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Copper-Zinc serum traces, physical activity and geriatric depression in older adults: a cross-sectional study

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Abstract

Aim: to evaluate serum levels of copper and zinc and to investigate the association between copper-to-zinc (Cu/Zn) ratios and age, self-reported depression, and physical exercise in a sample of adults aged 60 to 92 years. **Methods and materials**: 101 participants without prior cardiovascular disease were enrolled. Anthropometric measurements were taken, and blood samples were collected from the left arm. Serum copper and zinc levels were measured by atomic absorption spectroscopy, and Cu/Zn ratios were calculated by absorbance and colorimetric tests. Geriatric depression was assessed using the Yesavage scale index (GDS-15). Statistical analyses included chi-square tests and logistic regression models with a significance level of 5%. **Results**: of the 101 participants, 54 were physically active (i.e., engaged in at least 60 minutes of exercise, three times per week) with an average age of 67.5 ± 7.8 years, while 47 were sedentary with an average age of 74.8 ± 9.1 years. The results showed a high prevalence of Cu-Zn deficiency and alterations in Cu/Zn ratios in older adults and those with low physical activity. Multivariate analysis revealed an association between Cu/Zn ratios and physical exercise and age. Physical exercise was found to be a protective factor (odds ratio [OR] = 0.086; 95% confidence interval [CI]: 0.021-0.348; $p < 0.001$), while age was a risk factor (OR = 1.062; 95% CI: 1.005-1.123; $p < 0.034$) for high Cu/Zn ratios (≥2). **Conclusion**: in populations with a high prevalence of copper and zinc deficiencies, Cu/Zn ratios appear to be independent of nutritional parameters and more strongly associated with age and physical exercise.

Keywords: copper-zinc, serum traces, geriatric depression, physical activity.

Introduction

Copper (Cu) and Zinc (Zn) are essential trace elements that play a crucial role in various biological processes within the human body (Speich et al., 2001). These elements are necessary for maintaining nutritional status and regulating transport processes, acute phase reactions, and protection against oxidative (Kloubert & Rink, 2015; Malavolta et al., 2015; Mocchegiani et al., 2012; Ranasinghe et al., 2015). Furthermore, Cu and Zn are vital for the immunological response against infectious diseases (Stafford et al., 2013).

Zn is involved in cell growth and differentiation, energy metabolism, protein synthesis, and is a crucial component of many bodily metalloenzymes. It is also an integral part of antioxidant molecules and plays a role in the synthesis and stabilization of proteins, RNA, and DNA (Kloubert & Rink, 2015). Zn is also essential for the structure of ribosomes and membranes. On the other hand, Cu is a vital part of numerous antioxidant enzyme systems (Harrison & Kasper, 2015) and has several other crucial functions within the body.

Deficiencies of Cu and Zn are infrequent, but they are prevalent in developing or low-income countries (Gernand et al., 2016). Zn deficiencies can lead to decreased taste and smell, alopecia, dermatitis, diarrhea, immune dysfunction, growth retardation and development, gonadal atrophy, and congenital malformations. In contrast, Cu deficiencies can result in severe anemia, defects in keratinization and pigmentation of hair, hypothermia, degenerative changes in aortic elastin, osteopenia, and mental deterioration (Harrison & Kasper, 2015).

The CZr ratio is an essential indicator of the development and course of disability, mainly related to low-grade chronic inflammation independent of nutritional status (Malavolta et al., 2015). However, it is not precisely known how the inverse relationship between Cu and Zn operates. It is also worth noting that high levels of supplemented Zn (greater than 75 mg/day) can cause hematological and gastrointestinal effects, immunotoxicity, and lowering of cholesterol levels (HDL-c) by decreasing the absorption of copper (Saravanabhavan et al., 2017).

Age-related changes can also affect the study parameters, with the inverse CZr relationship being characteristic of diseases and disorders in advanced ages (Malavolta et al., 2015). Larger ratios of Cu-Zn than two in the elderly are associated with a reduced capacity of the organism to maintain or recover homeostasis after a destabilizing event due to deficient nutritional status, high oxidative stress, an inflammatory response, hormonal changes, or cellular senescence (Malavolta et al., 2015). Furthermore, high CZr levels have been linked to taste disorders and immune system dysfunction (Bahi et al., 2017; Yanagisawa et al., 2016), indicating that CZr can be a good indicator for various pathologies.

The present investigation aims to determine the serum levels of Cu-Zn and relate the proportions of CZr with age, psychological depression, physical activity, and lipid profile in a sample of adults over 60 years of age in Venezuela.

Methods and materials

This cross-sectional study was conducted in February 2018 among a group of elderly individuals residing in the Libertador Municipality of Mérida, Venezuela. The participants were divided into two groups based on their level of physical activity. The study was conducted in accordance with medical principles for research on human subjects, including the Declaration of Helsinki and the standards set by the Committee at the University of The Andes.

Sample size and selection criteria

The sample size was determined based on a significance level of 5%, a prevalence of 50% in physical activity (defined as more than 3 times per week), and an accuracy of 10%. This calculation resulted in a sample of 96 subjects. Ultimately, a total of 101 subjects were included in the study, with the following inclusion criteria: individuals over 60 years old, apparently free of acute illness or mental disorders as assessed by their treating physicians, able to perform daily activities, and non-smokers. Participation in the study was voluntary, and elderly individuals with reduced mobility were excluded from the study.

Data collection procedure

To collect anthropometric measurements, the researchers used specific instruments. Weight was measured using a D350K office balance with a maximum capacity of 160kg. Waist size and waist were measured using a metric tape with a maximum capacity of up to 2 meters. In addition, data on depression and physical independence were obtained through the use of two questionnaires. The Geriatric Depression Scale-Yasavege Test, which has good psychometric properties (Galeoto et al., 2018), was used to collect information on depression, while the Katz Index developed by Sidney Katz, which has been validated (Arik et al., 2015), was used to measure physical independence.

Blood samples were collected from participants following a specific protocol. Participants were evaluated while fasting at 7:00 a.m. The puncture kit used included a tray, cotton swabs, sterile forceps, 10 cc syringes, gloves, and a tourniquet. Blood samples were divided into two labeled test tubes, a red cap without anticoagulant, and transferred to the laboratory. The blood serum samples were left to rest for half an hour and then centrifuged at 2500rpm for 15 minutes before being stored in labeled Eppendorf tubes.

Determination of Total Cholesterol (TC) and High-Density Lipoprotein cholesterol (HDL-c)

The Laboratory of Clinical Biochemistry at the Faculty of Pharmacy and Bioanalysis of the University of The Andes (ULA) performed the determinations of total cholesterol (TC) and HDL-c. Blood was collected in a 13 x 100 test tube without anticoagulant, then centrifuged for 10 minutes at 2500 revolutions/min. The resulting serum was transferred into two test tubes for the corresponding determinations. The enzymatic method was used to determine TC in serum, using the StanbioTM LiquiColorTM Cholesterol Test (Trinder). The test utilized cholesterol oxidase of bacterial origin, followed by chemical saponification of cholesterol esters and HDL-c, which were determined in the supernatant fluid using a dilution factor derived from the calculation. The StanbioTM HDL-c kit was used as the precipitating reagent.

Four test tubes were labeled: white (W), standard or calibrator (S), control (C), and patient (P), and the working reagents were added. For the white tube, 500 ul of the working reagent and 5 ul of distilled water were used. For the standard or calibrator tube, 500 ul of the working reagent and 5 ul of the standard were used to calibrate the test in the equipment and measure its precision. For the control tube, 500 ul of the working reagent and 5 ul of the control were used to evaluate the reliability and precision of the method. For the patient tube, 500 ul of the working reagent and 5 ul of the patient's serum were mixed and brought to a water bath at 37 °C for 5 minutes for cholesterol and 10 minutes for the analyzer (Wiener lab. Metrolab 1600) to read at a wavelength of 500 nm.

Determinations of serum Cu-Zn levels

The Molecular Spectroscopy Laboratory at the Faculty of Sciences of the University of The Andes (ULA) was used for the determinations of Cu and Zn. An Atomic Absorption Spectroscopy system, coupled with a Flow Injection System (FIA-AAS) was used, which consisted of a Perkin-Elmer ASS 3100 spectrophotometer with a flame atomizer, coupled with an ISMATEC peristaltic pump model IPC, and the sources of Copper and Zinc hollow cathode lamp brand Varian.

Analytical grade reagents from the Merck house were used for the analysis of trace elements, and high-purity deionized water from Milli-Q (18 M´Ω cm-1 resistivity) was used for the preparation of solutions. Zinc metal (Zn metal with 99.9 % purity) and copper nitrate (Cu (NO_3)23H2O) with 99.5% purity were also used for the preparation of the solutions. A standard solution of 1000 mg/L of Copper was prepared by dissolving 3.7832 g of copper nitrate trihydrate in 5 mL of (1+1) nitric acid NHO3 65% (m/m) for analysis. Subsequently, 10 mL of HNO3 was added, and one liter of deionized water was added (dilution to 1 liter with 1% v/v HNO3). The working solution was prepared from this standard solution, by appropriate dilution with deionized water. The standard solution of 500 mg/L Zn was prepared by dissolving 0.500 g of metallic Zn in 5 mL of hydrochloric acid HCL 37% (m/m) for analysis. After that, 10 mL of HCL was added and one liter of deionized water was added.

For the determination of Cu and Zn, the serum was shaken, and 30 µL was taken using a Hamilton syringe, which was then introduced firmly and constantly into the flow injection system coupled to the atomic absorption spectrophotometer. The analytical conditions were verified with an aqueous pattern corresponding to each normal physiological value reported by the literature for Cu and Zn, following the recommendations of the Perkin-Elmer 3100 manual.

Statistical analysis

The results of this study were presented through various statistical analyses. Descriptive statistics were displayed in tables, including means ± standard deviation, absolute and relative frequencies, for variables such as age, sex, height, weight, self-reported pathologies, smoking habit, arterial hypertension, physical exercise, GDS, serum Zn, Cu, Total Cholesterol (TC), and High-Density Lipoprotein cholesterol (HDL-c). The measurements of central tendency, minimum, maximum deviations, and Relative risk (RR) for contingency table (2x2) were also presented. Indices such as CZr ratio (Cu/Zn), IBM Index Body Mass (kg/m^2), Castelli Risk Index I (CRI: TC/HDLc), Z-score (for CZr, Cu-Zn) and score of the GDS-15 were calculated and reported.

To analyze the effect of age on serum levels of Cu-Zn and CZr ratio, age was recoded into three categories: less than 70, between 70-80, and over 80 years old. Linear transformations were used for the dependent variables. Standard scores were calculated from the mean and group standard deviation to represent the typified variables (Z-score) with mean zero and standard deviation one (Z: 0;1) respectively. The statistical tests used included $\chi 2$ test, Kolmogorov-Smirnov test, Student's t-test, and Kruskal-Wallis test. Bivariate analysis was performed using categorical and continuous variables with a significance level of 5%. Binary logistic regression models were used in multivariate analysis to assess the association between dichotomized CZr levels, physical activity, and age, adjusted for the presence of depressive symptoms, total cholesterol, and the Castelli I index. Two models were adjusted to avoid overfitting, one with depressive symptoms and total cholesterol levels (model 1), and another with depressive symptoms and Castelli I index (model 2). All statistical calculations were performed using the SPSS program (Spss, 2011).

Results

The study included 101 participants, comprising of 68 women (67.3%) and 33 men (32.7%), with an average age of 70.95 ± 9.1 years (SD), a median age of 70 years, and an age range of 60 to 92 years. Of these participants, 47 individuals were classified as non-physically active (NPA), including 28 men and 18 women, whereas 54 individuals, including 5 men and 49 women, engaged in physical exercise at least three times per week and were classified as physically active (PA). Among these groups, the proportion of physically active women was higher (72%) compared to physically active men (15%). Notably, a significant difference was observed in the mean age between the physically active and non-physically active groups (PA: 67.6 ± 7.8 years vs. NPA: 74.8 ± 9.1 years; $p<0.0001$). Please refer to Table 1 for the descriptive characteristics presented as mean ± standard deviation.

Table 1. Descriptive characteristics of the participants in the study.

Variables	NPA (n=47)	PA (n=54)
Age (years)	74.8±9.1	67.5±7.8**
Body weight (Kg)	56.5±11.1	61.3±11.9
Height (m)	1.57±0.09	1.55±0.06
BMI	22.5 ± 3.5	23.0 ± 2.8
Waist-to-hip Ratio	0.87 ± 0.05	0.91 ± 0.05

PA: Physical Activities. NPA:Non-Physical Activities. BMI: Body Mass Indexes.

The study sample consisted of 101 participants, comprising 68 women (67.3%) and 33 men (32.7%), with an average age of 70.95 ± 9.1 years (standard deviation), a median age of 70 years, and age range from 60 to 92 years. Of these participants, 47 were classified as Non-Physically Active (NPA) (28 men and 18 women), while 54 were classified as Physically Active (PA) (5 men and 49 women). The frequency of physical exercise was higher in women (72%) than in men (15%).

The results revealed that the average values for Zn and Cu were 4.84 ± 0.79 µmol/L and 8.74 ± 1.66 µmol/L, respectively. These values were well below the lower limit of the international reference range for Zn (<10.70 µmol/L) and Cu (<14.16 µmol/L) established by Acosta et al. (2010), as well as the national reference range for Zn (<12.84 µmol/L) and Cu (<10.22 µmol/L) established by Burguera et al. (1986). The Student's T-Test confirmed the significant differences for Zn (p<0.0001) and Cu (p<0.0001), indicating a high prevalence of deficiencies in Zn and Cu in the participants.

Furthermore, significant differences were found in the average values of TC, HDLc, Cu, and CZr ratio between the NPA group and the PA group. The NPA group showed higher values for TC (229.3±45.1 vs. 161.1±39.0; p<0.0001), lower values for HDLc (24.4±6.4 mg/dl vs. 38.0±8.1 mg/dl; p<0.0001), lower values for Cu (8.74±1.66 µmol/L vs. 10.0±1.93 µmol/L; p<0.001), and lower values for CZr ratio (1.77±0.37 vs. 2.19±0.48; p<0.0001), as well as a higher TC/HDLc ratio (9.9±2.89 vs. 4.22±0.21; p<0.0001). Table 2 presented these results with mean ± SD values, and showed significant differences between the NPA group and the PA group of older adults.

In addition, comparisons between men and women revealed significant differences in TC, HDLc, and TC/HDLc (CRI I). Men had lower values for TC (158.56±36.8 mg/dl vs. 216.43±51.54 mg/dl; p<0.0001), higher values for HDLc (33.83±6.63 mg/dl vs. 29.25±10.92 mg/dl; p<0.029), and lower values for TC/HDLc ratio (5.0±2.41 vs. 8.35±3.51; p<0.0001). No significant differences were found between men and women in the other parameters studied.

Table 2. Quantitative variables by group according to the frequency of weekly physical activity.

Variables	Group (n=101)	NPA (n=47)	PA (n=54)
Total Cholesterol (mg/dL)	197.62±54.31	161.1±39.0	229.3±45.1**
HDL-c (mg/dL)	30.77±9.93	38.0±8.1	24.4±6.4**
TC/HDL-c (CRI I)	7.25±3.55	4.22±0,21	9.9±2.89**
Cu (µmol/L)	9.33±1.88	10.0±1.93	8.74±1.66**
Zn(µmol/L)	4.84±0.79	4.68±0.9	4.98±0.6
CZr (Ratio)	1.96±0.46	2.19±0.48	1.77±0.37**

CRI I. Castelli Risk Index I. PA: Physical Activities. NPA: Non-Physically Active . HDL: High density lipoproteins. ** diferencias significativas with NPA.

Significant differences were observed in the average TC levels between participants older than 80 years and those younger than 70 years (p<0.001). Although no differences were found in the raw scores of HDLc, Cu, Zn, and CZr between the age groups. The data suggests that in the studied sample, age is associated with a decrease in TC and Zn levels, accompanied by an increase in HDLc, Cu, and CZr levels.

Pearson's correlation analysis revealed a positive correlation between age and serum Cu levels (r=0.205; p<0.04) as well as CZr levels (r=0.206; p<0.039). However, the correlation between age and Zn levels was negative but not significant (r=-0.072). The impact of age on the means of the Z-scores for Zn, Cu, and CZr (ZZn, ZCu, and ZCZr) is illustrated in Figure 1. The distribution of ZZn for subjects younger than 70 years was above the group average, while ZCu and ZCZr were below the group average. Conversely, in the group of participants older than 80 years, the ZZn was below the group mean, while ZCu and ZCZr were above the group mean.

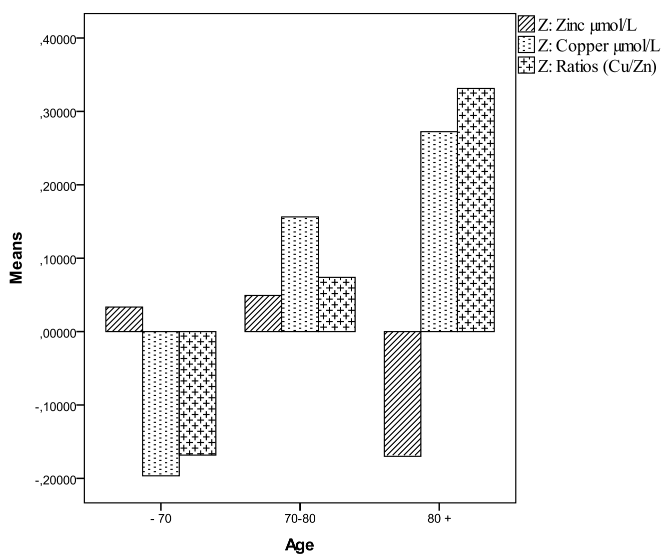
Table 3. Quantitative Variables by age groups.

		Age		
Variables	Average (n=101)	Less than 70 years olds (n=50)	Between 70-80 years olds (n=31)	More than 80 years olds (n=19)
Total Cholesterol	197.6±54.3	211.1±57.0	188.5±50.7	176.0±43.81**
HDLc	30.7±9.9	28.5±9.0	32.0±9.78	34.8±11.30
CRI I	7.2± 3.5	8.1± 3.4	6.5± 3.2	5.8± 3.7**
Copper µmol/L	9.3±1.8	8.9±1.5	9.6±2.1	9.8±1.9
Zinc µmol/L	4.8±0.8	4.8±0.8	4.8±0.71	4.7±0.7
CZr	1.9±0.4	1.8±0.4	2.0±1.2	2.1±1.9

CRI I. Castelli Risk Index I. Zn: Zinc, Cu: Copper, CZr: Ratios Cu/Zn, TC: Total Cholesterol, HDL: High Density Lipoproteins.**Significant differences with less than 70 years.

The study participants were divided into two groups based on their CZr ratio (CZr ≤ 2.0 and CZr > 2.0). Individuals with a lower CZr ratio are believed to have a decreased capacity of the body to maintain or restore homeostasis following a destabilizing event, as noted by Malavolta et al. (2015).

Figure1. The effect of the age variable on the Cu-Zn means Z-scores.



The chi-square test indicates significant associations between the following variables and CZr: Depression Geriatric DGS-15 ($p<0.0001$), PA ($p<0.0001$), TC ($p<0.031$), and Castelli Risk Index ($p<0.0001$). Table 4 presents the results of the associations between CZr and the dichotomized parameters studied.

Table 4. Relationship of the CZr with the parameters of geriatric depression, physical activity, Castelli Risk Index I, and total cholesterol.

		Ratio CZr		χ^2
		CZr >2 (n=38)	CZr≤2 (n=63)	*p*
Physical Active	< 3 days/week	29 (76.3%)	18 (28.6%)	*0.0001*
	+3 days/week	9 (23.7%)	45 (71.4%)	
Total Cholesterol	> 200 mg/dL	13 (34.2%)	35(55.6 %)	*0.031*
	≤ 200 mg/dL	25 (65.8%)	28 (44.4%)	
Geriatric Depression Scale	Depressed	36 (87.8%)	14 (23.3%)	*0.0001*
	Non-depressed	5 (12.3%)	46 (76.6%)	
CRI I	> 4.5	10 (26.3%)	45 (71.4%)	*0.0001*
	≤ 4.5	28 (73.7%)	18 (28.6%)	

CRI. Castelli Risk Index I.

The multivariate model was used to evaluate the association between CZr, physical exercise, and age, adjusted by GDS-15 and total cholesterol (model 1). The odds ratio (OR) for physical exercise was 0.086 (95%CI: 0.021-0.348, $p<0.001$), while the OR for age was 1.062 (95%CI: 1.005-1.123, $p<0.034$). In the adjusted model with GDS-15 and Castelli Risk Index, the OR for physical activity was 0.039 (95%CI: 0.005-0.284, $p<0.001$), and OR for age was 1.062 (95%CI: 1.004-1.124, $p<0.035$). In both models, physical activity and age remained significant. These findings indicate that physical activity is a protective factor for low CZr levels, while age is a risk factor.

Our study's results show that individuals who engage in physical activity more than three times a week have a lower likelihood of presenting CZr greater than 2.0, indicating greater capacity for homeostasis recovery after a destabilizing event. Furthermore, our findings reveal that CZr levels increase with age.

Discussion and conclusions

We observed a remarkably high prevalence of Cu and Zn deficiencies, exceeding those reported in the literature (Olivares et al., 2011). This may be attributed to the low protein and high carbohydrate diets prevalent in the socioeconomically challenged context of our study population. Although deficiencies in the trace elements studied have been previously reported in developing countries, our findings reveal a significantly higher prevalence than previously reported, thereby increasing the risk of disease and mortality among the studied population (Meertens et al., 2005). It is crucial to investigate the presence of deficiencies in other essential trace elements that were not studied.

Our results indicate that HDL-c levels are below the recommended levels, and total cholesterol levels in the elderly under 70 years of age are significantly higher, increasing their risk of cardiovascular events, particularly in women. Unadjusted mean comparisons show that participants over 80 have lower IBM, total cholesterol, and HDL-c levels than those under 70, despite being functionally independent. These findings are consistent with prior studies (Malavolta et al., 2010, 2015; Mocchegiani et al., 2012).

We observed that younger elderly individuals engaged in physical activity more frequently. This may be due to previous medical diagnoses requiring physical exercise to control cholesterol levels, and age may play an important role in performing exercise routines at least three times a week. Studies comparing the lipid profile in aging between men and women have shown that menopause has a significant impact on total cholesterol and triglyceride values, elevating the average values of women above those of men of a similar age (Campesi et al., 2016), explaining the differences found in our study. The hormonal deficit caused by ovarian failure that mainly affects women leads to changes in the lipid profile and significantly increases cardiovascular risk, particularly when menopause occurs prematurely (Anagnostis et al., 2015; Giacconi et al., 2017).

Our findings suggest that isolated levels of Zn and Cu are related to diets poor in these trace elements, particularly in the context we examined. However, the Cu-Zn relationship may be better explained by age and frequency of exercise, as the Cu-Zn relationship was more acute in older participants than younger ones, even in the presence of serum deficiencies. Cu-Zn ratio alterations are typically transient in young adulthood, but aging-related pathogen accumulation, immunosenescence, and chronic low-level inflammation can permanently disrupt the balance between Cu and Zn (Malavolta et al., 2010). Serum concentrations of Cu and Zn are tightly regulated by modulators and compensatory mechanisms that act to lower Zn concentrations while raising Cu concentrations in response to various stressors (Cousins, 1985).

Our study found that 37.6% of subjects had a Cu-Zn ratio greater than 2.0, with this imbalance being more prevalent in the population segment over 80 years of age. These results may be associated with decreased homeostasis recovery and increased inflammatory parameters in aging individuals, regardless of their physical condition and dietary Cu-Zn bioavailability (Malavolta et al., 2015). Such imbalances can significantly increase susceptibility to disease and mortality risks in the next 3-4 years (Malavolta et al., 2010) in our population.

We also examined the mean serum levels of Cu and Zn by sex and found no significant differences (Giacconi et al., 2017). However, caution is warranted when interpreting our results, as previous studies have reported various effects of menopause on these trace elements in women with normal values (Choudhary, 2017), whereas in our study, all women had significantly lower levels than the standard.

Previous research has suggested a link between Cu and Zn and cognitive function. Individuals with Alzheimer's disease tend to have low serum Zn levels and elevated Cu levels (Brewer & Kaur, 2013), while Zn deficiencies can accelerate cognitive decline (Prasad, 2009; Wani et al., 2017). Moreover, an imbalance in trace elements Fe, Cu, and Zn has been shown to have adverse effects on brain function in older adults (Alghadir et al., 2015).

In our bivariate analysis, we found a relationship between the Cu-Zn ratio and depression. However, we could not establish causality due to limitations in the design of our study, such as the lack of information on the timing of trace element deficiencies and depression onset. This association disappeared in our multivariate analysis, underscoring the need for longitudinal studies to identify health-compromising events in older adults and the apparent causal links between the Cu-Zn ratio and depression.

In conclusion, our findings suggest that the Cu-Zn ratio is independent of nutritional parameters, particularly in conditions of trace element deficiencies. Women's total cholesterol levels differ from men's, while age and physical exercise, when adjusted for depression, total cholesterol, and Castelli Risk Index I, are associated with the Cu-Zn ratio.

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Statement of ethics

The methods employed in this study were conducted in accordance with the Bioethics regulations and have been approved by the Bioethics Committee of the University of the Andes, Mérida-Venezuela, and adhere to the ethical standards outlined by the World Medical Association.

Disclosure statement

The authors declare no conflicts of interest regarding the publication of this article.

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