Effects of a Fixed Dietary Intake on Changes in Red Blood Cell Delta-Aminolevulinate Dehydratase Activity and Hemolysis

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This study was to assess the effect of a fixed dietary intake on biomarkers of red blood cell (RBC) biosynthesis and degradation. Over a two-year period, eight collegiate rhythmic gymnasts participated in this study. During the first year, they ate self-selected diets. During the second year, a fixed dietary intake involving consumption of common Japanese foods containing 15 mg iron and 1500 kcal energy was maintained for 4 wk at the beginning of the program. Fixed dietary intakes resulted in significantly increased intakes of protein, minerals and vitamins, and significantly decreased fat intake, but total energy and carbohydrate intakes were unchanged. Mean values of RBC, Hb, Ht, or TIBC were not affected by the intervention. A fixed dietary intervention appeared to enhance RBC turnover by increasing the capacity for erythrocyte biosynthesis and degradation, although the prevalence of iron-deficiency anemia remained unchanged.

Key Words: period of weight loss, iron deficiency anemia, haptoglobin, erythrocyte turnover

Competition in rhythmic gymnastics includes five hand implements recognized by the International Gymnastics Federation: ball, hoop, clubs, ribbon, and rope. The athletes must perform physically demanding routines of 1 to 1.5 min duration with each of the five implements. For rhythmic gymnasts to maximize performance, they strive to achieve an optimum sport-specific body size, low body mass and low body fat level (7, 11, 12, 17, 25). Leanness is particularly valued because the gymnast’s success is a function of appearance as well as technical performance.
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The strategies rhythmic gymnasts use to achieve optimum weight and body composition may, however, predisposes them to a variety of medical complications that include menstrual dysfunction, eating disorders, growth retardation, and nutritional deficiencies such as iron-deficiency anemia (4, 5, 11, 17, 25).

Iron deficiency and anemia are recognized as the most frequently developed nutritionally preventable health problems for female athletes, especially runners and gymnasts (5, 23). Iron depletion is related to quantities of food and inherent densities of iron in the diet, as well as a decision to exclude meat from a diet (2). Dietary supplementation with minerals such as iron and zinc could be needed (6). However, it is still unknown how common iron-deficiency anemia is among Japanese collegiate female rhythmic gymnasts and whether dietary iron would be of value in this population. Furthermore, it is not well understood how the metabolism and degradation of red blood cells (RBC) in well-trained gymnasts correspond with sessions of training.

In this study, we examined the prevalence of iron-deficiency anemia during two 8-week periods in female Japanese rhythmic gymnasts. In addition, we assessed what changes occur in the blood parameters of gymnasts over time when kept on the same energy and iron intakes but with higher intakes of protein and lower intakes of fat. Direct comparative measurements of key factors affecting the biosynthesis and degradation of RBC during training are not readily apparent. We then estimated delta-aminolevulinate dehydratase (δ-ALAD) activity (16) and haptoglobin (hp) concentration as the biosynthesis and degradation indices of RBC.

Materials and Methods

Subjects

Over a two-year period, eight collegiate rhythmic gymnasts participated in this study, which was approved by the Ethical Committee of the Japan Women’s College of Physical Education both in 2001 (No. 2001-1) and in 2002 (No. 2002-3). All subjects volunteered and were informed of all potential risks and procedures before giving their written informed consent to participate in this study. All had normal blood pressure and normal menstrual cycles. To create appropriate testing conditions, all participants were instructed to refrain from strenuous activity for 24 hours and were asked to fast overnight before the day of blood sampling.

Experimental Protocols

Each data observation took place at fixed intervals, as shown in Figure 1. Subjects were assessed at baseline (Pre-1), 4 weeks post-baseline (Pre-2), and 8 weeks post-baseline (Pre-3) during the pre-intervention year. One year later, intervention was initiated with observations at baseline (Int-1) and 4 weeks post-baseline (Int-2). Four weeks following the end of the dietary intervention, a post-intervention assessment took place (Post-1). Meal intervention was carried out as follows: a dietitian created 15 menus providing approximately 15 mg iron and approximately 1500 kcal energy intake per day. The athletes consumed these diets for 30 days. All experimental diets consisted of customary Japanese foods.
All data regarding the blood samples, body weight, and dietary intakes were collected at each observation period from 1 to 2 weeks after the menstruation (Figure 1). On the morning of the day of blood sampling, subjects were asked to arrive at the laboratory between 7 and 8 AM following an overnight fast. On arrival, subjects completed the dietary assessments and the health claims. Height, body mass and body fat mass were measured (Model TBF 210, TANITA, Co., Tokyo, Japan) prior to the blood sampling.

**Dietary Survey**

Dietary data for continuous 3 days before the blood sampling day were obtained from each subject by a registered dietitian throughout the experimental period. The intakes of individual nutrients were calculated based on the Standard Tables of Food Composition for the Japanese (5th edition; 27).

**Blood Analysis**

Extreme care was taken during collection of blood to avoid the possibility of hemolysis; a 21-gauge needle was used together with minimal stasis. Blood was expelled down each of three separate collection tubes. Two milliliters of blood were collected in an EDTA-2K tube to assess hematological parameters. Five milliliters of blood were transferred into a serum tube and allowed to clot at room temperature. After centrifugation at 3,000 rpm, 4° C for 10 min, serum was collected. Blood for assessing plasma, osmotic fragility, and the activity of δ-ALAD were collected in a 2-ml heparin tube.
Hematological parameters, including RBC counts, hemoglobin (Hb) concentration, and hematocrit levels (Ht) were measured using an automated system (Horiba LC-360, Horiba Seisakusho KK, Japan). Concentrations of serum iron, ferritin, total iron binding capacity (TIBC), unsaturated iron binding capacity (UIBC), serum erythropoietin (EPO) and hp were measured at the Medical Lab Co. Ltd. (Tokyo, Japan) as follows: iron by atomic absorption spectrophotometry (SHIMAZU AA 6400-F, Shimazu Co. Ltd., Japan), ferritin and EPO by enzyme-linked fluorescent assay, TIBC by the Nitroso - PSAP method, and hp by immune - nephelometry using a Nephelometry Analyzer II (Behring, Germany).

The osmotic fragility of RBC was run in duplicate and measured using the heparinized RBC fraction according to Beutler (3). δ-ALAD activity was run in duplicate, and assayed using the heparinized RBC fraction as described previously (13, 15, 16). Two ml of heparinized blood was centrifuged at 3,000 rpm for 10 min at 5 ºC and the resultant supernatant was discarded. The precipitated RBC were washed three times with 2 ml of cold phosphate buffered saline (pH 7.4), filled with the same buffer and stored at –80 ºC until assay. The resultant RBC was incubated at 37 ºC for 30 min with δ-aminolevulinic acid. The reaction was stopped with a TCA-HgCl₂ mixture and the color was developed using modified Ehrlich reagent. Enzyme activity was calculated using the molar absorption coefficient (6.1 x 10⁴) of the final Ehrlich color salt at 553 nm, and the results were expressed as porphobilinogen (nmol / mg Hb / hr).

Statistics

All values are presented as means ± standard deviations (SD). EXCEL version XP (Microsoft Corp., Japan) was used for data handling and graph generation. Data analysis by SPSS statistical software (version 11.5, SPSS Inc, JAPAN) was performed in three steps. Two-way (intervention x time) repeated measures analyses of variance (ANOVAs) were used to analyze dietary data, body iron status, hp, NaCl concentrations for osmotic fragility and δ-ALAD activity by means of Mauchly’s W test. Post hoc pairwise comparisons were by means of Friedman’s nonparametric ANOVA. After the finding of Friedman’s ANOVA was corroborated, the variables were submitted to a Wilcoxon’s signed-rank test. Statistical significance was accepted at P < 0.05.

Results

Physical Characteristics and Nutritional Intakes

Characteristics of the participants are shown in Table 1. The mean (± SD) age of the participants before the study was 18.6 (0.5) years. The mean weight, BMI and body fat percent did not change from Pre-1 to Pre-3 in the pre-intervention years, but those levels of the intervention year were significantly lower in Int-2 than those in Int-1 (both, P < 0.05).

Both intakes of energy and carbohydrate were unchanged throughout the two-year experimental period, respectively (Table 1). In the pre-intervention year, intakes of most of the nutrients did not change over the three months. However,
Table 1  Subject’s Characteristics and Nutritional Intakes

<table>
<thead>
<tr>
<th>Characteristics1</th>
<th>Pre-1</th>
<th>Pre-2</th>
<th>Pre-3</th>
<th>Int-1</th>
<th>Int-2</th>
<th>Post-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>51.1 ± 5.1</td>
<td>51.0 ± 5.5</td>
<td>51.8 ± 5.4</td>
<td>51.2 ± 3.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>48.9 ± 2.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>50.1 ± 3.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>BMI (kg/m&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>19.7 ± 1.5</td>
<td>19.7 ± 1.6</td>
<td>20.0 ± 1.6</td>
<td>19.9 ± 0.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.1 ± 0.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>19.6 ± 0.6</td>
</tr>
<tr>
<td>Body Fat (%)</td>
<td>21.2 ± 3.8</td>
<td>20.8 ± 4.2</td>
<td>20.7 ± 3.5</td>
<td>21.0 ± 4.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20.2 ± 3.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>20.5 ± 3.3</td>
</tr>
<tr>
<td>Dietary intakes*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy (kcal)</td>
<td>1596 (339)</td>
<td>1562 (394)</td>
<td>1590 (430)</td>
<td>1555 (330)</td>
<td>1465 (35)</td>
<td>1521 (398)</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>60.2 (16.4)</td>
<td>56.2 (19.4)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>57.2 (22.4)</td>
<td>65.4 (20.9)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27.6 (5.7)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>54.6 (17.2)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>49.2 (12.6)</td>
<td>49.6 (12.4)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>50.8 (13.4)</td>
<td>57.9 (15.2)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>72.4 (6.7)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>50.8 (20.8)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>212 (48)</td>
<td>211 (48)</td>
<td>214 (45)</td>
<td>230 (90)</td>
<td>233 (18)</td>
<td>200 (48)</td>
</tr>
<tr>
<td>Iron (mg)</td>
<td>7.3 (2.2)</td>
<td>6.4 (2.5)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.3 (1.8)</td>
<td>6.1 (1.5)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.2 (1.8)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.7 (2.6)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Vitamin C (mg)</td>
<td>84 (39)</td>
<td>77 (27)</td>
<td>78 (28)</td>
<td>73 (34)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>212 (45)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>55 (30)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup>All values are expressed as means ± SD. N=8. Abbreviation: BMI; Body Mass Index.
<sup>*</sup>All values are expressed as means (SD). Means with the different subscript letters a and b are significantly different from one another (P < 0.05) by Wilcoxon’s signed-rank test.
intakes of protein, calcium, magnesium, phosphate, iron, zinc, copper, vitamins A, E, K, B₁, B₂, B₆, B₁₂, and C were significantly increased in Int-2, and intakes of fat were significantly decreased in Int-2 as compared with Pre-2, Int-1, and/or Post-1, respectively. The coefficient of variation in nutritional intakes throughout the study periods was smallest for energy (8.6%) and largest for Vitamin A (65.5%) in Int-2, respectively. The athletes were able to meet the energy (1500kcal) and iron (15 mg) targets during the intervention period.

**Body Iron Status**

**Hematological Changes.** There were no significant differences observed in Hb, Ht, and TIBC levels among Pre-1, Pre-2, and Pre-3 (Table 2). MCV levels were significantly higher in Pre-1 and Pre-2 than in Pre-3. MCH and MCHC levels were significantly lower in Pre-2 and Pre-3 than in Pre-1. On the other hand, mean values of RBC, Ht, TIBC, and UIBC were significantly lower in Int-2 than in Int-1, respectively. Mean values of MCV, MCH, and MCHC were lower in Post-1 than in Int-2, respectively.

**Prevalence of Iron Depletion and Iron-Deficiency Anemia.** There was no significant effect of this fixed dietary intervention on the incidence of the iron-deficiency anemia over the experimental period (Figure 2). Iron depletion, defined by a ferritin level below 20 μg/L, was found in five, six, and three of the eight athletes in Pre-1, Pre-2, and Pre-3 of the pre-intervention year, and five, four, and five in Int-1, Int-2 and Post-1 of the intervention year, respectively. Iron-deficiency anemia, defined by the presence of anemia (Hb levels below 12 g/dL), ferritin levels below 12 μg/L, and transferrin saturation ratio below 16%, was found in one or two athletes in every experimental period.

Serum iron concentrations and transferrin saturation ratios were unchanged throughout these experimental conditions (Figure 3). Serum ferritin concentrations were significantly lower in Pre-2 than in Pre-1 (P < 0.05), but significantly increased in Int-2 compared with those in Int-1, Post-1, and Pre-2 (both P < 0.05), respectively.

**δ- ALAD Activity, EPO, hp and Osmotic Fragility.** The activity of δ-ALAD was significantly higher in Pre-3 than in Pre-1 (P < 0.05; Figure 4). The activity significantly increased in Int-2 as compared to Int-1 (P < 0.001), Post-1 (P < 0.01), or Pre-2 (P < 0.05), respectively.

The EPO concentrations were significantly higher in Pre-2 and Pre-3 than in Pre-1 (both P < 0.05), respectively, but those levels were unchanged among Int-1, Int-2 and Post-1. The EPO concentrations tended to be higher in the intervention year than in the pre-intervention year throughout the experimental periods.

There were no significant differences observed in the serum hp concentrations between Pre-1, Pre-2 and Pre-3, respectively (Figure 5). The hp concentrations were the lowest in Int-2 and significantly lower in Int-2 than those in Post-1 (P < 0.05), but no significant differences were observed between Pre-2 and Int-2.

The main effect of the intervention (P < 0.01), the main effect of the time (P < 0.01), and the intervention x time interaction effect (P < 0.05) on the osmotic fragility of RBC were all significant. Mean NaCl concentrations as indices of
Table 2  Hematological Changes

<table>
<thead>
<tr>
<th>Variables</th>
<th>Preintervention Year</th>
<th></th>
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<th>Intervention Year</th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Pre-1</td>
<td>Pre-2</td>
<td>Pre-3</td>
<td>Int-1</td>
<td>Int-2</td>
<td>Post-1</td>
<td></td>
</tr>
<tr>
<td>RBC (x10^{12} / μl)</td>
<td>456 ± 26^{a1}</td>
<td>474 ± 20^{b}</td>
<td>480 ± 26^{b}</td>
<td>491 ± 70^{a}</td>
<td>424 ± 22^{b}</td>
<td>461 ± 33</td>
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</tr>
<tr>
<td>Hb (g / dl)</td>
<td>12.9 ± 0.7</td>
<td>12.8 ± 0.9</td>
<td>12.9 ± 1.3</td>
<td>13.6 ± 2.1</td>
<td>11.9 ± 1.2</td>
<td>12.2 ± 1.2</td>
<td></td>
</tr>
<tr>
<td>Ht (%)</td>
<td>38.8 ± 1.8</td>
<td>39.9 ± 2.5</td>
<td>40.0 ± 3.2</td>
<td>41.5 ± 6.8^{a}</td>
<td>35.0 ± 2.2^{b}</td>
<td>36.9 ± 2.6^{a}</td>
<td></td>
</tr>
<tr>
<td>MCV (μ3)</td>
<td>84.9 ± 4.2^{a}</td>
<td>84.1 ± 4.5^{a}</td>
<td>83.1 ± 4.9^{b}</td>
<td>83.5 ± 4.3</td>
<td>82.2 ± 5.7^{a}</td>
<td>80.2 ± 6.3^{b}</td>
<td></td>
</tr>
<tr>
<td>MCH (rr)</td>
<td>28.5 ± 1.8^{a}</td>
<td>27.0 ± 1.6^{b}</td>
<td>26.9 ± 2.1^{b}</td>
<td>27.6 ± 2.1</td>
<td>28.0 ± 2.5^{a}</td>
<td>26.6 ± 2.8^{b}</td>
<td></td>
</tr>
<tr>
<td>MCHC (%)</td>
<td>33.5 ± 1.1^{a}</td>
<td>32.4 ± 0.5^{b}</td>
<td>32.4 ± 0.9^{b}</td>
<td>33.1 ± 1.3</td>
<td>34.1 ± 1.4^{a}</td>
<td>33.1 ± 1.4^{b}</td>
<td></td>
</tr>
<tr>
<td>TIBC (g/l)</td>
<td>314 ± 31^{#}</td>
<td>335 ± 41</td>
<td>341 ± 48^{#}</td>
<td>418 ± 38^{a}</td>
<td>394 ± 50^{b}</td>
<td>403 ± 51</td>
<td></td>
</tr>
</tbody>
</table>

^{1}Note. Values are means ± standard deviation. N = 8.

^{a,b}Means with the different subscript letters are significantly different from one another within the same intervention periods, P < 0.05 (Wilcoxon’s signed-rank test).

^{#}Means of the same time with or without intervention are significantly different, P < 0.05 (Wilcoxon’s signed-rank test).
osmotic fragility of RBC were 0.440±0.017%, 0.443±0.018%, and 0.429±0.023% in Pre-1, Pre-2, and Pre-3, respectively. NaCl concentrations were significantly higher in Pre-2 than in Pre-3 ($P < 0.05$). On the other hand, each levels in Int-1, Int-2, and Post-1 were 0.481 ± 0.010%, 0.447 ± 0.028%, and 0.453 ± 0.036%, respectively. NaCl concentrations were significantly lower in Int-2 than in Int-1 ($P < 0.05$), but not in Post-1.

Discussion

This study of rhythmic gymnasts during a period of weight loss shows that diets containing 1500 kcal energy and 15 mg iron for one month might result in significantly lowered body weights and body fat percent (both $P < 0.05$) with increases in serum ferritin concentrations but not serum free iron and TIBC, and they do not
Figure 3—Effect of the intervention on serum iron status. 1) Serum iron concentration (mean + SD), 2) Transferrin saturation ratio, and 3) Serum ferritin concentrations. No intervention x time interaction effect, no main effect of time, and no main effect of intervention were observed for either serum iron concentrations or transferrin saturation ratio (two-factor repeated-measures ANOVA). The main effects of the intervention ($P < 0.05$) and the time ($P < 0.05$) on serum ferritin concentrations were significant, as was the intervention x time interaction effect ($P < 0.01$; two-factor repeated-measures ANOVA). Values marked "*" in Int-2 indicate significantly higher concentrations than those in Pre-2 (Wilcoxon’s signed-rank test; $P < 0.01$). a,b: Values with different superscript letters are significantly different within the same year (Wilcoxon’s signed-rank test; $P < 0.05$).
Figure 4—Effect of the intervention on the activity of δ-ALAD and EPO concentrations. 1) Changes in the δ-ALAD activities (mean ± SD) and 2) EPO concentrations. The effect of time ($P < 0.001$) and the intervention x time interaction effect ($P < 0.001$) were significant in δ-ALAD activities (two-factor repeated-measures ANOVA). Values marked "*" in Int-1 or Int-2 indicate significantly lower or higher concentrations than in the Pre-1 or Pre-2, respectively (Wilcoxon’s signed-rank test; $P < 0.05$). a,b,c: Values with different superscript letters within the same year are significantly different (Wilcoxon’s signed-rank test; $P < 0.05$).

prevent the development of iron-deficiency anemia. Furthermore, a fixed dietary intake increased the δ-ALAD activity, but decreased the hp concentrations.

Rhythmic gymnastics is a competitive sport requiring speed, power, grace, and aesthetic appeal, so achievement of both a desired body shape and strength are necessary. Elite rhythmic gymnasts have been shown to be taller and leaner than average for their age (11, 12, 17). Rhythmic gymnasts sometimes consume less energy than the estimated requirements (6, 7).
Under our experimental conditions, there were no significant differences in total energy intakes over the experimental periods, with or without the intervention (around 1500 kcal/day). Thompson et al. (28) measured the resting metabolic rate in male endurance athletes who had either low energy intakes or adequate energy intakes. They found that the resting metabolic rates were significantly lower in the low energy intake-group compared to those of the adequate intake-group, while physical activity levels of both groups were similar. These observations indicate that decreased resting metabolic rates are related to lower energy intakes.

In the pre-intervention year, female athletes took 1500 kcal/day of energy for the 8 weeks and both their body weight and body fat percent were unchanged throughout the period, suggesting that the energy intakes are sufficient for the energy expenditures during the 8 weeks. On the other hand, athletes in Int-2 showed significantly lowered body weight and body fat percent than in Int-1. Body weight and body fat usually decrease when energy expenditure exceeds energy intake. Therefore, it seems likely that energy expenditures in Int-2 are higher than those in Pre-2 and Int-1, respectively.

Furthermore, dietary iron intakes in Pre-1, Pre-2, Pre-3, Int-1, and Post-1 were approximately 6-7 mg/day, indicating that iron intakes were much lower than those reported in the National Survey of Nutrition in 2002 (19) or the Dietary Reference Intakes for Japanese young women (20), respectively. Low energy intakes are associated with iron deficiency in female athletes (2, 5, 24), while sufficient iron intakes reduce iron-deficiency anemia risk (29). Since dietary intakes of iron (14.6 - 15.0 mg) were reported to be sufficient to prevent iron-deficient anemia.
(17), the minimum iron intake target in our present intervention program was established at 15 mg.

In the present study, iron depletion was found in 75% of the study population in Pre-1, 87.5% in Pre-2, and 62.5% in Pre-3, respectively. On the other hand, iron-deficient anemia was found in only 14% of female basketball players (8). The incidence of iron depletion and iron-deficient anemia in this study was much higher than in basketball players or endurance runners (8, 21), suggesting that rhythmic gymnasts may be more vulnerable to the iron-deficient anemia than other athletes. However, it is not clear whether a 4-week dietary intervention is sufficient to produce differences in the body iron profiles. Roughead et al. (22) examined the initial uptake and subsequent retention of heme and nonheme iron in human using isotopes, and found that at 2 weeks after the test meal intakes, ≈ 80% of the newly absorbed nonheme iron was incorporated into the erythrocytes of the subjects. This observation strongly suggests that the 4 week dietary intervention with Japanese ordinary foods in Int-2 provided enough substrate for increased erythrocytes biosynthesis. However, in this period, enhanced physical activity might promote the destruction of erythrocytes more than the biosynthesis. Accordingly, the dietary intervention could not improve iron-deficient anemia in Int-2.

Development of iron-deficient anemia is attributed to some factors such as menses, lower dietary intakes of iron, foot strike hemolysis, blood and iron loss through the gastrointestinal tract and urinary tract, and by excessive sweating (8). However, it is not well understood how the metabolism and degradation of red blood cells (RBC) in well-trained rhythmic gymnasts correspond with sessions of training.

In our study, the activity of δ-ALAD was significantly increased in Int-2 compared with Int-1 (P < 0.001) and Post-1 (P < 0.01), but hp concentrations were significantly lower in Int-2 than in Post-1. The increase of red cell turnover in athletes was reported by Ehn et al. (10), in which whole-body loss of radioactive iron occurred ~20% faster in female athletes than in non-athletes. Increased activity of δ-ALAD in our present study may correspond with increased RBC turnover, because δ-ALAD, an enzyme in the protoporphyrin pathway, is the marker enzyme for heme biosynthesis.

The concentrations of serum EPO in Pre-2 and Pre-3, and the activity of δ-ALAD in Pre-3 increased significantly compared with Pre-1, respectively. On the other hand, serum ferritin levels decreased significantly in Pre-2. The decrease of serum ferritin may correspond to an increased biosyntheses of RBC in Pre-2. Although serum iron concentrations were unchanged, serum ferritin concentrations and δ-ALAD activities increased significantly in Int-2 compared with both Int-1 and Post-1, respectively. There might be a possibility that serum iron is used as substrates for erythrocyte and ferritin biosyntheses and/or directly excreted in sweats and urine. The rise of serum ferritin concentration indicates that the iron of the meal origin has been absorbed.

Several studies have indicated that low hp levels are caused by the destruction of older RBC (18), and footstrike is the major contributor to hemolysis during running (26). Rhythmic gymnastic training involves extensive skipping and jumping with a club and/or a ball, and elite gymnasts would take advantage of a high anaerobic threshold in performing their competitive routines (1, 14). The vertical component of the grand reaction force had maximal amplitude of about 1900 N, equal to 3.5 times the force produced by the body weight of the gymnast (9). Under our
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Experimental condition, lower hp was apparent in Int-2 of the dietary intervention periods. This suggests that the intense rhythmic gymnastic training might induce footstrike hemolysis just like runners. Furthermore, osmotic fragility indicated by NaCl concentrations was lower in Int-2, suggesting that this fixed dietary intake might provide stronger and newer RBC against some changes in osmotic pressure and increased intensive activities.

In conclusion, the fixed dietary intake did not improve iron depletion in female rhythmic gymnasts in this study. Some limitations in our present study include the fact that we only had 8 subjects. It is also unclear which of the factors directly affect the body iron status: namely, the periods of dietary intervention or the quantity of dietary iron intake. Although this fixed dietary intake did not induce any changes in the incidence of iron depletion and/or iron deficiency anemia, this fixed dietary intervention reduces the RBC life span as indicated by an increased ALAD activity and a decreased hp concentration.

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References


