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## The effect of different doses of zinc supplementation on nail elements in elite female athletes

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### Abstract

Background: The trace elements concentration in metabolically inactive fingernails has been used to monitor the exposure to toxic metals or to assess associations between excess or deficiency of trace elements. Material and methods: The research protocol was applied to a total number of 20 female volleyball players. The subjects who were randomly allocated into two groups with an equal number of members were administered 220 mg/day (Group 1) and 440 mg/day (Group 2) of zinc sulfate. Results: The result of the analysis of nail samples collected after 2, 3 and 4 weeks of supplementation showed no significant difference between Mg, Ca, Fe, Cu, Se, P and Zn levels between the groups ( $p > 0.05$ ). It was determined that nail Ca, Fe, and Zn levels in the last week of zinc supplementation increased significantly compared to the level at the beginning of the supplementation in both groups ( $p < 0.05$ ). Conclusions: It was determined that two different applications significantly decreased Mg, P and Se levels for both groups regardless of the dose difference.

### Keywords

female athletes, nail, trace element, zinc supplementation

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# The effect of different doses of zinc supplementation on nail elements in elite female athletes

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## abstract

- Background:** The trace elements concentration in metabolically inactive fingernails has been used to monitor the exposure to toxic metals or to assess associations between excess or deficiency of trace elements.
- Material and methods:** The research protocol was applied to a total number of 20 female volleyball players. The subjects who were randomly allocated into two groups with an equal number of members were administered 220 mg/day (Group 1) and 440 mg/day (Group 2) of zinc sulfat.
- Results:** The result of the analysis of nail samples collected after 2, 3 and 4 weeks of supplementation showed no significant difference between Mg, Ca, Fe, Cu, Se, P and Zn levels between the groups ( $p > 0.05$ ). It was determined that nail Ca, Fe, and Zn levels in the last week of zinc supplementation increased significantly compared to the level at the beginning of the supplementation in both groups ( $p < 0.05$ ).
- Conclusions:** It was determined that two different applications significantly decreased Mg, P and Se levels for both groups regardless of the dose difference.
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## INTRODUCTION

In biochemistry, trace elements are also known as “micronutrients”. Trace elements form part of the daily diet and are well known to play vitally important roles in maintaining health [1]. Minimum alterations in their concentrations may induce critical alterations in a person’s physiological functions as well as in the physical performance [2].

Zinc is an essential trace element that has been implicated in numerous biological functions, including immunity, energy metabolism and antioxidative processes [3]. As zinc is necessary for many enzymes found in the metabolism, a serious zinc deficiency will have a negative impact on muscle functions [4]. Since zinc is necessary for the activity of several enzymes in the energy metabolism, low muscle zinc levels may lead to a reduction in the endurance capacity [5].

It is known that physical activity leads to many metabolic changes in the human organism; intense training may increase essential trace elements requirements, either by augmented degradation rates or by increased losses from the body [6]. Studies into the relation between zinc and exercise mainly focus on the distribution of this element in the body in response to exercise [7-9].

It was suggested that, since exercise increases zinc excretion from the body, and female athletes in particular do not get enough zinc through diet, zinc supplementation to athletes with zinc deficiency is essential [9]. Recent evaluations of the literature have provided evidence of significant acute changes in zinc metabolism following a bout of aerobic exercise [3].

Zinc and other elements affect absorption of each other when they are taken in excess [10]. Excessive intake of elements such as calcium, phosphorus, iron and copper reduces the absorption of zinc [11], while excessive intake of zinc also reduces absorption of some minerals (iron, copper, calcium, phosphorus and magnesium) [12-13].

Human nails are largely composed of keratin-rich proteins, which accommodate trace metals in proportion to their intake by various mechanisms, such as synthesis of proteins with sulfhydryl groups. Therefore, the nails are a useful marker for trace metals investigation and are increasingly used in clinical studies [14-15]. Their roots are highly influenced by the health status of the cells, whereas blood and other body fluids give transient concentrations, human nails provide a continuous record of elemental concentration [16-19].

The concentration of trace elements in metabolically inactive finger nails has been used to monitor exposure to toxic metals or to assess associations between excess or deficiency of trace elements. The individual’s nail’s mineral content varies between populations, and not all minerals are deposited in the nails at the same speed. The different elements present in nails have different metabolic roles in the body, and consequently, they could be used as indicators of their nutritional status [18].

An analysis of nail presents advantages over an analysis of biological fluids, such as sweat, urine or blood, because nails can be easily collected and stored at room temperature; moreover, the nail tissue is easier to handle and it also accumulates a large number of trace elements in relatively high concentrations [20-21].

The purpose of the present study was to determine the effect of oral zinc supplementation to elite athletes on the changes in nail element levels.

## MATERIAL AND METHODS

### PARTICIPANTS

The research was conducted on a total number of 20 female athletes playing in a volleyball team in the Super League of the Sport Club at Gazi University, whose age, bodyweight, and height were  $15.1 \pm 1.07$  years (14–17),  $59.9 \pm 6.4$  kg (50.4–72.2), and  $175.3 \pm 6.5$  cm, respectively. The subjects (and/or their parents) who were verbally informed also signed copies of the Helsinki declaration explaining who is conducting the study and why. The study protocol was approved by the Ethical Committee of High School of Physical Education and Sports Selcuk University (meeting number: 2009/005).

The athletes in the study were divided into two groups:

- Group 1: The group was administered 220 mg/day of zinc sulfate (each 220 mg of zinc sulfate contained 50 mg of elemental zinc).
- Group 2: The group was administered 440 mg/day of zinc sulfate (each 440 mg of zinc sulfate contained 100 mg of elemental zinc).

Zinc sulfate capsules were supplied by Berko Drug and Chemicals Industry Inc. in the form of capsules, each containing 220 mg of zinc sulfate.

### PROCEDURES

The study was conducted during the athletes' general preparation period of 8–10 weeks (consisting of 70–80% power, and 20–30% technical-tactical exercises).

As the subjects were female athletes, analyses were interrupted in the menstruation weeks. Daily nutrition of the athletes throughout study was kept under control with the nutrition program provided by a dietician. This helped them to have energy and nutrients depending on their personal requirement, and particular attention and care was given to prevent insufficient intake of zinc in the consumption of nutrients.

### NAIL MAGNESIUM, CALCIUM, IRON, COPPER, SELENIUM, PHOSPHORUS AND ZINC ANALYSES

Nail samples were collected from all athletes, who followed their daily training schedule (6 days/week), 5 times: during the resting period before zinc supplementation, and once a week during 4 weeks of zinc supplementation, and during resting period following the supplementation.

Approximately 5 mg of each nail sample was accurately weighed on a microbalance with an accuracy limit of 100 µg, and put in a teflon vessel, to which 1 mL of nitric acid was then added. The teflon vessels were placed in a pressure tight case and heated in a microwave oven with the power of 630 W for 10 minutes. After cooling to room temperature, the vessels were released, and the sample solutions were diluted with purified water to 10 mL. Standard solutions calibrations were prepared by mixing and diluting standard solutions of individual element. Mg, Ca, Fe, Cu, Se, P and Zn levels (µg/g) in the collected hair samples were determined through Inductively Coupled Plasma Mass Spectrometry (ICP-MS) device (AGILENT 7500 ce, Agilent Technologies, Santa Clara, CA 95051-7201 USA).

### STATISTICAL ANALYSIS

The data were evaluated using the Statistical Package for the Social Sciences (SPSS 15.0) where the means and frequency distributions were calculated. Further data analysis was conducted through t test, and the difference between the groups was determined using Mann Whitney U test. The difference that occurred by weeks was determined through Kruskal-Wallis test. The significance level was considered at  $p < 0.05$ .

## RESULTS

### PRE-SUPPLEMENTATION NAIL ELEMENT LEVELS OF THE GROUPS

Analysis of nail samples collected from the athletes before zinc supplementation, showed no significant differences between the groups in Ca, Se and P levels ( $p > 0.05$ ). A significant difference was found between the athletes' levels of Mg, Fe, Cu and Zn in Group 1 and 2 ( $p < 0.05$ ), where element levels in Group 1 were higher than those in Group 2 (Figures 1-7).

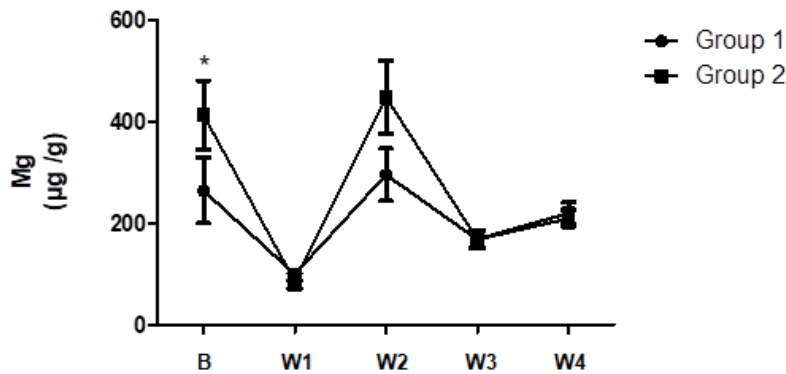


Fig. 1. Nail Mg levels of the groups before supplementation, during supplementation, and after 4 weeks of zinc supplementation

B - before supplementation; W1 - 1 week after supplementation; W2 - 2 weeks after supplementation; W3 - 3 weeks after supplementation; W4 - 4 weeks after supplementation.

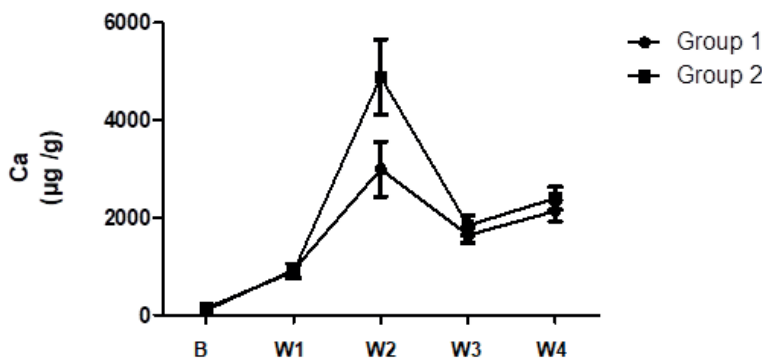


Fig. 2. Nail Ca level of the groups before supplementation, during supplementation, and after 4 weeks of zinc supplementation

B - before supplementation; W1 - 1 week after supplementation; W2 - 2 weeks after supplementation; W3 - 3 weeks after supplementation; W4 - 4 weeks after supplementation.

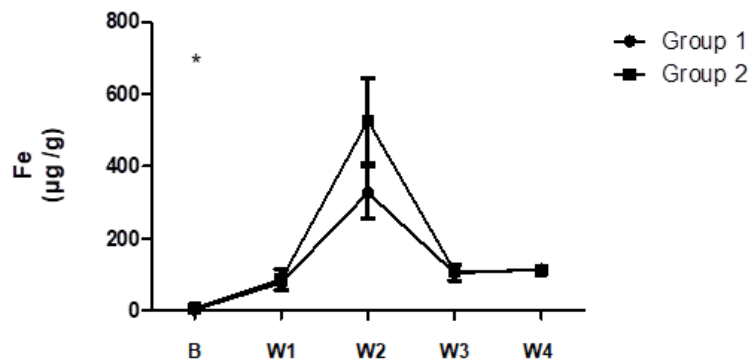


Fig. 3. Nail Fe level of the groups before supplementation, during supplementation, and after 4 weeks of zinc supplementation

B - before supplementation; W1 - 1 week after supplementation; W2 - 2 weeks after supplementation; W3 - 3 weeks after supplementation; W4 - 4 weeks after supplementation.

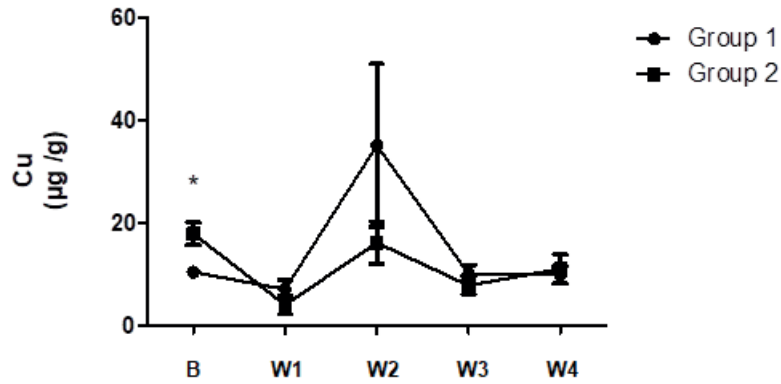


Fig. 4. Nail Cu levels of the groups before supplementation, during supplementation, and after 4 weeks of zinc supplementation

B - before supplementation; W1 - 1 week after supplementation; W2 - 2 weeks after supplementation; W3 - 3 weeks after supplementation; W4 - 4 weeks after supplementation.

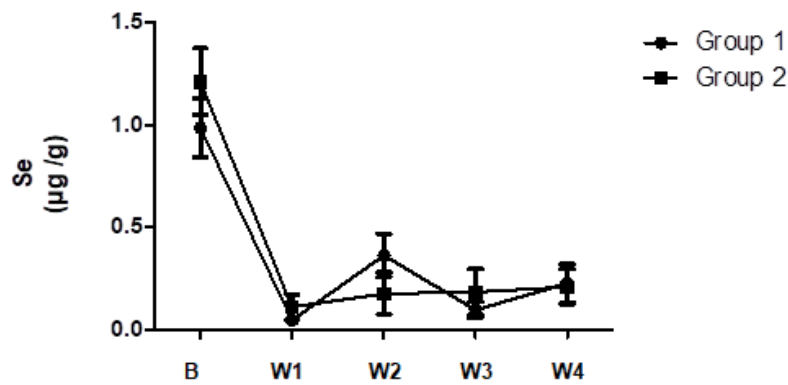


Fig. 5. Nail Se levels of the groups before supplementation, during supplementation, and after 4 weeks of zinc supplementation

B - before supplementation; W1 - 1 week after supplementation; W2 - 2 weeks after supplementation; W3 - 3 weeks after supplementation; W4 - 4 weeks after supplementation.

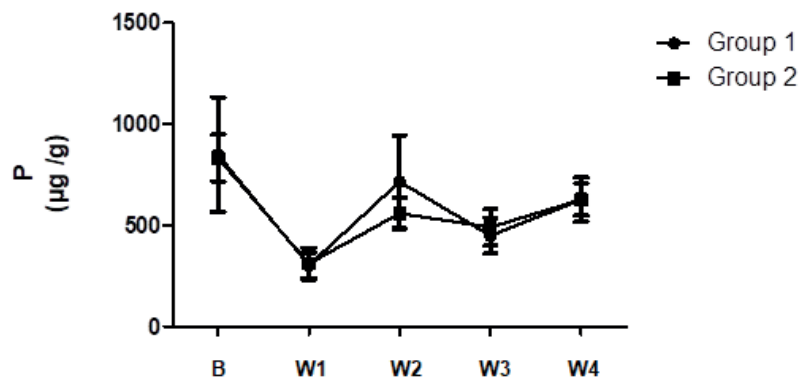


Fig. 6. Nail P levels of the groups before supplementation, during supplementation, and after 4 weeks of zinc supplementation

B - before supplementation; W1 - 1 week after supplementation; W2 - 2 weeks after supplementation; W3 - 3 weeks after supplementation; W4 - 4 weeks after supplementation.

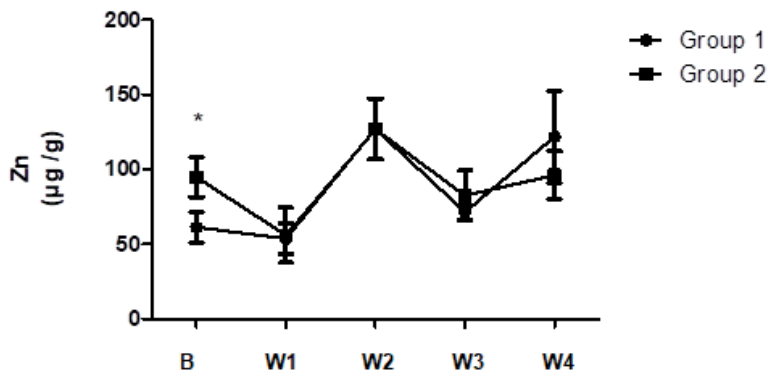


Fig. 7. Nail Zn levels of the groups before supplementation, during supplementation, and after 4 weeks of zinc supplementation

B - before supplementation; W1 - 1 week after supplementation; W2 - 2 weeks after supplementation; W3 - 3 weeks after supplementation; W4 - 4 weeks after supplementation.

### COMPARISON BETWEEN THE WEEKLY CHANGES CAUSED BY ZINC SUPPLEMENTATION IN NAIL ELEMENT LEVELS IN GROUPS 1 AND 2

The result of the analysis of nail samples collected after 1, 2, 3 and 4 weeks of supplementation showed no significant difference between Mg, Ca, Fe, Cu, Se, P and Zn levels between the groups ( $p > 0.05$ , Figures 1-7).

### COMPARISON OF THE CHANGES BY WEEKS IN NAIL ELEMENT LEVELS IN GROUP 1 AND GROUP 2 CAUSED BY ZINC SUPPLEMENTATION

Considering the change in the nail element level of the athletes in Group 1 by weeks, zinc supplementation led to a significant change in all elements level by weeks ( $p < 0.05$ ). Nail Mg, P, Cu, and Se levels in the last week of zinc supplementation were found to have significantly decreased compared to the level at the beginning of the supplementation, while Ca, Fe, and Zn levels have increased (Table 1).

Considering the change in the nail element level of the athletes in Group 2 by weeks, zinc supplementation led to a significant change in all element levels by weeks ( $p < 0.05$ ). Nail Mg, P and Se levels in the last week of zinc supplementation were found to have significantly decreased compared to the level at the beginning of the supplementation, while Ca, Fe, Cu, and Zn levels have increased (Table 2).

It was found that nail Ca, Fe, and Zn levels in the last week of zinc supplementation significantly increased compared to the level at the beginning of the supplementation in both groups (Tables 1 and 2).

Table 1. Changes by weeks in nail element levels in the athletes in group 1 caused by zinc supplementation

Elements	Supplementation Time	N	X ± SS	Kruskall Walls' z	p
Mg (µg /g)	Before supplementation pre exercise	10	413.86 ± 214.73	34.104	0.000*
	1 weeks after supplementation	10	85.69 ± 47.24		
	2 weeks after supplementation	10	449.23 ± 226.74		
	3 weeks after supplementation	10	168.76 ± 57.95		
	4 weeks after supplementation before exercise	10	220.44 ± 65.07		
P (µg /g)	Before supplementation pre exercise	10	833.04 ± 362.53	17.923	0.001*
	1 weeks after supplementation	10	310.72 ± 246.27		
	2 weeks after supplementation	10	559.82 ± 246.20		
	3 weeks after supplementation	10	491.00 ± 283.83		
	4 weeks after supplementation before exercise	10	627.08 ± 253.18		



Elements	Supplementation Time	N	X ± SS	Kruskall Walls' z	p
Se (µg /g)	Before supplementation pre exercise	10	142.76 ± 60.79	41.712	0.000*
	1 weeks after supplementation	10	893.45 ± 462.69		
	2 weeks after supplementation	10	4886.10 ± 2445.08		
	3 weeks after supplementation	10	1842.74 ± 629.85		
	4 weeks after supplementation before exercise	10	2398.30 ± 744.53		
Fe (µg /g)	Before supplementation pre exercise	10	6.64 ± 6.48	36.180	0.000*
	1 weeks after supplementation	10	86.72 ± 90.91		
	2 weeks after supplementation	10	526.34 ± 373.75		
	3 weeks after supplementation	10	110.63 ± 48.07		
	4 weeks after supplementation before exercise	10	111.68 ± 20.23		
Cu (µg /g)	Before supplementation pre exercise	10	17.88 ± 7.09	14.433	0.006*
	1 weeks after supplementation	10	3.97 ± 5.79		
	2 weeks after supplementation	10	16.14 ± 13.00		
	3 weeks after supplementation	10	7.78 ± 5.49		
	4 weeks after supplementation before exercise	10	11.03 ± 8.88		
Zn (µg /g)	Before supplementation pre exercise	10	94.87 ± 41.64	12.310	0.015*
	1 weeks after supplementation	10	56.11 ± 58.26		
	2 weeks after supplementation	10	126.98 ± 63.77		
	3 weeks after supplementation	10	82.56 ± 53.19		
	4 weeks after supplementation before exercise	10	96.22 ± 50.87		
Se (µg /g)	Before supplementation pre exercise	10	1.20 ± 0.51	22.369	0.000*
	1 weeks after supplementation	10	0.11 ± 0.20		
	2 weeks after supplementation	10	0.18 ± 0.32		
	3 weeks after supplementation	10	0.18 ± 0.36		
	4 weeks after supplementation before exercise	10	0.21 ± 0.27		

\*p < 0.05

Table 2. Changes by weeks in nail element levels in the athletes in group 2 caused by zinc supplementation

Elements	Supplementation Time	N	X ± SS	Kruskall Walls' z	p
Mg (µg /g)	Before supplementation pre exercise	10	264.75 ± 203.38	22.531	0.000*
	1 weeks after supplementation	10	96.23 ± 40.93		
	2 weeks after supplementation	10	296.12 ± 163.36		
	3 weeks after supplementation	10	168.30 ± 47.54		
	4 weeks after supplementation before exercise	10	209.69 ± 52.17		
P (µg /g)	Before supplementation pre exercise	10	849.54 ± 893.90	9.876	0.043*
	1 weeks after supplementation	10	302.53 ± 201.02		
	2 weeks after supplementation	10	716.15 ± 725.00		
	3 weeks after supplementation	10	449.42 ± 280.73		
	4 weeks after supplementation before exercise	10	627.24 ± 346.06		
Se (µg /g)	Before supplementation pre exercise	10	103.45 ± 64.60	35.361	0.000*
	1 weeks after supplementation	10	920.24 ± 432.54		
	2 weeks after supplementation	10	2995.50 ± 1783.32		
	3 weeks after supplementation	10	1641.00 ± 508.16		
	4 weeks after supplementation before exercise	10	2136.90 ± 700.77		

Elements	Supplementation Time	N	X ± SS	Kruskall Walls' z	p
Fe (µg /g)	Before supplementation pre exercise	10	3.08 ± 1.52	35.197	0.000*
	1 weeks after supplementation	10	78.29 ± 44.02		
	2 weeks after supplementation	10	327.24 ± 227.18		
	3 weeks after supplementation	10	104.85 ± 69.63		
	4 weeks after supplementation before exercise	10	114.41 ± 44.93		
Cu (µg /g)	Before supplementation pre exercise	10	10.36 ± 2.60	9.889	0.042*
	1 weeks after supplementation	10	7.04 ± 5.46		
	2 weeks after supplementation	10	35.12 ± 50.25		
	3 weeks after supplementation	10	10.05 ± 5.57		
	4 weeks after supplementation before exercise	10	12.33 ± 6.33		
Zn (µg /g)	Before supplementation pre exercise	10	61.45 ± 32.2	18.960	0.001*
	1 weeks after supplementation	10	53.82 ± 32.72		
	2 weeks after supplementation	10	127.32 ± 64.07		
	3 weeks after supplementation	10	71.52 ± 15.74		
	4 weeks after supplementation before exercise	10	121.89 ± 97.83		
Se (µg /g)	Before supplementation pre exercise	10	0.98 ± 0.45	29.614	0.000*
	1 weeks after supplementation	10	0.05 ± 0.08		
	2 weeks after supplementation	10	0.36 ± 0.33		
	3 weeks after supplementation	10	0.10 ± 0.13		
	4 weeks after supplementation before exercise	10	0.23 ± 0.29		

\*p < 0.05

## DISCUSSION

Macro and trace mineral behavior in whole body fluids during physical activity constitutes one of the poorest studied aspects in the field of exercise physiology. The precise link between physical exercise and organic mineral concentrations and redistributions is still unclear. There is a lot of research on the serum and urinary element distributions of exercise [9, 22-25]. In one study, it was found that zinc supplementation raised urinary zinc excretion in one-week intervals over the course of 4 weeks ( $p < 0.05$ ), and reduced selenium levels ( $p < 0.05$ ) [9]. Another study found that zinc supplementation increased serum Mg, Ca, Zn values [25].

Analysis of nail samples collected from the athletes before zinc supplementation showed no significant differences between the groups in the Ca, Se and P levels ( $p > 0.05$ ). A significant difference was found between Mg, Fe, Cu and Zn levels of the athletes in Group 1 and 2 ( $p < 0.05$ ), where element levels in Group 1 were higher than those of Group 2. The results of the analysis of nail samples collected after 1, 2, 3 and 4 weeks of supplementation showed no significant differences between Mg, Ca, Fe, Cu, Se, P and Zn levels between the groups ( $p > 0.05$ ). Nail and hair samples are markers of periods of longer exposure, while urine and blood reflect recent intake, on the order of several days for urine and several weeks for blood [26].

Considering the change in the nail element levels of the athletes in both groups by weeks, zinc supplementation led to a significant change in all elements level by weeks ( $p < 0.05$ ). Nail Mg, P, Cu, and Se levels in the last week of zinc supplementation was found to have significantly decreased compared to the level at the beginning of the supplementation, while Ca, Fe, and Zn levels have increased in group 1. Nail Mg, P, and Se level in the last week of zinc supplementation was found to have significantly decreased compared to the level at the beginning of the supplementation, while Ca, Fe, Cu, and Zn levels have in-

creased in group 2. Excessive intake of elements such as calcium, phosphorus, iron and copper reduces the absorption of zinc [11], while excessive intake of zinc also reduces the absorption of some minerals (iron, copper, calcium, phosphorus and magnesium) [12, 13]. This study determined that two different applications significantly decreased Mg, P and Ca levels for both groups regardless of the dose difference.

It was determined that nail Ca, Fe, and Zn levels in the last week of zinc supplementation significantly increased compared to the level at the beginning of the supplementation in both groups. In group 2, where the zinc dose was high, the level of nail copper was thought to increase depending on the dose. In another study, the trace element content in women's toenails, but not in men, were markedly influenced by physical activity, with higher levels of Ca and Fe and lower Hg among active females [18].

The role of toenails as a biomarker of supplement intake has been explored for several essential elements, especially for selenium. The use of Se supplements significantly increased toenail Se levels [27-30]. Se was the most studied element, with half of the reports showing a direct relationship of toenail levels with global Se intake [31, 32]. The relationship between the intake of Se supplements and toenail concentration was very consistent, probably due to the high doses of the element in supplements. For other elements (Zn, Mn, Cu), oral supplements were not related to toenail levels; however, there were no data for Fe, another element usually administered in supplements [31].

Among dietary factors, only toenail selenium showed clear associations with the intake of supplements or specific foods [31]. Deficiency in micronutrient can negatively affect athletic performance. Approximately 50% of athletes have reported consuming some form of micronutrient supplement; however, there is limited data confirming their efficacy for improving performance [33]. In this study, the athletes' food intake was kept under a dietician's control.

The data reveals that the level of elements in the nail can vary depending on gender and age [34]. Age is one of the main factors that could modify toenail metal levels; for example, some studies suggested that age could change the rate of incorporation of elements such as Se into toenails, but also that differences between younger and elder people could reflect age-related variations in the total amount of the element in the body [31]. Determination of inorganic elements in biological samples is important from the viewpoint of the nutritional status or scientific research [35].

Studies on nail element levels of athletes are quite insufficient. Similarly, data on the nail element distribution of zinc supplement application in athletes are insufficient. This situation limited a comparison of the results with other studies.

## CONCLUSIONS

At the end of the study, it was found that different doses of zinc sulfate supplementation in elite female athletes who conducted regular trainings on 6 days a week led to no significant difference of nail Mg, Ca, Fe, Cu, Se, P and Zn levels between the groups. The change in nail element level by weeks in athletes included in Group 1 (220 mg/day zinc sulfate) and Group 2 (440 mg/day zinc sulfate) was found to differ significantly with regard to all elements (Mg, Ca, Fe, Cu, Se, P, Zn) levels compared to the beginning of the study. Nail Mg, P, Cu, and Se levels in the last week of zinc supplementation was found to have significantly decreased compared to the level at the beginning of supplementation, while Ca, Fe, and Zn levels have increased in Group 1. Nail Mg, P, and Se levels in the last week of zinc supplementation was found to have significantly decreased compared to the level at the

beginning of supplementation, while Ca, Fe, Cu, and Zn levels have increased in Group 2. As a result of this study, it was determined that two different applications significantly decreased Mg, P and Se levels for both groups regardless of the dose difference.

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