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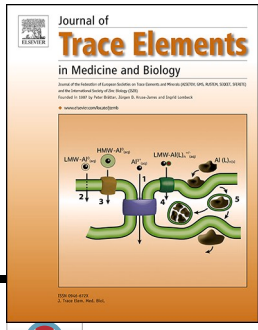

Effect of zinc supplementation on circulating concentrations of homocysteine, vitamin B_{12}, and folate in a postmenopausal population

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ABSTRACT

Introduction: The decrease in estrogen levels associated with menopause increases the risk of deficiencies of key micronutrients such as zinc and of disturbances in methylation cycle-related markers. The present study assesses the effect of 8-week Zn supplementation upon circulating concentrations of Hcy, B_{12}, and Fol levels in a population of postmenopausal women.

Methods: Fifty-one postmenopausal women aged between 44 and 76 years took part in the study. Two randomized groups (placebo and zinc [50 mg/day]) were treated during 8 weeks. Nutrient intake was assessed based on the 72-hour recall method. Zinc was analyzed by flame atomic absorption spectrophotometry. Clinical-nutritional parameters were determined by enzyme immunoassay techniques.

Results: Folate levels increased significantly ($p < 0.05$) in the zinc group on comparing the baseline versus follow-up values. Homocysteine decreased in the inter-group analysis ($p < 0.05$) after the intervention. Furthermore, higher folate ($r = -0.632$; $p = 0.005$) and vitamin B_{12} ($r = -0.512$; $p = 0.030$) levels were correlated to low homocysteine levels in the zinc group after the intervention, although the zinc intervention had the same effect on B_{12} levels in both groups.

Conclusion: Zinc supplementation enhanced circulating folate and homocysteine by improving the folate values in the zinc-supplemented group and decreasing homocysteine levels inter-groups. Further studies involving larger samples and optimizing the doses and intervention period are needed to reinforce our main findings.

1. Introduction

Menopause is the time in life when menstrual cycles cease due to a diminished secretion of ovarian hormones [1]. The decrease in estrogen production could lead to changes in lipid profile, which together with certain menopausal physiological disorders, could disturb clinical-nutritional cardiovascular health related parameters, deriving in cardiovascular diseases [2,3]. Moreover, if this menopausal situation is not monitored, the risk of deficiencies in the nutritional status of numerous key micronutrients, such as minerals, is seen to increase [4].

Zinc (Zn) is one of the most important trace elements, and its deficiency is a major health problem worldwide [5]. Zn is a cofactor of more than 300 enzymes, carrying out its cardiovascular protective functions by combating oxidative stress and inflammation, which are risk factors for cardiovascular diseases [6]. Abnormalities in Zn homeostasis have been reported in patients with metabolic disorders [7,8], since Zn deficiency may cause multiple systemic disturbances, including cardiovascular disease and dyslipidemia [9].

Supplementation of trace elements such as Zn with the aim of improving cardiovascular health is becoming increasingly popular [10]. Although several trials support the beneficial effects of Zn supplementation upon regulating the cardiovascular profile, there is a lack of clarity in the data reported on the impact of Zn supplementation upon human health, especially in postmenopausal women [11]. In this regard, Zn status may also influence the circulating amounts of clinically relevant cardiovascular biomarkers such as homocysteine (Hcy), by increasing folate (Fol) and vitamin B_{12} (B_{12}), which are inversely related to Hcy levels [12]. It is well documented that Fol and B_{12} deficiencies result in increased Hcy levels in aged populations such as postmenopausal women [13,14]. Recently, this relationship has been

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explained, albeit in a Zn-supplemented younger population, by the fact that Zn, Hcy, Fol and B_{12} play an important role in the methylation cycle. In the case of Zn deficiency, Hcy concentrations seem to be increased due to disturbances in the methylation cycle [15]. In this line, two methionine synthase (MS) and betaine Hcy methyltransferase (BHMT), which are enzymes in Hcy metabolism, are Zn-dependent, being Zn deficiency an important factor in the increase in Hcy levels [16]. It therefore would be interesting to assess the possible influence of Zn upon Fol and B_{12} levels in order to elucidate possible alterations in Hcy metabolism in the postmenopausal population.

To our knowledge, few data are available on the relationship between Zn supplementation and Hcy cycle-related parameters in postmenopausal women. The present study assesses the effect of 8-week Zn supplementation upon circulating concentrations of Hcy, B_{12}, and Fol levels, in a population of postmenopausal women.

2. Materials and methodology

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: Zinc Group – 50 mg/day of Zn (ZG: 26 women) and Placebo group (PG: 25 women). Zn capsules were provided by SM Natural Solutions, Sabadell, Spain (Number 0B62713821). Placebo capsules were made of the same color and size as Zn supplements for identical taste and appearance. The manufacturer recommended a period of eight weeks in order to ensure the supplementation effects. The supplementation period took place in winter from January 15th to March 15th.

Adherence to both Zn and Placebo interventions was checked as the percentage of capsules ingested during the intervention period. In order to verify the adverse effects, the safety and the efficacy of the product, two biochemical analytics were performed at baseline and follow-up. Written informed consent was obtained from all patients. The intervention 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. The study was registered at the US National Institutes of Health (ClinicalTrials.gov) NCT03672513.

2.2. Study participants

51 healthy postmenopausal women volunteers from the province of Granada, Spain, aged between 44 and 76 years, were recruited once informed consent was signed. All participants are derived from a larger cohort of postmenopausal women [17]. Inclusion criteria were (i) to present postmenopausal status (with at least 12 months of amenorrhea), (ii) to present baseline plasma Zn (one to five days before the intervention) determined by flame atomic absorption spectrophotometry (FAAS), (iii) to be non-sedentary, (iv) to smoke and drink alcohol in occasional and moderate quantities, (v) to present normal parameters of a routine hospital laboratory analysis. Exclusion criteria were (i) not to accept the randomization procedure, (ii) to take vitamin and mineral supplements, (iii) to present any pathology that could affect their nutritional status (i.e., the main components of metabolic syndrome, celiac disease, bulimia and anorexia), (iv) to be subjected to hormone replacement therapy, (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).

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 by not releasing 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). Both study participants and investigators were blinded to the group allocation.

2.3. Body composition analysis

We measured the participants height with a stadiometer (Seca, model 213, range 85–200 cm; precision: 1 mm; Hamburg, Germany). Body weight was assessed with bioelectrical impedance (Tanita MC-980 Body Composition Analyzer MA Multifrequency Segmental, Barcelona, Spain). The analyzer complies with the applicable European standards (93/42EEC, 90/384EEC) for use in the medical industry. BMI was obtained as weight (kg)/ height (m^2). Participants were informed in advance of the required conditions prior to the measurement: (i) no alcohol intake the previous 24 h, (ii) no vigorous exercise 12 h before the measurement, (iii) no food or drink intake less than 3 h prior to the measurement, and (iv) no urination immediately before the measurement.

2.4. Nutrient intake

Dietary nutrient intake was assessed and administered by a professional dietitian before and after intervention using a manual 72 h-recall, including one weekend day. Recall accuracy was recorded with a set of photographs of prepared foods and dishes that are frequently consumed in the region where the study was performed. Dietowin software (7.1. version, Barcelona, Spain) was employed to quantitatively convert food intake to both energy and micronutrients, determining their adequacy according to the Recommended Dietary Allowance (RDA) for the menopausal Spanish population [18].

2.5. Sample treatment

Plasma samples were obtained through a blood extraction which was centrifuged at 4 °C during 15 min at 3000 rpm in the morning in fasting conditions before and after intervention. To obtain erythrocyte aliquots, 3 mL of 0.9% sodium chloride solution was employed during 4 erythrocyte washes, centrifuging for 15 min at 3000 rpm after each wash. The samples were stored 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.

2.5.1. Measurement of biochemical parameters

Biochemical parameters such as glucose, urea, uric acid, albumin, prealbumin, creatinine, total bilirubin, transferrin, total proteins, triglycerides, Hcy, C-reactive protein (CRP), lactate dehydrogenase (LDH), triglycerides, high density lipoprotein (HDL), low density lipoprotein (LDL) and total cholesterol, were determined in the Analysis Unit at the Virgen de las Nieves Hospital, Granada (ECLIA, Elecsys 2010 and Modular Analytics E170, Roche Diagnostics, Mannheim, Germany). All reference values were provided by the Analysis Unit. Zn content was analysed by FAAS (Perkin Elmer A. Analyst'300 Norwalk, CT, USA), previous wet-mineralized way, in the Scientific Instrumentation Center (SIC) from the University of Granada. Accuracy of the method was evaluated by analysis of a Certified Reference Material (SeronormTM Trace Elements ref. MI0181 SERO AS, Billingstad, Norway), at different optimal wavelengths (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). The reliability was established by setting cut-off scores and considering a sample to be valid and reliable if it showed a coefficient of variation (CV) of less than 5% and an inter-class correlation coefficient (ICC) above 0.90. Fol and B_{12} were measured in SIC using a DxI® Autoanalyzer (Beckman Coulter, CA, USA) employing a competitive electrochemiluminescence immunoassay for quantitative determinations. The reference values of Zn, Fol and B_{12} were provided by the SIC.

2.6. Statistical analysis

All calculations were performed using the SPSS 22.0 Software for MAC (SPSS Inc. Chicago, IL, USA). Previously, a sample size calculation was estimated to determine the effect of a 50 mg/day Zn intervention upon Hcy levels in menopausal women. To our knowledge, no information is available on differences in means and standard deviations in Hcy response to Zn treatment compared to a PG. The sample size calculation was determined by applying a two-factor repeated measures ANOVA test being considered an effect size of 0.20 (effect size f, moderate), an alpha error of 0.05 and a power of 90%. Based on sample size calculations it was determined that a total sample size of 46 participants would be needed (G*Power, version 3.1.9.6; Universität Kiel, Germany). Descriptive analysis was presented as mean and standard deviation (X ± SD). 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. For the comparative analysis based intra-group and inter-group, the paired and unpaired *t*-tests for parametric samples were used, respectively. Correlation analyses and partial correlation coefficients were performed with Pearson test. Significance was set at p-value < 0.05.

3. Results

All participants self-reported 100% adherence to the intervention in both PG and ZG. The baseline characteristics of the postmenopausal population by group are presented in Table 1. In relation to the anthropometric parameters, the total population presented type I overweight on average. In terms of nutrient intake, energy consumption was seen to be below the reference values. With regard to the intake of micronutrients, Zn intake was below 50% of RDA, and reached over 100% of RDA after the Zn intervention in ZG. Moreover, two-thirds of Fol intake were not covered by 66% of the total population, and B_{12} intake was above the reference values in both groups. The biochemical parameters did not show mean differences at baseline.

The supplementation protocol has been published elsewhere. In summary, Zn status was assessed prior to the intervention, evidencing a deficient plasma and erythrocyte Zn status in 58.3% and 54.2% of all postmenopausal women, respectively. After Zn supplementation in ZG, the deficient plasma Zn levels were corrected by 50% of baseline Zn deficient intervened women ($p < 0.001$), and erythrocyte Zn deficiency was almost completely corrected ($p < 0.001$) [19]. Fig. 1 shows effect of placebo and zinc interventions upon main parameters of the study by groups. Fol values were within the reference ranges, increasing significantly (percentage change: 14.9% in ZG versus −6.48% in PG) after Zn supplementation in ZG ($p < 0.05$). In relation to B_{12}, although the levels were within normal limits in both PG and ZG, this parameter increased significantly after the intervention in both groups ($p < 0.05$). The Hcy levels in turn were above the reference values in PG, and significantly higher than in ZG after the intervention (percentage change: −5.41% in ZG versus 9.09% in PG) ($p < 0.05$).

Fig. 2 shows the bivariate Pearson correlation coefficients between Hcy-related parameters of the study by groups. With regard to the relationship between erythrocyte Zn and Hcy (Fig. 2A and B), no significant associations were observed by groups (all $p > 0.05$). When relating B_{12} and Hcy (Fig. 2C and D), a significant inverse correlation was found for ZG ($p = 0.030$). In case of the Fol and Hcy (Fig. 2E and F), the indirect significant correlation only was observed too for ZG ($p = 0.005$).

Table 1
Baseline characteristics of the study population.

Features	Reference values	Total population ($n = 51$)	ZG ($n = 26$)	PG ($n = 25$)	P-value
		Baseline (X ± SD)	Baseline (X ± SD)	Baseline (X ± SD)	
Age (Years)	–	58.3 ± 8.67	57.1 ± 8.34	59.7 ± 9.15	0.167
Weight (Kg)	–	68.1 ± 12.8	67.7 ± 14.4	69.2 ± 11.0	0.687
Height (cm)	–	158.8 ± 6.24	160.3 ± 6.31	157.2 ± 6.01	0.086
BMI (Kg / m^2)	22.0–25.0	26.9 ± 4.54	26.2 ± 4.56	28.0 ± 4.31	0.150
Energy intake (Kcal)	2000.0	1414.9 ± 341.8	1487.9 ± 385.5	1339.5 ± 283.1	0.130
CHO intake (g/day)	275.0	149.8 ± 39.7	154.1 ± 39.8	146.6 ± 40.3	0.520
Protein intake (g/day)	50.0	61.8 ± 14.2	63.8 ± 14.4	59.7 ± 14.2	0.316
Fat intake (g/day)	70.0	62.2 ± 22.6	67.7 ± 26.1	56.1 ± 17.2	0.073
Zn intake (mg/day)	12.0	6.24 ± 3.67	5.82 ± 1.39	6.74 ± 5.16	0.383
Folic acid intake (μg/day)	400.0	240.8 ± 85.4	246.1 ± 80.8	238.2 ± 92.2	0.750
Vitamin B_{12} intake (μg/day)	2.40	5.29 ± 4.79	5.75 ± 5.45	4.80 ± 4.14	0.496
Glucose (mg/dL)	70.0–110.0	93.2 ± 17.9	90.6 ± 16.0	96.0 ± 19.8	0.295
Urea (mg/dL)	10.0–50.0	34.7 ± 9.23	33.3 ± 8.20	36.3 ± 10.2	0.262
Uric acid (mg/dL)	2.40–5.70	4.39 ± 0.99	4.28 ± 1.00	4.50 ± 0.98	0.436
Albumin (mg/dL)	3.50–5.20	4.42 ± 0.22	4.37 ± 0.22	4.48 ± 0.21	0.070
Prealbumin (mg/dL)	20.0–40.0	25.4 ± 4.23	24.6 ± 3.50	26.6 ± 5.02	0.133
Total bilirubin (mg/dL)	0.10–1.20	0.48 ± 0.15	0.47 ± 0.17	0.49 ± 0.13	0.533
Transferrin (mg/dL)	200.0–360.0	278.0 ± 45.3	273.0 ± 48.6	285.8 ± 39.6	0.381
Total proteins (g/dL)	6.60–8.80	7.08 ± 0.53	7.05 ± 0.62	7.11 ± 0.43	0.705
CRP (mg/L)	< 5.00	0.29 ± 0.33	0.29 ± 0.39	0.29 ± 0.24	0.994
LDH (U/L)	110.0–295.0	183.0 ± 54.2	183.2 ± 69.8	182.8 ± 29.3	0.975
Triglycerides (mg/dL)	50.0–200.0	106.6 ± 76.1	98.2 ± 82.7	115.8 ± 68.9	0.419
HDL (mg/dL)	40.0–60.0	66.7 ± 16.2	70.4 ± 19.3	62.6 ± 11.2	0.089
LDL (mg/dL)	70.0–190.0	126.8 ± 33.8	119.6 ± 31.3	134.4 ± 35.3	0.123
Total cholesterol (mg/dL)	110.0–200.0	218.3 ± 36.5	212.8 ± 33.1	224.1 ± 39.7	0.279

n = 51. PG = Placebo Group. ZG = Zinc Group. BMI = Body Mass Index. CHO = Carbohydrates. Zn = Zinc. CRP = C-reactive protein. LDH = Lactate dehydrogenase. HDL = High density lipoprotein. LDL = Low density lipoprotein. Baseline values are expressed as mean (X) ± standard deviation (SD). For inter-groups p-value, unpaired t-student test was used. Intake reference values were obtained from the Reference Dietary Intakes (RDI) for the Spanish Population [18]. Reference values from biochemical parameters were provided from the Virgen de las Nieves Hospital and the Scientific Instrumental Center, Granada.

4. Discussion

The current study, which to our knowledge is the first of its kind in a postmenopausal population, was designed to investigate the effectiveness of an 8-week Zn intervention upon circulating concentrations of Hcy, vitamin B_{12}, and Fol levels in a population of postmenopausal women. The results of our study showed a decrease of Hcy levels at the end of the intervention inter-groups. Moreover, the postmenopausal women supplemented with Zn were seen to increase their Fol levels to within the reference ranges, compared to the baseline values. Interestingly, this improvement in Fol levels was correlated to a decrease in plasma Hcy in this group. Likewise, B_{12} was inversely related with Hcy

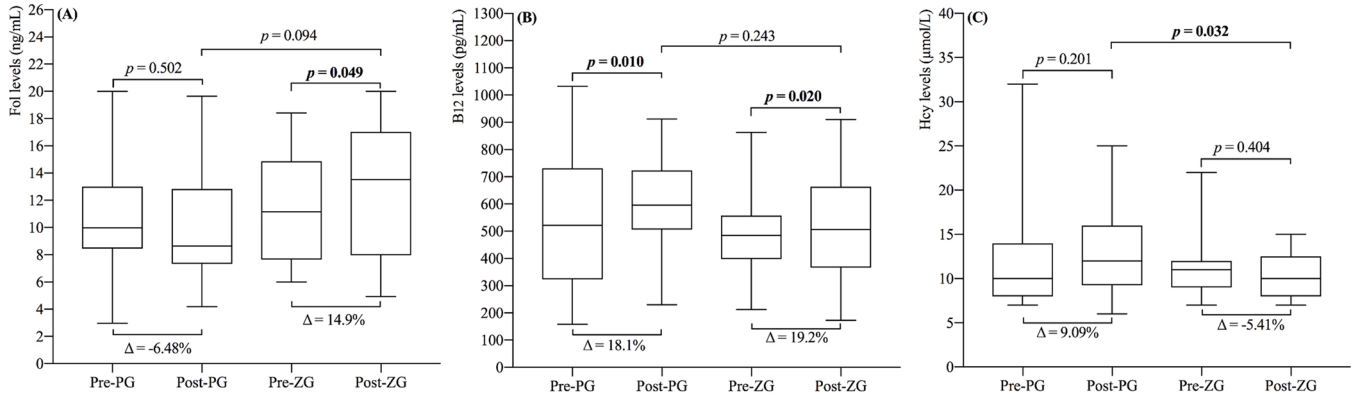

Fig. 1. Effect of placebo and zinc interventions upon main parameters of the study by groups. $n = 51$. Baseline (Pre) and follow-up (Post) values are expressed as mean (X) ± standard deviation (SD). Mean changes intra-group are expressed as mean change (%). Both for intra-group and inter-groups *p*-value, paired and unpaired *t*-student test was used. Reference values were provided from the Virgen de las Nieves Hospital and the Scientific Instrumental Center, Granada: Fol = 2.70–17.0 ng/mL; B_{12} = 190.0–900.0 pg/mL; Hcy = < 13 μmol/L. Abbreviations: Zn = Zinc. PG = Placebo Group. ZG = Zinc Group. Fol = Folate. B_{12} = Vitamin B_{12}. Hcy = Homocysteine.

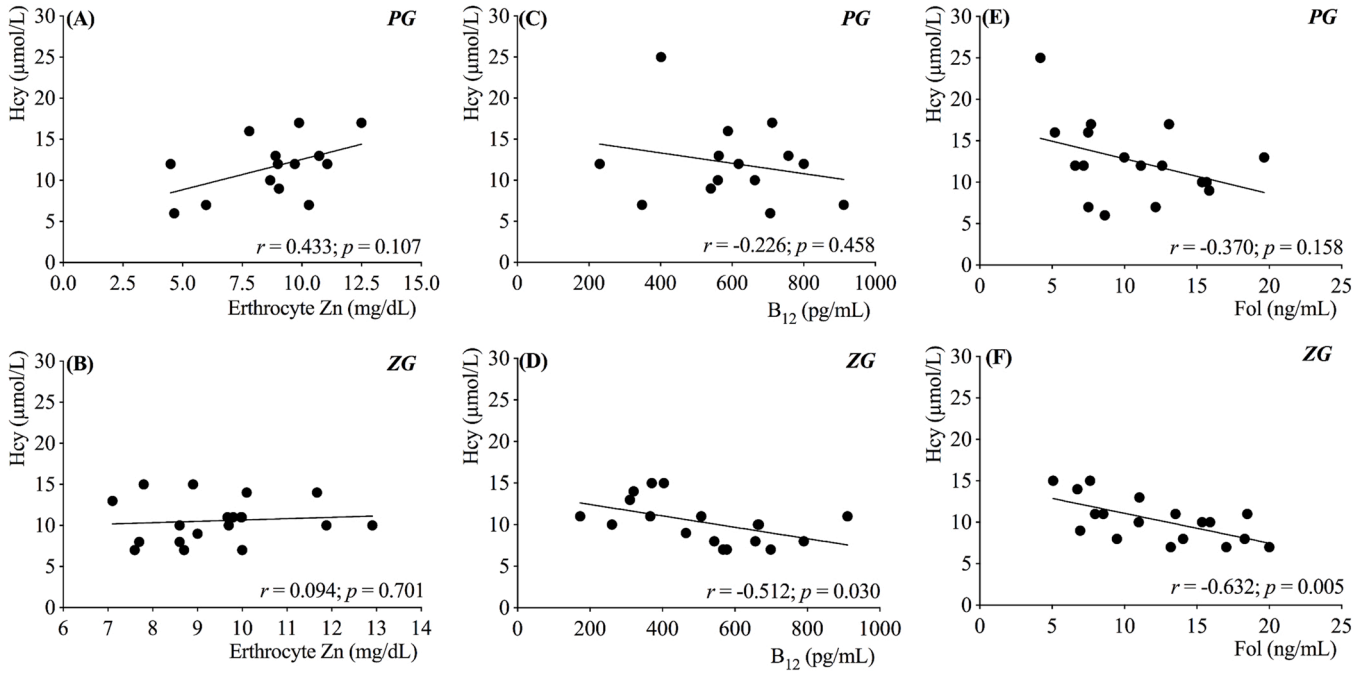

Fig. 2. Bivariate Pearson's correlation coefficients between homocysteine-related parameters of the study by groups. (A) Erythrocyte Zn and Hcy in PG. (B) Erythrocyte Zn and Hcy in ZG. (C) B_{12} and Hcy in PG. (D) B_{12} and Hcy in ZG. (E) Fol and Hcy in PG. (F) Fol and Hcy in ZG. Abbreviations: Hcy = Homocysteine. Fol = Folic Acid. B_{12} = Vitamin B_{12}. PG = Placebo Group. ZG = Zinc Group.

levels, but, regretfully, the intervention had the same effect upon B_{12} in both groups.

Zn status in humans depends on multiple factors (e.g., gender, age, physiological condition and diet), with Zn intake being the main contributor to Zn levels [20]. In our study, low Zn intake (below 50% of RDA) before the intervention was reported. The main cause of Zn deficiency is inadequate dietary intake, which is common in many parts of the world and is further increased in populations at risk, such as postmenopausal women [21]. Folic acid intake was found to be below two-thirds of RDA in 66% of our postmenopausal population. Some authors have found Fol intake to decrease with age, with inadequate nutrient intake being most notorious in postmenopausal women, with the RDA for Fol not being covered in 75% of the cases [22]. Some studies have reported lower rates of inadequate Fol intake in other European countries, where deficiency was found to range from 17.6% to 45.9% in women older than 64 years [23]. With regard to B_{12} intake, our results evidenced sufficient intake, which is not consistent with the above-mentioned study conducted in postmenopausal women, where lower B_{12} intakes were recorded [22].

Approximately 60% of the postmenopausal population in our study presented Zn deficiency in both the plasma and erythrocyte compartments. In this line, recent studies have found high rates of hypozincemia in elderly women [24]. This tendency seems to be less manifest in women of childbearing age, where lower deficiency rates (30%) have been found compared with the present study [25]. Moreover, our results showed a significant increase in both erythrocyte and plasma Zn levels in ZG after the Zn intervention, reversing most of the initial deficiency seen in both compartments. In this sense, a previous study reported that plasma Zn levels were significantly lower than when postmenopausal subjects were intervened with Zn [26]. Humans respond better to a Zn intervention when Zn status is low, as seen in a study performed in a cohort of Zn-intervened postmenopausal women who were adapted to a

Zn intervention within 8 weeks [27]. It should be noted that plasma Zn is characterized by rapid dynamics, increasing the risk of suffering several pathophysiological alterations in response to numerous conditions [28]. In this line, the regulation of cellular Zn is complex, and may involve several mechanisms to take into account for regulating cellular Zn turnover besides plasma and erythrocyte Zn [29]. Thus, cellular biomarkers such as erythrocyte Zn may not be a sensitive and a reliable marker of Zn status as previously described [30]. Accordingly, the observed increase in plasma and erythrocyte Zn levels, that could reflect genuine improvement of Zn status after Zn supplementation, should be interpreted with caution.

In our study, the plasma Fol values increased significantly in the Zn-supplemented group after the intervention. Published information on the effect of Zn supplementation upon Fol levels is scarce and controversial. The proposed mechanism underlying this increase was described by Manger et al. [31], who suggested that a Zn-deficient state reduces the activity of Fol Zn-dependent conjugase, which is necessary for the absorption of Fol; accordingly, an adequate Zn status could improve the Fol levels. To the best of our knowledge, there have been no Zn supplementation studies in postmenopausal populations assessing changes in Fol levels. A study carried out in an elderly Australian population (65–85 years of age) observed the same significant increase in plasma Fol levels after a 12-week Zn intervention [32]. However, in another study carried out in an elderly population supplemented with Zn during 6 months, the increase in plasma Fol was not accompanied by an increase in erythrocyte Fol. This would suggest that a Zn intervention would only be effective in correcting low plasma Fol levels, which reflect short-term Fol deficiency [33]. On the other hand, it is known that Zn could affect B_{12} through its effect upon Zn-dependent methionine synthase and therefore on the Hcy cycle also mediated by Fol [31]. In this line, we found the B_{12} levels to be within the reference ranges, but, regretfully, they increased significantly over follow-up in both ZG and PG in our postmenopausal population. Unfortunately, the limitations of 72-hour recall did not allow us to determine whether the regular diet was responsible for the increase observed in PG [34].

Studies that jointly examine Zn, Hcy, Fol and B_{12} status in humans are limited. In our study, a negative correlation was found between Hcy and both Fol and B_{12} levels after the intervention in our Zn-supplemented women. Previous studies involving animal models have found Zn-deficient status in rats to be related to increased Hcy levels and decreased Fol values [35]. Other studies in children and adolescents, involving a 15 mg/day Zn-intervention during three months, found the Hcy and B_{12} levels to decrease in the Zn-supplemented group, while the Fol values showed no significant changes [15]. Similar findings have been reported by other authors in diabetic Zn-supplemented individuals, with Zn supplementation leading to a significant rise in serum Fol and B_{12} levels, associated to a decrease in Hcy concentrations [36]. These findings moreover are in agreement with a previous study that evidenced no effect upon the plasma Hcy, B_{12} and Fol levels after 6 months of Zn intervention (15 mg/day and 30 mg/day) in a population of French healthy elderly individuals [37]. Since we observed these associations in menopausal women, we believe that the lack of significance in the abovementioned studies could be due to the administration of lower Zn supplementation doses in the general older population.

Some limitations must be considered in the interpretation of our findings. Firstly, the sample size, while appropriate for the present study, was small. Future studies involving larger samples are needed to confirm the validity of our results. Secondly, other biomarkers for identifying Zn homeostatic mechanisms triggered by Zn supplementation, such as Zn transporters, should be considered in order to elucidate the underlying processes. Thirdly, more 72-hour recalls should have been performed during the intervention, as this could have helped to elucidate the observed B_{12} increase in PG. Fourth, the present study included menopausal women with a wide age range, which makes it necessary to interpret the results with caution. Lastly, the Zn intervention period may have been insufficient to evidence more significant changes in cardiovascular risk factors. We thus suggest the need for Zn supplementation studies involving longer follow-up periods among postmenopausal women.

5. Conclusions

An 8-week Zn intervention reduced the initial high prevalence of Zn deficiency and improved Fol in the postmenopausal Zn-intervened group, together with a decrease in Hcy levels when comparing interboth intervened groups. The previous mentioned findings, and in accordance to the inverse correlations between the Hcy and Fol levels, could reflect an enhancement of some Hcy cycle-related parameters in the studied population after the Zn intervention, improving circulating Fol and Hcy. Regretfully, the intervention had the same effect in B_{12} levels for both intervened groups. Further studies involving larger sample sizes and long-term Zn interventions are needed to clarify the possible influence of a prolonged Zn supplementation upon circulating Hcy, B_{12} and Fol levels in a population at risk of Hcy cycle-related parameters disturbances, such as postmenopausal women.

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CRediT authorship contribution statement

Héctor Vázquez-Lorente: Formal analysis, Investigation, Resources, Writing – original draft, Writing – review & editing, Visualization. **Lourdes Herrera-Quintana:** Conceptualization, Investigation, Visualization. **Jorge Molina-López:** Methodology, Software, Data curation, Writing – review & editing, Supervision, Project administration. **Yenifer Gamarra:** Validation. **Elena Planells:** Conceptualization, Software, Resources, Writing – original draft, Supervision, Project administration, Funding acquisition. All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest

The authors declare no conflicts of interest.

Acknowledgments

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Trial registration

The study was registered at the US National Institutes of Health (ClinicalTrials.gov) NCT03672513.

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