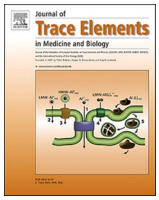
Journal of Trace Elements in Medicine and Biology 85 (2024) 127473

Contents lists available at ScienceDirect

Journal of Trace Elements in Medicine and Biology

journal homepage: www.elsevier.com/locate/jtemb



Zinc transporters expression profile in professional handball players supplemented with zinc

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ARTICLE INFO

Keywords:
Zinc Status
Zinc transporters
Team sports
Dietary Supplements
Athletes
Trace elements

ABSTRACT

Introduction: Zinc (Zn) deficiency has been described not only on general human health but also within the sports context –as negatively affecting performance–. Thus, Zn status assessment is of great interest for athletes, especially in order to correct deficiency states of this mineral.
Objective: The overall objective of this work was to assess Zn status in professional handball players during the competitive period (through plasma levels, dietary intake and gene expression of the Zn transporters), as well as to determine the effect of Zn supplementation.
Methods: A total of twenty-two participants were recruited, –twelve belonged to the Control Group (CG) and ten male handball players comprised the experimental group (ATH-G)–, being monitored over a 2-month period with 2 evaluation moments: baseline (i.e., initial conditions) and follow-up (i.e., after 8 weeks of training and competition). Zn intake, plasma Zn levels, and gene expression of Zn transporters were obtained.
Results: Plasma Zn levels were higher in ATH-G than in CG at the end of Zn intervention ($p \leq 0.010$). Moreover, differences in the gene expression profile of Zn transporters were observed in ATH-G –with the down-regulation of several Zn transporters–, compared to the CG at baseline ($p \leq 0.05$). Likewise, differences in the Zn transporters expression were observed in ATH-G at 8 weeks (all, $p \leq 0.001$) –with ZnT2, ZnT5, ZIP3, ZIP5, ZIP11, ZIP13 and ZIP14 transporters being up-regulated–.
Conclusion: Handball players seemed to have different nutritional needs for Zn, with differences in the gene expression of Zn transporters compared to controls. Zn intervention in our athletes may have influenced the expression of Zn transporters, indicating a potential increase in Zn transporters expression to mobilize Zn at the cellular level at 8 weeks of Zn intervention.

1. Introduction

Zinc (Zn) stands as the second most abundant nutritionally essential trace element in the human body, existing predominantly as a divalent cation (Zn^{2+}) [1,2], and presenting a key role in biological functions (e.g., metabolic, immune and oxidative processes, among others) [3,4]. Since Zn deficiency has been described as having negative consequences on human health [5], as well as its potential impact on athletic performance [6,7], the assessment of Zn status holds significant relevance for athletes.

Although existing scientific literature has linked Zn status to several factors including (I) anabolic adaptations of relevant hormones such as testosterone [8], (II) antioxidant response to combat exercise-induced oxidative stress [9], (III) muscle growth, (IV) tissue regeneration and synthesis, and (V) energy production and proper functioning of the immune system [10,11], the real impact of Zn homeostasis and its mobilization to other tissues during exercise remains unclear and requires further investigation [12]. In this regard, a decrease in serum Zn levels has been reported after aerobic exercise regardless of participant Zn status, type of exercise or blood collection time [12]; whereas

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https://doi.org/10.1016/j.jtemb.2024.127473
Received 29 February 2024; Received in revised form 8 May 2024; Accepted 13 May 2024
Available online 16 May 2024

moderate to vigorous exercise has been shown to promote an increase in plasma Zn levels immediately after exercise [13,14].

An unbalanced diet could be the main reason for the Zn deficiencies frequently found in athletes [15]. In situations of high metabolic demand as exercise or athletic training, inadequate circulating Zn levels may impair optimal physiological performance [16], making it might be advisable to use of sports supplements to maintain adequate enzyme activity and preserve athletic performance, especially in high-performance athletes [17]. Regarding Zn supplementation, prior research has shown that Zn supplementation reduced blood viscosity [6], being inconsistent with the increase in VO_{2peak} in active young adults [18]. Similarly, Zn supplementation could have indirectly contributed to the improvement of sports performance by positively modifying blood lipid levels [19]. Therefore, given that Zn requirements in athletes may be different and given the variability of exercise-induced responses, there is a need not only for Zn assessment but also the activity of proteins related with Zn to establish and clarify relationships with sports practice.

For Zn homeostasis, there are 24 Zn transporters, divided between 2 families: 10 ZnTs and 14 ZIPs [20,21]. ZnTs transporters reduce the cytoplasmic concentration of Zn by introducing Zn into the organelle lumens and expelling it into the extracellular space. In contrast, ZIP transporters have a reverse pathway, increasing the cytoplasmic Zn concentration by introducing Zn from the extracellular space or extracting it from the organelle lumens [21]. To the best of our knowledge, few studies have evaluated Zn transporters in athletes, noting existing studies in animal models, some of them with a previous pathophysiological condition. Specifically, the ZnT8 transporter, –whose main function is to maintain Zn homeostasis in beta cells–, reduced serum and pancreatic Zn levels and pancreatic ZnT8 expression, explaining in part the beneficial effects of training [22]. Moreover, impaired cytotoxic cell function in diabetes is partially corrected after chronic exercise, reversing the suppression of ZIP10 levels [23]. Also, ZIP7 modulates Zn efflux playing an important role in metabolic processes related to glycemic control in skeletal muscle and modulating other genes involved in carbohydrate metabolism and glycogen synthesis, being of interest for exercise [24]. Hence, analyzing the gene expression profile of Zn transporters could be highly beneficial in providing further insights into the cellular mobilization of Zn during exercise and after dietary interventions.

Based on the above, the scientific literature on the topic has been mainly conducted in animal models and hence, further research on Zn status and Zn transporters in athletes from different disciplines is needed. Moreover, most studies assessing Zn status have focused on predominantly aerobic or high-intensity disciplines, while intermittent activity disciplines such as team sports have not yet been analyzed. Therefore, the main objective of this study was to assess the plasma Zn status of Zn-supplemented professional handball players during the competitive period, as well as to determine the gene expression of the Zn transporter families involved in Zn mobilization in the organism.

2. Methods

2.1. Study design and participants

The present study adopts a prospective, longitudinal, case-control pre-experimental design evaluating a total of 22 subjects over a 2-month period (8-weeks) from February to April 2018, encompassing competitive period. Among these participants, twelve male belonged to the Control Group (CG), with an average of 20.9 ± 2.8 years, height of 1.8 ± 5.9 m, weight of 78.2 ± 7.5 kg, fat percentage of 17.2 ± 4.7 %. The experimental group (ATH-G) consisted of ten male handball players from the Puente Genil Handball Club (Córdoba, Spain), with an average of 22.9 ± 2.7 years, height of 1.9 ± 0.1 m, weight of 86.7 ± 5.4 kg, fat percentage of 10.7 ± 3.4 %. Throughout the 2-month supplementation period with 5 mg/day of Zn oxide (equivalent to 50 % of the Dietary Reference Intakes (DRI) for this nutrient) (Multicentrum, Pfizer, Barcelona, Spain), two evaluation moments were established for ATH-G: baseline (i.e., initial conditions) and follow-up (i.e., after 8 weeks of training and competition) took place. Adherence or compliance to the supplement intervention was assessed based on the percentage of all supplement capsules ingested over the study period.

During this period, athletes adhered to their usual training regime, comprising an average of 4–5 training sessions per week, culminating in a competitive match at the weekend. All athletes boasted extensive experience, with 8–12 years of involvement in the sport. All participants were actively engaged in the Spanish Professional Handball League. The study was conducted during the second competitive period of the handball season. The first competitive phase started with a total of 12 matches played, while the second phase started 2 weeks after the end of the first phase. During these 2 weeks, training sessions focused on integrated physical conditioning, spanning approximately 10 h per week.

The handball team's predetermined weekly training regimen comprised the following schedule: Monday: strength training in the gym, alongside individual technique development through modified game scenarios emphasizing equality, inferiority, and numerical superiority (1 × 1, 2 × 2); Tuesday: rest or gym-based strength work; Wednesday: integrated strength exercises on the court, focusing on collective tactics in both attack and positional defense (3 × 3, 4 × 4, 5 × 5, and 6 × 6), as well as drills in counter-attacking and defensive balance scenarios; Thursday: offensive and defensive technical-tactical training, with particular emphasis on simulating game situations and refining tactical strategies in preparation for upcoming competitions; Friday: activation training coupled with technical-tactical drills, tailored to prepare for Saturday's match while minimizing training intensity; Saturday: match; Sunday: rest and recovery. The CG reported to not to perform at least 150 min/week of moderate intensity or 75 min/week of vigorous intensity aerobic physical activity.

The inclusion criteria for ATH-G were: (I) to pass a recruitment medical evaluation consisting of a clinical examination, (II) to not to present injuries during and at least 6 months before the study, (III) non-smoker status, and (IV) the absence of medications use. The exclusion criteria were to consume nutritional supplements of any kind in the 6 weeks before and during the study period, and to present any pathological condition that could affect their nutritional status. The present study was approved by the Ethics Committee of the University of Granada (Granada, Spain), and was conducted in accordance with the last revised guidelines of the Declaration of Helsinki [25]. All players filled out an informed consent form prior the study, containing detailed information about the purpose of the study, the risks and the procedures involved throughout the study period.

2.2. Body composition and nutritional parameters

Body composition was assessed using bioelectrical impedance (Tanita MC-980MA Multi-frequency Segmental Body Composition Analyzer, Barcelona, Spain) in accordance with the manufacturer's procedures. Measurements included weight, body mass index (BMI) and body fat percentage. Nutritional intake assessment was conducted quantitatively by means of a 24-h recall by a qualified dietitian. Data from food intakes were obtained during individual interviews to request information from each participant about the types of foods and serving sizes. Recall accuracy was recorded with a set of photographs of prepared foods and dishes that are commonly consumed in Spain. The validated Nutriber® software package was used to estimate both qualitative and quantitative nutritional intake. Energy intake was represented both in absolute values and as an energy per weight ratio, while macronutrient intake was expressed as a percentage of nutrient per total energy ingested. Zn intake was calculated as nutrient density (i.e., the mass of micronutrient per 1000 kcal), with adherence to the dietary reference intakes (DRIs) set by the European Union were used [26]. Participants were instructed to maintain their usual dietary habits

throughout the study duration.

All participants samples were collected on Monday mornings between 8:00 and 10:00 under fasting conditions, following a minimum of 12-h abstention from physical exercise. Whole blood was drawn from the antecubital vein and plasma was separated by centrifugation at 4 °C for 15 min at 3000 × *g*. Samples were frozen until further analysis. Biochemical parameters, such as glucose (mg/dL), urea (mg/dL), creatinine (mg/dL), uric acid (mg/dL), total cholesterol (mg/dL), triglycerides (mg/dL), high density lipoprotein (HDL) (mg/dL), low density lipoprotein (LDL) (mg/dL), albumin (mg/dL), prealbumin (mg/dL) and hemoglobin (g/dL) and hematocrit (%) were determined in the analysis unit at Virgen de las Nieves Hospital from Granada (Spain), based on colorimetric and enzyme immunoassay procedures (ECLIA, Elecsys 2010 and Modular Analytics E170, Roche Diagnostics, Mannheim, Germany). All laboratory outcomes were obtained through standard techniques and adhered to established quality controls and procedures.

2.3. Plasma Zn determination

Before Zn determination, blood samples were mineralized by the wet method. For this purpose, plasma samples were placed in test tubes and 1 mL of nitric acid (65 %) was added in a sand bath. After eliminating nitrous vapors, 3 mL of 3 % nitric acid was added in a laminar flow hood. The resulting solution was transferred to plastic tubes for subsequent analysis. At the same time, a quality control was conducted using double distilled water to detect any potential contamination. Plasma samples were obtained in triplicate and Zn levels were measured using Inductively Coupled Plasma Mass Spectrometer (ICP MS, Nexion 300D, London, United Kingdom). Samples were collected at 8 weeks, calculating the mean difference and assuming a maximum margin of error of 5 % and a confidence limit of 95 %. The standard adult normal reference range for plasma Zn is 0.8–1.2 mg/L [27].

2.4. Determination of the Zn transporters' expression

Zn transporters' expression was determined using whole blood samples, initially collected in special test tubes (PAXgene blood RNA tube, Becton Dickinson, Germany) and stored at − 80 °C until further analysis. RNA was extracted and purified using the PAXgene™ Blood RNA Kit (Qiagen, Becton Dickinson, Germany) following the manufacturer's instructions. In order to determine RNA purity, 1 μL of RNA was measured on the NanoDrop® spectrophotometer (Thermo Scientific, London, United Kingdom) and Bioanalyzer 2100 (Agilent Technologies, London, United Kingdom) (absorbance 260–280 nm; RNA purity values > 1.9). The presence of organic contaminants (i.e., phenol or alcohol) was also considered (measured at 230 nm, with values < 1 being a possible cause of inhibition of the RT-PCR reaction). The RNA integrity number (RIN), indicative of 18S and 28S ribosomal RNA integrity ratio, was also analyzed (RIN = 7–10 was considered for good RNA integrity). Subsequently, 1 μg of total RNA was reverse transcribed to generate DNA by Thermo Script Ribonuclease H-Inverse Transcriptase (Invitrogen Life Technologies, Carlsbad, California), followed by dilution (1:10) with RNase-free water. Complementary DNA was subjected to Quantitative Polymerase Chain Reaction (qRT-PCR) analysis using SYBR Green PCR Master Mix in a 7900HT Fast Real-Time PCR System (Applied Biosystems; Foster City, California, United States of America). Primers for the Zn transporters were designed by Primer Design (Southampton, United Kingdom) (Suppl. Table 1). GAPDH, UBC and YWHAZ were used as housekeeping genes. These 3 genes were chosen using the geNorm software compared to 12 other reference genes by Primer Design (Southampton, United Kingdom). Relative gene expression was performed using the relative quantification method [28].

2.5. Data analysis

Statistical analysis was conducted using IBM SPSS version 25 for Windows (SPSS, Inc., Chicago, Illinois, United States of America). Means and standard deviation (SD) were calculated for quantitative variables. Normality of the variables was assessed using the Kolmogorov-Smirnov test. Student's t-test for independent and related samples were used to compare CG with ATH-G and to compare the evolution of athletes from week 0 to week 8. For Zn transporters gene expression analysis, delta crossing threshold (ΔCt) values were used, which were then transformed to a relative copy number (RCN) values to aid in data interpretation. RCN values for selected genes were determined through normalization of the expression of the three housekeeping genes: GAPDH, YWHAZ and UBC. The formula $RCN = E - \Delta Ct \times 100$ was employed, where E represents PCR efficiency, and ΔCt denotes Ct minus the reference Ct (the average of the 3 housekeeping genes). Zn transporters with $\Delta Ct \geq 13$ were considered as either not expressed or having expression levels too low to be detected, and thus were excluded from statistical analysis. The magnitude of Pearson's correlation was interpreted as follows: negligible (0.0–0.3), low (0.3–0.5), moderate (0.5–0.7), high (0.7–0.9), and very high (0.9–1.0) [29].

3. Results

Table 1 summarizes the descriptive, nutritional intake and biochemical characteristics of the study participants. Significantly higher energy and macronutrient intake, as well as absolute Zn intake, were observed in the ATH-G compared to the CG ($p \leq 0.001$). However, after adjusting for energy intake (mg Zn/1000 kilocalories), no statistically significant differences were observed for Zn intake. Regarding biochemical profile, all biochemical values fell within the normal range for both study groups. Nevertheless, lower levels of glucose, total cholesterol, LDL and albumin levels, and higher urea, creatinine, uric acid were observed in the ATH-G compared to the CG (all, $p \leq 0.011$).

Fig. 1 illustrates the concentrations of Zn in plasma in the CG and the ATH-G throughout the study period. The ATH-G exhibited higher concentrations of plasma Zn at 8 weeks ($p = 0.008$).

Fig. 2 presents the variation of Zn transporter gene expression, depicted in a heat map (Fig. 2**a**), the differences in Zn transporters expression between the CG and ATH-G at baseline (Fig. 2**b**), and the variation in Zn transporter expression in the ATH-G between week 0 and week 8, after the intervention with Zn (Fig. 2**c**). Altogether, a predominantly low or absent gene expression was observed for the transporters ZnT3, ZnT8, ZnT10, ZIP2 and ZIP12 (black, Fig. 2**a**). When comparing the expression of Zn transporters between groups under basal conditions (Fig. 2**b**), it was observed that the only up-regulated Zn transporter was ZIP2. Conversely, several transporters including ZnT1, ZnT3, ZnT4, ZnT7, ZnT8, ZIP3, ZIP4, ZIP5, ZIP8, ZIP9, ZIP10, ZIP11, ZIP13 and ZIP14 transporters were down-regulated in ATH-G compared to healthy controls at week 0 (all, $p \leq 0.001$). Finally, the gene expression of Zn transporters throughout the study period in athletes (from week 0 to week 8, Fig. 2**c**) revealed an up-regulation of ZnT2, ZnT5, ZIP3, ZIP5, ZIP11, ZIP13 and ZIP14 transporters (all, $p \leq 0.001$). No down-regulated Zn transporters were observed.

Fig. 3 depicts the correlation between daily Zn intake and gene expression associated with the ZnT and ZIP families in the study participants. Strong to moderate positive correlations were observed between Zn intake and the transporters ZnT7, ZIP3, ZIP4, ZIP5, ZIP8 and ZIP9 (all with r-values ranging from r = 0.45 to 0.83; $p \leq 0.047$). These associations were predicted in a range of 39.9–69.1 % of participants, except for ZIP9 which was predicted in 20 % of the sample.

Fig. 4 illustrates the correlations between Zn intake and plasma Zn levels with the expression of the ZnT4 in the total sample at baseline conditions (Fig. 4a) and after Zn intervention (Fig. 4b). Moderate correlations were observed between Zn intake and Zn in plasma with the expression of ZnT4 in total sample ($p \leq 0.018$). These associations were

Table 1
Nutritional intake and biochemical characteristics.

Characteristics	All (n = 22)		CG (n = 12)		ATH-G (n = 10)		*p Value*
	Mean	SD	Mean	SD	Mean	SD	
Energy (kcal/day)	2607	946	1811	365	3843	462	***0.001***
Carbohydrates (g/day)	310.9	104.4	225.5	57.4	404.7	43.3	***0.001***
Protein (g/day)	113.8	48.2	74.0	15.6	157.5	28.9	***0.001***
Fat (g/day)	103.8	44.2	71.3	28.2	139.5	27.6	***0.001***
Zn intake (mg/day)	11.1	3.65	8.52	1.01	15.1	2.18	***0.001***
Zn intake/1000 kcal of energy	4.7	1.16	4.91	1.37	4.33	0.68	*0.321*
Glucose (mg/dL)	82.5	23.1	93.5	25.3	69.4	10.4	***0.011***
Urea (mg/dL)	38.5	10.2	33.5	9.07	44.4	8.34	***0.009***
Creatinine (mg/dL)	0.97	0.28	0.78	0.17	1.21	0.19	***0.001***
Uric acid (mg/dL)	5.31	1.56	4.48	1.12	6.31	1.44	***0.003***
Total cholesterol (mg/dL)	191.5	43.7	217.0	34.4	160.8	33.0	***0.001***
Triglycerides (mg/dL)	117.5	60.2	101.6	38.9	136.7	76.7	*0.179*
HDL (mg/dL)	56.6	14.3	60.5	17.5	52.0	7.73	*0.171*
LDL (mg/dL)	111.6	34.8	131.1	28.5	88.2	26.7	***0.002***
Albumin (g/L)	4.74	0.28	4.59	0.22	4.91	0.26	***0.006***
Prealbumin (mg/dL)	28.1	7.43	25.4	9.78	30.0	4.96	*0.217*
Hemoglobin (g/dL)	14.1	3.46	14.2	1.74	13.9	4.92	*0.848*
Hematocrit (%)	40.6	9.78	41.2	4.41	39.9	14.1	*0.768*

Note: CG, Control group, ATH-G, Handball players. SD, standard deviation. HDL, High density lipoprotein. LDL, Low density lipoprotein. The quantitative variables data were expressed as the mean and standard deviation (SD). Independent t-test analysis was used to compare values for ATH-G and CG at baseline ($p < 0.05$ being considered statistically significant). References values: Glucose, 70–110 mg/dL; Urea, 10–50 mg/dL; Creatinine, 0.7–1.2 mg/dL; Uric acid, 3.4–7 mg/dL; Total Cholesterol, 110–200 mg/dL; Triglycerides, 50–200 mg/dL; HDL, 40–60 mg/dL; LDL, 70–150 mg/dL; Albumin, 3.5–5.2 mg/dL; Prealbumin, 20–40 mg/dL; Hemoglobin 13.8–17.2 g/dL; Hematocrit, 40.7–50.3 %.

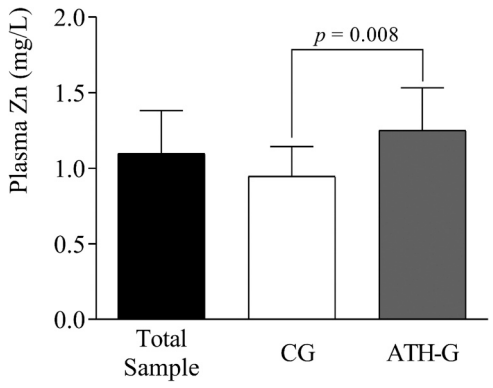

Fig. 1. Comparative analysis of plasma Zn concentrations throughout the study period. Note: CG, Control group, ATH-G. Handball players. Independent t-test analysis was used to compare values for ATH-G and CG 8 weeks after Zn intervention ($p < 0.05$ being considered statistically significant).

predicted in a range of 24.9–27.1 % of participants.

4. Discussion

The study aimed to evaluate the plasma Zn status of Zn-supplemented professional handball players during the competitive period, along with determining the gene expression of the Zn transporter families involved in Zn mobilization in the organism. Following supplementation, athletes exhibited higher plasma Zn levels compared to healthy controls. The expression profile of Zn transporters differed notably from non-athletes, suggesting distinctive Zn requirements. Additionally, Zn supplementation may have influenced Zn transporter expression, potentially enhancing cellular Zn mobilization.

In our investigation, despite variances in body composition, energy, macronutrient, and Zn intake, as well as certain biochemical parameters, which are typical when comparing athletes to healthy sedentary controls, both groups exhibited similar nutritional density for the mineral, meeting the estimated average requirement for a healthy population [30]. The primary determinant of these variances in biochemical parameters was consistent physical exercise and training. Previous studies have outlined these alterations, highlighting the impact of exercise on the reference ranges of biochemical and hematological parameters in elite athletes [31], suggesting the need for athlete-specific "pseudo" reference ranges. However, earlier research have also indicated altered Zn homeostasis in athletes, suggesting that they may have higher Zn requirements compared to sedentary individuals [32]. Given these findings, Zn supplementation has been proposed as a strategy to enhance athletic performance, owing to its catalytic, structural and signaling roles that contribute to the homeostasis of various cellular processes. In the context of exercise, these roles are particularly significant for tissues with high metabolic demands, such as skeletal muscle [1]. Despite athletes exhibiting higher total dietary Zn intake, they tend to have lower serum Zn concentrations, which could indicate increased Zn requirements due to stress of regular training and exercise [33]. This raises the concern that the observed Zn intake among our athletes might be insufficient to meet the sustained demands of their intensive exercise regimens.

During exercise, immediate changes occur in Zn concentrations, suggesting a connection between Zn metabolism and exercise related-functions [1]. Plasma Zn levels may rise due to muscle leakage caused by tissue and muscle soreness [34,35], returning to baseline values and normalizing within 24 h. Bordin et al. [13] suggested that this could result Zn redistribution between body compartments, namely blood and tissues, potentially leading to Zn deficiency if recurring over time. Additionally, in aerobic exercise, different authors have noted a reduction in serum Zn levels regardless of participants' status or the time of blood collection. Exercise may influence alterations in both serum and urine Zn levels [1], possibly attributable to muscle tissue repair processes [12]. Conversely, some studies have indicated an immediate increase in serum Zn concentration following aerobic exercise, suggesting acute changes in Zn homeostasis [14]. The scientific literature has been controversial in this regard, therefore, in our study, to mitigate the acute effect of exercise on plasma and/or cellular Zn status, blood samples were collected 12 h after exercise, aiming to assess the chronic effect of exercise on plasma Zn levels. Although we were unable to ascertain the prevalence of Zn deficiency at baseline, we observed higher plasma Zn levels in athletes at the end of the study. This phenomenon could be explained by the supplementation received during the study period. Specifically, upon analysing Zn status at this time point, we noted no cases of Zn deficiency among athletes at the plasma level, which leads us

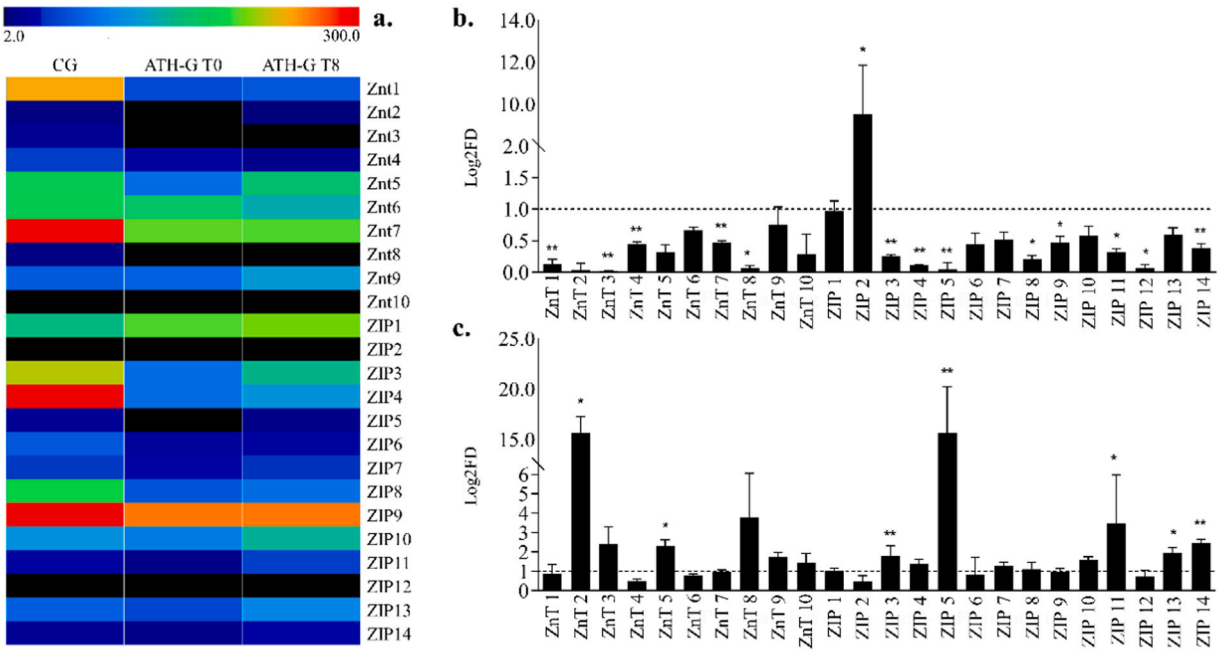

Fig. 2. Heat map corresponding to the gene expression of the different Zn transporter families (**a**). The categories correspond to red (high gene expression), green-blue (medium level of gene expression) and black (indeterminate to low gene expression). **b** shows the gene expression and its significance when comparing between groups at baseline conditions (Week 0). **c** shows the gene expression and significance when comparing the athletes throughout the study period (Week 0 vs. Week 8). * = p-value < 0.05; ** = p-value < 0.001.

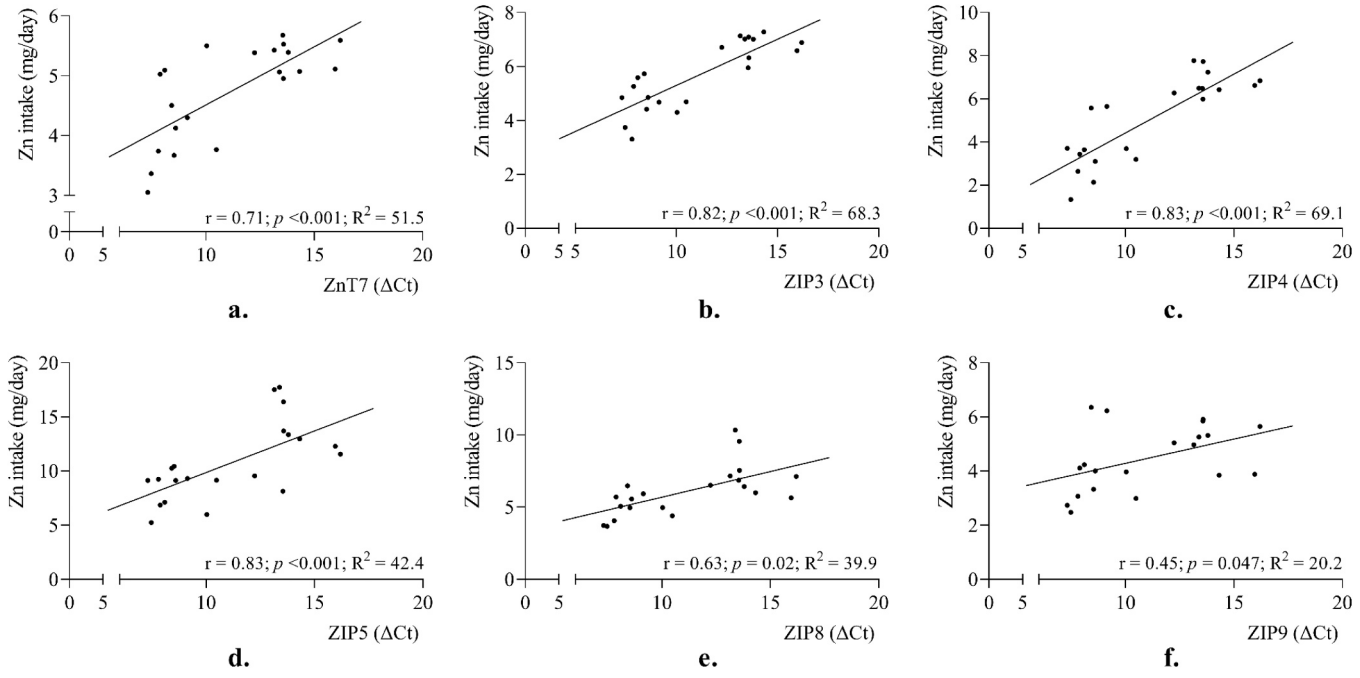

Fig. 3. Correlation matrix between Zn intake and the expression of the ZnT and ZIP families of Zn transporters in the total sample at baseline conditions.

to think that the Zn intervention may contributed normalizing plasma values.

Zn homeostasis is tightly controlled by a family of Zn transporters responsible for facilitating the movement of Zn into and out of cells. In recent years, there has been growing interest in the potential role of Zn and Zn transporters in athletes [23,36]. Our study revealed that among the Zn transporters examined, only ZIP2 exhibited up-regulation. In contrast, ZnT1, ZnT3, ZnT4, ZnT7, ZnT8, ZIP3, ZIP4, ZIP5, ZIP8, ZIP9, ZIP10, ZIP11, ZIP13, and ZIP14 were down-regulated in ATH-G compared to healthy controls at baseline. Although research on ZIP2 and exercise is limited, some evidence suggests that exercise could influence ZIP2 expression and Zn homeostasis, impacting immunity [37], though this effect may be more prominent under certain pathophysiological conditions. Studies suggest that ZIP2 up-regulation during reperfusion confers cardioprotective effects and may serve as an intrinsic protective mechanism against ischemia-reperfusion injury in animals [38]. Our study demonstrated a significant down-regulation of Zn transporter expression in athletes. The specific Zn transporters

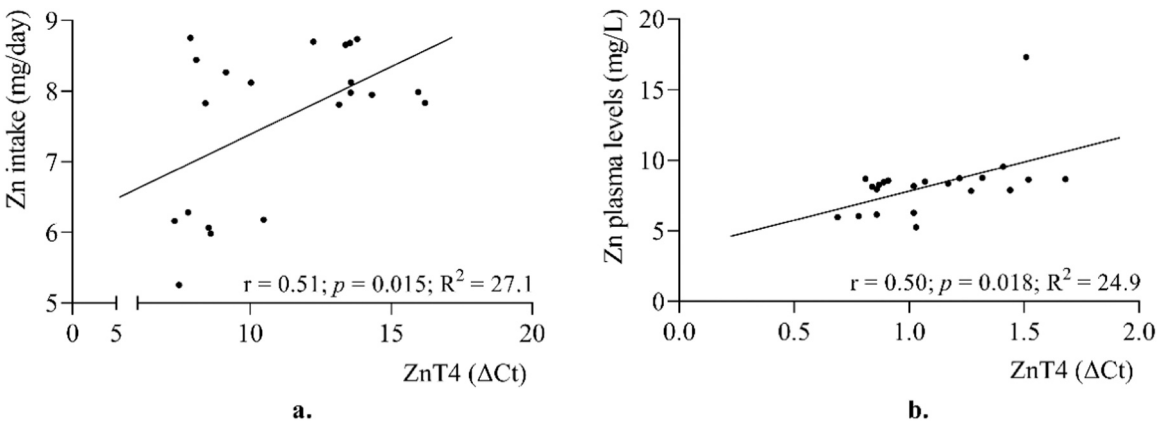

Fig. 4. Correlation matrix between Zn intake and plasma Zn levels with the expression of the ZnT4 in the total sample at baseline conditions (Fig. 4a) and after Zn intervention (Fig. 4b).

examined may play a crucial role in the metabolic functions necessary to meet the demands of exercise. For example, ZnT3 decreased levels in diabetic rats may be an indicator of hippocampal tissue damage after 4 weeks of chronic exercise [36]. Also, transporters such as ZnT7 and ZnT8 are involved in regulating insulin secretion, suggesting a potential link between Zn and insulin signaling pathways in skeletal muscle [39]. Additionally, ZIP7, ZnT8 and ZIP14 transporters are implicated in muscle proliferation and differentiation processes following exercise-induced muscle damage and recovery [40]. ZIP14, in particular, has been shown to promote muscle wasting through loss of myosin heavy chain and impaired regeneration [41], with alterations in expression during inflammatory responses and decreased serum Zn levels [42]. Moreover, the ZIP13 transporter plays a key role in the regulation of adipocyte biogenesis and energy released from fatty acids [20]. The observed differences in the expression of specific Zn transporters between our study groups may reflect acute and chronic exercise adaptations in handball players. These adaptations could be linked to specific metabolic pathways, which may vary due to the intermittent nature of their sport, or to specific tissues impacted by the physical of their athletic activities.

After Zn supplementation in athletes, we observed an up-regulation of Zn transporters, including ZnT2, ZnT5, ZIP3, ZIP5, ZIP11, ZIP13 and ZIP14, compared their baseline expression levels. This up-regulation potentially could enhance the utilization and mobilization of Zn for enzymatic reactions, cellular responses to physiological signals, and the maintenance of cellular homeostasis during exercise [43]. Furthermore, we found moderate to high positive correlations between Zn intake and the expression of specific Zn transporters, such as ZnT7, ZIP3, ZIP4, ZIP5, ZIP8 and ZIP9. These correlations suggest that higher baseline Zn intake might modulate the expression of these transporters, aligning with prior evidence that indicates increased Zn intake can reduce the expression of transporters like ZnT1, ZnT5, ZIP4, and ZIP8 [44,45]. The observed association between the ZnT4 transporter with both Zn intake and plasma Zn levels in athletes underscores the critical role of Zn in muscle function, where specific signaling pathways depend on adequate Zn [46]. Although zinc-deficient states were not identified at baseline in our athletes, interpreting the observed associations interpreting the observed associations requires caution. This is due to potential variations in gene expression, protein concentration, and degradation rates, which can occur in the absence of sufficient Zn as a cofactor [46]. These variations could influence the stability and functionality of zinc transporters, thereby affecting zinc homeostasis and its downstream physiological effects.

In our study, several associations between Zn transporters were observed (Suppl. Table 2), which could be due to the synergistic activity that these transporters play in controlling Zn influx and efflux. Notably, the up-regulated Zn transporters belonged to the ZIP family, responsible for increasing cytosolic Zn concentrations and thereby making it available for Zn cellular pathways [1,21]. Additionally, the correlations between zinc intake and certain zinc transporters support a previously established relationship in other studies. These studies showed that eight zinc transporters in peripheral blood mononuclear cells from healthy individuals with adequate zinc intake exhibited significant variations in their relative expression, with ZnT1, ZnT7, and ZIP1 being the most abundantly expressed [47]. Moreover, exercise might impact the expression Zn transporters and Zn homeostasis, although the underlying mechanisms remain incompletely understood. It is plausible that exercise-induced alterations in Zn transporters expression and utilization may play a role in athletic performance and recovery. Earlier studies have underscored the complexity of zinc transporter interactions in primary human tissue, even though associations between certain Zn transporters suggest a coordinated balance of zinc influx and efflux [47]. However, further research is needed to gain a comprehensive understanding of these effects and their implications for athletes and other active populations.

The present study has a number of limitations including: (I) there is little scientific evidence evaluating Zn transporters in athletes, since most of them focus on animal models; (II) the small number of participants, –given the difficulties of recruiting and intervening professional athletes–, limited the power of the observed relationships between Zn transporters and sports performance outcomes; (III) the focus on a single discipline of team sports and in male athletes in turn makes it difficult to extrapolate our results to other sports disciplines or female athletes; (IV) the utilization of healthy individuals as a control group at baseline, may potentially complicate the interpretation of the results and thus warrants cautious consideration; (V) the lack of plasma Zn levels at baseline, did not allow to assess the initial Zn status of the study participants and to determine the impact of Zn supplementation in each study group. As a strength, the present study is the first to evaluate the plasma and cellular Zn status in professional handball players, determining the activity of the two families of Zn transporters after Zn supplementation for 8 weeks, conducted during the competitive period, with the difficulty of access to professional athletes that this implies.

5. Conclusion

To sum up, plasma Zn levels were higher in athletes than in healthy controls at the end of Zn intervention, remaining within normal values. Our most important finding revealed differences in the expression profile of Zn transporters in athletes compared to our control subjects, suggesting distinct Zn requirements in handball players compared to non-athletes. Additionally, Zn supplementation in our athletes may have influenced the expression of Zn transporters, indicating a potential increase in Zn transporters expression to mobilize Zn at the cellular level

after 8 weeks. Further scientific evidence on the topic, larger samples and interventions over extended periods, and varying Zn intake, as well as research across different sports disciplines, are needed to clarify the cellular behavior of Zn in the organism and the actual requirements for this nutrient in athletes.

CRediT authorship contribution statement

Lourdes Herrera-Quintana: Writing – review & editing, Writing – original draft, Resources, Investigation, Formal analysis. **Yenifer Gamarra-Morales:** Writing – review & editing, Writing – original draft, Resources, Investigation, Formal analysis. **Christer Hogstrand:** Writing – review & editing, Writing – original draft, Software, Resources, Methodology, Investigation, Formal analysis. **Héctor Vázquez-Lorente:** Writing – review & editing, Writing – original draft, Resources, Investigation, Formal analysis. **Elena Planells:** Writing – review & editing, Writing – original draft, Supervision, Software, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Jorge Molina-López:** Writing – review & editing, Writing – original draft, Supervision, Software, Resources, Methodology, Investigation, Formal analysis, Conceptualization. **Diana Florea:** Writing – review & editing, Writing – original draft, Software, Resources, Methodology, Investigation, Formal analysis.

Ethics approval

This study abides by the Declaration of Helsinki on research involving human subjects and was approved by the Ethics committee of the University of Granada, Spain, for studies involving human subjects.

Consent to participate

Written informed consent was obtained from all individual participants after explaining the goals of the study.

Consent to publish

Consent to publish has been received from all participants of the study.

Authors contributions

Conceptualization, J. M.-L. and E. P.; Methodology, J. M-L., D. F., C. H. and E. P.; Software, J. M-L., D. F., C. H. and E. P.; Formal analysis, J. M-L., D. F., Y. G.-M., L. H.-Q., H. V.-L., C. H. and E. P.; Investigation, J. M-L., D. F., Y. G.-M., L. H.-Q., H. V.-L., C. H. and E. P.; Resources, J. M-L., D. F., Y. G.-M., L. H.-Q., H. V.-L., C. H. and E. P.; Writing – original draft, J. M-L., D. F., Y. G.-M., L. H.-Q., H. V.-L., C. H. and E. P.; Writing – review & editing, J. M-L., D. F., Y. G.-M., L. H.-Q., H. V.-L., C. H. and E. P.; Supervision, J. M-L and E. P.; Project administration, E. P.; Funding acquisition, E. P. All authors approved the final draft of the manuscript for publication. The authors declare that all data were generated in-house and that no paper mill was used.

Funding

This work was supported by the Spanish Ministry of Education, Culture and Sports [AP2009/3071] for J.M.-L. L.H.-Q. [REF. FPU18/03702] and H.V.-L. [REF. FPU18/03655] are under a FPU fellowship from the Spanish Ministry of Education.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be shared upon reasonable request to corresponding author: Héctor Vázquez-Lorente.

Acknowledgements

We thank COST Action TD1304 e-Network for the Biology of Zinc (Zinc-Net) for favoring our collaboration with experts on zinc biomarkers. This article is based upon work from COST Action TD1304 supported by COST (European Cooperation in Science and Technology). Funding for open access charge: Universidad de Granada /CBUA.

Conflict of interests

All authors declare that there are no conflicts of interest associated with this manuscript.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jtemb.2024.127473.

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