

Effect of exposure to high temperatures in the excretion of cadmium and lead

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ABSTRACT

Objective: This study aims to observe the effect on urine and sweat excretion levels of cadmium (Cd) and lead (Pb) in healthy men in a maximum incremental test until exhaustion and repeated exposure to heat.

Methods: twenty-nine adult men divided into control group (CG; n = 14) and experimental group (EG; n = 15) performing two maximum tests until exhaustion in normothermia (22 °C) and hyperthermia (42 °C). EG experienced 9 sessions of heat exposure at high temperatures (100 °C) (HEHT). After the nine sessions, the initial tests were repeated in both groups. Urine samples were collected before and after each test. After the hyperthermia tests, sweat samples were gathered.

Results: Urinary Cd increased after initial tests in GC and in hyperthermia in EG (p < 0.05). Urinary excretion of Pb rose after HEHT (p < 0.05). Pb in sweat was higher in EG than in CG after HEHT (p < 0.05).

Conclusion: Heat exercise and constant exposure to heat can be a valid method to increase the excretion of toxic metals.

1. Introduction

The levels of toxic elements in the organism have been increasing in recent decades (Benes et al., 2001). A rise in industrialization has increased the exposure to toxic metals such as cadmium (Cd) and lead (Pb) for general population (Ollson et al., 2017). Contact to these elements may occur either by water, air or food, therefore, they become harmful to health (Rehman et al., 2018).

Cadmium is a heavy metal of great industrial use. It is acknowledged as one of the most eco-toxic metals (Rizwan et al., 2017). Thus, this fact reveals an entirely damaging impact on the environment and the quality of food. Between 1050% of the Cd inhaled by humans is absorbed by the body. Once in the body, it is largely stored in muscles, bones, kidneys and liver with an average life of 10–30 years (Nordberg et al., 2015). This metal discloses adverse effects in all biological processes (Kabata-Pendias and Szeke, 2015). Cd produces oxidative stress, inducing tissue damage (Patra et al., 2011). The main routes of excretion of Cd are urine and sweat (Llerena et al., 2012). The latter has been considered the principal route of excretion for the elimination of this element from organism (Genuis et al., 2011).

Pb is applied to various industrialization and mining activities due to its distinctive physical and chemical properties (Mitra et al., 2017). This

toxic metal causes considerable environmental pollution as a result of its ecological persistence and transportability (Navas-Acien et al., 2007). Drinking water has been an important source of Pb entry into body during decades (DeWitt, 2017). Inhaled Pb is almost completely absorbed, whereas the absorption of ingested lead is approximately 10–15% (Mitra et al., 2017). Circulating Pb lives an average of 40 days, afterwards, it would be accumulated in the bone for decades and slowly released (Wani et al., 2015). A concentration higher than 5 µg/dl in blood is high for adults and infants (Mitra et al., 2017). Such as for Cd, the main excretion pathways are urine and sweat (Genuis et al., 2011).

Few studies have focused on assessing the effects of training and physical exercise on body concentrations of Cd and Pb, despite their ability to alter performance and their effects on health (Grijota et al., 2019; Llerena et al., 2012; Maynar-Mariño et al., 2018; Maynar et al., 2018; Rodriguez Tuya et al., 1996).

Currently, the elimination of toxic elements from the body through heat and exercise is being emphasized, along with sauna baths (Sears et al., 2012). Thus, since exercise and heat raise body temperature, they can facilitate the elimination of these elements through the skin by sweating. In addition, in some metals such as zinc, there seems to be a mechanism of action whereby a flow from red blood cell to plasma/serum during exercise is generated (Chu et al., 2016). A recent study

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discovered different levels of these toxic elements in erythrocytes among athletes, semi-professionals and sedentary, hence a relation between periodic training and homeostasis of these elements exists. Yet, a high volume of exercise is deemed to lead to an erythrocyte flow to the Cd plasma and it consequently eliminates them by sweating (Grijota et al., 2019). Another recent study suggests the flow of metals between plasma/serum and erythrocytes could be exacerbated in hot situations due to an increase in blood circulation resulting from the thermoregulation process (Siquier-Coll et al., 2019a).

Therefore, the purpose of this study is to observe how a maximum incremental test affects exhaustion to urinary and sweat concentrations in normothermic (22 °C) and hyperthermic (42°) conditions before and after nine sessions of heat exposure at high temperatures (HEHT).

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,a,b,c).

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 two 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.

The tests started with the collection of a urine sample obtained in fasting conditions. 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

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/m ²)	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 ₂ max (ml/min/kg)	44.24 ± 4.23	39.54 ± 5.93	0.029
VO ₂ max (L/min)	3.04 ± 0.29	3.06 ± 0.62	0.233

were performed in normothermic conditions and the second day in a hyperthermic environment. 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 nine sessions of heat exposure at high temperatures in the sauna for 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.

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 analysed 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 (T_c), measured in the buccal mucosa, and skin temperature (T_{sk}), 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. Familiarisation period

Before the start of the experimental period, one week of prior familiarisation 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}). Familiarisation 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 analyser "Geratherm Respiratory GMBH [Ergostik (Ref 40.400; Corp Bad Kissingen)]".

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 analyser 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)] along with 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 2 p.m.) and at the same time for each participant.

2.8. Sample collection

2.8.1. Urine samples

Urine samples were obtained from each participant before and after the test. 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.

2.8.2. 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 sweat 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 and urinary trace element determination

2.9.1. Sample preparation

Cd and Pb analyses were performed by inductively coupled plasma mass spectrometry (ICP-MS) according to the protocol followed by (Maynar et al., 2018). 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 HNO₃ and 0.4 mL H₂O₂ to 2 mL of 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 (Seronom, lot 0511545, Sero AS Billingstand, Norway) were prepared identically and used for accuracy testing. Qualquier 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 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%.

2.10. 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 referred to skin temperature (Tsk) (1 A), core temperature (Tc) (1 B) and sweat rate (1C) are represented in Fig. 1. An increase of Tc after hyperthermia trial before HEHT in both groups ($p < 0.01$) it can be observed. Tc was higher after the hyperthermia trial before HEHT (AHBHEHT) than in after the normothermic test before HEHT (ANBHEHT) in both groups ($p < 0.01$). Besides, both groups experimented a rise in Tc after hyperthermia trial after HEHT (AHAHEHT) with respect the value after the hyperthermic test before HEHT (AHBHEHT) ($p < 0.01$), and EG Tc was higher previous to hyperthermic trial after HEHT (PHAHEHT) than in the value pre-hyperthermic test before HEHT (PHBHEHT) ($p < 0.01$). Regarding Tsk, it increased significantly after each trial before HEHT in EG and CG. However, after HEHT, only was higher in each test in EG ($p < 0.01$) and the hyperthermia trial in CG ($p < 0.01$). Sweat Rate underwent a rise in hyperthermia trials in EG ($p < 0.01$) and CG ($p < 0.05$).

Table 2 presents the urine levels of Cd and Pb after each trial. Cd underwent changes in normothermia in both groups ($p < 0.05$) and hyperthermia in CG Before HEHT ($p < 0.05$). After HEHT, EG experimented a rise in Cd levels after normothermia test ($p < 0.05$). The value of Cd after the first normothermia trial was higher in EG than in CG ($p < 0.05$). Cd before the hyperthermic test was lower in comparison the same value before normothermic trial after HEHT in EG ($p < 0.05$). About Pb, it can be observed a higher concentration in CG after hyperthermia test before HEHT. In EG, Pb levels increased after hyperthermic trial after HEHT. The elimination of Pb was lower in hyperthermia test concerning normothermia trial in EG before HEHT. After HEHT, Pb pre-test was significantly lower in hyperthermia than in normothermia in both groups ($p < 0.05$). The Pb post-test value in EG in normothermia test after HEHT decreased with respect to the homologous trial before in the same thermic conditions ($p < 0.05$). It was differences between groups in the concentrations of Pb before the normothermia test after HEHT ($p < 0.05$).

Table 3 describes the values of sweat concentration after hyperthermic test with (C) and without the correction for sweat rate.

It can be observed significant differences in Pb between groups after the hyperthermic trial before HEHT ($p < 0.05$). Besides, it was a higher concentration in EG than CG of Pb after the hyperthermic test after HETH ($p < 0.05$).

4. Discussion

Exercise produces a thermoregulatory response in the body. This response is reflected in the increases in Tc and Tsk. The rise in the

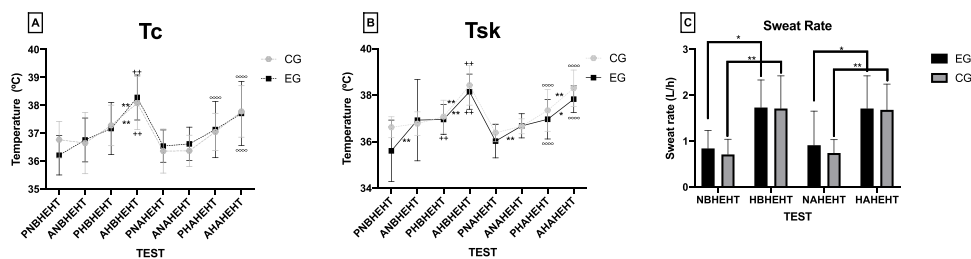


Fig. 1. Core temperature (Tc) and skin temperature (Tsk) before and after each test, and sweat rate in each trial. PNB HEHT = pre-normothermic trial before heat exposure at high temperature (HEHT); ANB HEHT = post-normothermic trial before HEHT; PHB HEHT = pre-hyperthermic trial before HEHT; AHB HEHT = post-hyperthermic trial before HEHT; PNA HEHT = pre-normothermic trial after HEHT; ANA HEHT = post-normothermic trial after HEHT; PHA HEHT = pre hyperthermic trial after HEHT; AHA HEHT = post-hyperthermic trial after HEHT; NB HEHT = Normothermic test before HEHT; HB HEHT = Hyperthermic test before HEHT; NAHEHT = Normothermic test after HEHT; HA HEHT =Hyperthermic trial after HEHT. A and B: *Pre-post differences between before and after each test ($p < 0.05$); ** Pre-post differences between before and after each test ($p < 0.01$); ++before-before and after-after differences before HEHT; ∞∞Before-before and after-after differences after HEHT. C: * Intra-groups differences between NB HEHT-HB HEHT test and NA HEHT-HA HEHT trials ($p < 0.05$); **Intra-groups differences between NB HEHT-HB HEHT test and NA HEHT-HA HEHT trials ($p < 0.01$).

Table 2
Cd and Pb concentration on urine before and after each trial.

		Pre-HEHT		Post-HEHT					
		Normothermic (22 °C)		Hyperthermic (42 °C)		Normothermic (22 °C)		Hyperthermic (42 °C)	
		Before	After	Before	After	Before	After	Before	After
Cd (µg/L)	Control	0,08 ± 0,05	0,10 ± 0,06*	0,05 ± 0,04	0,12 ± 0,04*	0,13 ± 0,14	0,12 ± 0,14	0,06 ± 0,04∞	0,13 ± 0,10
	Experimental	0,12 ± 0,04	0,21 ± 0,09#*	0,12 ± 0,06	0,11 ± 0,05+	0,12 ± 0,11	0,21 ± 0,14*	0,16 ± 0,14	0,24 ± 0,26
Pb (µg/L)	Control	0,36 ± 0,23	0,29 ± 0,21	0,23 ± 0,24	0,38 ± 0,24*	0,36 ± 0,19	0,25 ± 0,15	0,22 ± 0,17∞	0,33 ± 0,19
	Experimental	0,37 ± 0,36	0,52 ± 0,20	0,30 ± 0,19	0,21 ± 0,12+	0,22 ± 0,05#	0,19 ± 0,05‡	0,18 ± 0,22∞	0,57 ± 0,45*

* Pre-post differences of each test ($p < 0.05$); ‡ Differences after-after with respect to the test with the same thermal conditions ($p < 0.05$); # Differences with respect to the same parameter of the control group ($p < 0.05$); ∞ Differences before-before with respect to the normothermia after HEHT ($p < 0.05$).

Table 3
Sweat concentration of Cd and Pb in the hyperthermic condition with (C) and without correction for sweat excreted during the incremental test until exhaustion.

	CONTROL		EXPERIMENTAL	
	PRE-HEHT	POST-HEHT	PRE-HEHT	POST-HEHT
Cd (µg/L)	0,77 ± 0,54	1,13 ± 1,37	1,14 ± 1,37	1,18 ± 1,33
Cd-C (µg/h)	3,51 ± 5,32	2,30 ± 2,52	2,88 ± 3,82	3,1308 ± 4,37
Pb (µg/L)	21,50 ± 20,29#	24,30 ± 15,26	32,69 ± 13,11	37,86 ± 20,16
Pb-C (µg/h)	70,66 ± 77,04	58,08 ± 50,54	85,01 ± 48,46	89,40 ± 52,16#

#U Mann Whitney test ($p < 0.05$).

thermal load in the organism increases in the mechanism of heat loss, leading to an augmentation in peripheral circulation from 5-10% to 60-70% (Sears et al., 2012). This process is intensified in hyperthermic situations, as represented in Fig. 1. Accordingly, the thermoregulation process leads to a continuous homeostasis. This phenomenon also favours the production of homeostatic regulations at the level of trace elements during exercise and heat (Siquier-Coll et al., 2019b). Recent studies have observed changes in trace element concentrations in the different body compartments produced by acute heat exercise and

passive exposure to hyperthermia (Siquier-Coll et al., 2019a,b,c). Besides, acute exercise has been proved to alter the levels of toxic elements (Llerena et al., 2012; Maynar et al., 2018). Grijota et al. (2019) studied lower levels of Cd in subjects with different levels of physical activity in erythrocytes. In this research, the thermoregulation process during exercise could produce a mechanism of erythrocyte flow to the plasma facilitating its elimination via sweat and urine. Sears et al. (2012) reported sweating as a possible purifying process for toxic elements such as Cd and Pb. Omokhodion and Howard (1994) obtained a range of 1.1-3.1 µg/L of Cd in sweat, similar to those obtained in this study. In contrast, another study obtained an average of 5.7 µg/L after sauna-induced sweating (Genuis et al., 2011). The differences in sweat excretion in the above-mentioned studies regarding this research may be due to the industrialization of the geographical area and the age gap, since the older the subject is, the more levels of Cd and Pb arise (Jarup et al., 1998). About Pb, Genuis et al. (2011) reported a concentration of 31 µg/L, being higher than in this study such as the case of Cd. Elsewhere, Omokhodion and Howard (1994) detected concentrations in a range of 9-30 µg/L in controls, similar to the ones obtained in this research in Pb. However, no studies were found in order to be compared to the results obtained from toxic metal levels after acute heat exercise. Saran et al. (2018) observed a decrease in Pb after 14 days of training in cycloergometer. After HEHT, EG sweat concentrations did not suffer any change in the metals. Interestingly, a higher elimination of Pb-C was

found in sweat in EG with respect to CG after acclimation. This feature would indicate acclimation at high temperatures could induce a rise of the elimination of this element by sweat, unlike other trace elements decreasing its concentration in sweat after a heat acclimation (Siquier-Coll et al., 2019a,b,c). However, no studies in scientific literature observing the effects of heat exposure on Pb and Cd concentrations could be found in order to compare these results. Although the excretion rates of toxic elements by sweat exceed urinary excretion in 24 h (Sears et al., 2012), urine is also a critical excretion route. Urinary levels of Cd were similar to those obtained by Llerena et al. (2012), whereas the levels obtained in Pb were lower in this investigation, where the participants were from the same geographical area.

A recent study found no pre-post differences in urinary concentrations of Pb and Cd in either athletes or controls (Maynar et al., 2018). Conversely, in our study, significant differences were detected in both groups after the initial normothermic test and in CG in hyperthermia. This fact is relevant because changes produced in trace elements have been mentioned to be more significant in tests in treadmill than in cycloergometer (Chu et al., 2016; Soria et al., 2011). Another relevant characteristic is the growth in urinary excretion of Pb in exercise in hyperthermia in EG after HEHT, according to recent studies on the same subjects. The mentioned papers observed an increase in the elimination of minerals in heat exercise after acclimation in sauna at high temperatures (Siquier-Coll et al., 2019a,b,c; Siquier-Coll et al., 2020a, 2020b). Interestingly, in this study the rise in urinary excretion of minerals was accompanied by a decrease in excretion via sweat, whereas in this study the excretion increased in both excretion pathways.

Nevertheless, this research has limitations such as the number of participants. Another one is the concentrations of Cd and Pb in blood parameters not being able to be measured. Therefore, further research is required to perceive the consequences of repeated exposure to heat on blood parameters and counteract the results obtained.

5. Conclusions

Acute exercise in normothermia and hyperthermia causes an increase in the urinary excretion of Cd. Pb is excreted in higher concentrations after heat exercise and repeated exposure to heat. Additionally, repeated exposure to heat elicits a rise in urinary excretion of Pb in hyperthermia. Heat exercise and heat exposure are methods to increase the excretion of Pb and Cd.

In the light of the results, heat exercise and repeated exposure to heat seem to allow the elimination of toxic elements. This fact could be a route of detoxification for populations with high pollution and workers with a high degree of exposure to heavy toxic metals.

CRedit authorship contribution statement

J. Siquier-Coll: Investigation, Writing - original draft, Writing - review & editing, Formal analysis. **I. Bartolomé:** Investigation, Formal analysis. **M. Pérez-Quintero:** Investigation. **D. Muñoz:** Validation. **M. C. Robles:** Visualization. **M. Maynar-Mariño:** Conceptualization, Methodology, Writing - review & editing.

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