Iron Depletion Without Anemia Is Not Associated With Impaired Selenium Status In College-Aged Women

By: Lisa S. McAnulty, Sareen S. Gropper, **Steven R. McAnulty**, & Robert E. Keith

Abstract

Iron-deficiency anemia has been shown to alter body mineral concentrations and activities of iron- and non-iron-containing enzymes, especially those with antioxidant functions. These effects, however, have been less studied in nonanemic iron-depleted individuals. Thus, this study assessed indices of selenium status in 12 college-aged females with adequate iron stores and 15 college-aged females with low iron stores before and after iron therapy. Blood samples were drawn at baseline for both groups and following iron supplementation in the low-iron-stores group. Hematocrit, hemoglobin, and serum ferritin concentrations of the low-iron-stores group were significantly lower than those of the control group. The serum transferrin receptor-to-serum ferritin ratio in the low-iron-stores group was significantly greater than that of the control group. Serum selenium and glutathione peroxidase concentrations of the low-iron-stores group were not significantly different from those of the controls. Iron supplementation significantly increased hemoglobin, hematocrit, and serum ferritin concentrations and significantly decreased the serum transferrin receptor concentration and serum transferrin receptor:serum ferritin ratio in the low-iron-stores group posttreatment compared to pretreatment. Serum selenium and glutathione peroxidase concentrations did not differ significantly from pretreatment to posttreatment in the low-iron-stores group. Results of this study indicate that low iron stores without anemia are not associated with impaired selenium status in college-aged females.

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ABSTRACT

Iron-deficiency anemia has been shown to alter body mineral concentrations and activities of iron- and non-iron-containing enzymes, especially those with antioxidant functions. These effects, however, have been less studied in nonanemic iron-depleted individuals. Thus, this study assessed indices of selenium status in 12 college-aged females with adequate iron stores and 15 college-aged females with low iron stores before and after iron therapy. Blood samples were drawn at baseline for both groups and following iron supplementation in the low-iron-stores group. Hematocrit, hemoglobin, and serum ferritin concentrations of the low-iron-stores group were significantly lower than those of the control group. The serum transferrin receptor-to-serum ferritin ratio in the low-iron-stores group was significantly greater than that of the control group. Serum selenium and glutathione peroxidase concentrations of the low-iron-stores group were not significantly different from those of the controls. Iron supplementation significantly increased hemoglobin, hematocrit, and serum ferritin concentrations and significantly decreased the serum transferrin receptor concentration and serum transferrin receptor:serum ferritin ratio in the low-iron-stores group posttreatment compared to pretreatment.

Serum selenium and glutathione peroxidase concentrations did not differ significantly from pretreatment to posttreatment in the low-iron-stores group. Results of this study indicate that low iron stores without anemia are not associated with impaired selenium status in college-aged females.

Index Entries: Selenium; glutathione peroxidase; iron depletion; females.

INTRODUCTION

Iron deficiency is one of the most common nutritional deficiencies worldwide and affects an estimated 40% of the world's population *(1).* In the United States, iron deficiency is frequently observed in infants, young children, adolescents, females of childbearing age (especially female athletes), and pregnant women *(2–4).* Data collected as part of the National Health and Examination Survey (NHANES) III revealed that the prevalence of iron deficiency in children aged 1–2 yr was 9%, with iron-deficiency anemia occurring in 3% of the children. In adolescents and young adult women, 9–11% were iron deficient and 2–5% were found to have anemia *(4).*

Many adverse consequences are associated with iron deficiency. These manifestations generally occur in combination with each other and are more strongly related to anemia rather than just tissue iron deficits. Some effects of iron-deficiency anemia include impaired immune function and cognitive performance, and alterations in behavior, thermoregulation, energy metabolism, and exercise performance *(4–14).* Additionally, iron-deficiency anemia has been shown to alter tissue concentrations of minerals other than iron such as selenium and copper and to alter the activities or concentrations of non-iron-dependent enzymes, especially those with antioxidant functions *(15–27).* Moreover, iron-deficiency anemia has been found to increase red blood cell susceptibility to oxidation in vitro *(28).*

Although serum mineral concentrations and enzyme activities and concentrations appear to be affected by iron-deficiency anemia, few studies have examined the effects of iron deficiency prior to the anemia stage. Yet, as shown in NHANES data, two to three times more people have iron deficiency without anemia than with anemia *(4).* Iron deficiency without anemia is typically characterized by normal blood hemoglobin and hematocrit concentrations and below-normal serum ferritin concentrations. In addition to serum ferritin, which represents body iron stores, at least one other indicator of iron status is routinely measured and, if altered, can suggest iron deficiency without anemia. To date, only one other study has examined the mineral status in individuals with nonanemic iron deficiency. Gropper et al. *(29)*, as a substudy of the present study, found significantly lower serum copper and ceruloplasmin concentrations and erythrocyte superoxide dismutase activity in college-aged females with

nonanemic iron depletion versus a control group. Moreover, iron supplementation significantly increased indices of iron status as well as serum copper and ceruloplasmin concentrations. To date, no studies have examined selenium status in individuals with low iron stores in the absence of anemia. Thus, the objectives of the present study were to examine indices of selenium status, specifically serum glutathione peroxidase and selenium concentrations, in college-aged women with adequate and low iron stores and to examine the effects of iron supplementation in the low-iron-stores group on these same indicators of selenium status.

SUBJECTS AND METHODS

Subjects were recruited through posted advertisements in buildings on campus and through announcements in health-related classes. Twenty-seven females, aged 19–28 yr, participated in the study. Approval for this study was received from the Institutional Review Board for the Use of Human Subjects in Research at Auburn University. Each subject signed an informed consent form.

All subjects were weighed and measured using standard techniques, and the body mass index was calculated. Subjects also completed a medical questionnaire and a 24-h diet recall. Subjects who reported taking vitamin and mineral supplements on the medical questionnaire were allowed to participate in the study if they agreed to discontinue supplementation 1 mo prior to the start of the study and refrain from taking supplements for the duration of the study. In addition, subjects were excluded from the study if illness was reported on the medical questionnaire or if found to have anemia. Anemia was defined as having a hemoglobin concentration ≤ 120 g/L and a hematocrit concentration ≤ 36%. Dietary recalls were analyzed for energy, protein, meat, total iron, heme iron, selenium, and ascorbic acid intakes using the Food Processor® nutrition analysis software (ESHA Research, Salem, OR).

Subjects reported to the laboratory for blood collection between 7:30 and 9:00 AM after at least a 7-h fast. Blood samples were taken at baseline on all subjects and were drawn from an antecubital vein into unheparinized Vacutainer® tubes using 20-gage needles. Aliquots of blood were immediately taken from unheparinized tubes and transferred to heparinized microhematocrit capillary tubes and Microtainer Brand® tubes for analysis of hematocrit and hemoglobin, respectively. Remaining blood in Vacutainer tubes was placed on ice until centrifugation at 2000*g* for 10 min at 4°C (Beckman Model J-6B centrifuge; Palo Alto, CA). Aliquots of serum were stored in acid-washed tubes at –80°C until further analysis.

Based on serum ferritin concentrations, subjects were placed in either the low-iron-stores group (serum ferritin ≤ 20 μg/L) or the control group (serum ferritin > 20 μg/L). The value of 20 μg/L was selected because it is considered to represent the lower limit of normal body iron stores, although

an internationally recognized threshold value for ferritin has not been established and cutoff values range from 12 to 22 μg/L *(30–33).* Serum transferrin receptor concentrations were subsequently measured as an additional indicator of iron status. Subjects in the low-iron-stores group received 5 wk of iron supplementation as ferrous sulfate and were instructed to take one capsule (50 mg elemental iron) with meals per day. Compliance was monitored weekly by phone. Only one subject discontinued iron therapy for 5 d because of gastrointestinal problems but remained in the study. Subjects were instructed to maintain usual dietary and exercise routines during the supplementation period. At the end of the 5-wk supplementation period, subjects in the low-iron-stores group reported to the laboratory as earlier to repeat the blood draw and to complete another diet recall.

Several assays were run to assess iron status. Hematocrit concentration was measured using a microhematocrit centrifuge (Damon/IEC Division, Needham Heights, MA). Hemoglobin concentration was determined using a spectrophotometric assay (Sigma Diagnostics, St. Louis, MO). Serum ferritin and transferrin receptor concentrations were measured using enzyme-linked immunosorbent assays (ELISAs) (Ramco Laboratories, Houston, TX). To assess selenium status, serum glutathione peroxidase concentration was determined using ELISA (Oxis International, Inc., Portland, OR), and serum selenium concentration was analyzed using graphite furnace–atomic absorption spectrophotometry (Perkin-Elmer, Norwalk, CT). Standard Reference Material (SRM) 1598 (National Institute of Standards and Technology, Gaithersburg, MD), bovine serum certified for selenium, was employed as a standard. Analysis of SRM 1598 generated a mean serum selenium concentration of 40.5 μg/L, within the reported assay range of 39.9–45.9 μg/L.

Statistical Analyses

Statistical analyses were conducted using InStat version 4.10 (GraphPad Software, San Diego, CA). Unpaired Student's *t*-tests were used to determine baseline differences between the low-iron-stores and control groups with respect to all variables measured. Unpaired Student's *t*-tests also were used to determine baseline differences in selenium status indices between the control group and a subset of subjects in the low-iron-stores group that exhibited both low serum ferritin and a high serum transferrin receptor-to-serum ferritin ratio. Paired Student's *t*-tests were utilized to determine differences within the low-iron-stores group (total and a subset of the group with both low serum ferritin and a high serum transferrin receptor-to-serum ferritin ratio) at baseline (pretreatment) versus following iron supplementation (posttreatment) for all assays performed. Log transformation was applied to serum ferritin values and the ratio of serum transferrin receptor to serum ferritin before statistical analysis to normalize skewed distributions. Correlations between variables were examined using Pearson's *r* correlation.

Table 1
Dietary Energy, Protein, Iron, Heme Iron, Meat, Selenium, and Ascorbic Acid Intakes of the Control and Low-Iron-Stores Groups

	Subject groups	
	Control (n = 11)	Low Iron Stores (n = 15)
Energy (kJ)	7996 ± 3854	7226 ± 2506
Protein (g)	74.0 ± 46.7	74.2 ± 43.5
Iron (mg)	24.2 ± 23.5	14.5 ± 8.5
Heme Iron (mg)	0.5 ± 0.6	0.7 ± 0.6
Meat (g)	100 ± 105	137 ± 95
Selenium (ug)	60 ± 54	98 ± 75
Ascorbic Acid (mg)	69 ± 68	76 ± 76

Note: Data given as $\bar{X}$ ± SD.

RESULTS

Fifteen females had serum ferritin concentrations ≤ 20 µg/L and represented the low-iron-stores group. The low-iron-stores group had a mean (± SD) age of 21.7 ± 2.1 yr, height of 162.7 ± 6.1 cm, weight of 57.7 ± 7.2 kg, and body mass index of 21.8 ± 2.3 kg/m^2. Twelve females had serum ferritin concentrations > 20 µg/L and represented the control group. The control group had a mean age of 23.2 ± 2.0 yr, height of 163.4 ± 6.2 cm, weight of 60.0 ± 12.3 kg, and body mass index of 22.5 ± 4.6 kg/m^2. No significant (p>0.05) differences in mean age, height, weight, or body mass index were found between the two groups. Analysis of dietary information showed no significant (p>0.05) differences for intakes of energy, protein, iron, heme iron, meat, selenium, or ascorbic acid between the two groups (*see* Table 1).

Indicators of iron and selenium status are presented in Table 2. Compared to the control group, the low-iron-stores group had significantly lower mean hematocrit (p = 0.003), hemoglobin (p = 0.014), and serum ferritin (p < 0.001) concentrations. Differences in mean serum transferrin receptor concentrations between both groups approached statistical significance (p = 0.0768). The serum transferrin receptor-to-serum ferritin ratio in the low-iron-stores group was significantly (p < 0.0001) greater than that of the control group. Of the subjects in the control group, none had a serum transferrin receptor to serum ferritin ratio >500, whereas in the low-iron-stores group, 9 of the 15 subjects had a serum transferrin receptor-to-serum ferritin ratio >500. Serum transferrin receptor concentrations were signifi-

Table 2
Markers of Iron and Selenium Status in the Control and Low-Iron-Stores Groups[1]

	Subject groups	
	Control (n = 12)	Low Iron Stores (n = 15)
Hematocrit (%)	43 ± 3	39 ± 2[2]
Hemoglobin (g/L)	144 ± 11	134 ± 9[2]
Serum ferritin (ug/L)	38 ± 15	11 ± 5[2]
Serum transferrin receptor (mg/L)	5.1 ± 1.2	6.1 ± 1.6
Serum transferrin receptor : serum ferritin ratio	151 ± 61	890 ± 753[3]
Serum glutathione peroxidase (ug/L)	37.1 ± 13.7	41.4 ± 14.9
Serum selenium (ug/L)	112 ± 22	116 ± 19

[1] $\bar{X} \pm$ SD.
[2] Significantly ($p \leq 0.01$) lower than the corresponding value for the control group.
[3] Significantly ($p \leq 0001$) greater than the corresponding value for the control group.

cantly inversely correlated with serum ferritin concentrations in the low-iron-stores group ($r = -0.558$, $p = 0.03$). The mean serum glutathione peroxidase and selenium concentrations were not significantly ($p > 0.05$) different between the control group and the low-iron-stores group. Statistical analysis of serum selenium and glutathione peroxidase concentrations in the control group versus a subset of only the nine subjects in the low-iron-stores group who exhibited both a low serum ferritin and a high (>500) serum transferrin receptor-to-serum ferritin ratio also found no statistically significant differences in either parameter between the two groups. The mean serum selenium concentrations for both groups were above the concentration in which glutathione peroxidase activity has been reported to be suboptimal (<70–90 μg/L) and were within the reported range of normal (95–163 μg/L) *(34,35).*

The mean weight and body mass index in the low iron stores group after 5 wk of iron supplementation (57.2 ± 7.0 kg and 21.6 ± 2.3 kg/m^2, respectively) did not differ significantly from baseline (57.7 ± 7.2 kg and 21.8 ± 2.3 kg/m^2, respectively). The mean dietary energy, protein, and ascorbic acid intakes in the low-iron-stores group, after 5 wk of iron supplementation, were not significantly different from pretreatment values (*see* Table 3). As expected, the mean iron intake by the low-iron-stores group, which included the 50 mg elemental iron from the daily supplement, was significantly ($p < 0.001$) greater posttreatment than iron intake by the group pretreatment (baseline). The mean heme iron and meat consumption, however, were not significantly different between the base-

Table 3
Dietary Energy, Iron, Heme Iron, Meat, Selenium, and Ascorbic Acid Intakes by the 15 Subjects in the Low-Iron-Stores Group Pretreatment and Posttreatment[1]

	Treatment Period	
	Pre-Treatment	Post-Treatment
Energy (kJ)	7226 ± 2506	6853 ± 2331
Protein (g)	74.2 ± 43.5	62.9 ± 31.0
Iron (mg)	14.5 ± 8.5	65.4 ± 11.4[2]
Heme Iron (mg)	0.7 ± 0.6	0.6 ± 0.6
Meat (g)	137 ± 95	104 ± 92
Selenium (ug)	98 ± 75	57 ± 72[3]
Ascorbic Acid (mg)	76 ± 76	106 ± 95

[1] $\bar{X} \pm$ SD.
[2] Significantly ($p \leq 0.0001$) greater than the corresponding pretreatment value.
[3] Significantly ($p \leq 0.05$) less than the corresponding pretreatment value.

line/pretreatment and postsupplementation periods. The mean dietary selenium intake was significantly ($p = 0.038$) less at the end of the supplementation period than at baseline/pretreatment; however, mean intake both presupplementation and postsupplementation was in excess of the recommended intake of selenium of 55 µg/d *(35)*.

The indicators for iron and selenium status in the low-iron-stores group at baseline (pretreatment) and following 5 wk of iron supplementation (posttreatment) are shown in Table 4. The mean hematocrit ($p = 0.005$), hemoglobin ($p = 0.05$), and serum ferritin ($p < 0.0001$) concentrations were significantly increased in the low-iron-stores group following iron supplementation compared to values in this group at baseline. At the end of the supplementation period, both the mean serum transferrin receptor concentration ($p = 0.006$) and serum transferrin receptor-to-serum ferritin ratio ($p < 0.0001$) were significantly decreased compared to values at baseline in the group. At the end of the supplementation period, none of the subjects in the low-iron-stores group had a serum transferrin receptor to serum ferritin ratio >500. Iron supplementation had no significant effect on either serum glutathione peroxidase concentration or serum selenium concentration.

DISCUSSION

Although numerous studies in both humans and animals have demonstrated that iron-deficiency anemia negatively impacts selenium status, this

Table 4
Hematocrit, Hemoglobin, Serum Ferritin, Transferrin Receptor, Transferrin Receptor : Ferritin Ratio, Glutathione Peroxidase, and Selenium Concentrations in 15 Subjects in the Low-Iron-Stores Group Pretreatment and Posttreatment[1]

	Treatment Period	
	Pre-Treatment	Post-Treatment
Hematocrit (%)	39 ± 2	43 ± 3[2]
Hemoglobin (g/L)	134 ± 9	140 ± 10[2]
Serum ferritin (ug/L)	11 ± 5	27 ± 8[2]
Serum transferrin receptor (g/L)	6.1 ± 1.6	4.6 ± 1.5[3]
Serum transferrin receptor : serum ferritin ratio	890 ± 753	198 ± 114[3]
Serum glutathione peroxidase (ug/L)	41.4 ± 14.9	43.5 ± 14.3
Serum selenium (ug/L)	116 ± 19	119 ± 9

[1] $\bar{X} \pm$ SD.
[2] Significantly ($p \leq 0.05$) greater than the corresponding pretreatment value.
[3] Significantly ($p \leq 0.05$) less than the corresponding pretreatment.

study was the first to examine indicators of selenium status in humans with low iron stores but no anemia. Low iron stores were not associated with impaired selenium status as assessed by serum selenium and glutathione peroxidase concentrations in this group of college-aged females. Moreover, although 5 wk of iron supplementation significantly improved iron status, as demonstrated by increased hematocrit, hemoglobin, and serum ferritin concentrations and decreased serum transferrin receptor concentrations and serum transferrin receptor-to-serum ferritin ratio, serum selenium and glutathione peroxidase concentrations remained unchanged following iron therapy. No significant differences in intakes of energy, protein, total or heme iron, or meat consumption were found between the control and low-iron-stores groups. Additionally, intake of ascorbate, which is known to enhance nonheme iron absorption, was not significantly different between the control and low-iron-stores groups. Also, with the exception of the mean iron intake, which increased as a result of supplement use, and the mean selenium intake, which decreased but remained above recommended intakes, these nutrient intakes did not differ in the low-iron-stores group presupplementation versus postsupplementation.

The findings of normal selenium status in females with low iron stores are in contrast to most studies that have examined the effects of iron deficiency on tissue mineral concentrations and mineral-dependent enzymes involved in antioxidant functions in the body. However, most previous studies have been conducted in humans or animals with iron-deficiency

anemia and not in subjects in the early stages of iron deficiency (i.e., with depleted stores and no anemia). Iron-deficiency anemia has been demonstrated to decrease tissue selenium concentrations in rats *(20,25)* and humans *(36),* as well as to decrease the activity and/or concentration of selenium-dependent glutathione peroxidase in rabbits *(23),* rats *(20,25),* and humans *(19,22,27,36,37).* Thus, it appears that in states of iron-deficiency anemia, in which typically both storage and functional iron deficits are present, selenium metabolism is affected, whereas in the present study with low iron stores, functional or transport iron appears to have been sufficient to maintain normal selenium metabolism.

The mechanisms by which iron-deficiency anemia affects tissue mineral concentrations, such as selenium and non-iron-containing enzymes such as glutathione peroxidase, have not been elucidated. Factors that may be responsible for changes in selenium concentrations in tissues in iron-deficiency anemia include alterations in mineral absorption, uptake, distribution, or tissue metabolism. Additionally, effects of non-iron-containing enzymes may be impacted through iron-mediated regulation of gene expression. Moriarty et al. *(20)* reported diminished glutathione peroxidase synthesis and lower concentrations of glutathione peroxidase mRNA in rats with iron-deficiency anemia versus adequate iron status rats, suggesting decreased glutathione perioxidase gene transcription and/or decreased glutathione perioxidase mRNA stability. Thus, one possible reason for the observed differences in findings between the states of iron-deficiency anemia and iron deficiency without anemia is that in the present study, involving nonanemic iron depletion, enough iron may have been available to the cells to maintain glutathione peroxidase, whereas in more deficient states, enzyme synthesis diminishes.

The mean serum ferritin concentration of the low-iron-stores group was 11 ± 5 μg/L, below the lower limit of normal (20 μg/L). The mean serum transferrin receptor concentration of the low-iron-stores group (6.1 ± 1.6 mg/L) was similar to values for healthy individuals reported by Flowers et al. *(38)* (5.6 ± 1.4 mg/L) and Ferguson et al. *(39)* (5.4 ± 0.8 mg/L), suggesting that erythropoiesis had not yet been impaired. Typically, as the severity of iron deficiency progresses, serum transferrin receptor concentrations increase, representing the diminished availability of functional iron. An upper limit of normal of 8.5 mg/L for serum transferrin receptor concentrations has been suggested *(38,40).*

The serum transferrin receptor-to-serum ferritin ratio has been shown to be a sensitive indicator of iron deficiency in individuals with iron-deficiency anemia and nonanemic iron deficiency *(33,39–42).* The mean serum transferrin receptor-to-serum ferritin ratio for the nonanemic low-iron-stores group at baseline in this study was 890 ± 753. This value is similar to those of two other groups of nonanemic college-aged females, 842 ± 1015 and 553 ± 385 reported by Zhu and Haas *(43).* Skikne et al. *(40)* suggested that a serum transferrin receptor-to-serum ferritin ratio > 500 is indicative of depleted iron stores in adults.

Five weeks of iron therapy succeeded in raising serum ferritin concentrations above 20 μg/L in all but four subjects and in decreasing serum transferrin receptor concentrations in all but two subjects. The mean serum transferrin receptor-to-serum ferritin ratio in the present study decreased from 890 ± 753 to 198 ± 114 and more closely resembled the mean ratio of the control group (151 ± 61). Zhu and Haas *(43)* found that 8 wk of iron treatment (135 mg of elemental iron) in 20 nonanemic college-aged females significantly decreased the serum transferrin receptor to serum ferritin ratio to 180 ± 145. These findings suggest that because the serum transferrin receptor concentration depicts the overall balance between iron demand and iron supply in various body tissues, either the increase in iron supply from the iron capsules or a decrease in iron demand as iron stores were repleted was responsible for the observed decreases in concentrations posttreatment *(43).*

A significant (p = 0.03) inverse correlation between serum transferrin receptor concentrations and serum ferritin concentrations (r = –0.56,) was found in the low-iron-stores group in the present study. Similar correlations have been observed in healthy males *(44).*

Iron deficiency is known to affect millions of infants, children, and adults, especially females, and to impair health and well-being. Recent studies suggest that nonanemic iron depletion is associated with poor copper status and with adverse effects on physical performance *(29,45).* Although the results of this study showed that low iron stores without anemia are not associated with detrimental effects on serum selenium and glutathione peroxidase concentrations in college-aged females, the effects of iron depletion without anemia on body functions, especially those that are mineral dependent, require further investigation.

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