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RESEARCH ARTICLE

COMPARISON OF URINARY CONCENTRATIONS OF MACROELEMENTS AND TRACE METALS IN ATHLETES AND SEDENTARY SUBJECTS LIVING IN THE SAME AREA OF EXTREMADURA (SPAIN)

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ABSTRACT

In the last few years, progress in the biomedical evaluation of athletes has caused scientists to consider numerous factors that can be modified by intense physical activity, and at the same time influence in an important way the sportsmen's power output. An increasing emphasis has been placed on the role of macro and trace metals in human health and disease. The aim of the present study was to compare two macroelements Mg and P and the trace elements As, B, Cs, Li, Mg, P, Rb, Sn and Sr in urine samples in middle distance runners with the corresponding levels in referent sedentary subjects matched by age, sex, and geographic area. Twenty-one Spanish national middle distance runners were recruited before the start of their training period. Male students from the Sport Sciences Faculty of Extremadura University, who had not been regularly or systematically performing physical exercise volunteered for this study and constituted the control group. Mg, P, As, B, Cs, Li, Rb, Sr and Sn analysis of urine was performed by ICP-MS. Element analysis concentrations, expressed in µg/g creatinine were: Mg (44,92±36,8vs. 111,30±91,32, $p \leq 0.01$); Sn (0,67±0,92 vs. 2,49±3,29, $p \leq 0.05$), were lower in athletes urine; Cs (5,05 ± 2,65vs. 3,20±1,60,$p \leq 0.01$) in athletes urine have higher than the sedentary group. The results showed that there were not statistically differences between urinary concentrations in both groups of As, B, Li, P, Rb and Sr. We can conclude that the population in Extremadurahasnoab normal levels of these metals. But athletes have some modifications to the elimination of some of these metalsthat could be related to possible adaptation stophysical activity.

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INTRODUCTION

In the last few years an increasing emphasis has been placed on the role of macro and trace metals in human health and disease (Bertini and Cavallaro, 2008; Jomova and Valko, 2011). They are in contact with the man, who uses them for different purposes. Magnesium (Mg) is a cofactor in more than 300 enzymatic reactions in which food is catabolized and new chemical products are formed (Lukaski, 2000). Magnesium is also involved in cellular energy production, glycogen breakdown, and modulation of the activity of adenosine triphosphatase providing energy for cells; moreover, magnesium serves as a physiological regulator of neuromuscular functions (Ebel and Gunther, 1980; Lin *et al.*, 2002; Lukaski, 2000; Saris *et al.*, 2000). Phosphorous (P) stimulates renal tubular reabsorption of glucose through the same process, it joins to lipids and produce phospholipids, which are part of all cell membranes. Until the 1970s, about 80% of Arsenic (As) was used in the manufacturing of pesticides. Due to its toxicity, the use of As in pesticides has decreased to about 50%, but organic As compounds still dominate the pesticides production by 90% (Matschullat, 2000). The rest of the amount is used in wood preservation, photoelectric devices, glassware, and Pb-acid batteries. It is also used to improve corrosion resistance and tensile strength in Cu alloys (Zevenhoven *et al.*, 2007). Arsenic occurs in mammal tissue in the range from 10 to 500 µg/kg,being the lowest in heart and the highest in liver (Jørgensen, 2000). Li (2000) reported much lower as concentrations in soft tissues of human and estimated its average at 3.6 µg/kg (Kabata-Pendias & Mukherjee, 2007). Contents of Boron in mammalian tissues vary between <0.2 and <0.5 mg/kg, being the highest in kidneys and liver and the lowest in skin (Jørgensen, 2000). Its concentrations in soft tissues of humans range from 0.06 to 0.6 mg/kg, in brain and kidneys, respectively. The average content in tissues of the reference man is 0.3 mg/kg (Li, 2000). Borates have varied uses, and the principal sector (56%) is fibreglass and heat resistant borosilicate glass (e.g., Pyrex) production. It is used in flame retardant tools and textiles, agricultural fertilizers and pesticides, cosmetics, antiseptics, and leather tanning. Its great proportion is added to laundry products

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(WHO, 1998). The highest content of Cesium (Cs) is found in liver and heart tissues (Jørgensen, 2000). The mean concentration of Cs in soft tissues of reference man is given as 0.021 mg/kg (Li 2000). Radioactive 137Cs has been approved for the sterilization of some foods (e.g., wheat flour, potatoes), sewage, and surgical equipments, thus reducing the numbers of disease-causing bacteria. Rubidium (Rb) is present in all tissues of mammals within the range from 8 to 30 mg/kg, with the lowest value for skin and the highest for liver (Jørgensen, 2000). Total average soft tissue content of Rb in man has been calculated at 9.7 mg/kg (Li, 2000). It is used inelectronics, special glass and in the production of semi-conductors and photocells. Mammalian tissues contain tin (Sn) at the range of <0.2–0.85 mg/kg, being the lowest in brain and the highest in liver and kidneys (Jørgensen, 2000). According to Li (2000) total soft tissues of human contain Sn at 0.1 mg/kg, and its content of all tissues of a reference man averages at 0,24 mg/kg. Its uses are documented in the coating of steel (e.g., sheet metals) and for solder, glassmaking, PVC stabilizers (mainly organotin), pesticides and wood preservatives. Organotin is a common component of ship paints that has significant environmental effects (Hoch, 2001). Strontium (Sr) occurs in all mammalian tissues in the range from 0.09 to 0.24 mg/kg. Itshighest concentration is in kidneys and lowest in brain (Jørgensen, 2000). According to data presented by Li (2000), Srcontents range from 0,08 mg/kg in brain to 0,3 mg/kgin lymph. It is accumulated mainly in bones (138 mg/kg) and other hard tissues (e.g., hair 4.2 mg/kg). All color televisions in the USA are required by law to contain Sr in the faceplate glass ofthe picture tube to block X-ray emissions (ATSDR 2002a). Other uses of Sr compounds are in various technologies of metallurgy, luminescent paint pigments, and some medicines. At the present there are studies on the macrometals, however, practically nothing is known about the influence that these metals may have on the human body, and even less in sports subjects. The aim of the present study was to compare two macrometals Mg and P and the trace elements As, B, Cs,Li, Mg, P, Rb, Sn and Sr in urine samples in middle distance runners with the corresponding levels in referent sedentary subjects matched by age, sex, and geographic area.

MATERIALS AND METHODS

Subjects

Twenty-one Spanish national middle distance runners were recruited before the start of their training period. The athletes had performed training regularly for the previous two years and they have an average of 120 km per week of rigorous training aimed at high-level competition. Male students from the Sport Sciences Faculty of University of Extremadura, who had not been regularly or systematically performing physical exercise volunteered for this study and constituted the control group. Both groups lived in the same geographic area. The present work was carried out in accordance with the ethics standards of the Helsinki Declaration. All the participants were informed about the purpose of the study and gave informed consent. They all completed a questionnaire relative to their habits to ensure they were not taking any vitamins, minerals or other supplementation and to guarantee they all have a similar diet (answering the type and frequency of consumption of different food items).

Anthropometric measurements

The morphological characteristics of the participants were made in the afternoon and always at the same time. Bodyheight was measured to the nearest 0.1 cm using a wall mountedstadiometer (Seca 220), and body weight was measured to the nearest 0.01 kg using calibrated electronicdigital scales, (Seca 769) with subjects barefoot. Body massindex was calculated by dividing the weight (in kg) by the height squared (in m^2).Body fat content was estimated from sum of 6 skinfold. The 6 skinfolds thickness were measured with a Harpenden calliper. Measurements were made by the same operator, skilled in kinanthropometry techniques, in accordance with International Society for the Advancement of Kinanthropometry recommendations. Heart rate and blood pressure was determined using an automatic sphygmomanometer (Omron HEM-780) by a skilled technician, after a five-minuterest period in supine position.

Sample collection

Morning midstream urine samples obtained from all subjects were collected in polyethylene tubes previously washed with diluted nitric acid and frozen at -20°C untilanalysis. Prior to analysis, the samples were thawed and homogenised by shaking.

Experimental desing

Urinary metals

Mg, P, As, B, Cs, Li, Rb, Sn and Sr analysis was performed by ICP-MSin accordance with Sarmiento *et al*. for blood samples (Sarmiento-González *et al*., 2005). Decomposition of the organic matrix was performed by heating for 10 h to 90°Cafter the addition of 0.8 mL HNO_3 and 0.4 mL H_2O_2 to2 mL of urine. The samples were then dried at 200°C on ahot plate. Sample reconstitution was carried out by adding0.5 mL of nitric acid, 10 µL of In (10 mg/L) as internal standard and suprapure water to 10 mL. Reagent blanks, element standards and certified reference material (Seronorm, lot 0511545, Sero aS Billingstand, Norway) were prepared in the same way and used for accuracy testing. Prior to analysis, the commercial control materials were diluted according to the recommendation of the manufacturer. Digested solutions were assayed by ICP-MS in a PerkinElmer (Waltham, MA, USA) ELAN 9000 instrument equipped with a cross Flow nebulizer (resistant to HF and particle clogging), a Scott spray chamber, and a gold plated ceramic quadrupole mass analyzer. Instrumental parameters were as follows: RF power 1000 W, carrier gas flow rate 1 L/min, washing time 35 s, three replicates per sample. Quantification was performed by In as internal standard. The values of the standard materials of each element (10 µg/L) measured for quality controls were in good agreement with a intra and inter-assay variation coefficients of less than 5%.

Creatinine determination

Creatinine concentrations were measured in all urine samples to take into account the different degree of dilution (Shi *et al*., 1995), using Sigma's Creatinine 555–A kit and a UNICAM 5625 spectrophotometer.

Statistical evaluations

Statistical analyses were performed with the SPSS 16.0 computer program. The results are expressed as $x \pm s$, where x are the mean values and s the standard deviation. The normality of the distribution of the variables was assessed using the Shapiro-Wilks test. The student's *t* test was used to assess the significance of the differences between metals concentrations in the urine samples of athletes and sedentary people. A *p* value of $p<0.05$ was regarded as statistically significant.

RESULTS

Dietary habits

The dietary habits were comparable in the two groups. None of the participants followed any special diet like e.g., vegetarians and vegans. They reported a similar intake of milk, fish, meat, fruits, and vegetables.

Anthropometric and cardiovascular characteristics of participants

Table 1, describes anthropometric and some cardiovascular data for participants. A comparison of these values between both groups showed that, athletes have significantly lower BMI ($p<0.001$), body fat ($p<0.001$) and resting heart rate ($p<0.001$).

Table 1. Anthropometric and resting cardiovascular characteristics of athletes and sedentary people

	Athletes (n=21)	Sedentary subjects (n= 26)
Age (yr)	21.62 ± 4.27	22.65 ± 3.65
Height (m)	1.75 ± 0.06	1.77 ± 0.05
Weight (kg)	64.68 ± 7.25	76.94 ± 11.07**
BMI (kg /m^2)	18.25 ± 1.73	21.81 ± 3.14**
SBP (mmHg)	124.71 ± 6.90	125.36 ± 8.65
DBP (mmHg)	80.62 ± 6.77	84.64 ± 6.64
Heart rate (beats/ min)	53.76 ± 11.91	72.79 ±14.72**

SBP: systolic blood pressure. DBP: diastolic blood pressure. Data are expressed as mean ± SD

*$P<0.05$, **$P<0.001$. Comparison between both groups.

Table 2. Urinary concentration of face elements in athletes and sedentary people

	Athletes (n=21)	Sedentary people (n=26)
Mg (mg/L)	65,97±49,10	186,10±131,80 **
(mg/g creatinine)	44,92±36,81	111,30±91,32 **
P (mg/L)	2146±1162	1754±1116
(mg/g creatinine)	1407±909	1024±692
As (µg/L)	63,56 ± 78,84	109,46±199,7
(µg/g creatinine)	37,69 ± 41,29	56,08±92,64
B (µg/L)	936,79 ± 305,9	1256±838,1
(µg/g creatinine)	690,1 ± 442,5	738,7±568,9
Li (µg/L)	32,19 ± 16,82	26,31±13,56
(µg/g creatinine)	22,95 ± 16,39	15,38±9,25
Sn (µg/L)	1,146 ± 1,873	2,49±10,49 *
(µg/g creatinine)	0,675 ± 0,922	1,157±4,685 *
Cs (µg/L)	8,415 ± 5,942	5,552±2,573 *
(µg/g creatinine)	5,055 ± 2,653	3,200±1,601 **
Rb (µg/L)	1988 ± 1291	1791±1207
(µg/g creatinine)	1182,4 ± 563,9	1018±737,9
Sr (µg/L)	183,2 ± 87,8	210,6±103,47
(µg/g creatinine)	116,9 ± 45,01	121,96±72,23

Data expressed without or with creatinine adjustment as mean ± standard desviation.

*$P<0.05$, **$P<0.001$ t test. Comparison between both groups.

Urinary concentrations of metals

Table 2 shows urinary concentrations of each metal, without and with creatinine adjustment, in middle distance runners and in sedentary people. Element analysis concentrations, expressed in mg/L or µg/L and mg/g or µg/g creatinine were:no statistically differences between urinary concentrations in both groups of As, B, Li, P, Rb and Sr;Mg ($p<0.01$ and $p<0.01$), Sn ($p<0.05$ and $p<0.05$), were lower in athletes urine; Cs ($p<0.05$ and $p<0.01$) in athletes urine have higher than the sedentary group.

DISCUSSION

In our study all the studied metals values were within normal values submitted in other studies and expressed as in ours (mg/L or µg/L), made with a similar technique (Heitland, 2004, 2006). Accumulating evidence has shown a direct relationship between magnesium and exercise performance (Deuster and Singh, 1993; Laires and Alves, 1991; Laires *et al*., 1988, 1993; Lijnen *et al*., 1988; Mooren *et al*., 2005; Stendig-Lindberg *et al*., 1987). Casoni *et al*., 1990, reported that the serum magnesium levels are significantly lower in the Italian resistance athletes compared to sedentary, being the serum values within normal limits. These results would be in the same relationship to those found in our study on the urinary elimination in athletes and sedentary subjects. This reduced elimination in athletes of Mg in respect of sedentary subjects could be due to prevent a possible deficiency of Mg in the athlete, which could have great significance in their sporting performance. Phosphorus (P) plays an important role in the metabolism of the hydrates of carbon, contributing to the intestinal absorption of glucose through the process of phosphorylation, in which the phosphate is combined with glucose. In our study did not exist differences in the urinary elimination between the two groups for P, while normal values. Arsenicis known to be highly toxic to humans and animals. Both cations, As^{3+} and As^{5+} maycause similar toxicological effects, but the former is considered to be more mobile and toxic for living organisms (NRC, 1999). However, both specie are metabolized in humans, forming methylated As species which are excreted readily intotheurine. Hence, urine As is a good indicator to assess the As exposure (Akter and Naidu, 2006). In our study did not exist differences in the urinary elimination between the two groups for As. In 1996, the WHO Expert Committee on Trace Elements in Human Nutrition concluded that Bis “probably essential”. Pen land (1994), suggested that B may playaroleincell membrane function, mineral and hormone metabolisms, and enzyme reactions. Nielsen (1991,1994,2004) reported that B is required or beneficial in humans and animals for many life processes, such as embryo development, bone growth and calcification, immune function, psychomotor skills, and cognitive function. In our study did not exist differences in the urinary elimination between the two groups for B.

Cs exhibits an antagonistic reaction against potassium with respect to the biological availability. The daily dose of Cs with a regular adult’s diet is calculated as 10µg and its content inanaveragedietis9.4mg/kg. In our study highlights a greater urinary elimination of Cs for the athletes. The Cs exerts an antagonistic action with potassium, and this element is known as has a huge significance in the cellular functions during rest and exercise. This greater removal of the Cs in athletes would eliminate excesses of this element in the body of the athlete that could prevent a proper function of the body K. Lithium (Li) does not appear to serve a normal cofactor function in any enzyme or transport system, but is known to have an affinity for enzymes activated by Ca and Mg. Its deficiency apparently disturbs protein metabolism and may have an effect on several biological systems, such as endocrine, cardiovascular, neuromuscular, renal, and dermatological (Anke *et al*., 1995a). There are some observations that Li influences several hormonal, metabolic, neurological and immunological processes (Duda and Pasternak, 2003). In our study did not exist differences in the urinary elimination between the two groups. Rubidium is associated with the K cycle in organisms but does not substitute for it. There is some evidence that Rb is involved in brain functions, but specific roles have not yet been identified (Hameed and Vohora, 1990). This element is antagonistic to Li, and its increased level in food can be harmful to some metabolic processes related to Li. Amounts of Rb in food above 200 mg/kg might be harmful and its concentrations above 1000 mg/kg in human diet are of a health risk. In our study did not exist differences in the urinary elimination between the two groups. Humans are exposed to Sn from ingestion, inhalation, and dermal adsorption. However, the main source of Sn uptake is from food, with an exception of industrial areas where its concentrations in water and air are elevated (Biégo *et al*., 1999). Canned foods, especially fruits and vegetable products are considered to be the main source of Sn in the diet. Draws attention lower urinary elimination of Sn in athletes we cannot explain this situation because the two group has the same diet. It is possible that Sn could play an important function in the athlete for this athletes need it and loss it urinary elimination. Biochemical functions of Sr are not well known, but as reported D'Haese et al. (2002), its small quantities are needed for proper processes of the calcification of bones and teeth. It has been even used medicinally against various diseases, and its effect on bone formation/mineralization in the treatment of osteoporosis and osteoarthritis has been discussed (D'Haese et al., 2002). In our study did not exist differences in the urinary elimination between the two groups. For all the above we can conclude that the population in Extremadura has no abnormal levels of these metals. But athletes have some modifications to the elimination of some of these metals that could be related to possible adaptations to physical activity. It is necessary more studies to clarify this situation.

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