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DETERMINATION OF Br IN BLOOD OF AMATEUR RUNNERS USING NAA AND ITS CORRELATION WITH ADOPTED PHYSICAL TRAINING

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ABSTRACT

Bromine (Br) is one of the most abundant trace elements in the biosphere, in the human body is present in blood (as bromide), lungs, liver and hair. There is no evidence in humans of bromide concentration in any particular organ that might indicate a specific physiological function and not enough information is available on bromine metabolism but, some studies report its use by eosinophilic leukocytes for immune defense and electrolytic balance. In this study the Br levels were determined in Brazilian amateur athlete's blood that performing physical exercise at Laboratório de Bioquimica do Exercício (LABEX/UNICAMP - Brazil). The samples were collected from twenty six male athletes, ranging from 18 to 26 years old, at rest. The blood samples were irradiated in the nuclear reactor (IEA-R1, 3-4.5MW, pool type) at IPEN/São Paulo – Brazil and were analyzed using Neutron Activation Analyses (NAA) technique. These results were compared with the control group (subjects of same age but not involved with physical activities). The range established at rest for amateur runner (0.0040 – 0.0096 gL^{-1}) when compared with control group (0.0074 – 0.0306 gL^{-1}) suggests that there is a dependency of these limits in the function of the adopted physical training.

1. INTRODUCTION

Bromine (Br) is one of trace elements widely existing in biological and environmental materials [1]. Although Br is not considered to be an essential trace element, its normal distribution in tissue is similar to the distribution of essential trace elements [2]. Br is present in blood, lungs, liver and hair [3-4] and your physiological function is unclear [5-7], however some studies report its use by eosinophilic leukocytes for immune defense [8] and electrolytic balance [4]. Some studies conducted in animal models have suggested that Br is an essential element: its supplementation can alleviate growth retardation in mice, while its deficiency can be linked to depress growth and fertility in goats but, for humans, there is no consensus on whether Br is required to maintaining healthy nutrition [6].

The Br is found in significant dietary sources include grains, nuts, seafood, and sea salt [8]. After oral ingestion, bromide is rapidly and completely absorbed in the gastrointestinal tract and, distributed almost exclusively in the extracellular fluid [9]. Several hundred milligrams of bromide are filtered daily in the kidney and are likely to be recovered via chloride transporters and other carriers. Information on the precise nature of renal reabsorption of bromide is very limited and excretion with urine is the main route of bromine losses [8, 10]. The consequences of chronically low intake are uncertain, and growth retardation and insomnia have been suggested [8]. Tissue Br can be elevated by an increase in the dietary Br intake and also by respiratory and cutaneous exposures [2]. High acute exposure, may cause bronchospasm, gastrointestinal disturbances, headache, myalgia, reduced exercise tolerance, fatigue [8] and can also inhibit the absorption and bioavailability of iodine [10]. There are no recommendations for optimum dietary intakes.

In this study Br levels were determined, using Neutron Activation Analyses (NAA) technique, in amateur athlete's blood, which were submitted to a constant exercise on the treadmill at LABEX. The NAA procedure was applied due some advantages: it uses small quantities of blood (50μL); agile execution (it is not necessary waiting for blood coagulation procedure neither performing the serum-plasma separation) and it is not destructive (the blood sample can be storage for future reexamination, for a long period, without refrigeration) [11]. These data can be considered for a preparation of balanced diet and can also be useful for evaluating the performance of athletes during the preparation period for competitions, as well as to propose new evaluation protocols.

2. EXPERIMENTAL PROCEDURE

Twenty six male amateur runners specialized in 10 Km, age 18 to 36 years, participated of this study and have 3.3±1.6 years of training experience. They have a balanced diet without mineral supplements or fortified food. For the blood collection, a small capillary pin (Clinitubes®, Radiometer Copenhagen®) was inserted in the athlete's finger and exactly 50(±0.015) μL were dropped on to Whatman n° 41 filter paper (~2 cm^2) using a calibrated micropipette, and then dried for a few minutes using an infrared lamp. The same procedure was used for standard solution preparation. The control group samples were collected from 120 male healthy donators, with the same age (18-36 years) with no occupational physical activity, weighting from 50 to 85 kg, selected from Paulista Blood Bank at São Paulo, Brazil. Blood sample of 50 μL (in duplicate) was collected from each donor.

To determine the Br concentration each sample and standard (certified solution) were irradiated for 300 s in a pneumatic station in the nuclear reactor (IEA-R1, 3-4.5MW, pool type) at IPEN, with a thermal neutron flux ranged from 5 x 10^{12} to 9 x 10^{12} n cm^{-2} s^{-1}. After a decay time of 60 s, a gamma counting of 600 s was used to determine ^{80}Br ($T_{1/2}$ = 17,7 min, Eγ = 616 keV). The IAEA A-13 certified reference material was investigated for quality control. A γ-spectrometer system composed by a ORTEC HPGe detector (Model GEM-60195, FWHM=1.87keV for 1.33MeV of ^{60}Co), calibrated for energy through the measurements of standard sources of ^{56}Co, ^{137}Cs and ^{152}Eu, coupled to a MCA ORTEC Model 919E and a PC, were used to measure the induced gamma-ray activity. The filter paper (blank) was also analyzed using the same irradiation conditions. The Br concentrations were calculated using in-house software [12].

3. RESULTS AND DISCUSSION

For analytical-quality control IAEA A-13 animal blood was used and the results obtained in this study for Br 221.8 ± 1.7 mg kg^{-1} and certified values for Br 220 ± 20 mg kg^{-1}. The accuracy evaluation by Z-score test (Z\|0.10|) indicates the adequacy of the method for this element determined.

The Br concentration (mean value) determined in blood samples are presented in Table 1. The standard deviation (±1SD), median, mode, minimum and maximum values and the indicative interval are also presented for both groups: control group (CG) and amateur runner (AR) for comparison. In Fig. 1 Br concentrations for amateur runner and the mean value (MV) are showed considering ±1SD and ±2SD. The mean value for the control group was also included for comparison.

Table 1. Blood concentrations of Br for AR and CG.

Br gL^{-1}	CG n = 120	AR n = 26
Mean	0.0190	0.0068
±1SD	0.0058	0.0014
Indicative Interval*	0.0074 – 0.0306	0.0040 – 0.0096
Median	0.0173	0.0062
Mode	0.0157	0.0056
Minimum Value	0.0028	0.0039
Maximum Value	0.0410	0,0094
C_{AR}/C_{GC}		0.36

n: donors

*confidence interval of 95% for AR

C_{AR}/C_{CG}: concentration ratio comparison between AR and CG

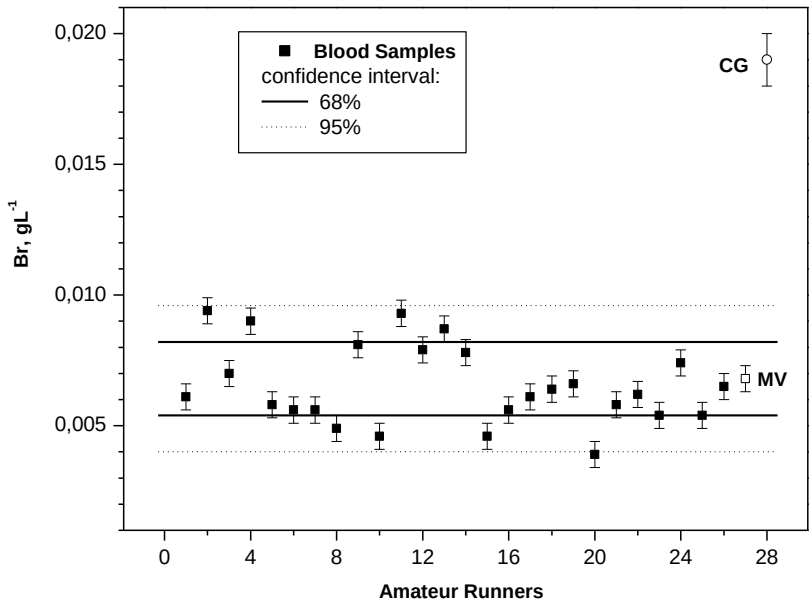

Figure 1. Results of Br concentrations for AR; the mean value for the CG was also include for comparison

According to Table 1 the results for Br show a decrease (64%) for AR suggesting a dependency of this limit in function of the adopted physical training. The variation Br may be related to diet, particularly by ingestion of grains and seafood rich bromides. However, a recent study [13] suggests that the high concentration in the control population may also be related to drug intake which contains in its formulation bromides (such as antidepressants, sedatives, analgesics, anti-inflammatory drugs) that are consumed by the Brazilian population [14-15]. Therefore, the marked decrease of Br in blood of athletes may be related to consumption rather controlled / limited these drugs.

4. CONCLUSION

The data from the present report give an indicative interval for Br in blood for AR. According to the Student's t-test this indicative interval is statistically different ($p < 0.05$) from CG and must be considered for checking the clinical status of these athletes. Related to the nuclear procedure adopted (NAA) it can be an alternative procedure to perform biochemistry analyses using small quantities blood.

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