	-0.3060	1.828	0.867
Gender (Female)	-6.8171	3.348	**0.043**

[1] Multiple model was built with variables related to serum zinc ($p < 0.1$) in a preliminary univariate analysis.
[2] Covariates were forced into the model to account for potential confounding/influence in the relationship between predictors and outcome depicted in table 3, and were: Age (years), total intake (Calories/day), BMI (Kg/m^2), and none physical activity per week (yes/no).
hs- CRP, high sensitivity C reactive protein.