Influence of Artistic Gymnastics on Iron Nutritional Status and Exercise-Induced Hemolysis in Female Athletes

Thaiz Mattos Sureira, Olga Silverio Amancio, and Josefina Aparecida Pellegrini Braga

This study evaluates the relationship between body iron losses and gains in artistic gymnastics female athletes. It shows that despite the low iron intake and exercise-induced hemolysis, iron deficiency or iron-deficiency anemia does not occur, but partial changes in the hematological profile do. The hypothesis that gymnasts’ nutritional behavior contributes to anemia, which may be aggravated by exercise-induced hemolysis, led to this cross-sectional study, conducted with 43 female artistic gymnasts 6–16 yr old. The control group was formed by 40 nontraining girls, paired by age. Hemogram, serum iron, ferritin, soluble transferrin receptor, haptoglobin, total and fractional bilirubin, Type I urine, and parasitologic and occult fecal blood tests were evaluated. The athletes presented mean hematimetric and serum iron values \( (p = .020) \) higher than those of the control group. The bilirubin result discarded any hemolytic alteration in both groups. The haptoglobin results were lower in the athlete group \( (p = .002) \), confirming the incidence of exercise-induced hemolysis. Both groups presented low iron intake. The results suggest that artistic gymnastics practice leads to exercise-induced hemolysis and partially changes the hematological profile, although not causing iron deficiency or iron-deficiency anemia, even in the presence of low iron intake.

**Keywords**: hemoglobin, haptoglobin, hemogram

Anemia affects 1.62 billion people in the world, which corresponds to 24.8% of the population. The highest prevalence is in preschool children, 47.4%, and the lowest prevalence is in men, 12.7%. The prevalence among pregnant women is 41.8% (World Health Organization, 2008). Therefore, this problem is mainly concentrated among women and children (Alleyne, Horne, & Miller, 2008). Anemia is normally associated with low iron intake, particularly from food sources of hemeiron (Kim, Kim, Kim, & Park, 2002). In athletes, anemia affects maximum oxygen consumption (reducing muscle oxidative metabolism), decreasing physical performance in short and intensive exercise. Reduced tissue iron leads to a reduction of myoglobin, reducing muscle aerobic capacity (Araújo, Moraes, Diniz, & Cosendey, 2004; Mercer & Densmore, 2005).

Adolescent female gymnasts present a higher risk of iron deficiency due to their greater requirements (Institute of Medicine [IOM], 2001), low energy consumption (related to weight control), inadequate iron intake and iron loss related to the practice of sport, prolonged training sessions, gastrointestinal losses, sporadic hematuria, intravascular hemolysis of erythrocytes (IOM, 2001), and also menstrual losses among others. Insufficient iron intake can lower performance and interfere in training, independent of the degree of deficiency (Reinke et al., 2012).

Another important concern is hemolysis in athletes, either through intravascular hemolysis caused by oxidative stress resulting from extremely exhausting activities or through impact hemolysis, which is characterized by erythrocyte rupture due to the high impact of the sports activity (Mohler, 1969). In regard to this question, studies have only been conducted in endurance and marathon runners and triathletes (Schumacher, Schmid, Grathwohl, Bültermann, & Berg, 2002; Shaskey & Green, 2000). Impact hemolysis already begins after 20 min of activity, and it can be more severe according to intensity and duration of training (Alayash, 2011).

Due to exposure, artistic gymnasts are considered at risk for iron deficiency, justifying this study, which had the objective of evaluating the influence of artistic gymnastics practice on iron nutritional status and exercise-induced hemolysis in female athletes.

**Methods**

A cross-sectional study was carried out involving 43 female artistic gymnastics, age 6–16 years, who trained at the Military Athletics Club, the São Paulo Olympic Training and Research Center, and the Guarulhos Olympic Center. This study was approved by the ethics committee at the Federal University of São Paulo, with previous written consent obtained from parents or guardians.
Participants
Inclusion criteria included regular participation in championships during the previous 12 months and training focused on national artistic gymnastics competitions. All athletes exercised regularly according to the program established by their coach, six times a week, 4–6 hr/day. Children aged 6–10 years trained 4 hr/day, including Sundays; other children trained 6 hr/day, excluding Sundays.

Exclusion criteria included irregular participation in competitions and use of iron supplements, which interfere in biochemical results (Zoller & Vogel, 2004).

The sample was determined by taking into consideration the mean reference value of haptoglobin, with a sampling error equal to 10% of the mean value and a confidence interval of 95%, resulting in 43 individuals. All the athletes, 50 in total, agreed to participate, but during the study 7 were transferred to other training centers.

The control group consisted of 40 girls, matched by age, from two public schools who did not practice any sports, except during curricular physical education.

The model adopted for sexual-maturation stages was that proposed by Marshall and Tanner (1969), employing the validated self-evaluation method (Matsudo & Matsudo, 1991). Female breast development and pubic hair growth were taken into consideration. The participants were questioned on the start of their menstrual cycle (yes/no) and menstrual-cycle intervals. A menstrual cycle of 28–30 days was considered regular.

Evaluation

Diet. A 7-consecutive-day food record was used (Karkek, 1987). Energy and iron intake were calculated using the validated and standardized Virtual Nutri software (Philippi, Szarfarc, & Latterza, 1996). The calculated dietary intake was compared with the Dietary Reference Intakes (IOM, 2001). For this comparison, age stratification was necessary.

Energy Expenditure. The validated questionnaire proposed by Bouchard et al. (1983) was used to estimate total daily energy expenditure. Subjects were accompanied during a 24-hr period (a weekday); data were recorded on resting and sleeping, as well as during other physical and sports activities.

Anthropometrics. Eight and height were recorded according to recommended techniques (Frisancho, 1981). Body-mass index (BMI) was calculated and the obtained values were compared with the data from the Centers for Disease Control and Prevention (2000). All individuals were classified as underweight, adequate weight, overweight, or obese (Himes & Dietz, 1994).

Percent Body Fat. Percent body fat was evaluated using the Slaughter equation (Slaughter et al., 1988), which is suitable for calculation at any maturational stage (Dezenberg, Nagy, Gower, Johnson, & Goran, 1999; Martin & Drinkwater, 1991). Triceps and subscapular skinfolds were recorded using a Sanny skinfold caliper (American Medical of Brazil Ltd.). All measurements were obtained from the dominant side in three nonconsecutive repetitions, for which we calculated the mean value (Frisancho, 1981).

Blood Chemistry. Blood samples were collected 2 weeks before the athletes’ participation in an artistic gymnastics competition and at least 12 hr after the end of training, between 8 and 9 a.m., after a minimum fasting time of 4 hr. However, fasting was not necessary for haptoglobin testing, so for this, blood samples were collected immediately after each athlete’s training session at the sporting facility. For the control group, the same collection method was applied—no fasting for haptoglobin analysis and a minimum 4-hr fasting period for the remaining determinations.

Hemoglobin was determined immediately after collection, using a hemoglobinometer previously calibrated with a standard 10-g/dL hemoglobin solution, using the cyanmethemoglobin method. Erythrogram analysis was conducted using the Cell-Dyn 3000 hematology analyzer (Abbott Diagnostics, IL, USA). Serum iron was determined with a Labtest Diagnostics Kit (Labteste Diagnóstica SA, Lagoa Santa, Brazil), which uses the Ferrozine method, with Control Serum N (human), previously reconstituted using 5 ml of Milli-Q deionized water. Serum ferritin concentrations were measured by a turbidimetric method using a Biosystem kit (Biosystems SA, Barcelona, Spain). Transferrin was determined by a turbidimetric method using a Biosystem kit (Biosystems SA). Soluble transferrin receptor was assayed using an R&D Systems kit (R&D Systems Inc., MN, USA) by means of ELISA technique. Haptoglobin was determined using a Belgian Apect diagnostic kit by means of a turbidimetric method, and controlled for accuracy with Protein Control Serum (Apect Diagnostik, Belgium). Total and fractional bilirubin were assayed using a Bioclin kit (Quiaba Ltd., Belo Horizonte, MG, Brazil), using a colorimetric method.

Urine and stool samples were collected by the athletes and delivered to the laboratory within 4 hr of collection, in special containers previously distributed by the laboratory, and collected on the same day as the blood sample. The girls were advised not to collect urine and feces during their menstruation. Urine tests conducted included chemical, physical, and sedimentoscopy measures. The Hoffman technique was used to detect parasites in the stool. Occult fecal blood was determined with a Hexagon OBTI Kit (Abbott Diagnostics, IL, USA), which adopts the immune chromatographic method.
Statistical Analysis

A comparison between groups was carried out using Student’s $t$ test and a Mann–Whitney test, according to the nature of the variables. Significance at $\alpha < .05$ was adopted.

Results

According to methodology, two age-homogeneous groups were established ($p = .925$). Regarding body weight, 15/19 athletes were below the 50th percentile versus the control group, in which 10/15 children were at or above the 50th percentile; the difference between groups was statistically significant ($p = .002$). For height, 14 athletes were below the 50th percentile compared with 14 controls who were equal to or above this percentile, with a significant difference between the groups ($p = .006$). BMI analysis, which does not discriminate muscle and adipose tissue, was lower in athletes than in controls ($p = .009$). In addition, percent body fat was different between the groups ($p < .001$; Table 1).

Regarding maturity stage, 48.8% of the athletes and 12.5% of the controls were at B1P1 (data not shown). The presence of menarche was observed in 12/43 athletes (27.9%) and in 20/40 controls (50.0%; Table 5). When comparing the two groups, energy intake by the athletes was lower ($p = .002$) and energy expenditure was greater ($p < .001$). No significant intake differences were observed between the control and athlete groups for iron ($p = .212$). In both groups mean iron intake was below recommended values, especially among the athletes age 6–8 years and 14–16 years (Table 2).

In the athlete group, in relation to control, significantly higher values were observed for mean hemoglobin ($p = .001$), hematocrit, mean corpuscular volume ($p < .001$), and serum iron ($p = .020$). A significantly lower value was observed for ferritin ($p = .038$) and a tendency for a higher mean value for transferrin ($p = .050$; Table 3).

Between the two groups, the mean value of haptoglobin was lower in the athlete group ($p = .002$); we observed that 11 of them had values below the lower reference limit. For mean bilirubin levels (total, direct, and indirect) there was no significant difference between the groups, $p = .205$, $p = .271$, and $p = .072$, respectively (Table 4). Occult fecal blood was observed in 2 athletes. Blood in urine and erythrocyte in urine above 10,000/ml were observed in 2 athletes (4.6%; Table 5).

Discussion

Children are beginning the practice of sports at an increasingly early age. The pursuit of physical and athletic perfection, the desire for record breaking, and political interests have taken sport to a level that requires effort and involvement from participants at an early age; this is especially evident in artistic gymnastics (Soares, 1997). A study conducted with athletes in rhythmic and artistic gymnastics reported a mean age of 11 ± 2.5 years, with initial training starting on average at age 6 (Viebig, Polpo, & Correa, 2006), values similar to those found in this study.

The impact of physical activity on growth is very controversial, since regular or even moderate practice along with environmental variables influences the genetically determined growth pattern (Viebig et al., 2006). Weight, height, and consequently BMI were significantly lower in the group of athletes than in the control group, especially in the age group over 10 years, when training sessions become more intense and frequent as technical demands increase. Similar results were reported by Ribeiro and

Table 1  Subject Characteristics, $M \pm SD$ (Range)

<table>
<thead>
<tr>
<th></th>
<th>Athletes, $n = 43$</th>
<th>Control, $n = 40$</th>
<th>$p$</th>
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</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>11.0 ± 2.4 (7.8–16.8)</td>
<td>11.0 ± 2.7 (7.1–15.3)</td>
<td>.925&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>30.2 ± 8.3 (19.6–55.0)</td>
<td>32.2 ± 11.6 (21.9–56.7)</td>
<td>&lt;.001&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.3 ± 0.1 (1.2–1.6)</td>
<td>1.4 ± 0.1 (1.2–1.6)</td>
<td>&lt;.001&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>BMI, kg/m&lt;sup&gt;2&lt;/sup&gt;</td>
<td>15.9 (13.5–22.3)</td>
<td>18.0 (13.1–28.7)</td>
<td>.009&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>11.1 (6.9–16.4)</td>
<td>19.8 (11.0–29.6)</td>
<td>&lt;.001&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
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</table>

<sup>Note</sup>. BMI = body-mass index.
<sup>a</sup> Descriptive level of Student’s $t$ test. <sup>b</sup> Median, descriptive level of the Mann–Whitney test.

Table 2  Mean Energy and Iron Intake and Energy Expenditure, $M \pm SD$ (Range)

<table>
<thead>
<tr>
<th></th>
<th>Athletes, $n = 43$</th>
<th>Control, $n = 40$</th>
<th>$p$</th>
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<tbody>
<tr>
<td>Energy intake, kJ/day</td>
<td>6,065.5 ± 2,028.8 (2,457.2–10,375.4)</td>
<td>7,711.1 ± 1,296.6 (4,838.7–9,072.5)</td>
<td>.002</td>
</tr>
<tr>
<td>Iron intake, mg/day</td>
<td>8.3 ± 4.3 (1.4–17.4)</td>
<td>9.6 ± 2.4 (5.4–13.0)</td>
<td>.212</td>
</tr>
<tr>
<td>Energy expenditure, kJ/day</td>
<td>7,857.12 ± 1,797.89 (5,608.82–12,287.58)</td>
<td>5,530.96 ± 508.18 (4,246.70–6,269.37)</td>
<td>&lt;.001</td>
</tr>
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</table>

<sup>Note</sup>. Reference values from the Institute of Medicine for energy intake: 3–8 years = 7,288.5, 9–13 years = 8,665.0, 14–18 years = 9,907.7 kJ. For iron intake: 4–8 years = 10, 9–13 years = 8, 14–18 years = 15 mg.
Table 3  Mean and Median Hematological Values, M ± SD (Range)

<table>
<thead>
<tr>
<th></th>
<th>Athletes, n = 43</th>
<th>Control, n = 40</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin, g/L [120–150]</td>
<td>144.5 ± 7.9 (131–160)</td>
<td>137.7 ± 10.3 (100–158)</td>
<td>.001a</td>
</tr>
<tr>
<td>Hematocrit, L/L [0.36–0.46]</td>
<td>0.439 ± 0.021 (0.387–0.480)</td>
<td>0.415 ± 0.026 (0.358–0.471)</td>
<td>&lt;.001a</td>
</tr>
<tr>
<td>Mean corpuscular volume, fl [80–100]</td>
<td>88.0 ± 4.1 (77.8–95.6)</td>
<td>84.2 ± 5.4 (69.5–93.2)</td>
<td>&lt;.001a</td>
</tr>
<tr>
<td>MCH, pg [27–32]</td>
<td>29.2 ± 1.6 (26.0–36.0)</td>
<td>27.6 ± 3.5 (23.2–31.0)</td>
<td>.008a</td>
</tr>
<tr>
<td>MCH concentration, g/L [305–320]</td>
<td>321.0 (300.7–330.7)</td>
<td>323.0 (307.4–368.6)</td>
<td>.074b</td>
</tr>
<tr>
<td>Serum iron, μmol/L [7.1–28.6]</td>
<td>18.7 ± 5.6 (8.9–37.0)</td>
<td>16.0 ± 5.3 (5.7–30.9)</td>
<td>.020a</td>
</tr>
<tr>
<td>Serum ferritin, pmol/L [22.4–651.6]</td>
<td>88.3 ± 31.9 (41.1–169.4)</td>
<td>112.5 ± 68.0 (13.7–328.5)</td>
<td>.038a</td>
</tr>
<tr>
<td>Transferrin, g/L [2.1–3.6]</td>
<td>2.61 ± 0.24 (2.26–3.13)</td>
<td>2.50 ±0.27 (2.02–3.16)</td>
<td>.050a</td>
</tr>
<tr>
<td>Serum iron, μmol/L [1.6–20]</td>
<td>4.2 ± 3.3a</td>
<td>7.0 ± 4.6b</td>
<td>.002</td>
</tr>
<tr>
<td>Haptoglobin, μmol/L [0.5–20.3]</td>
<td>(0.5–14.4)</td>
<td>(0.5–20.3)</td>
<td></td>
</tr>
<tr>
<td>Direct bilirubin, μmol/L [1.7–5.1]</td>
<td>3.4 ± 1.7</td>
<td>3.4 ± 1.7</td>
<td>.271</td>
</tr>
<tr>
<td>Indirect bilirubin, μmol/L [3.4–18.8]</td>
<td>8.5 ± 1.7</td>
<td>6.8 ± 3.4</td>
<td>.072</td>
</tr>
</tbody>
</table>

Note. MCH = mean corpuscular hemoglobin. Values in brackets ([]) are reference values from the Institute of Medicine.

aDescriptive level of Student’s t test. bDescriptive level of the Mann–Whitney test.

Table 4  Total Haptoglobin and Direct and Indirect Bilirubin, M ± SD (Range)

<table>
<thead>
<tr>
<th></th>
<th>Athletes, n = 43</th>
<th>Control, n = 40</th>
<th>p</th>
</tr>
</thead>
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<tr>
<td>Haptoglobin, μmol/L [1.6–20]</td>
<td>4.2 ± 3.3</td>
<td>7.0 ± 4.6</td>
<td>.002</td>
</tr>
<tr>
<td>Direct bilirubin, μmol/L [1.7–5.1]</td>
<td>3.4 ± 1.7</td>
<td>3.4 ± 1.7</td>
<td>.271</td>
</tr>
<tr>
<td>Indirect bilirubin, μmol/L [3.4–18.8]</td>
<td>8.5 ± 1.7</td>
<td>6.8 ± 3.4</td>
<td>.072</td>
</tr>
</tbody>
</table>

Note. Values in brackets ([]) are reference values from the Institute of Medicine.

aAfter training. bFasting.

Table 5  Distribution of Athletes and Control Individuals With Positive Results in the Urine and Feces, n (%)

<table>
<thead>
<tr>
<th></th>
<th>Athletes, n = 43</th>
<th>Control, n = 40</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood in urine</td>
<td>2 (4.6%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Erythrocytes in urine above 10,000/ml</td>
<td>2 (4.6%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Parasites in feces</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Occult fecal blood</td>
<td>2 (4.6%)</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Soares (2002), in a study with 46 gymnasts compared with an age-matched control group. We must consider the selective nature of the sport; for instance, short children and those with lower body weight have greater athletic possibilities. For best athletic performance, greater height and body weight have been considered a disadvantage, since artistic gymnastics requires agility, lightness, and strength (Juzwiak, Paschoal, & Lopez, 2000).

The lower percentage of body fat in athletes than in controls confirms the results observed in several studies (Deutz, Benardot, Martin, & Cody, 2000; Zanker, Osborne, Cooke, Oldroyd, & Truscott, 2004). Borgen and Oseid (2002) assert that for gymnasts, maintaining a low percentage of body fat is imperative for success, although Lohman (1992) highlights that body fat below 5% for men and 12% for women can be harmful to an athlete’s health. Other studies (Claessens & Lefevre, 1998; Deutz et al., 2000) also report lower percent body fat in groups of athletes. Bale, Doust, and Dawson (1996) report a similar percentage of body fat, 10.8%, to that found in this study, 11.1%.

Studies report that the practice of gymnastics causes delayed pubertal development for many reasons that involve the intensity of sport activities, genetics, body composition, inadequate energy intake, metabolic alterations, and stress (Rogol, Clark, & Roemmich, 2000, Weimann, Witzel, Shwidegall, & Böhles, 2000). Pubertal development is considered delayed when a teenager is older than 13 years and in B1P1 (Brazil Ministry of Health, 1993). Thus, different from literature, in this study only one athlete, 15.6 years of age, was in B1P1. The other athletes in B1P1 were 7.8–10.8 years old, consistent with this stage of maturity. For activities such as ballet and gymnastics, which require intense daily training coupled with a thin body, there is a higher incidence of delayed menarche (Theintz, Howald, Weiss, & Sizonenko, 1993; Weimann et al., 2000). This was not observed in the current study. Twelve athletes and 20 controls were postmenarche, with mean ages, respectively, of 11.4 ± 3.7 and 13.0 ± 2.2 years, with no statistical difference between them (p = .152; data not shown). These results suggest that the hypothalamic-pituitary axis is intact, maintaining adequate pubertal development; for instance, the organism probably uses the homeostatic mechanism, not allowing low energy intake and high energy expenditure to interfere with maturity stage.

Dietary assessment aims to identify food intake and evaluate it in relation to nutritional recommendations. Several methodologies in the form of dietary questionnaires can be used to obtain data on food intake, but there is still no method considered the gold standard to ensure that the information provided accurately reflects actual intake (Bonomo, 2000; Zulkifli & Yu, 1992). The ideal method would be to compare data on direct intake, but in
practice this is quite difficult; it would only be possible for 1–2 days, in confined spaces, without free choice of food. In addition, the presence of an observer may lead to modification of habitual intake, and one must also consider available resources, time, and cost (Zulkifli & Yu, 1992).

The number of days for the food record depends on the purpose of the investigation, but most commonly a 3-day record is used, consisting of 2 nonconsecutive weekdays and 1 weekend day (Bonomo, 2000; Langscheid, 1996). In this study, a 7-consecutive-day record was used, aiming to avoid possible errors. As an advantage, this method did not depend on memory, providing more accurate information on dietary intake (food type and quantity) and mealtimes (Bonomo, 2000; Kanimura, Baxmann, & Sampaio, 2002). As a disadvantage, the method can interfere with normal eating patterns, leading generally to underreporting. However, it has been shown that overweight and obese individuals underreport more than their lean counterparts (Dwyer, Picciano, & Raiten, 2003; Rennie, Coward, & Jebb, 2007).

In relation to energy intake, the results obtained in this study are in accordance with several studies on gymnasts that found significantly lower energy consumption than in controls (Cupisti, Alessandro, Castrogiovanni, Barale, & Morelli, 2000; Deutz et al., 2000). Few studies in literature refer to the energy requirements of adolescent athletes. It has been stated that young athletes have a high energy requirement, not only for their daily activities and physical training but also for their growth and development (Nova, Montero, López-Varela, & Ascensión, 2001).

Doubly labeled water is the gold-standard measure of energy expenditure (Schoeller, 2002), but it is very expensive to use in research. In this study, the method used for measuring energy expenditure analyzes the activities performed every 15 min throughout the day. It is important to mention that for the classification of sports activities, it was necessary to attend a week of training, since there is a weekly microcycle to be followed by the gymnastics coach. Although different methodology was used to evaluate energy expenditure, other studies (Cupisti et al., 2000; Juzwiak et al., 2000) also found greater energy expenditure than energy intake in athletes. It was suggested that rhythmic gymnastics athletes developed chronic adaptation to this energy restriction (Weimann et al., 2000). Nevertheless, it is worth remembering that a balanced energy consumption is essential for muscle-tissue maintenance, the proper functioning of the immunological and reproductive systems, and optimization of athletic performance (Cupisti et al., 2000).

Whenever there is a restriction in energy consumption, there is also an expected inadequacy in the intake of micronutrients. The low iron intake by both groups without a significant difference between them observed in this study is also reported in the literature (Alleyne et al., 2008). Cupisti et al. (2000) also found low iron intake, but their athlete group showed a higher consumption than their control. It was observed that the athletes’ iron intake was preponderantly of vegetable origin. Such a fact, associated with low energy intake, could well explain the low iron intake. In the control group, intake of iron of animal origin was higher than that of vegetable origin but still insufficient in quantity. This eating standard could contribute to the development of iron deficiency that could impair physical performance (Fallon, 2008) when taking into consideration the role of iron in building hemoglobin, myoglobin, lactic dehydrogenase, cytochromes, and some mitochondrial enzymes (Reinke et al., 2012).

The significantly higher hemoglobin level in the athlete group could be the result of adaptation to the practice of artistic gymnastics, which stimulates erythrocytosis. This adaptation effect ceases on average 3–5 days after the end of training (Fallon, 2008).

Significantly higher in the athlete group, mean corpuscular volume denotes the mean size of the cells (Fallon, 2008). One of the alterations caused by physical activity is an increase in mean corpuscular volume, which could originate from the physiological adaptation to the greater need for tissue oxygenation (Martinovic, Kotur-Stevuljevic, Dopsaj, Stefanovic, & Kasum, 2010). Nevertheless, 2/43 of the athletes and 9/40 of the controls presented results below the reference values, indicating microcytosis (Schumacher et al., 2002). The 2 athletes also presented low mean corpuscular hemoglobin, a fact also observed in 5 of the 9 controls, characterizing hypochromia.

Serum iron is used to complement the analysis of iron nutritional status. In the athlete group there were 2/43 results above the reference value. A possible explanation could be the occurrence of intravascular hemolysis by impact and/or oxidative stress (Ji & Meng, 2011), which can only point to an elevated training load (Alayash, 2011).

Normally, in healthy individuals a small quantity of ferritin circulates in the blood, whose plasmatic or serum concentration is proportional to the size of the iron store (Kim et al., 2002). Only 1 control presented a low ferritin value, indicative of iron deficiency. In the athlete group the ferritin results warranted attention, as they were lower than in the control group, when they should have been higher, since exercise can lead to increased ferritin. Thus, the mean serum ferritin within the reference limits could well be masking possible iron deficiency, since increased synthesis of ferritin due to erythrocyte hypercatabolism, caused by inflammatory processes or intravascular hemolysis, is reported in athletes (Graversen, Madsen, & Moestrup, 2001).

Transferrin, an iron-transport protein and a negative acute-phase protein whose concentration decreases in response to any damage, whether inflammatory, infectious, or traumatic (Mercer & Densmore, 2005), increases under conditions of iron deficiency (Kim et al., 2002). There was a tendency for a higher value in the athlete group, which probably would have been confirmed with a larger sample size, possibly indicating iron deficiency. In the control group, 3 girls showed low transferrin values; 1/3 already showed mean corpuscular volume and mean hypochromia.
corpuscular hemoglobin values below the reference values, characterizing possible iron deficiency.

The external surface of all cells expresses transferrin receptors proportionally to the need for intracellular iron. Therefore, the greater the degree of iron deficiency, the greater the expression of receptors on the surface of the cells. The receptor level is not altered by infectious or inflammatory processes (Fallon, 2008). As there was no statistical difference for this variable between the two groups ($p = .280$), this result, aligned with others on the biochemistry of iron, indicates that the athlete group did not present iron deficiency.

Haptoglobin constitutes a protein group that demonstrates linkage properties to hemoglobin, forming a stable complex, that is afterward metabolized by the liver and impedes the renal loss of protein and iron (Mohler, 1969). Levels of haptoglobin decrease in hemolysis and hepatic insufficiency, whereas with infections, malignant neoplasias, and cardiovascular illnesses they increase (Alayash, 2011). Lower values of haptoglobin after races were reported in male and female marathon runners by Davidson, Robertson, Galea, and Maughan (1987). In that study, there was a significantly lower value ($p = .002$) of haptoglobin in the athlete group, with a decrease in serum haptoglobin observed 10 min after an exercise session, despite the fact that the same result was not encountered 24 or 48 hr later (Alayash, 2011; Graversen et al., 2001).

Since haptoglobin is an acute-phase protein, with an increase in hepatic production as compensation, blood collection for this analysis occurred immediately after the completion of each athlete’s training session, to observe possible alterations in response to the practice of sport. There are no studies related to the practice of artistic gymnastics and the occurrence of hemolysis, which made a comparison of our results difficult. The great majority of studies of this nature were carried out on male athletes who practiced endurance sports.

Bilirubin is the principal product of protoporphyrin IX degradation, which forms hemoglobin, myoglobin, the cytochromes, and other hemoproteins. The mean values of total, direct, and indirect bilirubin were within the normal range in both groups, eliminating the possibility of hemolytic illnesses, which would increase bilirubin (Ji & Meng, 2011). Nevertheless, blood samples were collected during fasting and at least 12 hr after the end of training, with the objective of discarding hemolytic abnormalities that could have interfered in the interpretation of the haptoglobin values for the determination of hemolysis (Graversen et al., 2001). Since mean bilirubin values were within the reference range, discarding hepatic alterations, the lower mean haptoglobin value in relation to the control group could indicate exercise-induced hemolysis.

Daily excretion of basal iron is limited 0.9–1.02 mg/day in women who are not menstruating. Most iron is excreted in the feces, although losses also occur in the urine (0.06 mg/day), in the gastrointestinal tract (0.6 mg/day), and through the skin (0.2–0.3 mg/day). These values can decrease or increase due to a deficiency or excess of iron, respectively (Alayne et al., 2008). In an attempt to monitor the causes of iron loss, in the current study some laboratory tests were performed. Positive cases of blood in the urine, erythrocytes in the urine, and occult fecal blood in the athletes were most probably caused by vigorous physical activity, which can result in microlesions, in both the urinary and gastrointestinal tracts (Martinović et al., 2010). Stool examination excluded the possibility of intestinal parasites as an etiologic factor of blood loss, since results were negative for both groups, as well as those for occult fecal blood in most athletes, 41/43.

Two limitations need to be acknowledged and addressed regarding the current study. It was impossible to use a gold-standard method to assess energy expenditure, and the sample size was small, determined by inclusion criteria. A larger sample would probably more accurately present the interrelationship of high-performance physical activity in female adolescents with pubertal development and iron metabolism.

**Conclusions**

The practice of artistic gymnastics caused exercise-induced hemolysis and altered the hematological profile, in relation to the control group. However, this was without iron deficiency or iron-deficiency anemia, even in the presence of low iron intake.

**References**


