Effects of Iron and Folic Acid Supplements on Serum Zinc Levels Among a Cohort of Pregnant Women

The objective of this project was to identify associations between iron and folic acid supplementation and serum zinc levels among a cohort of women prior to and during pregnancy. A sample of 126 healthy women, who were attempting pregnancy with no history of chronic disease or infertility, completed food frequency questionnaires and had serum zinc levels drawn. Intake of nutrients from supplements and diet, and serum zinc levels were assessed using Pearson’s r correlations and multivariate analysis. No dietary variables were associated with serum zinc levels during pregnancy. Only two supplemental nutrient intakes, iron and folic acid, were related to serum zinc levels. An interactive association between iron and folic acid supplements on serum zinc levels was identified. Thus, supplemental folic acid appears to offer some protection against declines in serum zinc levels that may result from iron supplementation during pregnancy. Key words: folic acid supplements, iron supplements, pregnancy, prenatal nutrition, serum zinc

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PREGNANCY INCREASES a female’s nutritional requirements for energy, protein, and select vitamins and minerals. Although the preferred method for obtaining additional nutrients during pregnancy is through the selection of a nutritious diet, vitamin/mineral supplements are commonly prescribed during pregnancy to meet the additional micronutrient needs of pregnant women. Current recommendations for prenatal nutrient supplementation include the use of a 30 mg iron supplement after the twelfth week of pregnancy or at the time of the first prenatal visit for all women. An additional multivitamin/mineral supplement that consists of modest doses of four vitamins (B6, D, E, folic acid) and four minerals (iron, zinc, calcium, magnesium) is indicated for women who demonstrate inadequate dietary intakes that fail to improve with dietary counseling, for multifetal pregnancy, for pregnant adolescents, and for women who consume vegan diets. In addition, the Food and Nutrition Board has set the Dietary Reference

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Intake for folic acid during pregnancy at 600 mcg, and has recommended that 400 mcg be obtained through supplements or fortified foods by all women of childbearing age.4 It is estimated that 86 to 92% of pregnant women use vitamin/mineral supplements during pregnancy.5 The use of iron supplements at levels well in excess of the 30 mg recommendation is commonplace.6 Iron supplementation of 60–120 mg per day is recommended in the presence of iron deficiency anemia.7 Nutrient-nutrient interactions between iron and other minerals contained in vitamin/mineral supplements are almost certain to occur at these levels of supplementation.

Research suggests that iron-zinc ratios of > 2:1 result in decreased zinc absorption.78 Studies of pregnant women have shown that acute periods of iron supplementation adversely affect serum zinc status.9–11 While serum zinc levels appear to decline in most women during pregnancy, greater than normal declines in serum zinc during pregnancy have been associated with an increased risk of poor maternal outcomes such as pregnancy-induced hypertension, prolonged labor, preterm delivery, and maternal infection.12–15 Compromised zinc status has also been associated with an increased risk of poor fetal outcomes including reduced fetal size.14–16

Some research has suggested that folic acid supplementation might also compromise serum zinc status, however studies relating to potential interactions between supplemental intakes of folate and serum zinc status during pregnancy are inconsistent and potential mechanisms are not understood.14,17–23 Only two studies have examined effects of combinations of iron and folic acid supplements on serum zinc status.19,23 Neither study was able to assess individual as well as combined nutrient effects on serum zinc levels due to limitations in study design and statistical analysis methods utilized.

The purpose of this study was to identify associations between varying levels and combinations of iron and folic acid supplementation and serum zinc levels during pregnancy in a cohort of women, based on self-reported supplementation patterns. A second purpose was to determine whether dietary intakes of iron, folate, and other nutrients affect serum zinc levels during pregnancy. The overall goal is to improve prenatal outcomes through a better understanding of factors that affect nutritional status during pregnancy.

**METHODS**

Women included in this study were participants in the Diana Project, a population-based study designed to determine the effects of nutritional exposures on reproductive outcomes. Information on eligibility requirements, recruitment of subjects, and study methods has been previously published.24 Subjects were recruited from a random population of healthy women who were members of Group Health, Inc., a Health Maintenance Organization (HMO) serving the Minneapolis-St Paul metropolitan area of Minnesota. Selection criteria included plans to become pregnant within the enrollment period, age of 22–35 years, and no history of chronic disease, obstetric complications, or infertility. Of the 1,152 women enrolled in the Diana Project, 642 (66%) completed the study. A subgroup of participants who completed the Diana Project (n = 219) was sequentially selected for blood nutrient assays. These assays were performed once before and once during each trimester of pregnancy.
One hundred forty-four women provided serum zinc samples at least once prior to and at least twice during pregnancy. The final sample of women included in this study includes 126 of the 144 women; eighteen participants with at least three serum zinc values were excluded for incomplete dietary history or supplement use data.

Serum zinc levels were provided by 109 women prior to pregnancy, 100 in the first trimester, 97 in the second trimester, and 82 in the third trimester. Seventy-eight women provided serum zinc values during all four time periods. Non-fasting blood samples were drawn into vacutainers free of trace elements. Serum zinc was analyzed using flame atomic absorption spectrophotometry. The laboratory CV for serum zinc was 9.5%.

Dietary intake was assessed using the Willett Food Frequency Questionnaire (FFQ). The FFQ was completed approximately monthly for four months and then every three months until conception. The FFQ was completed each month during pregnancy. Results of the FFQ were compared to 4-day weighed food records in a subset of Diana Project participants. Correlations obtained by the two dietary assessment methods were \( r = 0.60 \) for zinc, \( r = 0.56 \) for folate, and \( r = 0.67 \) for iron intakes during pregnancy.

Nutrients and dietary substances thought to modify the bioavailability of zinc were examined in analyses. They include protein, dietary fiber, iron, folate, zinc, and energy.

Prenatal supplement use was assessed using detailed monthly questionnaires that asked participants to list the brand name, type, frequency of use, and amount of each supplement taken. Information on the time of day during which supplements were taken and whether they were taken with meals was not collected due to the potential for increased subject burden. Multivitamin, multivitamin/mineral and individual supplements were assessed. Information on vitamin and mineral supplement contents by brand name were obtained from pharmaceutical companies, the Nutrition Coordinating Center at the University of Minnesota, and from visual inspection of packaging provided by study participants.

Data on age, household income, employment status, education, race/ethnicity, and marital status were obtained by questionnaire. Height was recorded using a stadiometer at the initial clinic visit. Weights were obtained at clinic visits and on a weekly basis at home. This study was approved by the University of Minnesota Institutional Review Board and by the review board at Group Health, Inc.

**STATISTICAL ANALYSES**

Pearson’s r correlations between supplemental and dietary nutrient intakes were performed unadjusted, and then adjusted for potential confounding dietary variables, by computing partial correlations. A correlation of 0.2 or greater with serum zinc was considered to be the selection criteria for entrance into regression models.

Repeated measures univariate and multivariate mixed regression models (PROC MIXED) were used to determine levels and changes in serum zinc, before and during pregnancy. Interaction terms were entered into regression models where appropriate. When two variables produced a significant interaction term, each was divided into tertiles and plotted against the other to determine whether a dose-response relationship existed. Two-tailed t-tests were
used to determine if differences between mean serum zinc levels differed by tertile of nutrient intakes. All data were analyzed using SAS (version 6.10, 1996, SAS Institute, Cary, NC).

RESULTS

No significant differences were found between the women included in this study and those of the entire Diana Project with respect to age at conception, education level, employment status, race, marital status, parity, prepregnancy weight or height, pregnancy weight gain, or infant birthweight and gestational age (data not shown). Select characteristics of the 126 women included in this study and their infants are shown in Table 1.

Mean intakes of energy and select nutrients from supplement and dietary sources, consumed prior to and during pregnancy as well as averaged across pregnancy, are shown in Table 2. Dietary intakes of energy and all nutrients increased throughout pregnancy, peaking during the third trimester of pregnancy. Most subjects (97%) reported taking nutrient supplements during pregnancy. Only 26% reported consistent daily use of supplements, however. Supplemental intakes of folic acid and zinc peaked during the second trimester, while supplemental intakes of iron were highest during the third trimester of pregnancy.

Changes in nutrient intakes throughout pregnancy are shown in Table 3. The greatest changes in nutrient intake from supplements appeared to occur during the first trimester, while the greatest changes in nutrient intake from dietary sources occurred during the second trimester of pregnancy.

Significant decreases in serum zinc levels were noted during each trimester of pregnancy and as an overall change during pregnancy (Table 4). On average, serum zinc levels dropped $5.6 \pm 0.6 \mu$mol/L during pregnancy. Pearson product moment correlations between energy and select nutrient intakes from supplement and dietary sources were calculated prior to and during each trimester of pregnancy. Correlations were also run between changes in serum zinc and changes in nutrient intakes from supplement and dietary sources at each trimester of pregnancy. Because results of the correlations were very similar across each trimester of pregnancy and for change in nutrient intakes as well as estimated nutrient intakes, only estimated nutrient intake data averaged across pregnancy are reported here. None of the dietary nutrient intake variables (energy, protein, dietary fiber, iron, folate, zinc) correlated with serum zinc levels prior to or during pregnancy. Only folic acid and iron intakes

<table>
<thead>
<tr>
<th>Maternal and infant characteristics</th>
<th>Mean ± SD or %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at conception (yrs)</td>
<td>29.8 ± 3.2</td>
</tr>
<tr>
<td>Married at time of enrollment</td>
<td>100%</td>
</tr>
<tr>
<td>Caucasian</td>
<td>97%</td>
</tr>
<tr>
<td>Worked outside of home at time of enrollment</td>
<td>90%</td>
</tr>
<tr>
<td>College graduate or higher education level</td>
<td>64%</td>
</tr>
<tr>
<td>Household income &gt; $40,000</td>
<td>60%</td>
</tr>
<tr>
<td>Prepregnancy weight (kg)</td>
<td>62.7 ± 11.5</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.7 ± 0.1</td>
</tr>
<tr>
<td>Pregnancy weight gain (kg)</td>
<td>16.1 ± 5.3</td>
</tr>
<tr>
<td>Parity</td>
<td>0.44 ± 0.7</td>
</tr>
<tr>
<td>Infant gestational age (weeks)</td>
<td>37.7 ± 1.9</td>
</tr>
<tr>
<td>Infant birth weight (g)</td>
<td>3436.7 ± 351.8</td>
</tr>
<tr>
<td>Infant birth length (cm)</td>
<td>51.9 ± 2.8</td>
</tr>
</tbody>
</table>
from supplements were correlated with serum zinc levels during, but not prior to, pregnancy (r = 0.43 and 0.21, respectively, for data averaged across trimesters; p < 0.05).

Univariate and multivariate regression analyses revealed associations between iron and folic acid supplements and serum zinc levels during pregnancy. These associations were found to be unaffected by adjustment for energy, protein, or dietary fiber intakes. Table 5 illustrates associations between iron and folic acid supplements and serum zinc levels when averaged across all trimesters of pregnancy. In univariate analyses, supple-

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**Table 2.** Prepregnancy and pregnancy mean intakes of select nutrients from dietary and supplemental sources (± standard deviation)*

<table>
<thead>
<tr>
<th>Nutrients from Supplements</th>
<th>Pre-pregnancy mean</th>
<th>Mean intake trimester 1</th>
<th>Mean intake trimester 2</th>
<th>Mean intake trimester 3</th>
<th>Mean intake during pregnancy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Folic acid (µg)</td>
<td>212.7 ± 708.5</td>
<td>457.8 ± 335.9</td>
<td>581.9 ± 356.8</td>
<td>483.5 ± 365.0</td>
<td>512 ± 299</td>
</tr>
<tr>
<td>Iron (mg)</td>
<td>9.0 ± 15.8</td>
<td>39.1 ± 25.8</td>
<td>52.3 ± 28.4</td>
<td>54.1 ± 34.1</td>
<td>48 ± 24</td>
</tr>
<tr>
<td>Zinc (mg)</td>
<td>4.1 ± 6.9</td>
<td>15.5 ± 11.1</td>
<td>18.7 ± 8.5</td>
<td>17.2 ± 8.6</td>
<td>17 ± 7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Intakes from Dietary Sources</th>
<th>Folate (µg)</th>
<th>Iron (mg)</th>
<th>Zinc (mg)</th>
<th>Fiber (g)</th>
<th>Protein (g)</th>
<th>Energy (Kcals)</th>
</tr>
</thead>
<tbody>
<tr>
<td>268.4 ± 171.1</td>
<td>12.4 ± 6.6</td>
<td>12.5 ± 4.7</td>
<td>17.7 ± 7.9</td>
<td>73.3 ± 23.3</td>
<td>1779.9 ± 582.0</td>
<td></td>
</tr>
</tbody>
</table>

* n=126 for mean pregnancy, n=120 for prepregnancy, n=124 for trimester 1, n=123 for trimester 2, and n=121 for trimester 3 data

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**Table 3.** Changes in dietary and supplement nutrient intakes by trimester (± standard deviation)*

<table>
<thead>
<tr>
<th>Nutrients from supplemental sources</th>
<th>Prepregnancy to trimester 1 change</th>
<th>Trimester 1 to trimester 2 change</th>
<th>Trimester 2 to trimester 3 change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Folate (µg)</td>
<td>+251.3 ± 755.0</td>
<td>+116.9 ± 348.1</td>
<td>−90.7 ± 255.0</td>
</tr>
<tr>
<td>Iron (mg)</td>
<td>+29.6 ± 22.9</td>
<td>+12.7 ± 26.5</td>
<td>+2.2 ± 26.6</td>
</tr>
<tr>
<td>Zinc (mg)</td>
<td>+10.8 ± 9.2</td>
<td>+3.1 ± 11.2</td>
<td>−1.5 ± 6.9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Nutrients from dietary sources</th>
<th>Prepregnancy to trimester 1 change</th>
<th>Trimester 1 to trimester 2 change</th>
<th>Trimester 2 to trimester 3 change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Folate (µg)</td>
<td>+29.8 ± 154.8</td>
<td>+46.3 ± 147.0</td>
<td>+8.8 ± 152.5</td>
</tr>
<tr>
<td>Iron (mg)</td>
<td>+1.3 ± 5.4</td>
<td>+1.9 ± 5.7</td>
<td>+0.4 ± 6.3</td>
</tr>
<tr>
<td>Zinc (mg)</td>
<td>+0.2 ± 4.4</td>
<td>+1.2 ± 2.8</td>
<td>+0.4 ± 2.2</td>
</tr>
<tr>
<td>Fiber (g)</td>
<td>+0.7 ± 6.7</td>
<td>+1.6 ± 26.5</td>
<td>+0.5 ± 6.9</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>+10.0 ± 22.2</td>
<td>+9.4 ± 16.7</td>
<td>+1.6 ± 12.8</td>
</tr>
<tr>
<td>Energy (Kcals)</td>
<td>+139.1 ± 509.0</td>
<td>+246.7 ± 398.4</td>
<td>+37.8 ± 285.4</td>
</tr>
</tbody>
</table>

* n=120 for prepregnancy to trimester 1, n=123 for trimester 1 to trimester 2, and n=121 for trimester 2 to trimester 3 change data
mental folic acid was found to be positively associated with serum zinc levels, while supplemental iron was found to be negatively associated with serum zinc levels. Associations among iron and folic acid supplements and serum zinc levels appeared to be substantially modified by an interaction between iron and folic acid supplements, however, in that the negative association between supplemental iron intake and serum zinc level disappeared when folic acid supplement was included in the model. The interactive term suggests that the two nutrients together exert a moderately weak, but positive, effect on serum zinc levels.

Table 6 illustrates this interaction in greater detail, presenting observed mean serum zinc values within categories of supplemental iron and folic acid intake. As the combined dose of iron and folic acid from supplements increased, so did the mean serum zinc values. However, the association varied by

Table 4. Mean serum zinc levels before and during pregnancy, and changes in serum zinc levels during pregnancy*

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Mean serum zinc value (µmol/L) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prepregnancy</td>
<td>109</td>
<td>15.3 ± 4.0</td>
</tr>
<tr>
<td>Trimester 1</td>
<td>100</td>
<td>13.4 ± 2.8</td>
</tr>
<tr>
<td>Trimester 2</td>
<td>97</td>
<td>11.3 ± 3.1</td>
</tr>
<tr>
<td>Trimester 3</td>
<td>82</td>
<td>9.8 ± 3.4</td>
</tr>
<tr>
<td><strong>Mean serum zinc change (µmol/L) ± SD</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Change during trimester 1</td>
<td>85</td>
<td>−1.8 ± 0.5</td>
</tr>
<tr>
<td>Change during trimester 2</td>
<td>75</td>
<td>−2.2 ± 0.4</td>
</tr>
<tr>
<td>Change during trimester 3</td>
<td>63</td>
<td>−1.6 ± 0.4</td>
</tr>
<tr>
<td>Change during prepregnancy to trimester 3</td>
<td>69</td>
<td>−5.6 ± 0.6</td>
</tr>
</tbody>
</table>

* all values were significantly different from each other at p < 0.05

Table 5. Multivariate regression model including interaction terms* (n = 126)

<table>
<thead>
<tr>
<th></th>
<th>β</th>
<th>SE</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>73.36</td>
<td>5.13</td>
<td></td>
</tr>
<tr>
<td>Associations between subjects</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Folic acid supplement (100 µg)</td>
<td>0.37</td>
<td>1.04</td>
<td>0.01</td>
</tr>
<tr>
<td>Iron supplement (1 mg)</td>
<td>−0.31</td>
<td>0.12</td>
<td>0.02</td>
</tr>
<tr>
<td>Folic acid x iron supplement</td>
<td>0.05</td>
<td>0.02</td>
<td>0.01</td>
</tr>
<tr>
<td>Associations within subjects</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Folic acid supplement (100 µg)</td>
<td>1.67</td>
<td>0.70</td>
<td>0.03</td>
</tr>
<tr>
<td>Iron supplement (1 mg)</td>
<td>−0.24</td>
<td>0.08</td>
<td>0.01</td>
</tr>
<tr>
<td>Folic acid x iron supplement</td>
<td>0.05</td>
<td>0.03</td>
<td>0.12</td>
</tr>
</tbody>
</table>

*F = 4.32, R² = 0.23 This model explained 40.5% of the between person variation and 5.9% of the within person variation.
Table 6. Effects of an interaction of folic acid and iron supplements on serum zinc levels (µmol/L) averaged across pregnancy*

<table>
<thead>
<tr>
<th>Folic acid supplement (µg/day)</th>
<th>Iron supplement (mg/day)</th>
<th>0 mg/day</th>
<th>1 to ≤ 30 mg/day</th>
<th>&gt; 30 to ≤ 54 mg/day</th>
<th>&gt; 54 mg/day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>x ± SD</td>
<td>n</td>
<td>x ± SD</td>
<td>n</td>
</tr>
<tr>
<td>0 µg/day</td>
<td>4</td>
<td>12.0 ± 3.2</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>1 ≤ 400 µg/day</td>
<td>—</td>
<td>—</td>
<td>17</td>
<td>10.5 ± 2.9</td>
<td>16</td>
</tr>
<tr>
<td>&gt; 400 to ≤ 650 µg/day</td>
<td>—</td>
<td>—</td>
<td>5</td>
<td>11.2 ± 1.9</td>
<td>17</td>
</tr>
<tr>
<td>&gt; 650 µg/day</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>19</td>
</tr>
</tbody>
</table>

*To convert µmol/L to µg/dL, multiply by 6.54. To convert µg/dL to µmol/L, multiply for 0.153.

† p < 0.05 for differences between 1-400 µg folic acid at all iron levels and > 400 to ≤ 650 µg folic acid at 31-54 mg iron
‡ p < 0.05 for difference between 1-400 µg folic acid at all iron levels and > 650 µg folic acid at 31-54 mg iron
§ p < 0.001 for differences between 1-400 µg folic acid at all iron levels and > 650 µg folic acid with > 54 mg iron

level of folic acid supplementation; an increase in the dose of iron supplementation was associated with decreased serum zinc levels in women in the lowest tertile of folic acid supplementation. This decrease in serum zinc level did not appear to occur at higher levels of folic acid supplementation. Women who had moderate intakes of supplemental iron and folic acid (> 30 to ≤ 54 mg iron combined with > 400 to ≤ 650 µg folic acid) and those with the highest intakes of folic acid (> 650 µg) combined with moderate to high intakes of iron (> 30 mg) had significantly higher serum zinc levels than did other women. The four women who did not use iron or folic acid supplements appeared to have higher serum zinc levels than women taking any amount of supplemental iron and ≤ 400 µg folic acid. The small number of subjects in the nonsupplemented group precludes significant conclusions, however.

Tables 7 and 8 further detail the expected interaction between increasing increments of iron and folic acid supplements on serum zinc levels. First, women were divided into tertiles of average iron and folic acid supplement intake during pregnancy based on daily recorded intakes. Next, midpoints of the tertiles, as well as a zero supplement level, were used to evaluate the interaction. Evidence of

Table 7. Expected changes in serum zinc per 30 mg increase in supplemental iron at 4 levels of folic acid supplementation

<table>
<thead>
<tr>
<th></th>
<th>0 µg folic acid</th>
<th>175 µg folic acid</th>
<th>540 µg folic acid</th>
<th>860 µg folic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Changes expected between subjects (µmol/L)</td>
<td>-1.4</td>
<td>-1.0</td>
<td>-0.2</td>
<td>0.5</td>
</tr>
<tr>
<td>Changes expected within subjects (µmol/L)</td>
<td>-1.1</td>
<td>-0.7</td>
<td>0.1</td>
<td>0.9</td>
</tr>
</tbody>
</table>
Table 8. Expected changes in serum zinc per 400 μg increase in supplemental folic acid at 4 levels of iron supplementation

<table>
<thead>
<tr>
<th>Changes expected between subjects (μmol/L)</th>
<th>0 mg iron</th>
<th>19 mg iron</th>
<th>40 mg iron</th>
<th>70 mg iron</th>
</tr>
</thead>
<tbody>
<tr>
<td>Changes expected within subjects (μmol/L)</td>
<td>1.0</td>
<td>1.6</td>
<td>2.3</td>
<td>3.2</td>
</tr>
</tbody>
</table>

To convert μmol/L to μg/dL multiply by 6.54. To convert μg/dL to μmol/L multiply for 0.153.

a possible interaction between iron and folic acid supplements was seen: serum zinc decreased as iron increased when folic acid was low, but increased as iron increased when folic acid was high. For example, the expected change in serum zinc per 30 mg increment of iron supplement was −1.0 μmol/L at 175 μg folic acid, but was 0.5 μmol/dL at 860 μg of folic acid. Similarly, the expected increase in serum zinc per 400 μg increment of folic acid supplement was almost three times greater when supplemental iron was 70 mg than when it was 19 mg. In both cases, the expected differences between subjects and within subjects were in agreement with each other. However, the interaction of expected changes within subjects did not achieve statistical significance in the regression model.

**DISCUSSION**

Mean serum zinc levels were associated with supplemental intakes of iron and folic acid, but not with dietary intakes of these nutrients or other dietary constituents. Associations between iron and folic acid supplements and serum zinc levels appeared to be modified by an interaction between iron and folic acid supplements. High intakes of iron were associated with decreasing serum zinc levels when folic acid supplements were consumed at levels of < 400 μg/day. However, levels of folic acid of ≥ 400 μg/day appear to be positively associated with serum zinc levels at all levels of iron supplementation. Supplemental folic acid intakes of > 650 μg/day appear to provide the greatest degree of protection against declines in serum zinc associated with high levels of iron supplementation.

The mean serum zinc level noted in this study prior to pregnancy was 15.3 μmol/L, which is within published norms of 10.0–17.6 μmol/L and similar to levels found in other studies. Serum zinc data in this study appeared higher during the first and third trimesters than data collected by Lehti, while the mean third trimester serum zinc level in the current study was almost identical to that reported by Hunt. Mean serum zinc levels in this study exceeded plasma values reported by Mukherjee, but were lower than those found by Knight. Some of the differences in serum zinc levels between this study and others may be due to differences in study population; this study population was less socioeconomically and ethnically diverse than populations in previous studies.

Serum zinc levels of subjects dropped by approximately 37% during pregnancy. This is consistent with other research suggesting serum zinc levels decline 36% during pregnancy. The decline in serum zinc levels in
this study was gradual throughout preg-
nancy, with the greatest decrease in serum zinc
occurring during the second trimester of preg-
nancy. The pattern noted here differed some-
what from results of previous research\textsuperscript{32,33} in
that those studies found the greatest declines
in serum zinc during the first trimester, with
stabilization during the second trimester, and
further decrease during the third trimester.

Reported levels of folic acid and zinc sup-
plementation were somewhat lower in this
study than in a recently published study.\textsuperscript{34}
Intakes of folic acid and zinc supplements
during pregnancy among supplement users
in the current study ranged from 126–960 \(\mu\)g/
day and 4.1–24.2 mg/day, respectively, while
those reported by Berg and colleagues ranged
from 400–1000 \(\mu\)g/day for folic acid and 0–
25 mg/day for zinc.\textsuperscript{34} Differences be-
tween the current study and previous studies as to
how average supplemental nutrient intake
during pregnancy was estimated, should be
noted and may explain differences in report-
ed levels of supplement use. In the current
study, women reported daily consumption of
vitamin and mineral supplements each month
prior to and during pregnancy. Other studies
assessed nutrient supplement use for much
shorter periods of time and/or only at specific
points during pregnancy.\textsuperscript{10,19,34} Several stud-
ies did not monitor daily compliance.\textsuperscript{14,23}
Since only 26% of women in the current
study and 50% of women in another study\textsuperscript{14}
reported consistent daily use of vitamin and
mineral supplements, there is reason to be-
lieve that accurate estimates of supplemental
nutrient intakes may best be attained through
long-term daily monitoring of supplement
use throughout pregnancy. Another differ-
ence between this study and previously pub-
lished data on supplemental folic acid and
zinc intakes lies in the number of subjects
studied. The current study included 126 wom-
en while other research included much smaller
numbers of subjects.\textsuperscript{19,21,34}

At least two other studies have noted asso-
ciations between folic acid and iron supple-
ment use and serum zinc levels in pregnant
women.\textsuperscript{19,23} Both showed declines in serum
zinc levels with iron and folic acid supple-
mentation, however neither study separated
individual versus interactive effects of the
two nutrients. Although intakes of iron and
folic acid from supplements among women
in the current study were correlated due to the
common supplemental source (\(r = 0.56\)), we
were able to separate the effects of these
individual nutrients on serum zinc level in
the current study by utilizing univariate and
multivariate regression analyses which con-
trolled for other dietary and supplemental
nutrient intake variables.

A previous study of six male subjects showed
a non-significant tendency toward increased
serum zinc levels after feeding high levels of
folate.\textsuperscript{21} Those findings are in concurrence with
the current study. We were able to show statistically
significant differences in serum zinc level that varied by level of folic acid and iron
supplementation in the current study due to the
larger sample size and the use of multivariate
regression analyses. Future studies that include
a larger number of subjects and allow for more
controlled variation in supplemental nutrient
intake combinations could shed additional light
on the magnitude of the interaction noted be-
tween folic acid and iron on changes in serum
zinc levels.

Several limitations of this study should be
noted. While the current sample of women is
larger than found in many previous studies, the sample size of 126 may have been too small to quantify the true effect of iron and folic acid supplements on serum zinc levels. In addition, there was a great deal of variation in daily supplement use and in combinations of supplements used due to the lack of controlled conditions and the study design. We are unable to draw conclusions about the degree of difference in serum zinc levels noted between nonsupplement users and those who use varying levels of iron and folic acid from the current study since so few women (n = 4) reported no use of nutrient supplements. Further studies that include more women who voluntarily choose to not consume nutrient supplements during pregnancy and that include controlled levels of supplement combinations are needed to determine how the serum zinc status of these women differs from those who use varying combinations of folic acid and iron.

This study is able to shed additional light on potential associations between iron and folic acid supplements and serum zinc levels among pregnant women that previous studies were not. Women included in this study self-selected nutrient supplements and supplementation level, thus providing data on actual patterns of supplementation among pregnant women and how existent supplementation patterns affect serum zinc levels. Second, the number of women included in this study exceeds those included in most previous studies that examined iron and folic acid supplement effects on serum zinc status. Finally, the use of multivariate regression analyses allowed for the separation of iron and folic acid effects from each other and from other nutrients contained in prenatal vitamin/mineral supplements.

CONCLUSION AND CLINICAL APPLICATIONS

Pregnant women commonly use vitamin and mineral supplements that are prescribed by obstetric health care providers. Prenatal multivitamin-mineral supplements are formulated with little thought given to the possibility of nutrient-nutrient interactions, however. This study provides evidence of interactive associations between iron and folic acid supplements and serum zinc levels among pregnant women. Supplemental folic acid appears to offer some protection against declines in serum zinc levels that may result from iron supplementation during pregnancy. Because compromised serum zinc levels are thought to increase the risk of adverse maternal and fetal outcomes, precautions should be taken to prevent excessive declines in serum zinc levels during pregnancy. Nutritionists and other providers of obstetric care need to carefully consider the possibility of nutrient–nutrient interactions when counseling pregnant women who use prenatal nutrient supplements. The following interactions should be considered:

- Doses of supplemental iron should not exceed recommended levels of iron supplementation of 30 mg/day during pregnancy, unless indicated by the presence of iron deficiency anemia. Nutritionists should counsel patients to read labels for iron contents when choosing over-the-counter nutrient supplements and should make clients aware of cur-
rent recommended levels of iron intake during pregnancy.\textsuperscript{1,3,4}

- Counseling on obtaining nutrient intakes that meet the current Daily Reference Intakes\textsuperscript{4} for pregnancy should be provided to all pregnancy women. Folate intakes of 600 \( \mu \)g/day or more and zinc intakes of 11 mg or more should be stressed to women who take iron supplements to prevent excessive declines in serum zinc.

- Nutritionists should make other obstetric health care providers aware of subgroups of pregnant women for whom nutrient supplementation is warranted and should provide information about currently recommended formulations for prenatal supplements.

- Nutritionists should discuss the potential for nutrient-nutrient interactions with obstetrics health care providers who recommend prenatal nutrient supplements and with the women who choose to utilize them.

- Dietary intake of nutrients should be stressed during pregnancy to reduce the potential occurrence of nutrient-nutrient interactions.

REFERENCES


17. Milne DB, Canfield WK, Mahalko JR, Sandstead HH.


