Influence of prenatal iron and zinc supplements on supplemental iron absorption, red blood cell iron incorporation, and iron status in pregnant Peruvian women\textsuperscript{1–3}

Kimberly O O'Brien, Nelly Zavaleta, Laura E Caulfield, Dong-Xiao Yang, and Steven A Abrams

ABSTRACT

Background: It is estimated that 60\% of pregnant women worldwide are anemic.

Objective: We aimed to examine the influence of iron status on iron absorption during pregnancy by measuring supplemental iron absorption, red blood cell iron incorporation, and iron status in pregnant women.

Design: Subjects were 45 pregnant Peruvian women (33 ± 1 wk gestation), of whom 28 received daily prenatal supplements containing 60 mg Fe and 250 \(\mu\)g folate without (Fe group, \(n = 14\)) or with (Fe+Zn group, \(n = 14\)) 15 mg Zn, which were consumed from week 10 to 24 of gestation until delivery. The remaining 17 women (control) received no prenatal supplementation. Iron status indicators and isotopes were measured in maternal blood collected 2 wk postdosing with oral (\(^{57}\)Fe) and intravenous (\(^{59}\)Fe) stable iron isotopes.

Results: Maternal serum ferritin and folate concentrations were significantly influenced by supplementation (\(P < 0.05\)). Serum iron was also significantly higher in the Fe than in the Fe+Zn (\(P < 0.03\)) or control (\(P < 0.001\)) groups. However, the supplemented groups had significantly lower serum zinc concentrations than the control group (8.4 ± 2.3 and 10.9 ± 1.8 \(\mu\)mol/L, respectively, \(P < 0.01\)). Although percentage iron absorption was inversely related to maternal serum ferritin concentrations (\(P = 0.036\)), this effect was limited and percentage iron absorption did not differ significantly between groups.

Conclusions: Because absorption of nonheme iron was not substantially greater in pregnant women with depleted iron reserves, prenatal iron supplementation is important for meeting iron requirements during pregnancy. \textit{Am J Clin Nutr} 1999;69:509–15.

KEY WORDS Stable isotopes, iron, zinc, pregnancy, anemia, ferritin, folate, Peru

INTRODUCTION

Iron deficiency is believed to be the most prevalent nutrient deficiency in the world. Women have increased risk of iron deficiency due to their higher iron demands. Global estimates suggest that 47\% of nonpregnant and 60\% of pregnant women are anemic (1). Iron deficiency anemia during pregnancy has been related to several adverse health consequences, including increased risk of maternal mortality during the perinatal period, low birth weight, and preterm births (2–4). Anemia during pregnancy may also compromise maternal immunologic status (5) and in severely anemic women, increase placental weight (6).

To help meet the increased iron requirements of pregnancy, iron-containing prenatal supplements are routinely recommended for pregnant women. These supplements are likely to have a greater effect in regions of the world in which the iron bioavailability of the diet is poor and dietary sources of heme iron are limited.

Although the focus of most studies of mineral supplementation during pregnancy is on iron, dietary intakes of other minerals such as zinc are often limited in iron-deficient areas. Few studies, however, have addressed the potential effect of the addition of zinc to prenatal iron supplements in populations known to be at risk for the development of both nutrient deficiencies. Studies in healthy subjects found that Fe-Zn ratios > 2:1 can impair zinc absorption (7), and several studies in pregnant women indicated that acute periods of iron supplementation during pregnancy may negatively influence zinc status (8–10).

Currently, few data exist on the optimum amount and absorption of iron from commonly used prenatal supplements, and most were obtained in well-nourished women. The objective of this study was to determine the ability of pregnant women to absorb supplemental iron and incorporate it into red blood cells (RBCs) during the third trimester of pregnancy, and to address the effect of prenatal supplementation on these processes. We hypothesized that iron-deficient women would increase intestinal absorption of iron and incorporate more of an intravenously administered iron tracer into RBCs. To address these issues, we carried out dual stable-isotope studies of iron absorption in pregnant Peruvian women living in a low-income community with a high prevalence of iron deficiency anemia.

\textsuperscript{1}From The Johns Hopkins School of Hygiene and Public Health, Baltimore; the Instituto de Investigacion Nutricional, Lima, Peru; and the US Department of Agriculture/Agricultural Research Service Children's Nutrition Research Center, Baylor College of Medicine, Houston.

\textsuperscript{2}Supported by a grant from the Nestle Foundation.

\textsuperscript{3}Address reprint requests to K O'Brien, Center for Human Nutrition, The Johns Hopkins School of Hygiene and Public Health, 615 North Wolfe Street, Baltimore, MD 21205. E-mail: kobrien@jhshp.edu.

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SUBJECTS AND METHODS

Subject selection

Pregnant women between the ages of 18 and 35 y were recruited during their third trimester of pregnancy (30–36 wk gestation) from Villa El Salvador, a periurban shantytown on the outskirts of Lima, Peru. Subjects were recruited into this study if they had experienced no prenatal complications, had a parity of 0–2, and had no known underlying metabolic or disease process that might influence mineral metabolism. All women recruited for this study had lived in Villa El Salvador (which is at sea level) for ≥12 mo before the study, thus the influence of high altitude on hemodynamics and iron metabolism was avoided.

Three groups of women were recruited. Two groups were recruited from an ongoing US Agency for International Development–funded prenatal supplementation study designed to address the effect of zinc supplementation on labor and delivery complications in 1185 women from this community. The study comprised 2 groups of women who consumed daily prenatal supplements containing 60 mg Fe as ferrous sulfate and 250 µg folate (Fe group) or the same supplement with 15 mg Zn as zinc sulfate (Fe+Zn group). These women received prenatal supplements and were examined from week 10 to 24 of pregnancy until delivery.

In the 28 supplemented women who participated in this study, the total number of prenatal tablets consumed throughout gestation (as determined by pill counts) did not differ significantly between groups (141 ± 43 and 159 ± 38 tablets in the Fe and Fe+Zn groups, respectively). In addition, >90% of these women took their prenatal supplements ≥5 d/wk.

The third group of women (control) was recruited from the same community but did not receive prenatal supplementation because they did not seek medical attention until the third trimester of pregnancy. Women in this group were also questioned about their use of any iron-containing tonics that were available within the community, and were excluded if they consumed any form of supplemental iron during pregnancy. After the iron absorption study was completed, women in the control group were given prenatal supplements (containing 60 mg Fe and 250 µg folate) to take daily throughout the remainder of their pregnancy. Because the pregnancies of women in the control group had less reliable gestational ages, this group was recruited primarily at the low end of the gestational age range (32 wk) to avoid delivery before the end of the study.

Written, informed consent was obtained from each woman and the study was approved by the Committee for Human Research at The Johns Hopkins School of Hygiene and Public Health and by the Ethical Committee at the Instituto de Investigacion Nutricional, Lima, Peru.

Study dropout or loss to follow-up was low. Of the 49 women who participated in the isotope study, data from all but 4 women were included in the final analyses. Of these, one delivered her infant in another community and was lost to follow-up, one delivered before the study was completed, one delivered an infant with a neural tube defect (Fe+Zn group), and one subject in the Fe group was excluded because of an isotope dosing problem.

Isotope preparation

Iron isotopes of Russian origin were purchased as the metal (57Fe at 94.67% enrichment and 56Fe at 93.13% enrichment; Penwood Chemicals, Great Neck, NY). The oral 57Fe tracer was converted into ferrous sulfate by following the procedure of Kasnemayer et al (11) except that no ascorbic acid was added during tracer preparation to avoid the influence of this vitamin on iron absorption. The final 56Fe isotope concentration of the oral solution was 1.635 g/L. The intravenous 56Fe isotope was converted from the metal into a sterile, pyrogen-free solution of ferrous citrate by Merck Frosst Canada, Inc, Quebec. Isotopic composition of the final tracer solutions was validated by using magnetic sector thermal ionization mass spectrometry (MAT 261; Finnigan, Bremen, Germany).

Study design and isotope dosing

On the morning of the isotope dosing, fasted (≥1.5 h) women reported to the Maternal Infant Hospital, Cesar Lopez Silva (CLS) in Villa El Salvador and baseline height and weight measurements were taken. Baseline blood samples (10-mL) were collected and an intravenous tracer of 0.6 mg 56Fe was then infused over a 10-min interval. Each woman consumed 10 mg of the 57Fe tracer in 60–90 mL of a nonascorbic acid–containing flavored drink made from a powdered mix and deionized water. Women in the Fe and Fe+Zn groups also consumed a prenatal supplement at this time. The prenatal supplement was identical to the supplement normally consumed except that the total iron content was reduced by 10 mg to keep the total dose of supplement and tracer constant at 60 mg Fe. Women continued to fast for 1.5 h after dosing. All subjects were fed a standard breakfast meal consisting of 2 slices of white bread with margarine and jelly and coffee containing 0–15 mL condensed milk. Women remained in the clinic until lunchtime, when they were served a standard lunch of chicken, rice, vegetables, and canned apricots.

The women returned to the CLS clinic 2 wk postdosing and 5-mL blood samples were obtained for analysis of iron isotopes. Blood samples were collected 14-d postdosing because prior studies have indicated that RBC iron incorporation is largely completed in 14 d (12).

Isolation of iron from samples

Whole blood (1 mL) samples were digested with 15 mL HNO3 in 25-mL Erlenmeyer flasks by heating overnight on a hot plate. After the solutions were clear, they were transferred to beakers and evaporated to dryness. The digested residues were reconstituted in 2–4 mL 6 mol HCl/L, covered and heated slightly until the residue went into solution, and cooled.

Iron was extracted from the digested whole blood by using an anion exchange chromatography method (13). Briefly, small columns with 4-mL reservoirs were filled with anion exchange resin (AG1, AG MP-50; Bio-Rad Laboratories Ltd, Mississauga, Canada) and the resin was washed 2 times with deionized water before rinsing with 2 mL 6 mol HCl/L. Digested blood samples were then added to the column and columns were washed twice with 4–6 mL 6 mol HCl/L before eluting the iron with 4 mL 0.5 mol HCl/L. The eluate was dried on a hot plate and the sample was reconstituted with 30–50 µL 3% HNO3. All acids used in this study were ultrapure (Ultrax; JT Baker, Phillipsburg, NJ).

Mass spectrometry

Extracted blood samples (10 µL) were loaded onto a rhenium filament and isotopic ratios were measured by using a Finnigan MAT 261 magnetic sector thermal ionization mass spectrometer.
The total mass of the $^{58}$Fe tracer that was incorporated into RBCs was calculated as:

$$\text{Total incorporated }^{58}\text{Fe} = \text{circulating }^{58}\text{Fe} \times \text{natural abundance of }^{58}\text{Fe} \times 0.01 \times \text{total }^{58}\text{Fe dose}/100$$

The same equation was used for the $^{58}$Fe tracer that was contributed by the oral $^{57}$Fe tracer by substituting the $^{58}$Fe baseline and observed ratios into the equation. The natural abundance ratios of the $^{57}$Fe and $^{58}$Fe tracers used were 0.02326 and 0.00311, respectively.

### Calculation of RBC iron incorporation and iron absorption

Calculations for determination of iron absorption and RBC iron incorporation were reported extensively (15). Briefly, the total amount of circulating iron was determined as:

$$\text{Circulating }\text{Fe (mg)} = \text{blood volume} \times (70 \text{ mL/kg}) \times \text{hemoglobin} \times \text{wt (kg)} \times \text{[Fe]} \times (3.47 \text{ g/kg})$$

The total mass of the $^{59}$Fe tracer that was incorporated into RBCs was then determined as:

$$\Delta\% \text{ excess }^{59}\text{Fe} = \left( \frac{\text{natural abundance of }^{59}\text{Fe} \times \text{total }^{59}\text{Fe dose}}{\text{natural abundance of }^{59}\text{Fe}} \times \text{circulating }^{59}\text{Fe} \times 0.01 \times \text{total }^{59}\text{Fe dose}/100 \right)$$

Using this value, the percentage of the intravenous tracer incorporated into RBCs was calculated as:

$$\Delta\% \text{ excess }^{59}\text{Fe} = \left( \frac{\text{natural abundance of }^{59}\text{Fe} \times \text{total }^{59}\text{Fe dose}}{\text{natural abundance of }^{59}\text{Fe}} \times \text{circulating }^{59}\text{Fe} \times 0.01 \times \text{total }^{59}\text{Fe dose}/100 \right)$$

This value was corrected for the small fraction of the $^{59}$Fe tracer (0.20855%) that was contributed by the oral $^{57}$Fe tracer by using the calculations reported by Kastenmayer et al (11). Iron absorption was then determined by measuring the total incorporated $^{57}$Fe (as in equation 3), and measuring the fraction of the oral $^{57}$Fe dose incorporated into RBCs (equation 4). The amount of $^{57}$Fe absorbed from the oral dose was determined as:

$$\text{Fe absorption} = \left( \frac{\text{oral }^{57}\text{Fe incorporated into RBC}}{\text{total }^{57}\text{Fe incorporated into RBC}} \times 100 \right)$$

In this study, both the oral iron tracer and the prenatal iron supplement were administered as ferrous sulfate. However, the oral iron tracer was in liquid form whereas the ferrous sulfate in the supplement was in powdered form. Using this study design we are assuming that the 2 tracers would have comparable absorption from the gastrointestinal tract.

Total iron absorption was estimated for each group as the product of iron absorptive efficiency and the iron content of either the oral iron dose (control) or the sum of the oral tracer dose and prenatal supplemental iron (the Fe and Fe+Zn groups). In the supplemented groups these estimates represent the iron requirements fulfilled by the prenatal supplements. In the control group, however, these estimates reflect absorption from a 10-mg dose of nonheme iron. Actual iron absorption from dietary sources may be higher than that obtained from this tracer dose.

### Iron and zinc status indicators

Total iron was measured in digested serum samples by using a colorimetric procedure (Sigma Diagnostics, St Louis). Total iron binding capacity was then determined after saturating the serum with ferric chloride by using previously reported methods (16, 17). Hemoglobin was analyzed by using the cyanometMb method and the packed cell volume by using the microhematocrit method (18). Serum ferritin was measured by enzyme-linked immunosorbent assay using human antiferritin and antiferritin peroxidase antibodies purchased from DAKO (Santa Barbara, CA). Serum soluble transferrin receptors (sTIRs) were measured with a commercially available enzyme-linked immunosorbent assay (Quantikine; R&D Systems, Minneapolis). Serum folate and vitamin B-12 were measured in the same sample by using a radioimmunoassay (Diagnostic Products Corporation, Los Angeles). Atomic absorption spectrophotometry was used to measure zinc in digested serum samples (model 3100; Perkin Elmer, Norwalk, CT).

### Statistical analyses

Potential differences in measured variables between supplementation groups were analyzed by using analysis of variance; Scheffe’s test was used for post hoc comparisons. Linear regression analysis was used to examine the relations between iron status indicators, iron absorption, and RBC iron incorporation. Statistical analyses were completed using the STATVIEW 4.5 software program (Abacus Concepts, Berkeley, CA). All differences were considered significant if $P < 0.05$.

### RESULTS

The characteristics of the study population are shown in Table 1. No significant differences were found in any of the measured physical characteristics between groups. Women in the Fe and Fe+Zn groups began prenatal supplementation at the same stage of pregnancy (16.6 ± 5.1 and 15.6 ± 5.0 wk gestation, respectively) and hemoglobin concentrations at the start of supplementation did not differ significantly between groups (113.4 ± 13.4 and 114.6 ± 10.8 g/L in the Fe and Fe+Zn groups, respectively).

Iron status was significantly influenced by maternal iron supplementation as evidenced by significantly higher serum ferritin concentrations in supplemented (Fe and Fe+Zn groups) compared with nonsupplemented (control) women, of whom 65% had ferritin concentrations <12 μg/L (Table 2). The control group also had significantly higher sTIR concentrations than the Fe group (20.7 ± 9.7 and 13.5 ± 3.5 mg/L, respectively, $P < 0.05$). Furthermore, significantly higher serum iron concentrations were found in the Fe group than in the control group (20.9 ± 9.8 and 9.2 ± 3.8 μmol/L, respectively, $P < 0.0002$). However, the addition of zinc to the prenatal iron supplement given to the Fe+Zn group resulted in significantly lower serum iron concentrations in the Fe+Zn group than in the Fe group (13.1 ± 7.0 and 20.9 ± 9.8 μmol/L, respectively, $P < 0.003$). Furthermore, serum iron concentrations in the Fe+Zn group were not significantly different from those in the control group.

Serum folate concentrations were significantly influenced by prenatal supplementation and were significantly higher in the Fe and Fe+Zn groups than in the control group ($P < 0.003$). No differences in serum vitamin B-12 concentrations were observed between study groups. (Table 2)
Prenatal iron supplements had a negative effect on serum zinc concentrations as evidenced by significantly lower serum zinc concentrations in the Fe group than in the control group (8.4 ± 2.3 and 10.9 ± 1.8 µmol/L, respectively, P < 0.01). The addition of zinc to the prenatal iron tablet increased serum zinc concentrations to concentrations that did not differ significantly from those observed in the control group (9.2 ± 2.2 and 10.9 ± 1.8 µmol/L, respectively) (Table 2).

The fraction of the intravenous tracer taken up by RBCs was significantly influenced by iron supplementation. The Fe and Fe+Zn groups incorporated a significantly lower amount of intravenous iron tracer into their RBCs than did women in the control group (76.4 ± 13.1 and 91.5 ± 28.0, respectively, P = 0.02) (Table 3).

Percentage iron absorption did not differ significantly among treatment groups, nor was it significantly influenced by total doses of 10 or 60 mg nonheme Fe/d. Because of the larger supplemental iron intake, the estimated net iron absorption was significantly higher in the supplemented compared with the unsupplemented groups (P < 0.0001). The degree to which net iron absorption met the expected iron demands during pregnancy is shown in Table 3.

The only significant predictor of iron absorption was maternal ferritin status with this being inversely related to percentage iron absorption (Figure 1). Women with the highest ferritin concentrations (> 30 µg/L, n = 5) absorbed an average of 6.8 ± 4.4% of the supplemental iron whereas women whose serum ferritin concentrations were ≤ 30 µg/L absorbed an average of 12.2 ± 5.3% (n = 39). The subject with the highest ferritin concentration (61 µg/L) absorbed negligible (< 1.5%) iron from the supplement.

### DISCUSSION

Total iron requirements of an entire pregnancy are estimated to range between 480 and 1150 mg (19, 20). These demands are highest during the third trimester of pregnancy to support fetal erythropoiesis and placental iron accumulation. We found that absorption of supplemental iron during the third trimester of pregnancy was inversely related to maternal serum ferritin concentrations. However, the magnitude of increase in iron absorption was limited and increased by only 1.5% for every 10-µg/L decrease in serum ferritin concentrations. As a result, no significant differences in percentage iron absorption were evident among the study groups.

The mean percentage iron absorption observed from a 10- or 60-mg dose of nonheme iron averaged 11.7% and was similar to values reported in iron-replete pregnant women. In one such study, unsupplemented Swedish women absorbed an average of 14.3% of a 100-mg dose of nonheme iron at 35 wk gestation (21). Furthermore, in 466 unsupplemented women, absorption of 57Fe during the third trimester of pregnancy was ~15% from a 39-mg dose and was slightly less than 10% from a 120-mg iron dose (22). Higher absorptive efficiencies may occur when iron is administered in the presence of a meal (23). We chose to examine the absorption of supplemental iron in the fasted state

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**TABLE 2**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Supplemented group</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (g/L)</td>
<td>113.1 ± 9.8 [14]</td>
<td>111.3 ± 10.8 [14]</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>0.352 ± 0.038 [13]</td>
<td>0.345 ± 0.045 [14]</td>
</tr>
<tr>
<td>Serum soluble transferrin receptor (mg/L)</td>
<td>13.5 ± 3.5± [13]</td>
<td>16.6 ± 7.6± [14]</td>
</tr>
<tr>
<td>Folate (µmol/L)</td>
<td>12.6 ± 6.2± [9]</td>
<td>14.4 ± 6.5± [10]</td>
</tr>
<tr>
<td>Vitamin B-12 (pmol/L)</td>
<td>76.4 ± 28.4 [9]</td>
<td>101.9 ± 65.0 [10]</td>
</tr>
<tr>
<td>Serum iron (µmol/L)</td>
<td>8.4 ± 2.3± [14]</td>
<td>9.2 ± 2.2± [12]</td>
</tr>
</tbody>
</table>

†± SD; n in brackets. Data obtained in blood samples collected 2 wk after women received iron isotopes. Values within a row with different superscript letters are significantly different from each other, P < 0.05 by ANOVA and Scheffe’s test. Supplemented women consumed prenatal supplements containing 60 mg Fe and 250 µg folate from week 10 to 24 of pregnancy until delivery. Fe, iron and folate only; Fe+Zn, iron, folate, and 15 mg Zn. The control group received no supplements during pregnancy.
because pregnant women in this community are routinely advised to consume their prenatal supplements between meals. In our study population, typical dietary iron intakes average 13 mg/d in pregnant women (24), and dietary sources of heme iron are limited. This amount of iron intake is unlikely to meet iron needs during pregnancy unless a substantial amount of this iron is absorbed. We found no difference in percentage iron absorption from a 10- or 60-mg supplemental Fe dose. Because of the differences in the total iron dose, significant differences in net iron absorption were measured. Women who received 60 mg supplemental Fe/d (with or without the addition of zinc) absorbed an average of 6.9 mg/d. This amount of iron is consistent with the range of estimated iron demands during the third trimester of pregnancy (3.54–8.8 mg/d) (20). In contrast, estimated net iron absorption from a 10-mg nonheme iron dose (which is comparable with estimated daily dietary intakes of 13 mg/d) averaged 1.2 mg/d in unsupplemented women. To achieve the high end of the estimated iron demands, unsupplemented pregnant women would need to absorb ≈68% of the iron present in their diets.

In agreement with these estimates, prenatal iron supplementation did have a positive effect on maternal iron status in this population. Although there were no significant differences in mean hemoglobin concentrations between groups, those in unsupplemented women were below the cutoff point used by the World Health Organization to define anemia during pregnancy (<110 g/L) (25). Furthermore, iron reserves were depleted as evidenced by average serum ferritin concentrations <12 μg/L. In contrast, mean hemoglobin concentrations remained >110 g/L and significantly higher serum ferritin concentrations were observed in women who consumed prenatal iron supplements.

Concentrations of sTfR increase progressively in response to tissue iron deficiency and may be useful in assessing iron status during pregnancy. We found a significant increase in sTfR concentrations in unsupplemented compared with supplemented pregnant women. The observed difference in sTfR between groups (36%) was similar to results from a prior study of Jamaican women in which a 32% increase in sTfR was found in unsupplemented compared with iron-supplemented women (26). This indicator, however, was not significantly related to iron absorption during pregnancy; the finding was also reported in nonpregnant subjects (27).

Iron status was improved by consumption of iron-containing prenatal supplements, but zinc status may have been compromised, as evidenced by significantly lower serum zinc concentrations in iron-supplemented women. The addition of 15 mg Zn to these supplements increased serum zinc concentrations such that they did not differ significantly from nonsupplemented pregnant women. The Fe-Zn ratio of the zinc-containing prenatal supplements used in this study (4:1) was substantially greater than the ratio of 2:1 reported to influence zinc absorption (7). Several other studies in pregnant women also found that iron supplementation impaired zinc status (8, 9). A recent study has also reported that women consuming iron-containing prenatal supplements (contributing an additional 103 mg Fe/d) did not increase urinary zinc excretion to the same extent as did pregnant women consuming multivitamins containing only 18 mg Fe (10). Larger studies are needed to confirm this finding because of the variability observed in serum zinc concentrations.

RBC iron incorporation decreases from a prepregnancy value of ≈90% (28) to ≈65% (29) during pregnancy because of
increases in the size of the intravenous distribution pool and other physiologic changes of pregnancy. In our study, a significantly higher RBC iron incorporation (92%) was found in women who did not consume prenatal iron supplements compared with those who did. This may be because of an increased need to rely on exogenous sources for RBC formation because of limited reserves from which to draw on for this process. It is unlikely that this difference was due to differences in pool volume because no differences in weight, height, or body mass index were found between groups. Furthermore, plasma volume during pregnancy reaches a plateau by 28 wk; therefore all groups were studied after 30 wk of gestation, we do not think it changed markedly over the study interval (30). In contrast, women receiving prenatal supplements had an average RBC iron incorporation of 76%. This value is slightly higher but similar to that observed in a prior study in 12 pregnant women (23–36 wk gestation) in which RBC incorporation of \( ^{59}\text{Fe} \) was 64.7 ± 12.2% (29).

Using RBC iron incorporation as an estimate of iron absorption assumes that the oral and intravenous tracers are metabolized in the same fashion and that all iron absorbed from the gastrointestinal tract is incorporated into maternal RBCs. If, during pregnancy, a portion of absorbed iron was to be transferred directly to the fetus, the actual amount of absorbed iron would be underestimated with this technique. In one early human study, radioactive iron was detectable in cord blood only 40 min after it was given orally to the mother at term (31). We found no apparent differences in percentage iron absorption in our study, however potential differences in the transfer of oral tracer to the fetus remain to be examined.

In conclusion, absorption of nonheme iron did not substantially increase in pregnant women with depleted iron reserves. Prenatal supplements containing 60 mg Fe and 250 µg folate significantly improved the iron and folate status of pregnant women living in a population with a high prevalence of anemia. Despite the fact that iron supplementation had a positive effect on iron status, serum zinc concentrations were lower than those observed in women who did not consume prenatal supplements. Larger studies are needed to establish the optimal mineral composition of prenatal supplements and to address their impact on neonatal mineral status in populations at high risk of mineral deficiency.

We sincerely thank the women who volunteered to participate in this study. We also acknowledge Alberto Figueroa and Juanita Callalai for their excellent medical care of these women, the Cesar Lopez Silva (CLS) Hospital in Villa El Salvador for allowing us use of their medical facilities, and Lily Liang and Cindy Clarke for their technical help.

### REFERENCES


### TABLE 3

Percentage iron absorption and red blood cell iron incorporation compared with estimated maternal iron requirements

<table>
<thead>
<tr>
<th></th>
<th>Supplemented group</th>
<th></th>
<th>Control group</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>( n = 14 )</td>
<td>( n = 14 )</td>
<td>( n = 17 )</td>
</tr>
<tr>
<td>Iron absorption (%)(^2)</td>
<td>9.9 ± 5.7</td>
<td>13.0 ± 5.8</td>
<td>12.1 ± 4.9</td>
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<tr>
<td>Red blood cell iron incorporation (%)(^2)</td>
<td>74.5 ± 14.8</td>
<td>78.3 ± 11.4</td>
<td>91.5 ± 28.0</td>
</tr>
<tr>
<td>Total iron dose (mg)</td>
<td>60</td>
<td>60</td>
<td>10</td>
</tr>
<tr>
<td>Total iron absorbed (mg)(^2)</td>
<td>5.9 ± 3.4*</td>
<td>7.8 ± 3.5*</td>
<td>1.2 ± 0.5*</td>
</tr>
<tr>
<td>Estimated daily iron requirement (mg)(^2)</td>
<td>3.54–8.8</td>
<td>3.54–8.8</td>
<td>3.54–8.8</td>
</tr>
<tr>
<td>Percentage of estimated iron requirement met by supplemental intake (%)</td>
<td>67–167</td>
<td>89–220</td>
<td>14–34</td>
</tr>
</tbody>
</table>

\(^1\)Supplemented women consumed prenatal supplements containing 60 mg Fe and 250 µg folate from week 10 to 24 of pregnancy until delivery. Fe, iron and folate only; Fe+Zn, iron, folate, and 15 mg Zn. The control group received no supplements during pregnancy.

\(^2\)X ± SD. Values with different superscript letters are significantly different, \( P < 0.0001 \).

\(^3\)From reference 22.