



Influence of a physical exercise until exhaustion in normothermic and hyperthermic conditions on serum, erythrocyte and urinary concentrations of magnesium and phosphorus[☆]

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ABSTRACT

Aim: The aim of this study was to evaluate the effect of the performance of a maximal exercise test until exhaustion in normothermic and hyperthermic conditions on body concentrations of magnesium (Mg) and phosphorus (P).

Methods: 19 adult males (age: 22.58 ± 1.05 years) performed two maximum incremental exercise tests on a cycloergometer separated by 48 h. The first was performed in normothermia ($22 \pm 2^\circ\text{C}$) and the second in hyperthermic conditions induced with a sauna ($42 \pm 2^\circ\text{C}$). Blood and urine samples were taken before and after each test.

Results: The tests in hyperthermia did not produce ergospirometric alterations or a noticeable cardiovascular drift. Serum Mg concentrations underwent a reduction after the stress test in hyperthermia ($p > 0.05$) but not in normothermia. Nevertheless, urinary and erythrocyte concentrations of Mg, and urinary, erythrocyte and serum concentrations of P did not undergo alterations in either conditions.

Conclusions: It seems that exercise in hyperthermic conditions induces a tissue redistribution of Mg in the body, a fact which was not observed in normothermic conditions.

1. Introduction

Essential minerals are involved in several vital functions necessary for maintaining proper homeostasis during exercise. If the normal body values of these elements are altered, physical performance can be affected leading, in the worst cases, to several diseases (Day et al., 2018; Nielsen, 2014)

Magnesium (Mg) is a cofactor of lipid, protein and carbohydrate metabolism and is an enzymatic cofactor and substrate of the ATP pathway (Clarkson and Haymes, 1995). Adequate body values of Mg are essential in physical activity, and a fall in body Mg can induce a drop in exercise performance, and in the worst cases, can lead to inflammatory responses, and an increase of oxidative stress (Veronese et al., 2014). So, adequate body Mg content can be critical for physical activity. Mg depletion can be caused by inadequate intake, excessive alcohol intake and increased sweating rates during exercise (Nielsen

and Lukaski, 2006)

Phosphorus (P) is a critical mineral in energy metabolism, being essential in the synthesis of adenosine triphosphate (ATP) and 2, 3-diphosphoglycerate, a necessary molecule in the dissociation of oxygen from hemoglobin (Kiela et al., 2017). Furthermore, it has been reported that the body content of this element modulates the activity of several metabolic pathways (Shahsavari and Pourvaghari, 2010). In this respect, excessive P intake may induce hyperphosphatemia and may be harmful to the kidneys (Salomo et al., 2016). Hypophosphatemia is uncommon in humans as P is present in high concentrations in most common foods (Malliaropoulos et al., 2013).

Recently, several outcomes about the effect of exercise on trace mineral metabolism have been reported concluding that exercise can affect the body values of several minerals and that this effect could depend on the metabolic predomination of exercise (Maynar et al., 2018a, 2018b).

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Although it has been studied, current information about the precise influence of exercise on the metabolism of magnesium (Mg) and phosphorus (P) is still incomplete, especially in hyperthermic conditions.

It is known that heat stress may lead to a decrease in body magnesium and phosphorus due to increased sweat loss (Cheuvront et al., 2010). However, information about the combined effect of exercise and heat stress is still incomplete.

Thus, the aim of this investigation was to evaluate the changes in erythrocyte, serum, and urine levels of P and Mg induced by the performance of an acute incremental exercise test until exhaustion in hyperthermia (42 °C) and normothermia (22 °C).

2. Material and methods

2.1. Participants

Nineteen male university students voluntarily participated 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 at 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 of measurements separated by 48 h in order to ensure physical recovery. The order of the tests was: day 1-normothermia (23 ± 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 the circadian rhythms, all the tests were performed in the morning (from 9 a.m. to 14 p.m.) and at the same time for each participant.

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. Health security protocol

Previously to the experimental period, all participants were

Table 1
Anthropometric characteristics of the participants.

	Participants (n = 19)
Age (year)	22.58 ± 1.06
Weight (kg)	74.98 ± 9.08
Height (cm)	178.32 ± 5.93
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

examined by a physician in order to avoid any case of illness or contraindication to participating in the study. At this point the participants had to comply with the inclusion criteria: be a healthy male, not have taken any supplementation, medication or over-the-counter medication, drug or alcohol in the previous four weeks, have a healthy lifestyle, not to practice more than 3 h of physical activity per week and not to follow a specific training plan.

Once the first fitness screening was completed, the cardiocirculatory system of each participant was evaluated in resting conditions using an electrocardiograph (Sanro BTL-08 SD ECG) and a tensiometer (visiomat; comfort 20/40). Before the tests, the basal electrocardiograms were analyzed by a physician. Furthermore, heart activity was monitored in real time in the tests by mean of an electrocardiograph [Mortara; (Ref 9293-029-60)] during the exercise and recuperation times. Core temperature (T_c), measured in the buccal mucosa, and skin temperature (T_{skin}), measured in the frontal region of the head in triplicate, were monitored using an infrared thermometer [TAT 5000 "Exergen Temporal Scanner" (Corp., USA)] at the beginning and end of the tests.

In order to avoid cases of breathing difficulties, two forced spirometry tests were carried out before the exercise tests. A spirometer (Spirobank G) was used to measure respiratory capacity.

No diseases were reported during the whole study.

2.4. Familiarization period

Before the start of the experimental period, one week of prior familiarization was completed by all participants. During this week, each participant visited the laboratory and became acquainted with the physicians, the laboratory gear and tools and performed two sub-maximal tests on the cycloergometer (Ergoline 900; Bitz, Germany). Both tests started at 50 W, increasing intensity by 25 W every two minutes until reaching 75% of the estimated FC_{max}. Familiarization tests were performed in both normothermic (23 ± 2 °C, 40–60%RH) and hyperthermic (42 ± 2 °C, 40–60%RH) conditions, separated by 48 h.

During the tests, Heart Rate (HR) was measured with an ECG [Mortara; (Ref 9293-029-60)] and respiratory variables were measured using a gas analyzer "Geratherm Respiratory GMBH [Ergostik (Ref 40.400; Corp Bad Kissinguen)]".

2.5. Body composition

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 analyzer BF-350 (Tanita Corp. Japan).

2.6. Incremental exercise test until voluntary 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 two minutes, the power increased by 25 W until voluntary exhaustion. The tests ended when the subject was unable to sustain the power of the stage during 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.

Table 2
Hemoglobin, hematocrit, weight and temperature before and after the incremental exercise test until exhaustion, in both conditions.

	Normothermia (22 °C)		Hyperthermia (42 °C)	
	Before (n = 19)	After (n = 19)	Before (n = 19)	After (n = 19)
Hemoglobin (g/dL)	15.65 ± 1.16	15.59 ± 1.24	15.26 ± 0.92	17.09 ± 7.06*
Hematocrit (%)	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.

2.7. Sample collection

2.7.1. 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 and the second ones, just after it. 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 was 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.

Hematocrit was 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 means of the [Dill and Costill \(1974\)](#) equations.

2.7.2. Urine samples

Additionally, urine samples were obtained from each participant before and after the test, just after both blood extractions. 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 homogenized by shaking.

2.8. Serum, erythrocyte and urinary trace element determination

2.8.1. Sample preparation

Mg and P analyses were performed by inductively coupled plasma mass spectrometry (ICP-MS) following the protocol used by [Maynar et al. \(2018b\)](#). To prepare the analysis, the decomposition of the organic matrix was made by heating it for 10 h at 90 °C after the addition of 0.8 mL of HNO_3 and 0.4 mL of 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.

2.8.2. 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.

2.8.3. Sample analysis

Digested solutions were assayed in an ICP-MS Nexion analyzer 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 analyzed per sample. The sample quantifications were performed with indium (In) as the internal standard. The values of the standard materials of each element (10 $\mu\text{g/L}$) used for quality controls were in agreement with intra and inter-assay variation coefficients of less than 5%.

2.9. Statistical evaluation

Statistical analyses were carried out with SPSS 22.0 for Windows. The results are expressed as the mean and standard deviation ($\bar{x} \pm \text{sd}$). 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

The results on hemoglobin, hematocrit and body weight before and after the test in normothermia and hyperthermia are shown in [Table 2](#). A significant increase can be observed in the hematocrit ($p < 0.01$) after the tests in both thermal conditions in comparison to the pre-test values. Additionally, a significant weight decrease was reported after the tests ($p < 0.01$).

[Table 3](#) presents the maximum values of ergospirometric parameters and the sweat rate resulting from the tests in normothermia and hyperthermia. A significant decrease can be observed in VO_2max ($p < 0.05$) in hyperthermia in comparison to the results of the tests in normothermia. However, no significant changes were observed in the other ergospirometric parameters. There were statistical differences between before and after the test in Tskin in normothermia ($p < 0.05$) and hyperthermia ($p < 0.01$), and in Tc in both conditions ($p < 0.01$).

[Table 4](#) shows the concentrations of trace minerals before and after the tests in urine, serum and erythrocytes. Erythrocyte results are expressed in mg/gHb as Hb is the most abundant protein in red cells.

A significant decrease ($p < 0.05$) was observed in serum Mg after the test in comparison to the initial values in hyperthermic conditions while no significant differences were found in normothermic conditions.

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

	Normothermia (22 °C) (n = 19)	Hyperthermia (42 °C) (n = 19)
VO ₂ (L/min)	3.10 ± 0.49	2.89 ± 60
VO ₂ (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 (1/min)	47.00 ± 10.13	46.60 ± 8.50
HR (beats/min)	185.89 ± 10.94	188.42 ± 8.43
O ₂ Pulse (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 hyperthermia and normothermia conditions. VO₂ = oxygen consumption; VCO₂ = Dioxide of carbon; VE = Pulmonary ventilation; BF = Breath frequency; O₂Pulse = Oxygen pulse; RER = VCO₂/VO₂.

4. Discussion

As is known, exercise increases T_c and T_{skin}, this increase being more significant in hyperthermic conditions (Veltmeijer et al., 2017). Thus, Table 2 shows how this study corroborates this phenomenon, producing changes in homeostasis.

The hematocrit is the percentage of red cells and elements in water in the total blood. This value varies among individuals, but it normally ranges from 41% to 50% in adult men and between 36% and 44% in adult women (Kenney et al., 2015). Accordingly, the hematocrit of the participants was within the normal, healthy ranges. As can be observed, there were significant changes in the hematocrit at both temperatures, but not in hemoglobin. This fact may be because hemoglobin is a less susceptible parameter to changes due to dehydration than the hematocrit, which undergoes alterations due to the increase in hemoconcentration and blood viscosity during exercise in hyperthermia (Buono et al., 2016). As in the hyperthermia test the sweating rate is significantly higher than in normothermia, this can explain the significant changes in that parameter.

The significant decrease in body weight after the tests in both conditions was mainly caused by sweating. This body water loss induces changes in hematocrit levels due to the loss of plasma water content. Additionally, most of the hemoconcentration that occurs with maximal exercise is not due to fluid loss, but rather to the large increase in blood pressure causing fluid loss from the circulating plasma volume. These aspects generally induce hemoconcentration, which leads to an increase in the concentration of red blood cells (Alis et al., 2015). This phenomenon may affect the analysis of substances contained in the blood. In order to avoid this, the post-test values of the studied elements were corrected using the equations of Dill and Costill (1974) for hemoconcentration induced by dehydration.

Table 4
Urine, serum and erythrocyte concentrations of Mg and P, before and after the incremental exercise test until exhaustion. Serum concentrations were corrected for changes in plasma volume.

		Normothermia (22 °C)		Hyperthermia (42 °C)	
		Before (n = 19)	After (n = 19)	Before (n = 19)	After (n = 19)
Mg	Urine (mg/L)	72.47 ± 39.83	62.06 ± 37.97	75.15 ± 83.04	65.38 ± 87.98
	Serum (mg/L)	19.90 ± 1.48	19.40 ± 2.27	19.58 ± 1.89	18.31 ± 1.67*
	Erythrocyte (mg/g Hb)	26.89 ± 6.00	25.19 ± 4.95	26.04 ± 6.57	26.35 ± 8.55
P	Urine (mg/L)	620.30 ± 478.17	628.28 ± 474.20	615.36 ± 600.63	608.31 ± 733.62
	Serum (mg/L)	14.93 ± 1.87	14.76 ± 1.96	13.63 ± 1.96	13.92 ± 2.03
	Erythrocyte (mg/g Hb)	269.47 ± 57.70	260.67 ± 48.02	257.66 ± 69.01	268.16 ± 80.45

Wilcoxon Test.

* p < 0.05 Differences between pre and post test values.

Regarding the ergospirometric parameters, Sawka et al. (1985) found a significant drop in VO_{2max} after two tests in hyperthermia (49 °C) in comparison to normothermic conditions (22 °C) among cyclists. In a subsequent study, significant differences were reported in VO_{2max} between normal temperatures and hyperthermia in euhydrated and dehydrated subjects (Nybo et al., 2001). In contrast, in this study, there were no significant differences between hyperthermic and normothermic conditions in any of the parameters. In this respect, in the investigation carried out by Wingo et al. (2005a) no differences were found in the 15-min test between the control participants, who performed the test at 22 °C and the experimental group, which performed the same test at 40 °C. However, significant differences were found in the oxygen consumption between the 15- and 45-min tests. These authors based their findings on the phenomenon known as cardiovascular drift (Wingo et al., 2005a), a fact which was corroborated by his research group in subsequent studies (Wingo, 2015; Wingo et al., 2005b). Cardiovascular drift is defined as the decrease in systolic volume and the subsequent increase in heart rate (Coyle and Gonzalez-Alonso, 2001). However, increases in blood pressure and heart rate have also been documented with no changes in stroke volume. Thereby, dehydration caused by hyperthermia produces an increase of cutaneous blood flow to thermoregulate the body by means of increased sweating rates. Simultaneously, the amount of blood supplied to the muscles is reduced, inducing a rise in the heart rate to meet muscle demands (Coyle and Gonzalez-Alonso, 2001; Gonzalez-Alonso, 2012). Conversely, in this research, a significant increase in maximal heart rate or a decline in VO_{2max} were not found.

The erythrocyte and serum values of Mg and P obtained before the test were within normal ranges. It has been reported that the reference concentrations for Mg are 34.02 ± 8.64 mg/L (Buchman et al., 1998) for urine, 19 ± 1.2 mg/L for plasma and 47 ± 5.1 μ/L for erythrocytes (Lu et al., 2015). And the reference values of P have been documented as 0.18 ± 0.02 for plasma and 0.67 ± 0.04 g/L for erythrocytes (Lu et al., 2015). The urine values of P are similar to those obtained by Shinozaki et al. (2018) in adult men.

The number of reports in the literature of the acute response to exercise in body Mg is scarce. Lares and Alves (1991) observed changes in serum Mg but not in erythrocyte or urine after 30 min of swimming, a fact which was not observed in normothermia. These results reinforce the ones obtained in the present study.

Conversely, Soria et al. (2014) did not observe changes in plasma Mg after a submaximal intensity test in euhydrated subjects without a Mg deficiency. In this respect, an increase in Mg values in erythrocytes has been found in some studies (Doker et al., 2014; Lijnen et al., 1988; Stendig-Lindberg et al., 1987). This rise could be explained by the increase in blood osmolarity due to dehydration.

This increase of Mg in the erythrocytes may induce hypermagnesemia (Soria et al., 2011). In this research, no significant changes of Mg were observed in erythrocytes in either temperature condition. However, a significant decrease in plasma Mg was observed.

This fall in plasma could be explained by the cellular absorption of this element induced by free fatty acid mobilization, a fact which may ease the influx of Mg to the cell, leading to a diminution in the seric values (Laires and Monteiro, 2008; Soria et al., 2011) Due to the nature of physical exercise the main cells affected by this phenomenon should be the muscle and adipose cells. Additionally, a Mg flux from serum or plasma to erythrocytes, adipocytes and myocytes has been documented induced by the increased tissular metabolic demands of this element, in order to help in the processes of energy production or to counteract oxidative stress (Nielsen and Lukaski, 2006).

In contrast, as explained before, in this research no changes were observed in erythrocyte concentrations. So, the decline in seric Mg could also be due to sweat loss. The data on this phenomenon are contradictory. In this respect, Beller et al. (1975) reported a decline in serum Mg in a hot environment (49 °C), but without an increase in Mg excretion by sweat. In our knowledge, the effect of acute effort in the heat on magnesium concentrations is scarce, or non-existent, in the literature. This lack of information requires further research in this field.

The metabolism of phosphorus in exercise has not been fully studied. Maynar-Marino et al. (2015) observed differences in the body status of P among athletes of different metabolic modalities and control participants. In all cases, the values of their reports were similar to those obtained in serum in this study.

A recent investigation did not report any changes in serum or urine P content in female athletes after exercise (Eskici et al., 2016). These data reinforce the result obtained in this study in normothermic and hyperthermic conditions. This fact suggests that there was no muscle damage in the test, considering that an increase of seric P indicates muscle impairment (Knochel et al., 1974).

The results showed no significant increase in serum, so a test until exhaustion in hyperthermia should not produce muscle damage. It has to be highlighted that no studies which analyze the effect of exercise on the body values of P in hyperthermic conditions were found, manifesting the need for more studies in this area. Furthermore, this study was limited by the lack of knowledge of the participants' nutritional intake of P.

5. Conclusions

It can be stated that hyperthermia does not produce a decrease in VO_{2max} without cardiovascular drift accompanied by a seric diminution of Mg. Erythrocyte and urine values were not affected suggesting a tissular redistribution of this element.

P values did not experience changes during acute exercise in hyperthermia or normothermia.

Finally, although it seems that the performance of physical exercise in hot environments increases the body's needs, the lack of previous information on this effect suggests that further research is required.

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