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Erythrocyte Zn concentration and antioxidant response after supplementation with Zn in a postmenopausal population. A double-blind randomized trial

Héctor Vázquez-Lorente [a], Jorge Molina-López [b,a,*], Lourdes Herrera-Quintana [a], Yenifer Gamarra-Morales [a], Bartolomé Quintero-Osso [a], Beatriz López-González [a], Elena Planells [a,**]

[a] Department of Physiology, Faculty of Pharmacy, Institute of Nutrition and Food Technology "José Mataix", University of Granada, 18071 Granada, Spain
[b] Faculty of Education, Psychology and Sports Sciences, University of Huelva, 21007 Huelva, Spain

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ABSTRACT

Background & aims: Menopausal hormonal changes increase the risk of deficiencies of minerals such as zinc (Zn), which could further worsen the decreased antioxidant defense of postmenopausal women. This study assesses the effect of 8 weeks of Zn supplementation upon the antioxidant status and clinical nutritional parameters of a postmenopausal population.

Methods: Fifty-one postmenopausal women were divided into two groups: placebo (PbG) and zinc supplementation (ZnG). Mineral status was determined by Flame Atomic Absorption Spectrophotometry (FAAS). Total Antioxidant Capacity (TAC) and Superoxide Dismutase (SOD) were analyzed by kinetic colorimetric methods. Glutathione Peroxidase (GPx) was assessed by an enzymatic immunological method.

Results: Poor Zn status was initially observed in erythrocyte samples. Total antioxidant capacity showed a significant correlation ($r = 0.730$; $p < 0.05$) to erythrocyte Zn after the intervention (ZnG: $r = 0.96$; $p < 0.001$). Moreover, erythrocyte Zn concentration in ZnG was positively correlated to GPx activity after the intervention ($r = 0.61$; $p < 0.01$).

Conclusions: The postmenopausal women initially presented Zn deficiency, and the status of this mineral improved after the intervention. Zinc supplementation may be an effective approach for correcting the observed deficiencies, enhancing antioxidant defense in this risk population.

Clinical trial registration: The present study is registered at the US National Institutes of Health (ClinicalTrials.gov), NCT03672513.

1. Introduction

Menopause is a natural phase of the female aging process, triggered by the cessation of ovarian hormone secretion. It refers to the disappearance of menstrual cycles and the appearance of physiological changes, with an increase in the risk of chronic degenerative disorders (Ruediger et al., 2021). Due to the hormonal alterations, particularly estrogen depletion, this stage in life is characterized by an increased risk of deficiencies in the nutritional status of certain nutrients such as Zinc (Zn) (Nasiadek et al., 2020).

Over the last decades, researchers have postulated that menopause itself contributes to molecular oxidation caused by free radicals, particularly Reactive Oxygen Species (ROS) (Cervellati and Bergamini, 2016). Antioxidant status has been reported to be lowered during the menopausal period (Yang et al., 2014). Estrogens are sexual hormones that function as antioxidants. In this sense, during menopause, the lowered estrogen levels could cause a reduction of antioxidant status (Cervellati et al., 2013). On the other hand, it has been reported that a lower antioxidant status is involved in the deficient status of Zn (Davis et al., 2000). Moreover, Superoxide Dismutase (SOD), which depends on

Abbreviations: GPx, Glutathione Peroxidase; PbG, Placebo Group; SOD, Superoxide Dismutase; TAC, Total Antioxidant Capacity; ZnG, Zinc Group; Zn, Zinc.

* Correspondence to: J. Molina-López, Faculty of Education, Psychology and Sports Sciences, University of Huelva, 21007 Huelva, Spain.
** Correspondence to: E. Planells, Institute of Nutrition and Food Technology "José Mataix", University of Granada, 18071 Granada, Spain.
E-mail addresses: jorge.molina@ddi.uhu.es (J. Molina-López), elenamp@ugr.es (E. Planells).

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Zn, has been mentioned to be present at low levels in the menopausal process. In this regard, expression of SOD can be modulated by estrogen and progesterone, which are decreased in menopause (Unfer et al., 2015).

Zinc is widely deficient in the general population, and this constitutes a serious global public health problem (Sian et al., 2002). It acts as a cofactor of more than 300 enzymes in the human body, regulates thousands of genes, controls numerous cell-signaling pathways and is also a key mineral for the immune, nervous and reproductive systems (King et al., 2016). Zinc participates in antioxidant defense and counteracts the oxidative stress induced by ROS (Lu et al., 2020). In this line, Zn is an inhibitor of NADPH oxidase, a co-factor of SOD, and an inducer of metallothionein (Huang et al., 2018).

Cellular Zn regulation is complicated, and could imply several mechanisms to take into account for controlling cellular Zn turnover (Lowe et al., 2009). The World Health Organization (WHO) has suggested to further expand on already known clinical markers of Zn status as there is no universally accepted single measure to assess Zn status and findings from studies assessing Zn status biomarkers are often contradictory and inconsistent (Cheng et al., 2021). Although cellular biomarkers such as erythrocyte Zn concentrations may not be a sensitive and a reliable marker of Zn status over a long period of time (King et al., 2016), erythrocyte Zn analysis remains informative and easy to use (Andriollo-Sanchez et al., 2005), presenting a good sensitivity in the assessment of Zn status in premenopausal women (Ennes Dourado Ferro et al., 2011). Moreover, Zn in erythrocytes is not influenced by several factors (e.g., post-meal status, stress, physical efforts and hormones), revealing more precisely Zn deficiency and being suggested to be a good marker of Zn status (Arik Yilmaz et al., 2011; Whitfield et al., 2010), although an increase in erythrocyte Zn concentrations might not sometimes be a realistic indicator of enhanced Zn status through Zn supplementation, especially due to the lack of established reference values, making Zn concentration in erythrocytes to be interpreted with caution (King et al., 2016).

Previous studies have reported the beneficial effect of a Zn intervention on antioxidant enzymes in adult populations (Mohammadi et al., 2021). Estrogen depletion in postmenopausal women increases the risk of Zn deficiency, which could be prevented through Zn supplementation (Bednarek-Tupikowska et al., 2010). Aging is associated with an accumulation of free radical damage (Kumawat et al., 2012) and with a decrease in antioxidant minerals as Zn - which could lead to physiological and clinical modifications - in healthy elderly populations (Baudry et al., 2020). In fact, it has been demonstrated that a Zn intervention could have beneficial effects upon Total Antioxidant Capacity (TAC) in aged populations, but this effect has not been demonstrated in a population at risk of nutritional deficiency such as postmenopausal women (Hamedifard et al., 2020). Furthermore, due to the Zn mineral deficiency in postmenopausal women and the key role of this mineral in several physiological functions, supplementation with Zn in the menopausal setting is highly recommended (Suzuki et al., 2017).

Thus, given the lack of evidence on the effect of key mineral interventions with Zn upon antioxidant status in healthy postmenopausal women, the present study was carried out to assess the effect of 8 weeks of a Zn supplementation intervention upon antioxidant defense in this healthy yet risk population, and its association to nutritional, clinical and antioxidant parameters. We hypothesized that Zn in erythrocytes could be correlated to the analyzed antioxidant defense parameters in our postmenopausal population.

2. Methods

2.1. Study design and intervention

This is an eight-week, double-blinded, placebo-controlled, randomized intervention trial. Participants were randomly assigned to one of two treatment groups: Placebo Group (PbG: 25 women); Zinc Group (ZnG: 26 women) - 50 mg/day of Zn (600% of Recommended Dietary Allowances (RDA)). Zn supplements were supplied by SM Natural Solutions, Sabadell, Spain (Number 0B62713821) following the period of eight weeks, dosage and mode of application recommended by the manufacturer and based in previous studies with similar dosage and period of supplementation (Jamilian et al., 2016; Nazem et al., 2019). Placebo capsules contained lactose and were made of the same size and colour as Zn supplements for identical appearance and taste. The intervention was carried out in winter from January 15th to March 15th. The study was registered at the US National Institutes of Health (ClinicalTrials.gov) NCT03672513.

2.2. Study participants

Fifty-one healthy postmenopausal women volunteers from the province of Granada, Spain aged between 44 and 76 were recruited once had been informed about the protocol. Women were excluded if they were unwilling to accept the randomization procedure. Women were included according to the following criteria: (i) to present postmenopausal status (with at least 12 months of amenorrhea), (ii) not to present any pathology related to disorders in nutrients absorption and metabolism that could affect their nutritional status, (iii) not to be subjected to hormone replacement therapy (HRT), (iv) not to take vitamin and mineral supplements, (v) not to present systemic inflammatory status (C-reactive protein was included as a reference biomarker to assess inflammation status of the participants at baseline). The present study was conducted according to the principles of the Declaration of Helsinki and the approval by the Ethics Committee of the University of Granada (149/CEIH/2016), in accordance with the International Conference on Harmonization/Good Clinical Practice Standards. Written informed consent was obtained from all patients taking into account the approval of the Ethics Committee and the Research Committee of the Centre. Randomization was performed in a 1:1 ratio using a table of random numbers, prepared by a researcher who did not participate in the data collection. Allocation concealment was ensured, as the referred researcher did not release the randomization code until the participants were recruited into the trial after all baseline measurements were completed. Women were randomly assigned (simple randomization) to study groups (parallel design). In order to ensure comparable distribution across the treatment arms, women were stratified to balance baseline covariates including age ($\geq$ 58 or $<$ 58 years). Both study participants and investigators were blinded to the group allocation. Eligible participants of this study are represented in (Fig. 1).

2.3. Compliance evaluation

Adherence/compliance of nutritional intervention was determined as the percentage of all of the supplement capsules ingested throughout the study period. Moreover, biochemical and clinical-nutritional parameters were taken at baseline and follow-up to evaluate the safety of the product and to verify the adverse effects. In addition, subjects were asked to keep daily records about side effects or other problems related to the supplements.

2.4. Data collection

All qualitative data were obtained through the use of manual questionnaires administered by the interviewer that reflected information on personal data, sociodemographic aspects, an adequate diagnosis of the postmenopausal situation, smoking habits and physical activity.

2.5. Body composition analysis

Anthropometric recorded data were height (SECA® Model 274) and weight (by bioelectrical impedance (Tanita MC-980 Body Composition Analyzer MA Multifrequency Segmental, Barcelona, Spain). BMI was

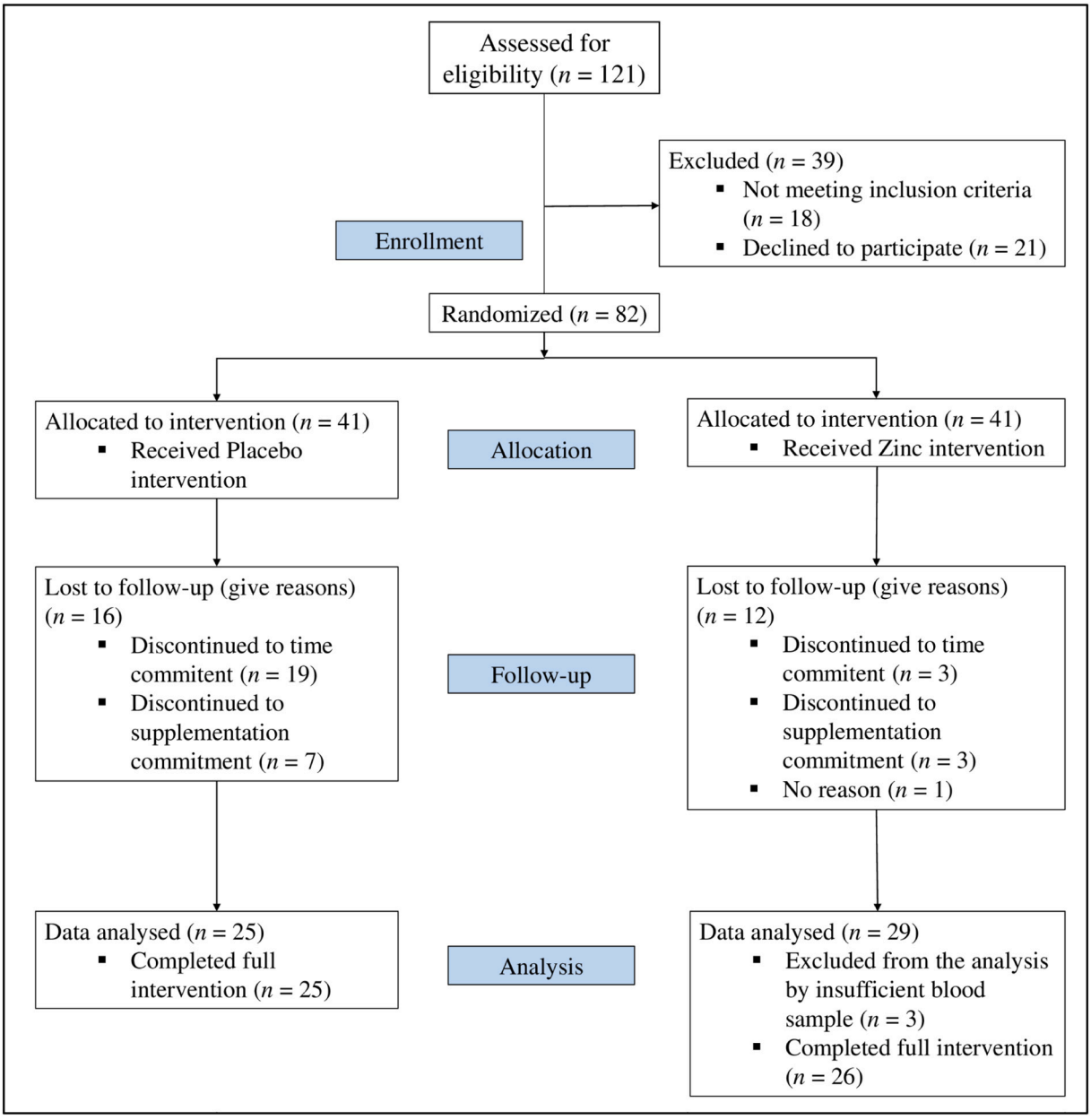

Fig. 1. Flowchart of participants recruited, enrolled, and involved in the clinical study.

them calculated as (weight in Kg/height in m^2). The analyzer complies with the applicable European standards (93/42EEC, 90/384EEC) for use in investigation. Participants were informed in advance of the required conditions prior to the measurement: (i) no alcohol less than 24 h before the measurement, (ii) no vigorous exercise less than 12 h prior to the measurement, (iii) no food or drink less than 3 h prior to the measurement, and (iv) no urination immediately before the measurement (Vázquez-Lorente et al., 2020a).

2.6. Intake rating

A manual 72 h-recall administered by a professional interviewer was used to assess nutrient intake, taking into account a holiday and two non-holidays days at baseline and follow-up. Recall accuracy was recorded with a set of photographs of prepared foods and dishes that are frequently consumed in Spain (Palma et al., 2008). The Dietowin software program (7.1. version, Barcelona, Spain) was used in order to adjust food intakes to absolute and percentage values of the Recommended Dietary Intake (RDI) of nutrient intended for individual subjects. Dietary intake was compared with the RDA for the female Spanish population within the age range included in our study (Cuervo et al., 2009).

2.7. Biochemical parameters analysis

A blood extraction performed in the morning in fasting conditions at baseline and follow-up was centrifuged at 4 °C for 15 min at 3000 rpm obtaining plasma and erythrocyte compartments. The erythrocytes were washed 4 times with 3 mL of 0.9% sodium chloride solution, centrifuging for 15 min at 3000 rpm after each wash. Then, the supernatant saline solution was removed from the last wash and the erythrocytes were obtained with a Pasteur Pipette. The samples were frozen at −80 °C for further analysis. All samples were measured in one run, in the same assay batch and blinded quality control samples were included in the assay batches to assess laboratory error in the measurements. Clinical parameters were glucose, urea, uric acid, triglycerides, total cholesterol,

total proteins, transferrin, albumin and C-reactive protein by routine analytical hospital assays. Zn content was analyzed by Flame Atomic Absorption Spectrometry (FAAS) (Perkin Elmer A. Analyst'300 Norwalk, CT, USA) previous wet-mineralised way in the scientific Instrumental Center (SIC) from the University of Granada. Accuracy of the method was evaluated by analysis of a certified reference material (Seronorm™ Trace Elements ref. MI0181 SERO AS, Billingstad, Norway). Levels of Zn were analyzed at different optimal wavelengths for each element (slit 0.7 nm), using a flow rate (Air/C_2H_2) of 10/1.9 L·min^{-1}, and a five-point calibration curves ($r^2 = 0.9997$) (Vázquez-Lorente et al., 2020b). 7.30 mg/dL was the cut-off point for determining a low erythrocyte Zn content (Vázquez-Lorente et al., 2021). Antioxidant status parameters measured were Total Antioxidant Capacity (TAC), GPx and SOD activities, analyzed by commercial kinetic colorimetric methods. TAC determination in plasma samples was carried out evaluating the reduction power of Cu^{2+} from the action of antioxidants present in samples (TAC kit, Jaica, Shizuoka, Japan). Variability was tested repeatedly conducting five samples and considering lower variability than 5% to be included (Vassalle et al., 2004). GPx activity was determined by enzymatic immunological method using the Bioxytech GPx-340™ kit (OxisResearch™), an indirect colorimetric assay of the activity of GPx (Chu et al., 1993). SOD activity was analyzed by colorimetric method based on cytochrome *c* reduction using the Randox Ransod kit (RANDOX Laboratories Ltd., United Kingdom) (Arthur and Boyne, 1985). The reference values for each antioxidant, clinical and mineral parameter were provided by the manufacturer, the hospital and the SIC, respectively.

2.8. Statistical analysis

Statistical analyses were completed using the Statistical Package for the Social Sciences Version 21.0 (SPSS Inc. Chicago, IL, USA). We performed sample size calculation for our primary aim of the double-blinded, placebo-controlled, randomized intervention based on the influence of oral Zn supplementation upon antioxidant status in a postmenopausal population by using G*Power software (version 3.1.9.6, Kiel, Germany). To the best of our knowledge, there were no available information on changes in TAC in postmenopausal women treated with Zn and based on their erythrocyte concentrations. Therefore, the number of participants to be included in the study was calculated based on the main statistical method used (unpaired *t*-test). An a priori power analysis indicated that at least a total of 82 participants were required. This calculation was based on a moderate effect size (effect size d = 0.65), an alpha level of 0.05, and a beta value of 0.80 for unpaired *t*-test calculating the difference between two independent means (two groups) (Faul et al., 2007). Categorical variables were summarized as frequencies, and continuous variables using mean and standard deviation. The hypothesis of normal distribution was accepted using the Kolmogorov-Smirnov test as a previous step to the execution of a parametric model or not. To compare the changes intergroup before and after intervention, unpaired *t*-test was used. To compare the differences in change over time among the two groups, paired *t*-test was employed. Correlation analyses and partial correlation coefficients were performed with Pearson test. A *p* value less than 0.05 was considered statistically significant.

3. Results

The women included in our intervention showed 100% adherence to supplementation and reported no side effects during the two-month intervention. Table 1 describes the baseline characteristics of the different postmenopausal groups. Based on the BMI, 37.9% of the postmenopausal women presented type I overweight before the intervention. With regard to the initial adequacy of mineral intake, 40.1% presented an insufficient intake of Zn (below 75% of the RDA). The rest of the nutrients were within the RDAs, though energy intake was rather below the reference values. In the case of Zn status, approximately 60% of the women in ZnG presented erythrocyte Zn deficiency, which was corrected to 4% after Zn supplementation. No intergroup differences were found for the studied parameters at baseline.

Table 1
Baseline characteristics of the study population.

	Reference values	PbG ($n = 25$)	ZnG ($n = 26$)	*p* value
		Mean ± *SD* (%RDA)	Mean ± *SD* (%RDA)	
Sociodemographic				
Age (years)	–	59.5 ± 9.00	57.0 ± 8.00	0.327
BMI (kg/m^2)	22.0–27.0	28.0 ± 4.30	26.2 ± 4.50	0.231
Blood pressure				
Normal blood pressure *n* (%)	–	11 (42)	16 (70)	–
High blood pressure *n* (%)	–	14 (58)	10 (30)	–
Physical exercise				
Sedentary *n* (%)	–	9 (36)	6 (23)	–
Non-sedentary *n* (%)	–	16 (64)	20 (77)	–
Smoking habit				
Non-smoker *n* (%)	–	18 (75)	21 (81)	–
Smoker *n* (%)	–	7 (25)	5 (19)	–
Educational level				
Basic educational level *n* (%)	–	11 (42)	8 (31)	–
Secondary or high level *n* (%)	–	14 (58)	18 (69)	–
Intake				
Energy (Kcal/day)	2000.0	1339.0 ± 277.1 (67.0)	1487.9 ± 385.5 (74.5)	0.121
CH (g/day)	275.0	145.5 ± 39.8 (53.2)	154.0 ± 39.8 (55.5)	0.452
Proteins (g/day)	50.0	59.7 ± 13.9 (145.7)	63.9 ± 14.4 (140.3)	0.307
Fats (g/day)	70.0	56.4 ± 16.9 (77.3)	67.7 ± 26.2 (93.0)	0.076
Zn (mg/day)	8.00	6.67 ± 5.06 (46.0)	5.80 ± 1.40 (40.2)	0.411
Biochemical parameters				
Glycemia (mg/dL)	70.0–110.0	96.0 ± 19.8	90.6 ± 16.0	0.295
Creatinine (mg/dL)	0.50–0.90	0.76 ± 0.18	0.66 ± 0.10	0.012
Urea (mg/dL)	10.0–50.0	36.2 ± 10.2	33.3 ± 8.20	0.262
Uric acid (mg/dL)	2.40–5.70	4.50 ± 1.00	4.36 ± 1.01	0.436
Triglycerides (mg/dL)	50.0–200.0	115.8 ± 68.9	98.2 ± 82.7	0.419
Cholesterol (mg/dL)	110.0–200.0	224.1 ± 39.7	212.8 ± 33.1	0.279
Transferrin (mg/dL)	200.0–360.0	289.9 ± 29.5	274.2 ± 49.2	0.381
Albumin (mg/dL)	3.50–5.20	4.48 ± 0.21	4.37 ± 0.21	0.069
Total proteins (g/dL)	6.60–8.70	7.10 ± 0.42	7.00 ± 0.62	0.705
Total bilirubin (mg/dL)	0.10–1.20	0.50 ± 0.13	0.47 ± 0.11	0.533
Prealbumin (mg/dL)	20.0–40.0	26.6 ± 5.03	24.8 ± 6.45	0.133
C-reactive protein (mg/L)	0.02–5.00	0.29 ± 0.25	0.29 ± 0.39	0.994
Erythrocyte Zn (mg/dL)	7.30–7.60	6.39 ± 2.12	6.13 ± 1.94	0.657

$n = 51$. Quantitative data are expressed as the mean ± standard deviation (percentage of RDA) unless specified otherwise. Qualitative data are expressed as n (%) of subjects. Abbreviations: RDA = Recommended Dietary Allowance; BMI = Body Mass Index; CH = Carbohydrates; Zinc = Zn; PbG = Placebo Group; ZnG = Zinc Group; Unpaired *t*-test was used for inter-groups analysis of quantitative variables. Statistical significance was considered for $p < 0.05$.

Fig. 2 shows the correlation analysis between Zn in erythrocytes and antioxidant parameters after two months of supplementation. Of the different antioxidant status parameters considered, TAC showed a significant correlation to erythrocyte Zn in the total sample ($r = 0.81$; $p < 0.001$) after the intervention (Fig. 2A), and also stratified by ZnG after the intervention ($r = 0.96$; $p < 0.001$) (Fig. 2B). Moreover, erythrocyte Zn in ZnG was positively correlated to GPx after the intervention ($r =$

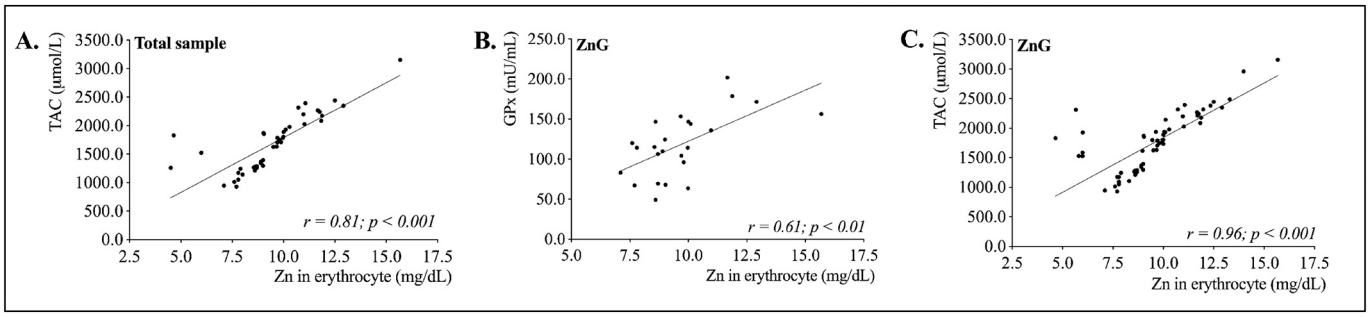

Fig. 2. Scatter plots of the relationship between erythrocyte zinc concentration after two months of supplementation and the different antioxidant parameters. Abbreviations: TAC = Total Antioxidant Capacity; GPx = Glutathione Peroxidase; Zn = Zinc; ZnG = Zinc Group. Correlation analyses and partial correlation coefficients were performed with Pearson test. A p value less than 0.05 was considered statistically significant. (A) Zn in Erythrocyte-TAC in total population; (B) Zn in Erythrocyte-TAC in ZnG; (C) Zn in Erythrocyte-GPx in ZnG.

0.61; $p < 0.01$) (Fig. 2C).

Fig. 3 shows the mean values of the three antioxidant parameters studied according to median age and the intervened groups following supplementation. Total antioxidant capacity was significantly lower ($p < 0.05$) in those postmenopausal women below the median age (58 years) in ZnG. No significant changes were observed after the intervention in both groups with regard to the rest of the antioxidant parameters.

4. Discussion

There is a growing interest in maintaining optimal trace element status in menopause. In this regard, Zn is associated to a number of indirect antioxidant functions, suggesting that it may play a preventive role in certain diseases associated with menopause (Sharif et al., 2015). The aim of this study was to investigate the effects of short-term Zn supplementation upon the antioxidant status of a cohort of healthy postmenopausal women. The results showed that a large percentage of postmenopausal women had alterations in Zn status that were partially corrected after the Zn intervention, observing significant improvement of antioxidant status in those Zn-supplemented postmenopausal women in the higher age range. Likewise, the observed relationship between the erythrocyte Zn cocnentrations after supplementation and antioxidant status (mainly TAC) may contribute to preserve antioxidant defenses during the postmenopausal process.

In line with other investigations (albeit to a lesser extent), the present study showed postmenopausal women to have insufficient Zn intake (Table 1), as previously reported accompanied by low biochemical levels of this mineral (Nielsen et al., 2011). According to our results, at baseline the mean erythrocyte Zn concentrations were below the reference values and improved significantly after the Zn intervention in ZnG. Regarding Zn supplementation, significantly increased erythrocyte Zn concentrations were recorded in ZnG over the course of the intervention, together with the achievement of optimum Zn intake. Supplementation had a major effect in restoring Zn homeostasis and consequently reducing the percentage of women who presented low Zn status. In this sense, a study involving a cohort of 387 middle-aged volunteers (55% women) aged 55–85 years showed Zn concentration - which increased significantly in the case of serum Zn following Zn supplementation - to be no different between supplemented and non-supplemented individuals in the case of erythrocyte Zn (Intorre et al., 2008). This observation could be explained in part by the implication of Zn in many metabolic pathways. Thus, the decrease in the concentration of the mineral in erythrocytes could be due to the larger demand for the mineral (King et al., 2016). Therefore, the significant increase observed in erythrocyte Zn after supplementation in ZnG in our study could suggest that the demand for this mineral is mainly focused on promoting the transport of blood Zn to different tissues and enzymatic systems before restoring the erythrocyte reserves. In this regard, taking into account that erythrocytes present a lifespan of 3 months, Zn content in erythrocyte could be a useful biomarker of Zn status according to our 2 months intervention period (Agte et al., 2004). In this line, another possible explanation could be the potential effect which metallothionein could perform upon Zn homeostasis, as it is the main regulator of the intracellular transport and mobilization, storage and transferring of Zn (Özcelik et al., 2012). Metallothioneins are more expressed when Zn is in higher amounts and bind Zn intracellularly, serving as markers of Zn status (Hennigar et al., 2016). Together with their well-known

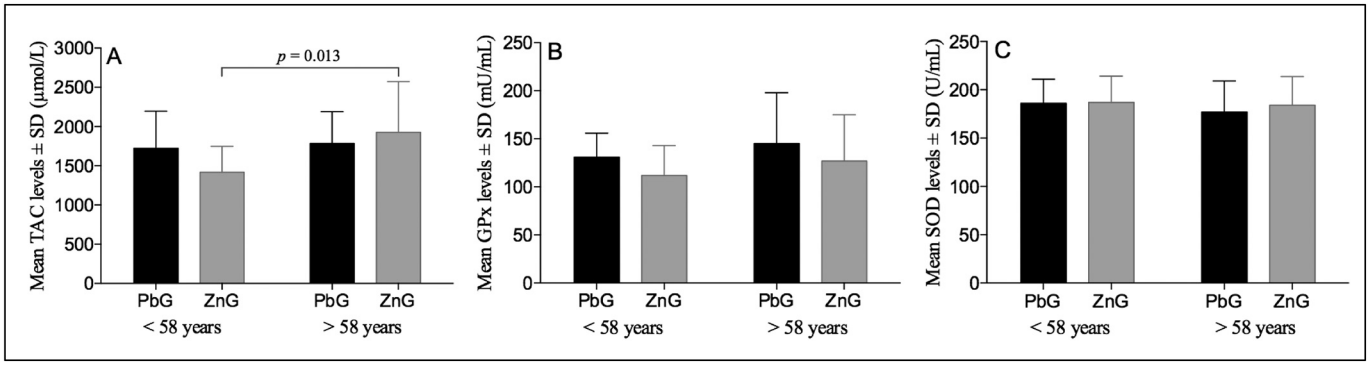

Fig. 3. Mean values of antioxidant parameters after intervention by groups and median age. All data are expressed as the mean (standard deviation). Abbreviations: PbG = Placebo Group; ZnG = Zinc Group; TAC = Total Antioxidant Capacity; GPx = Glutathione Peroxidase; SOD = Superoxide Dismutase. Paired t-test was used for comparing intra-groups and unpaired t-test for comparting inter-groups. The model was adjusted for erythrocyte Zn as covariate. Statistical significance considered for $p < 0.05$. (A) mean TAC levels by intervened groups and age; (B) mean GPx activity by intervened groups and age; (C) mean SOD activity by intervened groups and age.

relationship with Zn homeostasis, metallothioneins are considered too as antioxidant Zn-related factors due to their influence on decreasing oxidative damage (Cardova et al., 2017). Regretfully, metallothionein levels, that could have helped to clarify the assessment of Zn status and their potential involvement in increasing antioxidant defenses, were not assessed in this study.

Positive associations were found between erythrocyte Zn and TAC after the intervention in ZnG ($r = 0.96$; $p < 0.001$) (Fig. 2A and B), confirming the key role of Zn as an antioxidant. Previous evidence (Nasiadek et al., 2020) points to increased TAC levels after Zn interventions in menopausal populations compared to placebo, demonstrating the potential influence of Zn upon antioxidant status. Moreover, erythrocyte Zn was directly correlated to GPx activity in ZnG (Fig. 2C). Mariani et al. (2008) performed a Zn supplementation in healthy old population to assess its effect upon GPx activity. In their study, no age and gender-related differences in the activity of GPx was observed, but, contrary to our findings, they did not observe a direct relationship between the Zn intervention and GPx activity. Unfortunately, no relationship was found between erythrocyte Zn and SOD activity. Opposite to our findings, one study involving a Zn supplementation in postmenopausal women, evidenced increased SOD activity after the Zn intervention, considering SOD as a proper biomarker of Zn status in postmenopausal women (Davis et al., 2000). In this line, the abovementioned study only suggests short period interventions as the one performed in our study, because of the possible adverse effects of long-term Zn interventions.

Oxidative stress is a risk factor for clinical outcomes in menopausal women that could be ameliorated through enhanced antioxidant status parameters (Rezasoltani et al., 2021). In our study, TAC was significantly lower in those postmenopausal women below the median age in ZnG after the intervention. Metabolic changes occurring with advancing age may increase oxidative stress because of a decrease in antioxidant capacity (Ceci et al., 2020). In this sense, the increase in oxidative stress during menopause may be due to lower levels of hormones and their antioxidant role (Signorelli et al., 2006). It has recently been demonstrated that the decrease in antioxidant defense capacity depends on the time of the menopausal phase, possibly through the mentioned hormonal alteration (Kolesnikova et al., 2015). Alterations in metabolism, redox imbalance, and dysregulation of the inflammatory response in elderly women can be risk factors in the development, progression and clinical manifestations of different disease conditions (Bachi et al., 2019). In this regard, the observed differences in antioxidant status related to age could be due to the time of the menopausal phase. More trials are needed to further elucidate our findings.

The present study has strengths and limitations. As limitations, mention must be made of the use of questionnaires for assessing intake, without registering images and real weights of food (gold standard) (Pinheiro Fernandes et al., 2019), which could be affected by recall bias, since the results are conditioned by the memory and educational level of the subject. In this regard, the authors considered energy intake to be underestimated. One of the major limitations of the 72-hour quantitative recall is based on the fact that the subject tends to report lower intakes amounts. This could be one reason why no association between mineral intake and clinical parameters was found. In turn, the present study has a small sample size, recruiting less women than expected from the sample size estimation mainly due to the loss of participants during the follow up. Another limitation is the lack of established reference values for these cellular zinc concentrations makes interpreting the results difficult. Moreover, it could have been interesting to include in our study oxidative stress markers or metallothionein assessment with the aim of evaluating the influence of increased TAC in ZnG upon oxidative stress markers as ROS. On the other hand, it would be relevant to reproduce this study in the context of long-term supplementation. As strengths, this is a randomized, placebo-controlled study in which intakes of energy, macronutrients and related Zn mineral were controlled at baseline and over two months of follow-up. Ours is one of the few studies to have been conducted in this context, assessing the effect of a Zn intervention upon antioxidant parameters in a population such as postmenopausal women, at risk of suffering deficiencies of key micronutrients.

5. Conclusions

The results of our study show that an 8-week Zn intervention reduces the high percentage of postmenopausal women with low erythrocyte Zn concentrations observed in the analyzed population. Zinc in erythrocytes was related to TAC levels whether or not the women received Zn supplementation and was reinforced among those who were supplemented. Moreover, Zn in erythrocytes was directly related to GPx activity after the Zn intervention. These observations should be investigated more in depth, due to the lack of evidence of the influence of Zn supplementation upon antioxidant parameters. Further studies involving long-term antioxidant mineral interventions, including the evaluation of oxidative stress parameters, are needed in order to preserve the health of postmenopausal women and improve their quality of life.

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CRediT authorship contribution statement

Conceptualization, E.P., B.L.G and L.H.Q.; Methodology, J.M.L.; Software, J.M.L and E.P; Validation, Y.G.M. and B.L.G.; Formal Analysis, B.L.G. and H.V.L.; Investigation, H.V.L and L.H.Q; Resources, E.P and H. V.L; Data Curation, J.M.L; Writing - Original Draft Preparation, H.V.L, B. Q.O. and E.P.; Writing - Review & Editing, H.V.L. and J.M.L; Visualization, L.H.Q and H.V.L.; Supervision, J.M.L and E.P.; Project Administration, E.P., B.Q.O and J.M.L.; Funding Acquisition, E.P. All authors have read and agreed to the published version of the manuscript.

Declaration of competing interest

The authors have declared no conflicts of interest. Complete Declaration of Interest forms for each author has been uploaded at the time of manuscript submission.

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References

Agte, V.V., Nagmote, R.V., Tarwadi, K.V., 2004. Comparative in vitro uptake of zinc by erythrocytes of normal vs type 2 diabetic individuals and the associated factors. Diabetes Nutr. Metab. 17, 343–349.

Andriollo-Sanchez, M., Hininger-Favier, I., Meunier, N., Toti, E., Zaccaria, M., Brandolini-Bunlon, M., Polito, A., O'Connor, J.M., Ferry, M., Coudray, C., Roussel, A.-M., 2005. Zinc intake and status in middle-aged and older european subjects: the ZENITH study. Eur. J. Clin. Nutr. 59 (Suppl. 2), S37–S41. https://doi.org/10.1038/sj.ejcn.1602296.

Arik Yilmaz, E., Ozmen, S., Bostanci, I., Misirlioglu, E.D., Ertan, U., 2011. Erythrocyte zinc levels in children with bronchial asthma. Pediatr. Pulmonol. 46, 1189–1193. https://doi.org/10.1002/ppul.21501.

Arthur, J.R., Boyne, R., 1985. Superoxide dismutase and glutathione peroxidase activities in neutrophils from selenium deficient and copper deficient cattle. Life Sci. 36, 1569–1575.

Bachi, A.L.L., Barros, M.P., Vieira, R.P., Rocha, G.A., de Andrade, P.B.M., Victorino, A.B., Ramos, L.R., Gravina, C.F., Lopes, J.D., Vaisberg, M., Maranhão, R.C., 2019. Combined exercise training performed by elderly women reduces redox indexes and proinflammatory cytokines related to atherogenesis. Oxidative Med. Cell. Longev. 2019, 1–9. https://doi.org/10.1155/2019/6469213.

Baudry, J., Kopp, J.F., Boeing, H., Kipp, A.P., Schwerdtle, T., Schulze, M.B., 2020. Changes of trace element status during aging: results of the EPIC-Potsdam cohort study. Eur. J. Nutr. 59, 3045–3058. https://doi.org/10.1007/s00394-019-02143-w.

Bednarek-Tupikowska, G., Jodkowska, A., Antonowicz-Juchniewicz, J., 2010. Zinc, Cooper, manganese, and selenium status in pre- and postmenopausal women during sex hormone therapy. Adv. Clin. Exp. Med. 19, 337–345.

Cardova, A., Adam, P., Mariani, S., Richtera, L., Heger, Z., Labuda, J., Minunni, M., Adam, V., 2017. Electrochemical and optical study of metallothionein interactions with prion proteins. J. Pharm. Biomed. Anal. 140, 355–361. https://doi.org/10.1016/j.jpba.2017.03.044.

Ceci, R., Duranti, G., Di Filippo, E.S., Bondi, D., Verratti, V., Doria, C., Caporossi, D., Sabatini, S., Dimauro, I., Pietrangelo, T., 2020. Endurance training improves plasma superoxide dismutase activity in healthy elderly. Mech. Ageing Dev. 185, 111190 https://doi.org/10.1016/j.mad.2019.111190.

Cervellati, C., Bergamini, C.M., 2016. Oxidative damage and the pathogenesis of menopause related disturbances and diseases. Clin. Chem. Lab. Med. 54, 739–753. https://doi.org/10.1515/cclm-2015-0807.

Cervellati, C., Bonaccorsi, G., Cremonini, E., Bergamini, C.M., Patella, A., Castaldini, C., Ferrazzini, S., Capatti, A., Picarelli, V., Pansini, F.S., Massari, L., 2013. Bone mass density selectively correlates with serum markers of oxidative damage in post-menopausal women. Clin. Chem. Lab. Med. 51, 333–338. https://doi.org/10.1515/cclm-2012-0095.

Cheng, J., Bar, H., Tako, E., 2021. Zinc status index (ZSI) for quantification of zinc physiological status. Nutrients 13, 3399. https://doi.org/10.3390/nu13103399.

Chu, F.F., Doroshow, J.H., Esworthy, R.S., 1993. Expression, characterization, and tissue distribution of a new cellular selenium-dependent glutathione peroxidase, GSHPx-GI. J. Biol. Chem. 268, 2571–2576.

Cuervo, M., Corbal·n, M., BaladÌa, E., Cabrerizo, L., Formiguera, X., Iglesias, C., Lorenzo, H., Polanco, I., Quiles, J., Romero de Avila, M.D., Russolillo, G., Villarino, A., Alfredo MartÌnez, J., 2009. Comparison of dietary reference intakes (DRI) between different countries of the European Union, The United States and the World Health Organization. Nutr. Hosp. 24, 384–414.

Davis, C.D., Milne, D.B., Nielsen, F.H., 2000. Changes in dietary zinc and copper affect zinc-status indicators of postmenopausal women, notably, extracellular superoxide dismutase and amyloid precursor proteins. Am. J. Clin. Nutr. 71, 781–788. https://doi.org/10.1093/ajcn/71.3.781.

Ennes Dourado Ferro, F., Sousa Lima, V.B.de, Mello Soares, N.R., Franciscato Cozzolino, S.M.A., Nascimento Marreiro, D.do, 2011. Biomarkers of metabolic syndrome and its relationship with the zinc nutritional status in obese women. Nutr. Hosp. 26, 650–654.

Faul, F., Erdfelder, E., Lang, A.-G., Buchner, A., 2007. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav. Res. Methods 39, 175–191. https://doi.org/10.3758/bf03193146.

Hamedifard, Z., Farrokhian, A., Reiner, Ž., Bahmani, F., Asemi, Z., Ghotbi, M., Taghizadeh, M., 2020. The effects of combined magnesium and zinc supplementation on metabolic status in patients with type 2 diabetes mellitus and coronary heart disease. Lipids Health Dis. 19 https://doi.org/10.1186/s12944-020-01298-4.

Hennigar, S.R., Kelley, A.M., McClung, J.P., 2016. Metallothionein and zinc transporter expression in circulating human blood cells as biomarkers of zinc status: a systematic Review123. Adv. Nutr. 7, 735–746. https://doi.org/10.3945/an.116.012518.

Huang, T.-C., Chang, W.-T., Hu, Y.-C., Hsieh, B.-S., Cheng, H.-L., Yen, J.-H., Chiu, P.-R., Chang, K.-L., 2018. Zinc protects articular chondrocytes through changes in Nrf2-mediated antioxidants, cytokines and matrix metalloproteinases. Nutrients 10, 471. https://doi.org/10.3390/nu10040471.

Intorre, F., Polito, A., Andriollo-Sanchez, M., Azzini, E., Raguzzini, A., Toti, E., Zaccaria, M., Catasta, G., Meunier, N., Ducros, V., O'Connor, J.M., Coudray, C., Roussel, A.M., Maiani, G., 2008. Effect of zinc supplementation on vitamin status of middle-aged and older european adults: the ZENITH study. Eur. J. Clin. Nutr. 62, 1215–1223. https://doi.org/10.1038/sj.ejcn.1602844.

Jamilian, M., Foroozanfard, F., Bahmani, F., Talaee, R., Monavari, M., Asemi, Z., 2016. Effects of zinc supplementation on endocrine outcomes in women with polycystic ovary syndrome: a randomized, double-blind, placebo-controlled trial. Biol. Trace Elem. Res. 170, 271–278. https://doi.org/10.1007/s12011-015-0480-7.

King, J.C., Brown, K.H., Gibson, R.S., Krebs, N.F., Lowe, N.M., Siekmann, J.H., Raiten, D. J., 2016. Biomarkers of nutrition for development (BOND)-zinc review. J. Nutr. https://doi.org/10.3945/jn.115.220079.

Kolesnikova, L., Semenova, N., Madaeva, I., Suturina, L., Solodova, E., Grebenkina, L., Darenskaya, M., 2015. Antioxidant status in peri- and postmenopausal women. Maturitas 81, 83–87. https://doi.org/10.1016/j.maturitas.2015.02.264.

Kumawat, M., Sharma, T.K., Singh, I., Singh, N., Singh, S.K., Ghalaut, V.S., Shankar, V., Vardey, S.K., 2012. Decrease in antioxidant status of plasma and erythrocytes from geriatric population. Dis. Markers 33, 303–308. https://doi.org/10.3233/DMA-2012-00938.

Lowe, N.M., Fekete, K., Decsi, T., 2009. Methods of assessment of zinc status in humans: a systematic review. Am. J. Clin. Nutr. 89, 2040S–2051S. https://doi.org/10.3945/ajcn.2009.27230G.

Lu, X., Zhang, Q., Xu, L., Lin, X., Fu, J., Wang, X., Liu, Y., Lin, Y., Li, B., Wang, R., Liu, L., Mi, X., Wei, H., Tan, Y., Fang, Y., 2020. Zinc is essential for the transcription function of the PGC-1α/Nrf2 signaling pathway in human primary endometrial stromal cells. Am. J. Physiol.-Cell Physiol. 318, C640–C648. https://doi.org/10.1152/ajpcell.00152.2019.

Mariani, E., Mangialasche, F., Feliziani, F.T., Cecchetti, R., Malavolta, M., Bastiani, P., Baglioni, M., Dedoussis, G., Fulop, T., Herbein, G., Jajte, J., Monti, D., Rink, L., Mocchegiani, E., Mecocci, P., 2008. Effects of zinc supplementation on antioxidant enzyme activities in healthy old subjects. Exp. Gerontol. 43, 445–451. https://doi.org/10.1016/j.exger.2007.10.012.

Mohammadi, H., Talebi, S., Ghavami, A., Rafiei, M., Sharifi, S., Faghihimani, Z., Ranjbar, G., Miraghajani, M., Askari, G., 2021. Effects of zinc supplementation on inflammatory biomarkers and oxidative stress in adults: a systematic review and meta-analysis of randomized controlled trials. J. Trace Elem. Med. Biol. Organ Soc. Miner. Trace Elem. GMS 68, 126857. https://doi.org/10.1016/j.jtemb.2021.126857.

Nasiadek, M., Stragierowicz, J., Klimczak, M., Kilanowicz, A., 2020. The role of zinc in selected female reproductive system disorders. Nutrients 12, 2464. https://doi.org/10.3390/nu12082464.

Nazem, M.R., Asadi, M., Jabbari, N., Allameh, A., 2019. Effects of zinc supplementation on superoxide dismutase activity and gene expression, and metabolic parameters in overweight type 2 diabetes patients: a randomized, double-blind, controlled trial. Clin. Biochem. 69, 15–20. https://doi.org/10.1016/j.clinbiochem.2019.05.008.

Nielsen, F.H., Lukaski, H.C., Johnson, L.K., Roughead, Z.K.F., 2011. Reported zinc, but not copper, intakes influence whole-body bone density, mineral content and T score responses to zinc and copper supplementation in healthy postmenopausal women. Br. J. Nutr. 106, 1872–1879. https://doi.org/10.1017/S0007114511002352.

Özcelik, D., Nazıroglu, M., Tunçdemir, M., Çelik, Ö., Öztürk, M., Flores-Arce, M.F., 2012. Zinc supplementation attenuates metallothionein and oxidative stress changes in kidney of streptozotocin-induced diabetic rats. Biol. Trace Elem. Res. 150, 342–349. https://doi.org/10.1007/s12011-012-9508-4.

Palma, I., Farran, A., Cervera, P., 2008. Tables of food composition by household measures of common consumption in Spain. Rev. Esp. Nutr. Humana Dietética 12, 85.

Pinheiro Fernandes, A.C., Araneda Flores, J.A., Illanes Yamada, K., Pedraza Valencia, C., 2019. Evaluation of the precision and accuracy of a food registry survey with digital image support in real time. Nutr. Hosp. 36, 356–362. https://doi.org/10.20960/nh.2113.

Rezasoltani, P., Elliyoun, N., Ziaie, T., Sobhani, A., Kazemnezhjad Leyli, E., Kazemi Aski, S., 2021. Double-blind controlled trial of vitamin E effects on serum lipid profile in menopausal women. Diabetes Metab. Syndr. Obes. Targets Ther. 14, 1053–1060. https://doi.org/10.2147/DMSO.S233138.

Ruediger, S.L., Koep, J.L., Keating, S.E., Pizzey, F.K., Coombes, J.S., Bailey, T.G., 2021. Effect of menopause on cerebral artery blood flow velocity and cerebrovascular reactivity: systematic review and meta-analysis. Maturitas 148, 24–32. https://doi.org/10.1016/j.maturitas.2021.04.004.

Sharif, R., Thomas, P., Zalewski, P., Fenech, M., 2015. Zinc supplementation influences genomic stability biomarkers, antioxidant activity, and zinc transporter genes in an elderly australian population with low zinc status. Mol. Nutr. Food Res. 59, 1200–1212. https://doi.org/10.1002/mnfr.201400784.

Sian, L., Krebs, N.F., Westcott, J.E., Fengliang, L., Tong, L., Miller, L.V., Sonko, B., Hambidge, M., 2002. Zinc homeostasis during lactation in a population with a low zinc intake. Am. J. Clin. Nutr. 75, 99–103. https://doi.org/10.1093/ajcn/75.1.99.

Signorelli, S.S., Neri, S., Sciacchitano, S., Pino, L.D., Costa, M.P., Marchese, G., Celotta, G., Cassibba, N., Pennisi, G., Caschetto, S., 2006. Behaviour of some indicators of oxidative stress in postmenopausal and fertile women. Maturitas 53, 77–82. https://doi.org/10.1016/j.maturitas.2005.03.001.

Suzuki, T., Nakamura, Y., Kato, H., 2017. Changes of bone-related minerals during denosumab administration in post-menopausal osteoporotic patients. Nutrients 9, 871. https://doi.org/10.3390/nu9080871.

Unfer, T.C., Figueiredo, C.G., Zanchi, M.M., Maurer, L.H., Kemerich, D.M., Duarte, M.M. F., Konopka, C.K., Emanuelli, T., 2015. Estrogen plus progestin increase superoxide dismutase and total antioxidant capacity in postmenopausal women. Climacteric J. Int. Menopause Soc. 18, 379–388. https://doi.org/10.3109/13697137.2014.964669.

Vassalle, C., Petrozzi, L., Botto, N., Andreassi, M.G., Zucchelli, G.C., 2004. Oxidative stress and its association with coronary artery disease and different atherogenic risk factors. J. Intern. Med. 256, 308–315. https://doi.org/10.1111/j.1365-2796.2004.01373.x.

Vázquez-Lorente, H., Herrera-Quintana, L., Molina-López, J., Gamarra-Morales, Y., López-González, B., Miralles-Adell, C., Planells, E., 2020. Response of vitamin D after magnesium intervention in a postmenopausal population from the Province of Granada, Spain. Nutrients 12. https://doi.org/10.3390/nu12082283.

Vázquez-Lorente, H., Molina-López, J., Herrera-Quintana, L., Gamarra-Morales, Y., López-González, B., Planells, E., 2020. Association between body fatness and vitamin D3 status in a postmenopausal population. Nutrients 12. https://doi.org/10.3390/nu12030667.

Vázquez-Lorente, H., Molina-López, J., Herrera-Quintana, L., Gamarra-Morales, Y., López-González, B., Planells, E., 2021. Effectiveness of eight-week zinc supplementation on vitamin D3 status and leptin levels in a population of

postmenopausal women: a double-blind randomized trial. J. Trace Elem. Med. Biol. 65, 126730. https://doi.org/10.1016/j.jtemb.2021.126730.

Whitfield, J.B., Dy, V., McQuilty, R., Zhu, G., Heath, A.C., Montgomery, G.W., Martin, N. G., 2010. Genetic effects on toxic and essential elements in humans: arsenic, cadmium, copper, lead, mercury, selenium, and zinc in erythrocytes. Environ. Health Perspect. 118, 776–782. https://doi.org/10.1289/ehp.0901541.

Yang, S., Feskanich, D., Willett, W.C., Eliassen, A.H., Wu, T., 2014. Association between global biomarkers of oxidative stress and hip fracture in postmenopausal women: a prospective study. J. Bone Miner. Res. Off. J. Am. Soc. Bone Miner. Res. 29, 2577–2583. https://doi.org/10.1002/jbmr.2302.