

Iron supplementation improves progressive fatigue resistance during dynamic knee extensor exercise in iron-depleted, nonanemic women¹⁻³

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ABSTRACT

Background: Tissue iron depletion may negatively affect endurance performance and muscle fatigability.

Objective: We investigated tissue-level iron depletion and progressive fatigue of the quadriceps during dynamic knee-extension exercise in young women.

Design: Twenty iron-depleted (serum ferritin < 20 µg/L), nonanemic (hemoglobin > 110 g/L) women ($\bar{x} \pm \text{SEM}$ age: 29.1 ± 1.2 y) received iron (iron group) or placebo (placebo group) for 6 wk in a randomized, double-blind trial ($n = 10$ per group). A protocol integrating 2–3-s maximal voluntary static contractions (MVCs) with dynamic knee extensions was used to assess fatigue.

Results: No significant differences between the groups in baseline iron status, MVC at rest, or MVC at the end of the protocol were observed. After treatment, serum iron and transferrin saturation increased significantly in the iron group ($P = 0.02$ and $P = 0.03$, respectively). Serum transferrin receptor concentrations increased significantly in the placebo group ($P < 0.01$) but not in the iron group. After treatment, the rate of decrease in MVC was attenuated in the iron group but not in the placebo group ($P = 0.01$). In the iron group, MVC at the sixth minute of the fatigue protocol and MVC at the end of the protocol were $\approx 15\%$ ($P = 0.04$) and $\approx 27\%$ higher ($P < 0.01$), respectively, after treatment. These improvements were not related to changes in iron-status indexes or tissue iron stores, although power was low (< 0.50) to detect these relations.

Conclusions: Iron supplementation was associated with a significant improvement in muscle fatigability. Interpretation regarding the direct role of tissue iron status is limited by the study's low power to detect relations between tissue iron improvement and decreased muscle fatigue. *Am J Clin Nutr* 2003;77:441–8.

KEY WORDS Quadriceps femoris, leg kick, iron deficiency, endurance, oxidative capacity, mitochondrial respiration, Mexican women

INTRODUCTION

The worldwide prevalence of iron deficiency anemia in women is alarmingly high, and the prevalence of iron deficiency anemia in US women aged 18–44 y is 3–5% (1). Higher still is the prevalence of iron deficiency without anemia (IDNA), which ranges from 11 to 13% in this same population group (1). Female athletes, in particular, are at risk for IDNA because aerobic exercise

may result in a significant depletion of body iron stores (2–4). Although iron deficiency anemia decreases maximal oxygen consumption ($\dot{V}O_{2\text{max}}$) in direct proportion to a hemoglobin-mediated decrease in oxygen carrying capacity (5–11), the effects of IDNA on exercise performance remain obscure. IDNA has no effect on oxygen delivery or $\dot{V}O_{2\text{max}}$ (11–15), but possible effects of IDNA may become manifest because of tissue iron deficiency and decreased activity of iron-dependent mitochondrial oxidative enzymes and electron transport chain cytochromes (6, 16, 17). Reduction in tissue oxidative capacity may affect endurance capacity, a hypothesis that is well supported by animal studies (5, 6, 18). However, several human studies failed to replicate these findings in animals (11–13, 19), and only 2 studies support the hypothesis that tissue iron status mediates endurance capacity (20, 21). Discrepancies between animal and human studies may be attributable to several factors, including variation in the relative work level of the endurance protocol, or to problems in showing tissue-level iron sufficiency or deficiency in humans (22). Also, endurance tests in human subjects are difficult to apply because of the large motivational component necessary to reach a meaningful endurance-test endpoint.

Both treadmill running and cycle ergometry have been used to measure endurance, traditionally defined as the time to exhaustion at a constant, submaximal work intensity. The basic assumption of such a test is that it becomes increasingly more difficult to maintain a high-intensity constant power output because of factors that progressively limit performance. One such factor has been termed “the progressive fatigue of dynamic exercise,” defined as “. . . a failure to maintain the required or expected power output” (23; quoted in reference 24). By this definition, progressive fatigue during a complex dynamic activity occurs

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when a person is unable to continue at the prescribed power output, ie, when the person is at the point of exhaustion. Thus, progressive fatigue is a component of the overall phenomenon of exhaustion, but it is distinguished from other factors (such as motivation) by the fact that it relates principally to a failure of the muscle to maintain the required power output.

Until now, it has not been possible to measure progressive muscle fatigue during dynamic exercise. Conventional fatigue protocols are often based on repetitive static contractions of isolated muscles, with limited applicability to real-world exercise, which is complex and dynamic. To address this problem, Lewis and Fulco (24) developed a novel approach that quantifies progressive muscle fatigue during constant-work-rate, submaximal, dynamic exercise involving the isolated quadriceps femoris muscle group. In brief, their protocol alternates 1-min bouts of dynamic knee-extension work with short-duration (2–3 s) maximal voluntary static contractions (MVCs) until subject exhaustion. The protocol yields a reproducible measure of MVC decline over time (test-retest correlation = 0.94) that is largely independent of subject motivation (25, 26).

The purpose of this study was to determine whether tissue iron depletion mediates the progressive fatigue of muscle during dynamic knee-extension exercise by using the general approach of Lewis and Fulco. This question was addressed with a randomized, double-blind, placebo-controlled intervention trial (iron supplementation) that involved sedentary Mexican women who were identified as having IDNA on the basis of their hemoglobin and serum ferritin (sFer) concentrations.

SUBJECTS AND METHODS

Subjects

The subjects in this study were a subset of women participating in a larger study of iron deficiency and physical performance. Untrained women between the ages of 18 and 45 y ($n = 267$) were recruited for the screening phase of this study, and all were affiliated with the National Institute of Public Health, Mexico (Cuernavaca, Mexico), or the Universidad del Estado de Morelos (Cuernavaca, Mexico). One hundred forty-five of these women were identified as having iron depletion without anemia on the basis of a hemoglobin concentration > 110 g/L and an sFer concentration < 20 μ g/L in preliminary screenings. After physical examination, including a medical history, no subjects were found to have the following exclusion 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, or consumption of prescription medications that would interfere with dietary iron absorption. Of the 145 eligible women, 92 were willing to participate in the overall study. Sixty-eight of these women were scheduled for first-round (baseline) assessments, and the remaining 24 women decided not to continue for personal reasons. Fifty-six of these women completed the entire supplementation study. Of these, the first 20 were recruited to participate in the progressive muscle fatigue study. This subset of women did not differ significantly from the overall sample. Signed informed consent was obtained from each subject. The study was approved by the Cornell University Committee on Human Subjects and the Research Ethics Committee of the National Institute of Public Health, Mexico. The

progressive muscle fatigue test was approved by the Committee on Human Subjects of the State University of New York at Albany, where this protocol was developed.

Study design

The study was a randomized, double-blind, placebo-controlled intervention trial. Subjects were randomly assigned to 1 of 2 groups ($n = 10$ per group) who received either an iron supplement or a placebo. Subjects were supplemented twice a day with either 10 mg elemental Fe as ferrous sulfate or an identical placebo capsule for 6 wk. Previous work by our group showed that iron status improves after 4 wk of iron supplementation with a similar dose of ferrous sulfate (20, 27). The capsules were prepared on site with the use of gelatin capsules (Apothecary Products, Minneapolis), ferrous sulfate, and lactose filler. The mean (\pm SD) iron content was determined from a random sample of 10 capsules to be 9.04 ± 0.52 mg Fe_2SO_4 . The subjects were instructed to consume the capsules with citrus juice to enhance iron absorption and with meals to reduce side effects. They were also instructed to avoid consumption of other multivitamin and mineral supplements during the entire study period. Participants were provided with weekly supplies of capsules, and compliance was monitored on the basis of the number of capsules remaining at the end of each week. Unsolicited verbal reports from the subjects indicated that they were unable to detect whether they received iron or placebo.

Prestudy physical activity was assessed by a 24-h recall questionnaire that was used for the National Nutrition Survey of Mexico (1998). A food-frequency questionnaire was used to assess diet in the month before the study. It included questions about foods high in iron and enhancers or inhibitors of iron absorption. For all subjects, height, weight, and skinfold-thickness measurements were made according to conventional protocols; skinfold-thickness measurements included biceps, triceps, subscapular, and suprailliac sites. Skinfold thickness and body weight measurements were made immediately before and after the 6-wk treatment period.

Measurement of muscle fatigue

Fatigue in the isolated right quadriceps femoris muscle group was induced via a knee-extension exercise protocol on a specially designed apparatus according to a new paradigm developed by Lewis and Fulco (24) to measure progressive muscle fatigue during dynamic exercise. Our knee-extension apparatus is applied to one leg and is identical (in principle) to the apparatus of Lewis and Fulco. Briefly, subjects were seated on a platform and their right leg was fixed to a minimal-friction cable-pulley system that suspended a variable weight load. A padded foot harness was used for the connection of foot to cable at a point ≈ 1 cm above the robust tuberosity on the calcaneus, which marks the attachment site of the calcaneus (Achilles) tendon. This anatomic landmark was arbitrary but was easily recognized and represented a comfortable point of connection. Replication of the foot-to-cable connection between trials was critical to avoid variation in the distance between the knee pivot point and the foot-cable connection, which can affect force output. An LC105 250/S Omega beam load cell force transducer (Omega Engineering, Stamford, CT) was attached to the cable immediately behind the foot, and a continuous force signal was acquired by an REM/400M data-acquisition system (AD Instruments, Grand Junction, CO). In addition, a PT101 precision potentiometer position transducer (Nordisk Transducer Teknik, Hadsund, Denmark) was attached

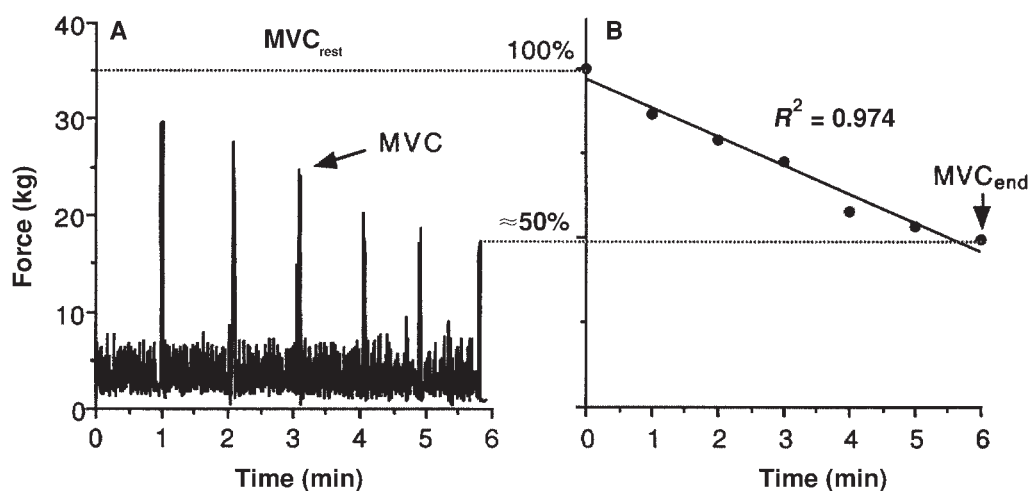


FIGURE 1. A: force curve for one subject showing alternating maximal voluntary static contractions (MVCs) and dynamic knee extensions at $\approx 20\%$ of MVC at rest (MVC_{rest}). B: derived MVC measures and regression line [force decline (%) = $96.8 - 8.5(\text{time})$] calculated from the data in A. MVC_{end} , MVC at the end of the protocol.

to the cable-pulley system to measure the distance of dynamic knee extension. A stop mechanism was introduced behind the force transducer to prevent cable movement. This stop mechanism was activated to fix the leg at a 90° angle for the measurement of force output during MVC of the quadriceps femoris (*see* below). The work rate [(kg · m)/s converted to joules] during dynamic knee extension was calculated by integration of the continuous force curve over time and the distance measurement.

Following the protocol of Fulco and Lewis, an MVC with the leg at a fixed position of 90° was measured ≥ 3 times with ≥ 1 min of rest between trials. MVCs lasted from 2 to 3 s until a plateau in force output was achieved. The highest force output was designated as the MVC in the rested state (MVC_{rest}). On the basis of the measured MVC_{rest} , weight was applied to the pulley system so that dynamic knee extensions produced a peak force of $\approx 20\%$ of MVC_{rest} . This produced a work rate during dynamic knee extension that ranged from 420 to 900 J/min (7–15 W) depending on the subject.

The fatigue test alternates 1 min of dynamic knee extension (from 90° to 150° and back every 2 s, ie, 0.5 Hz) with static measurements of MVC at the end of each minute until subjects have trouble maintaining cadence or knee-extension height. Cadence was measured by using a metronome, and adherence to cadence was facilitated by a researcher who counted out knee extensions to the test subject. Knee-extension height was standardized for all trials and was determined by the foot making contact with a flexible fixed marker. Time to fatigue was not considered an objective outcome of this protocol, and all the women were able to perform the same test with encouragement before and after treatment. What presumably changed between tests was the degree of comfort and the rate of MVC decline. The latter was taken as our measure of muscle fatigue rate and was independent of test length and subject motivation.

The force curve and the derived MVC measures from one subject during the dynamic fatigue protocol are shown in **Figure 1**, A and B, respectively. MVC_{rest} was measured in kilograms as previously described and represents 100% of muscle strength capacity. The last MVC measured during the protocol was designated MVC_{end} (in kg). Regression of MVC on time was used to assess the linearity of MVC decline for each subject, as shown. However,

the regression slope was not used as a measure of dynamic fatigue rate. This is because the rate of decline was not linear for all subjects and because slope calculations are sensitive to high leverage points, especially when < 10 points (MVC values) are used in the regression. Thus, to assess the effect of iron supplementation on dynamic fatigue rate, we compared average MVC values at specific time points (before and after supplementation) and across all time points by using a repeated-measures analysis (*see* below). The latter analytic approach has 2 advantages: 1) the rate of decline does not need to be linear to apply the statistical test, and 2) the attendant problems of regression analysis (ie, leverage and dependence on the number of points) are avoided.

Despite the application of a standardized relative work rate for dynamic knee-extension exercise, it was difficult to predict the individual degree of comfort with the protocol. Some women quickly fatigued, whereas others completed the first minutes of the protocol with little distress. For the latter, weight load was increased during the test with the goal of inducing muscle fatigue in ≈ 10 min. This was a necessary step because tests of long duration are difficult to reproduce and because preliminary studies showed that some women were capable of continuing dynamic knee extension at 20% of MVC_{rest} beyond 30 min after reaching an apparent plateau in MVC. All fatigue tests before and after supplementation were administered in exactly the same way, and subjects received verbal encouragement throughout the tests. Force and position transducers were calibrated before each test.

Anthropometry and iron-status measures

Anthropometric measurements (weight, height, and skinfold thicknesses) were made by using standard procedures described in Lohman et al (28). Percentage of body fat was calculated by using the equations of Durnin and Womersley (29). Iron status was measured at screening and before, at the midpoint of (3 wk), and after iron treatment (6 wk). While subjects were seated and in the fasted state, blood samples were obtained from the antecubital vein into 2 evacuated tubes: one containing EDTA and the other empty. Blood sampling for each subject was generally conducted before 0900 and at the same time of day at screening and at 3 and 6 wk to control for diurnal variability. No attempt was made to control for menstrual cycle phase. Hemoglobin and hematocrit

were assayed in whole blood immediately after sample collection. Coagulated blood was separated within 30 min of collection by centrifugation at $2010 \times g$ for 10 min at room temperature. Aliquots of serum samples were frozen at -70°C . To control for potential variation in assay conditions, each subject's serum samples [serum transferrin receptor (sTfR), sFer, and serum iron (sFe), and total-iron-binding capacity (TIBC)] from all 3 time points were analyzed concurrently at the completion of the study.

Hemoglobin, hematocrit, white blood cell count, and red blood cell count were determined by using a model 1700 Hemocultor (Abbott Diagnostics, Abbott Park, IL). sTfR and sFer concentrations were measured by using an enzyme-linked immunosorbent assay (Ramco Laboratories, Houston) according to the methods of Flowers et al (30, 31). Transferrin saturation (TS) was determined from the ratio of sFe to TIBC by using the method described by Persijn et al (32) (Sigma Diagnostics, St Louis). To control for day-to-day variation and to increase measurement precision, the averages of 2 independent measures of hemoglobin and sFer concentrations taken 3–7 d apart (at screening and before iron treatment) were used as the pretreatment concentrations.

Statistical analysis

All iron-status measures were evaluated for normality against a standard normal distribution by using the Kolmogorov-Smirnov test and the Lilliefors two-tail probability (SYSTAT, version 5.1a; SPSS Inc, Chicago). Log transformations of sTfR and TIBC were necessary to normalize these measures for statistical testing purposes, but nontransformed values are presented in the Results. All other iron-status indexes were not significantly different from normal values.

Independent Student's *t* test was used to test differences between the treatment groups in iron status at baseline. Repeated-measures analysis of variance was used to test time (baseline and 3 and 6 wk) and treatment group (supplementation) effects as well as time-by-treatment interactions for measures of iron status. A treatment effect of the iron supplementation protocol was determined if the linear term of the polynomial contrast used to examine the trend over time was significantly different between the placebo and iron groups. When significant iron treatment effects were detected, post hoc comparisons within each treatment group between iron status at baseline and that at 6 wk were evaluated with the use of a paired *t* test and Bonferroni correction.

Analysis of covariance was used to test for treatment effects on individual measures of MVC at any given time point during the leg kick protocol, with control for pretreatment values of these measures (eg, MVC_{end}). This is the suggested approach for dealing with "change" variables rather than performing *t* tests on percentages of change or absolute changes from baseline (33). Repeated-measures analysis of variance was used to test for time and treatment group effects and time-by-treatment group interaction effects on MVC over the first 6 min of the progressive muscle fatigue protocol. In all tests, statistical significance was indicated at $P < 0.05$ for main and interaction effects. Values are expressed as means \pm SEMs. All statistical analyses were performed by using SYSTAT version 5.1a (Macintosh) or version 9.0 (PC) (SPSS Inc).

RESULTS

Subject characteristics

The placebo and iron groups were not significantly different in age (30.0 ± 2.3 and 28.1 ± 1.1 y, respectively). At baseline,

TABLE 1

Serum indicators of iron status at baseline (0 wk) and after 3 and 6 wk of iron ($n = 10$) or placebo ($n = 10$) treatment¹

	Baseline	3 wk	6 wk
Hemoglobin (g/L)			
Placebo	140 \pm 3	138 \pm 3	134 \pm 4
Iron	140 \pm 3	130 \pm 4	139 \pm 2
sFer ($\mu\text{g/L}$)			
Placebo	14.90 \pm 1.91	13.33 \pm 2.48	16.18 \pm 2.29
Iron	12.41 \pm 3.29	13.93 \pm 3.01	15.02 \pm 2.22
STfR (mg/L) ²			
Placebo	4.52 \pm 0.64	5.56 \pm 0.58	6.30 \pm 0.67 ³
Iron	6.37 \pm 0.96	5.50 \pm 0.90	5.57 \pm 0.84
sFe ($\mu\text{mol/L}$) ⁴			
Placebo	15.4 \pm 2.3	19.8 \pm 3.7	15.8 \pm 3.4
Iron	11.3 \pm 2.0	16.7 \pm 2.6	22.7 \pm 3.3 ³
TIBC ($\mu\text{mol/L}$)			
Placebo	59.6 \pm 4.1	67.2 \pm 6.6	64.7 \pm 5.6
Iron	59.8 \pm 3.4	67.0 \pm 6.8	58.6 \pm 5.2
TS (%) ⁴			
Placebo	28.2 \pm 6.0	32.0 \pm 7.7	27.4 \pm 7.0
Iron	19.9 \pm 3.9	27.8 \pm 4.5	39.6 \pm 5.8 ³

¹sFer, serum ferritin; sTfR, serum transferrin receptor; sFe, serum iron; TIBC, total-iron-binding capacity; TS, transferrin saturation.

^{2,4}Significant time-by-treatment group interaction (repeated-measures ANOVA): ² $P < 0.01$, ⁴ $P < 0.05$.

³Significantly different from baseline, $P < 0.01$ (paired *t* test with Bonferroni correction).

the placebo and iron groups did not differ significantly in height (157.8 ± 3.0 and 158.9 ± 1.9 cm, respectively), body weight (61.9 ± 3.0 and 59.6 ± 3.6 kg, respectively), and percentage of body fat ($31.5 \pm 1.6\%$ and $28.7 \pm 1.5\%$, respectively). The placebo and iron groups did not differ significantly in intensity of physical activity before the study, as assessed by a questionnaire that was used to quantify the frequency of various habitual physical activities. Physical activities were categorized according to relative intensity level, ie, low, medium, and high intensity (data not shown). Similarly, the placebo and iron groups did not differ significantly in their food choices before the study, as assessed by a food-frequency questionnaire used to measure the frequency of ingestion of various common food items in the Mexican diet (data not shown).

Response to iron treatment

Measures of iron status at baseline and at 3 and 6 wk are shown in **Table 1**. At baseline, it was discovered that 4 women (2 in each group) had an sFer concentration $> 20 \mu\text{g/L}$, which was thus above our criterion for iron deficiency. This may have been because of measurement error at screening or improvement in iron stores from screening to baseline. Nevertheless, these women were included in data analyses because their sFer values were still close to the cutoff value. Exclusion of these subjects in separate analyses had no qualitative effect on study outcomes.

There were no significant differences between the groups in measures of iron status at baseline. Group differences in response to 6 wk of iron treatment or placebo were assessed via a repeated-measures analysis to gauge main effects (time and treatment group) and interaction effects (time-by-treatment group). There was a significant time-by-treatment group interaction for sTfR ($P < 0.01$), sFe ($P = 0.02$), and TS ($P = 0.03$).

Post hoc comparison with Bonferroni correction showed significantly higher sTfR concentrations at week 6 than at baseline in the placebo group ($P < 0.01$) but no significant change from baseline to week 6 in the iron group. Both sFe concentrations and TS values were significantly higher at week 6 than at baseline in the iron group ($P < 0.01$ for both indexes) but were unchanged in the placebo group. There were no significant intra- or intergroup differences in hemoglobin, sFer, or TIBC.

The response of sTfR to the iron treatment was dependent on the initial sTfR concentration. That is, the women who had a higher initial sTfR concentration (ie, lower iron stores) had a significantly greater decrease ($P < 0.01$) in sTfR concentration by the end of the study than did those who had a lower initial sTfR concentration. There was a significant negative correlation between sTfR and sFer at week 6 ($r = -0.50$, $P = 0.02$) but not at the 2 earlier time points. Hemoglobin was positively correlated with TS at baseline ($r = 0.51$, $P = 0.02$) but not at the 2 later time points.

Muscle fatigue

The initial set work rate for dynamic knee extension of the quadriceps femoris was not significantly different between the placebo and iron groups (708 ± 42 and 696 ± 42 J, respectively). The average test required 8.9 ± 0.6 min, ranging from 4 to 12 min. Test length did not differ significantly between the groups. By study design, the tests before and after supplementation were identical for each subject with respect to work rate and test length. The average MVC_{end} for all subjects before supplementation was $57.1 \pm 2.0\%$ of MVC_{rest} (Figure 2). This value may be considered as the average value of force decline that marks the point where subjects begin to have trouble achieving full knee-extension height or maintaining knee-extension cadence. As in Figure 1, the rate of MVC decline over time for an individual subject during the knee-extension protocol can be approximated by

linear regression. Regression variables showed an overall rate of decline of 4.8–7.3% of MVC_{rest}/min. R^2 values ranged from 0.44 to 0.99, with a mean R^2 for all tests of 0.86 ± 0.40 . Low R^2 values do not indicate poor data but simply reflect the fact that MVC decline was not linear for all subjects. For example, the lowest R^2 value observed (0.44) was from a fatigue test after supplementation in which the subject reached an MVC plateau after the first half of the protocol.

MVC_{rest}, the average MVC values over the first 6 min of the progressive muscle-fatigue protocol, and MVC_{end} in both study groups before and after supplementation are shown in Figure 2. To preserve sample size, paired comparisons before and after supplementation were made only over the first 6 min. That is, not all subjects completed tests longer than 6 min. Analysis of covariance showed no significant differences between the groups in changes in MVC after supplementation at any specific time point. Specifically, there was no significant difference between the groups in the increase in MVC_{end} ($P = 0.15$). However, a repeated-measures analysis over the first 6 min of the protocol (and including MVC_{end}) showed a significant time-by-treatment group interaction ($P = 0.01$). That is, the rate of MVC decline after treatment was attenuated in the iron group (Figure 2B) but not in the placebo group (Figure 2A). This is also supported by paired t test comparisons within the groups of MVC values at individual time points before and after supplementation. Although both groups tended to have higher MVC_{end} after treatment, the increase was only significant in the iron group (26.5% increase; $P < 0.01$). The increase in MVC in the iron group was also significant at minute 6 ($P = 0.04$) but not at minute 5 ($P = 0.09$). This attenuation in MVC decline after supplementation in the iron group means that after the fourth minute of the protocol, MVC values after supplementation were ≈ 10 –15% higher than those before supplementation.

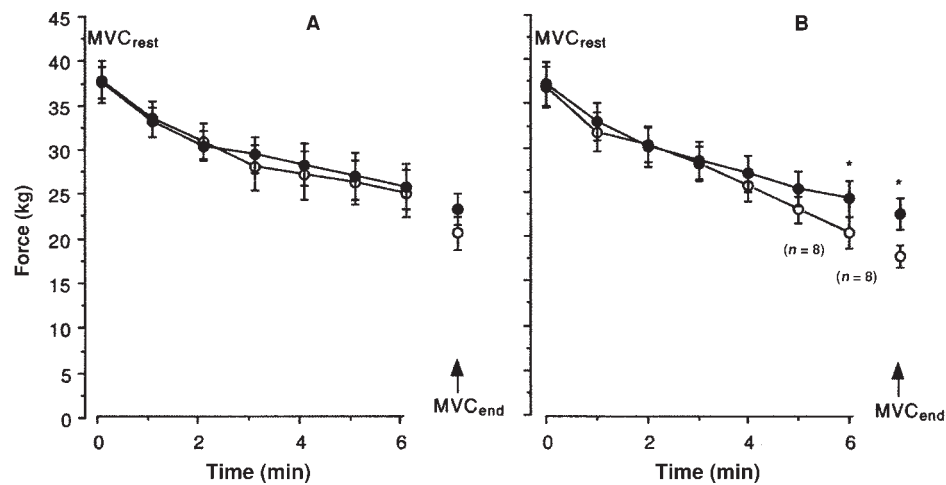


FIGURE 2. Mean (\pm SEM) maximal voluntary static contractions (MVCs) over the first 6 min of a progressive fatigue test before (\circ) and after (\bullet) supplementation in women who received supplementation with a placebo (A) or iron (B). The number of paired comparisons at each time point was 10 unless indicated otherwise. MVC_{end} is the last MVC of the protocol and occurred at different times for each study subject (ranging from 4 to 12 min). For 0–6 min of data and MVC_{end}, repeated-measures ANOVA showed a significant time-by-treatment group interaction ($P = 0.01$); ie, the decrease in MVCs was attenuated after treatment in the iron group but not in the placebo group. *Significantly higher MVC_{end} after supplementation than before supplementation, $P \leq 0.01$ (paired t test). However, analysis of covariance showed no significant difference between the groups in the change in MVC (from before supplementation to after supplementation) at any individual time point. The models with analysis of covariance controlled for the initial (before supplementation) value of MVC at each specific time point (see Subjects and Methods). MVC_{rest}, MVC at rest.

An alternative analysis was performed by sorting MVC data into 7 time blocks. Time block 1 consisted of MVC measures made during the first $\approx 14\%$ of the total time of the progressive fatigue test, time block 2 consisted of measures made during the next 14% of the time, and so forth. The advantage of this procedure was that all data were included, but the disadvantage was that adjacent MVC measures from the same subject could fall into the same time block, especially for subjects who performed tests longer than 7 min. When adjacent MVC measures fell into the same time block, the average of the 2 MVC values was used in the analysis. Results of this analysis (data not shown) were similar to those presented above and suggested attenuation of MVC decline starting at about the fourth minute of the test after treatment in the iron group but not in the placebo group.

Multivariate analyses showed no significant relation (within groups or across the entire study sample) between the change in sTfR concentration with iron supplementation and the increase in MVC at any point during the test or at the end of the test when the values of these variables before supplementation were held constant. In addition, when sTfR was entered as a covariate in analyses of differences in MVC between the placebo and iron groups, this did not attenuate the difference between the groups, suggesting that sTfR does not have a mediating effect on muscle fatigue. Similarly, there were no significant relations between changes in other iron-status indexes and changes in MVC measures.

Power analysis with PASS software, version 6.0 (NCSS, Kaysville, UT) was conducted for the multivariate models described above that were used to test for the effect of changing iron-status indexes on increases in MVC with supplementation. As an example, the measured SDs for changes in sTfR concentration and changes in MVC_{end} with treatment were 2.2 mg/L and 5.2 kg, respectively. With 10 subjects per group, the power to detect the effect of a 1-SD decrease in sTfR concentration producing a 10% increase in MVC was ≈ 0.50 , and the power to detect a 5% increase in MVC was < 0.25 . Thus, the power to relate iron-status indexes to improvements in muscle fatigue within the range observed as a consequence of iron supplementation was relatively low in this study.

DISCUSSION

This randomized, double-blind, placebo-controlled study of a small sample ($n = 10$ per group) of young, sedentary women with iron deficiency but without anemia showed that overall iron status improved significantly as a consequence of 6 wk of oral iron supplementation. sFe concentrations and TS values were both significantly higher in the iron group than in the placebo group after treatment, although sFer concentrations and TIBC were unchanged after iron treatment. Our inability to detect improvement in the latter 2 indexes may have been because of the relatively small sample size (see power analysis below), because our previous studies showed clear improvements in all of these indexes in placebo-controlled interventions with larger samples of study subjects (20, 27). Significant differences between the groups in sTfR response ($P < 0.01$) were evident and suggest that although iron supplementation did not necessarily improve tissue iron status in the present study, it did prevent further depletion (as evidenced by increased sTfR concentrations in the placebo group). This is an important consideration because the main study hypothesis postulates an improvement in physical performance as a consequence of improved tissue iron availability.

The principle finding of this study was that iron supplementation improved the fatigue resistance of the quadriceps femoris muscle group during dynamic knee-extension exercise. Specifically, we found that iron supplementation attenuated the decline of MVC after the first minutes of a progressive fatigue test to exhaustion (Figure 2). These results are consistent with those of animal and human studies suggesting that tissue iron status mediates the capacity for long-term aerobic exercise (5, 6, 18, 20, 21). However, we detected no direct relation between changes in sTfR concentrations and changes in MVC after supplementation; nor did the inclusion of sTfR as a covariate attenuate the supplementation effect to suggest a mediating role of tissue iron status on muscle fatigue. Similarly, changes in other iron-status indexes were also not directly related to improvement in muscle fatigability. Thus, the study does not provide direct evidence that muscle fatigue is related to tissue iron status. Again, this may be a consequence of sample size because power analysis showed relatively low power (< 0.50) to detect a relation between improvements in tissue iron status and the increases in MVC observed with supplementation. Alternatively, the effect on fatigue that was observed with supplementation may be related to other factors that were not measured in this study (see the discussion below of the mechanisms of fatigue).

To gauge muscle fatigue, we used dynamic knee-extension exercise, a testing modality that was originally developed as a model for the study of isolated exercising muscle in humans (34). Studies of dynamic knee-extension exercise show that 1) both pulmonary and leg $\dot{V}O_2$ increase linearly with increasing external work, 2) pulmonary $\dot{V}O_2$ at maximal levels of single-leg dynamic knee extension reaches $\approx 45\%$ of the $\dot{V}O_{2max}$ measured during conventional (2-leg) cycle ergometry, and 3) the specific mitochondrial $\dot{V}O_2$ of the quadriceps femoris during maximal knee extension is ≈ 2 -fold that achieved during cycle ergometry (34–36). Thus, in contrast with cycling or running, dynamic knee-extension exercise to a maximum level is characterized by very high rates of muscle-specific oxygen delivery and utilization that is not centrally limited by cardiac output or pulmonary gas exchange (37). In a similar group of college-aged women tested in our laboratory, 10 min of dynamic knee extension at 20% of MVC_{rest} (0.5 Hz) required a mean pulmonary $\dot{V}O_2$ of 0.77 ± 0.05 L/min (data not shown). This value is $\approx 43\%$ of the $\dot{V}O_{2max}$ of the Mexican women in the present study and is similar to values reported in the literature. Thus, it is likely that our dynamic knee-extension protocol produced high rates of muscle-specific oxygen consumption. As such, this type of exercise may represent the ideal way to examine tissue-level effects on exercise performance. If specific muscle work rates are high, without central limitation to oxygen delivery, then performance decrements probably reflect impairments in tissue-level oxygen utilization.

Dynamic knee-extension exercise to produce progressive muscle fatigue was applied according to a new approach developed by Lewis and Fulco (24). This approach is novel because muscle fatigue, quantified by the decrease in MVCs over time, is the direct result of dynamic exercise. Conceptually, this approach differs from conventional protocols in which fatigue is induced by repetitive (often isometric) contractions of an isolated muscle group, in situ muscle preparation, or isolated muscle fiber. Although standard fatigue protocols vary the strength, frequency, or duration of isometric muscle contraction, they often do not involve (to a large degree) the aerobic energy systems of the muscle. From this discussion, progressive fatigue (as measured here) should be considered as a component of overall endurance

performance. That is, progressive fatigue occurs when muscle force output during dynamic exercise is no longer sufficient to meet prescribed demands. This occurs, by definition, at the point of exhaustion, which (also by definition) marks the end of a typical endurance test. This interpretation is favored by Lewis and Fulco (24) and is consistent with results from their recent study using the knee-extension progressive-fatigue protocol to quantify the effect of hypobaria on muscle performance (26). That study showed that the rate of MVC decline in hypobaria (464 Torr) is nearly 2-fold that in normobaria (758 Torr). This is a large effect but corresponds well to the large decrease in endurance time during 2-leg cycle exercise typically seen with hypoxic exposure (38). As Fulco et al (26) note, previous studies that assessed the effect of hypoxia on fatigue rate by using conventional measures are equivocal, in part because of the inherent limitations of these approaches.


Several muscle force variables, including absolute MVC values at the beginning and end of the test and the rate of MVC decline over the duration of the test, may be analyzed with respect to progressive muscle fatigue. We detected no significant differences between the groups in improvement of MVC at any specific time point, but repeated-measures analysis clearly showed the attenuation of MVC decline after the first 4 min of the protocol with iron treatment in the iron group (Figure 2B). There are many ways in which tissue iron deficiency could affect the rate of progressive muscle fatigue. In general, muscle performance during dynamic exercise depends on tissue oxygen and fuel substrate delivery and the capacity and efficiency of mitochondrial respiration to produce ATP. Tissue iron deficiency clearly affects the latter, at least in animal studies, which show marked reductions in mitochondrial iron-containing respiratory enzymes and cytochromes (5, 17, 39, 40). The decrease in mitochondrial respiration capacity results in an altered metabolic response during dynamic exercise, which includes an increased reliance on glycolysis and a resultant increase in lactate production (6, 19, 39, 41–43). This may explain a part of the improvement in endurance capacity with tissue iron repletion, but the direct effect of these and other metabolic perturbations on muscle performance should also be considered.

The precise mechanisms underlying muscle fatigue have yet to be identified, but muscle failure probably occurs distal to the neuromuscular junction (44). That is, several studies suggest that neuromuscular communication is not the limiting factor in well-motivated subjects (45). Thus, fatigue is probably a peripheral phenomenon affecting ≥ 1 of the steps of the excitation-contraction coupling process. These steps include the transmission of a sarcolemmal action potential along the transverse tubules to the sarcoplasmic reticulum, the release of Ca^{2+} by the sarcoplasmic reticulum, the binding of Ca^{2+} by troponin, and finally the reabsorption of Ca^{2+} by the sarcoplasmic reticulum. Metabolic factors may induce muscle fatigue directly or indirectly at any step, and various metabolites, including ATP, creatine phosphate, glycogen, oxygen, fatty acids, lactate and muscle pH, calcium, ammonium, and various other electrolytes, have been implicated (46).

In 2 studies, muscle fatigue resistance was measured in iron-deficient rats (39, 47). Both studies used *in situ* preparations of the surgically isolated gastrocnemius-plantaris-soleus muscle complex, which was electrically stimulated to produce isometric muscle contractions. In the study by Finch et al (47), fatigue resistance did not differ significantly between the iron-deficient and control muscles, despite lower endurance and higher lactate

concentrations in the iron-deficient rats than in the control rats during a treadmill run. These results were strongly disputed by McLane et al (39), who showed a large ($\approx 50\%$) decrement in contractile force generation in iron-deficient muscle compared with control muscle over 10 min of electrical stimulation, as well as higher lactate concentrations in the iron-deficient muscle after the stimulation protocol. Importantly, 8 d of iron treatment significantly improved fatigue resistance in iron-deficient muscle and resulted in less lactate accumulation.

The discrepancy between these 2 studies may relate to oxygen delivery. In the study by Finch et al, leg blood perfusion was left to autoregulate via the intact circulation and may have been affected by the surgery or anesthesia. In the study by McLane et al, the hindlimb was perfused at a constant rate to control oxygen delivery to the muscle. Thus, we consider the study by McLane et al the better study in this regard. Their conclusion was that iron deficiency decreases skeletal muscle capacity for aerobic metabolism and, by that mechanism, increases susceptibility to fatigue. In particular, they pointed to lactate and muscle pH as possible mediators of fatigue resistance and endurance. Our results are consistent with this general conclusion, although we can say nothing about mechanisms of fatigue because of the noninvasive nature of the study.

In conclusion, the present study showed improvements in iron status, but no clear improvement in tissue iron stores, after 6 wk of iron supplementation in women with IDNA. Supplementation was associated with a significant improvement in muscle fatigue resistance, as measured by a novel protocol that induces fatigue via highly aerobic dynamic knee extensions performed over ≈ 8 min. Although this finding is consistent with the findings of human and animal studies suggesting that impaired tissue oxidative capacity impairs both the ability to perform and the ability to adapt to dynamic endurance exercise, we were unable to show a clear cause and effect in this regard. This inability may be because of a sample size limitation in the present study or because factors other than tissue iron status mediate muscle susceptibility to fatigue. 

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