Iron and zinc status in whole blood in long-distance runners by NAA

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ABSTRACT

Iron and zinc are cofactors in a variety of essential cellular processes for high performance athletes. High aerobic activity and dietary habits may result in depletion of body iron and zinc stores, which could decrease the aerobic performance, increasing the risks of fatigue and immune disorders. The purpose of this study was to investigate the iron and zinc levels in blood of elite athletes of long-distance running compared with a population. In this study, 10 elite athletes (6 men and 4 women) and 40 healthy individuals (20 men and 20 women) of the same age, but not involved in physical activities have participated. The samples were collected in the morning before physical training. Blood iron and zinc levels were measured using Neutron Activation Analysis (NAA) technique. The male control group exhibited higher blood Fe concentrations than female, while blood Zn levels for both genders were similar. The comparison for male control and elite athletes groups exhibited increased in blood Fe levels, while for female groups (control and athletes) there was a decrease in Zn concentrations. Furthermore, the blood Fe and Zn concentrations in athletes are higher in men than in women. Our data can be useful to a well-planned nutritional plan that could enhance the performance of endurance athletes.

Keywords: Iron, Zinc, Blood, Exercise, Elite athletes, Long distance running.

1 INTRODUCTION

Minerals are essential for metabolic and physiological processes in the human body [1]. Among them, iron and zinc play an important role for physical performance and health of the athlete. The human body contains approximately 3–5 g of iron [1,2], found in hemoglobin, myoglobin, cytochromes, and various enzymes involved in numerous metabolic processes, such as delivery of oxygen to tissues, cellular respiration, and energy production [3]. Furthermore, iron also contributes to regulate body temperature after physical exercise, control the cardiovascular system, immune defenses, and brain function [2,3]. There is no physiological mechanism for excretion of iron. Over half of the daily iron requirements are obtained from the breakdown of circulating senile red cells, while the remainder comes from feed [4]. After being absorbed by the intestines, iron is transported through the circulatory system via transferrin and then stored, mostly in the liver, as ferritin or hemosiderin [5]. Due to recycling of iron by senile red cells, only some percentage of iron is lost by sweat, skin, intestines, genitourinary tract, and in women by menstrual blood losses (child-bearing age) [1,4]. However, iron losses in athletes can also result from a series of mechanisms during exercise, such as foot strike hemolysis, hematuria, gastrointestinal blood loss and sweating [6,7].

Athletes, particularly those involved in endurance sports, are commonly diagnosed with iron deficiency [8-10], which can, therefore, limit the capture, transport, and utilization of oxygen by tissues, thereby reducing the cardiorespiratory function and endurance performance [11-13]. Severe iron deficiency result in anemia, characterized by decreased hemoglobin concentration that leads to higher risk of fatigue, overtraining syndrome, and vulnerability to infection [6]. Some studies [8,14] emphasize that iron deficiency anemia is higher in athletes, especially women, when compared with healthy individuals not involved in intense physical activities. On the other hand, a recent investigation on nutritional habits conducted with elite athletes, demonstrate a major (~ 87%) use of supplements, including iron, as part of their weekly nutrition because they believed that supplements are related to performance enhancements [15].

Excess of iron, when not maintained within the cells, may be associated with oxidative stress, DNA damage and increased risk of infections. All of them contributing to performance decrement [2,3,8]. The iron excess is toxic. It can deposit in the form of aggregates in tissues as liver, heart, pancreas, and joints leading to irreversible organ dysfunction [3,8]. Furthermore, the iron overload plays an important role in the increased risk of infections, pathogenesis of atherosclerosis, carcinogenesis, and neurodegenerative disorders [3].

Zinc is a co-factor for numerous enzymes implicated in several physiological processes, including DNA reproduction, cellular respiration, endocrine system, and immune response [11,12,16]. The human body has approximately 2 to 3 g of zinc, distributed in muscles, bones, skin, and liver. Only 0.1% of body zinc is found in plasma [11,17]. The body maintains zinc homeostasis by changes in absorption and excretion [18]. Zinc is absorbed in the small intestine and is transported through the circulatory system via proteins such as albumin and α2-macroglobulin and it is excreted mainly through the feces, and some percentage through urine, sweat, peeling skin, hair growth, semen, and menstruation [19]. Intense physical activity can rapidly redistribute zinc in the body, decreasing plasma zinc concentration, which could increase the risk of infections [18]. Athletes of both genders have lower serum zinc concentrations in comparison with sedentary individuals [8,12]. Probably, dietary habits contribute to the low serum zinc concentration in athletes, which generally consume less zinc than recommended (8 mg/day for female and 11 mg/day for male) [12,18]. Moreover, intense and prolonged endurance exercise may increase zinc losses through sweat and urine [20]. Therefore, low zinc intake associated with increased excretion may be responsible for the low plasma zinc concentration observed in many athletes. Zinc deficiency result in anorexia, body weight loss, decreased antioxidant protection and cardiorespiratory function, fatigue with decreased endurance and risk of osteoporosis [7,18,20].

The aim of this study was to investigate iron and zinc levels in blood of elite long-distance running athletes compared with lower and upper values of a reference population of healthy individuals not involved with physical activities. This study was done in cooperation with Exercise Biochemistry Laboratory (*Laboratório de Bioquímica do Exercício*, Labex) at UNICAMP (*Universidade de Campinas*) that investigates the metabolic interrelationship that occur with training (aerobic with high impact).

2 MATERIALS AND METHODS

2.1 COLLECTION AND PREPARATION OF THE SAMPLES

Ten elite athletes, 6 men and 4 women, ages 23.2 ± 4 years, weight 67.9 ± 8.7 kg, height 174 ± 0.10 cm, body mass index (BMI) 22.6 ± 2.5 kg/m^2, training time 10 ± 5.7 years and training/week 138.7 ± 20.3 km/wk participated in the study. The control group was 40 healthy subjects, 20 men and 20 women, donors selected from *Paulista* Blood Bank at Sao Paulo - Brazil, with the same range of age and weight, but not involved in intense physical activities. This study was performed with the approval of the Ethical Committee on Human Research (CAAE: 0200.0.146.000-08). The blood samples were obtained in the morning (8:00 AM), before the physical training (at rest) and under standardized conditions: 2 mL of total venous blood was collected in vacuum tube without anticoagulants (BD, Vacutainer). Aliquots of 500 µL were transferred to a polyethylene capsule and lyophilized (Thermo Electron Corporation, model Micro Modulyo 115). The athletes are regular participants in national and international competitions and reported a balanced diet intake, without multivitamin/mineral supplements consumption. The blood collection for control group was done by the same procedure.

2.2 EXPERIMENTAL PROCEDURE

The blood samples were irradiated for 4 h in the IEA-R1 nuclear reactor (3.5-4 MW, pool type) at IPEN using neutron flux (~5.10^{12} n.cm^{-2}.s^{-1}). After irradiation, blood samples were gamma-counted for 6 h using a HPGe detector (ORTEC GEM-60195) connected to an ADCAM multichannel analyzer (ORTEC 919E) and a computer. The iron and zinc concentrations were obtained using ^{59}Fe ($T_{1/2}$ = 44 d, E_γ = 1099 keV) and ^{65}Zn ($T_{1/2}$ = 244 d, E_γ = 1116 keV), respectively. Data reduction was done using an in-house software.

3 RESULTS

Figure 1 shows a comparison between the individual concentrations of iron and zinc (mg/dL), respectively, in blood of elite runners, separated by gender with the indicative interval of control group, considering ±1SD (95%) and ±2SD (68%). Almost all male elite runners present higher Fe concentrations

and no differences in female elite runners when compared to confidence interval of control group. Related to Zn concentrations the female elite runners, values are within the confidence interval, but close to the lowest values. Table 1 presents the ratios of these blood ions between mean value of elite runners and control group (C_{ER}/C_{CG}) and elite runners by gender (C_{ER}^{m}/C_{ER}^{f}). According to this table, there is an increase of iron and zinc levels in blood of male athletes when compared to control, while for female athletes there is a decrease, mainly for zinc.

4 DISCUSSION

The comparison of each runner with an interval of data (CI 95%) obtained from a greater number of blood samples (control group), as shown here, permitted a more individualized analysis. Several studies reported alterations in iron status in athletes of several sports [6,8]; but results vary. While some have reported an increase in iron status in athletes compared with sedentary individuals, others relate prevalence of iron deficiency and anemia in athletes, particularly those involved in endurance sports or compared with sedentary individuals [5,8,10,23,24].

In the present study, we have observed highest blood levels of iron in male elite runners (above 95% IC) when compared with female elite runners and when compared with people who are not involved in intense physical activities (Figure 1). Considering that athletes did not report an intake of iron supplement, this increase suggests the need for nutritional reassessment since iron overload may increase the risk of infection. On the other hand, the iron levels in female athletes are within the reference intervals of the control group. Our data are not in agreement with other studies that showed lower iron concentration in female athletes [8,25].

This study also observed decrease in blood zinc concentration in female athletes when compared with the control group. Some studies reported effects of exercise on zinc metabolism and severe zinc deficiency can affect muscle function. Since zinc is needed for the integration of many physiological systems, such as immunity, reproduction, wound healing, skeletal development, and others, it was proposed that low zinc levels can result in a reduction in endurance capacity and performance [18,26]. However, it is important to point out that the blood zinc concentration of the female athletes was within the 95% IC, which does not characterize severe zinc deficiency. Probably, the strenuous exercise contributed to this mild deficiency by increased sweat loss and urinary excretion [12,20,25].

In addition, we have shown higher ($p < 0.05$) iron and zinc concentrations in blood of male runners compared with female runners (Table 1). These results confirm the importance of different recommended daily intake based on gender, as well as different limits for Fe and Zn in blood.

The results presented in this study lead to speculation about adopting different recommendations of specifics minerals for athletes, mainly for endurance athletes. Therefore, knowing the mineral status of

athletes involved in high-intensity activity can contribute to a well-balanced diet planning and improvement of physical performance [27].

5 CONCLUSION

The results presented in this study reinforce the importance of blood monitoring for the maintenance of performance of endurance athletes. To know the mineral status of athletes involved in high-intensity activity can contribute to a well-balanced diet planning and improvement of physical performance. NAA is a powerful, non-destructive, and selective analytical technique which requires minimum amount of sample and presents low detection limits for most elements, being a complimentary analysis of elements in whole blood, opposite to extra or intracellular determinations of Fe [28]. The data presented here can be considered for evaluating the performance of the athletes during the competitive period and for the proposition of clinical protocols based on the limits established in blood for iron and zinc.

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ANEXO

TABLE 1. The range of control group with a confidence interval of 95% for both genders, means of ratios of iron and zinc in blood between elite runners (ER) and control group (CG) (C_{ER}/C_{CG}) and elite runners by gender ($C_{ER}{}^{m}/C_{ER}{}^{f}$). The percentage estimate between groups were also included for comparison.

Element	[range], mg/dL $n^m = 68$ $n^f = 52$		$C_{ER}/C_{CG}{}^{(*)}$	%		$C_{ER}{}^{m}/C_{ER}{}^{f}$	%
Fe	[28.90 – 55.30] ^{m} [27.70 – 51.30] ^{f}		1.38 ^{m} 0.97 ^{f}	> 38.5 ^{m} < 2.9 ^{f}		1.52	> 51.8
Zn	[0.40 – 0.80] $^{m, f}$		1.10 ^{m} 0.88 ^{f}	> 10.0 ^{m} < 11.7 ^{f}		1.24	> 24.0

n: blood samples
C_{ER}: mean value of elite runners (ER) concentration in the blood
C_{CG}: mean value of control group (CG) concentration in the blood
(*) concentration for control group taken as 100%
m: male
f: female
> increase
< decrease

FIGURE 1- Blood concentration results of iron (Fe) and zinc (Zn) for elite runner by gender