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Non-anemic iron deficiency, oral iron supplementation, and oxidative damage in college-aged females

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Abstract

Oxidative damage, as indicated by protein carbonyl and lipid hydroperoxide concentrations, was assessed in the plasma of college-aged females with adequate iron status and with non-anemic iron deficiency before and after eight weeks of iron supplementation. At baseline, the mean serum ferritin, iron, transferrin saturation, and total iron binding capacity of the iron deficient group (n = 13) was significantly different from the iron adequate controls (n = 24). Mean plasma lipid hydroperoxide and protein carbonyl concentrations did not differ between groups at baseline. Following eight weeks of iron supplementation, the mean serum ferritin, iron, and transferrin saturation significantly increased and the total iron binding capacity significantly decreased in the iron deficient group. No significant differences in plasma lipid hydroperoxide or protein carbonyl concentrations were found between groups at the end of the study period. When plasma lipid hydroperoxide and protein carbonyl concentrations of subjects within groups were compared at the start versus at the end of the study, no significant differences were found for either group. Neither non-anemic iron deficiency nor its treatment with oral iron supplements is associated with oxidative damage in the plasma of college-aged females.

Keywords: Non-anemic; Iron deficiency; Oxidative damage; Plasma protein carbonyl; Plasma lipid hydroperoxides; Females

1. Introduction

Iron deficiency is one of the most common nutritional deficiencies in the world as well as in the United States [1]. The condition impairs many aspects of health including the body's antioxidant system; however, the treatment of iron deficiency through oral ingestion of iron supplements also may be associated with oxidative damage. Iron deficiency anemia, for example, affects the absorption and metabolism of other antioxidant minerals including copper and selenium. Iron deficiency in rats promotes increased intestinal cell copper absorption, increased hepatic storage of copper, and reduced hepatic release of ceruloplasmin into the plasma [2–5]. Copper as well as iron, if unbound and especially if present in large quantities, are known to react with hydrogen peroxide as part of the Fenton and Haber-Weiss reactions. These reactions generate hydroxyl radicals, which are capable of damaging DNA, lipids, as well as proteins [6]. Iron deficiency anemia also has been shown in both humans and animals to diminish the concentrations and/or activities of glutathione peroxidase [7–13], catalase [7,11,14–16], and superoxide dismutase [15,17]. Recent studies also have shown that iron deficiency without anemia is associated with significant reductions in serum copper and ceruloplasmin, and erythrocyte superoxide dismutase activity, but not serum selenium or glutathione peroxidase concentrations, in college-aged females [18,19]. Reductions in antioxidant enzyme activities/concentrations may result in increases in substrates normally acted upon by the affected enzyme. Thus, diminished glutathione peroxidase and catalase may result in hydrogen peroxide accumulation, and diminished superoxide dismutase may lead to increased superoxide radicals. Superoxide radicals, one of many free radicals, and hydrogen peroxide, a reactive oxygen species, can cause oxidative damage if not removed from the environment.

To date, only a few studies, conducted in animals, have examined oxidative damage associated with iron deficiency anemia. The results of these studies demonstrate that iron

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deficiency anemia in rats is associated with lipid peroxidation and DNA damage in some but not all tissues [20–22]. Whether or not iron deficiency and the extent to which iron deficiency are associated with oxidative damage in humans has not been investigated.

While animal studies suggest that iron deficiency anemia increases oxidative damage to some body tissues, the treatment of iron deficiency appears to be associated with additional damage in rats. Iron deficient anemic rats treated with oral iron have been shown to exhibit in some but not all tissues significantly higher concentrations of malondialdehyde and protein carbonyls and exhibit more DNA damage than iron deficient anemic rats not treated with iron and/or control rats [20–22].

The treatment of iron deficiency in humans is usually accomplished through ingestion of oral iron supplements in dosages ranging up to about 120 mg/day. Whether or not ingestion of oral iron supplements is associated with oxidative damage in humans has not been investigated to date. This study is the first to examine associations between oxidative damage and non-anemic iron deficiency and its treatment in humans. The purposes of the study were to first determine if iron deficiency without anemia was associated with oxidative damage, and secondly to determine whether iron supplementation to treat iron deficiency impacts oxidative damage.

2. Methods and materials

2.1. Subjects

Subjects recruited for this study consisted of females aged 19 to 35 years. Subjects were recruited through announcements in Nutrition and Food Science, Health and Human Performance, and Nursing classes at Auburn University, Auburn, Alabama. Fliers also were posted in buildings and dorms at Auburn University, and at Cory Everson Aerobics and Fitness for Women, a local gym in Auburn, Alabama. Subjects were excluded from the study if taking medications or mineral supplements, or had a chronic illness, an infection, or a recent injury.

Subjects were considered "healthy" that is, "iron adequate" if subjects had normal concentrations of serum ferritin (>20 μg/L), serum iron (>11 μmol/L), transferrin saturation ($>15\%$), blood hemoglobin (>120 g/L) and hematocrit ($>36\%$). These subjects served as the control group. Subjects were considered to have non-anemic iron deficiency if hemoglobin and hematocrit concentrations were greater than 120 g/L and greater than 36%, respectively, and serum ferritin concentrations were ≤ 20 μg/L, serum iron concentrations were ≤ 11 μmol/L, and transferrin saturation was $\leq 15\%$ [22–25]. Subject participation in this study was voluntary. Each participant signed a letter of informed consent. Approval for this study was received from the Institutional Review Board for the Use of Human Subjects in Research at Auburn University.

2.2. Experimental methods and study design

Volunteers for the study reported on two different occasions. During the first visit, each subject completed a general medical questionnaire. The questionnaire provided information on subject health status, current medication(s) use, dietary supplement use, recent occurrences of injury and illness, and history of iron deficiency anemia. Subjects were measured for height and weight at the start and end of the study. All measurements were taken using standard techniques in a private room. All subjects were instructed to record a three-day diet record (two weekdays and one weekend day) at the start and end of the study. Diet records were analyzed for energy and nutrient intakes using Nutritionist V (First Data Bank, Inc., San Bruno, CA). Selected nutrient intakes were compared to current recommendations [23,27].

Blood samples (20 ml) were taken during the initial visit and during the second visit eight weeks later. Blood collection occurred between 7 am and 8 am after at least a seven-hour fast. Blood was drawn from an antecubital vein into two Vacutainer® tubes, one containing heparin and one containing no anticoagulant, using 21 gauge Vacutainer® needles by a certified phlebotomist.

Whole blood was analyzed within 6 hr of sampling for hemoglobin and hematocrit concentrations. Hematocrit concentrations were measured following centrifugation in a microhematocrit centrifuge (Stat Spin, Norwood, MA). Hemoglobin concentrations were measured using a spectrophotometric assay (Sigma Diagnostics, St. Louis, MO).

Vacutainer® tubes were placed on ice until centrifugation. Blood was allowed to clot before centrifugation. All tubes were centrifuged at 2000 x g for 20 min at 4°C (Beckman Model J-6B centrifuge, Beckman Instruments, Inc., Palo Alto, CA). Aliquots of serum and plasma were pipetted into vials, flash frozen in liquid nitrogen, and placed into an ultra-low temperature freezer at -80°C until further analysis.

Serum was analyzed for ferritin, iron, and unsaturated iron binding capacity (UIBC), to further assess iron status. Ferritin concentrations were analyzed using Coat-A-Count Ferritin Immunoradiometric assay (Diagnostic Products Corporation, Los Angeles, CA). Serum iron and UIBC were determined using colorimetric assays (Sigma Diagnostics, St. Louis, MO). Total iron binding capacity (TIBC) was calculated from total iron and UIBC. Transferrin saturation (Tsat) was calculated by multiplying serum iron by 100 and dividing by TIBC.

Two parameters, protein carbonyl and lipid hydroperoxide concentrations, were measured to determine the presence of oxidative damage. Protein carbonyls were measured in the plasma using an enzyme-linked immunosorbent assay (Zenith Technology Corp. Ltd, Dunedin, New Zealand). Lipid hydroperoxides were measured in the plasma using a

colorimetric method (Oxis Research, Portland, OR). Standards and controls were run with all assays to ensure accuracy and all assays were run in at least duplicate with a coefficient of variance of <7%.

2.2.1. Treatment (iron supplementation)

Subjects with non-anemic iron deficiency received free of charge an eight-week supply of ferrous sulfate supplements (Slow Release Iron, Leiner Health Products, Inc., Carson, CA), each providing 50 mg elemental iron (160 mg ferrous sulfate). Subjects were instructed to take one supplement daily with a meal for eight weeks and to not change dietary habits or exercise routines. Compliance with iron supplementation was monitored by follow-up phone calls, emails, and quantification (a counting) of remaining iron supplements at the end of the eight-week period.

Iron adequate subjects served as the control group, and were instructed to maintain their usual dietary habits and exercise routines for the eight-week duration of the study, and were not given iron supplements. At the end of eight weeks, all subjects (both control and iron deficient) returned to the laboratory for blood withdrawal and reassessment of all blood parameters, anthropometric reassessment, and completion of another three-day diet record.

2.3. Statistical analysis

Statistical analyses were conducted using InStat (GraphPad Software, San Diego, CA). Student's t-test was used to analyze mean differences for hemoglobin, hematocrit, ferritin, iron, transferrin saturation, TIBC, lipid hydroperoxides, and protein carbonyls and dietary intakes of energy, protein, vitamins A, C, and E, iron, copper, and zinc between the iron adequate control group and the non-anemic iron deficient group at baseline and after eight weeks. Paired t-tests were used to analyze mean differences for all measured parameters within each group at baseline versus at eight weeks. A Pearson correlation was used to assess the relationships between serum ferritin and both lipid hydroperoxides and protein carbonyls at baseline and the end of the study. In addition, Pearson correlation was used to assess correlations between calculated nutrient intakes and both protein carbonyls and lipid hydroperoxide concentrations at baseline and at the end of the study. Log transformation was applied to values if needed to normalize skewed distributions. Untransformed data are provided in tables. A P-value of <0.05 was considered significant.

3. Results

Thirty-seven subjects participated in the study. The iron adequate control group consisted of 24 subjects. The non-anemic iron deficient group consisted of 13 subjects. Following baseline assessments, subjects in the iron deficient group were given iron supplements (50 mg elemental iron), while the subjects in the control group did not receive iron supplements. Compliance with taking the iron supplements was 88%. The average number of iron supplements ingested was six supplements per week during each of the eight weeks.

Table 1
Mean ± standard deviation serum ferritin, iron, transferrin saturation, total iron binding capacity, blood hemoglobin and hematocrit, and plasma lipid hydroperoxides and protein carbonyl concentrations of iron deficient and iron adequate college-aged females at baseline and after eight weeks (end of study)

Parameter	Iron Deficient Group	Iron Adequate Control Group
Ferritin (μg/L)		
Baseline	11 ± 6[a]	43 ± 21[b]
End of study	25 ± 13[a]	53 ± 31[b]
Iron (μmol/L)		
Baseline	6 ± 2[a]	16 ± 5[b]
End of study	10 ± 5[a]	15 ± 5[b]
Transferrin saturation (%)		
Baseline	10 ± 4[a]	28 ± 8[b]
End of study	18 ± 9	25 ± 9
Total iron binding capacity		
Baseline	360 ± 52[a]	311 ± 36[b]
End of study	312 ± 26	319 ± 30
Hemoglobin (g/L)		
Baseline	140 ± 13	142 ± 7
End of study	142 ± 9	139 ± 11
Hematocrit (%)		
Baseline	43 ± 3	43 ± 2
End of study	42 ± 3	42 ± 3
Lipid hydroperoxides (μmol/L)		
Baseline	9.4 ± 4.9	9.9 ± 4.9
End of study	8.7 ± 3.8	10.1 ± 4.0
Protein carbonyls (nmol/mg protein)		
Baseline	0.35 ± 0.26	0.35 ± 0.26
End of study	0.32 ± 0.20	0.43 ± 0.24

[a,b] Values in rows with differing suprascripts differ significantly ($P < 0.05$)

No significant differences were found between groups at baseline for age, height, and weight. The mean age of the control group was 22.1 ± 3.9 years while that of the iron deficient group was 24.1 ± 5.1 years. The mean height and weight of the control group were 166.6 ± 4.7 cm and 63.2 ± 11.1 kg, respectively and for the iron deficient group were 166.7 ± 4.7 cm and 62.9 ± 10.9 kg respectively.

Baseline mean serum ferritin ($P < 0.0001$) and iron ($P < 0.0001$) concentrations and transferrin saturation ($P < 0.0001$) of the iron deficient group were significantly lower than that of the control group (Table 1). The mean baseline total iron binding capacity of iron deficient group was significantly ($P < 0.003$) greater than that of the control group (Table 1). No significant differences in mean hemoglobin or hematocrit were found between the two groups (Table 1), and the hematocrit and hemoglobin concentrations of all subjects were within the normal range.

Baseline mean energy and selected antioxidant nutrient intakes did not significantly differ between the two groups

Table 2
Mean ± standard deviation energy, protein, vitamins A, C, and E, and iron, copper and zinc intakes of iron deficient and iron adequate college-aged females at baseline and after eight weeks (end of study)

Nutrient	Iron Deficient Group	Iron Adequate Control Group
Energy (kcal)		
Baseline	1725 ± 567	1460 ± 342
End of study	1769 ± 645	1276 ± 401
Protein (g)		
Baseline	61 ± 27	66 ± 19
End of study	60 ± 17	62 ± 28
Vitamin A (μg)		
Baseline	1749 ± 1059	2536 ± 2374
End of study	894 ± 582	1391 ± 929
Vitamin C (mg)		
Baseline	142 ± 141	107 ± 155
End of study	35 ± 29	89 ± 68
Vitamin E (mg)		
Baseline	7.2 ± 4.6	6.9 ± 6.8
End of study	11.6 ± 5.3	6.6 ± 5.0
Iron (mg)		
Baseline	16.4 ± 13.1	11.6 ± 5.3
End of study	60.1 ± 7.6[a]	10.4 ± 5.0[b]
Copper (μg)		
Baseline	846 ± 441	815 ± 506
End of study	612 ± 432	650 ± 277
Zinc (mg)		
Baseline	7.5 ± 4.0	6.9 ± 3.6
End of study	5.5 ± 1.9	5.9 ± 4.5

[a,b] Values in rows with differing suprascripts differ significantly ($P < 0.0001$)

(Table 2). Mean baseline intakes of iron by both groups exceeded two-thirds of the recommended dietary allowance (RDA). Mean baseline intakes of vitamins A and C by both groups exceeded the RDA. Mean zinc and copper intakes by both groups at baseline were greater than 85% and 91%, respectively, of the RDA. Mean vitamin E intake was 46% of the RDA for controls and 48% of the RDA for the iron-deficient group. Four subjects in the iron deficient group reported smoking. Of the four, one subject averaged smoking a 0.5 pack/week while the other three smoked between 1 and 1.5 packs/week. No subjects smoked in the control group.

No significant differences in plasma lipid hydroperoxide or protein carbonyl concentrations were found between the groups at baseline (Table 1). When subjects who smoked were excluded from data analysis, statistical results did not change. No correlations were found between either plasma lipid hydroperoxides or protein carbonyl concentrations and baseline dietary intakes of vitamins A, C, or E, or copper, zinc, or iron. Similarly, no correlations were found between either lipid hydroperoxides or protein carbonyls and serum ferritin concentrations at baseline.

Eight weeks after the start of the study, 11 of 13 iron deficient subjects and 14 of the 24 controls returned. However, data from four control subjects were excluded because they became iron deficient (serum ferritin ≤20 μg/L, transferrin saturation ≤15%, serum iron ≤11 μmol/L) and thus end of the study data are reported based on 10 iron adequate controls. No significant differences were found between groups for weight or body mass index at the end of the study.

As shown in Table 1, at the end of eight weeks (end of study), no significant differences were found between groups in mean concentrations of serum iron, transferrin saturation, total iron binding capacity, hematocrit, or hemoglobin. At the end of the study, only the mean serum ferritin concentration of the iron deficient group (25 ± 13 ug/L) remained significantly lower than that of the control group (53 ± 31 ug/L).

Mean intakes of energy, protein, copper, zinc, and vitamins A and E did not significantly differ between groups at the end of the study (Table 2). As expected at the end of the eight weeks, the mean iron intake by the iron deficient group (60.1 ± 7.6 mg), which included supplemented iron, was significantly ($P < 0.001$) greater than the control group (10.4 ± 5.0 mg).

No significant differences in plasma lipid hydroperoxide or protein carbonyl concentrations were found between the two groups at the end of the study period (Table 1). No correlations were found at the end of the study between either lipid hydroperoxides or protein carbonyls and dietary intakes of vitamins A, C, or E, zinc, copper, or iron. Similarly, no correlations were found between serum ferritin concentrations and either lipid hydroperoxides or protein carbonyls. Lipid hydroperoxide concentrations were not found to correlate with protein carbonyl concentrations.

Analysis within each group, comparing parameters measured at baseline to the same parameter measured at the end of the study, found that the mean serum ferritin concentration of the iron deficient group at the end of the study was significantly ($P = 0.018$) greater than at baseline. Also within the iron deficient group, the mean transferrin saturation significantly ($P = 0.022$) increased and the mean total iron binding capacity significantly ($P = 0.012$) decreased at the end of the study versus at baseline. The mean increase in the serum iron concentration of the iron deficient group approached significance ($P = 0.083$) from baseline to the end of study. No significant differences were found within the iron deficient group in mean serum hemoglobin and hematocrit concentrations at baseline versus the end of the study. No significant differences in any of the assessed iron status parameters were found between baseline and the end of the study in the controls.

Analysis of baseline nutrient intakes versus end of study intakes within each group found the iron deficient group had a significantly ($P = 0.0014$) greater intake of iron and a significantly ($P = 0.032$) lower intake of vitamin C at the end of the study compared to baseline. No other differences in intake were found between baseline and the end of the study for either group. When baseline plasma lipid hydroperoxide and protein carbonyl concentrations of subjects within groups were compared at the start versus at the end

of the study, no significant differences were found in either of the groups.

4. Discussion

The results of this study showed that non-anemic iron deficiency was not associated with lipid peroxidation or protein oxidation in the plasma of college-aged females. While no other studies have examined oxidative damage in humans with iron deficiency, male rats (but not female rats) with iron deficiency anemia have been shown to have greater manifestations of oxidative damage in some tissues than male rats with adequate iron status. For example, male rats with iron deficiency anemia exhibit significantly greater hepatic mitochondrial DNA damage, liver and kidney (but not plasma) malondialdehyde concentrations, and breath pentane concentrations versus iron-adequate male rats [20,21]. Uehara and associates [4] reported differences in responses between male verus female rats whereby significantly higher serum and liver phosphatidylcholine hydroperoxides were found in male iron deficient rats versus male iron adequate and versus female iron deficient and female iron adequate rats. Similar to the findings of the present study and the findings in the female rats in Uehara and coworker's study [4], Srigiridhar and Nair [22] showed no significant differences in malondialdehyde and protein carbonyl concentrations in the intestinal cells of iron deficient versus iron adequate female rats. On the other hand, Rao and Jagadeesan [28] reported significantly lower malondialdehyde concentrations in the liver of iron deficient male versus iron adequate male rats.

Treatment of iron deficiency in the United States is predominantly accomplished through oral ingestion of iron supplements. In this study, eight weeks of iron supplementation significantly improved the iron status of the iron deficient group although the mean serum ferritin concentration of the iron deficient group was $>$20 ug/L it remained significantly less than that of the controls. Yet, at the end of the study period, the mean plasma protein carbonyl and lipid hydroperoxide concentrations did not differ significantly between the iron deficient and iron adequate groups. Moreover, mean plasma protein carbonyl and lipid hydroperoxide concentrations at the end of the study did not significantly change from respective baseline concentrations within either group. These findings suggest that daily oral iron supplementation providing 50 mg elemental iron for eight weeks did not result in increased oxidative damage as measured by protein carbonyls and lipid hydroperoxides in the plasma of college-aged females.

Studies in rats with iron deficiency anemia receiving oral iron supplements have reported mixed results. Srigiridhar and Nair [22] showed that oral iron supplementation significantly increased malondialdehyde production and protein carbonyl formation in the intestines of iron supplemented iron deficient female rats as compared to unsupplemented iron deficient and control female rats. No control rats were given iron supplements. Daily oral iron supplementation of male rats with iron deficiency anemia has been shown to result in significantly greater hepatic mitochondrial DNA damage than iron-adequate control male rats both receiving and not receiving iron supplementation [21]. Knutson and associates [20] found that malondialdehyde concentrations in the liver and kidney were significantly lower but breath pentane significantly higher in iron supplemented iron deficient male rats than in unsupplemented iron deficient male rats. Kidney malondialdehyde concentrations in iron deficient rats receiving iron supplements were significantly higher than in normal rats receiving supplements, but not different from unsupplemented normal rats.

Several factors could account for the observed differences in the response to iron deficiency and its treatment between studies in rats and the present study in humans. First, studies demonstrating significant increases in oxidative damage were conducted in rats with iron deficiency anemia, and the iron deficient subjects in the present study did not have anemia. Differences in antioxidative enzyme activities/concentrations between states (anemic versus non-anemic) of iron deficiency have been demonstrated in studies in animals and humans [7–19].

A second possible reason for the observed differences in findings may reflect the tissue sample in which indicators of oxidative damage were measured. In the present study, oxidative damage was measured in the plasma, and while no significant differences in plasma lipid hydroperoxides or protein carbonyl concentrations were found between iron deficient and control groups at the start or the end of the study period, it is possible that had indicators of oxidative damage been measured in tissues other than the plasma that oxidative damage may have been evident. Knutson and associates [20], for example, found significantly higher concentrations of malondialdehyde in the liver and kidney but not in the plasma of iron deficient versus control rats suggesting that damage is more apparent in the liver or kidney than in the plasma. Moreover, iron supplements given to iron deficient anemic rats increased oxidative damage to protein and lipids in intestinal cells but had variable effects on lipid peroxidation in the liver and kidneys [20–22].

A third possible reason for the difference in findings may relate to the differences in dosages of iron used between the studies. Subjects in the present study received 50 mg of elemental iron or about 2.8 times the recommended intake. In contrast, other studies [20–22] provided rats with iron in amounts 10 times higher than usual intake. Given the prooxidant properties of iron, higher dosages of iron would be expected to induce more oxidative damage than lower dosages.

In addition to differences in dosages, the time chosen to sample the tissues, the length of the supplementation period, and/or gender may have influenced the study results. In the present study, blood samples were collected after eight weeks of iron supplements. Tissues from most of the studies

conducted in rats were sampled 1 to 3 days after the last supplements were ingested and the iron supplementation period lasted from about 15 to 34 days. Rats provided with intermittent (every 3 days) iron supplements exhibited significantly less damage on the second and third day after the ingestion of the last iron dose versus rats receiving daily oral iron [21]. Moreover, Uehara et al [4] found differences in hydroperoxide production between male and female rats with males generating larger amounts than females.

Lastly, the difference in findings may reflect the markers used to assess oxidative damage. No single biological marker of oxidative damage is considered the most specific or accurate, and each method used to measure the biomarkers has its shortcomings. Protein carbonyls were measured in the present study, and in most other studies, to assess damage to protein. Protein carbonyls are stable and are formed during most types of oxidative stress. Biomarkers of lipid peroxidation include primary by-products such as lipid hydroperoxides, and secondary breakdown products such as hydrocarbons (pentane and ethane) and thiobarbituric acid-reactive substances such as malondialdehyde. Measurement of lipid hydroperoxides, used in the present study, is advocated as an accurate and reproducible marker of oxidative stress, but its use in human body fluids is cautioned because of the presence of conjugated dienes which may potentially confound peroxidation assessment [29]. The accuracy of breath ethane and pentane has been questioned because it is affected by oxygen concentration and metal ions in vivo [30]. In addition, exhalation rates may be diminished or exaggerated depending on units of expression such as per 100 g body weight. The accuracy of measurement of malondialdehyde also has been criticized as problematic for use in human fluid because other aldehydes have been shown to react with thiobarbituric acid and produce false positive results [30]. Moreover, malondialdehyde is extensively metabolized in vivo especially in the presence of metals [30]. Most of the studies examining lipid peroxidation measured malondialdehyde and breath ethane or pentane, whereas lipid hydroperoxides were measured in the present study.

While all the factors that cause oxidative damage are not known, attempts were made in the present study to control diet and exercise. Subjects were asked to maintain dietary habits and exercise routines throughout the study period. Dietary intakes of energy, protein, and antioxidant nutrients vitamins A and C, and copper, zinc, and iron at baseline did not differ significantly between the iron adequate and iron deficient groups. Mean vitamin E intake was low with intake of both groups at less than two-thirds of recommendations. However, any effects associated with the low vitamin E intake were likely to be similar as no significant differences in vitamin E intake were found between the two groups. At the end of the study, dietary energy, protein, vitamins A and E, and zinc and copper intakes did not differ among groups. As expected, mean iron intake by iron supplemented group was significantly greater than that of the control group. Mean vitamin C intake significantly decreased from baseline to the end of the study for the iron deficient group and was significantly lower than the control group at the end of the study. However, while mean vitamin C intake was significantly decreased, no significant changes in plasma protein carbonyl and lipid hydroperoxide concentrations resulted. Moreover, no correlations were found between dietary vitamin C intake and either plasma protein carbonyl or lipid hydroperoxide concentrations for either group at either baseline or end of the study. Thus, the observed decrease in dietary vitamin C intake for iron deficient group did not appear to have had an effect on the biomarkers of oxidative damage measured in this study. Moreover, given the numerous substances with antioxidant functions in the body and the body's vast mechanisms for homeostasis, daily fluctuations in nutrient intakes are less likely to directly impact oxidative damage, while extensive fluctuations leading to deficiencies are more likely to cause adverse effects on the oxidant/antioxidant balance.

While nutrient deficiencies can diminish the body's antioxidant system, environmental factors also can lead to increased free radical and reactive oxygen species generation. For example, drug metabolism, infection or inflammation, cigarette smoking, exposure to radiation including ultraviolet light, atmospheric pollution, and exercise are among the many factors that contribute to free radical/reactive oxygen species production in the body [31]. While subjects in the study were excluded if 1-on medications or if 2-they had a chronic illness or an infection or if 3- they had inflammation, exposure to pollution, ultraviolet light either through sun light or tanning beds, and other factors that may promote increased oxidative damage were not monitored [30,31]. Exercise is known to increase oxygen consumption that leads to the formation of reactive oxygen species and can cause muscle cell damage which has been shown to increase lipid peroxidation [30,32]. Generation of free radicals also is associated with macrophage or neutrophil invasion of the damaged muscle cell [30]. Subjects in the present study were asked to maintain their usual exercise routine; however, subjects did not keep activity logs. Cigarette smoking also is associated with oxidative damage. Cigarette smoke contains a large variety of oxidative compounds including superoxide, hydrogen peroxide, and hydroxyl radicals that cause oxidative damage to proteins and lipids in plasma and tissues such as heart, lung, and liver. These damaging effects, however, have been shown to be almost completely prevented in guinea pigs ingesting of 15 mg vitamin C/day (equivalent to 200 mg/day in humans) [33,34]. Moreover, Tsuchiya et al [35] recently found that smoking a single cigarette temporarily decreased plasma nitrate, nitrite, and serum antioxidant concentrations of vitamin C, cysteine, methionine, and uric acid, but these parameters returned to baseline values 60 min after smoking cessation. When the four subjects in the study who reported occasional smoking (usually on weekends) were excluded from data analyses, statistical outcomes did not change from those reported.

It is suggested that the body attempts to maintain redox homeostasis, a balance between oxidant production and antioxidant levels. Potentially this balance may be shifted toward overproduction of damaging oxidants if the antioxidant system becomes overwhelmed due to lack of antioxidant nutrients, inactivation of antioxidant enzymes, or excess production of pro-oxidant compounds. Based on the results of the present study, neither non-anemic iron deficiency nor its treatment with daily oral iron supplements is associated with oxidative damage in the plasma of college-aged females. However, based on the findings of studies in iron deficient anemic rats, the possibility clearly exists that oxidative damage may be present in tissues other than the plasma of non-anemic iron deficient college-aged females, and the use of daily oral iron supplements may result in oxidative damage. Given that iron deficiency anemia is the most prevalent nutritional deficiency worldwide and that it affects all segments of the population, additional studies are warranted.

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