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Effect of heat exposure and physical exercise until exhaustion in normothermic and hyperthermic conditions on serum, sweat and urinary concentrations of magnesium and phosphorus

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ABSTRACT

Aim: The aim of this survey was to ascertain the difference in the levels of Magnesium (Mg) and Phosphorus (P) after an exercise test in normothermia and hyperthermia before and after heat acclimation in comparison to their respective pre-test values.

Methods: Twenty-nine male university students were divided into an Experimental Group (EG) (n = 15) and a Control Group (CG) (n = 14). All of them voluntarily participated in this investigation. Both groups performed an incremental test until exhaustion on a cycloergometer in normothermia (22 °C) and hyperthermia (42 °C). EG underwent 9 sessions of heat acclimation (100 °C) in a sauna (Harvia C105S Logix Combi Control; 3–15 W; Finland). Once the experimental period was completed, all initial measurements were carried out again under identical conditions. Urine and blood samples were obtained before and after each trial. Sweat samples were collected at the end of every test performed in hyperthermia. The samples were frozen at −80 °C until further analysis by ICP-MS.

Results: Lower seric Mg levels were observed in both groups at the end of pre-acclimation tests. After acclimation, only EG experimented a decrease of Mg in serum after testing ($p < .01$). The urinary excretion was unaffected in the pre-acclimated period, but EG experimented an increase in Mg after trials in the post-acclimation evaluation ($p < .01$). Mg sweat loss decreased significantly after heat acclimation ($p < .05$). P did not undergo changes, except in its urinary excretion, which was elevated after the normothermia trial in the post-acclimation period ($p < .05$).

Conclusions: It seems that exercise in hyperthermia altered Mg status but not P homeostasis. Additionally, heat acclimation reduces Mg losses in sweat while increasing its loss in urine. Thus, Mg supplementation should be considered in unacclimated and acclimated subjects if physical exercise is going to be performed in hyperthermic conditions.

1. Introduction

Hyperthermia produces alterations in the body. Due to the thermoregulation process, there is an increase in blood flow, heart rate and sweat rate. In workers, continuous exposure to heat continues to put their health at risk, when high temperatures cause a severe increase in sweating. A recent review reported that 15% of workers suffer from kidney diseases in hot environments (Flouris et al., 2018). Heat stress induces electrolyte loss (Mao et al., 2001), so this phenomenon may lead to a decrease in minerals as has been suggested (Jacob et al., 1981). Likewise, Chinevere et al. (2008) reported an insufficient mineral intake in the working population in hot environments. Sweat losses due to heat and physical exercise are the most likely routes for the loss of minerals such as magnesium (Mg) and phosphorus (P) (National Academy of Sciences, US, 2006).

Mg stands out for being a cofactor in most of the phosphorylated reactions involved in cell growth, energy metabolism, protein synthesis and glycolysis (Zhang et al., 2017). Additionally, it is linked to bone

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formation through interactions with hormones involved in bone structure organization. Total body magnesium in adult humans is estimated at approximately 20–28 g. The Recommendatory Dietary Allowance is established at 400–420 mg/day for men and 310–320 for women (Zhang et al., 2017). Due to the high relationship of this element with physical activity, Mg loss through sweating can affect athletic performance.

Regarding Phosphorus, the essentiality of this mineral resides in signaling, energy transfer and cell structure (Chang and Anderson, 2017). The synthesis of 2,3-Diphosphoglycerate (2,3-DPG) carried out by phosphorus improves erythrocytic homeostasis and therefore the cardiovascular and respiratory capacity for exercise (Clarkson and Haymes, 1995; Kiela et al., 2017). The content of P in humans is around 700 g (Chang and Anderson, 2017). Calvo et al. (2014) reported a Recommended Dietary Allowance (RDA) of phosphorus of 1,655 mg/day for males and 1.199 for females. Hypophosphatemia due to physical activity in hot environments may impair the energy system.

We previously reported the influence of acute effects in hyperthermia (Siquier-Coll et al., 2019), but the chronic effect of heat stress on these elements is uncertain. Thus, the aim of this survey was to ascertain the homeostatic changes in magnesium and phosphorus produced by the acute effect and repeated exposure at high temperatures.

2. Material and methods

This section is similar to the one presented by Siquier-Coll et al. (2019).

2.1. Participants

Twenty-nine 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. The subjects were divided into an experimental group (EG) and a control group (CG). 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 and descriptive 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% relative humidity (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 urine after the test was also obtained from each individual. Once the first two tests were carried out, the sample was randomized, dividing the participants into EG (n = 15) and CG (n = 14). Sweat samples were collected at the end of the hyperthermia trials. EG performed 9 sessions of heat exposure at high temperatures in the sauna during three weeks. CG did not receive any heat exposure or specific training plan. After that, all the initial measurements were made in both groups to check the possible changes after exposure to heat.

Table 1
Descriptive characteristics of the sample.

	CONTROL (n = 14)	EXPERIMENTAL (n = 15)	p-value
Age (years)	22.04 ± 2.29	21.70 ± 1.99	0.097
Height (cm)	172.91 ± 3.76	176.65 ± 7.17	0.043
Weight (kg)	70.67 ± 5.69	74.47 ± 11.28	0.036
BMI (kg/m2)	23.61 ± 1.27	23.93 ± 3.01	0.311
Fat mass (kg)	11.15 ± 2.46	12.32 ± 5.14	0.122
Fat mass (%)	15.66 ± 2.36	16.05 ± 4.56	0.074
Fat-free mass (kg)	60.06 ± 4.33	62.49 ± 7.43	0.104
Fat-free mass (%)	84.34 ± 2.36	83.96 ± 4.55	0.067
VO_2max (ml/min/kg)	44.24 ± 4.23	39.54 ± 5.93	0.029
VO_2max (L/min)	3.04 ± 0.29	3.06 ± 0.62	0.233

2.3. Health security protocol

Previously to the experimental period, all participants were 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 recovery times. Core temperature (Tc), measured in the buccal mucosa, and skin temperature (Tsk), 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 submaximal tests on the cycloergometer (Ergoline 900; Bitz, Germany). Both tests started at 50 W, increasing intensity by 25 W every 2 min until reaching 75% of the estimated maximal heart Rate (HR_{max}). Familiarization tests were performed in both normothermic 823 ± 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 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 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.

2.7. Heat exposure

The sessions consisted of five series of 10 min in a sauna (Harvia C105S Logix Combi Control; 3–15 W; Finland) at 100 °C (20%RH) with a recovery of 5 min between series at ambient temperature (22 °C). In order to control the circadian rhythms, EG performed the session in the morning (from 9 a.m. to 14 p.m.) and at the same time for each participant.

2.8. Sample collection

2.8.1. Serum samples

Two extractions of 5 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, 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.

Hematocrit was obtained by centrifuging the whole blood into a glass capillary containing heparin in a Microcen microfuge (Alresa, Spain). Both hematocrits were used to correct the changes in plasma volume by means of the Van Beaumont (1972) equations.

2.8.2. Urine samples

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.

2.8.3. Sweat samples

The sweat was collected at the end of the hyperthermia tests. Prior to the trials, the participants' backs were washed following the guidelines of Ely et al. (2011) in order to avoid sample contamination. The backs of the participants were rinsed with a liberal amount of MQ distilled water. Additionally, just after the trials in hyperthermia, the sweats samples were collected and aliquoted into an Eppendorf tube (previously washed with diluted nitric acid) and conserved at − 80 °C until biochemical analysis. Sweat Rate was calculated with the equation proposed by Murray (1996) to calculate sweat loss after exercise.

2.9. Serum, sweat and urinary trace element determination

2.9.1. 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 organic matrix was decomposed 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.

2.9.2. Standard and reference material preparation

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

2.9.3. 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 µg/L) used for quality controls agreed with intra and inter-assay variation coefficients of less than 5%.

2.10. Statistical evaluation

Statistical analyses were carried out with SPSS 22.0 for Windows. The results are expressed as the mean and standard deviation ($x \pm sd$). 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, normothermia before and after intervention, hyperthermia before and after intervention and pre-post difference data were determined using the Wilcoxon test for paired samples in both groups. The Mann-Whitney U test was used to determine the differences between groups. A $p \leq .05$ was considered statistically significant.

3. Results

The results on hematocrit, Tc, and Tsk before and after the test in normothermia and hyperthermia, and the sweat rate in each test are shown in Table 2. A significant increase can be observed in the hematocrit ($p < .01$) after the tests in both thermal conditions before and after acclimation in comparison to the pre-test values in CG and EG.

Table 2
Hematocrit and temperature before and after the incremental test until exhaustion and sweat rate in each trial.

		PRE-ACLIMATION				POST-ACLIMATION			
		Normothermic (22 °C)		Hyperthermic (42 °C)		Normothermic (22 °C)		Hyperthermic (42 °C)	
		Before	After	Before	After	Before	After	Before	After
Hematocrit (%)	Control	46.57 ± 2.7	48.7 ± 2.1**	47.04 ± 2.92	48.63 ± 2.87**	48,1 ± 2,42	49,6 ± 2,8**	47,81 ± 2,98	49,07 ± 2,88**
	Experimental	47.06 ± 3.26	49.2 ± 3.28**	45.19 ± 3.2+	48.01 ± 3.06**	48,11 ± 2,98	50,03 ± 3,38**	45,63 ± 3,28∞∞	48,37 ± 3,77**∞
Tsk (°C)	Control	36.62 ± 0.45	36.77 ± 0.51	37.07 ± 0.71	38.42 ± 0.85**++	36,38 ± 0,39	36,66 ± 0,32	37,34 ± 0,91∞∞	38,3 ± 0,78**∞∞
	Experimental	35.6 ± 1.32¢¢	36.92 ± 1.75**	36.95 ± 0.64++	38.15 ± 0.76**++	36,02 ± 0,72	36,68 ± 0,52**	36,96 ± 0,85∞∞	37,83 ± 0,57*∞∞
Tc (°C)	Control	36.77 ± 0.65	36.65 ± 1.1	37.28 ± 0.73	38.08 ± 0.95**++	36,36 ± 0,79	36,37 ± 0,56	37,05 ± 0,067	37,78 ± 0,92∞∞
	Experimental	36.21 ± 0.71¢¢	36.76 ± 0.79	37.17 ± 0.94	38.28 ± 0.81**++	36,54 ± 0,58	36,62 ± 0,6	37,13 ± 1,01∞∞	37,71 ± 1,15∞∞
Sweat Rate (L/h)	Control	0.84 ± 0.39		1.73 ± 0.6*		0.91 ± 0.74		1.71 ± 0.71*	
	Experimental	0.71 ± 0.33		0.1.71 ± 0.71**		0.74 ± 0.29		1.68 ± 0.56**	

Pre-post differences of each test ($p < .05$); ** Pre-post differences of each test ($p < .01$); ¢ Difference with respect to the control group ($p < .05$); ¢ Difference with respect to the control group ($p < .05$); ¢¢ Difference with respect to the control group ($p < .01$); ++ Before-Before and after-after differences before acclimation ($p < .01$); ∞ Before-Before and after-after differences after acclimation ($p < .05$); ∞∞ Before-Before and after-after differences after acclimation ($p < .01$).

Table 3
Serum levels of Mg and P before and after the incremental test until exhaustion. The data are presented with and without correction (C) for possible hemoconcentration.

		Pre-acclimation				Post-acclimation			
		Normothermic (22 °C)		Hyperthermic (42 °C)		Normothermic (22 °C)		Hyperthermic (42 °C)	
		Before	After	Before	After	Before	After	Before	After
Mg (mg/L)	Control	19.26 ± 1.36	19.14 ± 1.33	19.43 ± 1.56	18.74 ± 1.67	19.13 ± 0.79	19.28 ± 1.01	19.53 ± 1.9	19.38 ± 1.5
	Experimental	19.89 ± 1.62	19.97 ± 2.32	19.44 ± 2.03	19.37 ± 2.47	18.59 ± 1.21##	18.57 ± 1.29#	18.19 ± 1.58#	18.59 ± 1.49
Mg-C (mg/L)	Control	19.26 ± 1.36	17.57 ± 1.38**	19.43 ± 1.56	17.62 ± 1.83**	19.13 ± 0.79	18.18 ± 1.21	19.53 ± 1.9	18.56 ± 3.04
	Experimental	19.89 ± 1.62	18.32 ± 2.09**	19.44 ± 2.03	17.27 ± 2.49**	18.59 ± 1.21	17.19 ± 1.54**#+	18.19 ± 1.58	16.67 ± 1.54**
P (mg/L)	Control	111.74 ± 26.72	121.77 ± 29.8*	113.62 ± 20.46	120.67 ± 22.75*	111.19 ± 16.13	118.39 ± 17.27	110.07 ± 22.1	118.76 ± 19.9*
	Experimental	136.28 ± 24.67+	144.17 ± 26.98	129.29 ± 26.58	139.83 ± 26.3*	124.27 ± 19.31	136.51 ± 20.44**#+	119.61 ± 22.59#	132.96 ± 19.52**
P-C (mg/L)	Control	111.74 ± 26.72	111.79 ± 26.43	113.62 ± 20.46	113.24 ± 21.73	111.19 ± 16.13	112.02 ± 19.18	110.07 ± 22.1	123.24 ± 22.62∞
	Experimental	136.28 ± 24.67	132.17 ± 23.65	129.29 ± 26.58	124.57 ± 25.28	124.27 ± 19.31	126.75 ± 21.77	119.61 ± 22.59	146.93 ± 25.85**#∞∞+

* Pre-post differences of each test ($p < .05$); ** Pre-post differences of each test ($p < .01$); # Differences after-after and before-before with respect to the test with the same thermal conditions ($p < .05$); ## After-after and before-before differences with respect to the test with the same thermal conditions ($p < .05$); ¢ Before-Before and after-after differences before acclimation ($p < .05$); ∞ Before-Before and after-after differences after acclimation ($p < .05$); ∞∞ Before-Before and after-after differences after acclimation ($p < .01$); + Differences with respect to the same parameter of the control group ($p < .05$); ++ Differences with respect to the same parameter of the control group ($p < .01$).

Additionally, the hematocrit before ($p < .01$) and after ($p < .05$) the test in hyperthermic condition post-acclimation was significantly lower respect to normothermic condition post-acclimation in EG. Regarding temperature, Tsk was always significantly higher after each test in EG, but only in hyperthermic trials in CG. Both groups experimented an increase in Tsk ($p < .01$) after the test in hyperthermia concerning the values after the test in normothermia. Tsk and Tc before, in normothermia, was significantly lower in EG with respect to CG before the acclimation. There were differences in pre-post in Tc in the hyperthermia pre-acclimation test in both groups ($p < .01$) but not after the acclimation period. However, Tc after the test in hyperthermia was higher in comparison to Tc in normothermia in both groups before and after the acclimation. The sweat rate was elevated in the hyperthermia tests with respect to the tests in normothermia before and after the acclimation period in CG ($p < .05$) and EG ($p < .01$).

The data related to serum concentration on Mg and P are presented in Table 3. The values are with and without correction for possible hemoconcentration after exercise. Mg values in EG were lower than CG after acclimation. However, in Mg-C there was only this difference after the test in normothermia post-acclimation ($p < .05$). A significant depletion of Mg-C after all trials could be observed in EG but in CG only after the test in the pre-acclimation period. Furthermore, a lower Mg-C in EG in the trial in normothermia post-acclimation could be observed with respect to the normothermia test pre-acclimation ($p < .05$) and the same parameter in CG ($p < .05$). Concerning P, an increment of P in CG ($p < .05$) could be observed after all the incremental tests until exhaustion except in normothermia post-acclimation. EG experimented a higher concentration of P after the hyperthermia trial pre-and post-acclimation and normothermia post-acclimation. Additionally, a significant increase in P after the normothermia test post-acclimation was observed in comparison with the data after the normothermia pre-acclimation test and with the same value in CG ($p < .05$). On the other hand, P-C only showed pre-post difference after the hyperthermia test post-acclimation in EG ($p < .01$). This value also underwent changes with respect to the same data in hyperthermia pre-acclimation ($p < .05$), normothermia pre-acclimation ($p < .01$) and CG ($p < .05$).

Table 4 shows the urine concentration of Mg and P, before and after each test. After the acclimation period, Mg underwent a significant increase after the normothermia test ($p < .01$). Additionally, the Mg concentration after the hyperthermia post-acclimation test was higher in EG than CG ($p < 0,05$). A lower P after hyperthermic test in comparison P after normothermic trial in pre-acclimation period could be observed in EG ($p < .05$). Table 5 presents the sweat values in the hyperthermia tests. The concentrations are divided by the sweat rate to adapt the data to the sweat rate (Chinevere et al., 2008). No significant difference was found in any of the minerals in CG. Mg and Mg-C values declined ($p < .05$) after acclimation in EG. P did not show statistical changes.

Fig. 1 shows the seric and urine concentrations of Mg and P. The values of seric Mg-C are illustrated in Fig. 1A. Fig. 1B shows the results in seric P-C. The values in urine Mg are represented in Fig. 1C. Fig. 1D displays the data referring to urine P.

Fig. 2 shows graphically the sweat values of Mg (A) and P (B) during the study.

4. Discussion

Increases in Tc because of physical exercise compromise all organism functions. Heat is produced by the human body due to the transformation of mechanical energy into thermal energy (Gonzalez-Alonso et al., 2008). This phenomenon induces an elevation of Tc and produces blood flow redistribution to peripheral areas to eliminate the heat, thus increasing Tsk. Recent research reported an elevation of Tc in hot environments (36.4–37.9 °C) in triathletes, whose initial Tc was similar to that obtained in heat (37 ± 0,3 °C) (Logan-Sprenger, 2019).

Table 4
Urine Concentration of Mg and P, before and after the incremental test until exhaustion.

		Pre-acclimation				Post-acclimation			
		Normothermic (22 °C)		Hyperthermic (42 °C)		Normothermic (22 °C)		Hyperthermic (42 °C)	
		Before	After	Before	After	Before	After	Before	After
Mg (mg/L)	Control	62.84 ± 24.9	58.8 ± 21.44	53.36 ± 44.47	70.04 ± 37.61	71.24 ± 50.75	70.2 ± 44.11	66.42 ± 46.68	86.24 ± 45.92
	Experimental	82.89 ± 52.93	87.33 ± 68.53	77.7 ± 63.07	63.22 ± 42.67	85.5 ± 38.57	110.47 ± 55.63**	96.29 ± 76.58	106.61 ± 68.11#
P (mg/L)	Control	467.62 ± 341.62	493.06 ± 243.34*	353.74 ± 238.83	631.18 ± 407.57	486.93 ± 261.26	605.61 ± 444.53	438.09 ± 390.83	641.53 ± 501.15
	Experimental	746.18 ± 535.92	815.53 ± 572.52	605.55 ± 409.65	472.01 ± 261.92¢	521.41 ± 307.91	623.25 ± 548.29	498.43 ± 381.4	633.24 ± 419.32

* Pre-post differences of each test ($p < .05$); ** Pre-post differences of each test ($p < .01$); ¢ Difference with respect to the control group ($p < .05$); # Differences after-after and before-before with respect to the test with the same thermal conditions ($p < .05$).

Table 5
Sweat concentration of Mg and P in the hyperthermic condition without and with correction (C) for sweat excreted after the incremental test until exhaustion.

	CONTROL		EXPERIMENTAL	
	PRE-ACCLIMATION	POST-ACCLIMATION	PRE-ACCLIMATION	POST-ACCLIMATION
Mg (mg/L)	12.95 ± 13.83	17.12 ± 17.43	22.5 ± 23.07	8.83 ± 3.48*
Mg-C (mg/h)	10.45 ± 12.55	10.41 ± 9.04	17.62 ± 18.64	5.92 ± 3*
P (mg/L)	2.27 ± 1.37	3.74 ± 4.49	3.18 ± 2.35	2.2 ± 0.92
P-C (mg/h)	1.98 ± 1.99	2.18 ± 2.32	3.27 ± 4.8	1.53 ± 0.94

* Differences between pre and post acclimation ($p < .05$).

Nevertheless, while in the mentioned paper Tc increased significantly with exercise, in this study there was no significant increase after the incremental test until exhaustion, the discrepancy perhaps being due to the fact that the measurement methods are different and the sample in the mentioned study is lower. In a subsequent study, in similar thermal condition as this survey (42 °C, 40–60% RH), they evaluated the Tc, measured in the rectum, and Tsk, observing a rise in temperature as the intensity of the exercise increased (Suvi et al., 2017). Since the test conducted in this investigation is of lesser duration, this could be the explanation of why there was no significant increase in Tc. On the other hand, there was an increase in Tsk, which would reflect the body's need to eliminate heat. In this survey, there was no decrease in the thermoregulatory response in the experimental group after 9 sessions of heat exposure, while Lorenzo and Minson (2010) found a significant decrease in Tc after 10 days of heat acclimation, but not in Tsk because acclimation did not alter the blood flow. Simultaneously, these authors did not observe changes in the hematocrit after 10 days of acclimation (Lorenzo et al., 2010) as in the present investigation. There was an increase in hematocrit in both groups after each test, but they were due to dehydration, reflected in the sweat rate, producing hemoconcentration (Buono et al., 2016). The sweat rate did not vary in EG in normothermia or in hyperthermia after 9 sessions of exposure to heat at high temperatures, contrary to what has been reported, where heat acclimation increases the sweat rate (Lorenzo and Minson, 2010). However, this did increase significantly in hyperthermia with respect to the test in normothermia before and after acclimation in both groups.

In relation to the minerals studied, serum was used to evaluate the state of Mg and P in the body, and urine and sweat as two of the three main routes of excretion, with feces. Serum concentrations are represented without and with correction for readjustment of mineral values after possible hemoconcentration through exercise (Maynar et al., 2018a). Additionally, the levels of Mg and P for the sweat were corrected by sweat rate. The control of sweating can be modified by acclimation to heat (Gagnon and Crandall, 2018), so, minerals can be diluted in a higher amount of sweat. In order to avoid this phenomenon, the values were corrected by the sweat rate, estimating the mg of minerals lost per hour, as proposed by Chinevere et al. (2008). The initial values of both minerals were found within the levels reported in previous studies in serum (Lu et al., 2015) and urine (Siquier-Coll et al., 2019) with the same technique. For a better discussion, each mineral

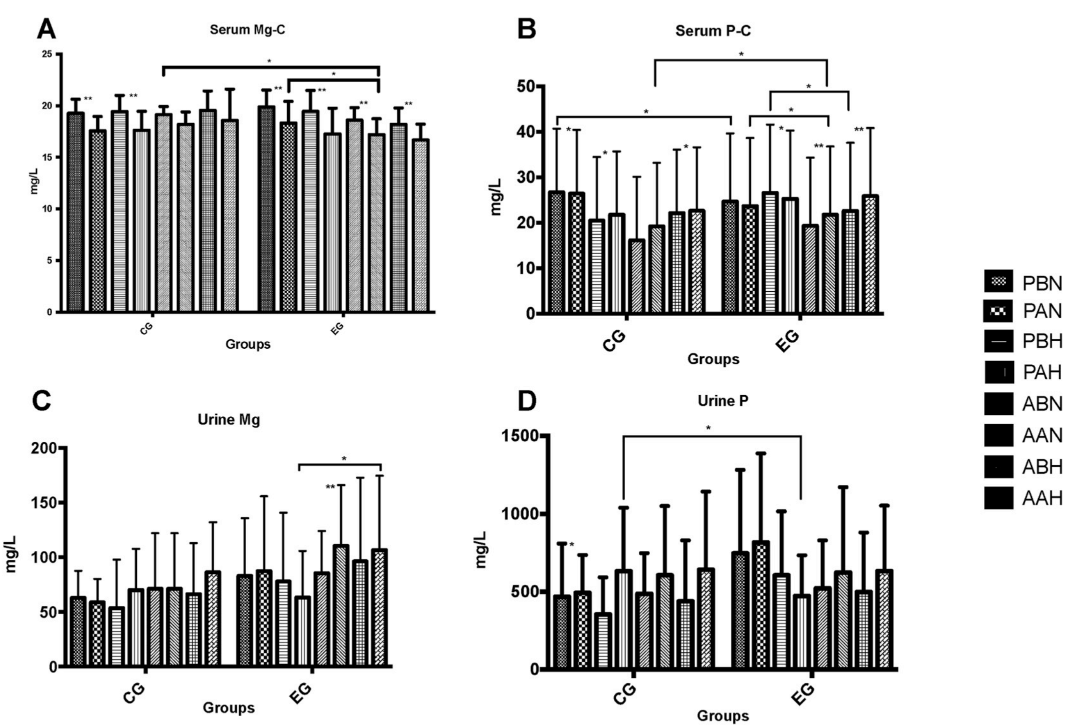

Fig. 1. Concentration of Mg and P in serum and urine in each test. A: Levels of Serum Mg-C. B: Concentrations of seric P-C. C: Levels of urine Mg. D: Urine P concentrations. *: $p < .05$; **: $p < .01$; PBN = Values before normothermic trial in pre-acclimation period; PAN = Levels after normothermic test in preacclimation period; PBH = Concentrations before hyperthermic test in pre-acclimation period; PAH = Concentrations before hyperthermic test in pre-acclimation period; ABN = Concentrations before normothermic trial in post-acclimation period; AAN = Levels after normothermic test in post-acclimation period; ABH = Concentrations before hyperthermic trial in post-acclimation period; AAH = Values after hyperthermic test in post-acclimation period.

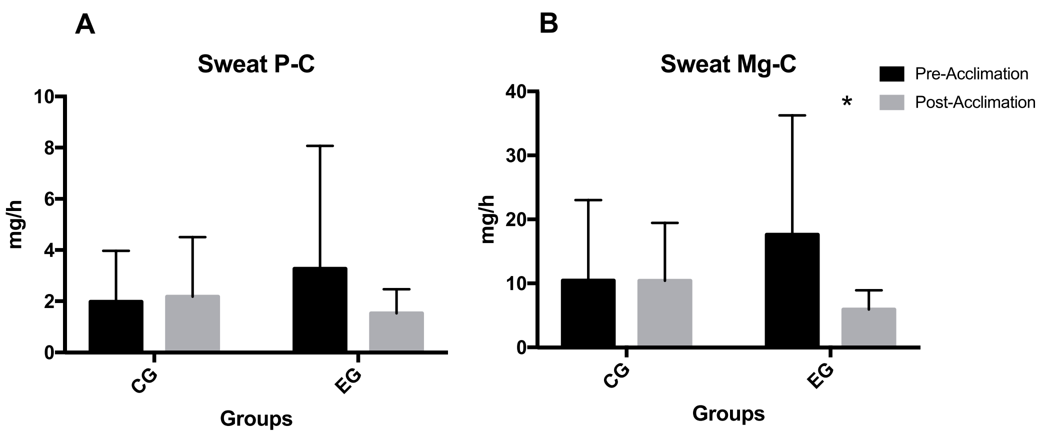

Fig. 2. Levels of P and Mg in Sweat. *: $p < .05$.

will be discussed separately in the three matrices. Likewise, the relevant findings are illustrating in Figs. 1 and 2.

Mg frequently presents a risk of deficiency in the diet, exacerbated by sweating in heat during exercise (Clarkson and Haymes, 1995). In the acute pre-acclimation effect there were no changes in serum Mg without correction. However, there was a fall in serum Mg-C in hyperthermia and in normothermia in both groups. The serum Mg decrease after exercise has been well documented. It seems that Mg losses occur after maximum exercise (Laires and Alves, 1991), but not after submaximal exercise (Laires and Monteiro, 2008; Soria et al., 2011). It was suggested that this depletion in Mg could be due to a cellular absorption by erythrocytes (Deuster et al., 1987; Doker et al., 2014; Resina et al., 1995). We did not find changes in erythrocyte Mg after the decrease of seric Mg in hyperthermia (Siquier-Coll et al., 2019). It has been reported that higher losses of Mg are produced by sweating after exposure to heat (Consolazio et al., 1964; Tang et al., 2016). This lack could be the cause of the reduction of serum Mg-C in both groups after the tests before the acclimation period, but not after the acclimation period where there were changes in EG, but not in CG (Fig. 1A). Beller et al. (1975) reported that the serum Mg decrease could not be explained by Mg changes in sweat. Thus, it could be due to Mg losses through the urinary tract, since strenuous and long-term exercise also increases the excretion of this mineral (Nielsen and Lukaski, 2006). Interestingly, neither group experimented alterations in the urinary excretion of Mg in the initial tests, however, after acclimation there was a rise in the Mg concentration after the test in normothermia and there was a significant elevation in urinary excretion in hyperthermia with respect to the test in the same conditions before acclimation in EG (Fig. 1C). Thus, acclimation produced an increase in the urinary excretion of Mg in EG. On the other hand, repeated exposure to heat caused a lower excretion of Mg by sweating (Fig. 2B). In a similar study, the participants performed an acclimation of 10 days walking in hyperthermic conditions (45 °C, 20% RH) for 100 min. They obtained lower levels of Mg in sweat, both in Mg (7.29 ± 4.43 mg/L) and in Mg-C (4.43 ± 3.64 mg/L), to those obtained in this survey. The mentioned study observed a significant decrease of Mg and Mg-C in the values of the tenth day in sweat (Chinevere et al., 2008), as in the present investigation. However, the same research group did not report changes in Mg after the collection of samples with the disinfection and skin cleansing protocol to avoid possible contamination (Ely et al., 2013). Conversely, in this study significant decreases were found after acclimation. In addition, our acclimation was different, since it did not require the realization of physical activity, but, on the other hand, the exposure to heat was at higher temperatures (45 °C versus 100 °C). This fact manifests the validity of the protocol of acclimation proposed for the lower excretion of Mg in sweat by this investigation. Notwithstanding, this adaptation produces an increase in the excretion of Mg in urine (Fig. 1C). Therefore, there is a decrease in Mg in both acclimated and non-acclimated subjects, possibly requiring Mg supplementation in hot conditions.

There are few studies in humans related to exercise and phosphorus. Nishimuta et al. (2004) reported that P can be strongly affected by factors external to the diet. In this line, Maynar-Marino et al. (2015) observed lower serum levels of P in athletes than in sedentary subjects, suggesting that there may be a loss of P through sweating. In a study of boxers, an elevation in plasma P was observed (Karakukcu et al., 2013). This process could be due to muscle damage, producing the release of P (Knochel et al., 1974). In this study, after the initial tests there was a significant increase in P, but once corrected, these statistical differences disappeared, as previously reported (Siquier-Coll et al., 2019). However, after acclimation there were significant changes in P-C in hyperthermic conditions (Fig. 1B), and there may be muscle damage in the said test. In urine, there were no differences in any of the tests as observed in a recent investigation after analyzing the effect of acute exercise (Eskici et al., 2016). Regarding sweating, the elimination of P does not decrease as sweating increases (Mitchell and Hamilton, 1949). Consolazio et al. (1964) investigated the effects of an acclimation at 37.77 °C for 16 days in minerals. This study observed a fall in P in sweat and an increase in P in urine after 9 days. Conversely, in this study there were no differences in P or P-C between before and after acclimation (Fig. 2A). No other studies were found in relation to phosphorus with exercise in thermal stress to be able to compare the results. Further research is needed to define P homeostasis during exercise in hot environments.

5. Conclusions

There is an increase in body temperature in response to exercise and exercise in hyperthermic conditions. Respecting minerals, the need to correct serum values through hemoconcentration and values in sweat with the sweat rate is revealed. The results obtained suggest that Mg supplementation may be necessary in hyperthermic or normothermic conditions, in both non-acclimated subjects and acclimated subjects due to the loss of this element through urine. Regular exercise does not produce changes in the homeostasis of P, but it does in hyperthermic conditions in acclimated subjects, and there may be a need for supplementation of this mineral.

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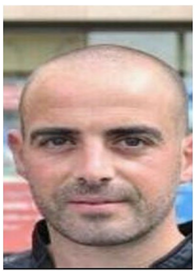

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