Supplementing Young Women with Both Zinc and Iron Protects Zinc-Related Antioxidant Indicators Previously Impaired by Iron Supplementation\(^1,2\)

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Abstract

Iron supplementation impairs antioxidant status, whereas zinc is recognized as an antioxidant micronutrient. We investigated the effect of supplementing both zinc and iron on iron, zinc, and antioxidant status in 18 women (22–31 y) studied during 2 sequential 8-wk periods. From wk 1 to 8, only iron (50 mg/d) (Fe period) was supplemented and from wk 9 to 16, zinc (25 mg/d) (Fe+Zn period) was also given but at a different time of the day. Indicators of iron (serum iron, iron-binding capacity, and serum ferritin), zinc (serum and urinary zinc), and antioxidant status (ferric-reducing ability of plasma (FRAP); erythrocyte osmotic fragility (EOF); erythrocyte aminolevulinic acid dehydratase (δ-ALAD) activity, and in vitro zinc-δ-ALAD activation (Zn-δ-ALAD%)) were measured at baseline and after each supplementation period. Fe period modified indicators of iron status as expected (\(P < 0.05\)) but did not affect indicators of zinc status. Fe+Zn period did not affect indicators of iron status but increased serum and urinary zinc (\(P < 0.02\)). Antioxidant status was impaired after the Fe period, as indicated by decreased FRAP (\(P < 0.005\)) and δ-ALAD activity (\(P < 0.05\)) and increased EOF (\(P < 0.01\)). After the Fe+Zn period, FRAP values tended to increase (\(P = 0.1\)), δ-ALAD activity and EOF returned to baseline values, and Zn-δ-ALAD% decreased (\(P < 0.001\)) compared with baseline. In conclusion, supplementing young women with both zinc and iron protects zinc-related antioxidant indicators previously impaired by iron supplementation without impairment of iron status.


Introduction

Iron deficiency is prevalent in women of reproductive age, mainly in developing countries (1); thus, WHO recommends preventive iron supplementation of 60 mg/d to women of child-bearing age (2). However, this practice may impair the antioxidant status of supplement users, particularly of those with an adequate iron status. Free iron participates in the Fenton reaction, leading to production of reactive oxygen species causing cellular damage (3,4). Studies with animals (5–7) and humans (8–10) have shown iron supplementation and oxidative stress are associated. Moreover, the use of iron supplementation may impair the nutritional status of other trace elements, such as zinc, by competitive interactions (11–13).

Differently than iron, zinc is present in biological systems as a stable ion that does not participate in redox reactions, although zinc is generally recognized as an antioxidant nutrient (14,15).

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The acute (short-term) antioxidant effects of zinc involve mainly 2 mechanisms, antagonism with redox-active metals such as iron and stabilization of sulfhydryl groups (14,15).

It has been suggested that zinc competes with iron for binding to cell membranes and certain proteins, displacing iron and reducing the formation of reactive oxygen species (14,15). This competitive interaction has been demonstrated by in vitro (16,17) and animal (6,7) studies, suggesting that zinc has a protective role against iron-mediated oxidative stress. Therefore, combining both zinc and iron supplementation could be a beneficial alternative to iron supplementation alone for women of reproductive age, protecting iron, zinc, and antioxidant status.

Zinc can also bind to sulfhydryl groups, protecting specific proteins from oxidation (14,15). This effect has been studied mainly in the enzyme δ-aminolevulinic acid dehydratase (δ-ALAD)\(^2\) (15). Zinc protects δ-ALAD from oxygen inactivation, preventing enzyme thiol oxidation and disulfide formation, whereas

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\(^3\) Abbreviations used: δ-ALAD, erythrocyte delta-aminolevulinic acid dehydratase; EOF, erythrocyte osmotic fragility; Fe period, iron supplementation period; Fe+Zn period, combined iron and zinc supplementation period; FRAP, ferric-reducing ability of plasma; TIBC, total iron-binding capacity; Zn-δ-ALAD%, δ-ALAD in vitro activation with zinc.
the removal of zinc increases sulfhydryl reactivity and results in the loss of enzyme activity (15,18). It has been shown that zinc is also essential for protecting sulfhydryl groups of critical plasma membrane proteins and ensuring erythrocyte membrane stability (19–21). Thus, the antioxidant effect of zinc supplementation could be investigated measuring erythrocyte δ-ALAD activity and erythrocyte membrane fragility.

The aim of this study was to investigate the effect of iron supplementation alone and of combined iron and zinc supplementation on iron, zinc, and antioxidant indicators in young women.

Subjects and Methods

Subjects. Eighteen female volunteers, 22–31 y of age, recruited among students and faculty at the Universidade Federal do Rio de Janeiro, participated in the study after giving their written informed consent. The women were nonsmokers, apparently healthy, without a recent use of mineral and/or vitamin supplements, of acceptable weight for height, and not engaged in heavy exercise. The study was approved by the Ethical Committee of Hospital Universitário Pedro Ernesto (Rio de Janeiro, Brazil).

Study design. The women were studied during 2 sequential 8-wk supplementation periods: from wk 1 to 8, only iron (50 mg/d as ferrous fumarate) was supplemented (Fe period); and from wk 9 to 16, volunteers were instructed to consume iron supplements at bedtime and zinc supplements at lunchtime. This regimen was used to minimize intestinal interaction between iron and zinc from supplements. The women were instructed not to change their dietary and other lifestyle habits during the study. A blood sample was obtained from the participants after an overnight fast as was a spot urine sample (50 mL) immediately before supplementation began (baseline, d 1) and after each supplementation period (Fe supplementation, d 56; Fe + Zn supplementation, d 112).

To monitor compliance, the iron and zinc capsules were supplied every 4 wk in different bottles containing 28 capsules each. Participants were requested to return any remaining capsules and the degree of apparent compliance was estimated.

Laboratory analysis. All materials employed for sample collection and analysis were either disposable or previously soaked overnight in nitric acid (1:4; v:v) and then carefully rinsed with high purity deionized water.

Blood samples were collected into heparin-containing tubes (5 mL), Na2EDTA-containing tubes (5 mL), and plain tubes (5 mL). Aliquots of heparinized blood were separated for immediate determination of hemoglobin and hematocrit by the cyanomethemoglobin method using a Hemoglobin kit (Bioclin) and by conventional capillary centrifugation, respectively.

Iron and zinc status indices. Serum iron and free iron binding capacity were determined by colorimetric assays using commercial kits (Labbtest Diagnostica) and total iron-binding capacity (TIBC) and transferrin saturation were calculated. Serum ferritin concentration was determined by immunoradiometric assay (Diagnostics Products). Serum and urinary zinc concentrations were determined by inductively coupled plasma atomic emission spectrometry (Perkin Elmer Plasma 1000). Urinary zinc was expressed relative to creatinine, which was measured by the Jaffe reaction, as previously described (22).

Antioxidant status indices. Ferric-reducing ability of plasma (FRAP) was measured in fresh Na2EDTA-plasma as described by Benzie and Strain (23). Erythrocyte δ-ALAD activity and δ-ALAD in vitro activation with zinc (Zn-δ-ALAD%), percent increment in δ-ALAD activity by added zinc in vitro) were determined in fresh heparinized whole blood as previously described (22). Enzyme activity was expressed as μmol of porphobilinogen(min·L) of erythrocytes and Zn-δ-ALAD as percentage. Erythrocyte osmotic fragility (EOF) was determined in freshly washed erythrocyte aliquots by an in vitro assay based on the degree of hemolysis in hypotonic saline solutions, as previously described (24). Conjugated dienes were analyzed in Na2EDTA-plasma using a spectrophotometric method as described by Lee (25). Urinary F2-isoprostanes were determined by competitive enzyme immunoassay (Cayman Chemical). Plasma and urine aliquots for conjugated dienes and F2-isoprostanes, respectively, were kept in liquid nitrogen until analysis.

Statistical analysis. The effects of iron supplementation alone and of combined iron-zinc supplementation on iron, zinc, and antioxidant indices were evaluated by 1-way ANOVA with repeated measures followed by Tukey’s test. Correlations between variables at baseline and at the end of each supplementation period were examined by Pearson’s correlation analysis. Values of P < 0.05 were considered significant. The statistical analyses were performed by using STATGRAPHICS (version 7 for DOS; Manugistics). Values in the text are means ± SD.

Results

The subjects were 25.5 ± 2.9 y of age and had an adequate BMI (22.8 ± 4.9 kg/m2) that did not change throughout the study.

At the beginning of the study (Table 1), the subjects were nonanemic and generally had iron indices within the normal range (26), although 2 women had serum ferritin concentrations <12 μg/L, indicative of depleted iron stores (26). Zinc status was also adequate at baseline, as indicated by the serum zinc concentration (27).

During the study, the estimated compliance of supplements use was considered satisfactory, because 97.6 ± 2.9% of the iron supplements and 98.0 ± 2.8% of the zinc supplements supplied were reported as consumed.

After the Fe period (Table 1), hematocrit (4%), serum iron (25%), and serum ferritin (40%) increased and TIBC decreased (5%) compared with baseline (P < 0.05). All subjects had serum ferritin concentrations >12 μg/L. The mean increment in serum ferritin concentration represented an increase of 127 mg in iron stores, assuming that each 1 μg ferritin/L plasma is proportional to ~8 mg of storage iron (26,28). Serum and urinary zinc did not change significantly from baseline.

After the Fe + Zn period (Table 1), hemoglobin, hematocrit, and iron indices remained unchanged compared with the Fe period. Moreover, serum iron, transferrin saturation, and serum ferritin were higher than baseline (P < 0.05). Serum and urinary zinc concentrations increased 11% and 88%, respectively, compared with the Fe period (P < 0.05).

Table 1 Effect of iron supplementation and of combined iron and zinc supplementation on biochemical iron and zinc indicators in young women

<table>
<thead>
<tr>
<th>Biochemical indicator</th>
<th>Baseline</th>
<th>Fe</th>
<th>Fe + Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematocrit, volume fraction</td>
<td>0.41 ± 0.03</td>
<td>0.43 ± 0.04</td>
<td>0.42 ± 0.03</td>
</tr>
<tr>
<td>Hemoglobin, g/L</td>
<td>167 ± 29</td>
<td>169 ± 21</td>
<td>175 ± 26</td>
</tr>
<tr>
<td>Serum iron, μmol/L</td>
<td>18.1 ± 5.6</td>
<td>22.6 ± 7.5</td>
<td>21.7 ± 7.4</td>
</tr>
<tr>
<td>TIBC, μmol/L</td>
<td>74.3 ± 13.1</td>
<td>70.6 ± 8.9</td>
<td>70.9 ± 9.4</td>
</tr>
<tr>
<td>Transferrin saturation, %</td>
<td>25.7 ± 8.0</td>
<td>31.1 ± 11.0</td>
<td>30.6 ± 9.0</td>
</tr>
<tr>
<td>Serum ferritin, μg/L</td>
<td>39.8 ± 31.9</td>
<td>55.7 ± 34.4</td>
<td>54.9 ± 31.5</td>
</tr>
<tr>
<td>Serum zinc, μmol/L</td>
<td>16.4 ± 2.0</td>
<td>15.7 ± 1.9</td>
<td>17.4 ± 2.7</td>
</tr>
<tr>
<td>Urinary zinc, μmol/mmol creatinine</td>
<td>0.42 ± 0.21</td>
<td>0.34 ± 0.19</td>
<td>0.64 ± 0.34</td>
</tr>
</tbody>
</table>

1 All values are means ± SD, n = 18. Means in a row with superscripts without a common letter differ, P < 0.05 (ANOVA with repeated measures followed by Tukey’s test).
Fe supplementation resulted in a reduction of 14% in FRAP ($P < 0.005$) and a decrease of 11% in erythrocyte δ-ALAD activity ($P < 0.05$), whereas EOF increased 12% ($P < 0.01$) (Table 2).

After the Fe+Zn period (Table 2), EOF decreased compared with the Fe period ($P < 0.001$), restoring values to baseline levels. Erythrocyte δ-ALAD activity also returned to baseline values. Zn-δ-ALAD% did not change after the Fe period but decreased by 30% after the Fe+Zn period compared with baseline ($P < 0.001$). After the Fe+Zn period, FRAP tended to be higher than after the Fe period ($P = 0.1$). Plasma conjugated dienes and urinary F2-isoprostanes did not change significantly during the study.

Erythrocyte δ-ALAD activity was correlated with plasma FRAP, hematoctrit, serum transferrin saturation, and serum ferritin at baseline and after the Fe+Zn period (Table 3).

### Discussion

The effectiveness of iron supplementation for the prevention of iron deficiency in women during reproductive age is well established (1,2). However, this practice could have a negative influence on zinc (12) and antioxidant (8,9) status. The present study provides evidence that, in nonanemic young women, supplementing both zinc and iron, at a different time of day, protects zinc-related antioxidant indicators previously impaired by iron supplementation without impairment of iron status.

Changes in the biochemical iron indicators after iron supplementation were consistent with a substantial (127 mg) increase in body iron (26,28), as shown in other studies of iron supplementation in nonanemic women (9,29,30). Despite evidence suggesting impairment of zinc status by iron supplements (12,31–33), iron supplementation did not modify serum and urinary zinc in our study. Our results possibly reflect the adequate zinc status of the women at the beginning of the study and the fact that iron supplements were administered with water without food at bedtime. When zinc was supplemented at lunchtime in addition to iron at bedtime, hematological and iron status indicators remained unchanged, but serum and urinary zinc increased, consistent with a higher zinc supply (34,35). Therefore, the supplementation scheme used in our study was adequate for protecting zinc and iron status.

The negative effect of iron supplementation on antioxidant balance (3–9) also occurred in our study. After 8 wk of daily iron supplementation (50 mg/d), changes in plasma FRAP, EOF, and erythrocyte δ-ALAD activity indicated impairment in the antioxidant status of the women. After combining zinc and iron supplementation, plasma FRAP tended to increase ($P = 0.10$) and EOF and erythrocyte δ-ALAD returned to baseline.

The decrease in plasma FRAP after iron supplementation may be due to a reduction in endogenous plasma-reducing components, such as uric acid, ascorbic acid, α-tocopherol, and bilirubin (23,36,37). Although we did not measure plasma contributors to FRAP, plasma iron-reducing components, mainly α-tocopherol and ascorbic acid, decreased in β-thalassemia patients treated with repeated blood transfusions (37), possibly as a response to the iron overload. After combining zinc with iron supplementation, the tendency of FRAP to increase suggests that zinc could protect plasma antioxidant components, such as α-tocopherol. Zinc deficiency has been shown to impair α-tocopherol status (38).

Increased in vitro EOF was observed after iron supplementation in our study, suggesting lipid and/or protein cell membrane damage (19–21,39). However, markers of lipid peroxidation (plasma dienes and urinary F2-isoprostanes) did not change with iron supplementation in our study, similarly to another study in women finding no effect of supplementing iron on plasma LDL oxidation and total dienes (29). In our study, the increased erythrocyte fragility could be due to oxidation of sulphhydril groups of cell membrane proteins by increased iron, as suggested by an ex vivo study in human atherosclerotic lesions where tissue iron levels correlated strongly with protein but not with lipid oxidation (40). However, the effect of iron supplementation on protein and lipid oxidative status of erythrocyte membrane needs to be specifically tested in further studies.

The reduction in erythrocyte δ-ALAD activity in our study after iron supplementation is consistent with animal studies relating δ-ALAD activity to antioxidant status (41,42). This reduction could be due to oxidation of sulphhydril groups by iron-mediated processes or loss of zinc protection (14,15,18). In our study, the decreased δ-ALAD activity could indicate iron oxidation, because Zn-δ-ALAD% did not change significantly (18).

After combining zinc and iron supplementation, the restoration of erythrocyte δ-ALAD activity and EOF and the reduced % Zn-δ-ALAD indicate improvement in zinc-related antioxidant indicators previously impaired by iron supplementation. Interestingly, several correlations between biochemical indicators observed at baseline were no longer significant after iron supplementation but were again significant after combining zinc and iron supplementation, suggesting that the antioxidant balance, previously impaired by iron, was restored by zinc. The use of other specific measurements related, for instance, to protein and δ-ALAD oxidation will be required in further studies to

### Table 2

<table>
<thead>
<tr>
<th>Biochemical indicator</th>
<th>Baseline</th>
<th>Fe</th>
<th>Fe + Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>FRAP, μmol Fe⁶¹/L</td>
<td>612 ± 116⁴</td>
<td>525 ± 101⁴</td>
<td>535 ± 116⁴</td>
</tr>
<tr>
<td>Erythrocyte δ-ALAD, μmol/(min·l)</td>
<td>38.4 ± 9.1⁴</td>
<td>34.0 ± 7.7⁴</td>
<td>36.5 ± 9.3⁴ab</td>
</tr>
<tr>
<td>Erythrocyte Zn-δ-ALAD, %</td>
<td>14.3 ± 4.8⁴</td>
<td>12.2 ± 8.7⁴ab</td>
<td>10.0 ± 2.5⁴b</td>
</tr>
<tr>
<td>EOF, %</td>
<td>59.2 ± 26.0⁴</td>
<td>66.2 ± 21.2⁴</td>
<td>57.2 ± 22.1⁴b</td>
</tr>
<tr>
<td>Plasma conjugated dienes, nmol/g total lipids</td>
<td>1.52 ± 0.29</td>
<td>1.55 ± 0.22</td>
<td>1.59 ± 0.20</td>
</tr>
<tr>
<td>Urinary F2-isoprostanes, ng/mmol creatinine</td>
<td>13.3 ± 5.2</td>
<td>11.2 ± 4.6</td>
<td>10.6 ± 1.2</td>
</tr>
</tbody>
</table>

1 All values are means ± SD, n = 18. Means in a row with superscripts without a common letter differ, $P < 0.05$ (ANOVA with repeated measures followed by Tukey’s test).

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**Table 3** Pearson correlations between biochemical indicators of the women in each study period

<table>
<thead>
<tr>
<th>Related variable</th>
<th>Baseline</th>
<th>Fe</th>
<th>Fe + Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td><strong>Fe</strong></td>
<td><strong>Fe + Zn</strong></td>
<td></td>
</tr>
<tr>
<td>Erythrocyte δ-ALAD and FRAP</td>
<td>0.56</td>
<td>0.01</td>
<td>—¹</td>
</tr>
<tr>
<td>Erythrocyte δ-ALAD and EOF</td>
<td>−0.51</td>
<td>0.01</td>
<td>—</td>
</tr>
<tr>
<td>Hematocrit</td>
<td></td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Erythrocyte δ-ALAD and serum transferrin saturation</td>
<td>0.56</td>
<td>0.01</td>
<td>—</td>
</tr>
</tbody>
</table>

¹ No significant correlations were found after the Fe period ($P > 0.10$).
better understand the underlying mechanisms and physiologic relevance of the potential zinc-antioxidant protection.

The present study demonstrates that supplementing healthy young women with combined zinc and iron protects zinc-related antioxidant indicators previously impaired by iron supplementation alone while maintaining the nutritional benefits. Therefore, our combined supplementation scheme could be an alternative to prevent iron deficiency in women of reproductive age. However, this needs confirmation by further studies in women with poor iron and zinc status.

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Literature Cited