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Concentrations of ions and metals in blood of amateur and elite runners using NAA

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Abstract Intense physical training is known to be associated with increased mineral losses through sweating (during the exercise) and also through urine (after the exercise). Nowadays physical training is recognized for adapting or damaging the muscles, depending on the intensity and duration of the effort, provoking detectable metabolic alterations in blood, mainly in the content of some ions. In this study Br, Ca, Cl, K, Mg, Na and S levels were investigated in blood of Brazilian athletes that were submitted to constant physical exercise, at Laboratório de Bioquímica do Exercício (LABEX/UNICAMP) using Neutron Activation Analyses technique (NAA). The blood samples were collected from male amateurs and elite athletes, ranging from 18 to 36 years old. The blood samples were irradiated in the nuclear reactor (IEA-R1, 3–4.5 MW, pool type) at IPEN/São Paulo-Brazil. The concentrations data were compared with the control group (subjects of same gender and age but not involved with physical activities). These data can be useful for evaluating the performance of endurance athletes during the period of competition preparation as well as to propose new evaluation of protocols not yet reported.

Keywords Blood · Ions · Athletes · NAA

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Introduction

The Exercise Biochemistry Laboratory (LABEX) at University of Campinas (SP, Brazil) is a group of national and international reference in a medical sport field. The LABEX investigates the interrelationships metabolic that occur with physical activity monitoring the effects of different training, through tests that evaluate their clinical status, aiming to propose new evaluation protocols.

The nutrition habits of athletes can cause severe disturbances in the body, restricting or even eliminating the athlete of his activity. A balanced diet of Na and K keeps the pH in body fluids, blood pressure and ionic balance controlled. The amount of Na required per day is unknown, but the recommend value is 1.5 g per day for young adults while for K, the amount required is 4.7 g per day [1]. However, for athletes undergoing long-term training, these evaluations may be above of the recommended values. According to Institute of Medicine's (IOM) Food and Nutrition Board the recommended values for people with no occupational physical activity can differ from athletes in intense sports performance, such as, marathons and other endurance athletic events [1, 2].

Table 1 summarizes the recommended values of some minerals for athletes undergoing intense training [3]. These elements are involved in important functions, such as: propagation of nerve impulses and in muscle and heart contraction (Ca, K, Mg and Na), control of vascular tonicity and blood coagulation (Ca), maintaining acid–base balance (Na, K and Cl), as well as the disorders associated with deficit or excess of them [4–6]. The estimative for people with no occupational physical activity (first column) are also presented [7, 8]. The variations observed in this Table emphasize the need to establish the control range for these elements in the blood of athletes undergoing intense

Table 1 Minerals requirement for athletes undergoing intensive training, deficit and excess

Elements	Athletes	Deficit/Symptoms	Excess/Symptoms
Na 1.5 g/d[a]	Max: 10 g/d[c]	Hyponatremia/Fatigue*	Hypernatremia/Dehydration
		Low blood pressure	Diarrhea
Cl 2.3 g/d[a]	Min: 2.3 g/d[c] (varies according to Na intake)	Hypochloremia/Bone pain	Hyperchloremia/Dehydration
			Hypertension
Ca 1.0 g/d[a] 0.8 g/d[b]	>1.3 g/d[c]	Hypocalcemia/Fatigue*	Hypercalcemia/Muscle weakness
		Broken bones (tibia and Standing)	Muscle and joint aches
K 4.7 g/d[a]	4.7 g/d[c]	Hypokalemia/Arrhythmias	Hyperkalemia/Arrhythmias
			Muscle weakness
Mg 0.3 g/d[a]	0.3 g/d[c]	Hypomagnesemia/Tremors	Hypermagnesemia/Dehydration*

Max maximum, *Min* minimum, *g/d* g/day

* in severe case leads to CFS (Chronic Fatigue Syndrome)

[a,b] Adequate intake, for people with no occupational physical activity [7, 8]

[c] Adequate Intake for athletes [3]

physical activity to better adapt the diet and the physical training intensity.

Although in the last decade, there was a growing interest in the athlete's health with a focus on continuous biochemical evaluation (in serum and urine) and controlled diet, based on high consumption of water, fruits, vegetables, salt restriction and processed foods, these habits have not decreased the rates of electrolyte dysfunctions, mainly of hyponatremia signs ($Na < 1.48\ gL^{-1}$) which may include weakness, dizziness, headache and nausea [9–12]. In severe case, athletes engaged in long exercise duration present digestive problems, muscle twitching or cramps and that can express an alert of Chronic Fatigue Syndrome (CFS) [6]. This syndrome is not well understood and can have many causes since of unbalanced diet, trauma injury and even thyroid imbalance. Data related to the Na blood balance during prolonged exercise emphasizes an increase of 30 % of hyponatremia cases per year in American athletes [12]. For Brazilians athletes there are no data related.

Moreover variations of K and Ca level in the athlete's blood may contribute to CFS. During the training K is released from muscles to blood which is directly related to exercise intensity. In the case of accumulation of K in the blood, which is caused by fatigue, muscle cramps can occur. Moreover, during the exercise, muscle cells store Ca ions and release them into the cytoplasm which triggers the muscle contraction. This transfer is also related to the exercise intensity, so if there is a reduction of the release of Ca, the muscles can be affected, resulting in fatigue. Regarding to K, low blood levels (hypokalemia) or high blood levels (hyperkalemia) can lead to arrhythmias (abnormal heart rhythms); in severe cases can lead to sudden death [13, 14].

In this study Br, Ca, Cl, K, Mg, Na and S levels were investigated in the athlete's blood, that were submitted to physical exercise at LABEX, using Neutron Activation Analyses (NAA) technique. The use of this technique presents some advantages, comparatively to conventional procedure (biochemical analyses in serum), when small quantities of biological samples are available, such as, non destructive procedure (samples can be stored without the need of refrigeration and they can be reexamined) which permits the evaluation of several elements of clinical relevance by using small quantities of blood (50–500 μL) [15].

In this study Ca, Cl, K, Mg and Na were selected due to their nutritional relevance, as well as the electrolyte disorders in the evaluated blood. Related to Br and S they were also investigated because they are present in the Brazilian diet, mainly in vegetables and fruits (rich in Sulfur), sea food (rich in Bromides), also due to the bromide presence in food products, like pesticide residue [16, 17].

Two groups of athletes were investigated: amateur runners (AR) and elite runners (ER). In the first step the AR blood samples were collected before, during and after the physical training. In the second step, the blood samples of ER were collected at rest (before the start of physical training).

Our goal was to compare the concentration results at rest from both runners groups (AR and ER) with the control group (CG) subjects of same age and gender but not involved with intense physical activities, so that it is possible to check the similarities between them. Besides that, we also performed a systematic investigation for the AR group during the exercise program. These data will be useful to propose new evaluation protocols.

Experimental

Collection and preparation of the samples

Fourteen male athletes, age 18–36 years, participated of this study. The blood collection was performed at LABEX, (SP, Brazil). Two groups of athletes were investigated: AR and ER. They have a balanced diet but no mineral supplements, or anti anemic formulas or even foods fortified were included.

Amateur runners

The blood samples were collected from 6 male athletes (AR) submitted to 10 km of constant exercise on the treadmill. For the blood collection, a small capillary pin was inserted in the athlete's finger which was collected before (0 km) and after (10 km) the physical exercise. Each sample, 500(±0.5 %) μL of blood, was dropped in a polyethylene capsule. As soon as the training started, the collection was performed every to 2 km: exactly 50(±0.5 %) μL were dropped on to Whatman n° 41 filter paper (~2.3 cm^2) using a calibrated micropipette, and then dried for a few minutes using an infrared lamp. This procedure was performed during the year of 2009. The same procedure was used for standard solution preparation.

Elite runners

The blood samples were collected from 8 male athletes (ER), before the physical training (at rest). The blood collection was performed by dropping 500(±0.5 %) μL in a polyethylene capsule.

Control group

Considering the population that was studied (male athletes, 18–36 years) for the CG, the whole blood samples were collected from 68 male healthy donators, with the same age, weighting from 50 to 85 kg, selected from Paulista Blood Bank at São Paulo, Brazil. Blood samples of 50 and 100 μL (dropped in filter paper) and 500 μL (dropped in polyethylene capsule) were collected from each donor.

Experimental procedure

Samples and standard were irradiated for 300 s in a pneumatic station in the nuclear reactor (IEA-R1, 3–4.5 MW, pool type) at IPEN, with a thermal neutron flux (ranged from $5.32 \cdot 10^{12}$ to $8.40\ 10^{12}$ n cm^{-2} s^{-1}). After a decay time of 60 s, a gamma counting of 120 s was used for determining ^{24}Na ($T_{1/2} = 15$ h, $E_\gamma = 1{,}369$ keV) and ^{38}Cl ($T_{1/2} = 37$ min, $E_\gamma = 1{,}642$ keV), while for ^{80}Br ($T_{1/2} = 18$ min, $E_\gamma = 616$ keV), ^{49}Ca ($T_{1/2} = 9$ min, $E\gamma = 3{,}084$ keV), ^{27}Mg ($T_{1/2} = 9$ min, $E\gamma = 844$ and $1{,}014$ keV) and ^{37}S ($T_{1/2} = 5$ min, $E_\gamma = 3{,}103$ keV) were gamma counted for 15 min. For ^{42}K ($T_{1/2} = 12$ h, $E_\gamma = 1{,}525$ keV) they were gamma counted by 3 h.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 = 1.87 keV for 1.33 MeV 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 background radiation was reduced by employing the iron shield [18]. The element concentrations were calculated using in-house software [19]. The filter paper (blank) and the polyethylene capsule were also analyzed using the same irradiation conditions.

Results and discussion

The Z-score values obtained for IAEA A-13 certified material indicated that our results are satisfactory considering 95 % confidence level. Some impurities such as Ca and Mg were identified in the filter paper but they do not interfere; Cl and Na were identified but in very low concentration (0.012–0.014 gL^{-1} for Na and 0.061–0.073 gL^{-1} for Cl). Also the plastic cylinder (polyethylene capsule) was investigated and impurities of Ca and Mg were identified but in low concentration, (i.e.), in the range of the uncertainty associated to the elements concentration (3.7 % for Ca and 7.5 % for Mg).

A summary of the concentrations results in blood samples for the AR_n ($n = 1$–6) group is shown in Table 2: the mean value and the associate uncertainty, as well as the minimum and maximum values during the training were included. Although the performance of each AR has fluctuated during the exercise training, the quantity of data (number of athletes x number of elements determined x number of blood collection/km) is too large to be presented. So, to illustrate, in Fig. 1 the behavior of the elements Br, Ca, Cl, K, Mg, Na and S in blood is showed, respectively, for one AR at rest and every 2-km over the 10-km run. The range established for CG, considering ±1SD and ±2SD, was also included for comparison as well as the mean value for the CG at rest.

According to Fig. 1 it is possible to evaluate the behavior of each element, during the exercise training, for each athlete. For this athlete, while for Br, Ca, Cl, K, Na and S no significant change was observed in blood during the exercise, for Mg there was a decrease (6 km) followed by recovery. Another aspect to be considered is the constancy of K in blood, an

Table 2 Blood concentrations of Br, Ca, Cl, K, Mg, Na and S for AR

Elements (σ%)	AR_1 0 km (rest)	AR_2 2 km	AR_3 4 km	AR_4 6 km	AR_5 8 km	AR_6 10 km
Br, 8 %						
MV	0.0080	0.0043	0.0093	0.0049	0.0070	0.0032
Min	0.0061	0.0029	0.0078	0.0039	0.0041	0.0026
Max	0.0105	0.0058	0.0127	0.0066	0.0081	0.0044
Ca, 10 %						
MV	0.289	0.271	0.288	0.339	0.313	0.250
Min	0.251	0.174	0.193	0.286	0.322	0.179
Max	0.310	0.397	0.381	0.380	0.333	0.277
Cl, 3 %						
MV	3.70	4.15	3.45	3.59	3.81	2.71
Min	3.53	3.92	3.15	2.82	3.46	2.01
Max	3.84	4.56	3.74	3.81	4.46	3.29
K, 2 %						
MV	1.71	1.82	1.79	1.74	2.12	1.15
Min	1.27	1.48	1.65	1.55	1.64	1.07
Max	1.91	1.96	2.02	1.84	2.59	1.32
Mg, 15 %						
MV	0.050	0.047	0.066	0.048	0.065	0.031
Min	0.037	0.031	0.039	0.033	0.044	0.025
Max	0.057	0.057	0.082	0.074	0.083	0.035
Na, 2 %						
MV	1.88	1.81	2.60	2.20	2.42	1.57
Min	1.65	1.54	2.38	1.72	2.04	1.26
Max	2.15	2.03	2.78	2.39	2.53	1.83
S, 19 %						
MV	0.91	0.59	0.75	0.55	0.61	0.49
Min	0.77	0.46	0.62	0.48	0.50	0.37
Max	1.01	0.70	0.93	0.69	0.66	0.51

The mean value (MV), minimum (min) and maximum (max) values are presented in gL^{-1}

σ: uncertainty (%)

important requirement for the heart muscle to perform properly. On the other hand, the high concentration of Mg and Na emphasizes the need to adopt different limits for athletes, depending on the physical activity performance and it also suggests that these elements must be constantly evaluated in blood during the training to avoid disturbances in the body (mainly hypermagnesemia and hypernatremia). The lower values for Br as well as the higher values for S may be associated with the diet; however, research on the athlete's nutritional needs should be done to confirm this hypothesis.

The concentration of the elements in blood samples for AR and ER at rest are shown in Table 3 as well as the range, considering a confidence interval of 95 % usually adopted in clinical practice. In this Table the results for the CG was also included for comparison. The significance of differences between these groups (CG, AR and ER) assessed by Student's t- test ($p < 0.05$) are presented in Fig. 2.

Conclusion

The use of the NAA technique allowed a quantitative estimation of Br, Ca, Cl, K, Na and S in blood samples of AR and ER using a small quantity of blood. The range (at rest) established for the three groups investigated (CG, AR and ER) show significant differences for some elements in blood, suggesting that a strong dependency of these limits in function of adopted physical training exist. Besides, these data can be considered in a preparation of a balanced diet and can also be used for evaluating the performance of athletes during the preparation period for competitions as well as to

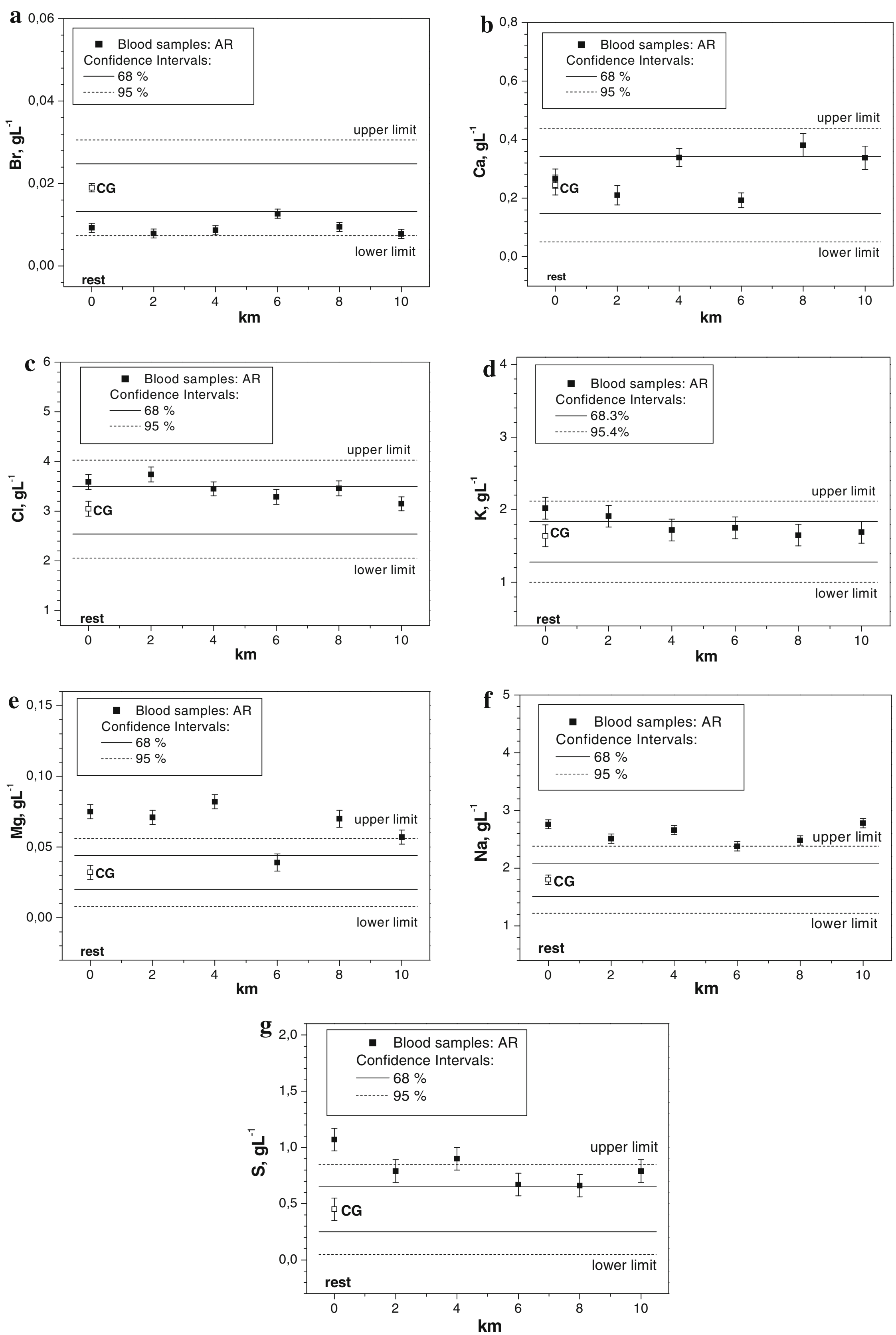

Fig. 1 Blood concentrations of Br (**a**), Ca (**b**), Cl (**c**), K (**d**), Mg (**e**) Na (**f**), S (**g**) for AR before, during (every 2-km) and after training the 10-km running; the results (mean value and confidence intervals) for the CG at rest were also include for comparison

Table 3 Blood concentrations of Br, Ca, Cl, K, Mg, Na and S for the CG, AR and ER at rest

Elements, gL^{-1}	MV ± 1SD [GR]*		
	CG	AR	ER
Br	0.0190 ± 0.0058 [0.0074 – 0.0306]	0.0068 ± 0.0014 [0.0040 – 0.0096]	0.0027 ± 0.0004 [0.0019 – 0.0035]
Ca	0.245 ± 0.097 [0.051 – 0.439]	0.286 ± 0.062 [0.162 – 0.410]	0.108 ± 0.022 [0.064 – 0.152]
Cl	3.05 ± 0.49 [2.07 – 4.03]	3.28 ± 0.54 [2.20 – 4.36]	3.63 ± 0.52 [2.59 – 4.67]
K	1.64 ± 0.26 [1.12 – 2.16]	1.62 ± 0.36 [0.90 – 2.34]	2.02 ± 0.27 [1.48 – 2.56]
Mg	0.032 ± 0.012 [0.008 – 0.056]	0.056 ± 0.016 [0.024 – 0.088]	0.034 ± 0.010 [0.014 – 0.054]
Na	1.80 ± 0.29 [1.22 – 2.38]	2.01 ± 0.50 [1.01 – 3.01]	2.35 ± 0.34 [1.67 – 3.03]
S	0.49 ± 0.14 [0.21 – 0.77]	0.65 ± 0.18 [0.29 – 1.01]	0.74 ± 0.09 [0.56 – 0.92]

The Groups Range (GR) for the groups were included for comparison

MV mean value, *SD* standard deviation

* for a confidence interval of 95 %

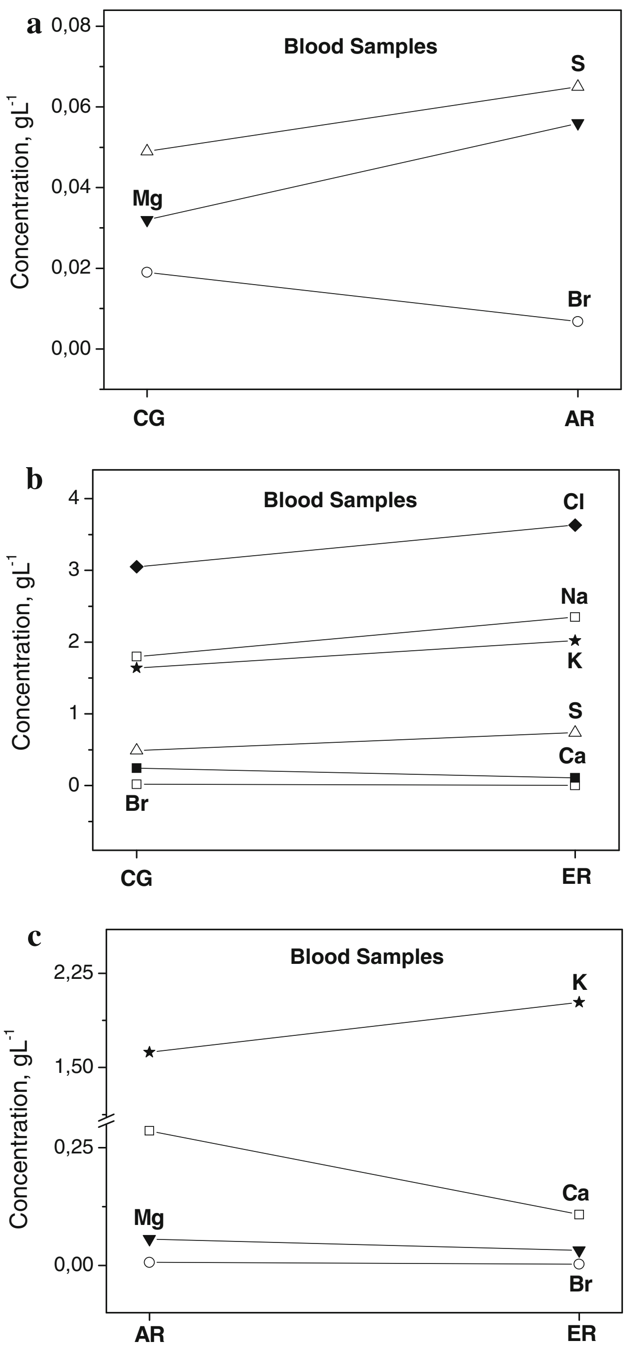

Fig. 2 Student's t-distribution between groups (**a** CG and AR; **b** CG and ER and **c** AR and ER)

propose a new evaluation of protocols. The NAA procedure for blood analysis also permitted to check the athlete's performance during the physical training, which is not the appropriate use of the conventional procedure (biochemistry analyses in serum) due to the quantity of blood that must be collected in a short period of time (one hour, or less).

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