

Tissue iron deficiency without anemia impairs adaptation in endurance capacity after aerobic training in previously untrained women¹⁻³

Thomas Brownlie IV, Virginia Utermohlen, Pamela S Hinton, and Jere D Haas

ABSTRACT

Background: We previously showed that iron supplementation significantly improves iron status and maximal work capacity in previously untrained, marginally iron-deficient women with a baseline serum transferrin receptor concentration > 8.0 mg/L. However, the effect of transferrin receptor status on adaptation in endurance capacity after aerobic training in these subjects has not been fully explored.

Objective: Our objective was to examine the effect of baseline serum transferrin receptor status on adaptations in endurance capacity.

Design: Forty-one untrained, iron-depleted, nonanemic women were randomly assigned to receive either 100 mg FeSO₄ or a placebo for 6 wk in a double-blind trial. All subjects trained on cycle ergometers 5 d/wk for the last 4 wk of the study. Endurance capacity was assessed at baseline and after treatment by using a 15-km time trial conducted on a cycle ergometer.

Results: Significant treatment effects were observed for time to complete the 15-km time trial, work rate, and percentage of maximal oxygen uptake in subjects with a baseline serum transferrin receptor concentration > 8.0 mg/L. No significant treatment effects were observed in subjects with a normal baseline transferrin receptor concentration.

Conclusions: Our findings suggest that, in the presence of overt tissue iron deficiency, iron deficiency without anemia impairs adaptation in endurance capacity after aerobic training in previously untrained women. This impairment can be corrected with iron supplementation. *Am J Clin Nutr* 2004;79:437-43.

KEY WORDS Iron deficiency without anemia, iron depletion, aerobic training, serum transferrin receptors, serum ferritin, endurance capacity, women

INTRODUCTION

Iron deficiency anemia has been shown to reduce maximal work capacity, endurance capacity, and voluntary activity (1). However, the functional consequences of iron deficiency without anemia are not fully understood, despite a high prevalence in the United States and worldwide (2).

Animal studies showed that concentrations and activities of iron-containing muscle oxidative enzymes and respiratory proteins decrease during iron deficiency without anemia (3-6). Moreover, iron deficiency without anemia has been shown to reduce endurance capacity and diminish the effects of training in animal models (3, 5-8). Conversely, findings from human studies examining the relation between iron deficiency without anemia and adaptation to training are equivocal (9-13).

Previous work in our laboratory showed that after work rate is controlled for, iron deficiency without anemia reduces the endurance capacity of untrained women by increasing energy expenditure (EE) and percentage of maximal oxygen uptake (% $\dot{V}O_{2max}$) (14). In a subsequent study, we examined the effects of iron deficiency without anemia on adaptations to aerobic training. We found that iron supplementation significantly enhanced adaptations in both maximal work capacity (15) and endurance capacity (13) after training. Additionally, we found significant interactions between baseline serum transferrin receptor (sTfR) concentrations and treatment group on improvements in iron status, maximal work capacity, and endurance performance (13, 15). These findings showed that initial sTfR concentrations modify the effects of iron supplementation on adaptation in $\dot{V}O_{2max}$ (15), time to complete a 15-km time trial, percentage of maximal capacity at which subjects work during a trial, and work rate (13).

Stratified analyses were conducted to further examine the relation between initial sTfR status and improvements in maximal work capacity. The stratified analyses showed that the significant treatment effects that were observed for iron status and maximal work capacity were due to improvements in the subjects who began the study with an elevated sTfR concentration (ie, in the subjects whose tissue iron status was more depleted) (15). No significant treatment effects were observed in the subjects who had a normal sTfR concentration. These findings prompted us to further examine the effect of baseline sTfR status on adaptations in endurance performance by using stratified analyses. We expected that subjects with an elevated baseline sTfR concentration would have the greatest improvements in endurance capacity after iron supplementation and training. Results from these analyses are reported in this article.

SUBJECTS AND METHODS

Subjects

Two hundred seventy-one physically active, untrained women aged between 18 and 33 y were recruited from the local community through fliers, newspaper announcements, and internet list-

¹ From the Division of Nutritional Sciences, Cornell University, Ithaca, NY.

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³ Reprints not available. Address correspondence to T Brownlie IV, Savage Hall, Cornell University, Ithaca, NY 14853. E-mail: tb22@cornell.edu. Received December 23, 2002.

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ings. After preliminary screenings, 51 women were identified as iron depleted and nonanemic on the basis of a serum ferritin concentration $< 16 \mu\text{g/L}$ and a hemoglobin concentration $> 120 \text{ g/L}$. Forty-nine women gave signed informed consent to participate in the study. After a physical examination, which included a medical history, none of the subjects were excluded from the study on the basis of the following criteria: current pregnancy or pregnancy within the previous year, recent infectious illness or fever, hemolytic anemia, asthma, musculoskeletal problems, recent history of eating disorders, smoking, excess alcohol consumption, recent use of recreational drugs, consumption of prescription medications that may interfere with dietary iron absorption, or participation in competitive athletics. The Cornell University Committee on Human Subjects approved this study.

Study design

The study was a randomized, double-blind, placebo-controlled intervention trial. Subjects were randomly assigned to receive either an iron supplement (50 mg FeSO_4 , 8 mg elemental Fe) or an identical placebo capsule twice daily for 6 wk. All supplements were prepared in our laboratory by using gelatin capsules (Apothecary Products Inc, Minneapolis). The iron supplements contained ferrous sulfate plus lactose filler, and the placebo supplements contained only lactose filler. From a random sample of 20 capsules, the mean (\pm SD) ferrous sulfate content of the iron capsules was determined to be $49.4 \pm 4.2 \text{ mg}$. The subjects were instructed to consume the capsules with citrus juice to enhance iron absorption and with meals to reduce possible side effects. They were also instructed to avoid consumption of any other multivitamin or mineral supplements during the entire study period. The subjects recorded capsule ingestion, consumption of medication, illness, menstrual status, gastrointestinal symptoms, physical activity, and musculoskeletal problems in a daily log. The subjects and the investigators were blinded to the group assignment until completion of the data collection.

Training

The subjects trained 5 d/wk for 4 wk beginning on the first day of week 3 of the study. To ensure that the subjects understood and complied with the training protocols, the first training session of each week was supervised. All other training sessions were self-reported. Training was performed in the research laboratory on a cycle ergometer (Ergociser E-3200; Cateye Co, Ltd, Osaka, Japan) equipped with a heart rate (HR) monitor and digital output of cadence (in rpm) and work (in W). The training sessions consisted of a 4-min warm-up at no resistance; a 25-min cycling session divided between workloads that were targeted at 75% and 85% of the subjects' maximum HR (HRmax), as determined during a previous test of peak work capacity (see below); and a 1-min cooldown. Over the 4 wk, time spent training at 85% HRmax increased weekly from 5 to 15 min with a corresponding decrease in the duration of cycling at 75% HRmax. The first week of training consisted of cycling for 20 min at 75% HRmax followed by 5 min at 85% HRmax. Week 2 consisted of cycling for 18 min at 75% HRmax and 7 min at 85% HRmax. By week 4 subjects cycled for 10 min at 75% HRmax and 15 min at 85% HRmax. Subjects recorded HR, average pedaling rate (cadence), and work (W) for each training session in a training log.

Prestudy habitual physical activity levels were assessed by a frequency questionnaire, which was analyzed by using the

method described previously (16) to obtain a physical activity score for each subject. This was done to ensure similar habitual physical activity levels between the groups after randomization. The subjects were asked to maintain the same nontraining activity level during the entire study period to ensure that the prescribed training regimen was the only additional source of training.

For all the subjects, body composition and physical performance were measured immediately before and after the 6-wk treatment period. In addition, dietary iron intake was assessed from a 4-d dietary record. All dietary records were analyzed by using NUTRITIONIST IV (version 3.0; First Databank, Inc, San Bruno, CA).

Physiologic measurements

Exercise tests were conducted on a mechanically braked, calibrated cycle ergometer (model 818E; Monark, Varberg, Sweden) with the use of a computerized metabolic cart (Physiodyne, Quogue, NY) in the Human Bioenergetics Laboratory at Cornell University. The ergometer was equipped with a digital readout of cadence (in rpm) and distance (in km) pedaled. Concentrations of oxygen and carbon dioxide in expired air were analyzed with Amotek gas analyzers (Pittsburgh); respiratory volume was analyzed with a Fitco Micro Flow respiratory pneumotachograph (Fitness Instrument Technologies, Farmingdale, NY). A Hans Rudolph T-Shape breathing valve was used for all tests (Kansas City, MO). Data output from the instruments was directed to an IBM 386 computer (Gateway, Inc, Poway, CA) for breath-by-breath calculation of oxygen consumption ($\dot{V}\text{O}_2$), CO_2 production ($\dot{V}\text{CO}_2$), respiratory exchange ratio (RER, $\dot{V}\text{CO}_2/\dot{V}\text{O}_2$), and minute ventilation. HR was monitored throughout the tests with an electrocardiograph (Burdick, Milton, WI). Electrocardiograph leads were connected at sites V1 and V6 and between intercostals 3 and 4 on the right side of the rib cage.

The subjects were asked not to perform any strenuous physical activities 2 d before the exercise tests. To control for the effects of dietary intake before exercise testing, the subjects were asked to start recording their food intake 3 d before baseline exercise testing and to continue the recording through the last day of baseline testing. They were instructed to consume the same diet for the baseline and posttreatment exercise tests. The subjects were instructed not to consume food or caffeinated beverages 3 h before exercise testing.

Peak maximal work capacity ($\dot{V}\text{O}_{2\text{max}}$) was assessed as previously described (15). Endurance performance was assessed from a 15-km time trial, which was divided into three 5-km bouts. Between each bout, the subjects were provided water and allowed to rest for 2 min. The subjects were asked to finish each bout as quickly as possible. The workload was standardized for each subject by setting the resistance to achieve 70% of $\dot{V}\text{O}_{2\text{max}}$ while the subject pedaled at 60 rpm (determined from the peak maximal work capacity test). The same resistance was used at the baseline and posttreatment endurance tests.

Performance was based on time to complete the time trial (TT time), mean oxygen consumption per minute (absolute $\dot{V}\text{O}_2$), mean oxygen consumption per minute per kilogram of fat-free mass (relative $\dot{V}\text{O}_2$), the ratio of mean $\dot{V}\text{O}_2$ to $\dot{V}\text{O}_{2\text{max}}$ (% $\dot{V}\text{O}_{2\text{max}}$), mean RER, mean EE, EE per minute, and external work performed per minute (work rate). Work rate (in W) was calculated from cadence (in rpm) and ergometer resistance (in kilopounds). EE in kilocalories was calculated from the

average $\dot{V}O_2$ and average nonprotein RER according to McArdle et al (17). EE in watts was calculated by multiplying EE in kilocalories by a conversion factor of 69.75. Efficiency (energy expended/external work performed) was calculated as the ratio of EE/min (in W) to work rate (in W).

Body size and composition were measured in the Human Body Composition Laboratory at Cornell University. Anthropometric measurements (weight, height) were made by using standard procedures described in Lohman et al (18). Percentage body fat and fat-free mass were determined by densitometry with the use of the technique described by Akers and Buskirk (19). The Siri equation adapted for females was used, and the sex-specific density of fat-free mass was assumed to be 1.096 g/mL (20).

Iron-status measurements

Iron status was assessed from nonfasting blood samples that were collected at screening, baseline, and the end of weeks 3 and 6. Because serum iron-status indicators are not immediately influenced by food intake, the subjects did not fast before having their blood drawn. Because of time and scheduling constraints, neither the time of day at which the samples were drawn (1000–1400) nor menstrual phase was standardized. However, any possible confounding effect that either of these factors may have had on iron status was controlled for through randomization.

Hemoglobin and hematocrit were assayed in whole blood immediately after sample collection. To control for potential variation in assay conditions, all plasma and serum samples were frozen at -20°C for ≤ 3 mo. Each subject's complete set of samples was then analyzed concurrently at the completion of the study.

Hemoglobin concentrations were measured by using the cyanomethemoglobin method described by van Assendelft and England (21) (Sigma Diagnostics, St Louis). Hematocrit was determined by using the microhematocrit method. Soluble sTfR and serum ferritin concentrations were measured by using enzyme-linked immunosorbent assays with commercial kits (Ramco Laboratories, Houston) according to the methods of Flowers et al (22, 23). Transferrin saturation was determined from the ratio of serum iron to total-iron-binding capacity by the method described by Persijn et al (24) (Sigma Diagnostics).

Statistical analysis

Data were examined to verify normality of distribution. Results are expressed as means \pm SEMs. Independent Student's *t* test was used to test group differences at baseline. Three-factor repeated-measures analysis of variance was used to assess the effect of baseline sTfR on iron and fitness outcomes. When significant group \times time \times baseline sTfR interactions were observed, the data were stratified by baseline sTfR to further examine the relations. To maximize the likelihood that subjects in the upper stratum were truly tissue iron deficient (normal range: 2.8–8.5 $\mu\text{g/L}$) (23) and to maintain an adequate sample size within each stratum, we chose a cutoff of 8.0 $\mu\text{g/L}$.

Repeated-measures analysis of variance was used to test group and time effects as well as group \times time interactions for both iron status and fitness outcomes. An exact *F* test statistic was used for all repeated-measures analyses. Statistical significance was indicated at $P < 0.05$. Statistical analyses were performed by using JMP version 3.1.5 (SAS Institute Inc, Cary, NC).

TABLE 1
Dietary profiles from 4-d dietary records completed by iron-deficient, nonanemic women¹

Variable	Value
Energy (kcal/d)	
Placebo group	1769 \pm 94
Iron group	1560 \pm 82
Carbohydrates (% of energy)	
Placebo group	63.4 \pm 1.8
Iron group	58.1 \pm 1.7 ²
Protein (% of energy)	
Placebo group	14.0 \pm 0.6
Iron group	13.7 \pm 0.6
Fat (% of energy)	
Placebo group	22.0 \pm 1.8
Iron group	28.0 \pm 1.7 ²
Iron (mg/d)	
Placebo group	14.8 \pm 2.6
Iron group	14.4 \pm 1.4
Vitamin C (mg/d)	
Placebo group	144 \pm 43
Iron group	118 \pm 18
Calcium (mg/d)	
Placebo group	883 \pm 123
Iron group	703 \pm 74
Fiber (g/d)	
Placebo group	16.9 \pm 2.1
Iron group	13.4 \pm 1.3

¹ $\bar{x} \pm$ SEM; *n* = 19 in the placebo group and 22 in the iron group.

² Significantly different from the placebo group, $P < 0.05$ (Student's two-tailed *t* test).

RESULTS

Subject characteristics

Forty-nine women participated in the study. Three women dropped out during the study for personal reasons not related to the study. Data for 4 additional women were excluded from analysis because of these women's high baseline serum ferritin concentrations ($> 16 \mu\text{g/L}$), which indicated that their ferritin values at screening had caused them to be misclassified as iron depleted; one subject was excluded because of missing final data for $\dot{V}O_{2\text{max}}$. The final sample included 19 subjects in the placebo group and 22 subjects in the iron group. Those who dropped out or were excluded were not different in baseline body composition, iron status, or physical performance from those who completed the study.

Diet analysis

Results from the 4-d dietary records are presented in **Table 1**. There were no significant differences in the amounts of calories, iron, vitamin C, calcium, or fiber consumed per day or in the percentage of energy derived from protein. However, the iron group reported consuming a significantly lower percentage of energy from carbohydrates ($P < 0.05$) and a significantly higher percentage of energy from fat ($P < 0.05$) than did the placebo group.

Training

The total number of training sessions (placebo group, 19.7 \pm 0.1 d; iron group, 19.9 \pm 0.1 d) and the total work performed

TABLE 2
Iron status results in iron-deficient, nonanemic women¹

	Baseline	Week 3	After treatment
Hemoglobin (g/L)			
Placebo group	132.2 ± 4.2	127.8 ± 3.9	130.8 ± 3.0
Iron group	134.2 ± 2.6	133.6 ± 3.6	135.2 ± 2.0
Hematocrit (%) ²			
Placebo group	37.9 ± 0.6	37.2 ± 0.7	38.0 ± 0.7
Iron group	38.5 ± 0.5	37.7 ± 0.6	39.8 ± 0.6
Ferritin (μg/L) ^{2,4}			
Placebo group	8.07 ± 0.77	9.01 ± 1.06	8.11 ± 0.90
Iron group	10.38 ± 0.82	13.71 ± 1.35	14.52 ± 1.5
sTfR (mg/L)			
Placebo group	7.94 ± 0.73	7.45 ± 0.85	7.93 ± 0.77
Iron group	7.92 ± 0.87	6.59 ± 0.53	6.78 ± 0.42
Serum iron (μmol/L) ^{2,5}			
Placebo group	13.4 ± 1.6	15.6 ± 2.0	12.2 ± 1.8
Iron group	12.2 ± 2.5	19.0 ± 2.9	19.4 ± 2.7
TIBC (μmol/L)			
Placebo group	65.0 ± 2.5	64.7 ± 3.5	60.7 ± 3.0
Iron group	64.9 ± 3.6	59.6 ± 3.7	62.1 ± 3.8
TS (%) ^{2,5}			
Placebo group	20.9 ± 2.0	24.1 ± 2.9	22.0 ± 3.7
Iron group	18.7 ± 3.7	30.9 ± 3.7	31.9 ± 4.2

¹ $\bar{x} \pm \text{SEM}$; $n = 19$ in the placebo group and 22 in the iron group. sTfR, serum transferrin receptor; TIBC, total-iron-binding capacity; TS, transferrin saturation.

² Significant time effect, $P < 0.05$ (two-factor repeated-measures ANOVA).

³ Significant group effect, $P < 0.05$ (two-factor repeated-measures ANOVA).

⁴ Marginally significant group \times time interaction, $P = 0.09$ (two-factor repeated-measures ANOVA).

⁵ Significant group \times time interaction, $P < 0.05$ (two-factor repeated-measures ANOVA).

(placebo group, 59 805 ± 2621 W; iron group, 54 625 ± 2094 W) did not differ significantly between the groups, nor were there significant differences between the groups in habitual physical activity (data not shown). Weekly meetings between the subjects and the research staff, along with a daily training log in which the subjects recorded their average HR, cadence, and ergometer resistance settings required to achieve the target HRs, confirmed training compliance.

Supplementation compliance

Supplementation compliance was assessed from a personal log in which the subjects recorded the number of capsules taken daily. These data indicated that the subjects had a high rate of compliance (placebo group, 88.6%; iron group, 91.4%) and that compliance did not differ significantly between the groups (placebo group, 74.4 ± 3.0 capsules; iron group, 76.8 ± 1.6 capsules). The frequency and severity of reported side effects (eg, tiredness, stomach pain) due to supplementation were very low and did not differ significantly between the groups.

Iron responses

Iron responses in the complete sample are presented in Table 2. Significant group \times time interactions were observed for serum iron and transferrin saturation. Significant time effects were observed for hematocrit, serum ferritin, serum iron, and transferrin

TABLE 3
Physiologic responses before and after 6 wk of supplementation with iron or placebo and 4 wk of training in iron-depleted, nonanemic women¹

Variable	Baseline	After treatment
Absolute $\dot{V}O_2$ (L/min)		
Placebo group	1.89 ± 0.07	1.99 ± 0.09
Iron group	1.79 ± 0.07	1.93 ± 0.07
Relative $\dot{V}O_2$ (mL · min ⁻¹ · kg FFM ⁻¹)		
Placebo group	43.3 ± 1.2	45.1 ± 1.7
Iron group	41.7 ± 1.4	44.9 ± 1.1
HR (bpm)		
Placebo group	172 ± 3	170 ± 3
Iron group	176 ± 2	170 ± 3
RER		
Placebo group	0.94 ± 0.02	0.89 ± 0.02
Iron group	0.93 ± 0.01	0.87 ± 0.02
Total EE (kcal)		
Placebo group	298 ± 10	294 ± 10
Iron group	292 ± 12	282 ± 10
EE (kcal/min)		
Placebo group	9.4 ± 0.4	9.8 ± 0.4
Iron group	8.8 ± 0.4	9.4 ± 0.3
Efficiency (%)		
Placebo group	18.5 ± 0.4	19.0 ± 0.5
Iron group	17.7 ± 0.5	18.4 ± 0.5

¹ $\bar{x} \pm \text{SEM}$; $n = 19$ in the placebo group and 22 in the iron group. $\dot{V}O_2$, oxygen uptake; FFM, fat-free mass; HR, heart rate; RER, respiratory exchange ratio; EE, energy expenditure. There were no significant group \times time \times baseline serum transferrin receptor interactions. There was a significant main effect of time ($P < 0.05$, repeated-measures ANOVA) for all variables except total EE and efficiency. For efficiency there was a marginally significant main effect of time ($P = 0.09$, repeated-measures ANOVA).

saturation. A significant group effect was observed for serum ferritin. No significant treatment effects were observed for sTfR, total-iron-binding capacity, or hemoglobin. Because significant differences were observed in the percentage of energy derived from carbohydrates and fat, these variables were included as covariates in statistical models to exclude possible confounding of iron responses by diet. None of the variables were significant in the models and were subsequently removed.

Physiologic responses

After posttreatment % $\dot{V}O_2$ max was controlled for, three-way repeated-measures analysis of variance showed a significant group \times time \times baseline sTfR interaction ($P = 0.027$) for change in TT time. Similarly, significant interactions were observed for change in work rate after posttreatment % $\dot{V}O_2$ max was controlled for ($P = 0.026$) and for change in % $\dot{V}O_2$ max after change in work rate was controlled for ($P = 0.028$).

No significant three-way interactions were observed for absolute $\dot{V}O_2$, relative $\dot{V}O_2$, HR, RER, total EE, EE/min, or efficiency. However, there were significant time effects for all but total EE and efficiency. Results for these responses are presented in Table 3.

Physiologic responses stratified by baseline sTfR concentration

Stratified analyses were performed to further evaluate the three-way interactions between group, time, and baseline sTfR

TABLE 4

Physiologic responses before and after 6 wk of supplementation with iron or placebo and 4 wk of training stratified by baseline serum transferrin receptor (sTfR) concentration in iron-depleted, nonanemic women¹

Variable	sTfR \leq 8.0 mg/L		sTfR $>$ 8.0 mg/L	
	Baseline	After treatment	Baseline	After treatment
TT time (min)				
Placebo group	31.3 \pm 0.7	29.2 \pm 0.6	33.2 \pm 0.6	32.6 \pm 1.2
Iron group	32.8 \pm 0.6	29.4 \pm 0.8	33.4 \pm 0.9	30.0 \pm 0.6
% $\dot{V}O_2$ max (%)				
Placebo group	79.6 \pm 2.6	75.2 \pm 2.2	81.6 \pm 4.2	83.9 \pm 2.4
Iron group	84.9 \pm 1.8	78.8 \pm 2.0	87.7 \pm 5.0	78.2 \pm 4.3
Work rate (W)				
Placebo group	125 \pm 6	136 \pm 7	113 \pm 11	116 \pm 11
Iron group	111 \pm 7	123 \pm 7	105 \pm 8	117 \pm 7

¹ $\bar{x} \pm$ SEM. For the subjects with an sTfR concentration \leq 8.0 mg/L, $n = 12$ in the placebo group and 15 in the iron group; for the subjects with an sTfR concentration $>$ 8.0 mg/L, $n = 7$ in the placebo group and 7 in the iron group. TT time, time to complete the time trial; $\dot{V}O_2$ max, maximal oxygen uptake. There was a significant main effect of time ($P < 0.05$, repeated-measures ANOVA) for all variables in the subjects with an sTfR concentration \leq 8.0 mg/L. There were significant group \times time \times sTfR interactions ($P < 0.05$, repeated-measures ANOVA) for all variables in the subjects with an sTfR concentration $>$ 8.0 mg/L. Also in the subjects with an sTfR concentration $>$ 8.0 mg/L, there was a significant main effect of time ($P < 0.05$, repeated-measures ANOVA) for TT time and work rate. There was also a significant group \times time interaction ($P < 0.05$, repeated-measures ANOVA) for $\dot{V}O_2$ max and marginally significant group \times time interactions ($P = 0.07$, repeated-measures ANOVA) for TT time and work rate in the subjects with an sTfR concentration $>$ 8.0 mg/L.

on the physiologic responses. The stratified analyses are presented in Table 4. There were no significant differences in baseline endurance performance between the sTfR strata.

In the subjects with an elevated sTfR concentration, a significant group \times time interaction ($P < 0.05$) was observed for % $\dot{V}O_2$ max. The iron group also tended to have greater improvements in TT time (group \times time interaction, $P = 0.07$) and work rate (group \times time interaction, $P = 0.07$) than did the placebo group; however, these differences were not significant.

Significant effects of time ($P < 0.05$) were observed for TT time, absolute $\dot{V}O_2$, relative $\dot{V}O_2$, % $\dot{V}O_2$ max, HR, RER, EE/min, and work rate in subjects with a normal baseline sTfR concentration. However, there were no significant group \times time interactions.

Similar to the iron analyses, percentages of energy derived from carbohydrates and fat were included as covariates in preliminary statistical models to exclude possible confounding by diet. None of the variables were significant in the models and were subsequently removed.

Physiologic responses stratified by 5-km bout

Because the time trial was conducted as three 5-km bouts separated by 2-min breaks, the data for each 5-km bout were also analyzed separately. Results from these analyses are depicted in Figures 1–3. For clarity of presentation, results for subjects with normal and elevated baseline sTfR concentrations are depicted separately.

As shown in Figure 1A, there were no significant group effects or group \times time interactions for TT time during any of the posttreatment 5-km bouts in the subjects with a normal baseline sTfR concentration. However, there were significant effects of time for all of the 5-km bouts. Conversely, as shown in Figure 1B, a group \times time interaction was observed for TT time during the second 5-km bout in the subjects with an elevated baseline sTfR concentration; however, the effect was not significant ($P < 0.10$). A significant group \times time interaction ($P < 0.05$) was observed for TT time during the third 5-km bout.

As shown in Figure 2A, there were no significant group effects

or group \times time interactions for change in work rate during any of the 5-km bouts in the subjects with a normal baseline sTfR concentration. However, there were significant effects of time

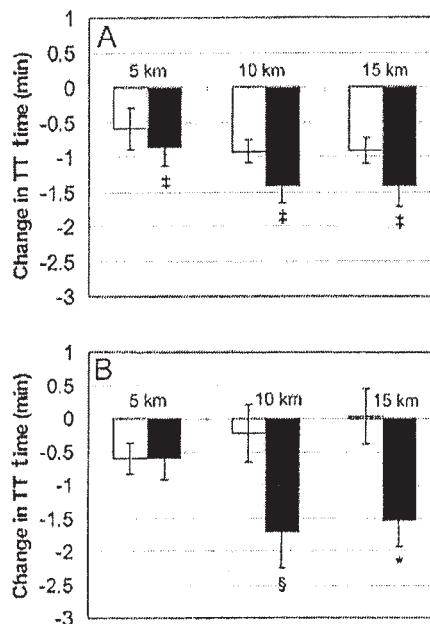


FIGURE 1. Mean (\pm SEM) changes in time to complete each 5-km bout of the 15-km time trial (TT time) from baseline to after treatment in (A) subjects with a normal baseline serum transferrin receptor (sTfR) concentration [\square , placebo group ($n = 12$); \blacksquare , iron group ($n = 15$)] and (B) subjects with an elevated baseline sTfR concentration [\square , placebo group ($n = 7$); \blacksquare , iron group ($n = 7$)]. *Significant group \times time interaction, $P < 0.05$ (two-factor repeated-measures ANOVA). [§]Marginally significant group \times time interaction, $P < 0.10$ (two-factor repeated-measures ANOVA). [‡]Significant effect of time, $P < 0.05$ (two-factor repeated-measures ANOVA). A significant group \times time \times baseline sTfR interaction ($P = 0.027$) for change in TT time after posttreatment % $\dot{V}O_2$ max was controlled for prompted the stratified analysis presented in this figure.

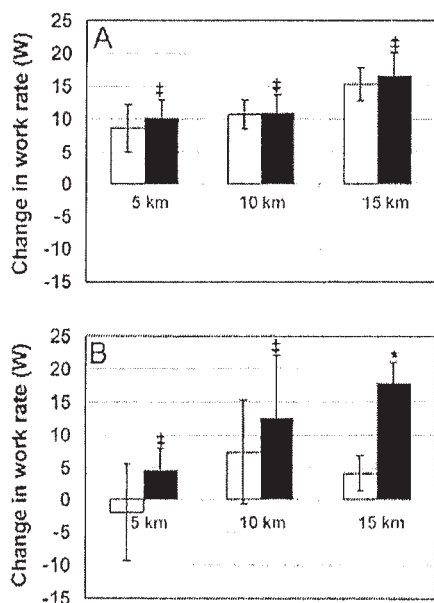


FIGURE 2. Mean (\pm SEM) changes in work rate from baseline to after treatment for each 5-km bout of the 15-km time trial in (A) subjects with a normal baseline serum transferrin receptor (sTfR) concentration [\square , placebo group ($n = 12$); \blacksquare , iron group ($n = 15$)] and (B) subjects with an elevated baseline sTfR concentration [\square , placebo group ($n = 7$); \blacksquare , iron group ($n = 7$)]. *Significant group \times time interaction, $P < 0.05$ (two-factor repeated-measures ANOVA). †Significant effect of time, $P < 0.05$ (two-factor repeated-measures ANOVA). A significant group \times time \times baseline sTfR interaction ($P = 0.026$) for change in work rate after posttreatment $\% \dot{V}O_{2\max}$ was controlled for prompted the stratified analysis presented in this figure.

during all of the 5-km bouts ($P < 0.05$). As shown in Figure 2B, there were significant effects of time for change in work rate during the first and second 5-km bouts in the subjects with an elevated baseline sTfR concentration. A significant group \times time interaction was observed during the third 5-km bout ($P < 0.05$).

As shown in Figure 3A, there were no significant group effects or group \times time interactions for $\% \dot{V}O_{2\max}$ during any of the 5-km bouts in the subjects with a normal baseline sTfR concentration. However, there were significant effects of time for the first and second 5-km bouts ($P < 0.05$). Conversely, as shown in Figure 3B, there were significant group \times time interactions for change in $\% \dot{V}O_{2\max}$ during the first and second 5-km bouts ($P < 0.05$) in the subjects with an elevated baseline sTfR concentration after change in work rate and posttreatment work rate, respectively, were controlled for. Moreover, $\% \dot{V}O_{2\max}$ during the third 5-km bout also tended to decrease more in the subjects in the iron group than in those in the placebo group; however, the group \times time interaction was not significant ($P < 0.10$).

DISCUSSION

Animal studies have consistently shown that iron deficiency without anemia reduces iron-containing oxidative enzyme concentrations and activities and, in turn, impairs endurance capacity (3–6, 8). Human studies have yielded suggestive results (9, 14), but none have shown a strong, consistent relation between iron deficiency without anemia and alterations in endurance performance or adaptations in endurance performance. One possible

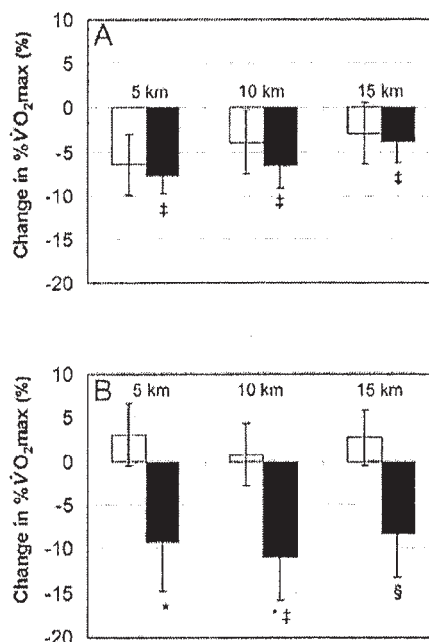


FIGURE 3. Mean (\pm SEM) changes in percentage of maximal oxygen uptake ($\% \dot{V}O_{2\max}$) from baseline to after treatment for each 5-km bout of the 15-km time trial, with control for change in work rate for the first and third 5-km bouts and control for posttreatment work rate for the second 5-km bout, in (A) subjects with a normal baseline serum transferrin receptor (sTfR) concentration [\square , placebo group ($n = 12$); \blacksquare , iron group ($n = 15$)] and (B) subjects with an elevated baseline sTfR concentration [\square , placebo group ($n = 7$); \blacksquare , iron group ($n = 7$)]. *Significant group \times time interaction, $P < 0.05$ (two-factor repeated-measures ANOVA). †Significant effect of time, $P < 0.05$ (two-factor repeated-measures ANOVA). ‡Significant group \times time \times baseline sTfR interaction ($P = 0.028$) for change in $\% \dot{V}O_{2\max}$ after change in work rate was controlled for prompted the stratified analysis presented in this figure.

explanation for this discrepancy may be that the human studies have failed to show alterations in tissue iron status or iron-containing oxidative enzymes in their subjects. Consequently, the conclusions that can be drawn from these studies are limited.


The present study was the first training study to use sTfR as an indicator of tissue iron status. Before accounting for sTfR, we found one significant group \times time interaction effect on endurance: TT time (13). However, stratifying the data by baseline sTfR concentration and by time trial bout showed significant group \times time interactions for time, work rate, and $\% \dot{V}O_{2\max}$ in the subjects with an elevated baseline sTfR concentration (Table 4, Figures 1–3). Specifically, the subjects who began the study with a more depleted tissue iron status and received iron supplements worked at a significantly lower percentage of their maximal work capacity during the first and second 5-km bouts of the posttreatment time trial than did those who began the study with normal tissue iron status and received placebos.

In contrast, there were no group differences between the iron and placebo groups in posttreatment performance in the subjects who began the study with normal tissue iron status. In fact, subjects in both groups who began the study with normal tissue iron status had significant improvements in almost all physio-

logic responses, which suggests that iron status did not impair their ability to adapt to aerobic training.

One possible explanation for the greater improvements in fitness among the more tissue iron-depleted subjects is functional anemia, such that tissue depletion served as a proxy for impaired hemoglobin production. Although none of our subjects at baseline were anemic on the basis of the conventional cutoff (< 120 g/L), hemoglobin production may have been impaired in the more depleted women and thus may have resulted in reduced oxygen-carrying capacity. However, if functional anemia were the underlying cause of this greater adaptive response, one would expect both greater changes in hemoglobin and fitness adaptation among those classified as more depleted at baseline, and changes in hemoglobin would be correlated with changes in fitness. Our data do not support this explanation. Subjects with an elevated sTfR concentration at baseline did not have greater improvements in hemoglobin concentration during the study, nor were changes in hemoglobin concentration correlated with improvements in fitness.

Alternatively, greater mitochondrial and myoglobin adaptations may explain why tissue iron-deficient subjects had greater improvements in fitness. Training is known to increase iron-dependent mitochondrial constituents in the presence of sufficient cellular resources (25). Thus, the subjects who had the largest improvements in tissue iron status were also more likely to have the largest increases in concentrations of myoglobin and iron-containing oxidative enzymes in muscle tissue.

The present study provides additional evidence that sTfR status can be used to distinguish between iron depletion and functional iron deficiency and that functional iron deficiency impairs adaptation in endurance capacity after aerobic training. Furthermore, iron depletion without tissue iron deficiency does not impair adaptation after training, and improvements in iron status in these subjects do not enhance their ability to adapt to training. Future research would benefit from a more detailed characterization of the relation between iron-status indicators and from the development of a cutoff value for sTfR that is sensitive to functional changes in iron status. 

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JDH designed the study. JDH, TB, and PSH coordinated the study. TB and PSH collected the data. TB performed the statistical analyses and wrote the manuscript. VU provided statistical and analytic advice and support. All researchers reviewed the final version of the manuscript. None of the authors had any conflicts of interest to report.

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