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EVALUACIÓN DE LOS NIVELES DE ADIPOCINAS Y SU RELACIÓN CON LOS NIVELES DE COBRE Y ZINC EN MUJERES ADULTAS CON OBESIDAD

TESIS QUE PRESENTA

L.N. LUIS CÉSAR VÁZQUEZ VÁZQUEZ

PARA OBTENER EL GRADO DE MAESTRO EN CIENCIAS BIOMÉDICAS BÁSICAS

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Tesis que presenta:

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RESUMEN:

La obesidad es un estado patológico que se caracteriza por alteraciones en la homeostasis de elementos traza como el cobre (Cu) y el zinc (Zn). Este trabajo evalua las asociaciones entre los niveles de Zn, Cu y la relación Cu/Zn con adipocinas, marcadores de estrés oxidativo, así como marcadores cardiometabólicos en mujeres mexicanas adultas con normopeso, sobrepeso y obesidad. Los niveles de leptina y resistina se midieron mediante un ensayo por inmunoabsorción ligado a enzimas (ELISA). Para evaluar el estrés oxidativo, se analizó el nivel de peroxidación lipídica mediante el ensayo de sustancias reactivas al ácido tiobarbitúrico (TBARS). La capacidad total antioxidante sérica (sTAC) se midió a través del método DPPH. Las concentraciones séricas de Zn y Cu se determinaron mediante espectrometría de masas con plasma acoplado inductivamente (ICP-MS). Los resultados mostraron que el Cu sérico se asociaba positivamente con el colesterol total ($p = 0,045$). El sobrepeso y la obesidad se relacionaron con niveles séricos más bajos de Zn ($p = 0,003$) y una relación Cu/Zn más alta ($p = 0,021$). La obesidad por sí sola se asoció con un aumento de los TBARS ($p = 0,013$) y una reducción de la sTAC ($p = 0,030$). Las concentraciones de leptina y resistina aumentaron con el sobrepeso y la obesidad, pero no mostraron asociación con los TBARS, el sTAC ni los niveles de Zn y Cu. En conclusión, entre las mujeres adultas mexicanas, el sobrepeso y la obesidad se asocian con mayores TBARS, menor sTAC, menor Zn sérico y una relación Cu/Zn elevada, junto con alteraciones en los parámetros cardiometabólicos y las adipocinas. La obesidad puede actuar como un factor determinante que favorece la desregulación del equilibrio de elementos traza y el aumento del estrés oxidativo e inflamación.

Serum zinc, copper status and Cu/Zn ratio in overweight and obesity: Associations with oxidative stress, adipokines, and cardiometabolic traits in adult women

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Abstract

Obesity is associated with alterations in zinc (Zn) and copper (Cu) homeostasis, including the Cu/Zn ratio, and with changes in adipokines, which may influence oxidative stress. This study evaluated associations among nutritional status, serum Zn and Cu, the Cu/Zn ratio, adipokines, oxidative stress markers, and cardiometabolic traits in adult women. In this observational cross-sectional study, 123 adult women from Mexico City were evaluated. Anthropometric measures and cardiometabolic traits—fasting serum glucose (FSG), total cholesterol (TC), triglycerides (TG), and the triglyceride–glucose (TyG) index—were assessed using a clinical chemistry analyzer. Leptin and resistin were quantified by enzyme-linked immunosorbent assay (ELISA). Oxidative stress was evaluated by measuring serum total antioxidant capacity (sTAC) using the DPPH method and by assessing lipid peroxidation via thiobarbituric acid reactive substances (TBARS). Serum Zn and Cu concentrations were quantified by inductively coupled plasma mass spectrometry (ICP-MS). Our results show that serum Cu concentrations were positively associated with TC (p = 0.045), and overweight and obesity were associated with lower serum Zn concentrations (p = 0.003) and a higher Cu/Zn ratio (p = 0.021). Additionally, obesity was associated with increased TBARS (p = 0.013) and reduced sTAC (p = 0.030). Leptin and resistin concentrations increased across overweight and obesity but were not associated with TBARS, sTAC, or Zn and Cu concentrations. In conclusion, in Mexican adult women, overweight and obesity are associated with increased TBARS, reduced sTAC, lower serum Zn concentrations, and an altered Cu/Zn ratio, together with changes in cardiometabolic traits and adipokines.

Keywords: obesity, trace elements, total antioxidant capacity, lipid peroxidation, cardiometabolic traits, adipokines

Introduction

Obesity is a complex chronic disease considered a global epidemic because of its high worldwide prevalence and metabolic complications (Ng et al. 2025; Bae et al. 2025). Among the main processes involved in the pathophysiology of obesity, low-grade chronic inflammation and oxidative stress are consistently recognized as central mechanisms in the development of metabolic complications. In this regard, oxidative stress is a particular concern in adult women, given the higher prevalence of abdominal obesity (88%) and a more adverse cardiometabolic risk profile than in men (Barquera et al. 2024; Flores-Luna et al. 2025).

In obesity, low-grade chronic inflammation is associated with elevated circulating levels of adipokines, such as leptin and resistin, which have been reported to promote inflammatory signaling, enhance reactive oxygen species (ROS) generation, and contribute to metabolic dysregulation (Fernández-Sánchez et al. 2011; Frühbeck et al. 2017; Ottobelli et al. 2016). Moreover, reduced antioxidant defense capacity leads to oxidative stress, which is also associated with cardiometabolic impairment. In parallel, alterations in trace element homeostasis, particularly copper (Cu) and zinc (Zn), have been implicated in the redox imbalance associated with obesity (Franco et al. 2024; Vazquez-Moreno et al. 2023; Gu et al. 2020). Higher serum Cu concentrations have been reported in individuals with obesity (Gu et al. 2020), which may promote ROS formation and further enhance oxidative damage (de Paiva et al. 2023). Excessive ROS production triggers lipid peroxidation, which damages cellular membranes, alters protein function, and compromises genomic integrity, thereby impairing metabolic homeostasis (Flores-Luna et al. 2025; Juan et al. 2021).

In non-enzymatic antioxidant activity, lower serum total antioxidant capacity (sTAC) has been associated with increased body weight in many studies (Anaya-Morua et al. 2023; El Frakchi et al. 2024). The main mechanisms proposed to explain the decrease in sTAC in obesity include reduced intake of exogenous antioxidants from fruits, vegetables, and legumes. Furthermore, excessive ROS production observed in obesity has been suggested to accelerate the consumption of non-enzymatic antioxidant compounds, further compromising the redox balance (Savini et al. 2013).

Chronic low-grade inflammation in obesity may reduce expression of antioxidant enzymes, including superoxide dismutase (SOD), catalase, and glutathione peroxidase (Fernández-Sánchez et al. 2011; Furukawa et al., 2004). In this context, Zn plays a critical role as a structural and functional component of Cu/Zn-SOD, and reduced dietary intake or low serum Zn concentrations have been associated with diminished antioxidant

enzyme activity, thereby favoring oxidative stress (de Oliveira et al., 2024; Ozata et al., 2002). Furthermore, the Cu/Zn ratio has emerged as a sensitive biomarker of trace element imbalance, reflecting a relative excess of prooxidant Cu and a deficiency of antioxidant Zn, and has been associated with oxidative stress and metabolic dysfunction in obesity (Cui et al. 2025).

In Mexico, according to the 2023 National Health and Nutrition Survey, the combined prevalence of overweight and obesity among adults was 74.5%, with obesity more prevalent among women than men (Barquera et al., 2024). This epidemiological scenario underscores the need to identify the biological mechanisms underlying obesity-related cardiometabolic risk in women. Oxidative stress and disturbances in trace element homeostasis, particularly Zn and Cu, have been implicated in the development of dyslipidemia and insulin resistance, making them a significant public health concern. Nevertheless, studies that simultaneously evaluate serum Zn and Cu concentrations, the Cu/Zn ratio, oxidative stress markers, and the lipid profile within the same population remain limited, especially among Mexican adult women. Therefore, the present study aimed to analyze the relationship between altered Zn and Cu status, increased Cu/Zn ratio, adipokines, oxidative stress markers, and cardiometabolic traits in adult women from Mexico City.

Subjects and Methods

Study sample

Mexican women aged 18-60 years from Mexico City were randomly selected and invited to participate in this observational cross-sectional study, conducted between June 2024 and May 2025, to evaluate the association between trace element status, adipokines, oxidative stress markers, and cardiometabolic traits. Exclusion criteria included women with renal disease, hypo- and hyperthyroidism, cancer, hypertension, liver disease, and those with fasting serum glucose (FSG) concentrations higher than 126 mg/dL. Women who had taken vitamin and mineral supplements in the 3 months prior to the study were also excluded.

Ethical approval

This observational study was approved by the Bioethics Committee of the National School of Biological Sciences of the National Polytechnic Institute (approval registration: ENCB/CEI/064/2021). The protocol was conducted in accordance with the Declaration of Helsinki and the Regulations of the General Health Law on Health Research in Mexico. Written informed consent was obtained from all participants.

Anthropometric measurements

All participants were weighed with a digital scale (SECA) and measured for height with a portable stadiometer (SECA). Body mass index (BMI) was calculated as weight (kg) divided by height squared (kg/m^2). Body weight categories were defined according to World Health Organization criteria: normal weight (BMI <25 kg/m^2), overweight (BMI ≥25 and <30 kg/m^2), and obesity (BMI ≥30 kg/m^2).

Blood sampling and biochemical measurements

After at least 8 h of fasting, a peripheral blood sample was collected from each participant into a 6 mL clot activator tube and centrifuged at 2,000 x g for 15 min. Serum aliquots were stored at –80 °C until use.

A clinical chemistry analyzer (ILAB 300, Instrumentation Laboratory, Barcelona, Spain) was used to determine FSG, triglycerides (TG), and total cholesterol (TC). The triglyceride-glucose index (TyG) was calculated and used as a surrogate marker of insulin resistance (Guerrero-Romero et al. 2010; Rios-Lugo et al. 2020).

Assessment of adipokine levels

Adipokine levels were measured to characterize the inflammatory profile associated with nutritional status. Leptin and resistin levels were quantified using enzyme-linked immunosorbent assay (ELISA) with specific commercial kits (Thermo Fisher Scientific, WA, USA). Absorbance was measured at 450 nm using a microplate reader (WHYM201; Poweam Medical Co., Ltd, Nanjing, China).

Trace elements quantification by ICP-MS

The concentrations of Cu and Zn in serum samples were measured by inductively coupled plasma mass spectrometry (ICP-MS iCAP Q, Thermo Scientific, Germany) under helium kinetic energy discrimination (He-KED) mode to minimize polyatomic interferences. Samples were thawed from –80°C to 4°C before use. The total serum volume was 0.2 mL, spiked with an internal standard [Indium In; 50 $\mu g\ L^{-1}$], and acid-digested using a microwave digestion system (MARS 6, CEM, USA) with 5 mL of concentrated HNO3. The acid digestion consisted of a temperature ramp to 200 °C, maintained for 15 min. After digestion, samples were evaporated to dryness in teflon containers, and the residues were diluted with 2% v/v HNO_3 and transferred to 5 mL Class A glassware. Cross-contamination was assessed by monitoring the contribution of Zn and Cu in reagent and cleaning blanks throughout the analytical sequence. Quality control was performed using a 100 $\mu g\ L^{-1}$ dissolution prepared from certified standard dilutions. Cu and Zn concentrations were determined using an external calibration curve (10, 25, 50, 100, 150, 200, 500, and 1000 $\mu g\ L^{-1}$). Concentrated high-purity HNO_3

(Milestone Duopur system by Milestonesrl, Italia), high-purity water with 18 MΩ cm (Milli-Q® system by Millipore, Mexico), and Cu and Zn certified standard solutions (High–Purity Standards, North Charleston, USA) were used in sample preparation and analysis.

Measurement of oxidative stress markers

Lipid peroxidation

Following the methodology described by Jentzsch et al. 1996, lipid peroxidation was measured using the thiobarbituric acid reactive substances (TBARS) assay. This assay quantifies the complex formed between lipid peroxidation products, such as malondialdehyde (MDA), and thiobarbituric acid (TBA, ≥98%; Sigma-Aldrich, Darmstadt, Germany). The samples were prepared by adding 0.3 mL of serum and 0.2 mL of phosphoric acid (H_3PO_4 ≥99.99%, Sigma-Aldrich, Darmstadt, Germany), then homogenized. Subsequently, 0.025 mL of TBA was added, and the mixture was vortexed. The mixture was incubated at 90 ± 2°C for 60 min. The samples were cooled in an ice bath, and 0.4 mL of butanol (Sigma-Aldrich, USA) was added for precipitation, then vortexed and centrifuged at 15,000 x g at 25 °C for 10 min. Finally, 0.25 mL of supernatant was collected, and the 96-well plates were read at 535 nm using a Multiskan FC spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

A standard calibration curve was prepared using an MDA stock solution and the reagent 1,1,3,3-tetramethoxypropane (TMP, ≥98%; Sigma-Aldrich, Darmstadt, Germany). Dilutions were prepared to obtain seven concentration points from 0.25 to 5.0 nmol mL^{-1}, along with a blank control sample. Serum samples and MDA standards were processed under identical conditions. The results were calculated from the calibration curve and expressed in nmol mL^{-1}.

Total antioxidant capacity

The sTAC was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH; Sigma-Aldrich, Darmstadt, Germany) assay, as described by Rumpf et al. (2023) and Huang et al. (2005). The assay measures the reduction of the DPPH free radical by antioxidants in serum, producing a purple color. DPPH was prepared at 0.1 mM in 80% methanol (≥99.9%; Sigma-Aldrich, Darmstadt, Germany) as the control (A0). 0.025 mL of serum was diluted in 0.725 mL of DPPH solution (A1). Blanks were prepared by adding 0.025 mL of serum to 0.725 mL of 80% methanol (A2). The mixed samples were incubated at room temperature in total darkness for 1 h, then centrifuged at 9,000 x g at 4 °C for 10 min. The supernatant (0.250 mL) was transferred to 96-well plates, and

absorbance was measured at 540 nm using a Multiskan FC spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Absorbances A0, A1, and A2 were used to calculate the percentage of DPPH scavenging using the formula % DPPH scavenging = (A0 − [A1 − A2]/A0) x 100. A standard calibration curve was prepared by serially diluting a 1 mM Trolox stock solution into DPPH solution to yield seven concentration points (5.0 to 50.0 μM) and a blank control. Results were expressed in mmol L^{-1} of the Trolox

Data analysis

The Shapiro–Wilk test was used to assess the normality of continuous data. Variables that were not normally distributed were normalized using the rank-based inverse normal transformation (Supplementary Table 1). A one-way ANOVA was performed to compare continuous data across nutritional status groups: normal weight, overweight, and obesity. Linear regression models were used to evaluate associations among trace element status, oxidative stress markers, cardiometabolic traits, and adipokines, with adjustment for age and nutritional status. Statistical analyses were conducted using SPSS (version 21.0), and p-values < 0.05 were considered statistically significant.

Results

ICP-MS analytical performance

The analytical performance of the ICP-MS system was rigorously evaluated to ensure data reliability. Instrumental parameters, limits of detection (LOD), calibration performance, precision, and accuracy are summarized in the Supplementary Material. Calibration curves for Cu and Zn demonstrated excellent linearity, with regression coefficients (r^2) of 0.9994 and 0.9992, respectively. LODs were established following the procedure recommended by Thermo Scientific (Al-Hakkani, 2019), where values of 0.295 ± 0.017 μg L^{-1} for Cu and 0.333 ± 0.021 μg L^{-1} for Zn were obtained. Method recovery exceeded 89.7 ± 15.9% for In. Both precision and accuracy were < 2% at a reference concentration of 100 μg L^{-1} for Cu and Zn, confirming the robustness and reproducibility of the analytical procedure. These results confirm the suitability of the analytical method for evaluating serum trace element status in this study.

General characteristics of the study sample

The general characteristics of the study sample, categorized by BMI-based nutritional status, are presented in Table 1. A total of 123 women were included in the study: normal weight (n = 26), overweight (n = 48) and obesity (n = 49). While the FSG level was higher in the normal weight group ($p < 0.001$), BMI, serum

leptin and resistin levels increased progressively with increasing body weight ($p < 0.001$). No significant differences were observed in age, lipid profile variables (TC and TG), TyG index, and serum Cu ($p \geq 0.212$). Serum Zn concentrations decreased significantly across weight categories ($p = 0.003$), while the Cu/Zn ratio increased ($p = 0.041$).

Association between nutritional status and oxidative stress markers

Figure 1 shows the association between nutritional status and oxidative stress markers, adjusted for age. Overweight and obesity were positively associated with serum TBARS levels ($\beta = 0.217 \pm 0.086$, $p = 0.013$). In contrast, overweight and obesity were negatively associated with serum sTAC ($\beta = -0.081 \pm 0.025$, $p = 0.030$).

Association between nutritional status and trace elements

The associations between nutritional status and serum Zn and Cu concentrations were evaluated using linear regression models adjusted for age (Table 1). Overweight and obesity status were negatively associated with serum Zn concentrations ($\beta = -10.880 \pm 3.621$, $p = 0.003$). No significant association was observed between nutritional status and serum Cu concentrations ($p = 0.318$). In contrast, a positive association was observed between overweight and obesity status and the Cu/Zn ratio ($\beta = 0.481 \pm 0.214$, $p = 0.021$).

Association of oxidative stress markers and trace elements with cardiometabolic traits

Associations between oxidative stress markers, trace elements, adipokines, and cardiometabolic traits, adjusted for age, are presented in Table 2. Serum Cu concentrations were positively associated with TC ($\beta = 0.211 \pm 0.104$, $p = 0.045$). Marginal inverse associations were observed between TBARS and the TyG index ($\beta = -0.114 \pm 0.058$, $p = 0.053$) and between the Cu/Zn ratio and resistin ($\beta = -0.161 \pm 0.084$, $p = 0.058$). No statistically significant associations were observed between TBARS, sTAC, adipokines, serum Zn concentrations, or the Cu/Zn ratio and the remaining cardiometabolic traits.

Association between trace elements and oxidative stress markers

The association analysis of serum Zn and Cu levels, as well as the Cu/Zn ratio with serum TBARS and sTAC adjusted for age and body weight, is presented in Table 3. Serum Cu concentrations did not show any significant association with TBARS ($\beta = 0.003 \pm 0.002$, $p = 0.197$) and sTAC ($\beta = 0.001 \pm 0.001$, $p = 0.615$). Similarly, serum Zn concentrations and the Cu/Zn ratio were not significantly associated with TBARS and sTAC ($p \geq 0.449$).

Discussion

In the present study, we evaluated the associations of overweight and obesity with cardiometabolic traits and inflammatory biomarkers, as well as with serum levels of oxidative stress markers and Zn and Cu in adult women from Mexico City. Overweight and obesity were associated with increased TBARS levels. Conversely, sTAC and serum Zn concentration were negatively associated with overweight and obesity. In contrast, the Cu/Zn ratio was positively associated with overweight and obesity. Additionally, serum Cu level was positively associated with TC concentration.

Several studies have demonstrated a relationship between obesity and increased oxidative stress (Choromańska et al. 2021; Adnan et al. 2019). A recent analysis showed that postmenopausal women with obesity had significantly higher TBARS levels than those with normal weight (Leanza et al. 2023). Another study in women found a strong association between obesity and increased serum MDA levels, a recognized marker of oxidative damage (Amin et al. 2020). Elevated lipid peroxidation results from the damage of polyunsaturated fatty acids by hydroxyl radicals in cell membranes, leading to structural damage and disruption of cellular function (Hauck et al. 2016).

The level of sTAC reflects the synergistic activity of non-enzymatic antioxidants in the serum. This parameter provides insight into the body's redox state and the balance between oxidizing species and antioxidant defense (Silvestrini et al. 2023). In our study, overweight and obesity were negatively associated with sTAC. These findings are consistent with previous studies linking central obesity and abdominal adiposity to lower antioxidant capacity (Amirkhizi et al. 2010; Chrysohoou et al. 2007). The reduction in total non-enzymatic antioxidant capacity in serum is attributed to a dual mechanism involving increased ROS and decreased circulating non-enzymatic antioxidants (Choromańska et al., 2021). Obesity increases mitochondrial ROS production, and adipose tissue remodeling and hypoxia trigger endoplasmic reticulum stress, which, in turn, enhances ROS generation. Additionally, chronic inflammation activates immune cells, such as macrophages, which can exacerbate low-grade systemic inflammation and may deplete protective antioxidant defenses (Cinti, 2023). Persistent oxidative challenge in obesity progressively depletes the antioxidant defense system (Hosseini et al. 2027).

Although we did not identify associations between leptin or resistin and the oxidative stress markers and trace elements analyzed, the progressive increase in these adipokines across body weight categories

supports their role as indicators of the adiposity-related inflammatory milieu. However, in this dataset, their relationships with TBARS, sTAC, Zn, Cu, and Cu/Zn were not statistically supported by the adjusted models and should be interpreted cautiously (Fernández-Sánchez et al. 2011; Frühbeck et al. 2017; Ottobelli et al. 2016).

Epidemiological studies have reported associations between serum Cu levels and overweight and obesity (Knazicka et al. 2024). However, this relationship was not significant in our study. Cu is a trace element involved in lipid metabolism (Blades et al. 2021). Nevertheless, altered Cu homeostasis is linked to metabolic disturbances and has been hypothesized to exert a prooxidant effect. In our study, serum Cu levels were positively associated with TC levels, consistent with studies in both adult and pediatric populations (Song et al. 2018; Zang et al. 2018). The biological mechanism underlying the relationship between Cu and altered blood lipids remains uncertain. Elevated Cu levels may promote the formation of hydroxyl radicals, which oxidize LDL cholesterol (Bo et al. 2008). Excess Cu has been shown in both in vitro and in vivo studies to impair cholesterol metabolism, specifically synthesis, and to induce hepatic damage, which, in turn, disrupts liver function in cholesterol processing and export (Svensson et al. 2033; Hou et al. 2023).

Although body weight was significantly associated with oxidative stress markers, no significant associations between trace elements and these markers were found in the present study. Nonetheless, overweight and obesity were negatively associated with serum Zn levels, consistent with previous studies reporting lower serum Zn levels in Mexican individuals with higher BMI (Rios-Lugo et al. 2020; Hernández-Mendoza et al. 2022). In this context, low serum Zn levels in overweight and obesity may compromise antioxidant defenses. While Zn does not directly contribute to total antioxidant capacity, it is a crucial component of the enzymatic antioxidant defense system, such as Cu/Zn-superoxide dismutase and metallothionein, and contributes to ROS elimination due to its high sulfhydryl content (Chen et al. 2024).

Regarding the observed positive association between overweight and obesity and the Cu/Zn ratio, this index has also been considered a marker of trace-element imbalance related to oxidative stress in women (Cui et al. 2025). An increase in the Cu/Zn ratio may reflect a relative increase in Cu, which acts as a prooxidant by generating ROS, and a reduction in Zn, which functions as an antioxidant due to its key role in Cu/Zn-SOD activity. Consequently, redox homeostasis is impaired, potentially contributing to the metabolic imbalance characteristic of obesity (Michalczyk et al. 2023).

Overall, our findings suggest a pattern of obesity in Mexican women characterized by increased markers of oxidative damage, reduced sTAC, and altered serum Zn levels, highlighting the relevance of this trace element in the oxidative imbalance associated with overweight and obesity.

Limitations of this study include a small sample size and the lack of variables related to nutritional status and dietary habits. Furthermore, we did not collect data on hormonal treatments, which have been reported to contribute to changes in Cu levels and the oxidative response (Berg et al. 1998; Ansar et al. 2015). Despite these limitations, one of the strengths of the study is the analysis of both sTAC and TBARS levels, markers of antioxidant defense and lipid peroxidation, respectively, which together provide a more comprehensive assessment of the redox status. To our knowledge, this is one of the first studies to comprehensively evaluate the associations among nutritional status, cardiometabolic traits, oxidative stress markers, and trace elements in Mexican women.

Conclusion

The results of this study indicate that higher body weight in Mexican adult women is associated with increased lipid peroxidation, reduced serum sTAC, and lower serum Zn concentrations. In addition, an increased Cu/Zn ratio was observed across higher body weight categories, although no direct associations were identified between Zn and Cu status and oxidative stress markers. These findings underscore the relevance of oxidative imbalance and trace element homeostasis, particularly reduced Zn levels, in the context of overweight and obesity in women. While the observed associations do not establish causality, they suggest a potential link between altered Zn status and cardiometabolic alterations, as reflected in lipid-related traits. However, future studies involving larger cohorts and more comprehensive clinical, nutritional, and redox assessments are warranted to confirm these associations and to clarify the role of Zn and the Cu/Zn ratio in impaired antioxidant defense and cardiometabolic risk.

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Statements and Declarations

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Conflicts of interest statement

No potential conflicts of interest relevant to this article were reported.

Author contributions

M.J.R.L., J.S.S., H.H.M., M.V.M., A.N.C., M.C., and L.C.V.V. designed the study, performed the statistical analyses, wrote the manuscript, and designed tables and figures. J.S.S., M.J.R.L., H.H.M., M.V.M. A.N.C., M.C., A.R.C., E.L.E. and P.E.C.T., collected the data and critically reviewed the manuscript. M.J.R.L., H.H.M., and L.C.V.V. handled sample treatment and analysis by ICP-MS, while M.V.M., A.N.C., A.R.C., and L.C.V.V. performed sample treatment and analysis of TBARS and sTAC. All authors read and approved the final manuscript.

Ethics approval

This observational study was approved by the Bioethics Committee of the National School of Biological Sciences of the National Polytechnic Institute (approval registration: ENCB/CEI/064/2021). The protocol was conducted according to the guidelines established in the Declaration of Helsinki and based on the Regulations of the General Health Law on Health Research in Mexico.

Data availability

The dataset generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent to participate

Written informed consent was obtained from all participants before their inclusion.

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Table 1. General characteristics of the study sample.

Trait	Normal weight (n= 26)	Overweight (n= 48)	Obesity (n= 49)	p-value
Age (years)	31.55 ± 12.15	33.04 ±11.39	33.27 ± 12.54	0.817
BMI (kg/m^2)	22.85 ± 1.68	27.79 ± 1.51	34.48 ± 3.59	**<0.001**
FPG (mg/dL)	103.43 ± 20.44	90.09 ± 13.48	90.20 ± 12.07	**<0.001**
TC (mg/dL)	142.18 ± 54.15	164.12 ± 67.97	160.38 ±37.02	0.212
TG (mg/dL)	143.14 ± 36.72	148.35 ± 58.40	146.94 ± 60.21	0.920
TyG index	8.87± 0.34	8.720 ± 0.46	8.710 ± 0.430	0.271
Leptin (ng/mL)	13.06 ± 4.57	27.17 ±5.54	31.56 ± 7.96	**<0.001**
Resistin (ng/mL)	9.76 ± 1.62	12.72 ± 2.66	13.96 ± 3.18	**<0.001**
Zn (µg/dL)	141.44 ± 48.59	107.63 ± 48.41	101.79 ± 51.40	**0.003**
Cu (µg/dL)	123.91 ± 41.01	137.49 ± 53.60	140.06 ± 53.90	0.388
Cu/Zn ratio	0.95 ± 0.47	2.20 ± 3.12	2.68 ± 3.48	**0.041**

Data are expressed as mean ± standard deviation. Abbreviations: BMI, body mass index; FPG, fasting plasma glucose; TC, total cholesterol; TG, triglycerides; TyG, triglycerides glucose index; Zn, Zinc; Cu, Copper. Student t and Chi-square tests were used to compare means and frequencies, respectively. Analysis by one-way ANOVA. Significant *p*-values ($p<0.05$) are represented in bold.

Table 2. Association of oxidative stress markers and trace elements with cardiometabolic traits.

Traits	TBARS (nmol/mL)	sTAC (mEq Trolox)	Cu (µg/dL)	Zn (µg/dL)	Cu/Zn ratio
FPG (mg/dL)	-3.329 ± 2.389 (0.168)	12.262± 21.623 (0.574)	-0.044 ± 0.031(0.157)	-0.008± 0.028 (0.779)	0.101 ± 0.470 (0.830)
TC (mg/dL)	6.397± 8.813(0.470)	-32.720 ± 67.108 (0.629)	0.211 ± 0.104 **(0.045)**	0.053 ± 0.099 (0.591)	-0.778 ± 1.636 (0.635)
TG (mg/dL)	10.326± 7.427 (0.169)	7.600 ± 55.327 (0.889)	0.154 ± 0.106 (0.151)	-0.084 ± 0.100 (0.493)	2.368 ± 1.662 (0.157)
TyG index	-0.114 ± 0.058 (0.053)*	0.159 ± 0.419 (0.706)	0.001 ± 0.001(0.435)	-0.001 ± 0.001(0.493)	0.019 ± 0.013 (0.143)
Leptin (ng/mL)	0.543 ± 1.086 (0.619)	-6.657 ± 7.527 (0.382)	0.006 ± 0.014 (0.660)	-0.014 ± 0.014(0.323)	-0.087 ± 0.227(0.704)
Resistin (ng/mL)	0.385 ± 0.338 (0.258)	0.082 ± 2.983(0.987)	0.001 ± 0.005(0.853)	0.007 ± 0.005 (0.161)	-0.161 ± 0.084 (0.058)*

Data are presented as β ± standard error (p-value). Abbreviations: TBARS, thiobarbituric acid reactive substances; sTAC, serum total antioxidant capacity; Zn, zinc; Cu, copper. Analysis by a linear regression model adjusted for age and nutritional status. Significant p-values ($p<0.05$) are represented in bold, and * indicates that it does not reach statistical significance but shows a trend close to the significance threshold.

Table 3. Association between trace elements and oxidative stress markers.

Traits	Cu (µg/dL)	Zn (µg/dL)	Cu/Zn ratio
TBARS (nmol/mL)	-0.003 ± 0.002 (0.197)	0.001 ±0.002 (0.515)	-0.029 ± 0.042 (0.449)
sTAC (mEq Trolox)	0.001 ± 0.001 (0.615)	0.001 ± 0.001 (0.512)	0.007 ± 0.010 (0.520)

Data are presented as β ± standard error (p-value). Abbreviations: TBARS, thiobarbituric acid reactive substances; sTAC, serum total antioxidant capacity; Zn, zinc; Cu, copper. Analysis was performed using a linear regression model adjusted for age and nutritional status.

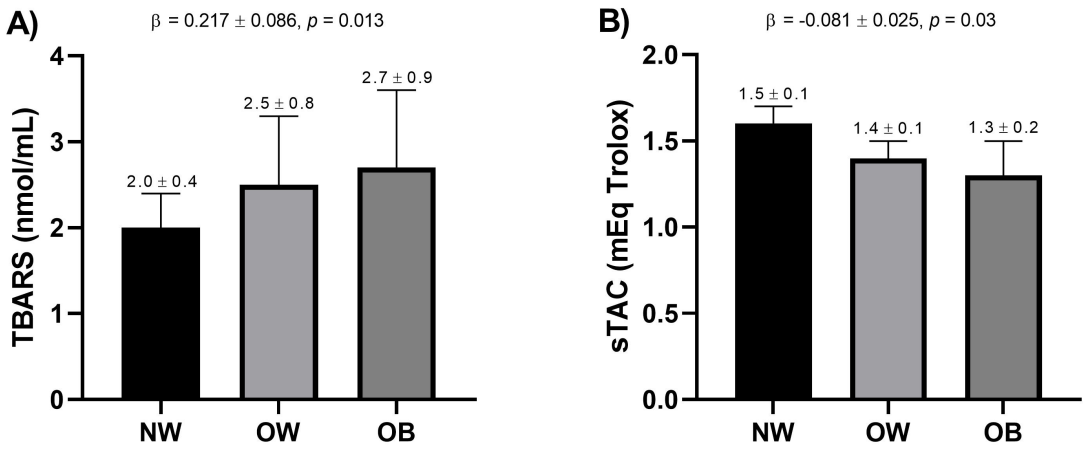


Figure 1. Association of nutritional status with oxidative stress markers: A) thiobarbituric acid reactive substances (TBARS); B) serum total antioxidant capacity (sTAC). Data are presented as β ± SE, p-value. Analysis by linear regression, adjusted for age. Sample size: normal weight (n = 26), overweight (n = 48), obesity (n = 49).

Supplementary Table 1. Normality evaluation.

Trait	Shapiro-Wilk Test (*P*-value)	
	Untransformed	Rank-based inverse normal transformation
BMI, kg/m2	**0.037**	0.999
FPG, mg/dL	**<0.001**	0.999
TC, mg/dL	**0.001**	0.999
TG, mg/dL	**<0.001**	0.999
TyG index	0.927	-
Leptin, ng/mL	0.301	-
Resistin, ng/mL	**0.002**	0.999
Zn, µg/dL-1	**0.023**	0.997
Cu, µg/dL-1	**<0.001**	0.982
Cu/Zn ratio	**0.001**	0.999
TBARS (nmol/mL)	**<0.001**	0.999
sTAC (mEq Trolox)	**0.912**	-

Shapiro-Wilk tests were performed on continuous variables before and after rank-based inverse normal transformation to determine whether they are normally distributed. Significant *P*-values (<0.05) are represented in bold. Abbreviations: BMI, body mass index; FPG, fasting plasma glucose; TC, total cholesterol; TG, triglycerides; TyG, triglycerides glucose index; Zn, Zinc; Cu, Copper; TBARS, thiobarbituric acid re-active substances; sTAC, serum total antioxidant capacity.

Supplementary Table S2. Instrumental conditions of Cu and Zn analysis by ICP-MS.

Parameter	Value
Plasma Power (kW)	1550
Energy Discrimination KED (V)	5.0
Cell Gas Flow (mL min-1)	4.53
Nebulizer Flow (mL min-1)	1.04
Spray Chamber Temperature (ºC)	2.7
Sampling depth	5
Detector Analog (V)	1770
Detector Counting	975
Plasma gas (L min^{-1})	15
Angular Deflection	– 265
CCT bias	– 21
CCT Entry Lens	– 95.95
CCT Exit Lens	– 40
CCT Focus Lens	– 1.92
Run	10
Nebulize type: MicroMist mL min^{-1}	0.2
Internal standard	^{89}Y
External standard	^{115}In
Isotopes	^{63}Cu, ^{66}Zn

Supplementary Table S3. Results obtain sensitivity and stability tests (n= 600).

Sensitivity		Stability	
Bkg4.5 (cps)	0.030	^{59}Co (%)	0.8
Bkg220.7 (cps)	0.01	^{209}Bi (%)	0.9
$^{140}Ce^{16}O/^{140}Ce$	0.0045	^{238}U (%)	1.0
$^{59}Co/^{35}Cl^{16}O$	24.0	^{115}In (%)	0.5
^{59}Co (cps)	72905		
^{209}Bi (cps)	270291		
^{238}U (cps)	463737		
^{115}In (cps)	75664		

Supplementary Table S4. Results obtained in the linear regressions, LOD and LOQ

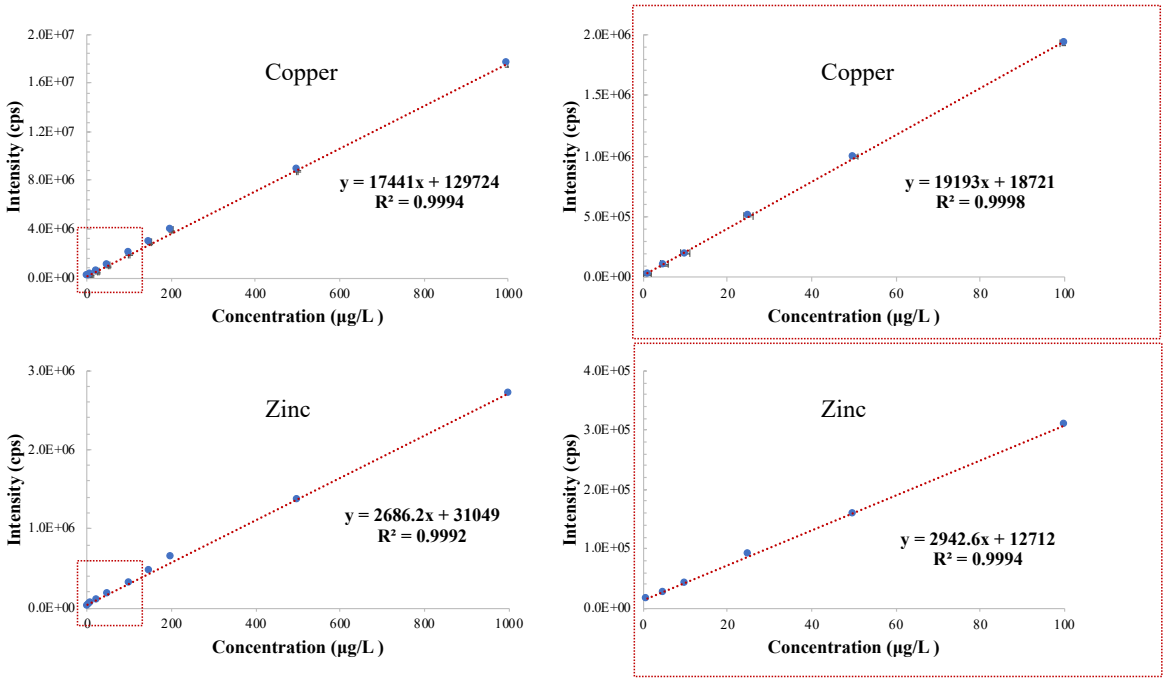
Element	Blank (cps)$^{+}$	linear regression	r^2	LOD (µg L^{-1})$^{++}$	LOQ (µg L^{-1}) $^{+++}$
^{63}Cu	5199.4 ± 603.5	y=17441x +	0.9994	0.295 ± 0.017	0.295 ± 0.172
^{66}Zn	4461.0 ± 492.6	y=2686.2x +	0.9992	0.333 ± 0.021	3.331 ± 0.214
^{115}In	150.9 ± 23.6	y=135052x –	0.9996	0.0012 ± 0.0002	0.0116 ± 0.0016

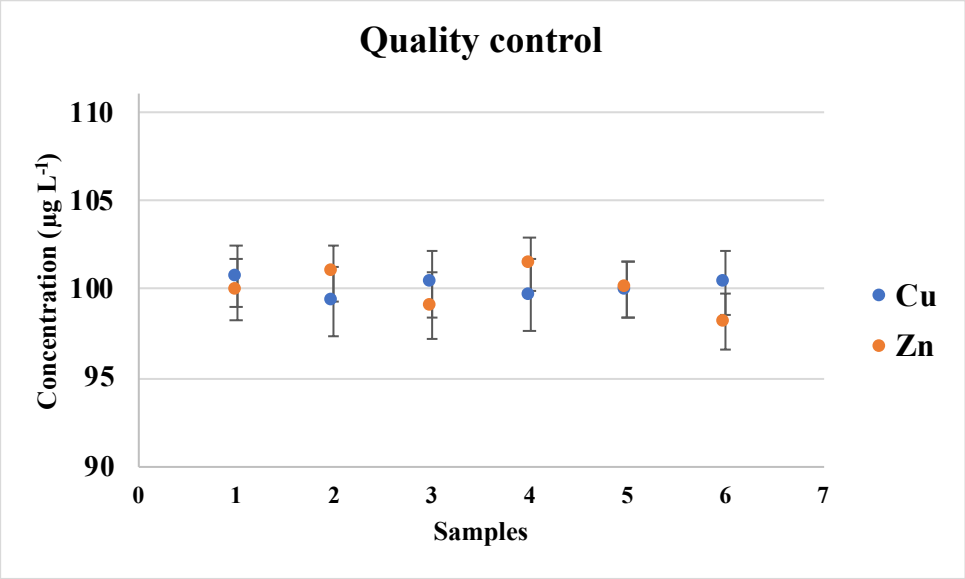
$^{+}$Average of reagent blank intensities (n= 6).

$^{++}$The detection limits (LOD) of Cu, Zn and In were determined following the methodology reported by Thermo Scientific (Al-Hakkani, 2019).

$^{+++}$ The quantification limits (LOQ) of Pb isotopes, Ir, Bi and In were obtained with LOQ= LOD*10 equation.

Supplementary Figure S1. Linear regressions of Cu and Zn.





Supplementary Figure S2. Average the Cu and Zn concentrations that were obtained in the quality control by ICP-MS (n= 6).