Sulfur status in long distance runners

L Kovacs[1], C Zamboni[1], T Lourenço[2] and D Macedo[2]

[1]Instituto de Pesquisas Energéticas e Nucleares (IPEN / CNEN - SP)
Av. Professor Lineu Prestes 2242, 05508-000, São Paulo, SP, Brasil.
[2] Laboratório de Bioquímica do Exercício (LABEX), Universidade de Campinas
Cidade Universitária 13083-970 - Campinas, SP, Brasil, P.O Box: 6109

E-mail: lksantos@ipen.br

Abstract. In sports medicine, sulfur plays an important role and its deficiency can cause muscle injury affecting the performance of the athletes. However, its evaluation is unusual in conventional clinical practice. In this study the sulfur levels were determined in Brazilian amateur athlete's blood using Neutron Activation Analyses (NAA) technique. Twenty six male amateur runners, age 18 to 36 years, participated of this study. The athletes had a balanced diet, without multivitamin/mineral supplements. The blood collection was performed at LABEX (Laboratório de Bioquimica do Exercício, UNICAMP-SP) and the samples were irradiated for 300 seconds in a pneumatic station in the nuclear reactor (IEA-R1, 3-4.5MW, pool type) at IPEN/CNEN-SP. The results were compared with the control group (subjects of same age but not involved with physical activities) and showed that the sulfur concentration was 44% higher in amateurs athletes than control group. These data can be considered for preparation of balanced diet, as well as contributing for proposing new protocols of clinical evaluation.

1. Introduction

Minerals are essential for metabolic and physiologic processes in the human body [1]. Among them sulfur is the third most abundant element found in human body after calcium and phosphorus and it is present in every cell [2]. A small percentage of sulfur comes in the form of inorganic sulfates and the other forms of organic sulfur presents in foods. They are important sources of sulfide in human. Sulfur plays important roles in the body, but is an element usually not analyzed in conventional clinical. According to references [3,4,5] the amount of sulfur levels in human blood ranges from 0.1 to 1.0 g/L.

Sulfur participates in the structure and activities of molecules playing important roles in growth, energy transfer mechanism, metabolism, defense, transport, and detoxification processes [2] and it is also necessary for formation of collagen (the protein found in connective tissue in our bodies). Sulfur is also present in keratin, which is necessary for the maintenance of the skin, hair, and nails, helping to give strength, shape, and hardness to these protein tissues [6].

[1]lksantos@ipen.br

Sulfur performs a number of functions in enzyme reactions and protein synthesis. It is present mainly in four common amino acids, i.e., methionine, cysteine, homocysteine and taurine (Figure 1), but only methionine and cysteine are incorporated into proteins [7]. Sulfur present in cysteine and methionine amino acids helps regulate carbohydrate metabolism, while sulfur - contained in taurine (found in bile acids) helps in the digestion process. Methionine cannot be synthesized by the body and needs to be supplied by the diet while the cysteine may, but this process requires a steady supply of sulfur [8].

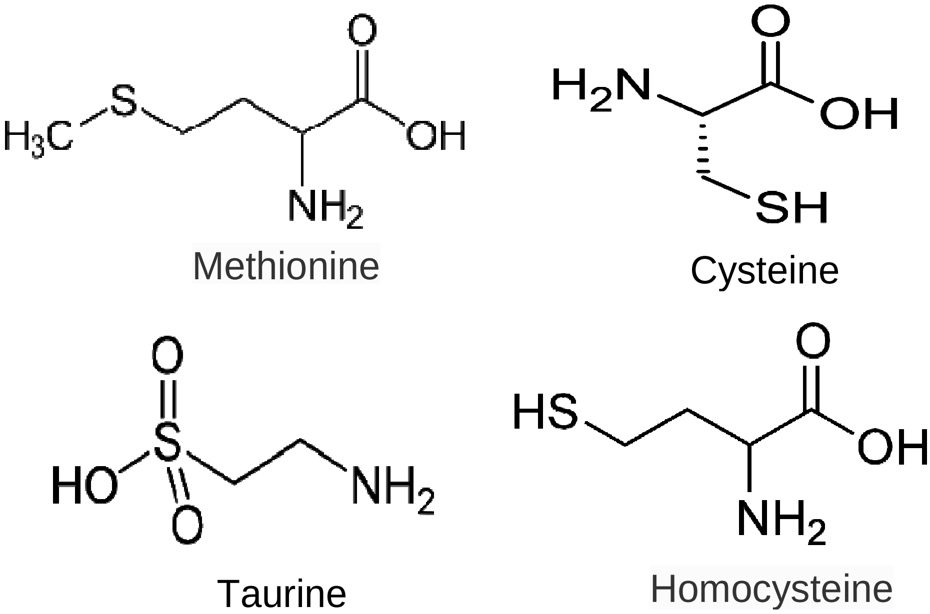

Figure 1. Structures of the sulfur-containing amino acids.

The sulfate absorption occurs in the stomach, small intestine and colon. The excess inorganic sulfur, generated as a result of liver or kidney metabolism, is excreted in urine as sulfates. There is not a recommended amount of sulfur intake but its toxicity may occur if sulfur-containing amino acids are ingested in large amounts.

In sports medicine, the sulfur intake plays a very important role: while its deficiency can cause muscle injury affecting the performance of the athletes [6], its excess can cause intoxication. However, its evaluation is unusual in conventional clinical practice.

In this study the sulfur levels were determined in blood of amateur athlete's using Neutron Activation Analyses (NAA) technique. This technique measures the total amount of sulfur present in sample independent of its chemical formula and without any pre-treatment of the sample [9]. This study is a partnership with Exercise Biochemistry Laboratory (LABEX) at University of Campinas (UNICAMP/SP, Brazil), 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.

2. **Materials and Methods**

2.1 Collection and preparation of the samples

Twenty six male amateur athletes from LABEX, age 18 to 36 years, participated of this study. The athletes had a balanced diet, without multivitamin/mineral supplements. The blood samples were collected before (at rest) the physical training. For the blood collection, a small capillary pin

(Clinitubes®, Radiometer Copenhagen®) was inserted in the athlete's finger and exactly 50(± 0.5%) μL were dropped on to Whatman nº 41 filter paper (~2.3 cm^2) using a calibrated micropipette, and then dried for a few minutes using an infrared lamp (Figure 2). Considering the population of the study (male with 18-36 years) for the control group the whole blood samples were collected from 68 male healthy donators, with the same age and not involved with physical activities, selected from Paulista Blood Bank at São Paulo, Brazil. The sample preparation was performed as described for amateur athletes.

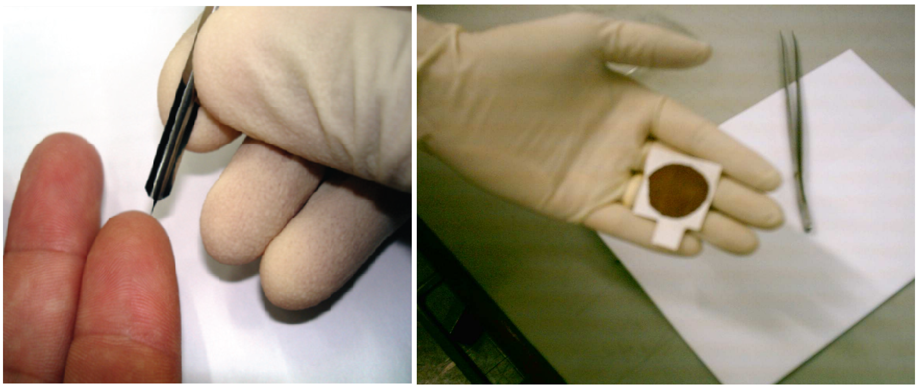

Figure 2. Blood collection and sample preparation (blood dropped in filter paper).

2.2 Experimental procedure
Blood sample and standard were irradiated for 300 s in a pneumatic station of the IEA-R1 nuclear reactor (3-4.5 MW, pool type) at IPEN. The thermal neutron flux utilized of ~$8 \cdot 10^{12}$ $n \cdot cm^{-2} \cdot s^{-1}$. After the irradiation, followed by a 60 s decay time, the activated materials were gamma-counted for 300 s using a high resolution HPGe detector (FWHM = 1.89 keV) connected to an ADCAM multichannel analyzer (ORTEC 919E) and a PC computer. The concentrations of sulfur were obtained using S^{36} ($T_{1/2}$ ~ 5 min, E_γ = 3104 keV). The filter paper (blank) was also analyzed using the same irradiation conditions. Data reduction was done using in-house software [10]. The IAEA A-13 certified reference material was investigated for quality control.

3. Results
Partial gamma-ray whole blood spectrum is presented in Figure 3; the energy used for sulfur (3104 keV) is highlighted.

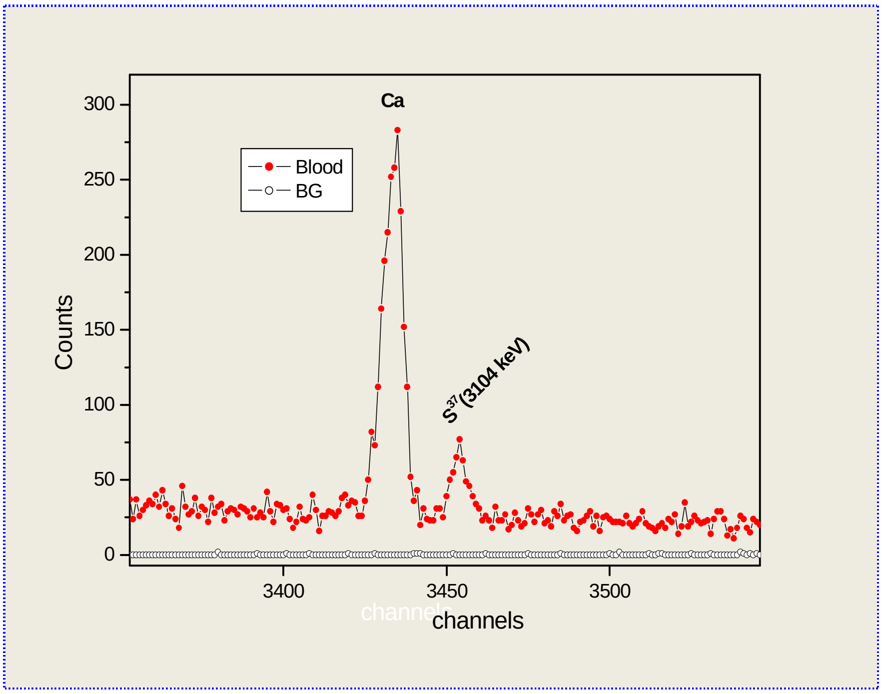

Figure 3. Gamma ray spectrum.

For analytical quality control Animal Blood (IAEA-A13) certified reference material was used. Table 1 shows the certified value for sulfur, the result obtained in this study, relative standard deviation (RSD), relative error (Er) and Z-score. The |Z score| < 2, indicated that our result was satisfactory and is within the range of certified data at the 95% confidence level. Sulfur blood concentration results, mean value, standard deviation (±1SD), median, mode, minimum value, and maximum value for amateur runner and control group are show in Table 2.

Table 1. Element concentrations obtained in the analysis of AIEA A-13 reference material.

Element gkg^{-1}	**Certified value** mean ±1 SD	**This work** mean ±1SD	**RSD** %	**Er** %	**Z- score**
S	6.50 ± 0.52	6.62 ± 0.92	13.89	1.8	0.23

Table 2. Sulfur blood concentration results, mean value, standard deviation (±1SD), median, mode, minimum value, and maximum value for amateur runner. The results for the control group were also included for comparison.

S, g/L^{-1}	Amateur Runners	Control Group
Medium value	0.65	0.45
± 1SD	0.23	0.20
Median	0.66	0.44
Mode	0.50	0.47
Minimum	0.21	0.12
Maximum	1.24	0.88

Figure 4 shows the sulfur concentration in whole blood of amateur runners (AR) and control group (CG) by Box Plot and Figure 5 shows a comparison between the individual concentrations of sulfur in whole blood of amateur runner. In this figure the indicate interval of control group, considering ± 1SD and ± 2SD were also included for comparison. According to the statistical analysis (Student *t*-test) there is a significant difference ($p < 0.05$) between these groups.

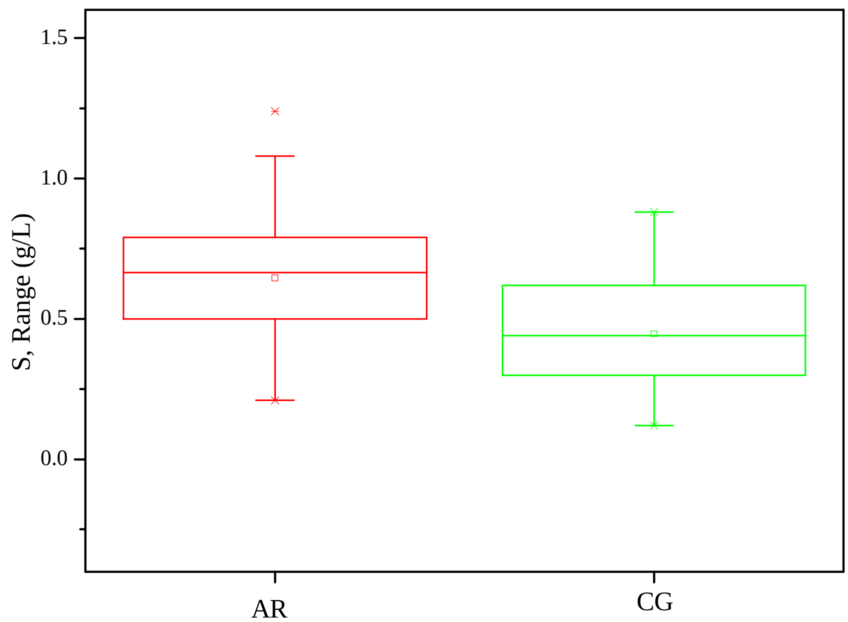

Figure 4. Sulfur concentration in blood of amateur runners (AR) and control group (CG) by Box Plot.

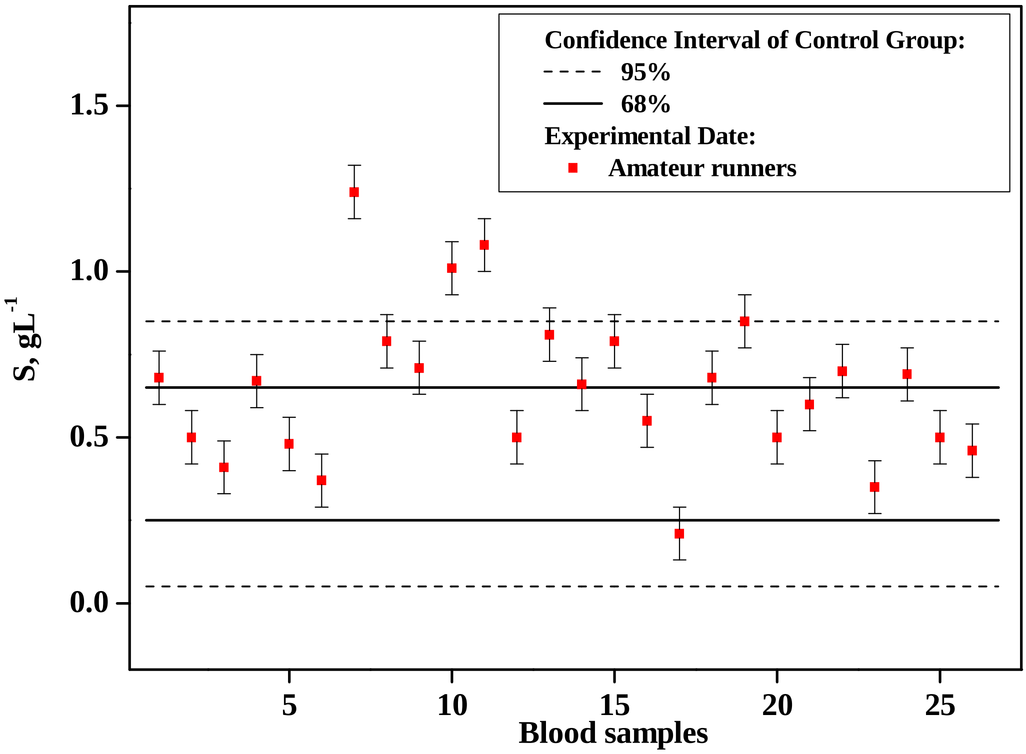

Figure 5. Sulfur concentration in blood of amateur runner.

4. Discussion

In the present study, we have demonstrated high sulfur level in the blood of amateur runners when compared to people not involved in intense physical activities. These alterations can be associated mainly with athlete's diet that is very rich in amino acids, such as, the use of amino acids in the formulations of beverages, bars cereals and supplements between other varieties of food [11].

For long-distance runners, sulfur present in some amino acids is essential, mainly the sulfur contained in methionine because it helps the body to eliminate fat and reduce the level of inflammatory histamines. Furthermore, the sulfur contained in taurine can act as a catabolic preventing the loss of muscle mass and to assist the entry of glucose into muscle.

Although all the benefits of these amino acids for the athlete the intake must be done carefully. The intake of sulfated amino acids and other sulfur compounds in excess, may contribute to the increase sulfur in the blood, which can result in intoxication.

5. Conclusion

The results showed significant differences and suggest that they can be considered for preparation of balanced diet, as well as to contribute for proposing new protocols of clinical evaluation. Moreover, these results may be useful in other areas of research such as health and nutrition.

Acknowledgments

The authors would like to acknowledge the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the financial support.

References

[1] Maughan R J 1999 *Br Med Bull* **55**(3) 683
[2] Ingenbleek Y and Kimura H 2013 *Nutr Rev*.**71**(7) 413

[3] Denis W 1921 J Biol Chem **49** 311
[4] Mousavi-Yeganrh S et al 1984 NIM **B**(3) 364
[5] Oliveira L C and Zamboni C B 2013 AIP **1529** 73
[6] Mahan L K and Stump S E 2008 *Krause's Food & Nutrition Therapy*. (USA, Saunders/Elsevier)
[7] Brosnan J T and Brosnan M E 2006 *J Nutr* **136**(6) 16365
[8] Nimni M E et al 2007 *Nutr Metab* **4** 24
[9] Greenberg R R et al 2011 *Spectrochim. Acta B* **66** 193
[10] Medeiros J A G, Zamboni C B, Zahn G S, Oliveira L C, Dalaqua L. 2005 *Proc. 39º Congresso Brasileiro de Patologia Clinica (Brazil)* CD ROM
[11] Vally H et al 2009 *Clin Exp allergy* **39**(11) 1643