Micronutrient requirements of physically active women: what can we learn from iron?\textsuperscript{1–3}

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ABSTRACT
The health benefits of physical activity are well established and there is increasing recognition of the importance of fitness as a key modulator of chronic disease. The impact of physical activity on micronutrient requirements is a topic of tremendous interest to the lay public, but the interest is in sharp contrast to data from well-designed studies. Research in this area is poorly controlled for nutritional status of the participants, standardized exercise protocols, markers and cutoff points for measurement of micronutrient status, and variability in subject characteristics. The micronutrient status of women in the general population is of concern, but it is not clear that physical activity increases the requirement of most micronutrients. When dietary intake is adequate, the results of most studies are either equivocal or show no benefit to performance of supplementation. In the few instances where exercise does appear to increase an individual’s requirement, the increase can be obtained within the additional calories required for energy balance. In the absence of consistent data, micronutrient supplementation is often indiscriminate without regard to nutrient status. Because iron is such a key nutrient for physical activity, and the status in women is often compromised, it serves as a useful example of how current research limits the ability to make recommendations regarding the impact of exercise on micronutrients requirements in women. With the recent recognition of the importance of physical activity to the prevention and treatment of chronic diseases through the life span, more attention should be focused on the impact of exercise on micronutrient requirements, especially in the context of weight loss regimens. Am J Clin Nutr 2005;81(suppl):1246S–51S.

KEY WORDS Exercise, physical activity, iron, micronutrients, women, supplementation, nutritional status, dietary intake, exercise protocol, markers

INTRODUCTION
Though the health benefits of physical activity are indisputable (1–8), its impact on micronutrient requirements is much less well established. In the case of many micronutrients, women have inadequate intake (9), and a deficiency has a known detrimental impact on physical performance. Less clear is whether physical training and physical activity increase the requirement of particular micronutrients, and whether amounts in excess of the dietary reference intakes (DRIs) should be recommended for physically active individuals. In the few instances where exercise does appear to increase an individual’s requirement, the increase can be obtained within the additional calories required for energy balance (10–12). A large gap on micronutrient requirements exists in the literature regarding the impact of a weight loss regimen’s that include exercise.

Much of the uncertainty regarding micronutrient requirements and exercise stems from the lack of standardization or assessment of the nutritional status of the participants at entry, failure to assess subject’s dietary intake, lack of standardization of the exercise protocol or program, lack of standardization of markers and cutoff points, difficulty comparing one subject population (sex, menstrual status, age) to another, and failure to differentiate between nutritional requirements during the initial training period and those of the period that follows the adaptive phase.

In the absence of clear recommendations, micronutrient supplementation is often indiscriminate without regard to nutrient status, due to the belief that it cannot hurt and may help. This assumption is erroneous. In some instances, additional micronutrient ingestion beyond the DRI not only has no effect on exercise performance (12–14) but may have negative impact on general health (15, 16).

Many of these issues have been comprehensively addressed in recent reviews of the topic of exercise and micronutrients (13, 14, 17). This article uses iron as an example of how the limitations in current research constrain the ability to make clear recommendations regarding the impact of exercise on micronutrients requirements in women. The article concludes with the practical implications of the ambiguity, and suggestions for future research.

BRIEF OVERVIEW OF IRON PHYSIOLOGY AND REQUIREMENTS
Iron is a critical nutrient for active individuals, male and female alike. It plays a key role in energy production as a carrier of oxygen, both in the form of hemoglobin in the blood and myoglobin in the muscles. Additionally, iron is a part of the cytochromes found in the electron transport system.

The recommended dietary allowance (RDA) for premenopausal women is 18 mg daily. While additional iron is recommended for pregnancy and lactation, an increased intake is not

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recommended for physically active women. A tolerable upper level (UL) has been set at 45 mg daily for all adults to avoid the gastrointestinal distress often experienced with doses above this amount (18).

Menstruating women carry an increased risk for iron deficiency regardless of training status due to monthly blood loss. Adding to this concern, survey data shows that female athletes often underconsume calories (19–21), making it less likely that they will consume adequate dietary iron.

As iron clearly plays a critical role in athletic performance and deficiencies are not uncommon, female athletes are often advised to supplement iron without prior determination of hematological parameters. This practice is discouraged by medical practitioners due to the danger of toxicity from iron overload.

Iron depletion occurs in 3 stages. The first stage, depletion of stores, is identified by serum ferritin below 12 µg/L. The second stage, iron-deficient erythropoiesis, is identified by increased concentrations of transferrin and reduced transferrin saturation. Stage 3, anemia, is characterized by microcytic hypochromic red blood cells and diagnosed as hemoglobin below 12 mg/dL in females and below 13 mg/dL in males.

While it is clear that iron deficiency anemia impairs athletic performance, it is less clear whether iron deficiency without outright anemia will have this effect. Thus a focus of current research is whether iron deficiency without anemia will impair performance and, if so, whether and at what point iron supplementation should be initiated.

The choice of markers of iron deficiency has complicated clarification of this issue. Serum ferritin, the most commonly used marker, may become elevated in response to inflammation, infection, liver disorders, malignancies, and exercise-induced hemolysis (22). Iron deficiency may therefore be masked in certain individuals. Also clouding this issue is the lack of standardization in cutoffs used for diagnosis. Though serum ferritin concentrations below 12 µg/L is typically considered to be the marker for depletion of stores, researchers have use varying levels in developing inclusion criteria for their studies.

Measurement of serum levels of soluble transferrin receptor (sTfR) has recently become the gold standard for identifying iron deficiency in its earliest stage. First described in 1963, sTfR is bound to the cell membrane and mediates the endocytic transfer of iron from transferrin into erythroid cells. Levels increase when iron stores are depleted or turnover stimulated. Small amounts appear in the blood and can be measured. This marker, therefore, reflects both iron stores and the rate of erythropoiesis, and has been found to be more sensitive to iron deficiency than serum ferritin as well as more indicative of the functional pool of iron (23). At this time, this marker is the most accurate indicator of iron stores, and least confounded by factors such as inflammation (24). Values may be confounded by muscle growth, as this will result in a rise in sTfR levels due to a need for increased erythropoiesis. Thus muscle growth must be taken into account during analysis of changes in sTfR. In addition, the most appropriate cutoff point must be clearly established to allow a meaningful consensus to emerge regarding physical activity and iron requirements.

DOES EXERCISE INCREASE IRON REQUIREMENTS?

Proposed mechanisms for an increased requirement for iron include increased losses in sweat, feces and urine, intravascular hemolysis, and impaired absorption. Endurance athletes are known to experience sports anemia, a dilution of ferritin and hemoglobin due to the plasma volume expansion that occurs with training. This is a transient effect that occurs when plasma volume increases more rapidly than the increase in red blood cell mass, and typically results in a dilution of ferritin and hemoglobin of 15% (25).

Long distance runners may experience greater gastrointestinal losses through the feces. Though female data have not been provided, Telford and colleagues (26) studied hemolysis in male triathletes after a 1-hr cycle and run. They found that plasma free hemoglobin and serum haptoglobin concentrations were increased after both exercise bouts, but the increase was 4 times greater after the run than after the cycle. The authors concluded that footstrike is the major contributor to hemolysis during running.

Iron losses may occur through sweat and desquamated epithelial cells. One early study conducted in healthy males, in which the skin was carefully cleaned to remove desquamated epithelial cells and thus isolate sweat, found sweat iron losses to be small (22 µg per liter sweat) and thus unlikely to have an impact on iron requirements (27). A more recent study of female athletes found that sweat losses of iron declined with time (28). The greatest concentration of iron in sweat occurred during the first 30 min of exercise, and was lower in a hot environment than a neutral environment. Sweat iron concentration in this study was related to ferritin concentration, suggesting that conservation of iron may occur with reduced stores. The authors estimated that 5.7% of daily absorbed iron, or 1.2 mg/dL, would be lost by exercising females during the first hour of exercise, and that this could contribute to depletion of iron stores.

The effect of exercise on iron absorption has been questioned. While female data are not provided in this area either, moderate intensity exercise did not impair iron absorption in a study on male cyclists exercising at 60% VO2max for one hour (29). To the contrary, absorption of 100 mg ferric sodium citrate led to a 48.2% increase in serum iron concentrations when taken 30 min before exercise as compared with an increase of 8.3% when taken at rest.

Based on the available research, it is difficult to arrive at conclusions regarding the impact of exercise on iron requirements in women. If there is an increase in requirements, it will most likely be for women engaged in long distance running due to gastrointestinal losses and footstrike hemolysis. To fully clarify the confusion, future research protocols must control for diet, menstrual status, standardize exercise protocols, and use sTfR as the primary marker of iron status.

EFFECT OF TRAINING ON IRON STATUS

Strength training

Numerous studies have looked at changes in iron status that occur with training. In a study designed to examine the effects of resistance training on iron status, Murray-Kolb and colleagues tested 17 older, postmenopausal women (54–71 y old) and 18 men (56–69 y) before and after a 12-wk resistance training program (30). Diet including bioavailability of iron was evaluated with a 3-day food log, supplements accounted for, and compliance monitored. All hematological measures, including iron, transferrin, ferritin, and sTfR were within normal limits for both
genders at baseline and after training. However, women did experience a significant decrease in ferritin and a trend toward increased TIBC, whereas men experienced a rise in sTfR, probably related to an increase in lean mass, which was not achieved by the women. Thus the researchers found that men and women experienced sex-specific changes in iron status, and noted that a rise in sTfR during resistance training may indicate muscle growth rather than iron deficiency. Deruissseau et al (31) designed a similar study with collegiate men and women participating in a 12-wk weight training program. Diet and adherence were monitored. Contrary to the previous study, only the men experienced a decline in ferritin, and no change in sTfR was observed in subjects of either sex.

Despite the care taken by both research teams to control for diet, and to use state of art assessment of iron status, these 2 studies fail to resolve the question of whether strength training increase iron requirements, perhaps due to differences in exercise protocols. Clarification is needed regarding whether the observed rise in sTfR during resistance training indicates muscle growth or iron deficiency.

Endurance training

The iron status of female triathletes was assessed before and after a competition consisting of a 1.5 km swim, 40 km cycle, and 10 km run (32). Of the 12 athletes studied, 2 presented with anemia based on hemoglobin below 12 mg/dL and 4 met the researcher’s criteria for iron deficiency of ferritin below 10 μg/L before the event. Serum transferrin receptor was elevated in one subject before the race. After the race, ferritin concentration remained elevated after correction for hemoconcentration, while sTfR levels did not change. The dietary intake of iron in these athletes was not assessed, leaving it unclear whether iron deficiency and anemia occurred as a result of increased requirements or inadequate intake.

Ashenden et al (33) conducted a retrospective review of hematological status from 6 y of data on female rowers, basketball players, and netball players to establish changes that occur through training seasons and how the mode of training might effect those changes. Mean serum ferritin concentrations for all athletes experienced a decline in serum ferritin of about 25% during the training season. While the means did not drop below normal at any point, a sub-group presented with low concentrations (7.5 ± 2.7 μg/L). Rowers (non-weight-bearing) maintained higher levels than basketball and netball players (weight-bearing) throughout the season. Again, dietary intake was not evaluated and sTfR was not measured.

Based on these few studies, one of which focused on an acute exercise bout, the other on long-term training, it is once again too difficult to arrive at a conclusion that could serve as the basis for a recommendation for or against iron supplementation in response to training. A major obstacle to arriving at a conclusion is the failure of each study to assess dietary intake and correlate with hematological changes.

EFFECT OF IRON SUPPLEMENTATION ON NONANEMIC, IRON-DEFICIENT WOMEN

Earlier studies supplementing iron in non-anemic women have been equivocal. Reasons for this include lack of control of iron status and dietary intake, variability in subject characteristics (trained versus untrained), exercise protocols, parameters used for cutoffs, and use of different markers for measuring iron depletion.

A series of studies from the lab of Brownlie and colleagues tested the hypothesis that iron supplementation would help deficient but non-anemic women progress through a training program by improving endurance capacity (34–36). Subjects in these studies were 18- to 33-y-old untrained but active women who presented with normal hemoglobin (>120 mg/L) and low serum ferritin based on a cutoff of 16 μg/L. Subjects were given 8 mg elemental iron BID (35, 36) or 10 mg BIO (34) versus placebo for 6 wk. A 4-wk training protocol was carried out using cycle ergometers. The studies were all carefully controlled for diet using 4-d food logs and compliance with the protocol was monitored.

In the first of this series (34), subjects in the iron-supplemented group experienced an increase in serum ferritin, iron, and transferrin saturation, while sTfR concentration decreased. The placebo group was without significant changes in these parameters.

Endurance capacity and exercise performance were tested with a 15-km time trial at a level of resistance consistent with 70% VO2 max. While both groups improved their finish time in a time trial after the training program, the supplemented group was found to improve twice as much as the placebo group. Further analysis demonstrated that the effect of supplementation on endurance capacity was most pronounced in those who exhibited higher sTfR levels at baseline, suggesting an increased efficiency of oxygen utilization at the tissue level.

A subsequent study was conducted by the same laboratory (35) to further elucidate the effect iron depletion without anemia would have on women’s ability to improve aerobic capacity during a 4-week training program. The exercise protocol was the same as the previous study. Here, however, though serum ferritin, iron, and transferrin saturation increased in the supplement group, sTfR did not significantly change. Exercise testing revealed greater improvements in both absolute and relative VO2 max in the supplement group as compared with placebo.

Using sTfR to further delineate iron status, results were stratified between those with initially elevated levels indicating decreased stores and erythropoiesis and those with normal levels using a cutoff of 8.0 mg/L. Those with higher baseline sTfR were found to respond the most to iron supplementation, exhibiting the greatest improvements in VO2 max. Exercise performance was not tested in this study.

In the third study of this series (36), a time trial was again introduced as a performance measure. Physiologic responses were stratified by baseline sTfR concentrations using a cutoff of 8.0 mg/L, revealing a significant effect of supplementation on % VO2 max in the time trial in those subjects with baseline elevated sTfR. Improved performance in the time trial approached significance in this group compared to placebo.

The results of these studies demonstrate the value of including sTfR as a marker for functional iron deficiency. As the authors note, further research is needed to develop the most appropriate cutoff value so recommendations for supplementation can be accurately made.

Using another population, Friedmann and colleagues (37) studied the effect of iron supplementation in adolescent male and
female trained athletes with serum ferritin levels <20 μg/L and normal Hb levels. Athletes were given 100 mg elemental iron twice a day or placebo for 12 wk. A treadmill performance test was administered before and after the treatment period. Ferritin concentration increased significantly in the supplement group, while blood volume, red blood cell volume, and total body hemoglobin did not change in either group. VO_{2}\text{max} and O_{2} consumption increased significantly in the iron group, although it should be noted that the increase was slight, and relative VO_{2}\text{max} did not reach significance. sTfR was not measured, perhaps preventing the researchers from demonstrating an increase in erythropoiesis and masking a more significant improvement in a subgroup of their population. Furthermore, previous studies were successful at improving aerobic capacity and iron status with a much smaller dose of supplemental iron, leaving open the question of just how much iron is necessary to obtain the desired results.

Brutsaert and colleagues (38) used maximal voluntary contractions (MVCs) to test progressive muscle fatigue in 18- to 45-y-old women with normal hemoglobin (>110 g/L) and low ferritin (<20 μg/L) given a supplement of 10 mg elemental iron or placebo. Serum transferrin receptor, serum iron, and total iron binding capacity were measured. After a 6-wk training period, sTfR concentrations were found to rise in the placebo group, indicating a decrease in available iron. Serum iron and transferrin saturation rose in the treatment group, indicating an improvement in iron status. The rate of decline in MVCs was less rapid in the supplement group as compared with the placebo group, suggesting improved iron status at the tissue level.

Non-iron deficient adolescent male and female swimmers were studied for a 6-mo period while training for competition (39). A strength of this study was the attention paid to menstrual cycle and consequent blood loss. All subjects presented with normal levels of hemoglobin and ferritin (cutoff of normal was 7 ng/mL), but sTfR was not measured. One group was given an iron supplement of 47 mg, a second group was counseled on a high iron diet plan providing 26 mg of iron and a third group was included as a control without either intervention. Compliance with diet and supplementation was monitored. Dietary analysis showed that all groups met the RDA for iron. No performance benefit was found with supplementation in the absence of iron depletion.

Given the low cutoff used for serum ferritin, combined with the apparent adequate intake for all participants, it is not surprising that supplementation did not have an impact. In fact, this study was designed to deter unnecessary and indiscriminate supplementation that is common among female athletes.

These studies suggest that iron supplementation in nonanemic, iron-deficient women improves endurance performance. Although the range of supplemental iron was tremendous (8 mg/d to 100 mg/BID), it appears that 8 mg/d may be sufficient to achieve improvements. Three of these more recent studies (34-36) demonstrate that by controlling for dietary iron intake, reducing the variability in chosen markers, and by choosing a marker that is not confounded by inflammation, it is more likely to reach a consensus regarding physical activity and iron. The study that showed no improvement is an affirmation that supplementation is only warranted in the presence of iron deficiency (39).

HIGH-RISK GROUPS

Certainly specific populations are at an increased risk for iron deficiency, including adolescents experiencing a growth spurt especially once they begin menstruating. Inadequate energy intake has been identified from numerous surveys of female athletes (20–21), which increases the likelihood of inadequate iron intake. Individuals adhering to a strict vegetarian diet are hampered by the diminished bioavailability of non-heme iron. In addition, they are likely to consume food substances that impair absorption such as phytic acid, polyphenols, calcium and phosphate salts, and soy protein, placing them at further risk. Due to these factors, the RDA for menopausal women is 32 mg per day, almost twice the RDA for nonvegetarians (18).

In a recent review, Barr and Rideout noted that iron intake is similar among vegetarian and nonvegetarian athletes (40). The failure to meet the increased RDA for vegetarians is probably responsible for the higher incidence of functional anemia (normal Hb with low ferritin) reported in this population. Another review also concluded that a vegetarian diet may be a risk factor in iron deficiency, particularly for female runners (41).

Active women frequently remain physically active during pregnancy or may be unaware that they have become pregnant. Iron requirements increase significantly during pregnancy, and maternal anemia has been associated with an increased risk of preterm delivery (42). Therefore, it is imperative that women of childbearing ages, particular those with multiple pregnancies, monitor iron status carefully.

RECOMMENDATIONS FOR IRON SUPPLEMENTATION

The degree to which exercise itself increases iron requirements is unclear. While long-distance runners may experience gastrointestinal bleeding, it is likely that these losses will be compensated for by an increase in iron absorption (25). Sweat losses of iron do not appear to be of a degree to cause concern (27).

It is clear that iron supplementation should never be initiated without prior determination of iron status, as iron overload presents serious health issues. Differences in the male French elite cyclists were found in 1 study to have hyperferritinaemia as a result of excessive enteral and parenteral iron supplementation (43), which may increase the risk of liver disease. Hemochromatosis is a hereditary disorder that can result in iron overload. Supplementation might lead to toxic levels in athletes with this disorder. Those who supplement need to be aware that the body does not have a mechanism by which to excrete excess iron, and that excessive iron will act as a pro-oxidant, carrying with it a risk of liver cancer and cardiovascular disease. Additionally, dietary iron intake has been positively associated with the incidence of type 2 diabetes in postmenopausal women (44).

Governing groups must set protocols for evaluating iron status and initiating iron supplementation. A recent survey of NCAA Division I-A schools found that screening for iron deficiency was not routine, and that there is a wide degree of variability in the criteria used for diagnosis and treatment (45). Perhaps most interesting, hemoglobin and serum ferritin together were most often used to determine iron deficiency in the institutions responding to the survey, demonstrating that iron deficiency without anemia is not routinely identified. Serum transferrin receptor concentration is currently considered the most reliable way to
identify iron deficiency and should, in the opinion of some scientists, become the standard for assessing the iron status of athletes (16).

In an intriguing recent study of 321 early postmenopausal women (46), dietary iron was positively associated with bone mineral density in those women who had mean calcium intakes ranging from 800 to 1200 mg calcium daily. The relation remained after protein, which is also positively associated with bone mineral density, was factored out. The authors suggest that iron may play a role in the prevention of stress fractures in both the elderly and elite female athletes. Given the incidence of disordered eating in this population and its effect on bone mineral density, this study provides more ammunition for convincing female athletes to forgo energy and nutrient deficient diets in the quest for leanness.

Protocols will be difficult to develop unless testing and treatment become more standardized. Nielsen and Nachtigall (47) recommend using a pharmaceutical iron preparation of 100 mg/d with a known high bioavailability for a period of 3 mo, with the conditions for testing standardized and reported. They feel that supplementation should be recommended for those athletes with serum ferritin <35 μg/L to reverse up-regulation of mineral absorption and prevent an increased absorption for other potentially toxic metals along with iron. The need for such high dosages should be assessed in future studies, especially when in cases of mild deficiency, 8–10 mg/d may be sufficient for repletion.

In the studies reviewed above, diagnosis of iron deficiency has been made with a range of serum ferritin levels (7 μg to 35 μg). Some of the more recent studies measured sTfR, others did not. Few controlled for diet or initial iron status. Some studies were conducted on trained individuals, others on individuals initiating an exercise program, and exercise protocols varied in intensity, duration, and frequency. As a result, clear recommendations are difficult to make.

PRACTICAL APPLICATIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH

Despite an upsurge in interest in physical activity, for most vitamins and minerals, current research is not conclusive enough to provide specific micronutrient recommendations to physically active women. It is clear that they need to get at least the RDA for micronutrients, and that many women fail to do so for several vitamins and minerals. Therefore, a primary practical implication is to assess the micronutrient intake of any women engaged in a physical activity program, or about to embark on a program, and make sure that her intake at least meets the RDAs.

With regard to iron and many other micronutrients, several articles in this supplement stress the importance of a woman being replete at the outset of her pregnancy. Although young female athletes may be focused on performance, and the idea of pregnancy may seem remote, the importance of educating them, their physicians, and their parents about starting pregnancy “with a full tank” of micronutrients, cannot be overemphasized. As to whether or not exercise increases micronutrient requirements, future studies should be designed to address the gaps that currently exist:

• variability of nutritional status of the participants at entry
• lack of control and standardization of subject’s dietary intake of the micronutrients of interest.

REFERENCES


