Voluntary Resistance Exercise Improves Blood Hemoglobin Concentration in Severely Iron-Deficient Rats

Tatsuhiro MATSUI1,*, Hyung-Sook KANG1, Hiroo SUZUKI1 and Masashige SUZUKI2

1 Faculty of Agriculture, Kagawa University, Ikenobe, Mikicho, Kitagun, Kagawa 761–0795, Japan
2 Institute of Health and Sport Sciences, University of Tsukuba, Tsukuba, Ibaraki 305–8574, Japan

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Summary

To evaluate the effects of long-term voluntary resistance exercise (climbing) compared with aerobic exercise (swimming) on iron status in severely (4 mg Fe/kg diet) and mildly (18–29 mg Fe/kg diet) iron-deficient rats, we trained male Wistar rats for 8 wk to climb a wire-mesh tower (Ø20 cm × 200 cm, CLIMB) and to swim in a plastic pool (Ø50 cm × 50 cm, SWIM). These rats were compared with sedentary (SED) rats. After the experimental period, blood hemoglobin level, plasma iron concentration, and transferrin saturation were significantly lower in the 4 mg Fe/kg diet rats than in the 18, 29, and 40 mg Fe/kg diet rats. In 4 mg Fe/kg diet rats, the hemoglobin level was significantly higher in the CLIMB group than in the SED and SWIM groups. On the other hand, neither exercise affected iron status in mildly iron-deficient rats. Bone marrow δ-aminolevulinic acid dehydratase activity was significantly higher in the CLIMB group than in the SWIM and SED groups. These results suggest that long-term resistance exercise is more effective than aerobic exercise in improving blood hemoglobin concentration in severely iron-deficient rats, probably because of an increase in heme biosynthesis. Resistance exercise may be a useful therapy for iron-deficient anemia.

Key Words resistance exercise, aerobic exercise, iron deficiency, hemoglobin, heme biosynthesis

Iron deficiency continues to be a significant nutritional problem in the world (1, 2). Previous studies suggest that chronic exercise can detrimentally alter body iron physiology. Decreased hematocrit, hemoglobin, and serum iron and increased erythrocyte fragility can occur with aerobic exercise (3–6). Thus far, animal studies (7, 8) examining iron deficiency and aerobic exercise interactions have demonstrated that exercise lessens the impact of moderate iron deficiency on essential body iron components, such as hemoglobin. Hisaoka and Shibuya (9) demonstrated that hemoglobin, hematocrit, and red blood cell volume were significantly higher in swimming rats than in control rats. Tobin and Beard (10) reported that running failed to alter hemoglobin, hematocrit, and red blood cell mass in iron-deficient and control rats.

On the other hand, few studies have examined the effects of resistance exercise on body iron status. We previously demonstrated that mild resistance exercise improved nonanemic iron deficiency without iron supplementation in young women (11). We recently designed a new voluntary resistance training model in which rats climbed a vertical tower (12). Three weeks of climbing increased the activity of δ-aminolevulinic acid dehydratase, the marker enzyme for heme biosynthesis, in the bone marrow of severely iron-deficient rats, whereas blood hemoglobin concentration was not affected (13, 14). These findings suggested that 3 wk of resistance exercise could increase heme biosynthesis, but neither exercise improved severe iron deficiencies (13, 14). However, we assumed that the length of the experiment and the level of iron deficiency influenced the iron status.

The purpose of the present study was to determine whether resistance exercise for a longer period (8 wk) would induce improvement in essential body iron components in severely and mildly (marginal) iron-deficient rats and, if so, to determine how that improvement would increase δ-aminolevulinic acid dehydratase (ALAD) activity in bone marrow. We also compared the effectiveness of resistance versus aerobic exercise (swimming).

Experimental

Animals and experimental design. Seventy-two male Wistar rats (3 wk old) were obtained from Japan SLC, Inc. (Shizuoka, Japan). The rats were fed CE-2, commercial rodent diet (CLEA Japan, Tokyo), and water ad libitum through age 5 wk. All animals were individually housed in an animal room at 24±1°C with lights on from 8 a.m. to 8 p.m. The rats were randomly assigned.

* To whom correspondence should be addressed.
E-mail: matsuo@ag.kagawa-u.ac.jp
to one of four dietary groups. The difference in dietary treatment was the level of dietary iron as follows: 4, 18, 29, and 40 mg of elemental iron as ferrous sulfate heptahydrate per kilogram diet. Except for iron, the purified diet met the recommendations or the American Institute of Nutritional Ad Hoc Committee on Standards for Nutritional Studies (15, 16). The diet composition was as follows (g/kg): casein, 200.0; cornstarch, 649.8; corn oil, 50.0; cellulose, 50.0; AIN-76 vitamin mixture, 10.0; AIN-76 mineral mixture without iron, 35.0; choline chloride, 2.0; dl-methionine, 3.0; butylated hydroxytoluene, 0.2. Four groups of rats were randomly divided into three subgroups: sedentary (SED), swimming exercise (SWIM), and climbing exercise (CLIMB). Each group (n=6/group) was meal-fed the diet at 8:00 to 9:00 h and 17:00 to 19:00 h and given free access to water for 8 wk. After the 8-wk experimental period, the rats were fasted overnight and killed by decapitation under light ether anesthesia. Liver and femoral bone marrow were quickly removed and stored at -40°C until analysis. The voluntary resistance training model, CLIMB, was a modification of the one described by Yarasheski et al. (17) and Duncan et al. (18). Rats of the CLIMB group were housed in metal cages containing wire-mesh towers (φ20 cm×200 cm) with water bottles set on the tower tops (12-14). On the other hand, the rats of the SWIM group were trained from 8 a.m. to 9 a.m. everyday in a plastic pool (φ50 cm×50 cm) with water maintained at 33-35°C. The voluntary swimming exercise was performed in the manner described previously (19, 20).

Analysis. Blood hemoglobin concentration was determined colorimetrically with a hemoglobin B-Test kit purchased from Wako Pure Chemical Industries (Osaka, Japan). Plasma iron concentration and total iron-binding capacity (TIBC) were determined by the method of the International Nutritional Anemia Consultative Group (21). Transferrin saturation was calculated by plasma iron concentration and TIBC (23). Iron content in the liver was measured after acid digestion with an atomic absorption spectrophotometer (Model Z-5000, Hitachi, Tokyo, Japan). ALAD activity in the bone marrow was measured by the method of Sassa (22), with the Ehrlich's reagent being made as reported by Tomokuni (23).

Statistics. Data were expressed as means±SE. All data were analyzed by a two-way analysis of variance (ANOVA) and Fisher’s PLSD tests. The differences were considered statistically significant at p<0.05.

Results and Discussion

Swimming exercise reduced body weight gain in the 4 mg Fe/kg diet and the 18 mg Fe/kg diet groups (Fig. 1), though food intake was approximately the same in all experimental groups (SED, SWIM, CLIMB: 10.1±0.3, 10.7±0.4, 10.2±0.5 g/d for the 4 mg Fe/kg diet group; 9.9±0.3, 10.9±0.4, 10.1±0.5 g/d for the 19 mg Fe/kg diet group; 9.3±0.4, 10.2±0.2, 10.1±0.5 g/d for the 29 mg Fe/kg diet group; and 10.2±0.5, 10.7±0.4, 10.9±0.5 g/d for the 40 mg Fe/kg diet group). The blood hemoglobin level, plasma iron concentration, and transferrin saturation were significantly lower (p<0.05), and TIBC was significantly higher (p<0.05) in the 4 mg Fe/kg diet rats than in the 18, 29, and 40 mg Fe/kg diet rats for the SED, SWIM, and CLIMB groups (Table 1 and Fig. 1). In the 4 mg Fe/kg diet-fed rats, hemoglobin levels were significantly higher (p<0.05) in the CLIMB group than in the SED and SWIM groups (Fig. 1). Liver iron concentration increased linearly as dietary iron increased, whereas neither exercise influenced iron levels in liver (Fig. 1).

Only severe iron deficiency increased bone marrow ALAD activity (Fig. 2). The bone marrow ALAD activity was significantly higher (p<0.05) in the CLIMB group than in the SWIM and SED groups in the 4, 18, 29, and 40 mg Fe/kg diet rats (Fig. 2).

This study demonstrates that long-term resistance exercise (CLIMB) training increases iron status indices such as blood hemoglobin level more effectively than aerobic exercise (SWIM) training does in severely iron-deficient rats. These results suggest that the capacity for heme biosynthesis may be dependent on resistant loads to the animal body. Hemoglobin is dependent on heme synthesis in the bone marrow because iron-containing porphyrin constitutes the ring structure to which the hemoglobin is conjugated with apoprotein. We therefore hypothesized that the heme pathway would be accelerated by exercise-induced increases in hemoglobin concentration. Tobin and Beard (10) found that training in iron-deficient animals led to a higher percentage of ^57^Fe associated with red cells as compared to iron deficient sedentary rats within 1 h after an intravenous injection of ^59^Fe. The present study supports these findings. On the other hand, neither resistance nor aerobic

| Group   | Plasma iron (µg/mL) | Total iron binding capacity (µg/mL) | Transferrin saturation (%) |
|---------|---------------------|-------------------------------------|--------------------------|
| SED-4   | 1.4±0.2ab           | 9.2±0.6a                           | 15.2±0.9b                |
| SWIM-4  | 1.5±0.2ab           | 8.9±0.5a                           | 16.8±1.2b                |
| CLIMB-4 | 1.5±0.1ab           | 9.0±0.6a                           | 16.7±1.3b                |
| SED-18  | 3.2±0.4a            | 5.2±0.6a                           | 61.5±6.9b                |
| SWIM-18 | 3.2±0.3b            | 5.8±0.5b                           | 56.8±4.9b                |
| CLIMB-18| 2.9±0.2b            | 5.1±0.6b                           | 56.9±5.0b                |
| SED-29  | 2.8±0.1a            | 4.7±0.4a                           | 60.0±4.2a                |
| SWIM-29 | 3.3±0.2a            | 5.5±0.5a                           | 60.0±4.9b                |
| CLIMB-29| 3.4±0.3a            | 5.6±0.5a                           | 60.7±3.2b                |
| SED-40  | 3.1±0.4a            | 5.4±0.5a                           | 57.4±3.2b                |
| SWIM-40 | 3.0±0.3a            | 5.1±0.5a                           | 58.2±3.2b                |
| CLIMB-40| 3.0±0.2a            | 5.5±0.5a                           | 54.5±3.2b                |

1 Values are means±SE for 6 rats. Means with different superscripts within a column are significantly different at p<0.05, determined by ANOVA and Fisher’s PLSD tests.

2 SED, sedentary; SWIM, swimming exercise; CLIMB, climbing exercise. Numbers indicate dietary iron levels (mg Fe/kg diet).
Fig. 1. Effects of swimming or climbing exercise on body weight gain, blood hemoglobin concentration, and liver iron content in rats receiving 4, 18, 29, and 40 mg Fe/kg diet for 8 wk. Values are means and SE for 6 rats. Means with different superscripts are significantly different at p<0.05, determined by ANOVA and Fisher’s PLSD test. SED, sedentary; SWIM, swimming exercise; CLIMB, climbing exercise.

In this study, liver iron content increased significantly and linearly as dietary iron content increased. Since blood hemoglobin levels are normal in mildly iron-deficient rats, 18–29 mg Fe/kg diets caused nonanemic “marginal” iron deficiency. Borel et al. (24) reported that as dietary iron intake increased above 16 mg Fe/kg diet, liver iron concentrations steadily increased, and hemoglobin concentrations were maintained at normal levels. Slimes et al. (25) determined that a dietary iron intake of less than 25 mg Fe/kg diet resulted in hemoglobin concentrations of 12 mg/100 mL or below. Our findings agree with the results obtained by Borel et al. (24) and Slimes et al. (25). On the other hand, neither exercise influenced liver iron content or plasma iron concentration for any dietary iron level. Strause et al. (26) demonstrated that sprint-exercised rats absorbed more iron than sedentary rats did in radioiron tracer studies. In our study, iron absorption may be higher in the CLIMB group than in the other groups. Climbing exercise may stimulate the distribution of hemoglobin iron rather than storage iron in the liver. These mechanisms remain unknown, so further studies will be required.

In conclusion, our study demonstrates that long-term resistance exercise improves iron status in severely iron-deficient rats more effectively than aerobic exercise does because of an increased heme biosynthesis in rat bone marrow. These results suggest that resistance exercise may be a useful therapy for iron-deficient anemia.

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