

Randomized, double-blind, placebo-controlled trial of iron supplementation in female soldiers during military training: effects on iron status, physical performance, and mood^{1–5}

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ABSTRACT

Background: Decrements in iron status have been reported in female soldiers during military training. Diminished iron status adversely affects physical and cognitive performance.

Objective: We wanted to determine whether iron supplementation could prevent decrements in iron status and improve measures of physical performance and cognitive status in female soldiers during basic combat training (BCT).

Design: In this 8-wk randomized, double-blind, placebo-controlled trial, soldier volunteers ($n = 219$) were provided with capsules containing either 100 mg ferrous sulfate or a placebo. Iron status indicator assays were performed pre- and post-BCT. Two-mile running time was assessed post-BCT; mood was assessed by using the Profile of Mood States questionnaire pre- and post-BCT.

Results: The BCT course affected iron status: red blood cell distribution width and soluble transferrin receptor were elevated ($P < 0.05$), and serum ferritin was lowered ($P < 0.05$) post-BCT. Iron supplementation attenuated the decrement in iron status; group-by-time interactions ($P < 0.01$) were observed for serum ferritin and soluble transferrin receptor. Iron supplementation resulted in improved ($P < 0.05$) vigor scores on the Profile of Mood States post-BCT and in faster running time ($P < 0.05$) in volunteers reporting to BCT with iron deficiency anemia.

Conclusions: Iron status is affected by BCT, and iron supplementation attenuates the decrement in indicators of iron status in female soldiers. Furthermore, iron supplementation may prove to be beneficial for mood and physical performance during the training period. Future efforts should identify and treat female soldiers or athletes who begin training regimens with iron deficiency or iron deficiency anemia. *Am J Clin Nutr* 2009;90:124–31.

INTRODUCTION

Iron deficiency (ID) and ID anemia (IDA) affect billions of people worldwide. Recent studies suggest that ID could affect up to 50% of the world's population (1). Although those in the developing world are at the greatest risk of ID and IDA, data from the United States and the United Kingdom indicate that ID affects up to 16% and 21% of premenopausal women, respectively (2, 3). Premenopausal women are at the greatest risk of ID because suboptimal iron consumption and menstrual bleeding lead to negative iron balance (4). Furthermore, premenopausal women who engage in regular physical activity may be at even greater

risk of poor iron status because physical activity appears to have a negative effect on iron stores (5, 6).

The US military comprises >15% women, which amounts to >341,000 individuals (7). These women often serve in occupations that are physically and cognitively demanding, especially when operationally deployed or during field training. The prevalence of ID and IDA in women entering military service has been reported to be similar to that of the corresponding demographic within the US population (8), although both cross-sectional and longitudinal studies indicate that iron status declines during initial military training (8, 9). Maintaining optimal iron status in this population is critical because of the known contribution of iron nutrition to physical and cognitive performance. The effect of IDA on physical performance is well described: reduced hemoglobin results in a diminished ability to do physical work (10, 11). Although the effect of ID without anemia on physical performance is not as well described, it has been reported that ID without anemia results in impaired aerobic adaptation and endurance capacity in women (12, 13) and that iron supplementation improves exercise endurance (14) and maintains ventilatory threshold (15) in athletes with ID. Optimal iron nutrition is also essential for cognitive function; Murray-Kolb and Beard (16) recently reported that iron supplementation

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of women with ID and IDA resulted in marked improvements in time to completion on a series of cognitive tasks.

Previous work has not determined the functional effect of diminished iron status, or of iron supplementation, on soldier health and performance during military training. The primary objective of this randomized, double-blind, placebo-controlled trial was to determine whether iron supplementation could prevent the longitudinal decrement in iron status previously observed in female soldiers undergoing 8–9 wk of military training (8, 9). We hypothesized that daily oral iron supplementation would serve to maintain iron status during military training, which could affect physical performance and mood at the end of the training period.

SUBJECTS AND METHODS

Subjects

This study was approved by the Human Use Review Committee at the US Army Research Institute of Environmental Medicine and conducted at Fort Jackson, South Carolina. Human volunteers participated in these studies after providing their free and informed voluntary consent. Investigators adhered to US Army Regulation 70–25 and US Army Medical Research and Materiel Command Regulation 70–25 on the use of volunteers in research. The initial date of recruitment for the study was 9 August 2007.

A total of 219 female soldiers volunteered to participate in this randomized, double-blind, placebo-controlled trial. Baseline data were collected within 1 wk of arrival at basic combat training (BCT). Demographic characteristics of the volunteer population appear in **Table 1**. Relations between iron status indicators and anthropometric measures collected at the pre-timepoint have been reported elsewhere (17). After baseline data collection, volunteers were randomly assigned to treatment groups. Volunteers assigned to the placebo group were provided with cap-

sules containing cellulose; volunteers assigned to the iron supplementation group were provided with capsules containing 100 mg of ferrous sulfate. This dose was selected on the basis of previous work describing the beneficial effects of 100 mg of ferrous sulfate on iron status and performance with minimal side effects (12, 13). Capsules were provided by Compounded Solutions in Pharmacy (Monroe, CT) and were identical in color and appearance. Volunteers were instructed to consume the capsules with water immediately before breakfast each day throughout the 8 wk of BCT. No other multivitamin or mineral supplements were consumed by the volunteers during BCT in accordance with army regulations. From a random sample of 5 capsules, the mean ($\pm$ SD) measured elemental iron content of the capsules was 15.0 ± 0.2 mg. Capsules were provided by blinded study staff on a daily basis, and compliance data were recorded. A volunteer was considered compliant when she was observed consuming the capsule. Overall compliance in the placebo group was 94% (4378 of 4675 total capsules); compliance in the iron-treated group was 93% (4391 of 4730 total capsules). Chi-square analysis indicates no significant difference in compliance between the treatment groups.

Final data (post) were collected at the end of the BCT course. This course involves aerobic activities, such as road marching, distance running, and sprinting, as well as muscle strength training that includes activities such as calisthenic exercises, sit-ups, and push-ups (18). Military activities such as rappelling, obstacle courses, prolonged standing in formation, and didactic classroom instruction are also required during the BCT course. Estimates of the ambulatory activity experienced during BCT (19) and the average fitness levels of female soldiers reporting to BCT have been reported elsewhere (9). A total of 171 volunteers completed the trial. There were no reports of adverse medical events. In the placebo group, 85 of 107 total volunteers (79%) completed the trial; in the iron-treated group, 86 of 112 total volunteers (77%) completed the trial. Common reasons for attrition included injuries and separation from the army unit included in the trial. After the baseline data collection, volunteers were informed if they met our definition of IDA (*see below*) and were given the opportunity to visit with health care providers and/or withdraw from the research trial. In both the placebo and iron-treated groups, 22 volunteers were notified that they met the definition of IDA. If women with IDA were provided supplemental iron by health care providers, they were excluded from the trial.

Biological sample collection and analysis

Blood samples were collected following an overnight fast at pre- and post-timepoints. Samples were obtained by using antecubital venipuncture and collection tubes containing the appropriate anticoagulants, including EDTA for the collection of samples used in whole-blood assays (Vacutainer; Becton-Dickinson, Franklin Lakes, NJ). Assays using whole blood, including hemoglobin and red blood cell distribution width, were performed by using a hematology analyzer (Medonic CA 620; Boule Medical AB, Stockholm, Sweden). Serum was isolated, frozen, and shipped to the Pennington Biomedical Research Center (Baton Rouge, LA) for iron status indicator assays. Serum ferritin was measured by using an automated immunoassay instrument (Siemens Medical Solutions, USA, Inc, Malvern, PA).

TABLE 1
Demographic characteristics¹

	Placebo (<i>n</i> = 85)	Iron (<i>n</i> = 86)
Age (y)	20.8 $\pm$ 4.4 ²	20.4 $\pm$ 4.2
Height (cm)	161.9 $\pm$ 6.1	161.7 $\pm$ 6.2
Weight, pre (kg)	62.2 $\pm$ 8.5	61.8 $\pm$ 9.4
Weight, post (kg)	61.9 $\pm$ 6.9	61.8 $\pm$ 8.2
Ethnicity (<i>n</i>)		
Not Hispanic	72	72
Hispanic	13	14
Race (<i>n</i>)		
White	55	60
Black	21	19
Native American/Alaskan Native	0	2
Asian	4	1
Native Hawaiian/Pacific Islander	1	2
Other	3	2

¹ pre, baseline data collected within 1 wk of arrival at basic combat training; post, data collected at the end of the basic combat training course. Age, height, and weight comparisons were performed by using Student's *t* tests. Ethnicity and race comparisons were performed by using chi-square tests. There were no significant differences

² Mean $\pm$ SD (all such values).

Serum iron and total iron-binding capacity were measured by using the Beckman Coulter DXC 600 Pro (Fullerton, CA). Transferrin saturation was calculated by dividing serum iron by total iron-binding capacity. Soluble transferrin receptor (sTfR) was determined by using a commercially available immunoassay (Quantikine IVD; R&D Systems Inc, Minneapolis, MN).

A 3-variable model was used to identify volunteers with ID or IDA (8). We selected this model on the basis of previous work indicating that multivariable models avoid misclassifications that may occur because of errors or variability in single iron status indicators. Multivariable models have been used to determine the prevalence of ID and IDA in many large studies, including the National Health and Nutrition Examination surveys (3, 20, 21). In our model (8), volunteers were categorized as ID if they presented with ≥ 2 of the following 3 indicators of abnormal iron status: serum ferritin < 12 ng/mL, transferrin saturation $< 16\%$, or red blood cell distribution width $> 15\%$. Volunteers were categorized as IDA if they had ID as well as hemoglobin concentrations < 12 g/dL.

Physical performance and mood

Two-mile run times were measured at the end of BCT as an indicator of physical performance. This measure was selected because soldiers are required to run 2 miles within an allotted period of time to successfully graduate from the training course, and a 2-mile run time has been shown to be an accurate predictor of maximal aerobic capacity (22). The maximum allotted 2-mile run time required for successful completion of BCT depends on age; for soldiers of the mean age included in this study (21 y), the standard required for graduation is 1182 s. During testing soldiers were not asked to stop the test if they had exceeded the period of time required to complete BCT. Mood state was assessed both pre- and post-BCT by using the Profile of Mood States (POMS) questionnaire. The POMS is a standardized, widely used inventory of self-reported mood states (23). The POMS is sensitive to a variety of nutritional manipulations, environmental factors, and sleep loss (24–26). The test presents 65 mood-related adjectives that are rated on a 5-point scale in response to the question, “How are you feeling right now?” Six subscales are derived as well as an aggregate total mood scale.

Statistical analysis

Statistical analysis was performed by using commercially available statistical software (SPSS 16.0; SPSS Inc, Chicago, IL). Descriptive statistics are presented as means $\pm$ SDs. Comparisons of demographic characteristics were performed by using Student's *t* tests and chi-square tests. Two-way repeated measures analysis of variance was used to test treatment and time effects in addition to treatment-by-time interactions for both iron status indicators and POMS indexes. Normal distribution of variables was determined by using the Kolmogorov–Smirnov test. When variables were not normally distributed, results of the 2-factor repeated-measures analysis of variance were verified by using nonparametric *t* tests. In cases in which interactions were identified, post hoc comparisons were adjusted by using Bonferroni corrections. Two-factor analysis of variance was used to test treatment and iron status effects on 2-mile run time with

post hoc comparisons adjusted by using Bonferroni corrections. For all tests, significance was set at $P \leq 0.05$.

RESULTS

Iron status

Participation in the 8-wk BCT course had an effect on iron status, as shown by the elevation ($P < 0.05$) of hemoglobin, red blood cell distribution width, and sTfR in both the placebo and iron-treated groups following the BCT course (Table 2). Serum ferritin was diminished ($P < 0.05$) in the placebo but not in the iron-treated group after BCT. Significant ($P < 0.05$) group-by-time interactions were observed for serum ferritin and sTfR values. In the placebo group, serum ferritin values at the end of BCT were 66% of the starting values; in the iron-treated group, the post-BCT values were 87% of the starting values. Likewise, in the placebo group, sTfR values at the end of BCT were 125% of the starting values; in the iron-treated group, the post-BCT values were 110% of the starting values.

When volunteers were stratified by iron status, the prevalence of ID and IDA was similar at the start of the study: there were 14 ID volunteers in both the placebo and iron-treated groups, and 17 and 18 IDA volunteers, respectively, in the placebo group and the iron-treated group. At the end of the study, the number of ID volunteers in the placebo group climbed to 28, a 100% increase, whereas the number of ID volunteers in the iron-treated group was 19, a 36% increase. As hemoglobin increased over the course of BCT, 13 volunteers had IDA in the placebo group at the end of the study and 9 volunteers had IDA in the iron-treated group. The effects of BCT and the intervention on iron status indicators in volunteers stratified by pre-BCT iron status appear in Table 3. Group-by-time interactions ($P < 0.05$) were observed for hemoglobin in volunteers who were IDA at the start of the study; in the placebo group, hemoglobin concentrations rose by 6%, and in the iron-treated group, hemoglobin concentrations rose by 11%. Group-by-time interactions ($P < 0.05$) were observed for serum ferritin in volunteers who were iron-normal and IDA at the start of the study. In placebo-treated, iron-normal volunteers, post-BCT serum ferritin values were 65% of the pre-BCT values; in iron-treated volunteers, post-BCT serum ferritin values were 84% of the pre-BCT values. In volunteers with IDA, post-BCT serum ferritin values were 69% of the starting values in the placebo group and 139% of the starting values in the iron-treated group. A similar protective effect of the intervention was observed for sTfR. In placebo-treated, iron-normal volunteers, post-BCT sTfR values were 124% of the pre-BCT values; in iron-treated volunteers, post-BCT sTfR values were 111% of the pre-BCT values. Likewise, in volunteers with IDA, post-BCT sTfR values were 126% of the starting values in the placebo group and 104% of the starting values in the iron-treated group.

Physical performance and mood

When physical performance was assessed post-BCT by using the 2-mile run time, there was no difference between the placebo and the iron-treated groups in volunteers who were iron-normal or ID at the start of BCT (Figure 1). However, in volunteers with IDA at the start of BCT, iron treatment had a beneficial effect because mean run time was 110 s faster ($P < 0.001$) at the end

TABLE 2
Longitudinal changes in iron status during military training¹

	Baseline	After treatment	Effect
Hemoglobin (g/dL)			
Placebo	12.3 ± 1.3	12.8 ± 1.1	T
Iron	12.3 ± 1.1	13.0 ± 0.9	
RDW (%)			
Placebo	16.1 ± 1.5	16.8 ± 1.5	T
Iron	16.1 ± 1.6	16.9 ± 1.6	
Ferritin (ng/mL)			
Placebo	39.6 ± 30.7	26.0 ± 18.3 ²	T, G × T
Iron	37.0 ± 29.4	32.0 ± 22.1	
TS (%)			
Placebo	18.5 ± 8.8	17.8 ± 9.4	
Iron	18.8 ± 9.8	20.6 ± 10.3	
sTfR (nmol/L)			
Placebo	21.8 ± 8.0	27.2 ± 10.5 ²	T, G × T
Iron	21.4 ± 9.0	23.6 ± 9.6 ^{2,3}	

¹ All values are means ± SDs; *n* = 82 in the placebo group and 83–84 in the iron group. RDW, red blood cell distribution width; TS, transferrin saturation; sTfR, soluble transferrin receptor; T, main effect of time; G × T, group-by-time interaction (*P* < 0.01, all such occurrences). Two-factor repeated-measures ANOVA with post hoc Bonferroni corrections were used for comparisons.

² Significantly different from baseline, *P* < 0.01.

³ Significantly different from placebo group, *P* < 0.05.

of BCT in the iron-treated as compared with the placebo-treated volunteers (1081 ± 125 s compared with 1191 ± 96 s).

The BCT course resulted in a significant (*P* < 0.05) improvement in mood state as shown by effect of time on all subscales of the POMS questionnaire, as well as on the total score for both the placebo and iron-treated groups (Table 4).

There was a group-by-time interaction (*P* < 0.05) for vigor, which indicated that iron supplementation improved this indicator of mood over the course of BCT. When volunteers were stratified by iron status at the start of BCT, the beneficial effects of time on mood remained significant (*P* < 0.05) for all indicators, with the exception of anger in the ID groups (Table 5).

DISCUSSION

The major finding of this randomized, double-blind, placebo-controlled trial of iron supplementation in female soldiers during military training was that iron supplementation could attenuate the decline in iron status observed during BCT. Secondary findings indicate that iron supplementation over the course of BCT resulted in faster running time in women with IDA and that iron supplementation improved vigor, an important indicator of cognitive state. These findings are of importance to female soldiers because the period following BCT includes physically and cognitively demanding activities, which include advanced individualized training and the potential for operational deployment. Furthermore, these findings can be generalized to include all physically active premenopausal women because our data indicate that it is likely that iron status is diminished by moderate-to-heavy physical activity, which may affect physical performance and mood, especially in women who begin these activities with diminished iron status.

The decrement in indicators of iron status observed during military training, including serum ferritin and sTfR, have been described in previous studies of female soldiers (8, 9). Although the mechanism has not been identified, less-than-optimal iron intake has been reported in female military personnel (27, 28). Furthermore, the increase in physical activity experienced by

TABLE 3
Longitudinal changes in iron status during military training stratified by iron status at baseline¹

	Normal (placebo, <i>n</i> = 51; iron, <i>n</i> = 52)			ID (placebo, <i>n</i> = 14; iron, <i>n</i> = 14)			IDA (placebo, <i>n</i> = 17; iron, <i>n</i> = 18)		
	Baseline	After treatment	Effect	Baseline	After treatment	Effect	Baseline	After treatment	Effect
Hemoglobin (g/dL)									
Placebo	12.6 ± 0.9	13.2 ± 0.7	T	12.9 ± 1.6	12.8 ± 0.7		11.0 ± 1.0	11.6 ± 1.3 ²	T, G × T
Iron	12.6 ± 0.9	13.1 ± 0.7		12.7 ± 0.6	13.3 ± 0.6		11.0 ± 0.8	12.2 ± 1.2 ²	
RDW (%)									
Placebo	15.5 ± 1.1	16.2 ± 0.9	T	16.8 ± 1.1	17.0 ± 1.1	T	17.5 ± 1.8	18.3 ± 1.9	T
Iron	15.5 ± 0.9	16.2 ± 0.9		16.3 ± 1.1	17.0 ± 1.2		17.9 ± 2.2	18.8 ± 1.8	
Ferritin (ng/mL)									
Placebo	51.9 ± 30.3	33.8 ± 18.2 ²	T, G × T	26.0 ± 23.7	17.1 ± 10.4	T	14.0 ± 10.3	9.7 ± 5.5 ²	G × T
Iron	47.9 ± 30.6	40.3 ± 23.5 ²		30.8 ± 16.2	23.6 ± 8.3		10.4 ± 8.1	14.5 ± 8.7 ^{2,3}	
TS (%)									
Placebo	23.3 ± 6.0	21.0 ± 8.3		13.7 ± 8.6	15.4 ± 10.9		7.7 ± 2.8	10.3 ± 6.2	T
Iron	24.0 ± 8.3	23.8 ± 10.0		12.1 ± 2.0	16.7 ± 8.9		8.7 ± 5.1	14.6 ± 8.7	
sTfR (nmol/L)									
Placebo	18.7 ± 4.3	23.1 ± 5.3 ²	T, G × T	23.3 ± 7.5	29.4 ± 9.4	T	29.8 ± 11.0	37.6 ± 15.1 ²	T, G × T
Iron	18.5 ± 4.2	20.6 ± 5.2 ^{2,3}		22.3 ± 6.8	25.8 ± 4.5		29.6 ± 14.8	30.9 ± 16.6 ³	

¹ All values are means ± SDs. Volunteers were categorized as iron deficient if they presented with ≥2 of the following 3 indicators of abnormal iron status: serum ferritin <12 ng/mL, transferrin saturation (TS) <16%, or red blood cell distribution width (RDW) >15%. Volunteers were categorized as iron deficient with anemia if they had iron deficiency (ID) and hemoglobin concentrations <12 g/dL. IDA, iron deficiency with anemia; sTfR, soluble transferrin receptor; T, main effect of time; G × T, group-by-time interaction (*P* < 0.01, all such occurrences). Two-factor repeated-measures ANOVA with post hoc Bonferroni corrections were used to compare interactions within iron status grouping.

² Significantly different from baseline, *P* < 0.05

³ Significantly different from placebo group, *P* < 0.05.

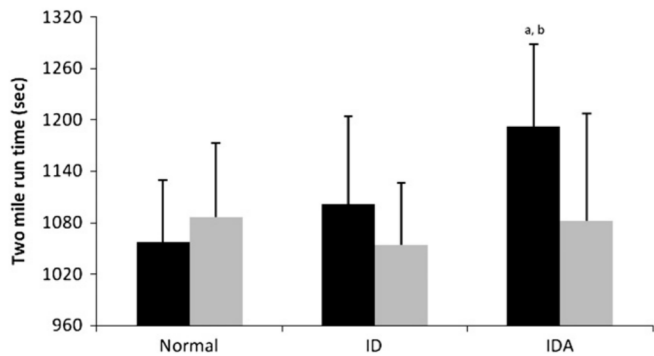


FIGURE 1. Two-mile run time after military training stratified by iron status at baseline. ■: Placebo group [*n*: normal = 46, iron deficiency (ID) = 12, ID anemia (IDA) = 17]. ■: Iron group (*n*: normal = 48, ID = 13, IDA = 14). Analyses were conducted by using 2-factor ANOVA with post hoc Bonferroni corrections. There was significant group-by-iron status interaction ($P \leq 0.001$). ^aWithin-group difference between iron-normal and ID, $P < 0.05$. ^bBetween-group difference between placebo and iron-treated group, $P \leq 0.001$.

soldiers beginning a training regimen could result in the production of proinflammatory cytokines, including interleukin-6 and tumor necrosis factor- α , which subsequently affect the synthesis of hepcidin by the liver (29, 30). Hepcidin has been identified as an important regulator of iron status that is secreted by the liver and other tissues in response to iron status and pro-inflammatory cytokines (31, 32). Although few studies have explored the role of hepcidin in the regulation of iron status in athletes or others participating in heavy physical activity, one study reports increased urinary hepcidin excretion in female

athletes following a marathon (33). Other potential explanations for the decrement in iron status experienced by female soldiers during BCT could include gastrointestinal bleeding (34), increased whole-body iron turnover (35), exercise-induced hematuria (36), or iron loss through sweat (37).

In this study, we selected ferrous sulfate (100 mg) as the dietary intervention with the objective of preventing the decrement in iron status observed during military training. We selected this dose and form of iron on the basis of previous work showing the efficacy of ferrous sulfate in improving iron status indicators with minimal side effects in studies of similar duration (12, 13). In our study, daily consumption of ferrous sulfate was effective in preventing the decrements in serum ferritin and sTfR observed in the placebo-treated group. Furthermore, iron supplementation resulted in a significant elevation in hemoglobin concentrations post-BCT in volunteers who reported to BCT with IDA. Interestingly, consumption of the iron supplement by individuals reporting to BCT with ID or with normal iron status did not result in an increase in any iron status indicator, excluding hemoglobin. In individuals reporting to BCT with IDA, iron supplementation resulted in improvements in serum ferritin, transferrin saturation, and hemoglobin. Although all other iron status indicators showed reduced iron status in the placebo group throughout the study, hemoglobin concentrations were moderately elevated ($\approx 4\%$) over the course of the study. This elevation in hemoglobin concentrations during physical training has been reported in the past and may indicate an increased mobilization of iron away from ferritin for the increased production of erythrocytes (6, 9).

Our findings suggest that supplementation with iron at concentrations (15 mg/d) approaching the current Recommended Dietary Allowance (RDA) for this demographic (18 mg/d; 38) may be effective for attenuating the decrement in iron status observed during military training, but not for restoring iron status in ID individuals. This finding was somewhat unexpected because female soldiers reporting to BCT with normal iron status or ID may experience level or negative iron balance during the course of the training period, even while consuming a daily iron supplement containing $\approx 85\%$ of the current RDA. Assuming that female soldiers consume a minimum of 3 mg of iron at the BCT dining facilities, which provide cafeteria-style meals with a variety of food choices, including foods containing bioavailable sources of iron, it is possible that RDA amounts of iron do not effectively maintain iron status during military training activities.

Iron supplementation resulted in a faster 2-mile running time post-BCT in volunteers that reported to BCT with IDA, but not in individuals who were ID or iron-normal, which suggests a better training adaptation in iron-treated individuals who present with IDA. It seems likely that faster run times are the result of improved hemoglobin concentrations in the IDA volunteers who consumed the iron supplement during BCT. Correlations between hemoglobin concentrations and running performance have been reported previously (9, 39).

Scores on the vigor subscale of the POMS increased substantially more (ie, double) in iron-treated volunteers compared with those receiving the placebo. Vigor, as assessed by the POMS, is a sensitive indicator of cognitive status. A variety of disease states, such as Parkinson's disease, multiple sclerosis, and depression are associated with loss of vigor and increased fatigue (40–42). Several nutritional deficiencies, such as thiamine or

TABLE 4
Longitudinal changes in Profile of Mood States indexes during military training¹

	Baseline	After treatment	Effect
Tension			
Placebo	11.3 $\pm$ 5.4	7.5 $\pm$ 5.4	T
Iron	12.8 $\pm$ 6.4	8.9 $\pm$ 6.6	
Depression			
Placebo	10.6 $\pm$ 8.8	5.5 $\pm$ 8.2	T
Iron	13.5 $\pm$ 10.6	7.6 $\pm$ 10.3	
Anger			
Placebo	7.8 $\pm$ 7.6	5.3 $\pm$ 8.1	T
Iron	9.4 $\pm$ 9.0	7.0 $\pm$ 8.7	
Vigor			
Placebo	10.5 $\pm$ 4.9	11.5 $\pm$ 6.1 ²	T, G $\times$ T
Iron	10.8 $\pm$ 4.9	13.1 $\pm$ 6.3 ²	
Fatigue			
Placebo	13.0 $\pm$ 5.8	9.3 $\pm$ 6.4	T
Iron	13.4 $\pm$ 5.6	9.8 $\pm$ 7.0	
Confusion			
Placebo	8.0 $\pm$ 3.6	5.6 $\pm$ 3.7	T
Iron	8.6 $\pm$ 4.6	6.3 $\pm$ 4.8	
Total			
Placebo	40.1 $\pm$ 27.7	21.7 $\pm$ 29.0	T
Iron	47.0 $\pm$ 33.6	26.5 $\pm$ 35.2	

¹ All values are means $\pm$ SDs; *n* = 85 in the placebo group and 86 in the iron group. Two-factor repeated-measures ANOVA with post hoc Bonferroni corrections were used for comparisons. T, main effect of time; G $\times$ T, group-by-time interaction ($P < 0.05$).

² Significantly different from baseline, $P < 0.05$.

TABLE 5

Longitudinal changes in Profile of Mood States indexes during military training stratified by iron status at baseline¹

	Normal (placebo, <i>n</i> = 52; iron, <i>n</i> = 54)			ID (placebo, <i>n</i> = 14; iron, <i>n</i> = 14)			IDA (placebo, <i>n</i> = 17; iron, <i>n</i> = 18)		
	Baseline	After treatment	Effect	Baseline	After treatment	Effect	Baseline	After treatment	Effect
Tension									
Placebo	11.8 ± 5.4	7.9 ± 5.4	T	9.6 ± 5.8	6.9 ± 5.5	T	10.6 ± 5.3	6.3 ± 4.8	T
Iron	12.7 ± 6.4	8.7 ± 6.6		9.0 ± 4.8	5.1 ± 4.1		16.4 ± 6.0	12.4 ± 6.9	
Depression									
Placebo	11.6 ± 9.1	6.2 ± 9.1	T	6.7 ± 6.9	3.8 ± 5.9	T	10.8 ± 9.4	4.4 ± 7.2	T
Iron	13.4 ± 10.2	8.1 ± 10.5		9.7 ± 10.4	3.4 ± 6.6		16.8 ± 11.5	9.3 ± 11.4	
Anger									
Placebo	8.4 ± 7.5	5.9 ± 8.0	T	7.6 ± 10.4	6.4 ± 11.2		6.3 ± 5.2	2.6 ± 4.8	T
Iron	9.2 ± 8.7	7.4 ± 9.0		6.5 ± 7.8	3.9 ± 6.1		12.6 ± 10.0	8.3 ± 9.4	
Vigor									
Placebo	9.9 ± 4.5	11.0 ± 5.5	T	9.9 ± 4.3	10.1 ± 5.9	T	13.2 ± 6.2	14.2 ± 7.6	T
Iron	11.1 ± 5.1	13.1 ± 6.6		10.7 ± 5.1	13.9 ± 6.2		9.9 ± 4.2	12.4 ± 5.5	
Fatigue									
Placebo	13.5 ± 5.4	9.6 ± 6.2	T	13.5 ± 6.0	10.7 ± 6.9	T	11.3 ± 7.0	7.0 ± 6.8	T
Iron	13.6 ± 5.3	9.6 ± 7.1		12.8 ± 6.5	8.9 ± 7.0		13.1 ± 6.0	10.5 ± 7.2	
Confusion									
Placebo	8.3 ± 4.1	5.6 ± 4.0	T	7.6 ± 2.6	6.0 ± 2.8	T	7.2 ± 3.0	4.8 ± 3.2	T
Iron	8.6 ± 4.6	6.5 ± 4.8		6.6 ± 4.2	4.4 ± 3.8		10.2 ± 4.6	7.5 ± 5.4	
Total									
Placebo	43.7 ± 28.3	24.1 ± 29.7	T	35.1 ± 29.8	23.6 ± 30.5	T	33.0 ± 24.7	10.9 ± 24.9	T
Iron	46.3 ± 32.3	27.3 ± 35.7		34.0 ± 32.8	11.9 ± 26.8		59.2 ± 35.9	35.7 ± 37.8	

¹ All values are means ± SDs. ID, iron deficiency; IDA, iron deficiency with anemia. Two-factor repeated-measures ANOVA were used for comparisons. T, main effect of time ($P < 0.01$).

vitamin C deficiency states, also cause decrements in this variable (43, 44). In addition, factors known to acutely degrade cognitive performance, such as sleep deprivation and treatment with hypnotics, adversely affect the POMS vigor scale (45–48). Improvement in a fundamental cognitive state, such as vigor, resulting from iron supplementation suggests that iron status is a critical nutritional variable that, when inadequate, degrades key aspects of cognitive function in at-risk populations and is in agreement with previous work in this area (16). This finding is consistent with the effects of iron supplementation on the productivity of workers in a variety of occupations (49) and suggests that the maintenance of iron status in female soldiers should be considered not only for the well-described physical performance benefits but also for the optimization of cognitive status.

Perhaps the greatest strength of this study was the randomized, double-blind, placebo-controlled design. Placebo or iron capsules that were the same in appearance were delivered to each volunteer on a daily basis, allowing for direct study-team interface with the soldier volunteers, which proved to be an effective method for tracking compliance and side effects. Compliance was generally high, at >90%, which likely resulted in reduced variability in our treatment effects. Weaknesses in our study include the difficulty in estimating the effects of BCT on plasma volume, which could affect iron status indicators. Studies with long-distance runners (50) and other endurance athletes (51) indicate that plasma volume may expand with long-term endurance training, which would affect the interpretation of iron status indicator data. However, the use of multivariable models with iron status indicators that provide a dynamic response to changes in iron status, including sTfR (52), may have overcome this potential confounder. A second shortcoming of this study

was the inability to estimate iron intake from food sources in our volunteer population; this level of contact with soldiers is typically not possible in the BCT environment. Currently, army regulations require the menus at the BCT cafeteria to include choices from each food group as described in the US Department of Agriculture food guide pyramid such that the military Dietary Reference Intakes for each nutrient may be consumed. The current military Dietary Reference Intake for iron is 15 mg/d, although a recent Institute of Medicine report recommended up to 22 mg/d (53). Although iron intake has not been assessed in army BCT, it is interesting to note that one study found that consumption of meat products, which generally contain bioavailable iron, did not change over the course of BCT, remaining consistent at ≈9 oz/d in female soldiers (54). Other shortcomings of this study include a lack of data regarding the effect of menstrual blood losses on iron status, the effect of BCT on other micronutrients, including folate and vitamin B-12, and the relative contribution of inflammation to the mechanism by which iron status declines during the training course. Future studies should attempt to collect menstrual cycle, food consumption, and inflammatory biomarker data in conjunction with the assessment of iron status indicators in an effort to determine the mechanism by which iron status declines during BCT and whether the current RDA for iron is appropriate for physically active premenopausal women, including soldiers.

In summary, this study shows that daily supplementation with ferrous sulfate capsules (100 mg) attenuates the decrement in iron status resulting from BCT. Furthermore, iron supplementation results in improved physical performance in volunteers reporting to BCT with IDA and improves a key aspect of mood in the combined cohort. These findings suggest that female soldiers

should be screened for iron status on initially reporting to BCT and that individuals with ID and IDA should be identified and provided with supplemental iron throughout the course. The prevention and treatment of IDA is especially critical for female soldiers participating in BCT because the period following the course includes the potential for physically and cognitively demanding activities, including operational deployment. Educational guidance regarding the consumption of foods containing bioavailable iron should be provided to female soldiers, and efforts to provide and identify foods rich in iron in military cafeterias should be initiated. It is likely that these findings and recommendations apply to other at-risk female populations, including those engaged in regular physical activity.

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