Efficacy of iron supplementation may be misinterpreted using conventional measures of iron status in iron-depleted, nonanemic women undergoing aerobic exercise training

Laura M Pompano and Jere D Haas

Division of Nutritional Sciences, Cornell University, Ithaca, NY

ABSTRACT

Background: Despite its known detrimental effects, iron deficiency remains the most common micronutrient deficiency in the world. Many interventions that aim to improve iron status involve physically active populations. Intense aerobic exercise training negatively affects iron status; however, the impact of regular moderate aerobic exercise on the effectiveness of iron supplementation remains unclear.

Objective: This study aimed to determine whether aerobic training modifies the assessment of the effectiveness of iron supplementation in improving conventional iron status measures.

Design: Seventy-two iron-depleted, nonanemic Chinese women [serum ferritin (sFer) <25 μg/L and hemoglobin >110 g/L] were included in an 8-wk, partially blinded, randomized controlled trial with a 2 × 2 factorial design including iron supplements (42 mg elemental Fe/d) or placebo and aerobic training (five 25-min sessions/wk at 75–85% of maximum heart rate) or no training. Linear mixed models were used to evaluate the relation between supplement type, training, and changes in iron status over time, measured by sFer, hemoglobin, soluble transferrin receptor (sTfR), and estimated total body iron.

Results: After treatment, both the iron-supplemented trained and untrained groups showed significantly improved sFer, sTfR, and body iron values compared with either of the placebo groups. Similarly, trained participants had significantly higher aerobic fitness measures than untrained participants. Training modified the sFer response to supplementation (training by supplement interaction, $P = 0.07$), with the iron-supplemented trained group having significantly lower sFer than the iron-supplemented untrained group at week 8 (mean ± SD: 31.8 ± 13.5 and 47.6 ± 15.7 μg/L, respectively; $P = 0.042$), whereas there was no significant difference between the placebo trained and untrained groups (21.3 ± 12.2 and 20.3 ± 7.0 μg/L, respectively; $P = 1.00$).

Conclusions: Regular aerobic training reduces the apparent effectiveness of iron supplementation in improving sFer and calls into question whether conventional measures of iron status accurately reflect iron metabolism in physically active, nonanemic women. This trial was registered at clinicaltrials.gov as NCT03002090. *Am J Clin Nutr* 2017;106:1529–38.

Keywords: iron deficiency, supplementation, aerobic training, serum ferritin, body iron

INTRODUCTION

Iron deficiency anemia affects 20–34% of Chinese women of childbearing age (1, 2). This rate likely underestimates the prevalence of iron deficiency (ID) in the population because it does not account for ID without anemia [IDNA; hemoglobin >110 g/L and serum ferritin (sFer) <15 μg/L] (3). ID with anemia and IDNA have negative consequences on physical performance and exercise capacity (4, 5). However, there is also evidence that aerobic training may have a negative influence on iron status, which is potentially more concerning when considering that iron interventions are frequently targeted toward physically active populations (6–8).

Intense physical exercise lowers several measures of iron status, including sFer, and increases soluble transferrin receptor (sTfR), indicative of either compromised iron status (6–9) or redistribution of iron to be used in erythropoiesis or muscle formation (10–13). Female soldiers who underwent 9 wk of basic combat training showed declining iron status (7, 14). The provision of an iron supplement (14) during basic combat training attenuated some of these changes compared with placebo. Although these findings occurred in highly intensive training programs, recreational physical activity can also negatively affect iron status. In a study by Hinton et al. (4) in women with IDNA who underwent aerobic training, iron supplementation

Supported by Fulbright US student fellowships, the Cornell Lehman Fund for Scholarly Exchange with China, Foreign Language Area Studies fellowships, the Cornell Human Ecology Alumni Association, the Cornell College of Agriculture and Life Sciences Alumni Association, the Mario Einaudi Center for International Studies at Cornell University, and a Nancy Schlegel Meinig Professorship.

Supplemental Table 1 is available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.

Address correspondence to LMP (e-mail: lmp262@cornell.edu).

Abbreviations used: AGP, α1-acid glycoprotein; CRP, C-reactive protein; FeTr, iron trained; FeUn, iron untrained; ID, iron deficiency; IDNA, iron deficiency without anemia; PLTr, placebo trained; PLUn, placebo untrained; sFer, serum ferritin; sTfR, soluble transferrin receptor; $\dot{V}O_2$max, maximal oxygen consumption.

Received January 11, 2017. Accepted for publication October 5, 2017.

First published online November 1, 2017; doi: https://doi.org/10.3945/ajcn.117.152777.

resulted in a modest increase in sFer (4.1 μg/L) compared with those who received placebo. Interestingly, the improvement in sFer in the iron-supplemented trained women in the Hinton and Sinclair study (15) was less than half of that observed in a separate study in fit IDNA women who received similar iron supplementation but who did not undergo training. These findings suggest that improvements in sFer from iron supplementation may differ if women train during supplementation. This difference may be a result of an exercise-induced prioritization of erythropoietic demands and muscle growth over storing iron in the liver, as reflected by sFer concentration. Although the current literature supports this idea of iron redirection away from the stores to support more functional uses of iron, most interventions that aim to improve iron status or anemia measure only hemoglobin, sFer, and sometimes sTfR or estimated body iron, calculated from the ratio of sTfR to sFer, and do not measure other body iron pools. This oversight could potentially lead to erroneous conclusions of the effectiveness of the intervention.

Further complicating the relation between IDNA and exercise, research also suggests that women who are actively training perform better when given iron supplementation than do those who train without supplementation (6, 14), which suggests an intricate, but currently undefined, relation between iron status, total body iron, exercise, and physical performance capacity. This relation must be understood before we can properly design effective interventions to measure and target IDNA in physically active populations, such as laborers, athletes, or nonathletes who want to benefit from improved fitness. Although several studies have shown that training worsens traditional measures of iron status, to our knowledge no study has directly examined the interaction between training and iron supplementation to determine how training affects the effectiveness of iron supplementation, as assessed by sFer and hemoglobin concentrations. Therefore, the goal of this study was to determine whether 8 wk of regular aerobic exercise on a cycle ergometer modifies the apparent effectiveness of concurrent iron supplementation in improving the traditional measures of iron status. We hypothesized that those women who received both iron and training would have smaller changes in sFer than women who did not train while taking iron.

METHODS

Participants

The sampling scheme for this study is shown in **Figure 1**. A total of 379 physically active, untrained 18- to 26-y-old women were recruited in 2 waves (September–December 2014 and March–June 2015) from the student population at Kunming Medical University in Yunnan, China. Of these, 98 women were identified in the fall wave and 281 were identified in the spring

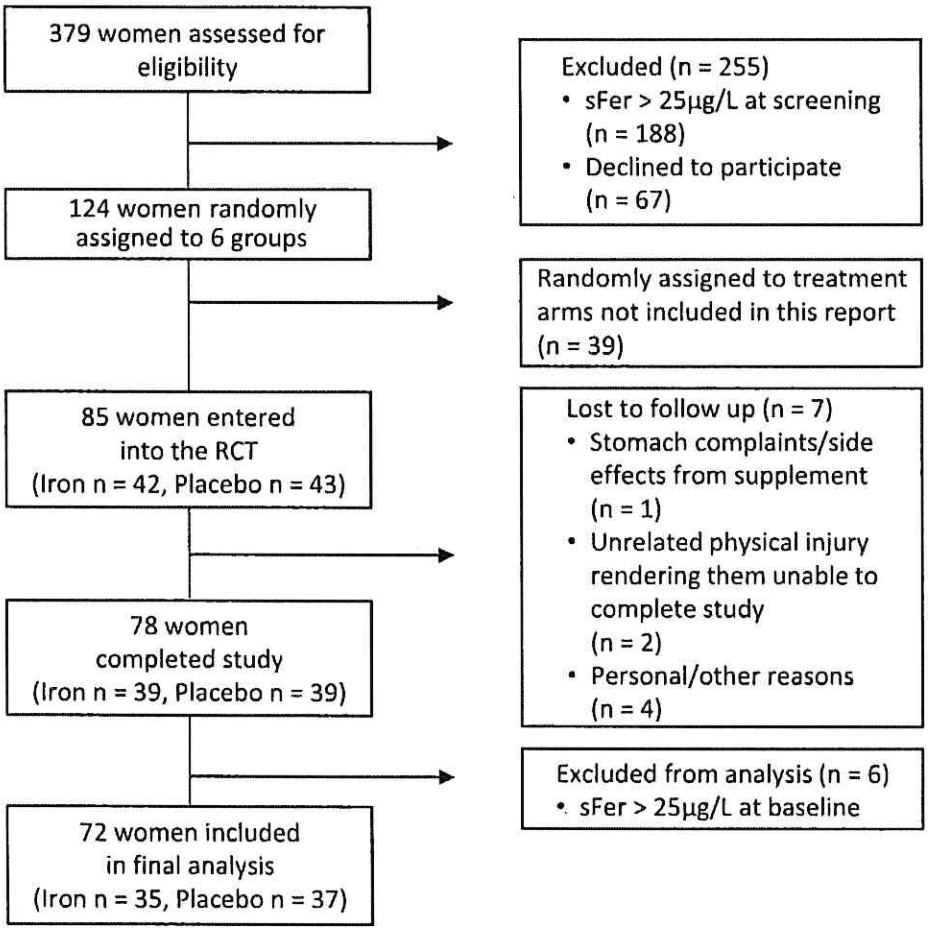

FIGURE 1 Consolidated Standards of Reporting Trials diagram. RCT, randomized controlled trial; sFer, serum ferritin.

wave. In total, 191 were identified as iron depleted without anemia, defined as hemoglobin >110 g/L and sFer <25 μg/L. Anemia was defined as <110 g/L to align with the hemoglobin cutoff used by the First Affiliated Hospital of Kunming Medical University where the screening analyses were performed. The cutoff for iron depletion was set at 25 μg/L, because previous research has shown that physical performance changes occur even in women who are iron depleted and not clinically iron deficient (16–20). Screening, which included a medical history questionnaire, was conducted to identify and exclude women who met any of the following criteria: anemia (hemoglobin <110 g/L), current pregnancy or pregnancy within the previous year, current lactation or lactation within the previous year, recent infectious illness or fever, current inflammation or chronic inflammatory diseases, hemolytic anemia, chronic respiratory disease, musculoskeletal problems, history of eating disorders, smoking, BMI (in kg/m^2) <17 or >25, or recent consumption of iron supplements, vitamin supplements, or medications that may affect dietary iron intake or absorption or that had anticoagulant properties. Participants also filled out a physical activity questionnaire that indicated they did not participate in regular exercise or organized sports, were interested in increasing their level of physical fitness, and were willing to comply with the full 8-wk training program. Of the eligible women, 124 agreed to participate in the trial (45 in the fall wave and 79 in the spring wave). The recruitment process for this study was designed to recruit for 2 additional arms of the study. This article focuses on the first 4 arms of the study, which included the iron- and placebo-supplemented groups. The other arms of the study included 2 groups who received a Chinese herbal supplement. Of the 124 women in the total study, 85 were randomly assigned to receive the treatments relevant to this study.

Data from 6 women were excluded from statistical analyses because their sFer values at week 0 increased above the screening cutoff of 25 μg/L, whereas their original screening values from the previous 2 wk indicated that they were iron depleted. In addition, 7 women dropped out of the trial. The final sample size for this analysis was 72 women. Signed informed consent was obtained from each participant. The study was approved by the Cornell University Institutional Review Board and the Kunming Medical University Ethical Committee and registered under clinicaltrials.gov as NCT03002090.

Study design

The experimental design of the study was a 2 × 2 randomized, double-blind, placebo-controlled intervention trial. Participants were randomly assigned by one of the authors (LMP) via a random-number generator to treatment groups. Participants received either 100 mg ferrous sulfate or an identical placebo capsule 2 times/d for 8 wk. Participants and all of the investigators were blinded to supplement type until after the per-protocol analyses were completed. Sample size was based on consumption of a minimum elemental iron concentration of 12 mg/capsule over 8 wk. The final capsule concentration met this requirement. Previous work has shown that iron status improves after 4 wk of iron supplementation at a similar dose of ferrous sulfate (4, 21). The capsules were prepared by LMP with the use of gelatin capsules, ferrous sulfate, and dextrose filler (Professional Compounding Centers of America). At weeks 0, 2, 4, 6, and 8 of each wave of the study, 20 capsules of each type were randomly selected from the batch and stored in a sealed container in a cool, dry place until analyzed poststudy by inductively coupled plasma mass spectrometry. After the study, iron concentrations of the iron and placebo capsules were determined from this random sample to be 21.1 and 0.00 mg elemental Fe, respectively.

Participants were instructed to consume the capsules with citrus juice during their morning and evening meals and to avoid consuming the capsules on an empty stomach. Thirty capsules were distributed to each participant in a bottle labeled with their unique subject identification number every 2 wk (weeks 0, 2, 4, and 6). Each participant was instructed to complete a daily log to record capsule ingestion as well as information on medication use, illness, menstrual cycle status, any gastrointestinal complaints, physical activity, and musculoskeletal problems. In addition, they were instructed to return their capsule bottles every 2 wk with any remaining capsules, which were then counted as an independent confirmation of the number of capsules reported as consumed on the daily logs.

One-half of each supplement group were further randomly assigned to an exercise training program or to no training to create 4 treatment groups: iron trained (FeTr), iron untrained (FeUn), placebo trained (PLTr), and placebo untrained (PLUn). The training protocol was adapted from a protocol previously published by Hinton et al. (4) that has produced measurable improvements in endurance and maximal oxygen consumption ($\dot{V}O_2$max). The intensity of training increased each week according to each participant's heart rate. Training sessions lasted 25 min and were divided into 2 target heart rates of 75% and 85% of age-estimated maximum, calculated as 220 (beats per minute) minus age (years). The workload was adjusted as needed to maintain the target heart rate throughout the session. Minutes spent at 85% of maximum heart rate increased each week to increase the difficulty of the training. The training protocol is shown in **Table 1**. Participants trained Monday through Friday during each of the 8 wk of the study. Optional training days were offered on weekends to allow participants to make-up any days they had missed during the week. The maximum number of training days was 40.

Training was performed on a stationary exercise bicycle (model B8.4E; KangLe Exercise Products Company) equipped with digital output of work (watts) and cadence (rotations per minute). Participants wore a T31 Polar heart rate monitor, which was read by using a Polar A5 heart rate watch (Polar Electro Inc.).

TABLE 1
Daily training schedule, by week

Week	75% of maximum heart rate, min	85% of maximum heart rate, min
1	20	5
2	19	6
3	18	7
4	17	8
5	15	10
6	13	12
7	11	14
8	10	15

Trained research assistants recorded the watts, heart rate, and speed of each participant in a training log every 5 min throughout each session.

Daily physical activity, defined as minutes spent performing discretionary exercise, was assessed at weeks 0, 4, and 8 by using the data from a daily log completed each day over the 56-d trial. Minutes of self-reported physical activity were totaled at each time point. In addition, a physical activity frequency questionnaire was administered at week 0 to assess similarity in habitual physical activity levels between groups. Participants were asked to maintain their normal prestudy activity patterns for the duration of the 8-wk study period, regardless of whether they were in the trained or untrained groups.

Body composition and physical performance were measured before and after the study as well as at the 4-wk midpoint. Dietary iron intake was assessed at week 0 by using a 4-d diet record that spanned from Thursday to Sunday. These records were analyzed for daily iron, inhibitors and enhancers of iron absorption, and macronutrient content by using Nutrition Data System for Research Software (2016; University of Minnesota).

All of the participants were compensated for study participation with a gift. In addition, to increase compliance to the training program, trained participants were offered incentives in the form of small gifts when they completed a target number of training days (25, 32, and 40 d). Although all of the participants received the same gift for participation in the study, the smaller training-based incentives were only offered to the trained group.

Iron status measurements

Iron status was assessed at weeks 0, 4, and 8. Whole blood was collected from the antecubital vein into 4-mL EDTA-coated tubes by a phlebotomist at the Kunming Medical University campus hospital. A small sample of whole blood was removed for hemoglobin analysis. The remaining samples were stored at 4°C until centrifugation at 1600 × *g* for 10 min at room temperature (23°C) within 24 h. The serum was collected and separated into 0.5-mL aliquots and frozen at −80°C until analysis. Blood was analyzed for sFer, sTfR, α1-acid glycoprotein (AGP), and C-reactive protein (CRP) at the First Affiliated Hospital of Kunming Medical University in the first wave and at the Shanghai Fenglin Clinical Laboratory in the second wave. In addition, 75 samples from the second wave were analyzed at both laboratories to allow for comparison between the laboratories. Hemoglobin was determined by using a Coulter LH 750 Hematology analyzer (Beckman Coulter Inc.). sFer, sTfR, AGP, and CRP were analyzed by using a Siemens Advia 2400 automated analyzer (Siemens Healthcare). Estimated total body iron was derived from the ratio of sTfR to sFer by using the equation reported by Cook et al. (22) as follows:

$$\text{Total body iron} = -[\log(\text{sTfR}/\text{sFer}) - 2.8229]/0.1207 \quad (1)$$

This equation uses sTfR values from Ramco ELISA kits (Ramco Laboratories). Therefore, we converted the sTfR values derived from the Chinese laboratories to Ramco-adjusted sTfR values with the prediction equation below, which was derived from 35 random duplicate samples analyzed by using Ramco Laboratories sTfR ELISA kits.

$$\text{sTfR Ramco} = (3.779 \times \text{sTfR}_{\text{lab}}) + 0.400,\ R^2 = 0.93 \quad (2)$$

Physiologic measurements

Height and weight were measured with the use of previously described standard protocols (23). Body composition was estimated from skinfold thicknesses measured with Lange calipers at the biceps, triceps, subscapular, and suprailiac sites. The Durnin and Womersley (24) equation was used to calculate body density and percentage of body fat.

Physical performance was assessed by using a $\dot{V}O_2$max test at weeks 0 and 8 on a mechanically braked and calibrated cycle ergometer (Monark 884E; Monark Exercise AB). Oxygen consumption was determined while cycling at levels of effort ranging from rest to ~100% of participants' maximum level of exertion. Metabolic variables were assessed with a portable metabolic measurement system (Cosmed K_4B^2; Cosmed), which analyzes heart rate, the volume of respiratory air, and concentrations of oxygen and carbon dioxide in expired air. The participants breathed room air through a 2-way valve connected to a facemask worn during the testing sessions. Oxygen consumption per kilogram of body weight ($\dot{V}O_2$, milliters per kilogram per minute) was calculated from the portable metabolic unit's internal program.

Participants were asked not to perform any strenuous physical activities or exercise for 1 d before the exercise tests, excluding daily training for the trained group. Trained participants did not train on the day of their performance tests. In addition, participants were told not to consume food or caffeinated products for 3 h before performance testing.

The $\dot{V}O_2$max protocol was adapted from that used by Brownlie et al. (25). The test started when the participant's heart rate was <100 beats/min. The test began with a 5-min warm-up with a workload of 1 kg, cycling at 50 × *g*. Workloads were then increased by 0.4 kg every 2 min, cycling at 50 × *g*, until $\dot{V}O_2$ did not increase by >150 mL/min from the previous workload, suggesting that the participant was working at her $\dot{V}O_2$max. If this condition could not be observed, the test proceeded until the participant was unwilling or unable to continue. The participant was considered to have achieved $\dot{V}O_2$max if 2 of the following conditions were met: reaching a respiratory exchange ratio >1.10, a heart rate within 10 beats/min of their age-predicted maximum (220 − age), or blood lactate >8.0 mM. Blood lactate was assessed by finger stick with the use of a Lactate Plus portable blood lactate analyzer (Nova Biomedical) after completion of the final workload.

Compliance analyses

Compliance to capsule consumption was assessed from 2 sources of documentation: capsule counts from returned bottles and daily logs. The Pearson correlation coefficient between the capsules reported as missing in the daily logs and the capsules counted from the returned bottle was 0.39 ($P = 0.01$), suggesting some discrepancies. To create a more reliable assessment of

capsules consumed, 2 compliance variables were created: a conservative estimate and an average estimate. The conservative estimate was calculated by comparing the information from the capsule counts and the log for each 2-wk period and taking the higher number of missed capsules (reported or physically returned). The average estimate was created by taking the average of the log and bottle counts. There were no group differences in the number of capsules consumed for either the conservative or the average estimates.

Statistical analyses

Sample size calculations were based on the Hinton et al. (4) and Hinton and Sinclair (15) studies discussed previously, which suggested that a smaller change in sFer is observed when iron supplementation is given to women who are training. Sample size was determined to require 25 participants in each of the 4 groups, which was expanded to 29 participants/group after considering potential loss to follow-up to detect a 4.1-μg/L change in sFer or an effect size of ~0.8 SDs after 8 wk with 80% power and $\alpha = 0.05$ (4). Data were examined to verify normality of distribution by using the Kolmogorov-Smirnov test, histograms, and qq-plots. Statistical analyses were performed on log-transformed variables for sFer and sTfR, which were found to have non-normal distributions. Measures of iron status at weeks 4 and 8 were analyzed by using linear mixed models with fixed effects of supplement, training group, time, all 2- and 3-way interactions, and a random effect at the participant level. Baseline was included as a covariate for each measure of iron status. If the 3-way interaction was not significant in the full model, the 3-way interaction term was removed from the model and the model was reanalyzed with all of the 2-way interaction terms. Any 2-way interaction terms that were not significant were also removed and a final model was analyzed that included only the significant 2-way interaction or interactions. Post hoc pairwise comparisons were made, with Bonferroni corrections for multiple comparisons. Secondary analyses were performed by using linear models to test for relations between variables. Residuals were examined for normality for all linear mixed models by using the Kolmogorov-Smirnov test, histograms, and qq-plots. For a subgroup of those participants who had a baseline sFer <20 μg/L, the RR of resolving one's iron depletion (being iron depleted at baseline and iron replete at week 8) was calculated for all pairwise comparisons between groups. For all of the analyses, an α level of 0.05 was used to indicate significance. For each result for which a Bonferroni correction was indicated, the original P value was multiplied by the number of comparisons made and considered significant if ($P \times k$ comparisons) < 0.05. All of the statistical analyses were performed in SAS 9.4 (SAS Institute).

RESULTS

Participant characteristics

There were 19 FcTr, 16 FeUn, 18 PLTr, and 19 PLUn participants included in the final analyses. Background characteristics, including age, anthropometric measures, and fitness level as assessed by $\dot{V}O_2$max at baseline, blood biomarkers, and dietary intake at baseline, are shown in **Table 2**. At baseline, all

TABLE 2
Characteristics of total sample at week 0[1]

Characteristics	Iron trained (n = 19)	Iron untrained (n = 16)	Placebo trained (n = 18)	Placebo untrained (n = 19)
Age, y	20.6 ± 1.1	20.3 ± 0.9	20.7 ± 1.2	20.5 ± 1.7
Height, cm	156.8 ± 5.7	156.9 ± 6.2	157.3 ± 4.7	157.4 ± 5.0
Weight, kg	51.2 ± 6.8	51.4 ± 7.9	53.5 ± 6.1	49.7 ± 5.6
BMI, kg/m^2	20.8 ± 2.8	21.0 ± 3.5	21.6 ± 2.1	20.0 ± 2.1
Body fat, %	25.2 ± 2.5	25.5 ± 3.2	26.0 ± 2.0	24.7 ± 2.0
sFer, μg/L	14.0 ± 5.8	17.1 ± 4.2	14.4 ± 4.7	15.2 ± 5.7
Hemoglobin, g/L	135 ± 10.3	141 ± 5.2	132 ± 12.8	133 ± 9.7
sTfR, mg/L	6.8 ± 3.2	5.3 ± 0.8	5.8 ± 1.5	6.1 ± 1.7
Body iron, mg/kg	1.1 ± 2.6	2.7 ± 0.9	1.8 ± 1.8	1.7 ± 1.9
$\dot{V}O_2$max, mL · min^{-1} · kg^{-1}	42.7 ± 4.8	42.4 ± 5.6	41.4 ± 5.3	42.8 ± 5.0
Physical activity,[2] min	59.8 ± 91.1	56.1 ± 60.5	52.9 ± 71.7	49.0 ± 65.9
AGP, g/L	0.61 ± 0.08	0.61 ± 0.09	0.64 ± 0.10	0.59 ± 0.09
CRP,[3] mg/L	0.04 (0.00, 0.01)	0.22 (0.00, 0.01)	0.20 (0.00, 0.30)	0.10 (0.00, 0.21)
Daily dietary intake				
Energy, kcal	1536 ± 278	1573 ± 333	1609 ± 324	1582 ± 318
Fat, g	51.5 ± 12.6	57.2 ± 15.0	56.6 ± 13.3	54.8 ± 16.9
Protein, g	59.5 ± 20.3	60.2 ± 18.4	59.5 ± 17.5	58.2 ± 20.0
Carbohydrate, g	213.5 ± 43.5	204.3 ± 49.3	215.2 ± 47.2	216.0 ± 51.6
Iron, mg	11.7 ± 4.5	13.0 ± 5.8	11.5 ± 3.6	12.3 ± 3.7
Calcium, mg	323.4 ± 116.4	340.0 ± 145.5	322.5 ± 158.4	388.8 ± 190.2
Ascorbic acid, mg	79.5 ± 40.8	60.2 ± 37.5	63.1 ± 41.8	67.0 ± 37.9
Phytic acid, mg	534.0 ± 196.9	597.8 ± 251.5	519.1 ± 246.9	598.3 ± 252.5

[1] Values are means ± SDs unless otherwise indicated; n = 72. AGP, α1-acid glycoprotein; CRP, C-reactive protein; sFer, serum ferritin; sTfR, soluble transferrin receptor; $\dot{V}O_2$max, maximal oxygen consumption.
[2] Minutes of physical activity per week, defined as discretionary exercise on the basis of a self-reported questionnaire.
[3] Values are means with first and third quartiles in parentheses, due to skewed distribution.

TABLE 3
Total capsules and iron consumed by iron-supplemented groups over 8 wk[1]

	Iron trained (n = 19)		Iron untrained (n = 16)	
	Conservative estimate	Average estimate	Conservative estimate	Average estimate
Capsules consumed,[2] n	95 ± 13	98 ± 11	97 ± 10	101 ± 7
Iron consumed, g/8 wk	2.0 ± 0.3	2.1 ± 0.2	2.1 ± 0.2	2.1 ± 0.1

[1] Values are means ± SDs. No group differences were observed for number of capsules consumed or total iron consumed for either estimate by using 1-factor ANOVA with $P < 0.05$.
[2] Maximum possible capsules = 112.

72 participants were iron depleted but not anemic. Of these 72 women, 39 (54.2%) were clinically iron deficient defined as sFer <15.0 μg/L, 5 women (6.9%) had sTfR values >8.3 mg/L, and 12 women (16.7%) had body iron <0 mg/kg. Consistent with discretionary physical exercise of slightly <1 h/wk, $\dot{V}O_2$max levels indicated that participants had a moderate to average fitness level, with no differences between the groups. There were no significant differences in body weight or composition between the 4 groups before or after the study, nor were there significant changes during the 8-wk study period (data not shown). Likewise, there were no differences in reported symptomology between the 4 treatment groups at any time point. In addition, there were no differences between groups for daily iron, calcium, ascorbic acid, phytic acid, fat, carbohydrate, protein, or total caloric intake (Table 2). Of the 72 participants, 13 women (18.1%) had a daily iron intake under the current US Estimated Average Requirement of 8.1 mg/d (26). There were no significant differences in inflammation, measured as CRP and AGP, between the 4 groups at any time point in the study, nor were there significant changes between time points during the 8-wk study period (data not shown). During the 8-wk study, there were no participants who indicated inflammation on the basis of AGP >1.0 g/L. Only 1 participant was determined to have inflammation on the basis of CRP >5.0 mg/L, which occurred at week 4. All of the models were analyzed including this participant and again excluding this participant, but no differences were found in the results of these analyses, so the participant was included for all analyses reported here.

Compliance to treatments

The iron and placebo groups returned 81% and 80% of their capsule bottles, respectively, and completed 96% and 92% of their daily capsule logs, respectively. There were no significant differences between the number of bottles returned or the percentage of daily logs completed by the combined iron and combined placebo groups (t test, $P = 0.87$) or in the 4 individual treatment groups (1-factor ANOVA, $P = 0.31$). Estimates of the amount of iron consumed by the 2 iron groups are shown in **Table 3**.

Details of the training sessions attended by each group are shown in **Table 4**. The average number of training sessions attended by participants in the FeTr and PLTr groups was not significantly different (t test, $P = 0.76$). The FeTr and PLTr groups achieved mean ± SD percentages of 90.5% ± 7.8% and 91.9% ± 5.6%, respectively, of the target heart rate over the 8 wk of training, which was not significantly different (t test, $P = 0.52$). In the combined training groups, 80% of the target heart rate was achieved on 91% of all days trained. In addition, minutes spent in self-reported physical activity outside of the assigned training groups did not differ between groups at any time point. At baseline and week 8, 88% and 92% of participants, respectively, reached $\dot{V}O_2$max. There was no difference in the percentage of each of the groups who reached $\dot{V}O_2$max at baseline or week 8 (chi-square test: $P = 0.92$ and $P = 0.13$, respectively), suggesting there was equal compliance to the testing protocol between groups.

Response to iron treatment

Table 5 shows the week 8 values for each iron biomarker as well as $\dot{V}O_2$max after 8 wk of treatment. Unadjusted means and SDs for each group, by time point, are shown in **Supplemental Table 1**. No significant 3-way interaction effects (supplement type by training group by time) were observed for any of the outcome measures in the linear mixed models (data not shown), nor were there significant interaction effects between time and training in any model. Significant training by supplement type interactions were observed for sFer and hemoglobin, which will be discussed separately.

There was a significant interaction effect between time and supplement type for sTfR (P-interaction = 0.039). At week 8, the iron group had a significantly lower sTfR concentration than did the placebo group (4.9 ± 0.9 and 5.9 ± 1.8 mg/L, respectively; $P < 0.001$). No significant 2-way interaction effects were observed for body iron. However, the iron group had significantly higher body iron than the placebo group (5.7 ± 1.9 and 2.8 ± 2.5 mg/kg, respectively; $P < 0.001$).

Response of $\dot{V}O_2$max to aerobic training

There was no interaction observed between training group and supplement type for $\dot{V}O_2$max; however, a significant training

TABLE 4
Training sessions attended, by group[1]

Group	n	Days trained	Range[2]	Attended ≥30 d, % (n)	Percentage of target heart rate
Iron trained	19	33.2 ± 5.7[3]	21–40	73.7 (14)	90.5 ± 7.8
Placebo trained	18	32.7 ± 3.7	28–40	77.8 (14)	91.9 ± 5.6

[1] No significant differences were observed between training groups for any variable by using a t test, $P > 0.05$.
[2] The maximum possible number of training sessions was 40 sessions.
[3] Mean ± SD (all such values).

TABLE 5
Effects of training and supplement type[1]

Group	sFer, μg/L	Hemoglobin, g/L	sTfR, mg/L	Body iron, mg/kg	$\dot{V}O_2$max, L · min^{-1} · kg^{-1}
FeTr (n = 19)	31.8 ± 13.5	139.4 ± 8.0	5.3 ± 1.0	4.7 ± 1.9	43.3 ± 4.1
FeUn (n = 16)	47.6 ± 15.7	143.3 ± 6.7	4.4 ± 0.7	6.9 ± 1.3	40.8 ± 4.3
PLTr (n = 18)	21.3 ± 12.2	137.3 ± 9.4	5.9 ± 1.3	2.6 ± 2.8	42.4 ± 3.5
PLUn (n = 19)	20.3 ± 7.0	135.3 ± 10.3	5.9 ± 2.3	2.9 ± 2.2	38.8 ± 4.5
P[2]					
Supplement	<0.001	0.003	<0.001	<0.001	0.28
Training	0.034	0.66	0.018	0.023	<0.001
Interaction	0.072	0.030	0.86	0.17	0.50
Supplement effect,[3] P					
FeTr compared with PLTr	<0.001	1.00			
FeUn compared with PLUn	<0.001	0.003			
Training effect,[3] P					
FeTr compared with FeUn	0.042	0.43			
PLTr compared with PLUn	1.00	1.00			

[1] Values are unadjusted means ± SDs at week 8. FeTr, iron trained; FeUn, iron untrained; PLTr, placebo trained; PLUn, placebo untrained; sFer, serum ferritin; sTfR, soluble transferrin receptor; $\dot{V}O_2$max, maximal oxygen consumption.
[2] Results of linear mixed models, adjusted for baseline. Interaction P values represent the interaction of supplement type by training group.
[3] P values derived by post hoc pairwise comparisons with Bonferroni corrections, reported for measures in which a significant training-by-supplement-group interaction was observed.

effect was observed. The trained group had a significantly higher $\dot{V}O_2$max after treatment than did the untrained group (42.8 ± 3.8 and 39.7 ± 4.5 mL · min^{-1} · kg^{-1}, respectively; linear mixed model $P < 0.001$). Within the training group, the number of training sessions attended (range: 21–40 sessions) was not significantly associated with the 8-wk change in $\dot{V}O_2$max (linear regression: $r^2 = 0.01$, $P = 0.42$).

Interaction of supplement and training

Significant interaction effects between supplement type and training group were observed for sFer and hemoglobin ($P = 0.072$ and 0.030, respectively; Table 5). The study was not powered at the 0.05 level to detect a clinically relevant 2-way interaction between training and supplement type, such as that observed for sFer. In addition, the calculated sample size of 25 women/group was not achieved due to research conditions in the field. Therefore, the P value for the interaction (P = 0.07) was treated as being significant, even though it did not reach the prespecified 0.05 level. A reduced model was analyzed that included only the supplement-by-training group interaction term, where it retained its significance (P = 0.07). Post hoc pairwise comparisons between supplement and training groups were adjusted for multiple comparisons with the use of a Bonferroni correction (Table 5).

In examining the training-by-supplement interaction for sFer, shown in **Figure 2**, both the FeTr and FeUn groups showed significantly higher sFer values than either of the placebo groups. However, sFer in the participants who received training in addition to iron supplementation was significantly lower than that in those participants who did not train. For the training-by-supplement interaction for hemoglobin, the FeUn group had significantly higher hemoglobin concentration after treatment than the PLUn group. There were no other significant post hoc comparisons for the training-by-supplement interaction for hemoglobin.

Academic semester of testing was included as a covariate in all linear mixed models but was not significant. The interaction between training and supplementation for the sFer model was maintained when baseline age, height, weight, and $\dot{V}O_2$max were included as covariates in the models predicting sFer. None of these covariates were significant at $P < 0.05$. To determine whether the changes observed in sFer were independent of hemoglobin, a model was analyzed including hemoglobin as a covariate. The significance of the training-by-supplement interaction and all trends in the post hoc comparisons were maintained when hemoglobin was included as a covariate

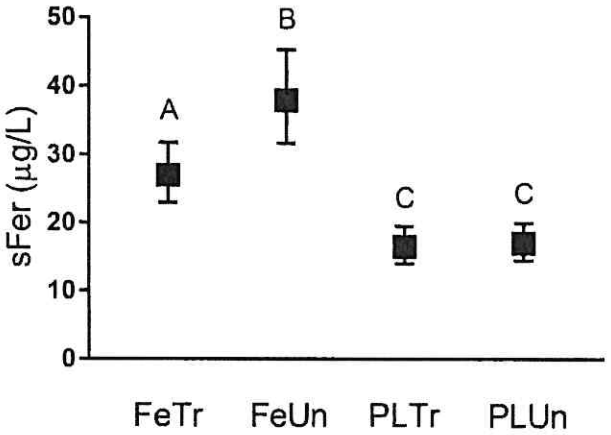

FIGURE 2 Least-square means for the training-by-supplement-type interaction for serum ferritin. Values are post hoc pairwise comparisons between supplement and training groups, with 95% CI bands for the reduced model that did not include time point. n = 19, 16, 18, and 19 for FeTr, FeUn, PLTr, and PLUn groups, respectively. $P = 0.07$ for the supplement-by-training-group interaction. Uppercase letters indicate significant differences between groups with differing letters. Results are from linear mixed models controlling for baseline, in which Bonferroni corrections for multiple comparisons were applied to the post hoc pairwise comparisons between groups. FeTr, iron trained; FeUn, iron untrained; PLTr, placebo trained; PLUn, placebo untrained; sFer, serum ferritin.

(P-interaction = 0.09), whereas hemoglobin was not significant in the model (P = 0.81).

To examine the biological plausibility of the main findings, several secondary analyses were conducted. No relation was found between the number of days trained and change in sFer in either of the supplement groups. In addition, there was no significant association between the number of capsules consumed and the change in sFer in either of the supplement groups, even when sFer status at baseline was included as a covariate. The same result was seen with the use of the more conservative compliance measure for the number of capsules consumed. However, when only those participants who returned 100% of their capsule bottles and daily logs were examined (n = 25), there was a positive relation between the number of capsules consumed and the change in sFer for the iron group (r^2 = 0.39, P = 0.04) but no relation in the placebo group (data not shown).

Participants who had the highest sTfR concentrations at week 0 (i.e., the lowest tissue iron concentrations) showed the largest decreases in sTfR over the 8-wk study period when given iron supplements, but no change was observed in those given the placebo (supplement by week 0 sTfR concentration interaction, linear model $P < 0.001$). This same trend was observed for body iron, with those participants starting with the lowest body iron showing the largest improvements in body iron over the 8-wk study (supplement-by-week 0 body iron concentration interaction, P = 0.03).

In a subgroup analysis of participants with more severe iron depletion (sFer <20 μg/L) at baseline, 100% of the FeUn group repleted their iron stores by 4 wk, and this resolution persisted until 8 wk. Conversely, after 4 wk, only 53.5% ± 12.9% of the FeTr group had resolved their iron depletion and only 73.3% ± 11.4% had resolved their iron depletion by week 8. **Figure 3** shows the prevalence of iron depletion within each group at each time point. RR calculations were performed to determine pairwise differences in percentage resolution between the 4 treatment groups. At the end of the 8-wk study period, the FeTr group was no more likely to be iron replete than the PLTr or PLUn group (FeTr/PLTr: RR = 1.83; 95% CI: 0.92, 3.66; P = 0.51; FeTr/PLUn: RR = 2.05; 95% CI: 0.95, 4.42; P = 0.39); however, the FeUn group was 2.5 times more likely to be replete than the PLTr group and 2.8 times more likely to be replete than the PLUn group [RR: 2.50 (95% CI: 1.35, 4.65; $P < 0.05$) and 2.80 (95% CI: 1.39, 5.65; $P < 0.05$), respectively].

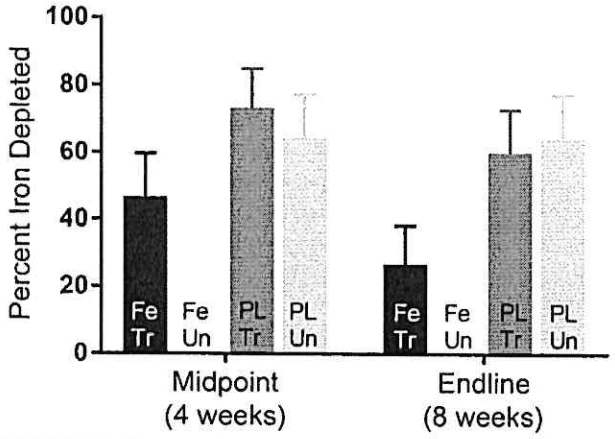

FIGURE 3 Women with iron depletion (sFer <20 μg/L) by treatment group at 4 and 8 wk. All of the participants were iron depleted (sFer <20 μg/L) at baseline. n = 19, 16, 18, and 19 for FeTr, FeUn, PLTr, and PLUn groups, respectively. Values are percentages ± SEs. No FeUn participants were depleted at week 4 or 8 (i.e., there was total resolution of iron depletion in this group). FeTr, iron trained; FeUn, iron untrained; PLTr, placebo trained; PLUn, placebo untrained; sFer, serum ferritin.

DISCUSSION

Improvements in iron status measures in both of the iron-supplemented groups show that the supplementation regimen was adequate to elicit changes in iron status. After 8 wk of treatment, the iron-supplemented groups had significantly higher sFer and body iron and significantly lower (indicating better iron status) sTfR concentrations than the placebo groups. Previous studies have shown that 100 mg ferrous sulfate/d is sufficient to cause changes in iron status markers of similar magnitudes as those observed in this study (4). Similarly, post-training improvements in $\dot{V}O_2$max in the trained groups confirm that the training program was sufficient to induce physiologic adaptations. The high compliance to the training program (89% of participants attended >28 d) likely explains this effect, although the narrow range in the number of sessions attended likely limits our ability to find a dose-response effect of training on $\dot{V}O_2$max.

Aerobic training lowered the effectiveness of iron supplementation in improving traditional measures of iron status, as evidenced by the significant training-by-supplement interaction in linear mixed models of sFer effects. Although both iron-supplemented groups showed significant improvements in sFer, the improvements in the trained participants were significantly smaller than those in the untrained group. One interpretation of this finding is that participants who trained while taking iron supplements did not benefit from the supplements as much as those who did not train.

In addition, by week 8, 100% of the FeUn group had resolved their iron depletion, as defined by sFer <20 μg/L, compared with only 73% of the FeTr group. These findings are consistent with those of McClung et al. (14), who found that participation in basic combat training decreased iron status in female soldiers (27).

Although the modifying effect of aerobic training on iron status is shown by the results of this study, the physiologic mechanism explaining this effect remains unclear. Possible explanations include reduced absorption of iron due to exercise-induced inflammation, increased demand for iron in the oxidative pathways, or increased production of total body hemoglobin or myoglobin, which could be supported by redirecting iron from iron stores toward erythropoietic processes (10, 28–30).

There is currently conflicting evidence about whether long-term moderate exercise results in a chronic inflammatory state that could affect iron status. Both acute and chronic inflammation results in increased concentrations of hepcidin, the primary regulator of iron absorption in the body. Although studies have shown a relation between increased hepcidin expression and lower iron status after acute exercise (31, 32), few studies have shown a long-term impact (32, 33). Outside of the role of hepcidin, several studies have shown that inflammation induced by exercise (34) or chemical injection (35, 36) results in decreased expression of proteins required for iron absorption into the enterocyte. Although this study did not detect inflammation with the use of CRP or AGP, inflammation may still have been

present in this population. This study did not measure hepcidin, its mediator IL-6, or proteins involved in iron absorption. Therefore, we cannot determine whether inflammation or absorption differed between the FeTr and FeUn groups.

There is evidence that exercise-induced increases in erythropoiesis draw iron from the other iron stores in the body to meet the increased iron demand (10, 37), which could explain the smaller increase in sFer observed in the FeTr group than in the FeUn group. However, the details of muscle iron homeostasis in conditions of high erythropoietic demand, such as long-term aerobic training, remain unclear. Some studies have shown that the increased iron demand for erythropoiesis draws iron from myoglobin (10), whereas others suggest that it comes from the liver (11, 37). It is possible that the iron entering the body in the FeTr group was used directly for erythropoiesis or muscle growth. In this study, the FeUn group showed significantly higher hemoglobin after iron supplementation than the PLUn group; however, the FeTr group did not have significantly higher hemoglobin than the other groups. This finding may support the idea that training increased the erythropoietic demand for iron and that the supplemented iron was used directly for this process rather than being stored as ferritin. However, if training were increasing the demand for iron for erythropoiesis, it would therefore be expected that the PLTr group would show decreases in their serum ferritin, which was not observed. It is possible that iron contained in muscle myoglobin was diverted for use in erythropoiesis in the PLTr group; however, this study was unable to assess myoglobin and thus cannot determine whether this was the case.

Finally, it is possible that there was an increased iron demand in skeletal muscle mitochondrial bioenergetics pathways, because many enzymes and cofactors in energy production are iron dependent. It has been shown that ID impairs mitochondrial function (10) and that aerobic exercise training in combination with iron supplementation improves mitochondrial function and density (38–40). Therefore, it is also feasible that the iron in the FeTr group was used to synthesize the enzymes needed for increased energy production or skeletal muscle growth known to occur with training (10, 41).

One limitation of this study is its short duration, which may have been insufficient to observe any long-term effects. In addition, blood and exercise data were not collected for women who dropped out, thus limiting our ability to perform a true intention-to-treat analysis. The participants who dropped out or were excluded from analyses due to discrepancies in their sFer values at baseline may have been systematically different from those who were retained; however, there were no baseline differences between those participants who dropped out or were excluded and those who were retained. The study may have had increased type I error due to the assessment of multiple biomarker variables. In addition, the training group was not blinded, potentially introducing bias. Finally, this study was unable to measure other, potentially more appropriate measures of iron status, such as those involved in inflammation, muscle growth, or erythropoiesis. Further investigation requires more invasive or more expensive analysis techniques, such as metabolomics, that would provide a more comprehensive view of iron metabolism. However, our results support the idea that sFer and hemoglobin may not be the best biomarkers for iron status in nonanemic, physically active populations.

In conclusion, we have shown that regular aerobic training diminishes the effectiveness of iron supplementation on improving sFer in iron-depleted, nonanemic women compared with untrained women. Iron supplementation was still able to increase sFer in trained women, but at a slower rate. This finding could have implications for interventions aiming to improve iron status in physically active populations, such as in women who train casually to improve fitness in developed countries or in developing countries where heavy manual labor is necessary for economic livelihoods. Typically, iron supplementation interventions can have durations of 4–6 wk at the dose used in this study (4, 42). This dose can be sufficient in that time frame, as evidenced by the 100% resolution of iron depletion in the FeUn group. However, our study suggests that longer interventions may be necessary to improve iron stores in active women. Further research should explore whether a larger dose of iron can counteract the modifying effect that daily exercise has on improvements in sFer and whether this effect has any long-term impacts. In addition, further research should determine an optimal iron intervention dose and duration for active populations and develop other, more appropriate biomarkers that are more indicative of iron involved in muscle metabolism and erythropoietic processes.

We thank Lynn Johnson at the Cornell Statistical Consulting Unit for her help with the statistical analyses for this article. We also thank Yijing Gong, Wanhui Kang, Guanchen Liu, and Cindy Wu for their contributions to translating and analyzing the dietary record data and He Yuxu and Cai Le from Kunming Medical University and Yunnan University of Traditional Chinese Medicine for their assistance in data collection.

The authors' responsibilities were as follows—LMP: conducted the research, analyzed the data, and had primary responsibility for final content; and both authors: designed the research, wrote the manuscript, and read and approved the final manuscript. Neither of the authors reported a conflict of interest related to the study.

REFERENCES

1. Qin Y, Melse-Boonstra A, Pan X, Yuan B, Dai Y, Zhao J, Zimmermann MB, Kok FJ, Zhou M, Shi Z. Anemia in relation to body mass index and waist circumference among Chinese women. Nutr J 2013;12:10.
2. Luo R, Wang X, Zhang L, Liu C, Shi Y, Miller G, Rozelle S, Yu E, Martorell R. High anemia prevalence in western China. Southeast Asian J Trop Med Public Health 2011;42:1204–13.
3. Yue A, Marsh L, Zhou H, Medina A, Luo R, Shi Y, Zhang L, Kenny K, Rozelle S. 2016. Nutritional deficiencies, the absence of information and caregiver shortcomings: a qualitative analysis of infant feeding practices in rural China. PLoS One 2016;11:e0153385.
4. Hinton PS, Giordano C, Brownlie T, Haas JD. Iron supplementation improves endurance after training in iron-depleted, nonanemic women. J Appl Physiol 2000;88:1103–11.
5. Brownlie T, Utermohlen V, Hinton PS, Haas JD. Tissue iron deficiency without anemia impairs adaptation in endurance capacity after aerobic training in previously untrained women. Am J Clin Nutr 2004;79:437–43.
6. McClung JP, Karl JP, Cable SJ, Williams KW, Young AJ, Lieberman HR. Longitudinal decrements in iron status during military training in female soldiers. Br J Nutr 2009;102:605–9.
7. Karl JP, Lieberman HR, Cable SJ, Williams KW, Young AJ, McClung JP. Randomized, double-blind, placebo-controlled trial of an iron-fortified food product in female soldiers during military training: relations between iron status, serum hepcidin, and inflammation. Am J Clin Nutr 2010;92:93–100.
8. Di Santolo M, Stel G, Banfi G, Gonano F, Cauci S. Anemia and iron status in young fertile non-professional female athletes. Eur J Appl Physiol. 2008;102:703–9.

9. McClung JP, Murray-Kolb LE. Iron nutrition and premenopausal women: effects of poor iron status on physical and neuropsychological performance. Annu Rev Nutr 2013;33:271–88.
10. Buratti P, Gammella E, Rybinska I, Cairo G, Recalcati S. 2015. Recent advances in iron metabolism: relevance for health, exercise, and performance. Med Sci Sports Exerc 2015;47:1596–604.
11. Díaz V, Gammella E, Recalcati S, Santambrogio P, Naldi AM, Vogel J, Gassmann M, Cairo G. Liver iron modulates hepcidin expression during chronically elevated erythropoiesis in mice. Hepatology 2013; 58:2122–32.
12. Robach P, Cairo G, Gelfi C, Bernuzzi F, Pilegaard H, Vigano A, Santambrogio P, Cerretelli P, Calbet JAL, Moutereau S, et al. Strong iron demand during hypoxia-induced erythropoiesis is associated with down-regulation of iron-related proteins and myoglobin in human skeletal muscle. Blood 2007;109:4724–31.
13. Robach P, Recalcati S, Girelli D, Gelfi C, Aachmann-Andersen NJ, Thomsen JJ, Norgaard AM, Alberghini A, Campostrini N, Castagna A, et al. Alterations of systemic and muscle iron metabolism in human subjects treated with low-dose recombinant erythropoietin. Blood 2009;113:6707–15.
14. McClung JP, Karl JP, Cable SJ, Williams KW, Nindl BC, Young AJ, Lieberman HR. Randomized, double-blind, placebo-controlled trial of iron supplementation in female soldiers during military training: effects on iron status, physical performance, and mood. Am J Clin Nutr 2009;90:124–31.
15. Hinton PS, Sinclair LM. Iron supplementation maintains ventilatory threshold and improves energetic efficiency in iron-deficient nonanemic athletes. Eur J Clin Nutr 2007;61:30–9.
16. Clénin G, Cordes M, Huber A, Schumacher YO, Noack P, Scales J, Kriemler S. Iron deficiency in sports—definition, influence on performance and therapy. Swiss Med Wkly 2015;145:w14196.
17. Borrione P, Spaccamiglio A, Rizzo M, Termine A, Chierto E, Campostrini N, Quaranta F, Di Gianfrancesco A, Pigozzi F. Urinary hepcidin identifies a serum ferritin cut-off for iron supplementation in young athletes: a pilot study. J Biol Regul Homeost Agents 2011;25: 427–34.
18. Yu D, Huo J, Xie L, Wang L. [Meta-analysis of studies on cut-off value of serum ferritin for identifying iron deficiency.] Wei Sheng Yan Jiu 2013;42:228–35.
19. Peeling P, Sim M, Badenhorst CE, Dawson B, Govus AD, Abbiss CR, Swinkels DW, Trinder D. Iron status and the acute post-exercise hepcidin response in athletes. PLoS One 2014;9:e93002.
20. WHO. Iron deficiency anaemia: assessment, prevention and control. Geneva (Switzerland): WHO; 2001. [cited 2016 Mar 10]. Available from: http://www.who.int/nutrition/publications/micronutrients/anaemia_iron_deficiency/WHO_NHD_01.3/en/.
21. Zhu YI, Haas JD. Response of serum transferrin receptor to iron supplementation in iron-depleted, nonanemic women. Am J Clin Nutr 1998;67:271–5.
22. Cook JD, Flowers CH, Skikne BS. The quantitative assessment of body iron. Blood 2003;101:3359–64.
23. Lohman TG, Roche AF, Martorell R. Anthropometric standardization reference manual. Champaign (IL): Human Kinetics Books; 1988.
24. Durnin JV, Womersley J. Body fat assessed from total body density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16 to 72 years. Br J Nutr 1974;32:77–97.
25. Brownlie T IV, Utermohlen V, Hinton PS, Giordano C, Haas JD. Marginal iron deficiency without anemia impairs aerobic adaptation among previously untrained women. Am J Clin Nutr 2002;75:734–42.
26. Institute of Medicine. Dietary Reference Intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc [Internet]. 2001. [cited 2017 May 31]. Available from: http://www.nationalacademies.org/hmd/Reports/2001/Dietary-Reference-Intakes-for-Vitamin-A-Vitamin-K-Arsenic-Boron-Chromium-Copper-Iodine-Iron-Manganese-Molybdenum-Nickel-Silicon-Vanadium-and-Zinc.aspx.
27. Yanovich R, Karl JP, Yanovich E, Lutz LJ, Williams KW, Cable SJ, Young AJ, Pasiakos SM, McClung JP. Effects of basic combat training on iron status in male and female soldiers: a comparative study. US Army Med Dep J 2015;67–73.
28. Scheuer J, Tipton CM. Cardiovascular adaptations to physical training. Annu Rev Physiol 1977;39:221–51.
29. Blomqvist CG, Saltin B. Cardiovascular adaptations to physical training. Annu Rev Physiol 1983;45:169–89.
30. Sawka MN, Convertino VA, Eichner ER, Schnieder SM, Young AJ. Blood volume: importance and adaptations to exercise training, environmental stresses, and trauma/sickness. Med Sci Sports Exerc 2000; 32:332–48.
31. Peeling P, Dawson B, Goodman C, Landers G, Trinder D. Athletic induced iron deficiency: new insights into the role of inflammation, cytokines and hormones. Eur J Appl Physiol 2008;103:381–91.
32. Dzedzej A, Ignatiuk W, Jaworska J, Grzywacz T, Lipińska P, Antosiewicz J, Korek A, Ziemann E. The effect of the competitive season in professional basketball on inflammation and iron metabolism. Biol Sport 2016;33:223–9.
33. Skarpańska-Stejnborn A, Basta P, Trzeciak J, Szcześniak-Pilaczyńska Ł. Effect of intense physical exercise on hepcidin levels and selected parameters of iron metabolism in rowing athletes. Eur J Appl Physiol 2015; 115:345–51.
34. Liu Y-Q, Chang Y-Z, Zhao B, Wang H-T, Duan X-L. Does hepatic hepcidin play an important role in exercise-associated anemia in rats? Int J Sport Nutr Exerc Metab 2011;21:19–26.
35. Barrett TD, Palomino HL, Brondstetter TI, Kanelakis KC, Wu X, Yan W, Merton KP, Schoetens F, Ma JY, Skaptason J, et al. Prolyl hydroxylase inhibition corrects functional iron deficiency and inflammation-induced anaemia in rats. Br J Pharmacol 2015;172:4078–88.
36. Deschemin J-C, Vaulont S. Role of hepcidin in the setting of hypoferremia during acute inflammation. PLoS One 2013;8:e61050.
37. Kautz L, Jung G, Nemeth E, Ganz T. Erythroferrone contributes to recovery from anemia of inflammation. Blood 2014;124:2569–74.
38. Willis WT, Jones PH, Chengson R, Dallman PR. Hepatic adaptations to iron deficiency and exercise training. J Appl Physiol 1992;73:510–5.
39. Willis WT, Dallman PR. Impaired control of respiration in iron-deficient muscle mitochondria. Am J Physiol 1989;257:C1080–5.
40. Johnson JA, Willis WT, Dallman PR, Brooks GA. Muscle mitochondrial ultrastructure in exercise-trained iron-deficient rats. J Appl Physiol 1990; 68:113–8.
41. Willis WT, Dallman PR, Brooks GA. Physiological and biochemical correlates of increased work in trained iron-deficient rats. J Appl Physiol 1988;65:256–63.
42. DellaValle DM, Haas JD. Iron supplementation improves energetic efficiency in iron-depleted female rowers. Med Sci Sports Exerc 2014; 46:1204–15.