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Serum, erythrocyte and urinary concentrations of iron, copper, selenium and zinc do not change during an incremental test to exhaustion in either normothermic or hyperthermic conditions

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ABSTRACT

Aim: The aim of this study was to evaluate the effect of the performance of an incremental exercise test until exhaustion in normothermic and hyperthermic conditions on serum, erythrocyte and urine concentrations of Iron (Fe), Copper (Cu), Selenium (Se) and Zinc (Zn).

Methods: Nineteen adult males (age: 22.58 ± 1.06 years) performed two maximum incremental exercise tests on a cycloergometer in normothermia (22 ± 2 °C) and hyperthermia (42±2 °C) separated by 48 h. Urine, serum and erythrocyte samples were collected before and after each test.

Results: Serum Se ($p < 0.01$) and Cu ($p < 0.05$) levels were altered after each test, but the significance disappeared with the correction for haematocrit. The rest of the values did not undergo alterations in either condition.

Conclusions: It seems that a higher stimulus is necessary to obtain changes in these minerals. The study reveals the need to correct serum concentrations concerning possible changes in these volumes after an acute effort.

1. Introduction

Iron, Copper, Selenium and Zinc (Fe, Cu, Se, Zn) are essential trace elements for cardiorespiratory fitness, taking part in oxygen transport, and antioxidant pathways so that a deficit can reduce aerobic performance.

Fe is an essential metal for cellular proliferation and DNA synthesis, and is linked to necessary proteins for the integrity of human cells. This metal is distributed in the human body in haemoglobin (≈65%), myoglobin (≈15%) and hepatocytes (≈25%) (Casarrubea et al., 2013). During exercise, Fe is crucial for aerobic capacity due to its oxygen transport functions (Hinton, 2014). Inadequate Fe status leads to anaemia, a decline of maximal oxygen consumption (VO2 max), poor control of body temperature, and consequently, a decline in performance (Kang, 2018). Exercise induces changes in the Fe-status (Auersperger et al., 2013), and can be aggravated in hot environments due to the possible losses of this metal by sweat (DeRuisseau et al., 2002; Saran et al., 2018).

Cu is a cofactor of multiple enzymes (cuproenzymes) involved in aerobic energy (Collins, 2016). Cu stands out for its catalytic functions for ferroxidases, superoxide dismutase, cytochrome oxidase, among them (Chen et al., 2010; Oberley-Deegan et al., 2009; Robinson and Winge, 2010). Therefore, Cu participates in haemoglobin synthesis (Myint et al., 2018), an essential protein for aerobic endurance. In hot environments, Cu takes part in thermal regulation and glucose metabolism (Bousquet-Moore et al., 2010). Cu deficiency can impair the immune and cardiovascular systems. Furthermore, alterations in Cu levels affect the haematocrit and iron-copper status (Scheiber et al., 2013). It is unclear if acute exercise increases or decreases the copper concentration (Baydil, 2013). Therefore, it would be interesting to know how heat stress affects the copper levels.

Selenium (Se) is vital for health, antioxidant systems, selenoprotein functions and metabolic processes (Baltaci et al., 2016). Se is linked to glutathione peroxidase (GPx), an important enzyme for antioxidant

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status. The oxidative stress caused by physical exercise is well known (Maynar et al., 2018b), so a Se decline can affect the mentioned status. The antioxidant response increases in hot environments (Quindry et al., 2013). This process can alter selenium homeostasis.

Zinc (Zn) is classified as a trace element involved in numerous biological processes such as protein transport and takes part in the activity of more than 200 enzymes. These enzymes are involved in the metabolism of DNA (Chu et al., 2016). Additionally, they influence the synthesis of proteins, participate in glycolysis and neoglucogenesis, in the synthesis of prostaglandins, in the metabolism of cholesterol, prevent lipid peroxidation and maintain membrane structures (Jomova and Valko, 2011; Kabata-Pendias and Mukherjee, 2007). The post-exercise Zn concentration in plasma/serum increases due to the redistribution flow from erythrocytes (Chu et al., 2016). The thermoregulation process can increase the mentioned flow. This flow can exacerbate the losses of this metal by sweat and urine due to the circulation of the plasma/serum towards sweat and the kidneys in heat stress.

Thus, these elements are essential for athletes (Lukaski, 2004). However, the acute effects of physical activity and hyperthermia on the concentration of the elements in the organism is poor studied. Nowadays, it is observed how the participation in aerobic and long-term aerobic activities practice (cycling, half and full marathon) has increased in our society, looking for health and performance benefits (Niemela et al., 2016). However, the engagement in these activities could have pose a risk to health especially in response to derived from adverse environmental conditions, for health such as hyperthermia (Maughan et al., 2007). In heat exercise, electrolytes are lost by sweat, so there may also be a loss of minerals (Mao et al., 2001). Sweating due to heat stress can lead to changes in the content of minerals (Montain et al., 2007). Besides, as mentioned above, increase flow due to thermoregulation process can alter the homeostasis of the elements. Thus, it would be relevant to know the acute effect of physical activity in hyperthermia on the essential elements Fe, Cu, Se and Zn. Therefore, the objective of the present study was to observe the impact of an incremental test until exhaustion in healthy young subjects in normothermia (22 °C) and hyperthermia (42 °C) in the concentration of these elements in serum, erythrocytes and urine.

2. Materials and methods

The data presented in the current study were collected as part of a previously published study. For further details consult Siquier-Coll et al. (2019).

2.1. Participants

Nineteen male university students participated voluntarily in this study. Previously to the experimental period all of them were informed about the aim, characteristics and risks of the research. Before beginning the experiments, all the participants provided their written consent and accepted their voluntary participation. This work was approved by the bioethics committee of the University of Extremadura under the Helsinki Declaration ethic guidelines of 1975, updated in the World Medical Assembly in Seoul (2008), for research involving human subjects. The anthropometric characteristics of the participants are presented in Table 1.

2.2. Experimental protocol

The testing was carried out on 2 different days separated by 48 h in order to ensure physical recovery. The order of the tests was: day 1-normothermia (22 ± 2 °C, 40–60%RH); day2-hyperthermia (42 ± 2 °C, 40–60%RH). The participants were exposed to 15 min of heat (42 ± 2 °C, 40–60%RH) before starting the measurements on the second day. In order to control for circadian rhythms, all the tests were performed in the same time (from 9 a.m. to 2 p.m.) and at the same time for each participant. Additionally, the participants did not take any medication or supplement before the experimental protocol.

Table 1
Descriptive characteristics of the participants.

	Participants (n = 19)
Age (year)	22.58 ± 1
Weight (kg)	74.98 ± 9.08
Height (cm)	178.32 ± 5.9
BMI (kg/m^2)	23.63 ± 1.83
Fat mass (kg)	11.21 ± 3.40
Fat mass (%)	14.75 ± 2.85
Fat free mass (kg)	63.57 ± 6.42
Fat-free mass (%)	85.24 ± 2.84

The tests started with a blood extraction from the antecubital vein of each participant and with the collection of a urine sample. Both samples were obtained in fasting conditions. Then the participants had a similar breakfast consisting of a 250 ml glucosaline drink which did not contain any of the elements studied. One hour after the breakfast, every participant performed an exercise test until exhaustion (described below). The protocol of the tests was the same for both days of measurements, but the first day the tests were performed in normothermic conditions and the second day in a hyperthermic environment. Once finished, another blood sample was drawn from each participant. The first urination after the test was also obtained from each individual.

2.3. Body composition determination

The anthropometric measurements were taken in the morning, in fasting conditions, and at the same time for each participant. Body height was measured using a wall stadiometer (Seca 220). Body weight, fat-free mass and fat mass were measured by electric bioimpedance, using a body composition analyser BF-350 (Tanita Corp. Japan).

2.4. Incremental exercise test until exhaustion

Each participant performed two maximal exercise tests in laboratory conditions. The subjects performed a 50 W warm-up for 5 min. The first test was carried out at room temperature, and the second one in a sauna (Harvia C105S Logix Combi Control; 3–15 W; Finland). Both tests were performed on the same cycloergometer, starting at an initial power of 50 W (W). Every 2 min, the power increased by 25 W until voluntary exhaustion. The tests ended when the subject was unable to sustain the power of the stage for more than 15 s or if the subject reached exhaustion. During the test, HR [Mortara; (Ref 9293-029-60)] as well as respiratory variables [Geratherm Respiratory GMBH, Ergostik (Ref 40.400; Corp Bad Kissinguen)] were recorded in real time. Sweat rate was calculated with the equation proposed by Murray (1996) to calculate sweat loss after exercise.

2.5. Sample collection

2.5.1. Blood samples

Two extractions of 10 mL of venous blood were drawn from the antecubital vein of each participant using plastic syringes fitted with a stainless-steel needle. The first samples were extracted before the exercise test, the participants were sitting on the cycle ergometer, and the second ones, just after it, in the same way as in the first extraction. In hyperthermic conditions, the first extraction was drawn after 15-min of heat exposure. Once extracted, the samples were collected in a metal-free polypropylene tube (previously washed with diluted nitric acid).

Later, 5 mL of the blood samples were 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 °C until biochemical analysis.

Five ml of the blood extraction were deposited in glass tubes with

ethylenediaminetetraacetic acid (EDTA) as an anticoagulant factor and centrifuged at 1800 rpm for 8 min to separate the plasma from the erythrocytes. The erythrocytes, previously separated from the plasma, were washed three times with a 0.9% sodium chloride solution in ultrapure water and stored at −80 °C until biochemical analysis.

Haematocrit was obtained by centrifuging the whole blood into a glass capillary containing heparin in a Microcen microfuge (Alresa, Spain). Haemoglobin (Hb) was determined using a Hb analyser (HemoCue, Sweden). Both haematocrit and Hb were used to correct the changes in plasma volume by means of the Dill and Costill (1974) equations.

2.5.2. Urine samples

Additionally, urine samples were obtained from each participant before and after the test, just after both blood extractions. The post-test urine collection time was 15.21 ± 7.34 min for normothermic conditions and 18.74 ± 8.23 min for hyperthermic conditions. The urine samples were collected in polyethylene tubes previously washed with diluted nitric acid and frozen at − 80 °C until analysis. Before the analysis, the samples were thawed at room temperature and homogenised by shaking.

2.6. Serum, erythrocyte and urinary trace element determination

2.6.1. Sample preparation

Fe, Cu, Se and Zn analyses were performed by inductively coupled plasma mass spectrometry (ICP-MS) according to the protocol followed by Maynar et al. (2018b). To prepare the analysis, the decomposition of the organic matrix was achieved by heating it for 10 h at 90 °C after the addition of 0.8 mL HNO_3 and 0.4 mL H_2O_2 to 2 mL of serum, erythrocyte or urine samples. The samples were then dried at 200 °C on a hot plate. Sample reconstitution was carried out by adding 0.5 mL of nitric acid, 10 µL of indium (In) (10 mg/L) as the internal standard, and ultrapure water to complete 10 mL.

2.6.2. Standard and reference material preparation

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

2.6.3. Sample analysis

Digested solutions were assayed in an ICP-MS Nexion analyser 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 (KED) with helium as the collision gas; and in reaction mode (DRC) with ammonia as the reaction gas. 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 analysed per sample. The sample quantifications were performed with indium (In) as the 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%.

2.7. Statistical evaluation

Statistical analyses were carried out with SPSS 22.0 for Windows. The results are expressed as the mean and standard deviation (x ± sd). The Kolmogorov– Smirnov test was applied to examine the distribution of the variables, and Leven's test was used to verify their homogeneity. The difference between normothermia and hyperthermia, and pre-post difference data were determined using the Wilcoxon test for paired samples. A $p \leq 0.05$ was considered statistically significant.

3. Results

Table 2 shows the haemoglobin, haematocrit and weight data before and after the test in normothermia and hyperthermia. There were significant differences in haematocrit between before and after the tests in normothermia and hyperthermia. Similarly, weight decreased significantly after the test ($p < 0.01$).

The results obtained in ergospirometric parameters referring to the test in normothermia and hyperthermia are shown in Table 3. No significant alterations were found in the parameters. Sweat rate was significatively ($p < 0.01$) lower in normothermia than hyperthermia.

Table 4 shows the data on serum concentration before and after the test in normothermic conditions. The results are expressed with and without haemoconcentration correction. There were significantly different between before and after in Copper and Selenium in normothermic ($p < 0.05$; $p < 0.01$) and hyperthermic conditions ($p < 0.01$; $p < 0.01$) only without correction. No significant changes were found in the rest of the values.

Table 5 shows the levels of trace elements before and after the test in erythrocytes. The results with correction are expressed in mg or ug/gHb. No significant differences were observed in the elements.

Urinary concentrations of each element are presented in Table 6. No statistically significant differences were observed.

4. Discussion

A significant decrease in body weight accompanied by increases in sweat rate were observed during the tests. This fact, consequence of the increase in blood pressure due to the rise in temperature and sweat loss, reflected in Table 2, leads to haemoconcentration and consequently, changes in the haematocrit. To avoid the changes due to haemoconcentration, the data on the elements was corrected with the equation of Dill and Costill (1974). While haematocrit increased in both normothermic and hyperthermic conditions, haemoglobin concentration only increased in hyperthermia. This situation may be due to a higher haemoconcentration in hyperthermia, reflected in the sweat rate as we reported in a previous investigation (Siquier-Coll et al., 2019).

Cardiovascular drift has been reported in the majority of studies on thermal stress due to a decline in VO_2 max, and an increase in HR after exercise in hyperthermia because of dehydration (Cheuvront et al., 2010; Coyle and Gonzalez-Alonso, 2001; Wingo et al., 2005). Conversely, this study, despite obtaining a significant increase in the rate of sweating in hyperthermia did not find the previously mentioned effects with regard to normothermia.

It has been proposed that Fe losses in athletes may be higher than those that occur in the general population (Williams, 2005), due to

Table 2
Haemoglobin, Haematocrit, weight, and temperature before and after the Incremental exercise test until exhaustion, in both conditions.

	Normothermia (22 °C)		Hyperthermia (42 °C)	
	Before	After	Before	After
	(n = 19)	(n = 19)	(n = 19)	(n = 19)
Haemoglobin (g/dL)	15.65 ± 1.16	15.59 ± 1.24	15.26 ± 0.92	17.09 ± 7.06*
Haematocrit (%)	46.37 ± 2.95	48.24 ± 2.99**	44.90 ± 3.25	47.40 ± 3.56**
Weight (kg)	74.62 ± 9.11	74.37 ± 8.92**	74.81 ± 9.15	74.22 ± 9.12**
Tskin (°C)	35.47 ± 1.15	36.12 ± 0.64*	36.09 ± 1.4	37.23 ± 0.69**
Tc (°C)	35.88 ± 0.81	36.6 ± 0.93**	36.04 ± 1.32	37.52 ± 1.2**

Wilcoxon test: *p < 0.05; **p < 0.01 Differences between before and after.

Table 3
Maximum ergoespirometric and power parameters in an incremental exercise test until exhaustion.

	Normothermia (22 °C)	Hyperthermia (42 °C)
	(n = 19)	(n = 19)
VO_2 max (L/min)	3.10 ± 0.49	2.89 ± 0.60
VO_2 max (ml/min/kg)	41.66 ± 5.60	39.03 ± 7.74
VCO_2 (L/min)	3.27 ± 0.87	3.24 ± 0.63
VE (L/min)	115.29 ± 23.92	108.32 ± 17.01
BF (breaths/min)	47.00 ± 10.13	46.60 ± 8.50
HR (beats/min)	185.89 ± 10.94	188.42 ± 8.43
O_2Pulse (ml/beat)	15.47 ± 2.42	15.12 ± 2.45
RER	1.13 ± 0.04	1.12 ± 0.06
Power (W)	247.36 ± 37.16	243.42 ± 35.19
Sweat Rate (L/h)	0.89 ± 0.56	1.85 ± 0.75**

Wilcoxon Test: *p < 0.05; **p < 0.01 Differences between hyperthermic and normothermic conditions. VO_2 max = oxygen consumption; VCO2= Carbon dioxide; VE=Pulmonary ventilation; BF=Breath frequency; O_2Pulse = Oxygen pulse; RER = VCO2/VO2.

factors such as increased haemolysis (DellaValle and Haas, 2011), gastrointestinal bleeding (Suedekum and Dimeff, 2005), overtraining or excessive sweating (Deruisseau et al., 2004). Nevertheless, the losses of iron through sweating does not warrant increasing the intake of Fe in the diet (Hinton, 2014). Exercise produces haemolysis, increasing serum Fe. This phenomenon has its origin in flow redistribution during exercise (Babic et al., 2001). However, in the present investigation, an iron decrease was not observed after testing at either temperature. Thus, Skarpanska-Stejnborn et al. (2015) reported an insignificant decline in serum and erythrocyte Fe after a maximal exercise test in rowers, but found a significant increase in systematic iron metabolism parameters (TIBC, UIBC, sTfR). In a subsequent study (Doker et al., 2014), there were no significant differences in serum Fe between pre-post exercise and 1 h later in sedentary controls, and amateur and elite swimmers. In contrast to these results, a recent study showed a significantly lower blood Fe after high-intensity exercise in elite male and female dragon boating athletes (Bauer et al., 2018). However, the mentioned papers did not correct the data for haemoconcentration in serum and erythrocyte.

In extreme environments, concretely in hypoxia, Fe metabolism is unaffected in moderately trained endurance athletes (Govus et al., 2014), as in hyperthermia in this investigation. Conversely, Wang et al. (2012) observed lower plasma Fe after 1-week high-intensity basketball training in hot and humid environments. However, this could be because the high intensity and increased sweating during exercise in the heat can decrease iron levels. In rats, Bloomer et al. (2014) suggest an increase in hepatic Fe after heat exposure (Bloomer et al., 2014).

Concerning Copper, the concentration of Serum Cu was similar as established by Maynar et al. (2018a) for sedentary males. Additionally, the mentioned paper suggests a major concentration of Cu in serum in aerobic-anaerobic sport-men. The acute effect on Cu concentration is varied. On the one hand, Granell (2014) found an increase in serum Cu (p = 0.002) and urine (p < 0.05) concentrations after 40 min of aerobic

Table 4
Serum concentrations of trace elements before and after the exercise test in hyperthermic and normothermic conditions, without and with corrections (C) for possible haemoconcentration.

	Normothermia (22 °C)		Hyperthermia (42 °C)	
	Before	After	Before	After
Fe (μg/L)	1017.87 ± 264.56	1044.61 ± 309.06	1019.02 ± 272.20	1149.29 ± 425.63
Fe–C (μg/L)	1017.87 ± 264.56	985.65 ± 353.07	1019.02 ± 272.20	1128.68 ± 430.18
Cu (μg/L)	762.96 ± 132.22	796.57 ± 122.16*	724.59 ± 138.13	790.58 ± 138.07**
Cu–C (μg/L)	762.96 ± 132.22	763.06 ± 113.50	724.59 ± 138.13	746.87 ± 142.82
Se (μg/L)	119.13 ± 14.24	127.62 ± 16.79**	117.57 ± 3.29	127.19 ± 4.93**
Se–C (μg/L)	119.13 ± 14.24	123.58 ± 21.74	117.57 ± 3.29	119.06 ± 22.42
Zn (μg/L)	857.67 ± 145.60	904.87 ± 201.46	856.09 ± 176.58	902.18 ± 267.88
Zn–C (μg/L)	857.67 ± 145.60	803.72 ± 220.49	856.09 ± 176.58	853.14 ± 266.47

Wilcoxon Test: *p < 0.05; **p < 0.01 Differences between pre and post-test values.

Table 5
Erythrocyte concentrations of trace elements before and after the exercise test in hyperthermic and normothermic conditions, without and with corrections (C) for possible haemoconcentration.

	Normothermia (22 °C)		Hyperthermia (42 °C)	
	Before	After	Before	After
Fe (mg/L)	460.77 ± 112.89	444.44 ± 134.37	427.13 ± 138.23	469.56 ± 133.04
Fe–C (mg/gHb)	293.42 ± 71.43	290.80 ± 89.80	289.75 ± 89.61	292.01 ± 101.63
Cu (μg/L)	461.29 ± 138.26	427.78 ± 130.87	419.61 ± 135.67	456.15 ± 148.67
Cu–C (μg/gHb)	286.27 ± 88.14	275.04 ± 91.15	276.97 ± 137.37	281.96 ± 110.36
Se (μg/L)	89.76 ± 29.13	85.83 ± 32.31	86.43 ± 35.11	92.62 ± 38.10
Se–C (μg/gHb)	56.94 ± 19.52	55.67 ± 23.84	56.61 ± 24.54	57.77 ± 27.77
Zn (μg/L)	8.39 ± 1.79	7.93 ± 1.92	7.99 ± 1.87	8.23 ± 1.87
Zn–C (μg/gHb)	5.30 ± 1.21	5.14 ± 1.42	5.22 ± 1.35	4.89 ± 1.49

Table 6
Urinary concentrations of trace elements in athletes before and after the exercise test in hyperthermic and normothermic conditions.

	Normothermia (22 °C)		Hyperthermia (42 °C)	
	Before	After	Before	After
Fe (μg/L)	14.24 ± 7.13	11.41 ± 4.98	11.11 ± 10.92	12.31 ± 12.73
Cu (μg/L)	6.57 ± 5.18	7.15 ± 4.33	6.36 ± 6.25	7.46 ± 6.78
Se (μg/L)	19.34 ± 7.78	18.09 ± 6.74	18.44 ± 9.77	20.39 ± 13.87
Zn (μg/L)	448.65 ± 206.73	432.10 ± 200.92	333.15 ± 242.48	391.48 ± 300.04

exercise. Simultaneously, Anderson et al. (1995) observed changes in urine and serum Cu levels after acute exercise.

On the other hand, recent research reveals a statistically significant change in urine Cu after exercise in female athletes (Eskici et al., 2016). This investigation presented significant changes in serum Cu without correction in hyperthermia and normothermia ($p < 0.01$), nonetheless, the data with correction showed no significant differences. Thus, the importance of correcting the results for haemoconcentration is highlighted.

In warm environments, a significant copper decrease was observed after ten days of observation in heat stress (37.8 °C, 50% humidity) (Consolazio et al., 1964). Copper in hyperthermic conditions may be lost through sweating (Campbell and Anderson, 1987). Conversely, Montain et al. (2007) did not reveal significant differences in sweat in treadmill exercise in hyperthermia (35 °C, 30% relative humidity) and normothermia (27 °C, 40% relative humidity). This fact leads us to deduce that in the absence of changes in sweat, there are no changes in serum, as in this study. Future investigations will have to clarify copper metabolism during heat stress.

Regarding Selenium, the literature on the acute effect of exercise on this element is limited. Singh et al. (1991) reported a significant decrease in plasma ($p < 0.05$) after a five-day rigorous training programme. This effect can be due to a change in selenium storage owing to a vital necessity for antioxidant protection of tissues during exercise (Lamprecht et al., 2009). Recently, Maynar et al. (2018b) reported serum Se changes after acute exercise but not with the values with haematocrit correction as in this study. In hyperthermia, a loss of 340 μg of selenium was detected after 8 h of heat exposure at 37.8 °C in men (Consolazio et al., 1964). A gradual increase of plasma Se was seen after 1-week high-intensity basketball training in a hot humid environment due to a major reactive oxygen species (Wang et al., 2012). Nevertheless, in this study, plasma Se was not altered after the exercise test. No current report about selenium after exercise in hyperthermia was found in the literature to compare with the data from this investigation.

A flow of Zn from the plasma/serum to the erythrocyte after acute exercise has been suggested (Volpe et al., 2007). Conversely, an inflammatory process during exercise may lead to a zinc flux from the erythrocyte to serum. In the present survey, like that of Maynar et al. (2018b) changes were not observed in serum and erythrocyte Zn with and without correction before and after the test in normothermia in sedentary males. A recent review (Chu et al., 2016), concluded that Zn changes occur at maximum intensities, but not in submaximal exercise. The data from the present study do not reveal differences in zinc after maximum intensities of exercise. Exposure to extreme heat temperatures may stimulate changes in Zn metabolism (Research and Marriott, 1993). Ten days of heat acclimation at the rectal temperature of 39.5 °C walking on a treadmill at 3.5 mph, 4% grade, for 100 consecutive minutes did not alter the zinc content of sweat ($p = 0.1$) (Chinevere et al., 2008) suggesting no changes in serum as in this survey.

Previous studies reported (Maynar et al., 2018a,b; Munoz et al., 2018) the necessity of correcting the data after an acute exercise which lead to changes in plasma volume in order to obtain reliable parameters. Correction should be applied to physiological parameters which are sensitive to plasma changes, in this case through Hb and haematocrit.

The present study has a limitation which should be mentioned. The tests were not randomized because a full recovery after physical exercise under hyperthermic conditions would need more than 48 h to be completed.

5. Conclusions

Changes in plasma volume caused by physical activity should be considered when conducting studies on the concentration of mineral elements in different matrices after acute physical activity conditions.

In this regard, a maximal incremental test until exhaustion in both normothermic and hyperthermic conditions does not produce significant changes in the concentration of the elements after applying corrections to plasma volume changes.

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