J Radioanal Nucl Chem (2016) 307:1641–1643
DOI 10.1007/s10967-015-4511-9

Determination of Fe in blood using portable X-ray fluorescence spectrometry: an alternative for sports medicine

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Received: 31 May 2015 / Published online: 9 October 2015

Abstract An alternate methodology based on a portable X-ray fluorescence spectrometry (PXRFS) for determination of Fe in blood was evaluated. The iron concentrations was determined in whole blood of 18 male amateur athletes (runners) using this portable XRF spectrometer and compared with a control group (54 male donors at the same age but not involved with physical activities) obtained by XRF and NAA techniques. The Fe concentration in the blood of runners is an important factor in sports medicine contributing to the performance of endurance athletes as well as for proposing new protocols of clinical evaluation.

Keywords Iron · Blood · Sport medicine · X-ray fluorescence · Neutron activation analysis

Introduction

In the last decade, there has been a growing interest in the athlete's health, with a focus on continuous biochemical evaluation in serum and urine and controlled diet. Moreover, it is also recognized that intensity and duration of the physical training can provoke metabolic alterations in blood, mainly in the content of some ions. Particularly, high aerobic activity and poor dietary habits may results in depletion of body iron stores, which could decrease aerobic performance and increase the risks of fatigue and immune disorders. Athletes, particularly those involved in endurance sports, are commonly diagnosed with iron deficiency, which leads to higher risks of fatigue, overtraining syndrome and vulnerability to infection [1–5]. On the other hand, excess of iron is toxic: it can deposit in the form of aggregates in tissues such as liver, heart, pancreas and joints leading to irreversible organ dysfunction [4, 6]. Adequate iron intake recommended for adults are 8 mg/d for men and 18 mg/d for women and the tolerable upper intake levels for adults are 45 mg/d [7]. As a result, the use of supplements by athletes must be strictly controlled. Recently, elements of clinical relevance (Ca, Cl, K, Mg and Na) in blood of amateur and elite runners (long distance runners) were analyzed using neutron activation analysis (NAA). A comparison with subjects of the same gender and age but not involved with physical activities revealed significant differences among them [8]. In this study, we intend to continue this analysis, to complement the blood investigation, performing measurements to determine Fe, using energy dispersive portable X-ray fluorescence spectrometry (PXRFS). Recent investigations in whole blood, using the PXRFS, showed it to be appropriate for clinical end points [9, 10]. The major advantage for using the portable spectrometer is the viability to use small quantities of whole blood (0.1 mL), compared to conventional analyses performed in serum or plasma (using at least 5–10 mL of biological fluid), and the short irradiation time (few minutes). The present study is a partnership with Exercise Biochemistry Laboratory (LABEX) at University of

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Campinas (UNICAMP/SP, Brasil). The iron status determined in an amateur athlete's whole blood can be applied to the preparation of a balanced diet and can be useful also for evaluating the performance of athletes during the preparation period for competitions, as well as to propose new evaluation protocols.

Collection and preparation of the samples

Eighteen male amateur athletes (runners) from LABEX, age 26–39 years and mean weight 69 ± 10 kg participated of this study. The athletes had a balanced diet, without multivitamin/mineral supplements and training time of 37 ± 13 km/week. 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.

Experimental

A MINI-X spectrometer (Amptek XR-100SDD model) was used to perform the XRF measurements. The characteristics of the fluorescence intensities of X-rays (K_α lines) were measured with a Si Drift detector (25 mm^2 × 500 μm/0.5 mil) with Be window (1.5″). Each whole blood sample and standard (certified iron solution) was irradiated for 300 s using 30 kV and 5 μA excitation conditions and the analysis of the spectra was performed using the WinQxas Software. For NAA, whole blood samples and certified reference material (IAEA-A-13) were irradiated for 4 h in the IEA-R1 nuclear reactor (IPEN) and gamma counted for 6 h using HPGe detector (FWHM = 1.92 keV). Fe concentration was determined using the *Activation* software [11]. The IAEA-A-13 was used for analytical quality control.

Results and discussion

The Z-score test indicated that the results were satisfactory ($|Z| < 2$) considering 95 % confidence interval. The relative standard deviations were lower than 5.7 % and relative errors were expressed by 4.1 % for XRF and 2.9 % for NAA. The results for Fe in whole blood samples for the runners by XRF analysis is shown in Table 1 and is expressed as Fe concentration (Mean Value), standard deviation (±1 SD), median, minimum and maximum values. The detection limit (DL) and range (for a confidence interval of 95 %) for Fe in whole blood of the control group (CG) obtained by both XRF and NAA techniques are also included for comparison.

Table 1 Iron whole blood concentration results for amateur runners (athletes) and the control group

Fe (mg L^{-1}) Groups	XRF Runners	XRF Control	NAA
Mean value	443	354	409
±1 SD	76	54	60
Median	454	343	407
Minimum	290	267	315
Maximum	563	482	495
Range (±2 SD)		246–46	289–553
DL		13	22

Figure 1 shows the individual results for Fe in whole blood of the runners compared to the normal range established for the control group (considering ±1 SD and ±2 SD) by the XRF technique.

According to the Table 1, the Fe results obtained for the control group by both techniques are in agreement considering a confidence interval of 68 %, confirming the viability of using a portable X-ray fluorescence spectrometry (PXRFS) for iron concentration determination in whole blood samples. However, the results of runners when compared with the control group showed an increase of 30 %. Statistical analysis (*t* test) demonstrated significant differences ($p < 0.05$) among these groups (AR and CG), showing that the blood iron levels for long distance runners may differ from sedentary people or from people not involved with intense physical exercise. According to

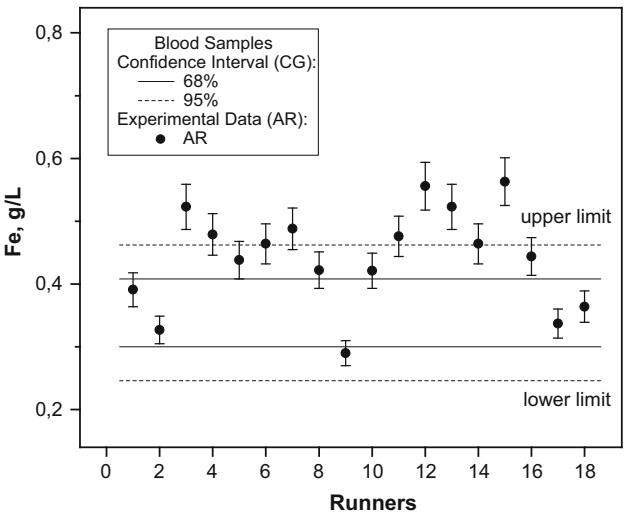

Fig. 1 Fe blood concentration of amateur runners (AR) by XRF analysis

Fig. 1, considering a confidence interval of 95 % usually adopted for clinical practice, it was observed that Fe status in runners' blood was kept near upper limit and it some cases above the normal range, suggesting a slight tendency of iron overload (>0.462 gL^{-1}). This increase may cause risk of infection decreasing the athlete performance and in more severe case can evolve to neurodegenerative disorders [5]. Considering that the athletes did not report the intake of supplement, this increase suggests the need of nutritional reevaluation. Furthermore, the individual evaluation of iron in blood of these athletes (Fig. 1) reinforce the importance of periodic clinical evaluations for assessing the adequacy of the daily needs of the athletes during their sports performances.

Related to the alternative procedure (PXRFS) applied in this work some positive aspects can be emphasized: the simplicity involved in the blood collection and sample preparation, the storage of the sample without the need for refrigeration as well as the speed to perform the XRF measurements (few minutes).

Conclusion

The portable spectrometer (PXRFS) has shown itself to be appropriate for Fe blood analyses and offers a new contribution for studies in sports Medicine related to biochemical analyses of blood. The results of the analysis of Fe in blood of runners reinforce the importance of periodic clinical analysis, creating greater understanding of nutritional requirements and adequacy of the daily needs as well as suggesting the possibility of adopting different recommendations of iron for athletes, mainly for long distance runners.

Acknowledgments The authors would like to acknowledge the clinical staff at blood bank for technical assistance given in the blood collection, the voluntary athletes and the financial support the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

References

1. Hurrell R, Egli I (2010) Iron bioavailability and dietary reference values. Am J Clin Nutr 91(suppl):1461S–1467S
2. Beard J, Tobin B (2000) Iron status and exercise. Am J Clin Nutr 72:594S–597S
3. Wilkinson J, Martin DT, Adams AA (2002) Iron status in cyclists during high-intensity interval training and recovery. Int J Sports Med 23:544–548
4. Lukaski HC (2004) Vitamin and mineral status: effects on physical performance. Nutrition 20:632–644
5. Schumacher YO, Schmid A, König D et al (2002) Effects of exercise on soluble transferrin receptor and other variables of the iron status. Br J Sports Med 36:195–200
6. Mettler S, Zimmermann MB (2010) Iron excess in recreational marathon runners. Eur J Clin Nutr 64(5):490–494
7. Institute of Medicine (2009) The National Academies, Washington, DC. http://www.iom.edu/Activities/Nutrition/SummaryDRIs/DRI-Tables. Accessed 18 Dec 2014
8. Kovacs L, Zamboni CB, Nunes LAS, Lourenço TF, Macedo DV (2013) Concentrations of ions and metals in blood of amateur and elite runners using NAA. J Radioanal Nucl Chem 297:393–398
9. Zamboni CB, Metairon S, Rizzutto MA, Santos SB (2013) The use of portable X-ray fluorescence spectrometry (PXRFS) for clinical practices. PoS (X LASNPA). 1–6 Dec 2013
10. Zamboni CB, Metairon S, Sato IM, Rizzutto MA (2014) New strategy to evaluate the effectiveness of new treatments using animal models. Rev Appl Phys 3:40–43
11. Medeiros JAG, Zamboni CB, Zahn GS, Oliveira LC, Dalaqua LJ (2005) Software for clinical practices using radioanalytical procedure. In: Proceeding of 39° Brazilian Congress of Clinical Pathology/Medicine Laboratorial CD ROM