



# Effect of an Acute Exercise Until Exhaustion on the Serum and Urinary Concentrations of Cobalt, Copper, and Manganese Among Well-Trained Athletes

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## Abstract

The current information about the effect of physical exercise on the body concentrations of several minerals is still limited, both in the acute (short-term) and adaptive (long-term) responses. So, this manuscript aims, on the one hand, to assess the possible differences on basal levels of cobalt (Co), copper (Cu), and manganese (Mn) concentrations in serum and urine between athletes and sedentary participants and, on the other hand, to evaluate the effect of an acute progressive physical exercise until voluntary exhaustion on the serum and urinary concentrations of Co, Cu, and Mn. Two groups participated in this survey, one was formed by untrained, sedentary males (CG;  $n = 26$ ), and the other group was constituted by national endurance (long and middle distances) athletes (AG;  $n = 21$ ). All participants were from the same region of Spain. Participants of both groups performed a physical test on a treadmill, reaching voluntary exhaustion. Blood and urine samples of each participant were collected before and after the tests. Once obtained and processed, the concentrations of Co, Cu, and Mn elements were analyzed by inductively coupled plasma mass spectrometry (ICP-MS). The differences in the studied variables were evaluated using a mixed model by means of an ANOVA and Bonferroni post hoc tests. In the comparison of the pre-test values between groups, the results showed that serum concentrations of Mn were significantly lower in CG than in AG ( $p < 0.01$ ). In urine, Co and Mn levels were significantly higher among CG participants ( $p < 0.01$ ) than among AG ones, while in the case of Cu, the values were lower ( $p < 0.01$ ) in the CG than in the AG. Regarding the effects of the effort tests, no significant changes were found among the participants of the CG. It was observed that the serum concentrations of Co ( $p < 0.05$ ) and Cu ( $p < 0.01$ ) decreased after the test among the AG participants. Also, the results showed that there were no statistical differences in Co and Mn values (expressed in  $\mu\text{g/g}$  creatinine). However, the urinary post-test Cu concentrations were lower ( $p < 0.05$ ) among AG participants. In basal conditions, serum concentrations of Mn were significantly lower in CG than in AG. In urine, Co and Mn levels were significantly higher among CG participants and Cu level was significantly lower in CG, a fact which may reflect adaptive responses to exercise. An incremental exercise to exhaustion in AG produces a decrease in Co and Cu serum concentrations, as well as in urinary excretion of Cu.

**Keywords** Physical activity · Performance · Minerals · Blood · Urine

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## Introduction

Essential trace minerals are elements necessary for the human body due to the large number of biological functions in which they are involved, especially those linked to physical exercise. However, the current information about the effect of physical exercise in the body mineral levels is still limited, both in the acute (short-term) or adaptive (long-term) responses.

Cobalt (Co) is an essential element which enters in the composition of vitamin B12 and increases erythropoiesis [1]. Soluble cobalt supplements are increasingly being marketed for consumers interested in healthy practices, with recommended daily doses up to 1000 µg/day. In the field of endurance sports, some athletes may consider the use of Co supplements as blood doping agents, due to the role of Co in erythropoiesis [2, 3].

Copper (Cu) is an active compound in two antioxidant enzymes: ceruloplasmin and Cu-Zn superoxide dismutase (Cu-Zn-SOD) [4, 5]. The antioxidant function reduces the oxidative damage caused by free radicals, which is thought to induce fatigue and to delay muscle recovery [6, 7].

In this sense, the production of reactive oxygen species and endogenous tumor necrosis factor (TNF) alpha and interleukin (IL) 1 beta induced by exercise leads to the activation of manganese superoxide dismutase (Mn-SOD), which develops critical roles in the cardioprotective biphasic responses against ischemia/reperfusion injury [8, 9]. Mn is an integral compound of Mn-SOD, an essential antioxidant enzyme. Mn is also part of other important metabolic enzymes such as pyruvate carboxylase, a key compound in the process of gluconeogenesis, and arginase, an important enzyme in the metabolism of urea [10].

So, the aim of the present study was to determine the acute effect of a controlled physical test until extenuation on the serum and urinary concentrations of cobalt (Co), copper (Cu), and manganese (Mn) among athletes and untrained participants.

## Materials and Methods

### Participants

Twenty-one national long-distance male athletes (AG) (age  $23.21 \pm 3.89$  years) and 26 male students who had not been practicing any daily physical training (CG) (age  $22.10 \pm 3.41$  years) participated in this survey. All of them had been living in the same region of Extremadura (Spain) for at least 2 years before the beginning of the survey. The athletes had been performing daily physical training for, at least, 2 years, recording a weekly average distance of 120 km competitive aerobic physical training. During this period, their physical training routines were monitored weekly, being 15–25 km/

week above anaerobic threshold and 90–100 km/week in aerobic conditions. Best and worst records of athletes were 3:37.79 and 4:09.57 in 1500 m and 13:11.01 and 15:10.35 in 5000 m. The weekly training routines consisted of 3–4 days/week of continued aerobic running or fartlek and 2–3 days/week of intense series and interval training [11] depending on whether or not there was a competition at the weekend. The survey was carried out after the beginning of the training period.

All participants were informed about the aim and procedures of the study; they gave their informed consents and participated voluntarily. The study was approved by the Ethics Committee of the University of Extremadura in accordance with the latest version of the Helsinki declaration for human research.

### Anthropometrics Measurements

The morphological characteristics of the participants were measured in the morning, always at the same time and in identical conditions. Body height was measured to the nearest 0.1 cm using a wallmounted stadiometer (Seca 220, Hamburg, Germany). Body weight was measured to the nearest 0.01 kg using calibrated electronic digital scales (Seca 769, Hamburg, Germany) in nude, barefoot conditions.

Body fat content was estimated from the sum of four skinfolds (abdominal, suprailiac, tricipital, and subscapularis:  $\Sigma 4$ ) and the sum of six skinfolds ( $\Sigma 4$  plus thigh and calf skinfolds:  $\Sigma 6$ ). Skinfold thickness was measured with a Harpenden caliper (Holtain Skinfold Caliper, Crosswell, UK) and converted to % of body fat using the equations of Jackson and Pollock (1985) [12]. All anthropometric measurements were made by the same operator, skilled in kinanthropometry techniques, in accordance with the International Society for the Advancement of Kinanthropometry guidelines. Heart rate and blood pressure were determined using an automatic sphygmomanometer (Omron HEM-780, Osaka, Japan) after a 5-min rest period in supine position.

### Nutritional Evaluation

In order to ensure that all participants were not taking any vitamins, minerals, or other supplements and, in order to guarantee that all of them were following a similar diet, all participants completed a nutritional questionnaire. The questionnaire consisted of a daily nutritional record of duration of 3 days, completed in two weekdays and in one weekend day. In each day, participants indicated the type, frequency, and quantity (in grams) of every food consumed in every meal. The results of their responses were transformed to values of macro and micronutrients using different nutritional databases [13–15].

## Physical Activity

An exercise test was used to evaluate the physical performance of each participant. The test consisted of running on a treadmill (Powerjoc, UK) until voluntary exhaustion. The treadmill was equipped with a gas analyzer (Metamax, Cortex Biophysik, GmbH, Germany) and a Polar pulsometer (Polar, Norway). In order to guarantee a pre-warming phase before the test, all participants ran progressively for 15 min. Controls completed 5 min at 6 km/h, 5 min at 7 km/h, and 5 min at 8 km/h while athletes ran at 8, 9, and 10 km/h respectively. All participants started the tests just after the warm-up phase.

The protocol of the tests consisted in running incrementally in stages, until voluntary exhaustion (no chance to keep running), starting at an initial speed of 8 km/h for controls and 10 km/h for athletes, increasing the speed in 1 km/h every 400 m, with a stable slope of 1%. Anaerobic threshold was determined by ventilatory parameters. The tests were configured in order to guaranty a distance enough to achieve the same physiological changes induced by a standardized field test. All tests were performed in the morning (between 10 am and 12 pm) within the recommended parameters [16]. Training intensity and volume were reduced the two previous days, applying regenerative load in order to avoid fatigue in the physical evaluations.

## Sample Collection

### Blood Samples

Two extractions of 5 mL of venous blood were extracted from the antecubital vein of each participant, after a fasting period of 8 h. All extractions were made with a plastic syringe fitted with a stainless-steel needle. The first sample was extracted before the exercise test and the second one just after it. Once extracted, the samples were collected into a metal-free polypropylene tube (previously washed with diluted nitric acid). The day of the test, after the first blood extractions, all participants ingested a similar breakfast, consisting of a bottle of glucosamine solution drink, which did not contain any of the minerals studied.

Once obtained, the blood samples were allowed to clot, then centrifuged at 2500 rpm for 10 min at room temperature to isolate the serum. The serum was aliquoted into an Eppendorf tube (previously washed with diluted nitric acid) and conserved at  $-80^{\circ}\text{C}$  until biochemical analysis.

**Determination of Hematocrit and Hemoglobin** The hematocrits were obtained by centrifuging the whole blood into a glass capillary containing heparin in a Microcen microfuge (Alresa, Spain). Hemoglobin (Hb) was determined using a Hb analyzer (HemoCue, Sweden). Both hematocrit and Hb

were used to correct the changes in plasma volume by mean of the Dill and Costill equations [17].

### Urine Samples

Additionally, urine was obtained from each participant before and after the test, simultaneously to the blood extractions. The urine samples were collected in polyethylene tubes previously washed with diluted nitric acid and frozen at  $-80^{\circ}\text{C}$  until analysis. Before the analysis, the samples were thawed at room temperature and homogenized by shaking.

**Creatinine Determination** Creatinine concentrations were measured in all urine samples to determine different dilution degrees [18], using Sigma's Creatinine 555-A kit and a UNICAM 5625 spectrophotometer.

### Serum and Urinary Trace Elements Determination

**Samples Preparation** Co, Cu, and Mn analyses were performed by inductively coupled plasma mass spectrometry (ICP-MS). To prepare samples for analysis, the decomposition of the organic matrix was made by heating it for 10 h at  $90^{\circ}\text{C}$  after the addition of 0.8 mL  $\text{HNO}_3$  and 0.4 mL  $\text{H}_2\text{O}_2$  to 2 mL of the serum or the urine samples. The samples were then dried at  $200^{\circ}\text{C}$  on a hot plate. Sample reconstitution was carried out by adding 0.5 mL of nitric acid, 10  $\mu\text{L}$  of indium (In) (10 mg/L) as internal standard, and ultrapure water to complete 10 mL.

**Standard and Reference Materials Preparation** Reagent blanks, element standards, and certified reference material (Seronorm, lot 0511545, Sero AS Billingstand, Norway) were prepared identically and were used for accuracy testing. Before the analysis, the commercial control materials were diluted according to the recommendations of the manufacturer.

**Samples Analysis** Digested solutions were assayed by an ICP-MS Nexion model 300D (PerkinElmer, Inc., Shelton, CT, USA) equipped with a triple quadrupole mass detector and a reaction cell/collision device that allows operation in three modes: without reaction gas (STD), by kinetic energy discrimination with helium as the collision gas (KED), and in reaction mode with ammonia as the reaction gas (DRC). Both collision and reaction gases such as plasmatic argon had a purity of 99.999% and were supplied by Praxair (Madrid, Spain). Two mass flow controllers regulated gas flows. The frequency of the generator was free-swinging and worked at 40 Mhz. Three replicates were analyzed per sample. The sample quantifications were performed with indium (In) as an internal standard. The values of the standard materials of each element

(10 µg/L) used for quality controls were in agreement with intra and inter-assay variation coefficients of less than 5%.

## Statistical Analysis

Statistical analyses were carried out with the software IBM SPSS Statistics 22.0 for Windows. The results are expressed as  $x \pm sd$ , where  $x$  is the mean value and  $sd$  the standard deviations. The statistical evaluation consisted of a Kolmogorov–Smirnov test to verify the normality of the data, and to analyze the differences between groups in basal conditions, an ANOVA test was used. To evaluate the differences before and after the tests, we used a post hoc test of Bonferroni. A  $p < 0.05$  was considered statistically significant.

## Results

### Anthropometric Characteristics of Participants

Table 1 shows the anthropometric data of CG and AG in rest conditions. Total weight ( $p < 0.01$ ), body mass index ( $p < 0.01$ ), percentage of body fat ( $p < 0.01$ ), and skinfold ( $p < 0.01$ ) differed between CG and AG groups at baseline.

### Nutritional Parameters

Table 2 shows the intake of the studied elements the days before the survey. For each element, its reference daily intake is shown. Not significant differences were present in the study.

### Ergoespirometrics Parameters

Table 3 shows the results of the ergoespirometrics parameters at the beginning and at the end of the effort tests. A significant increase in all variables was observed in both groups after the test. Maximal distance, oxygen uptake ( $VO_2$ ), and minute

**Table 1** Resting anthropometric and cardiovascular characteristics of all participants

	Controls ( $n = 26$ )	Athletes ( $n = 21$ )
Age (years)	22.65 ± 3.65	21.62 ± 4.27
Height (m)	1.77 ± 0.05	1.75 ± 0.06
Weight (kg)	76.94 ± 11.07	64.68 ± 7.25**
BMI (kg/m <sup>2</sup> )	21.81 ± 3.14	18.25 ± 1.73*
% body fat	12.28 ± 2.87	8.45 ± 1.03**
∑6 skinfold (mm)	62.76 ± 12.23	47.57 ± 10.68**

\* $p < 0.05$ ; \*\* $p < 0.01$ , differences between the initial values of groups (ANOVA test)

**Table 2** Food intake of trace elements with essential functions demonstrated: cobalt (Co), copper (Cu), and manganese (Mn) in controls and athletes throughout the study

Trace elements	Controls ( $n = 26$ )	Athletes ( $n = 21$ )
Co (200–300 µg/day)	253.71 ± 136.3	220.71 ± 146.53
Cu (2000–300 µg/day)	1842.1 ± 636.21	1715.0 ± 615.01
Mn (2500–5000 µg/day)	3053.6 ± 1222.1	3216.8 ± 1360.2

ventilation (VE) were significantly lower among CG in comparison to AG. The lowest values of maximal HR and RER in basal conditions were found among AG.

### Weight, Hemoglobin, Hematocrit, and Urinary Creatinine

Table 4 shows the results of the parameters linked to the changes in blood and urine concentrations during exercise. Total weight significantly decreased after the tests in CG ( $p = 0.001$ ) and AG ( $p = 0.001$ ), while post-test hemoglobin (CG  $p = 0.021$ ; AG  $p = 0.011$ ) and hematocrit (CG  $p = 0.009$ ; AG  $p = 0.010$ ) values increased in both groups. Urinary creatinine remained unchanged after the test in both groups.

### Serum Concentrations of Minerals

Table 5 shows serum concentrations of each metal before and after the performance of the effort tests. The results are expressed with and without hemoconcentration corrections.

When the initial basal concentrations of elements were compared between the two groups, it can be observed that only Mn ( $p < 0.01$ ) concentrations were significantly lower in CG than in AG.

For the comparisons between pre- and post-test values, it can be observed that Cu increased only in the CG group ( $p < 0.05$ ). But if the corrections for hemoconcentration are applied, this difference was no longer significant.

Among AG participants, no statistical differences were observed in the case of Mn, both for corrected and uncorrected values. In the cases of Co ( $p = 0.043$ ) and Cu ( $p = 0.017$ ), the serum values suffered a significant decrease after the test, being observable just when the hemoconcentrations were applied.

### Urinary Concentrations of Elements

Table 6 shows the urinary concentrations of each metal before and after the exercise tests in both groups. Results are expressed with and without creatinine corrections.

In the case of the differences in the initial basal concentrations between the two groups, it can be found that the values of Co without ( $p < 0.01$ ) and with ( $p < 0.05$ ) corrections and Mn ( $p < 0.001$  and  $p < 0.01$  respectively) were higher among

**Table 3** Ergospirometric results of controls and athletes at the beginning and at the end of the exercise test

	Controls ( <i>n</i> = 26)			Athletes ( <i>n</i> = 21)		
	Before	After	<i>p</i> value	Before	After	<i>p</i> value
Distance (m)	0 ± 0	3112 ± 417	0.000	0 ± 0	4429 ± 557	0.000
HR (bpm)	62.76 ± 12.8	195.4 ± 8.7	0.000	53.76 ± 11.91**	192.7 ± 7.6	0.000
VO <sub>2</sub> (mL/kg/min)	10.46 ± 8.47	46.14 ± 5.16	0.000	12.55 ± 7.12	66.9 ± 9.7	0.000
VE (L/min)	18.46 ± 11.47	99.26 ± 16.63	0.000	23.48 ± 17.36	136 ± 22.5	0.000
RER	0.78 ± 0.18	1.18 ± 0.05	0.000	0.75 ± 0.17	1.036 ± 0.04	0.000

HR heart rate, VO<sub>2</sub> oxygen uptake, VE pulmonary ventilation, RER respiratory exchange ratio

\*\**p* < 0.01, differences between the initial values of groups (ANOVA test)

*p* value: post hoc Bonferroni test

the CG participants than in the AG ones. Corrected (*p* < 0.001) and uncorrected (*p* < 0.01) Cu concentrations were lower among in the CG than in the AG.

For the comparison of the values before and after the tests, no statistical changes can be observed in the cases of Co, Cu, and Mn, both in the corrected and uncorrected forms among CG participants. In case of the AG, no statistical changes were found for Co and Mn. Oppositely, urinary Cu decreased after the effort tests, both for corrected (*p* = 0.021) and uncorrected variables (*p* = 0.049).

## Discussion

For a better understanding, the discussion will be ordered in two parts. First, the differences between initial basal concentrations of minerals in CG and AG will be discussed. Then, the effects of the exercise tests in serum and urinary mineral concentrations will be analyzed.

On the first hand, the nutritional analysis (Table 2) showed that the intake of all studied minerals was similar in both groups of participants, as well as similar basal serum concentrations of Co and Cu. However, Mn basal concentrations were higher (*p* < 0.001) among athletes. For the case of urine, the concentrations of Co and Mn were higher in the CG than in the AG, losing statistical significance if the corrections for

creatinine are applied. Oppositely, the urinary Cu values were lower among CG participants.

In all cases, all serum and urinary metal concentrations were within the normal values reported in previous surveys [19–21], which were developed with a similar technique and expressed in the same units (µg/L).

In this sense, Berger et al. (2002) found similar concentrations in the whole blood of Co, in marathon runners [22]. In the present survey, the minor urinary Co elimination among AG participants, with normal serum levels, may be due to an adaptive response of the body to maintain its concentrations. This fact may be due to the essential role of this element in one of the most important adaptations to endurance training, red blood cell synthesis, especially since it is known that inorganic Co is a non-specific stimulant of erythropoiesis [1, 23]. For this reason, cobalt chloride can be utilized for sportsmen. In this sense, Krug et al. (2014) found in their study that the urinary elimination of Co yielded concentrations between 40 and 318 ng/mL during the first 6 h post-administration of Co, being the levels remained elevated (> 22 ng/mL) up to 33 h [24]. In the present study, the AG concentrations of Co were 43 ± 35 ng/mL, indicating no illicit use of cobalt among the athletes.

Similar to our study, Gropper et al. (2003) and Resina et al. (1990) found that mean serum Cu concentrations among aerobic sportsmen were within the normal range

**Table 4** Total body weight, hemoglobin, and hematocrit, before and after the exercise test

	Controls ( <i>n</i> = 26)			Athletes ( <i>n</i> = 21)		
	Before	After	<i>p</i> value	Before	After	<i>p</i> value
Weight (kg)	75.12 ± 8.17	73.21 ± 9.32	0.001	65.05 ± 7.11*	64.09 ± 7.06	0.001
Hemat (%)	45.72 ± 4.40	46.31 ± 3.97	0.009	43.71 ± 4.19	44.76 ± 6.19	0.010
Hb (g/dL)	14.68 ± 1.31	14.81 ± 2.07	0.021	14.57 ± 1.39	14.92 ± 2.07	0.011
Creat (mg/dL)	1.69 ± 0.43	1.72 ± 0.48	0.143	1.61 ± 0.54	1.66 ± 0.59	0.152

Hemat hematocrit, Hb hemoglobin, creat urinary creatinine

\**p* < 0.05, differences between the initial values of groups (ANOVA test)

*p* value: post hoc Bonferroni test



**Table 5** Serum concentrations of trace elements in controls and athletes before and after the exercise test, without and with corrections (-C) for possible hemoconcentration

	Controls ( <i>n</i> = 26)			Athletes ( <i>n</i> = 21)		
	Before	After	<i>p</i> value	Before	After	<i>p</i> value
Co (μg/L)	0.810 ± 0.40	0.803 ± 0.41	0.087	0.752 ± 0.25	0.716 ± 0.14	0.100
Co-C (μg/L)	0.810 ± 0.40	0.790 ± 0.39	0.345	0.752 ± 0.25	0.655 ± 0.12	0.043
Cu (μg/L)	750.46 ± 113.47	796.22 ± 122.16	0.044	739.45 ± 127.24	722.38 ± 123.49	0.091
Cu-C (μg/L)	750.46 ± 113.47	766.26 ± 124.63	0.175	739.45 ± 127.24	664.74 ± 127.28	0.017
Mn (μg/L)	1.18 ± 0.41	1.21 ± 0.39	0.290	3.50 ± 2.40**	3.77 ± 1.58	0.150
Mn-C (μg/L)	1.18 ± 0.41	1.099 ± 0.45	0.225	3.50 ± 2.40**	3.44 ± 1.49	0.125

\*\**p* < 0.001, differences between the initial values of groups (ANOVA test)

*p* value: post hoc Bonferroni test

and did not differ significantly to the values of the control participants [25, 26]. However, Resina et al. (1990) found in 19 soccer players lower serum ceruloplasmin levels and lower serum ceruloplasmin biological activity, in comparison to the respective levels of the control group. These results suggest that more attention should be paid to serum copper and ceruloplasmin levels among sportsmen. Therefore, normal levels of Cu in serum do not necessarily exclude a deficiency in the activity of other Cu-proteins such as Cu-Zn SOD, being the main cause of this situation the possible urinary elimination of this mineral. One previous study indicated that physical exercise results in large increases in urinary excretion of Cu [27]. The increased urinary Cu obtained in this survey among AG participants may be related to the biological mobilization of this mineral induced by physical training, as it was described by Kikukawa and Kobayashi in 2002 [28].

The high serum Mn concentration found among AG participants could also be caused by a possible iron deficiency in athletes, fact that would increase Mn absorption, as indicated by Park et al. (2013).

Regarding the effect of the effort test, it can be appreciated that the efforts performed by participants induced a high physiological stress (Table 3), which lead to changes in post-test hematocrit and hemoglobin values (Table 4), as well as in body weight, possibly due to sweat losses. This fact can also have affected the concentrations of blood and urine minerals. So, in terms of reliability, when physical effort is performed, it is desirable to use the hemoglobin and hematocrit corrections [17] for post-test blood samples and the urinary creatinine corrections [18] for post-test urine values.

In the case of post-test serum Co values, it was found that in the AG, the concentrations decreased significantly (*p* < 0.05) after the acute physical effort only if a hemoconcentration correction was applied. No significant changes were reported in the urinary concentrations of this element.

In the case of Cu, the serum concentrations among CG participants increased (*p* < 0.05), being this change significant only without corrections for plasma volume, so it could be possible that this increase would be due to a hemoconcentration after the test. In the AG, a decrease (*p* < 0.01) was observed in the serum concentrations of Cu

**Table 6** Urinary concentrations of trace elements in controls and athletes before and after the exercise test, without and with (-C) corrections for urinary creatinine

	Controls ( <i>n</i> = 26)			Athletes ( <i>n</i> = 21)		
	Before	After	<i>p</i> value	Before	After	<i>p</i> value
Co (μg/L)	0.707 ± 0.96	0.729 ± 0.92	0.185	0.043 ± 0.065 **	0.083 ± 0.19	0.153
Co-C (μg/g creat)	0.434 ± 0.65	0.430 ± 0.70	0.176	0.038 ± 0.08*	0.055 ± 0.12	0.278
Cu (μg/L)	34.37 ± 16.81	36.14 ± 13.19	0.155	82.23 ± 23.44***	69.53 ± 26.82	0.049
Cu-C (μg/g creat)	19.66 ± 8.93	21.26 ± 14.63	0.278	55.18 ± 18.94**	43.96 ± 14.57	0.021
Mn (μg/L)	8.46 ± 13.85	8.41 ± 14.40	0.105	2.86 ± 3.83***	1.88 ± 3.35	0.175
Mn-C (μg/g creat)	5.58 ± 1.46	4.88 ± 0.46	0.210	1.64 ± 0.98**	1.10 ± 0.73	0.087

\**p* < 0.05; \*\**p* < 0.01; \*\*\**p* < 0.001, differences between the initial values of groups (ANOVA test)

*p* value: post hoc Bonferroni test

after the test, only observable when the hemocorrections are applied.

In urine, a greater elimination of Cu ( $p < 0.05$ ) among AG participants was observed at the end of the test, being greater when the urinary corrections are applied. However, a physical activity of this type did not modify the serum or urinary concentrations of Mn in either group.

Berger et al. [22] reported no changes in whole blood Co concentrations after running a marathon. No more research concerning serum Co and acute exercise has been found. The lower post-test Co values reported in the present survey can be explained by two different hypotheses. On the one hand, as exercise produces sweat losses of Co, in AG, this mineral could be lost via sweating, and the high-level training programs performed by these athletes can induce daily sweat losses which may affect the obtained results. On the second hand, the results could be due to a Co redistribution from body fluids to cells and tissues involved in exercise metabolisms, as it has been described in the case of other elements [29].

The outcomes of previous studies about changes in the blood Cu concentrations induced by physical efforts are sometimes discordant and seem to be related to the type of the exercise performed. Granell (2014), in control participants, observed that after 40 min of running on a treadmill at an intensity corresponding to the anaerobic threshold and after a muscular resistance test until exhaustion, a significant increase of serum Cu was observed [27]. This report is similar to the results obtained in our study but only in the CG and for uncorrected values. It should be pointed out that these authors did not make corrections for hemoconcentration, fact which could have affected their results, as occurred in the present survey. Oppositely, Pourvaghar and Shahsavar (2009) did not find changes in serum Cu after a Bruce test on a treadmill [30]. However, Savas (2009) found in trained subjects, as in the present survey, a significant decrease in serum Cu after a maximal aerobic effort test [31]. These conclusions were also pointed out by Hazar (2009) in rowers [32]. These results could indicate a different response to maximal efforts, depending on the training status.

In relation to the urine, Rodriguez and Diaz (1995) found that people who practiced daily physical activity presented a diminution in the urinary Cu elimination [33]. In this sense, Marrella et al. (1993) found a decrease in the urine Cu values after running a marathon [34]. This diminution in urinary excretion among trained subjects could be produced as an adaptive measure to avoid a decrease in the body Cu content, which may induce a diminution in performance or an increase in muscle tissue damage.

The present results manifest the importance of hemoconcentration and dehydration corrections in the analysis of blood and urinary values after a physical effort test. The explanation of this idea is based on the body water losses suffered as consequence of an increased sweating rate induced

by physical exercise, especially in hyperthermic conditions. These losses decrease the total amount of body water and, consequently, diminish blood volume and increase urinary density, fact which, as have been observed in our results, may affect the post-exercise body concentrations.

## Conclusions

This study indicates that the performance of high-level aerobic training can produce significant increases in serum concentrations of Mn and in urinary Cu values, as well as decreases in the urinary concentrations of Co and Mn that may be related to adaptive responses to exercise.

An incremental strenuous exercise in middle- and long-distance athletes produces a decrease in Co and Cu serum concentration as well as a decrease in urinary Cu.

Our results show possible adaptations to exercise which may be reflected in the concentrations of metals that can be of great importance for the performance of athletes and which could require, in some cases, specific nutritional supplementation.

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## Compliance with Ethical Standards

**Ethics Approval** This research was carried out under the Helsinki Declaration ethic guidelines, updated at the World Medical Assembly in Seoul in 2008, for research with human subjects. The experimental design was approved by the Ethic Committee of University of Extremadura.

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

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