Biol Trace Elem Res (2014) 158:15–21
DOI 10.1007/s12011-014-9912-z

Influence of Training Frequency on Serum Concentrations of Some Essential Trace Elements and Electrolytes in Male Swimmers

Serhat Döker · Muhsin Hazar · Musa Uslu · İlyas Okan · Emin Kafkas · İ. İpek Boşgelmez

Received: 30 September 2013 / Accepted: 3 February 2014 / Published online: 16 February 2014

Abstract Elemental fluctuations during physical performances have been a point of interest. This study was designed to investigate the effect of swimming frequency on serum concentrations of some trace elements (chromium, iron, copper, zinc, selenium) and electrolytes (sodium, magnesium, potassium, calcium). Three groups of different-level male swimmers were included in the study, as elite swimmers (n=14), amateur swimmers (n=11), and sedentary individuals (n=10). Elite and amateur swimmer groups followed a 3-week training program. At the end of the period, all volunteers were subjected to a controlled swimming test, and blood samples were collected at the beginning of (pre-test), immediately after (post-test), and 1 h after this activity. Element concentrations were determined by inductively coupled plasma mass spectrometry using a dilute and shoot procedure. Apart from the swimming test applied, pre-test calcium and potassium levels were higher in elite swimmers compared to amateurs and controls. The difference in pre-test levels of these elements can be associated with adaptive mechanisms emerged by the frequent training. Regarding the test applied, changes in magnesium, calcium, copper, zinc, and selenium levels exhibited a common pattern in all study groups, with higher post-test serum concentrations. Another point of note was a drop of copper, zinc, and selenium levels at 1 h after the test in elite swimmers. The decrease in serum zinc was also observed in the other groups. Results highlight the value of regular control of elemental status to provide insight into transient effects and deficiencies.

Keywords Serum trace elements · Electrolytes · Male swimmers · Exercise · Sports · Collision/reaction cell-ICP-MS

S. Döker (✉) · M. Uslu
Faculty of Science, Department of Chemistry, Çankırı Karatekin University, 18100 Çankırı, Turkey
e-mail: serhatdoker@gmail.com

M. Hazar · İ. Okan
School of Physical Education and Sports, Gazi University, 06500 Ankara, Turkey

E. Kafkas
School of Physical Education and Sports, İnönü University, Malatya, Turkey

İ. İ. Boşgelmez
Faculty of Pharmacy, Department of Toxicology, Erciyes University, 38039 Kayseri, Turkey

Introduction

Today the importance of inorganic entities of life is being more clearly understood through research in different areas of life sciences. Some elements such as sodium (Na), potassium (K), magnesium (Mg), and calcium (Ca) are the main cations acting as electrolytes and essential constituents for life. Trace elements such as iron (Fe), copper (Cu), zinc (Zn), selenium (Se), and chromium (Cr) perform unique roles and act in numerous metabolic tasks such as electron transport (e.g., Cu, Fe), catalysis (e.g., Cu, Zn, and Se), and maintenance of structural stability of proteins. Concentrations of trace elements and electrolytes in various biological samples (e.g., serum, tissues, urine) are of interest [1-4], since their levels should be maintained within physiological limits as well as at the proper location in a timely fashion. Homeostasis of trace elements has been associated with diverse factors such as aging [1], exposure to xenobiotics [2], and exercise [4, 5].

Regular and moderate physical activity has been widely recognized as an integral component of a healthy lifestyle. A recent study suggested that exercise is so effective that it may be considered as a drug and emphasized the importance of dosing and individual variations [6]. Moderate activity is generally considered better than none at all [7]; however,

vigorous or intense physical activity may sometimes cause a disturbance in the homeostasis [7-9]. Elemental fluctuations in the body during physical performances have been a point of interest [10, 11]. In a recent report [12], plasma Zn concentrations have been measured below normal levels, suggesting a possible Zn deficiency in elite swimmers. It has been demonstrated that urinary excretion of Zn and Cu has been increased during physical exercise [13]. In another study conducted in rats, plasma Zn and Se levels have been found to decrease immediately after acute swimming [14]. It has been reported that decrease in Cu and Zn levels in kidney tissue of aged rats may be recovered by long-term swimming exercise [15].

This study was designed at two dimensions: First one probes the effect of training frequency on the serum concentrations of some trace elements and electrolytes in different-level swimmer groups (i.e., the elite swimmers, amateurs, and sedentary controls), while the second one focuses on the elemental alterations at three different sampling time points, i.e., pre-test, post-test, and 1 h later (within-group comparisons). To the best of our knowledge, this time-dependency, influence of training frequency, and different proficiency levels have not been examined previously in the same study with regard to serum element levels.

Materials and Methods

Study Design and Groups

A total of 35 male volunteers were assigned into three groups (Table 1) according to their swimming frequencies: elite swimmers (n=14), amateur swimmers (n=11), and sedentary individuals (n=10). The elite and amateur swimmer groups were subjected to a controlled 3-week training program before blood collection, while sedentary individuals (control subjects) were not included in the training period. The schedule for elite swimmers was double training 6 days per week, while the amateur swimmers had a training frequency of 3 times a week. A typical training consisted of a free warming up process for 10 min, followed by 2,000–2,500 m (depending on the condition of the swimmer on the day) interval swimming (100 m loaded freestyle swimming and 20 s resting). The uninterrupted swimming capacities of swimmers were determined as 800 m for elite swimmers and amateurs, whereas 400 m for sedentary control group, and the test was performed accordingly. Volunteers in all groups were subjected to the test, and serum element levels were monitored at three different time points (i.e., pre-test, post-test, and 1 h later). The 1-h interval was chosen to ensure hemodynamic stabilization [16]. The dietary and supplementation information has been given by the volunteers, and all volunteers were asked to stop taking any supplementation at least 1 week before sampling. All participants were informed about the objectives and methods of study and were asked to give written voluntary informed consent. The study protocol was approved by the local ethics committee of Selcuk University Meram Medical School, Konya-Turkey (protocol number: 2009/032).

Sampling and Sample Preparation

Blood samples were collected from the antecubital vein of volunteers into venous blood vacuum serum collection tubes, and allowed to stay for 30 min at room temperature before serum separation. After centrifugation at 3,000 rpm for 10 min, serum samples were pipetted out into 2.0-mL volume clean storage tubes and stored at −80 °C until analysis. All glassware and plastic ware were cleaned with 10 % HNO_3 at least overnight and gently rinsed with ultrapure water. Serum samples were allowed to thaw at room temperature and vortexed for 2 min. Samples were diluted 14-fold in polyethylene tubes for determination of trace elements, while 100-fold dilution was applied for determination of electrolytes prior to analysis. The tubes were vortexed and placed in the autosampler to be analyzed immediately.

Instruments and Working Conditions

An Agilent 7700x inductively coupled plasma mass spectrometry (Tokyo, Japan) with a collision/reaction cell (CRC-ICP-MS) was used throughout the study. The calibration standards were prepared by dilution of multi-element stock standard solution of trace elements (Agilent Technologies, Santa Clara, CA, USA) and electrolytes (Jenway, Essex, England). The internal standards containing ^{203}Bi, ^{72}Ge, ^{115}In, ^{6}Li, ^{175}Lu, ^{103}Rh, ^{45}Sc, and ^{159}Tb (Agilent Technologies) were on-line merged into the sample flow to negate the possible mixing problems. Nitric acid (69 % TraceSELECT®) was purchased from Sigma (St. Louis, MO, USA). Ultrapure water was produced from Milli-Q system (Millipore, Billerica, MA, USA). The instrumental conditions were set as follows: plasma power 1,600 W; plasma gas flow rate 15 L/min; carrier gas flow rate 1.15 L/min; and sampling depth 8 mm; isotopes monitored ^{52}Cr, ^{57}Fe, ^{63}Cu, ^{66}Zn, ^{78}Se, ^{23}Na, ^{24}Mg, ^{39}K, and ^{43}Ca; and repetition three. Collision cell was pressured with helium at a flow rate of 5.0 mL/min. The octopole and quadrupole voltages were adjusted to −18 and −15 V, resulting in a kinetic energy discrimination of 3 V.

A graphite furnace atomic absorption spectrometry (GF-AAS) system (Perkin Elmer AAnalyst 100, Norwalk, CT, USA) with pyrolytically coated graphite tubes (Perkin Elmer Part no. B010-9322) was used as a comparative instrumental technique. The analysis of serum samples by GF-AAS were performed in the recommended conditions of the manufacturer. Samples were diluted 50 times for Cu, Zn, and Fe; and 20 times for Se determination using 1 % HNO_3 containing 0.1 % Triton X-100. Serum Cr determination was performed as

described [17] with a slight change. As diluent, 0.1 % Triton X-100 solution was used instead of 0.02 % CTAC, and the injection volume was applied to be 50 μL to increase absorbance signal [18], giving a limit of detection of 0.03 ng/mL.

Statistical Analysis

Statistical analyses were performed using GraphPad Prism 6.01 (La Jolla, CA, USA). Data were analyzed for significant differences between the three study groups using one-way ANOVA followed by Tukey's post hoc test. Repeated measures ANOVA followed by post hoc test was performed for the assessment of intraindividual changes in elements at different time points (pre-test, post-test, 1 h later). Differences were accepted statistically significant when the p value was <0.05.

Results

In the present study, as shown in Table 1, there were no significant differences in elite swimmers, amateur swimmers, and controls relating to age and body mass index. However, as a consequence of the study design, the elite swimmers were engaged in regular swimming training for a longer period as compared to amateur swimmers. Serum electrolyte and trace element levels of study groups are presented in Figs. 1 and 2, respectively. The elements were within the reference values reported [19] with minor exceptions. Among all the elements analyzed, Na presented only one result close to upper limit of the reference range [19]. Three individuals in the elite swimmer group had K levels higher than the reported [19] reference values (136.5–198.9 μg/mL, corresponding to 3.5–5.1 mmol/L). Regarding the serum Cr concentrations, although all results were within the reported ranges [19], low (0.109 ng/mL) and high (0.276 ng/mL) values were found in pre-test data of elite swimmers resulting in a wider distribution in this group. Exclusion of these data revealed no change in mean values (0.187±0.01 ng/mL, n=12 vs 0.188±0.03 ng/mL, n=14) as well as in group comparisons. Besides, Zn levels were lower than the limits of the reference range [19] in the samples obtained 1 h after the test in all groups. It should be mentioned that there was neither Fe deficiency nor Fe overload in any of the volunteers included in the study throughout the period (Fig. 2).

Regarding the inter-group comparisons, serum post-test Na levels were significantly higher than the 1-h-later-sampling time point in the control group ($p<0.05$); however, there was no statistical difference in other groups in this regard (Fig. 1). In all groups, serum Mg concentrations were insignificantly elevated after the swimming test (post-test mean values) as compared with the corresponding pre-test results, whereas the concentrations were significantly reduced ($p<0.05$) at 1-h-later samples in elite and amateur swimmer groups. There were no significant differences in the Mg concentrations within control group results (Fig. 1). The pre-test serum K and Ca levels were significantly higher in the elite swimmer group than the amateur swimmers and sedentary controls (Fig. 1, $p<0.05$). With regard to K levels, the post-test values in elite swimmers were significantly decreased as compared to the pre-test status ($p<0.05$); however, the samples obtained 1 h after the test in this group showed a significant increase when compared to the post-test results ($p<0.05$), exhibiting a rebalancing effect. On the other hand, amateur swimmers and control group showed elevated K levels 1 h after the test as compared to pre- and post-test results ($p<0.05$). With respect to within-group differences for Ca, the post-test serum Ca levels of elite and amateur swimmers were significantly higher than 1-h-later-sampling results ($p<0.05$); however, there was no statistical difference in the control group in this regard (Fig. 1).

As depicted in Fig. 2, post-test serum Cr concentrations were significantly lower in elite swimmers than in amateur swimmers and sedentary controls ($p<0.05$). Similarly, the serum Cr levels of elite swimmers obtained 1 h later were significantly lower than the corresponding value of amateur swimmers ($p<0.05$). Interestingly, serum Cr levels revealed no within-group differences. When the within-group Fe concentrations were compared, it was found that the levels were slightly higher in the post-test results than the pre-test and 1-h

Table 1 Demographic characteristics of the study groups

	Elite swimmers	Amateur swimmers	Sedentary controls
Number of subjects (n)	14	11	10
Body mass index (kg/m^2)	23.9±1.8	23.1±1.9	23.8±1.7
Age (years)	23±4	25±4	22±2
Regular swimming experience (years)	9±4	5±2	–
Training schedule (controlled 3-week training program)	Double training daily (09:00 am, 06:00 pm) 6 days per week	Training once a day (09:00 am), 3 days per week	None

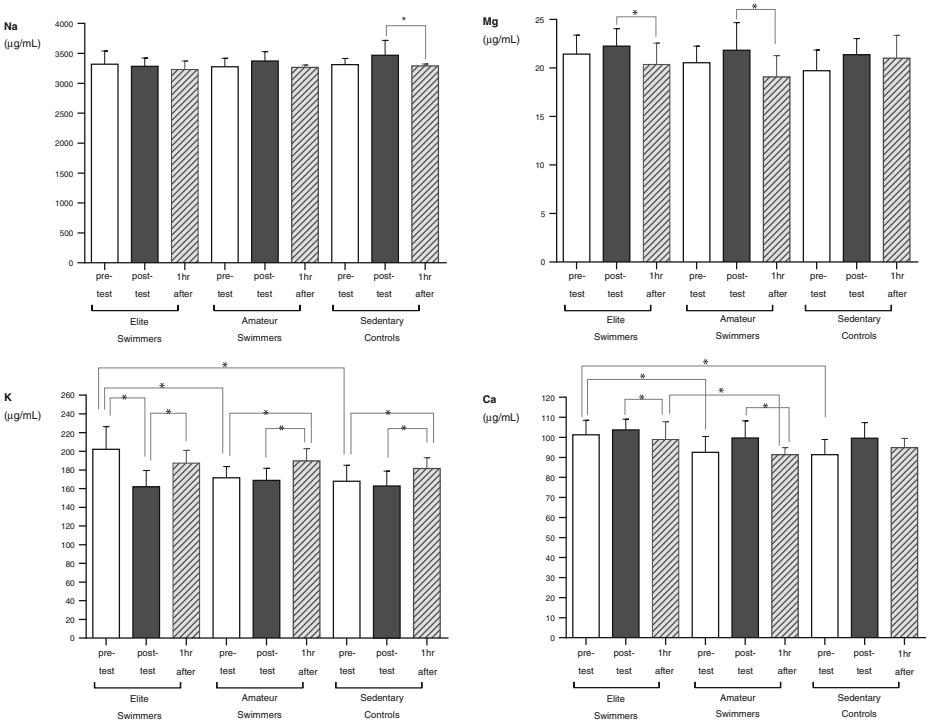

Fig. 1 Serum concentrations (mean±standard deviation) of some electrolytes (Na, K, Mg, Ca) evaluated in study groups set as elite swimmers (n=14), amateur swimmers (n=11), and sedentary controls (n=10). Three different sampling time points were selected: pre-test (before swimming), post-test (immediately after swimming), and 1 h after the activity. The p values were obtained with one-way ANOVA for inter-group comparisons and repeated measures ANOVA for within-group differences followed by post hoc test (Tukey's), and differences between groups were judged to be statistically significant when the p value was <0.05 (GraphPad Prism 6.01)

later values; however, there was no statistical difference in this regard (Fig. 2). It is of interest that the mean serum Cu concentrations of elite swimmers obtained 1 h after the test were significantly lower than the pre-test and post-test levels (p<0.05), whereas in the other groups, pre-test and 1-h-after results did not significantly differ. The mean pre-test Cu concentration was lower in amateur swimmers as compared with elite swimmers (p<0.05), while there was no statistical difference between elite swimmer and control groups (Fig. 2). In amateur swimmer and control groups, the mean post-test Cu levels were higher than pre-test and 1-h-after values (p<0.05). Regarding Zn concentrations (Fig. 2), the post-test Zn levels were significantly higher than pre-test and 1-h-after results (p<0.05), while the mean Zn concentrations obtained 1 h later were significantly lower than the pre-test levels (p<0.05) in all groups. As for serum Se concentrations, in elite swimmers, 1-h-after levels were lower with regard to pre-test and post-test values (p<0.05). On the other hand, in the amateur swimmers group, post-test values were higher than the pre-test and 1-h-later levels (p<0.05); despite a lack of statistical significance, a similar profile was noted in the control group for Se concentrations.

In regard with analytical methodology, some figure of merits including limit of detection (LOD) values of the studied elements, together with the internal standards giving most accurate results, are shown in Table 2. The spike recovery experiments showed that the recovery values were acceptable. An excess value in Se recovery, probably due to the increased ionization of Se in carbon-containing samples [20], was simply corrected after data acquisition. In addition, comparative analysis of pooled sera was performed by ICP-MS and GF-AAS for trace elements. The results (mean±confidence interval, expressed as nanogram per milliliter; n=4) obtained by ICP-MS vs. GF-AAS were highly consistent with each other at 95 % confidence level: Cr (0.185±0.006 vs. 0.182±0.020), Fe (970.4±8.1 vs. 975.3±13.6), Cu (735.3±5.7 vs. 736.8±14.5), Zn (667.5±6.4 vs. 669.0±10.7), and Se (133.5±1.0 vs. 133.8±3.6). Recovery values of internal standards were very stable during the analysis of 136 samples for 5 h, underpinning the robust plasma conditions (data not shown). A remarkable point of the method is the direct "dilute and shoot" procedure, allowing negligible contamination and low limit of detection values.

Discussion

In this study, the differences in serum element levels between study groups could obviously be attributed to different training schedules and intensity followed. Between-group comparisons revealed higher pre-test levels of serum Ca and K in elite swimmers as compared to other groups (Fig. 1, p<0.05) which may be related to adaptive mechanisms. Swimming has been shown to alter the expression of duodenal genes pertaining to transport of some ions including Ca and K as well as water homeostasis [21]. Therefore, higher pre-test serum Ca levels in elite swimmers (Fig. 1), at least in part, may be related with the enhanced intestinal Ca absorption via upregulation of Ca transporter genes as shown in rats [21]. In order to retain a long-term effect in Ca absorption, scheduled

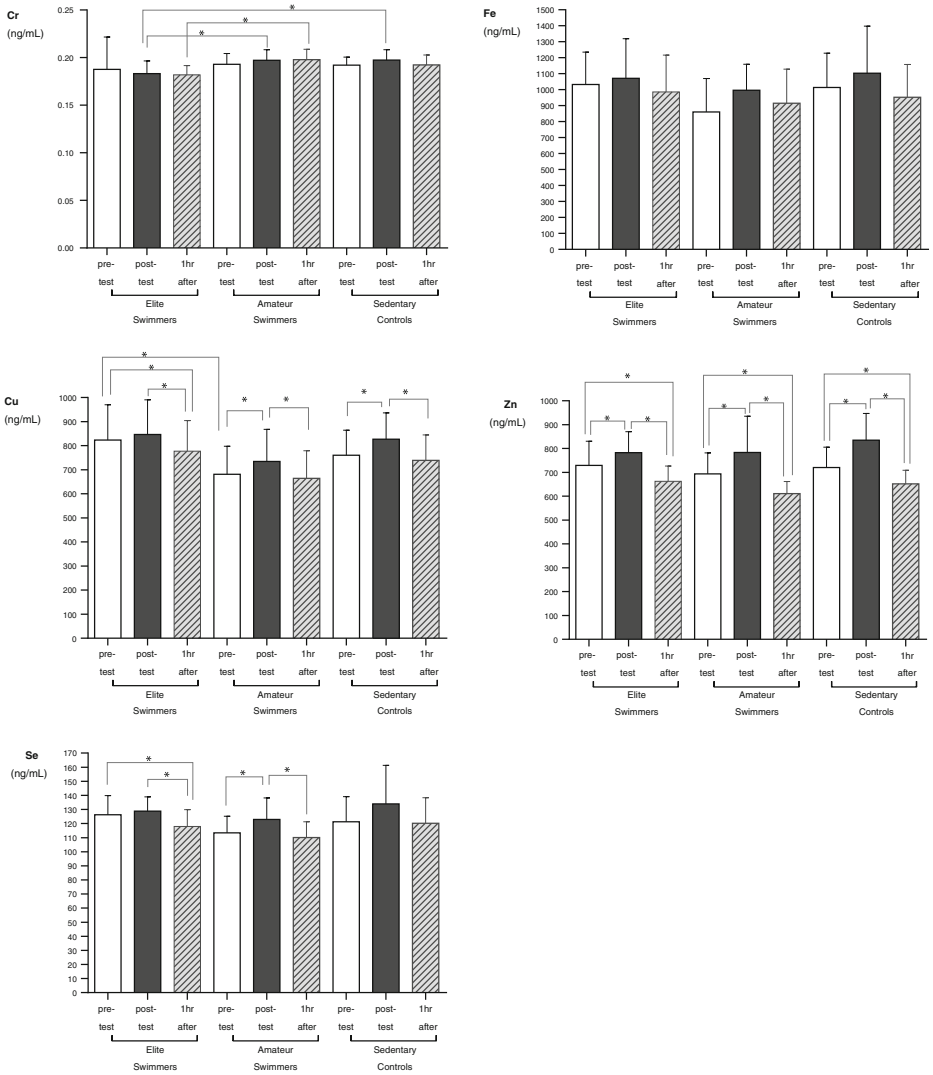

Fig. 2 Serum concentrations (mean±standard deviation) of some trace elements (Cr, Fe, Cu, Zn, and Se) evaluated in study groups set as elite swimmers (n=14), amateur swimmers (n=11), and sedentary controls (n=10). Three different sampling time points were selected: pre-test (before swimming), post-test (immediately after swimming), and 1 h after the activity. The p values were obtained with one-way ANOVA for inter-group comparisons and repeated measures ANOVA for within-group differences followed by post hoc test (Tukey's), and differences between groups were judged to be statistically significant when the p value was <0.05 (GraphPad Prism 6.01)

Table 2 Some figure of merits for determination of the analytes in serum samples using internal standards

Analyte isotope	Internal standard	Spike (ng/mL)	Recovery[a] (%)	RSD (%)	Correlation coefficient	LOD[b] (ng/mL)
^{52}Cr	Ge	1	98	3.7	0.9998	0.028
^{57}Fe	Ge	5	95	3.8	0.9991	0.140
^{63}Cu	Ge	5	99	1.8	0.9999	0.098
^{66}Zn	Ge	5	99	2.9	0.9998	0.994
^{78}Se	Ge	5	110	3.1	1.0000	0.350
^{23}Na	Sc	200	100	1.7	1.0000	3
^{24}Mg	Sc	200	97	2.0	1.0000	3
^{39}K	Sc	200	100	2.4	0.9999	22
^{43}Ca	Sc	200	98	2.4	0.9995	168

[a] Mean of three experiments

[b] Limit of detection for the method

training program may be suggested; however, the stimulatory effect of swimming on duodenal calcium absorption in rats has been found to be hindered by forced training or prolonged stress [22]. Consequently, a key point with this regard may be to consider exercise as a drug and the importance of dosing and individual variations [6]. The Cr levels also exhibited inter-group variances, especially in post-test results of elite swimmers, with lower levels, as compared to amateur swimmers and sedentary controls (Fig. 2). A decrease in mean Cr concentrations in blood samples after the marathon has been reported in athletes [5]. An extensive review in this field [23] indicates that endurance activity may exert a negative influence on Cr status possibly through enhancing urinary Cr excretion.

On the other hand, the serum element levels were evaluated at three sampling time points with regard to test (i.e., pre-test, post-test, and 1 h later). The significant fall in post-test K levels of elite swimmers (Fig. 1, p<0.05) and a very slight change in the other groups may be attributed to improved

uptake of plasma K^+ into muscle cells by training-induced upregulation of Na^+,K^+ pump [24-27]. Serum K levels have been shown to decrease immediately after training in male field hockey players, while higher concentrations have been observed 1 h later [16]. The significantly higher concentrations of K after 1 h as compared to post-test levels in all groups (Fig. 1) may be a consequence of test [28]. Since human skeletal muscles contain the largest single pool of K^+ in the body, even modest relative release may cause marked changes in plasma K concentrations [24].

Another interesting point regarding within-group differences was the similar pattern in Mg, Ca, Fe, Cu, Zn, and Se levels, showing higher post-test values in all groups. This finding might be explained as a consequence of exhaustion and water loss. As shown in Fig. 1, elite swimmer and amateur swimmer groups exhibited increased post-test Mg values which are consistent with previous studies underlining higher Mg levels after exercise [4, 10]. Westmoreland et al. have reported that high-intensity exercise induces an increase in plasma Mg, possibly as a compensatory mechanism that involves rapid mobilization of Mg from apatite crystals in bone [10]. Our finding regarding the lower serum Mg concentrations at 1-h-later samples of elite and amateur swimmer groups ($p<0.05$) was in line with a previous report in male field hockey players [16]. On the other hand, the only observed alteration in regard to Na in the control group 1 h later ($p<0.05$) may be a consequence of its stringent control in healthy individuals.

Present results highlight lower serum Cu, Zn, and Se concentrations in elite swimmers at 1 h after the test as compared with the pre-test and post-test levels (Fig. 2). Synergic role of Cu, Zn, and Se has been well known, due to their essential functions in antioxidant enzymes. Supplementation of Zn at physiologic doses to athletes, particularly in periods of intense exercise, has been suggested to be beneficial to antioxidant system and to physical performance [29]. Plasma Zn concentrations below normal levels in elite swimmers have been previously reported [12]. It is of note that in all groups, Zn concentrations 1 h after the test (Fig. 2) were found below reference range [19], pointing out a generalized potential Zn deficiency. In young amateur boxers, decline in plasma Cu and Zn levels after acute exercise and decrease in Zn concentrations after 4 weeks of regular boxing training have been shown [30]. This effect may be related to element loss since an increased urinary excretion of Cu and Zn during exercise has been demonstrated [13]. Other mechanisms such as unbalanced diet or Zn redistribution between plasma and erythrocytes may also contribute to this alteration. With regard to redistribution, significant decreases in the erythrocyte content of Zn as a consequence of acute effects of prolonged exercise and increase in hepatic Zn levels after daily physical training have been reported [31]. Moreover, the change is more elevated at the end of a competitive season [31].

It can be concluded that, apart from the swimming test applied, observed inter-group variations between pre-test levels of study groups may be a consequence of training frequency. On the other hand, within-group variations might be related to dehydration and compartmental element shifts due to swimming test. Although the elemental homeostatic alteration might be usually transient, it could be sufficient to predispose the individual to become more susceptible to potential health concerns. Therefore, regular control of elemental status to be aware of the potential impacts of strenuous exercise can be suggested to individuals especially engaged professionally in sports. Integration of the present methodology with approaches such as metallomics [32, 33] to probe such stimuli is among our future work.

Acknowledgments The Scientific and Technological Research Council of Turkey, TUBITAK (project no. 112T365), State Planning Organization, DPT (contract no. 2010 K120720), and Çankırı Karatekin University Scientific Research Project Unit, BAP (contract no: 2011/26) are acknowledged for partial financial support for this work.

Conflict of Interest The authors declare that they have no conflict of interest.

References

1. Letsiou S, Nomikos T, Panagiotakos D, Pergantis SA, Fragopoulou E, Antonopoulou S, Pitsavos C, Stefanadis C (2009) Serum total selenium status in Greek adults and its relation to age. The ATTICA study cohort. Biol Trace Elem Res 128:8–17
2. Döker S, Mounicou S, Dogan M, Lobinski R (2010) Probing the effects of the administration of chromium (VI) to mouse on the metal homeostasis by ICP MS and size-exclusion chromatography-ICP MS. Metallomics 2:548–555
3. Love ST, Di Bona KR, Sinha SH, McAdory D, Skinner BR, Rasco JF, Vincent JB (2013) Urinary chromium excretion in response to an insulin challenge is not a biomarker for chromium status. Biol Trace Elem Res 152:57–65
4. Navas FJ, Martin JF, Cordova A (1997) Compartmental shifts of calcium and magnesium as a result of swimming and swimming training in rats. Med Sci Sports Exerc 29:882–891
5. Berger CE, Kröner A, Kluger R, Baron R, Steffan I, Engel A (2002) Effects of marathon running on the trace minerals chromium, cobalt, nickel, and molybdenum. J Trace Elem Exp Med 15:201–209
6. Vina J, Sanchis-Gomar F, Martinez-Bello V, Gomez-Cabrera MC (2012) Exercise acts as a drug; the pharmacological benefits of exercise. Br J Pharmacol 167:1–12
7. Fagard RH (2012) Physical activity, fitness and mortality. J Hypertens 30:1310–1312
8. Naziroğlu M, Kilinç F, Uğuz AC, Celik O, Bal R, Butterworth PJ, Baydar ML (2010) Oral vitamin C and E combination modulates blood lipid peroxidation and antioxidant vitamin levels in maximal exercising basketball players. Cell Biochem Funct 28:300–305
9. Aydin C, Ince E, Koparan S, Cangul IT, Naziroglu M, Ak F (2007) Protective effects of long term dietary restriction on swimming exercise-induced oxidative stress in the liver, heart and kidney of rat. Cell Biochem Funct 25:129–137

10. Westmoreland D, Anderson D, Porta S (2006) Effects of maximal and submaximal exercise on plasma electrolyte shifts. Trace Elem Electrolyte 23:312–317
11. Wang L, Zhang J, Wang J, He W, Huang H (2012) Effects of high-intensity training and resumed training on macroelement and microelement of elite basketball athletes. Biol Trace Elem Res 149:148–154
12. Giolo De Carvalho F, Rosa FT, Marques Miguel Suen V, Freitas EC, Padovan GJ, Marchini JS (2012) Evidence of zinc deficiency in competitive swimmers. Nutrition 28:1127–1131
13. Kikukawa A, Kobayashi A (2002) Changes in urinary zinc and copper with strenuous physical exercise. Aviat Space Environ Med 73:991–995
14. Baltaci AK, Uzun A, Kilic M, Mogulkoc R (2009) Effects of acute swimming exercise on some elements in rats. Biol Trace Elem Res 127:148–153
15. Kuru O, Sentürk UK, Gündüz F, Aktekin B, Aktekin MR (2003) Effect of long-term swimming exercise on zinc, magnesium, and copper distribution in aged rats. Biol Trace Elem Res 93:105–112
16. Hazar M, Sever O, Otag A (2012) Physiological responses of macro-elements to maximal aerobic exercise among elite women and men field hockey players. Health Med 6:3084–3090
17. Nunes BR, Magalhaes CG, da Silva JBB (2002) Fast determination of chromium in human serum by electrothermal atomic absorption spectrometry. J Anal At Spectrom 17:1335–1338
18. Döker S, Uzun L, Denizli A (2013) Arsenic speciation in water and snow samples by adsorption onto PHEMA in a micro-pipette-tip and GFAAS detection applying large-volume injection. Talanta 103:123–129
19. Wu AHB (ed) (2006) Tietz clinical guide to laboratory tests. 5th edition. Saunders, St. Louis
20. Labat L, Dehon B, Lhermitte M (2003) Rapid and simple determination of selenium in blood serum by inductively coupled plasma-mass spectrometry (ICP-MS). Anal Bioanal Chem 376:270–273
21. Teerapornpuntakit J, Dorkkam N, Wongdee K, Krishnamra N, Charoenphandhu N (2009) Endurance swimming stimulates transepithelial calcium transport and alters the expression of genes related to calcium absorption in the intestine of rats. Am J Physiol Endocrinol Metabol 296:E775–E786
22. Charoenphandhu N, Teerapornpuntakit J, Lapmanee S, Dorkkam N, Krishnamra N, Charoenphandhu J (2011) Long-term swimming in an inescapable stressful environment attenuates the stimulatory effect of endurance swimming on duodenal calcium absorption in rats. J Physiol Sci 61:473–486
23. Speich M, Pineau A, Ballereau F (2001) Minerals, trace elements and related biological variables in athletes and during physical activity. Clin Chim Acta 312:1–11
24. Clausen T (2010) Hormonal and pharmacological modification of plasma potassium homeostasis. Fundam Clin Pharmacol 24: 595–605
25. Nielsen JJ, Mohr M, Klarskov C, Kristensen M, Krustrup P, Juel C, Bangsbo J (2004) Effects of high-intensity intermittent training on potassium kinetics and performance in human skeletal muscle. J Physiol 554(Pt3):857–870
26. Clausen T (2003) Na^+-K^+ pump regulation and skeletal muscle contractility. Physiol Rev 83:1269–1324
27. Nordsborg NB, Kusuhara K, Hellsten Y, Lyngby S, Lundby C, Madsen K, Pilegaard H (2010) Contraction-induced changes in skeletal muscle Na^+, K^+ pump mRNA expression—importance of exercise intensity and Ca^{2+}-mediated signalling. Acta Physiol 198:487–498
28. González-Haro C, Soria M, Lopez-Colon JL, Llorente MT, Escanero JF (2011) Plasma trace elements levels are not altered by submaximal exercise intensities in well-trained endurance euhydrated athletes. J Trace Elem Med Biol 25S:S54–S58
29. Kara E, Gunay M, Cicioglu I, Ozal M, Kilic M, Mogulkoc R, Baltaci AK (2010) Effect of zinc supplementation on antioxidant activity in young wrestlers. Biol Trace Elem Res 134:55–63
30. Karakukcu C, Polat Y, Torun YA, Pac AK (2013) The effects of acute and regular exercise on calcium, phosphorus and trace elements in young amateur boxers. Clin Lab 59:557–562
31. Micheletti A, Rossi R, Rufini S (2001) Zinc status in athletes: relation to diet and exercise. Sports Med 31:577–582
32. Mounicou S, Szpunar J, Lobinski R (2009) Metallomics: the concept and methodology. Chem Soc Rev 38:1119–1138
33. Döker S, Bosgelmez II, Guvendik G (2011) Metallomics as a junction between life sciences. Hacet J Biol Chem 39:173–188