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CONCENTRATIONS OF IONS IN BLOOD OF ATHLETES USING NAA

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ABSTRACT

Sodium (Na), chlorine (Cl) and potassium (K) are widely distributed in the body and are the mainly of body fluids electrolytes. K is the major intracellular ion. Na and Cl are the major extracellular ions. Therefore, Na and Cl can be regarded as the most important osmotically active electrolytes. The concentrations of these ions in body fluids are very tightly controlled. These electrolytes play central roles in electrolytic balances and current, in osmotic control, in the transport of organic metabolites by cells, and stabilization of polyelectrolytes in cells. In this study Na, Cl and K levels were investigated in blood of athletes submitted to physical exercise at Laboratório de Bioquimica do Exercicio (LABEX/UNICAMP - Brazil) using Neutron Activation Analyses (NAA) technique. The blood samples were collected from six male athletes, ranging from 18 to 26 years old, before and after the physical training. These results were compared with the rest condition (before start the physical exercise), as well as with the control group (subjects of same age but not involved with physical activities), for checking the performance of the athletes during and after the exercise. The nuclear procedure adopted as NAA, it can be an alternative procedure to perform biochemistry analyses in blood, mainly when the biological material is scarce.

1. INTRODUCTION

Sodium, chlorine and potassium exist largely as free hydrated ions that bind only weakly to organic molecules. They function largely in the maintenance of electrolytic and osmotic balances [1]. Sodium, the major cation of the extracellular fluid, functions primarily in the control of water distribution, fluid balance, and osmotic pressure of body fluids. Hypernatremia is defined by an elevated sodium level in the blood (greater than 2.06 gL^{-1}) and hyponatremia is when the sodium concentration in the blood is lower than normal (less than 1.48 gL^{-1}) [3]. Sodium is also associated with chloride and bicarbonate in the regulation of acid-base equilibrium of body fluid [2, 4]. Potassium, the principal cation of intracellular fluid, is critical in the regulation of nerve conduction and muscle contraction, particularly in the heart. When the concentration of K in the blood exceeds 1.89 gL^{-1} hyperkalemia [3], the membrane depolarizes, causing muscular weakness, flaccid paralysis and cardiac arrhythmias. In contrast, in hypokalemia, when the concentration of K in the blood is less

than 1.33 gL^{-1}[3], the membrane hyperpolarizes, and this can interfere with the normal functioning of nerves and muscles, resulting in muscle weakness and decreased smooth muscle contractility. Severe hypokalemia can lead to paralysis, metabolic alkalosis and death [4, 5]. Chlorine, the major extracellular anion, closely follows the metabolism of sodium, and changes in the acid-base balance of the body are reflected by changes in the chlorine. [6].

The serum levels variation, including excess or deficiency of metals and ions in the organism, have been used as a told for diagnosis or prognostic of diseases [4]. Currently, these analyses are also useful to monitor training effects. The physical training can adapt or damage the muscles, depending on the intensity and duration of the effort, provoking detectable metabolic alterations in blood. The concentration of some elements in the blood depends of both the modality and the amount of muscular mass involved in the execution of physical exercises. In this study Cl, K and Na levels were investigated in blood of athletes submitted to physical exercise at Laboratório de Bioquimica do Exercício (LABEX/UNICAMP) using Neutron Activation Analyses (NAA) technique. The use of this technique presents some advantages when limited material must be analyzed: it uses small amounts (25μL); it permits simultaneous evaluation of these elements; the samples can be stored without refrigeration and they can be reexamined (non destructive procedure) [7].

2. EXPERIMENTAL PROCEDURE

The blood samples were collected from six male athletes, ranging from 18 to 26 years old, before and after the physical training. For the control group, blood samples were collected from healthy male donors (n=33) selected from Paulista Blood Bank at São Paulo, ranging from 18 to 26 years old. For blood collection small capillary pins was inserted in the athlete's finger and immediately (before blood coagulation) exactly 25μL (±0.5%) were dropped on to Whatman filter paper (~1.5 cm^2), using a calibrated micropipette, and dried using an infrared lamp. For this investigation the whole blood samples were prepared in duplicate. Aliquots of standard solutions of Cl, K and Na were also transferred to filter paper and prepared in the same manner as the biological samples.

The blood samples and standards were irradiated for 120s in the pneumatic station in the nuclear reactor (IEA-R1, 2-4MW, pool type) at IPEN. The thermal neutron flux utilized ranged from $4.5 \cdot 10^{12}$ to $8.4\ 10^{12}$ n cm^{-2} s^{-1}. For ^{38}Cl ($T_{1/2}$ = 37 min, E_γ = 1642 keV) and ^{24}Na ($T_{1/2}$ = 15 h, E_γ =1369 keV) a decay time of 60s and 240s of counting were used. For, ^{42}K ($T_{1/2}$ ~ 12 h, E_γ = 1525 keV) after a decay time of 60s they were counted for 4 hours. The measurements of the neutron induced activity of the samples were carried out using an ORTEC HPGe detector (Model GEM-60195, FWHM = 1.9 keV), calibrated for energy through the measurements of standard sources of Co^{56}, Cs ^{137} and Eu^{152}, coupled to a MCA ORTEC Model 919E and connected to a PC. The background radiation as well as the escape peaks was reduced by employing the iron shield described by Medeiros et al [8]. The element concentrations were calculated using in-house software [9]. For analytical - quality control IAEA A-13 Animal Blood was used. The filter paper (blank) was also analyzed using the same irradiation conditions.

3. RESULTS AND DISCUSSION

The Z-score values obtained for IAEA A-13 certified material are presented in Table 1. They indicated that our results are satisfactory considering 95% confidence level. The

concentration of the elements in blood samples are shown in Table 2. In this table was included: the mean value (gL^{-1}) before and after the physical training, the standard deviation (1SD), the normal range (gL^{-1}), from the control group, for a confidence interval of 68%.

Table 1. Results of the present analysis (NAA) compared to the IAEA A-13 certified values

Element	NAA MV ± 1SD	Certified values	|z-score|
Br, gkg^{-1}	0.0211 ± 0.0025	0.0220 ± 0.0024	0.37
Ca, $mgkg^{-1}$	263 ± 44	286 ± 54	0.43
Mg, $mgkg^{-1}$	101 ± 32	98 ± 26	0.11
Na, gkg^{-1}	13.0 ± 0.31	12.6 ± 1.01	0.50
S, gkg^{-1}	7.1 ± 0.64	6.5 ± 0.52	1.15
K, gkg^{-1}	2.03 ± 0.43	2.50 ± 0.35	1.34

MV: Median Value
SD: Standard Deviation

Table 2. The Cl, K and Na concentrations data in blood samples for the control group and for the athletes before and after the physical training.

Samples	Cl, gL^{-1} MV ± 1SD Before After	K, gL^{-1} MV ± 1SD Before After	Na, gL^{-1} MV ± 1SD Before After
Athlete 1	3.28 ± 0.10 3.89 ± 0.12	1.23 ± 0.31 1.60 ± 0.33	2.52 ± 0.06 2.91 ± 0.06
Athlete 2	3.82 ± 0.09 4.08 ± 0.09	1.93 ± 0.30 1.96 ± 0.31	2.51 ± 0.07 2.63 ± 0.06
Athlete 3	3.35 ± 0.0 8 3.26 ± 0.0 9	2.02 ± 0.18 1.69± 0.15	1.78 ± 0.05 2.07 ± 0.06
Athlete 4	2.67 ± 0.06 3.22 ± 0.06	1.46 ± 0.33 1.46 ± 0.31	1.81± 0.04 2.20 ± 0.04
Athlete 5	1.58 ± 0.08 1.94 ± 0.08	1.30 ± 0.31 1.72 ± 0.37	1.20 ± 0.05 1.36± 0.05
Athlete 6	1.99 ± 0.11 2.41 ± 0.11	1.11 ± 0.11 1.29 ± 0.10	1.26 ± 0.07 1.52 ± 0.08
Control Group	[2.41 – 3.33]*	[1.28 – 1.84]*	[1.48 – 2.06]*

*control group (confidence interval of 68%).
MV: mean value
SD: standard deviation

In Figures 1, 2 and 3 the behavior of Cl, K and Na concentration in blood, respectively, for the control group, before and after the exercise program, for all the athletes, are presented.

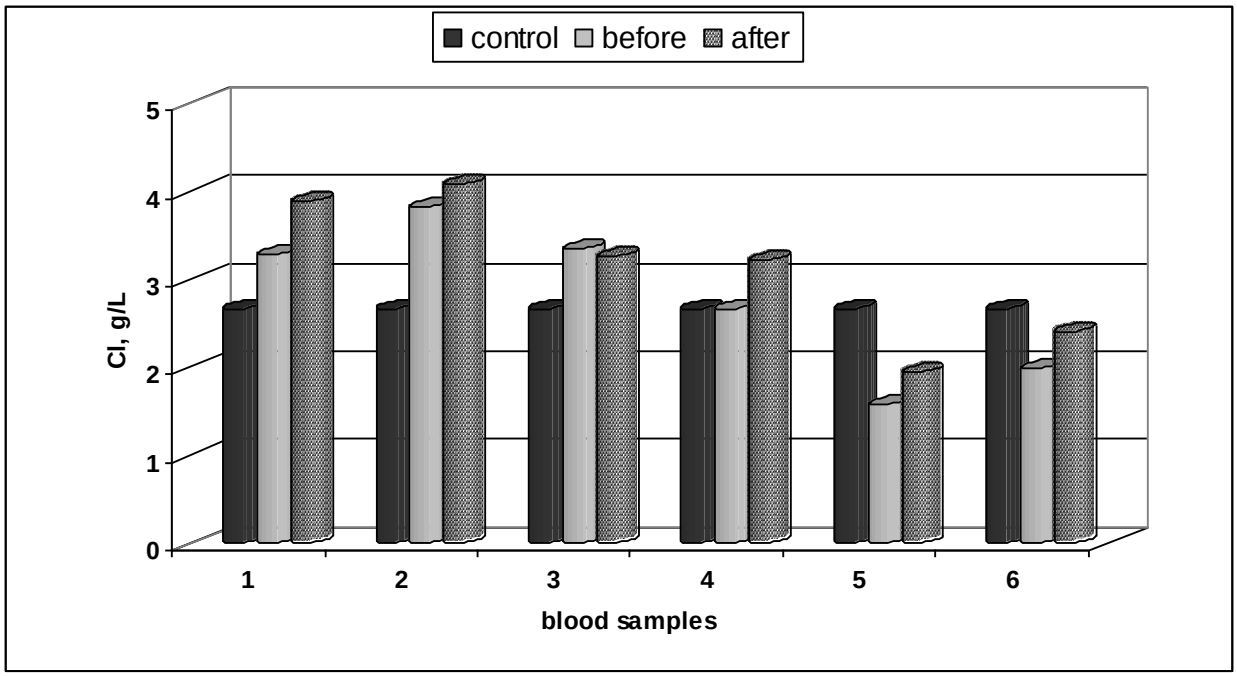

Figure 1. The Cl data before and after the exercise; the mean value for the control group (CG) was also include for comparison.

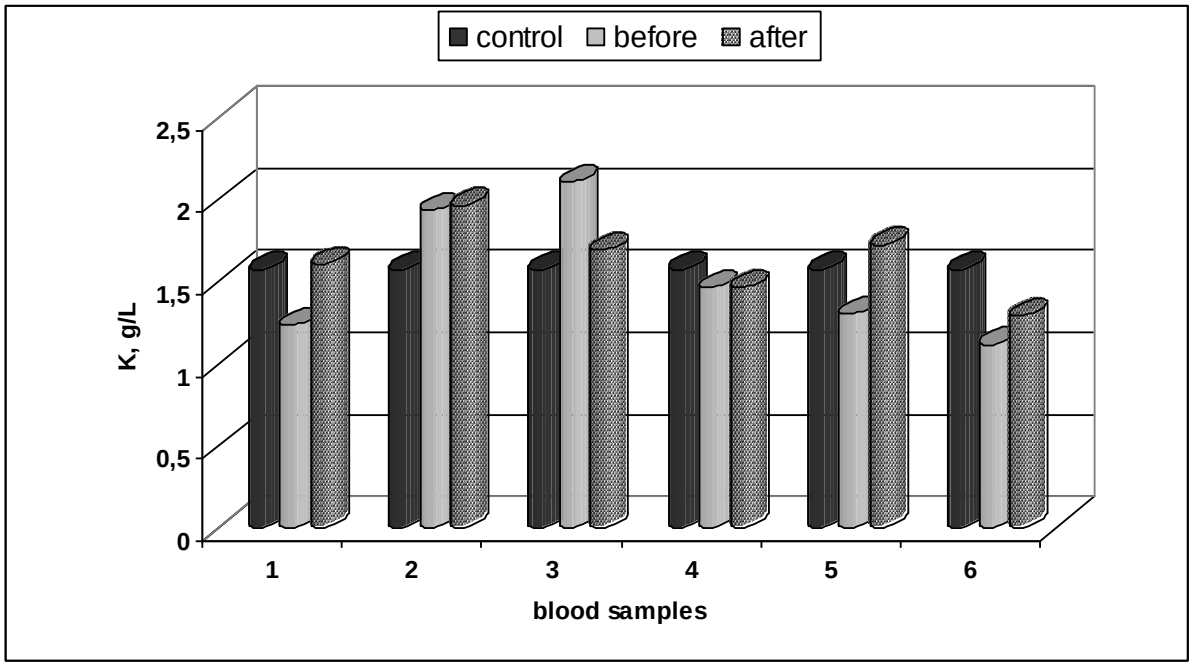

Figure 2. The K data before and after the exercise; the mean value for the control group (CG) was also include for comparison.

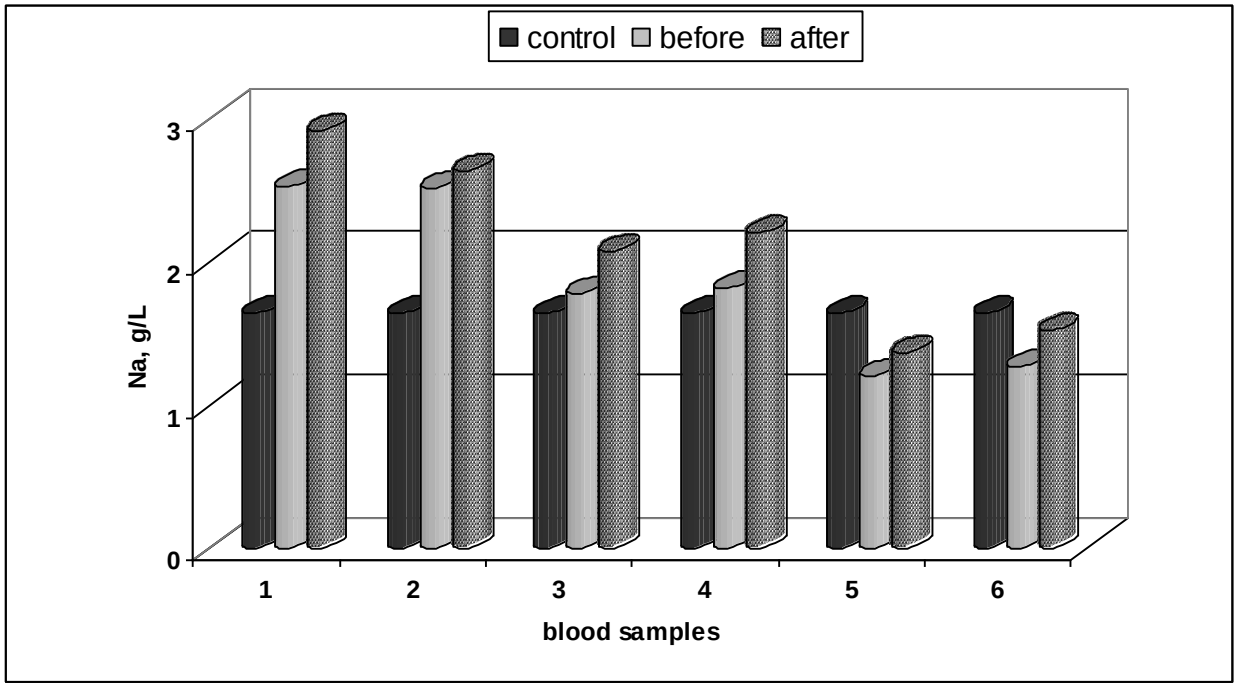

Figure 3. The Na data before and after the exercise; the mean value for the control group (CG) was also include for comparison.

According to Figure 1, for Cl considering a confidence interval of 95% usually adopted for clinical practice, only one subject (athlete 5) shows Cl concentration value lower to the normal range before and after the exercise. Related to K levels (Figure 2), all the athletes finished the physical exercise with no symptoms of severe fatigue, but it was observed that in the case of athlete 3, the K level in the rest condition (before the training) is near to the upper limit suggesting a tendency of Hyperkalemia; however, after the physical exercise, it is inside the normal range. The concentration values for Na, before and after the exercise program, when compared with the normal range (control group) show that athlete 5 and athlete 6 are out of the confidence interval of 95%. Also, athlete 1 presents systematic increase in the Na levels, suggesting a light tendency of Hypernatremia. The Na behavior also suggests the need of its evaluation as routine

4. CONCLUSIONS

The use of the NAA technique has allowed a quantitative estimation of Cl, K and Na in blood samples of athletes before and after the physical exercise using small quantities of blood (25µL). This procedure dicrease the stress during the conventional collect (few mL of blood) and furthermore, these results may also, in the future, with more statistical analysis, help to evaluate and compare the athlete performance during their training.

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