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Blood elements concentration in cyclists investigated by instrumental neutron activation analysis

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Abstract In this study Br, Ca, Cl, Fe, K, Mg, Na, S and Zn levels in blood samples of cyclists were investigated using neutron activation analysis technique. The results were compared to individuals of the same age and gender, but not involved with physical activities (control group), which showed considerable differences. A decrease mainly in Br (91 %) and Ca (78 %) and an increase in Fe (26 %), S (82 %) and Zn (22 %) levels were evidenced. These results emphasize the importance of blood monitoring for the maintenance of endurance athletes performance, particularly for Br, Ca and S.

Keywords Blood · Cyclists · NAA · Endurance athletes

Introduction

Cycling is one of the most efficient forms of human locomotion requiring low energy per unit mass per distance traveled. It is considered an aerobic activity that helps metabolic functions of the whole body, leading to health benefits [1]. Cycling has been recognized as a means of promoting public health [2] and some large population studies have shown the effects of commuting by bicycle on reducing mortality and cardiovascular risk and lower rates of obesity (in regions with high rates of cycling) [3–5].

Good athlete's performance is characterized by a combination of factors among which stand out physical training and proper nutritional intake. Although physical activities provide health benefits, cyclists impose considerable demands on their body during both training and competition period, which can result in muscle injury depending on the intensity and duration used in the efforts. Similarly, a balanced nutrition should provide nutrients to support growth, development and maintenance of several metabolic and physiological processes [6]. A balanced nutrition reduces fatigue, increases the willingness and prevents the occurrence of injuries and infections, improving performance and recovery post-exercise [7, 8]. Several clinical analysis, mainly in serum and urine, are performed regularly in athletes to check their physical condition, such as, oxygen intake, lactate, total protein, albumin, serum glucose, non-esterified fatty acids, thyroid hormones and insulin levels as well as plasma ions [9, 10]. Recently an investigation involving the determination of element concentrations in the blood of long-distance runners revealed significant differences before and after training, as well as when compared with subjects not involved with physical activities [11]. This suggests the need to adopt different intake recommendations for athletes involved with intense aerobic activity.

The purpose of this study is to perform an investigation in blood of five cyclists in constant training for the last 6 years. The number of athletes who are prepared within a specific program is restricted, for training on specific conditions (for long periods, often years) and are accompanied by a team (coach, doctor, nutritionist, among others) which searches for improvements that can benefit and

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improve the performance of each athlete through out of individual and routine assessments. Noteworthy that in this area of activity "little is a lot", which means that any benefit that may add advantages in performance of an athlete should be considered for a research. Therefore, the present study was designed to determine whether each of the athletes of the same category, subjected to intensive training and same size, are appropriate physical condition of the clinical point of view before and post workout. At the same time a comparison with a control group was conducted. These comparisons have been proposed based on the differences that were found in the blood runner's investigation [11].

In this study the Br, Ca, Cl, Fe, K, Mg, Na, S and Zn levels in blood samples of cyclists were investigated using neutron activation analysis (NAA) technique. This technique was applied due to its advantage in evaluating elements of clinical relevance (Ca, Cl, Fe, K, Mg and Na) as well as some not so usual (Br, S and Z) [12] using small quantities of whole blood (0.5 mL), comparatively to conventional analyses performed in serum, using at least 1.0 mL by element determination [13]. We performed a comparison before and after the high-intensity training and with individuals not involved with physical activities (control group). These data may be useful to propose new evaluation protocols besides improving the performance of athletes.

Materials and methods

Athlete group

Five physically healthy male professional trained-cyclists that were in constant training for the last 6 years, ages 32 ± 6.2 years, weight 76.6 ± 4.4 kg, height 179 ± 0.06 cm and training volume of 8–13 h week^{-1} participated in the study. This study was performed with the approval of the Ethical Committee on Human Research of the University of Campinas (SP, Brazil). The blood samples were collected in the morning, in two different situations: before (at rest) and after the physical exercise. About 2 mL of venous blood was collected (before and after the training) in vacuum tube without anticoagulants (BD, Vacutainer). Aliquots of 0.5 mL were transferred to a polyethylene capsule. The blood collection procedure was performed by a trained professional and monitored by a professional of Physical Education in the Pharmacy laboratory of the University Center FIEO—UNIFIEO (FIEO—Fundação Instituto de Ensino para Osasco at São Paulo city). The athletes have a balanced diet elaborated by a professional nutritionist.

Control group

The control group was composed of 65 healthy subjects, male donors selected from Paulista Blood Bank (at Sao Paulo city, Brazil) with the same range of age and weight, but not involved in intense physical activities. The sample preparation was performed as described for the athletes. All the samples (athletes and control) were prepared in duplicate. All uncertainties are based on statistical counting and calculated at one standard deviation. Samples and reference material (IAEA-A-13, Animal Blood) were irradiated for 5 min in a pneumatic station at the nuclear reactor (IEA-R1, 3.5-4.5 MW, pool type), IPEN/CNEN-SP, Brazil, with a thermal neutron flux (ranged from 4.6×10^{12} to 6.8×10^{12} cm^{-2} s^{-1}). After a decay time of 1 min, a gamma counting of 20 min was used for determining ^{80}Br ($T_{1/2} = 18$ min, $E_\gamma = 616$ keV), ^{49}Ca ($T_{1/2} = 9$ min, $E_\gamma = 3084$ keV), ^{38}Cl ($T_{1/2} = 37$ min, $E_\gamma = 1642$ keV), ^{27}Mg ($T_{1/2} = 9$ min, $E_\gamma = 844$ and 1014 keV), ^{24}Na ($T_{1/2} = 15$ h, $E_\gamma = 1369$ keV) and ^{37}S ($T_{1/2} = 5$ min, $E_\gamma = 3104$ keV) following by 6 h of counting time for ^{42}K ($T_{1/2} = 12$ h, $E_\gamma = 1525$ keV). For determining ^{59}Fe ($T_{1/2} = 44$ days, $E_\gamma = 1099$ keV) and ^{65}Zn ($T_{1/2} = 244$ days, $E_\gamma = 1116$ keV) samples and reference material were irradiated in the core of the IEA-R1 nuclear reactor ($\sim 8.4 \times 10^{12}$ cm^{-2} s^{-1}) for 4 h, followed by 10 days decay time at least and a counting time of 8 h. A γ-spectrometer system composed of an ORTEC HPGe detector (Model GEM-6019), coupled to a MCA ORTEC Model 919E and a PC, were used to measure the induced gamma-ray activity. The concentration of each element was calculated using in-house software [14].

Results

The NIST SRM-1577b Bovine Liver and IAEA-A-13 Animal Blood reference materials were used for analytical quality control. According to Table 1 the evaluation of the Z-score test, obtained using these reference materials, are satisfactory for the determination of all elements ($|Z| < 2$). In Table 2 the element concentrations in the cyclists blood, before (C_{BT}) and after (C_{AT}) the training and for the control group (CG) are presented as the mean value (MV) and one standard deviation (±1 SD). The range from the control group for a confidence interval of 95 % (usually adopted for checking the clinical status of the organism) was also included for comparison. To visualize, in Fig. 1 is presented the results for each athlete (A1, A2, A3, A4 and A5) by element. The mean values (of three experiments) for the athlete group at rest (before start the training, expressed by MV) and for the control group (expressed by CG) were also included.

Table 1 Mass fraction and associated uncertainly measured in the reference materials

Elements	This work MV ± 1 SD	NIST SRM 1577b Bovine Liver	IAEA-A-13 Animal Blood	|Z-score|
Br (mg kg^{-1})	8.4 ± 0.6[a]	9.7*	22 ± 2	0.5[b]
	20.8 ± 1.7[b]			
Ca (mg kg^{-1})	119 ± 11[a]	116 ± 4	286 ± 54	0.75[a]
	292 ± 32[b]			0.11[b]
Cl (g kg^{-1})	2.73 ± 0.11[a]	2.78 ± 0.06	13.40 ± 1.07	0.83[a]
	13.61 ± 0.41[b]			0.20[b]
Fe (g kg^{-1})	0.195 ± 0.012[a]	0.184 ± 0.015	2.40 ± 0.14	0.74[a]
	2.52 ± 0.21[b]			0.71[b]
K (g kg^{-1})	9.91 ± 0.52[a]	9.94 ± 0.02	2.50 ± 0.35	1.50[a]
	2.62 ± 0.21[b]			0.36[b]
Na (g kg^{-1})	2.38 ± 0.11[a]	2.42 ± 0.06	12.60 ± 0.101	0.67[a]
	12.91 ± 0.52[b]			0.31[b]
S (g kg^{-1})	7.90 ± 0.74[a]	7.85 ± 0.06	6.50 ± 0.52	0.83[a]
	6.62 ± 0.90[b]			0.23[b]
Zn (mg kg^{-1})	133 ± 0.08[a]	127 ± 16	13.00 ± 1.04	0.25[a]
	13.72 ± 1.52[b]			0.69[b]

SD Standard deviation

* Value not available

[a] NIST-SRM-1577b

[b] IAEA-A-13

Table 2 Elements concentration in blood of cyclists before (C_{BT}) and after (C_{AT}) the training and for the control group (C_{CG})

Elements (g L^{-1})	C_{BT} MV ± 1 SD	C_{AT}	C_{CG} [range][a]
Br	0.0017 ± 0.0006	0.0018 ± 0.0012	0.0190 ± 0.0058 [0.0074–0.0306]
Ca	0.053 ± 0.006	0.056 ± 0.003	0.245 ± 0.097 [0.051–0.439]
Cl	2.56 ± 0.16	2.52 ± 0.22	3.05 ± 0.49 [2.07–4.03]
Fe	0.531 ± 0.100	0.537 ± 0.122	0.421 ± 0.066 [0.289–0.553]
K	1.33 ± 0.13	1.41 ± 0.26	1.64 ± 0.26 [1.12–2.16]
Mg	0.021 ± 0.032	0.021 ± 0.039	0.032 ± 0.012 [0.008–0.056]
Na	1.52 ± 0.09	1.53 ± 0.13	1.80 ± 0.29 [1.22–2.38]
S	0.89 ± 0.14	0.83 ± 0.10	0.49 ± 0.14 [0.21–0.77]
Zn	0.0073 ± 0.014	0.0070 ± 0.016	0.0060 ± 0.0010 [0.0040–0.0080]

The range for the CG was also included for comparison

MV Mean value, *SD* standard deviation

[a] Confidence interval of 95 %

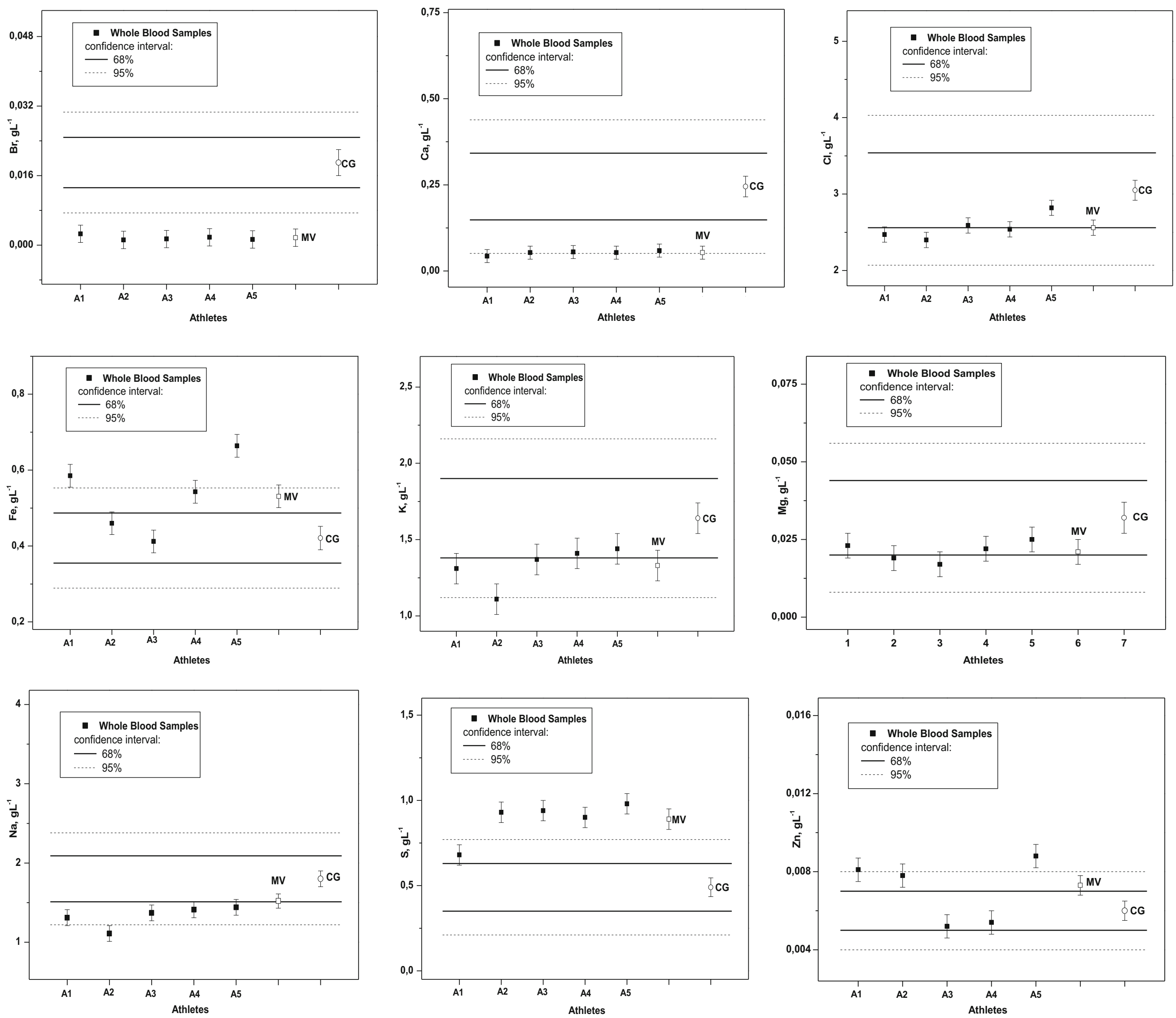

Fig. 1 Cyclists blood concentrations at rest. The mean value for the athletes at rest (MV) and for the control group (CG) were included for comparison

The Student's *t* test for unequal variances was applied for results comparison between athlete's blood concentrations before and after training. The results for all elements are considered statistically equal ($p > 0.05$). However, the results of the cyclists when compared with the control group showed a decrease of Br (91 %), Ca (78 %), Cl (16 %), K (19 %), Mg (34 %), Na (16 %) and an increase of Fe (26 %), S (82 %) and Zn (22 %). According to the Student's *t* test, there are differences between these groups ($p < 0.05$) for Br, Ca, Fe, S, and Zn.

Among elements with significant differences are Br and S. They are usually not evaluated by clinical tests. Br physiological function is not very clear [15–19], however some studies have reported its relevance for the immune defense and balanced electrolytes [15, 17–20]. Although Br concentrations in blood samples of cyclists are significantly low, it is questionable whether this can really be a status of deficiency. Studies with the Brazilian population have shown that high consumption of drugs (rich in bromides, such as antidepressant and somniferous) [21] and a diet rich in seafood contributes to high levels of Br in blood [22]. A comparison with other populations (0.0079 ± 0.0027 g L^{-1} [23] and 0.0140 ± 0.0014 g L^{-1} [24]) also emphasis high Br levels in sedentary subjects comparatively to the results in blood samples of cyclists (0.0017 ± 0.0006 g L^{-1}). Therefore, additional studies regarding the importance of Br nutrition should be made to establish the limits for daily intake for aerobic endurance athletes. Related to S, it is present in all cells, plays an important role in growth as well as in the metabolism and defense of the body [25]. In sports medicine, the sulfur intake is very important because its deficiency can cause muscle injury affecting the performance of the athletes [26]. However high concentration of S in blood, as is observed in cyclist's blood, can lead to the development of cardiovascular diseases [27, 28]. Therefore, the supplementation and consumption of proteins rich in amino acids, mainly methionine ($C_5H_{11}NO_2S$), cysteine ($C_3H_7NO_2S$), homocysteine ($C_4H_9NO_2S$) and taurine ($C_2H_7NO_3S$), must be strictly controlled. In cases were S levels are extremely high can cause intoxication.

Related to Ca, its need increases with age because the body becomes less efficient at absorbing calcium from food. The Ca blood levels can be moderately low without causing any severe dysfunction, as observed in this investigation. However, if the levels are low for long periods, muscle cramps become common and its chronic deficiency can cause osteoporosis [29]. In cases were Ca levels are extremely low (hypocalcemia), the brain can be affected causing neurological or psychological symptoms (such as, memory loss and depression) as well as muscle aches, stiffening and abnormal heart rhythm [30, 31]. These facts suggesting that a conventional clinical test for Ca evaluation (calcemia), at regular intervals (at least semiannually), is recommended to prevent in long term possible dysfunctions.

Particularly, in sport medicine, Fe and Zn have been widely investigated in blood (serum and plasma) of endurance athletes [32–46]. Iron levels in blood contribute to the regulation of the body temperature after physical exercise, control of the cardiovascular system, immune defenses, and brain function [32, 33], while Zn is a cofactor for numerous enzymes implicated in several physiological processes, including DNA reproduction and immune response [8, 30]. For Fe, some studies have reported that some endurance athletes have prevalence of Fe deficiency and anemia [32, 34–38] and others indicate excess [33, 39–41] comparatively with sedentary individuals. According to these investigations, the Fe serum levels in endurance athletes range from 0.088 ± 0.033 mg L^{-1} [36] to 0.937 ± 0.298 mg L^{-1} [41]. In terms of Zn, intense physical activity can rapidly redistribute it in the body, decreasing its plasma concentration, which could increase the risk of infections. In cases of its severe deficiency muscle functions can be affect [42, 43]. On the other hand, Zn supplementation may affect plasma cupper concentration, thyroid-stimulating hormone, thyroid hormones and glucose homeostasis [45]. A recent study indicated differences in blood elements and vitamin levels in high physical activity groups. However, the vitamins were less variable in comparison to some elements, such as Fe and Zn [46]. In this study, Fe and Zn were also evaluated in whole blood samples of the cyclists and the results were compared with the control group. Taking into account that the reference values in blood have connections between the environment, dietary habits, life-style and urban and industrial exposure we performed a comparison with the data from this study, for both CG (control group) and AG (athlete group), and others data available in literature for several populations [23, 24, 47–50]. These results were summarized in Fig. 2 as the mean values (MV) and one standard deviation (± 1 SD). The Fe data of the CG from the present study is in agreement with all estimative [23, 24, 47–50] while for AG is above of the adequate ($p < 0.05$) for most of the cases [23, 24, 47–49]. For Zn there are some severe alterations ($p < 0.05$), for both groups CG [23, 46, 48] and AG [23, 24, 48, 50], even considering 2 standard deviations (± 2 SD). These comparisons intensify the need to establish limits for specific populations (i.e., for both, control and athletes).

Finally, when the evaluation is performed individually (for each athlete, Fig. 1) it is observed that the elements Fe and Zn do not have a systematic behavior. Particularly to the athlete A5, the concentrations for these elements (664 ± 28 mg L^{-1} for Fe and 8.8 ± 7 mg L^{-1} for Zn) are at levels well above expected (289–553 mg L^{-1} for Fe and 4.0–8.0 mg L^{-1} for Zn). While for Fe the excess can be associated with oxidative stress and increase the risk of infections [39, 40], for high levels of Zn the absorption of other elements can be inhibited [44], emphasizing that Fe and Zn supplementation must be strictly controlled.

Conclusion

The elements Br, Ca, Cl, Fe, K, Mg, Na, S and Zn in blood samples of cyclists (competitive senior athletes engaged in this modality during the past 6 years) were investigated in two situations: (i) at rest and compared with the control group and, (ii) before (i.e., at rest, before start the training) and after high-intensity training.

Cyclists and control group

For all the athletes the elements Br, Ca, Cl, K, Mg, Na and S in blood have systematic behavior and the concentrations are close to the lower limits, except for S whose concentrations are above the upper limit. Only for Fe and Zn the concentration behavior is not systematic suggesting the need to perform a clinical evaluation before the supplementation of minerals and vitamins.

The elements Br, Ca and S investigated in the cyclist's blood presented significant differences induced probably by the exercise intensity and diet. That can be considered for a proposition of new clinical protocols based on the range established by the control group. Particularly, for Ca,

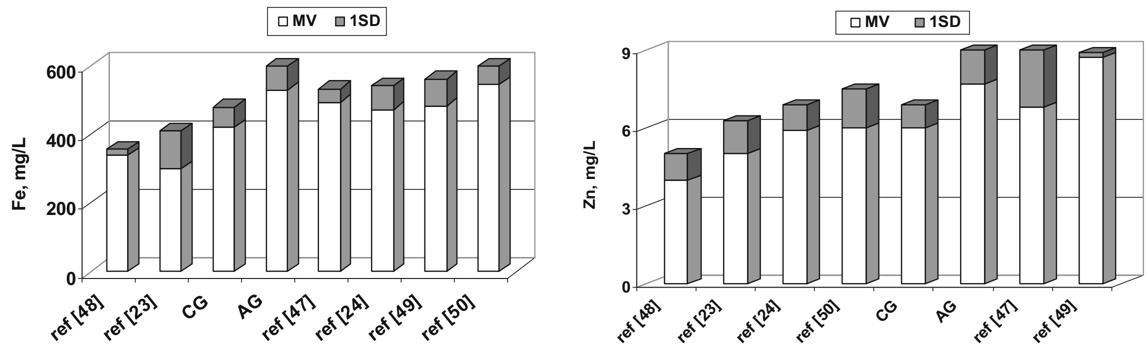

Fig. 2 Fe and Zn concentration results in whole blood. The data are presented as mean value (MV) and standard deviation (SD)

the athlete's blood levels are close to the lower limit suggesting that high-intensity training for long periods (years) can reduce Ca stores. It is possible that this reduction over time can affect the performance of the cyclists. As a precaution, its clinical evaluation at regular intervals is recommended to prevent in long term possible dysfunctions. Moreover, Br and S concentration results suggest the need to investigate different intake recommendations for aerobic endurance athletes.

Cyclists (at rest) and cyclists (after training)

No differences of the elements concentration in blood were observed for the athletes submitted before and after to a constant and intense physical exercise.

The observed tendencies in this investigation suggest that more wide studies are required to specify in more detail the elements behavior in blood as well as the intake recommendations for aerobic athletes with intense and controlled physical activity. Moreover, the differences highlighted in the athlete's blood can help the development of clinical protocols (specific) by sport category and a correct dimensioning of the nutrient intake.

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