Association between erythrocyte concentrations of magnesium and zinc in high-performance handball players after dietary magnesium supplementation

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Abstract. Currently, research on athletes focuses on optimizing their nutritional status in order to adjust their minerals requirements. This study was designed to evaluate baseline nutritional status and the effect of a nutritional intervention based on magnesium (Mg) supplementation, on plasma and erythrocyte concentrations of Mg and zinc (Zn), and their relationship with training load. We analyzed training load by recording the training volume, intensity and rating of perceived exertion (RPE) during a four-month period, in 14 high-performance handball players. Intensity was studied in different levels of residual heart rate (RHR). We analyzed nutrient intake and plasma and erythrocyte concentrations of Mg and Zn by FAAS. All biomarkers were measured at baseline, after two months of dietary supplementation with Mg, and after two months without supplementation. RPE was associated with training volume at different intensities of RHR. Mg supplementation significantly increased plasma Mg levels during the supplemented period and preserved for subsequent changes in the non-supplemented period. Erythrocyte concentrations of Mg and Zn show associations between baseline and Mg supplementation. Mg levels were associated with training volume at different intensities after supplementation. In conclusion, our findings in high-performance handball players show that during competition, there is a relationship between erythrocyte Zn and Mg levels, regardless of Mg supplementation or Zn intake. Mg dietary supplementation tended to preserve changes in mineral levels during training and competition.

Key words: nutritional status, handball player, magnesium, zinc, erythrocyte

Considerable research has focused on the search for strategies to optimize nutritional status in athletes. One current trend is to use nutritional supplements for this purpose [1]. Several minerals have been studied because of their implication in sports performance and the potential benefits of supplementation. In this connection, other authors have reported [2] that athlete nutrition...
education programs usually aim to rectify dietary inadequacies and promote optimal health and athletic performance.

Magnesium (Mg) is required for a variety of fundamental cellular activities involved in different physiological systems [3, 4]. Research has shown that Mg insufficiency diminishes physical performance, and that Mg status can affect exercise capacity [4-6]. Some studies have found that extreme exercise reduces Mg concentrations and the number of erythrocytes or monocytes, and that the decrease persists after the training session had ended [7, 8]. Several authors have reported that brief, intense exercise is associated with hypermagnesemia, whereas moderate, prolonged exercise appears to be associated with hypomagnesemia [7, 9-12]. Erythrocyte concentrations of Mg may be low as a result of inadequate dietary intake or an imbalance in Mg metabolism [13].

Zinc (Zn) has considerable effects on metabolism during exercise [14-16], and plays a fundamental role in cell division, growth and maturation [3]. Moreover, Zn is needed to ensure the function of many enzymes that can affect physical performance [17]. Zinc deficiency may be involved in the development of insulin resistance and increased susceptibility to oxidative damage [18-20]. Recent studies have tried to elucidate the relationship between Zn and other elements and exercise, by focusing mainly on how this element is distributed in the body in response to exercise [3, 14].

The findings of different studies to date have been unclear regarding the effects of Mg supplementation on physical performance [21]. Research that investigated the changes in plasma Zn after exercise has likewise yielded contradictory results [22]. In light of these uncertainties, we attempted a new approach to studying the behaviour of these minerals. To date, there appear to be no studies that recorded changes in the status of these nutrients during prolonged periods of intense competition in terms of specific variables such as volume, intensity and rating of perceived exertion (RPE). In this respect, it should be borne in mind that the actual sports season for professional athletes involves a succession of stimuli and responses triggered by repeated sessions of training and competition. To our knowledge, the present study is the first to evaluate nutritional status and the effect of Mg supplementation on plasma and erythrocyte concentrations of Mg and Zn in a group of high-performance athletes, taking into account the training load parameters mentioned above. The present study was thus designed to evaluate nutritional status at baseline and the effect of dietary Mg supplementation, on plasma and erythrocyte concentrations of Mg and Zn in a group of high-performance handball players, and to determine the relationships between these variables and training load.

Subjects and methods

Participants

The study was performed during the sports season (10/2009 to 02/2010), and all participants were members of a handball team (n = 14) sponsored by the Club Deportivo Puente Genil de Balonmano (Granada, Spain), in the Honor B Division of the Spanish professional handball league. The sample comprised 14 men (mean age 22.9 ± 2.7 years) who trained for an average of four days per week in addition to competing in matches at weekends.

Participation in the study was voluntary. All players provided their informed consent in writing, and were given detailed information at the beginning and end of the study regarding the aims and procedures involved. The study was approved by the Research Ethics Committee of the University of Granada.

Dietary intake

To evaluate dietary intakes we used a procedure consistent with a 72-h recall system over three consecutive days (two working days and one nonworking day). Three time points were used during a four-month period: baseline, followed by two months of dietary supplementation followed by two months without supplementation. Food intakes were recorded with the help of a manual containing photographs of standard amounts of different foods and prepared dishes. The participants were asked to identify the foods consumed and describe the size of the portions as accurately as possible.

Food intakes were analyzed with Nutriber® software [23] to convert them into data for absolute nutrient intakes and percentage values of appropriate intakes according to individual needs. Total energy expenditure was calculated as basal metabolism for each participant multiplied by an
activity factor of 1.7. Macronutrient intakes (carbohydrates, proteins and fats), as well as Mg and Zn intakes were compared to reference intakes [4, 24, 25]. Percentage macronutrient intakes in relation to total energy intake were compared with recommended dietary allowances (RDA) [26].

Supplementation

The dietary intervention consisted of daily supplementation with 100 mg Mg as MgO for two months starting immediately after baseline measurements.

Training program

To record training parameters, we used three variables that defined training load: volume, intensity and RPE. All participants trained for a mean of four-to-five days per week, in addition to participating in competition matches at weekends.

Training volume was recorded during a four-month period covering the professional handball competition season, and divided into four, one-month mesocycles. In each training session, we recorded the number of minutes spent on each type of exercise during warm-up and actual training in order to evaluate training load in each weekly microcycle and each of the four-monthly mesocycles.

Training intensity was recorded with Polar S610 and Polar Team pulse meters (Polar Electro Ibérica, Barcelona, Spain) once per training week. Finally, 22 records of training sessions, 11 for each training period, were obtained. To calculate maximum heart rate (HR max), we used the “course navette” test of maximum aerobic power. We also recorded baseline heart rate over one week to obtain an accurate mean value. Heart rate reserve or residual heart rate (RHR) was calculated as HR max minus basal heart rate to establish the level of intensity and the time each athlete spent at each level. We assessed the volume training at three ranges of intensity: < 60%, between 60% and 80%, and > 80% RHR [27].

The RPE was used to determine whether the amount of exertion was consistent with actual intensity of exertion [28], defined in this study as overall RPE as determined by each participant at the end of each training session once per training week, (finally 22 recorded training sessions, 11 for each training period). We calculated RPE as the mean ± standard deviation to evaluate perceived load in each microcycle (week of training), and mesocycle (month of training).

Nutritional education intervention

The educational intervention was designed ad hoc by a team of nutrition specialists for this type of study population. The intervention consisted of three phases. First, the nutrition team explained aspects related to nutrition in general, with emphasis on the different types of nutrients and their importance for maintaining good health in basically healthy persons. This was followed by education focusing more specifically on nutrition and physical activity. In this second phase, the emphasis was on specific nutritional requirements in persons who perform continuous physical activity, and on the frequent errors in nutrition in this population. In the third phase, team members responded to the questions participants raised at any time throughout the study period, in order to provide additional information and clarification.

Biochemical analyses

To separate blood cells and plasma, blood samples were centrifuged in Venoject® tubes for 15 min at 3,000 rpm. The blood cell samples were washed three times in saline solution, divided into aliquots and stored at -80°C until analysis.

Samples were mineralized by wet acid digestion using the method of Palacios et al. [29], and Merck GR for analysis grade reagents (Merck, Darmstadt, Germany). Concentrated erythrocyte or plasma samples (0.4-0.4 mL) were placed in a beaker, covered with an inverted watch glass and placed in a preheated sand bath, after which NO3H/ClO4H (1:1) was added. When the solution had cooled, 5 N HCl was added.

Plasma concentrations of Zn and Mg were determined in all samples by FAAS (Perkin Elmer® Analyst300, Norwalk, CT, USA). The background correction used was based on the D2 method, which required a single-element, hollow cathode lamp for the element-specific absorption and a deuterium lamp for the background absorption. The deuterium arc background correction provided simultaneous correction for molecular absorption and light scattering. The source of energy for free atom production was heat in
the form of an air-acetylene flame (Air Liquide, Granada, Spain). The mixture was ignited in a flame ranging in temperature from 2,100 to 2,800°C. Plasma and erythrocyte levels of Mg and Zn were determined in samples that were diluted in water (Milli Q quality) before the analysis.

The calibration line was calculated from a stock solution of Mg or Zn at 1,000 mg/L ($r^2 = 0.9995$). For quality control we used human serum Ca, Mg, Li BCR304-BCR® Certified Reference Material for Mg (Fluka, Madrid, Spain), and human serum Al, Se, Zn BCR63-BCR® Certified Reference Material, for Zn (Fluka, Madrid, Spain). The value obtained for Mg was 12.22 ± 0.05 mg/L (certified value 12.25-14.32 mg/L), and the value for Zn was 1.12 ± 0.17 μg/L (certified value, 1.18-1.42 μg/L). All samples were analyzed in triplicate.

Mg in plasma and erythrocyte samples was analyzed at a wavelength of 285.2 nm (slit 0.7 nm) with a flow rate (air/C$_2$H$_2$) of 10/1.9 L/min. We used a six-point calibration curve for Mg in the concentration range of 0-0.3 mg/L established by Perkin Elmer for quantification, and obtained $r^2 = 0.9998$. Plasma and erythrocyte levels of Zn were analyzed at a wavelength of 213.9 nm (slit 0.7 nm), using a flow rate (air/C$_2$H$_2$) of 10/1.9 L/min. We used a six-point calibration curve for Zn in a concentration range of 0-1 μg/L established by Perkin Elmer for quantification, and obtained $r^2 = 0.9997$.

Statistical analyses

The data are reported with descriptive statistics. For numerical variables, we used the arithmetic mean, standard deviation and standard error of the mean. For categorical variables, we used percentage frequencies. To determine whether the data fitted a parametric model, the Kolmogorov-Smirnov test was used to verify the normal distribution. For comparisons, we used single-factor analysis of variance. Linear regression analysis was used to identify correlations by calculating Pearson’s bivariate correlation coefficient. All statistical analyses were performed with SPSS v. 16.0 for Windows.

Results

Table 1 summarizes the characteristics of the participants. There were no statistically significant differences in any of the parameters between any of the pairs of time points.

Training characteristics

Table 2 shows the results for volume, RPE and the levels of intensity based on RHR for each of the four-monthly mesocycles. Training volume in mesocycles 2 and 3, with dietary Mg supplementation differed significantly from volume in mesocycles 1 and 4 without dietary Mg supplementation ($p < 0.05$). The mean overall RPE during mesocycles 1 and 2 during the intervention (dietary supplementation period) was significantly lower than in mesocycles 3 and 4 ($p < 0.05$), when dietary supplementation was not used. There were no significant differences between medium training volumes with or without dietary Mg supplementation.

During mesocycles 1 and 2, 30.35% of the training volume was in the 60%-80% range of

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Subjects characteristics</th>
<th>Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td></td>
<td>22.9 (2.7)</td>
</tr>
<tr>
<td>Height (m)</td>
<td></td>
<td>1.87 (0.06)</td>
</tr>
<tr>
<td></td>
<td>Bp</td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td></td>
<td>86.72 (5.36)</td>
</tr>
<tr>
<td>Body mass index (kg/m$^2$)</td>
<td></td>
<td>24.72 (1.12)</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td></td>
<td>11.58 (2.53)</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>86.47 (5.59)</td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td>86.38 (4.81)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>24.61 (1.30)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>24.62 (1.14)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11.60 (2.45)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11.57 (2.34)</td>
</tr>
</tbody>
</table>

Results are shown as mean and standard deviation (SD)

Bp: baseline point; S: after training with dietary supplementation; and NS: after training without dietary supplementation.

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Table 2. Volume, intensity and rating of perceived exertion in each mesocycle of the dietary intervention and non-intervention period.

<table>
<thead>
<tr>
<th>Volume (min)</th>
<th>With dietary Mg supplementation</th>
<th>Without dietary Mg supplementation</th>
<th>Mean ± SD</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mesocycle 1</td>
<td>1,215</td>
<td>1,347.5</td>
<td>1,155</td>
<td>1,308.5</td>
</tr>
<tr>
<td>Mesocycle 2</td>
<td>1,480</td>
<td>1,462</td>
<td>1,155</td>
<td>1,308.5</td>
</tr>
<tr>
<td>Overall rating of perceived exertion</td>
<td>15.27 (1.26)</td>
<td>16.58 (1.12)</td>
<td>16.57 (1.13)</td>
<td>16.71 (1.03)</td>
</tr>
<tr>
<td>Residual heart rate &lt;60%</td>
<td>55.60 (7.38)</td>
<td>46.32 (7.32)</td>
<td>50.67 (10.1)</td>
<td>48.30 (8.51)</td>
</tr>
<tr>
<td>Residual heart rate 60%-80%</td>
<td>29.70 (2.96)</td>
<td>36.94 (2.76)</td>
<td>34.60 (4.92)</td>
<td>35.87 (3.87)</td>
</tr>
<tr>
<td>Residual heart rate &gt;80%</td>
<td>14.68 (4.52)</td>
<td>16.74 (6.90)</td>
<td>14.74 (5.26)</td>
<td>15.83 (6.00)</td>
</tr>
</tbody>
</table>

Results are shown as mean and standard deviation (SD)

a Statistically significant difference (p<0.05) mean of mesocycles 1 versus mesocycles 2, mesocycles 3 and versus mesocycles 4.
b Statistically significant difference (p<0.05) mean of mesocycles 2 versus mesocycle 3 versus mesocycles 4.
c Statistically significant difference (p<0.05) mean of mesocycles 1 and 2 versus mean of mesocycles 3 and 4.

RHR, whereas during mesocycles 3 and 4 this percentage increased to 35.87% (p<0.05). In addition, our results show significant differences (p<0.05) between mesocycle 3 and mesocycles 2 and 1. Bivariate analysis with Pearson’s correlation coefficient revealed a statistically significant correlation between overall RPE and the proportion of training volume in the intensity range of 60%-80% RHR (p<0.01, r = 0.64), and between the former and the intensity range of >80% of RHR (p<0.01, r = 0.76).

Evaluation of nutrient intakes

Our analysis of energy and nutrient intakes was based on current RDA [24, 25]. We found statistically significant differences (p<0.05) in energy intake between baseline levels and both with and without dietary Mg supplementation, with a significant increase in energy intake following the nutritional education program (table 3).

The percentage protein intake referred to the RDA was 129.25 ± 18.75% at baseline, and increased to 147.04 ± 25.51% during the period without dietary Mg supplementation. For carbohydrates, the percentage of the RDA covered at baseline was 69.76 ± 6.92% at baseline, with the highest percentage of the RDA for this macronutrient recorded as 81.34 ± 9.63% during the period with dietary Mg supplementation. For fats, the percentage RDA covered initially was 152.99 ± 31.52% at baseline, with this figure increasing to 169.92 ± 21.38% during the dietary Mg supplementation period. Carbohydrate and fat intakes were significantly higher during the supplementation period than at baseline (p<0.05), and carbohydrate and protein intakes were significantly higher during the non-supplemented period than at baseline (table 3).

The percentage, optimal, macronutrient intake based on total energy intake, as calculated in accordance with current recommendations [26], showed that protein and fat intakes were above the RDA. The only exception was fat intake during the non-supplemented period, which was within the range of recommended values.

Magnesium intake was 89.12% of the RDA at baseline and 91.88% of the RDA during the period without dietary Mg supplementation, compared to reference values [4]. Mineral intake surpassed 100% of the RDA only during the supplementation period, as a result of the dietary supplementation. Significant differences in Mg intake were found (p<0.05) at baseline compared with the period with dietary Mg supplementation, and between the baseline and the period without dietary Mg supplementation.

Zinc intakes were above the RDA [4]. Significant differences in Zn intake were found (p<0.05) between baseline and supplementation periods.
Table 3. Mean intake and percentage of the RDA covered for macronutrients, magnesium and zinc at three time points.

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>Recommended daily allowance</th>
<th>Bp Mean (SD)</th>
<th>S Mean (SD)</th>
<th>NS Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal/kg/day)</td>
<td>44</td>
<td>34.45 (3.56)</td>
<td>38.91 (4.15)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>38.54 (2.94)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Macronutrients (g/day)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>104-147</td>
<td>133.43 (14.32)</td>
<td>146.64 (35.64)</td>
<td>147.04 (25.51)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>519-865</td>
<td>360.91 (27.64)</td>
<td>421.50 (49.24)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>416.80 (38.82)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fat</td>
<td>78-95</td>
<td>118.57 (22.52)</td>
<td>132.22 (17.75)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>129.57 (21.79)</td>
</tr>
<tr>
<td><strong>Macronutrients (g/kg/day)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>1.2-1.7</td>
<td>1.54 (0.22)</td>
<td>1.70 (0.44)</td>
<td>1.70 (0.33)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>6-10</td>
<td>4.17 (0.41)</td>
<td>4.88 (0.60)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.82 (0.36)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fat</td>
<td>0.9-1.1</td>
<td>1.37 (0.28)</td>
<td>1.53 (0.19)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.49 (0.21)</td>
</tr>
<tr>
<td><strong>Macronutrients (% E)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>12-15</td>
<td>17.97 (1.83)</td>
<td>17.47 (3.73)</td>
<td>17.65 (2.54)</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>45-65</td>
<td>48.66 (4.10)</td>
<td>50.21 (2.54)</td>
<td>50.20 (3.62)</td>
</tr>
<tr>
<td>Fat</td>
<td>20-35</td>
<td>35.71 (4.88)</td>
<td>35.51 (3.81)</td>
<td>34.92 (4.01)</td>
</tr>
<tr>
<td><strong>Minerals (mg/day)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Magnesium</td>
<td>400</td>
<td>374.30 (122.62)</td>
<td>488.73 (79.61)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>388.73 (79.61)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Zinc</td>
<td>11</td>
<td>18.06 (9.91)</td>
<td>26.13 (12.50)</td>
<td>23.70 (10.16)</td>
</tr>
<tr>
<td><strong>Macronutrients (% RDA)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>-</td>
<td>129.25 (18.75)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>141.85 (35.93)</td>
<td>142.39 (28.54)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>-</td>
<td>69.76 (6.92)</td>
<td>81.34 (9.63)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>80.33 (6.30)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fat</td>
<td>-</td>
<td>152.99 (31.52)</td>
<td>169.92 (21.38)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>166.18 (24.30)</td>
</tr>
<tr>
<td><strong>Minerals (% RDA)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Magnesium</td>
<td>-</td>
<td>89.12 (29.19)</td>
<td>116.37 (18.95)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>91.88 (21.93)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Zinc</td>
<td>-</td>
<td>164.18 (90.09)</td>
<td>237.54 (113.53)</td>
<td>215.45 (92.36)</td>
</tr>
</tbody>
</table>

Results are shown as mean and standard deviation (SD)

<sup>a</sup> Statistically significant difference (p<0.05) Bp versus S and NS

<sup>b</sup> Statistically significant difference (p<0.05) S versus NS

Bp: baseline point; S: after training with dietary supplementation; NS: after training without dietary supplementation.

**Relationship between training and nutritional status**

Bivariate analysis, showed no correlation between training volume and biochemical Mg and Zn levels.

We found significant correlations (p<0.01) at 60-80% of RHR volume training between plasma Mg levels during the periods with and without supplementation (r = 0.36) and (r = 0.38) respectively. Furthermore, there were significant correlations (p<0.01) at >80% of RHR training volume between plasma (r = -0.28) and erythrocyte (r = 0.52) Mg levels (figure 1). Zn plasma levels were associated (p<0.01, r = 0.26) with 60-80% of RHR volume training during the supplementation period. There were no correlations at <60% of RHR volume training between Mg and Zn levels.

Finally, RPE correlated significantly with erythrocyte Mg levels (r = -0.29; p<0.01).

**Biochemical analyses**

Table 4 summarizes the findings for the biochemical parameters analyzed here. Clinical parameters for nutritional status were within the standard reference values at all three time points.

Plasma Mg concentrations were within the reference range in all participants. However, comparisons of the values at different time points showed significant differences (p<0.01) between baseline and the periods with supplementation, and without supplementation.

As regards Zn intake, plasma Zn concentrations were above the reference values at all three time points. However, we found no correlation between
Zn intake and plasma concentrations at any time during the study.

In erythrocytes, neither Mg nor Zn concentrations differed significantly between any two time points. Bivariate analysis with Pearson’s correlation coefficient revealed a positive correlation \( (p < 0.001) \) between Mg and Zn at baseline \( (r = 0.83) \) (figure 2) and during the supplementation period \( (r = 0.87) \) (figure 2).

Discussion

The anthropometric profiles of the athletes who participated in this study were similar for all parameters, and our sample was consequently highly homogeneous in this aspect.

Our results for macronutrient intakes showed that protein and fat accounted for a large proportion of the total energy intake. Many earlier studies have reported similar findings and noted imbalances in macronutrient intakes. For example, Zalcman et al. [30] reported a protein intake of 17.6 ± 6.4% and a fat intake of 32.3 ± 5.7%, the latter value being slightly lower than in our sample of handball players. Carbohydrate intake in our sample was highest during the supplementation period (4.88 ± 0.60 g/kg/day), but was below the recommended allowance \[24, 26\] of 6-10 g/kg/day, and was lower than the 5.9 ± 1.8 g/kg/day value in the adventure racers studied by Zalcman et al. [30]. In their sample of swimmers, Lukaski et al. [15] obtained a carbohydrate intake of 440 ± 33 g/day, a protein intake of 120 ± 7 g/day (both lower than in our sample), and a fat intake

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**Figure 1.** Correlation between training volume in >80% of RHR intensity range and plasma and erythrocyte Mg concentrations at points without supplementation (NS), in high-performance handball players.

**Table 4.** Biochemical parameters at three time points compared to reference values.

<table>
<thead>
<tr>
<th>Biochemical parameters</th>
<th>Reference (mg/dL)</th>
<th>Bp Mean (SD)</th>
<th>S Mean (SD)</th>
<th>NS Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transferrin</td>
<td>200-360</td>
<td>261.21 (27.82)</td>
<td>261.71 (33.00)</td>
<td>265.50 (28.67)</td>
</tr>
<tr>
<td>Prealbumin</td>
<td>20-40</td>
<td>26.76 (3.53)</td>
<td>27.19 (3.12)</td>
<td>26.76 (2.77)</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>110-200</td>
<td>147.86 (26.74)</td>
<td>149.71 (27.68)</td>
<td>154.57 (26.80)</td>
</tr>
<tr>
<td>Plasma Mg</td>
<td>1.7-2.6</td>
<td>2.06 (0.15)</td>
<td>2.27 (0.18)</td>
<td>2.28 (0.13)</td>
</tr>
<tr>
<td>Plasma Zn</td>
<td>0.07-0.1</td>
<td>0.36 (0.16)</td>
<td>0.38 (0.13)</td>
<td>0.48 (0.12)</td>
</tr>
<tr>
<td>Erythrocyte Mg</td>
<td>-</td>
<td>48.21 (9.62)</td>
<td>43.22 (17.15)</td>
<td>43.30 (17.24)</td>
</tr>
<tr>
<td>Erythrocyte Zn</td>
<td>-</td>
<td>1.13 (0.33)</td>
<td>1.01 (0.33)</td>
<td>1.04 (0.31)</td>
</tr>
</tbody>
</table>

Results are shown as mean and standard deviation (SD)

\( ^a \) Statistically significant difference according to Student’s \( t \) test \( (p < 0.05) \) Bp versus S and NS.

Bp: baseline point; S: after training with dietary supplementation; NS: after training without dietary supplementation.

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of 149 ± 9 g/day (higher than in our sample). These results suggest that different nutritional strategies are needed to achieve a balanced diet in different types of athletes.

Our analysis of mineral intakes showed, unsurprisingly, that Mg intake increased significantly ($p<0.05$) during the nutritional intervention period as a result of the dietary Mg supplementation. No significant changes were found in Mg intake in relation to diet without Mg supplementation. Some earlier studies of athletes found that dietary intake did not cover 100% of the RDA for Mg intake (400 mg/day), but led to Zn intakes above 100% of the RDA (11 mg/day) [4]. In our sample, as in other studies, the RDA for Mg was covered only during the supplementation period, whereas Zn intakes surpassed the RDA at all time points [4].

A noteworthy aspect of nutritional research in athletes is that most of the studies we reviewed reported plasma and erythrocyte concentrations of Mg and Zn measured at baseline, immediately after a specific exercise test used to measure macronutrient levels, and in some cases a only a few minutes after the test had been completed. We aimed to improve upon this methodology by measuring mineral concentrations 12 h after training sessions had ended, since during exercise, nutrient concentrations can be expected to vary. As a result, the most appropriate time to evaluate nutritional status and test for possible deficiencies in athletes is not immediately after exertion, but after the body has had time to respond to changes in nutrient levels and recover from transitory deficits.

We monitored hydration status in our participants to avoid possible interference by this factor. Biochemical analysis detected no deficiencies for Mg or Zn at any time point according to reference values for the adult population. After the nutritional intervention and training period (during both supplemented and non-supplemented periods), we found that plasma Mg concentration increased significantly ($p<0.05$) compared to baseline. Cinar et al. [9] reported significant differences in plasma Mg levels ($p<0.05$) in a group of athletes after Mg supplementation. Earlier studies without dietary supplementation failed to find changes in plasma concentrations of either mineral in athletes after training [11, 31, 32]. In our study, we found no changes in plasma Zn levels between baseline and supplementation periods. Cinar et al. [9] found no changes in plasma Zn levels in Mg-supplemented athletes.

During the non-supplemented period, there were no changes in Mg and Zn levels, despite significant increases in their concentrations ($p<0.05$) caused by training load in this last period. These results may reflect the fact that the body’s high Mg requirements prevent storage of this mineral in tissues such as the liver or muscle, so that it remains readily available in the bloodstream during exercise, as described by Elin [33], who attributed the lack of change to the long, biological half-life of magnesium. However, Córdova [34] noted that both plasma and erythrocyte concentrations of Mg increased immediately after completion of three different types of exercise or test, and suggested that this change reflected the redistribution of Mg. However, the possible influence of dehydration may also have contributed to these increases.

Several authors have noted that brief, high-intensity exercise is associated with a hypermagnesemic state as measured in plasma, whereas moderate exercise for prolonged periods appears to be associated with hypomagnesemia [7, 9-12].
The participants in our study performed strenuous exercise, and we measured both plasma and erythrocyte concentrations of Mg. Our long-term follow-up documented the effects of subsequent recovery from 12 hours of strenuous exercise. During training sessions, the plasma concentration of Mg decreased while erythrocyte levels of the mineral increased, probably because of the anaerobic nature of the exercise [7, 9]. During recovery, this process was inverted as a result of the redistribution of Mg in different body compartments due to the increased demand during exercise [7]. Associations found in our study, confirm this redistribution process supported by the evidence that exercise caused temporal, intercompartmental Mg changes [35].

Newhouse et al. [1] criticized earlier studies because of differences in the amount of dietary supplements given to athletes, and because supplements were given without establishing pre-supplementation intakes. This made it impossible to evaluate changes in Mg levels that might have reflected actual deficiencies in this nutrient [8]. We determined Mg nutritional status at baseline, and found that before the dietary intervention, athletes in our study had inadequate dietary Mg intakes based on the reference value for the healthy adult population. Nielsen and Lukaski [8] suggested that the best method to document Mg status may be to evaluate whether mineral intake is adequate. However, other authors have pointed out the difficulties arising from the lack of consensus on adequate Mg intake in athletes [8, 36].

On the other hand, associations found in our study between Mg and Zn levels in erythrocytes were possibly caused by co-transport mechanism into the red blood cells [37].

In conclusion, our findings in high-performance handball players show that during competition, there is a relationship between erythrocyte Zn and Mg levels regardless of Mg supplementation or Zn intake. Mg dietary supplementation tended to preserve changes in mineral levels during training and competition.

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athletes with no magnesium deficiency. *Magnes Res* 2011; 24: 36-44.


