Effect of high-dose iron supplements on fractional zinc absorption and status in pregnant women


ABSTRACT

Background: Women have an increased risk of iron deficiency during pregnancy because of the demands of the developing fetus. Iron supplements are commonly advocated as a prophylactic treatment and are generally taken with meals to reduce side effects, but iron can interfere with the absorption of zinc.

Objective: The aim was to determine the effect of consuming an iron supplement (100 mg Fe/d as ferrous gluconate) with meals from 16 wk gestation to term on zinc status and absorption.

Design: Stable-isotope techniques were used to measure zinc status (exchangeable zinc pool, EZP) and fractional zinc absorption (FZA) in early and late pregnancy from a meal consumed at a different time from that of iron supplement or placebo consumption in 6 women given iron supplements and 7 given a placebo.

Results: FZA increased during pregnancy, independent of iron supplementation. FZA was significantly higher ($P < 0.001$) at week 34 than at weeks 16 and 24, and urinary zinc excretion was higher at weeks 16 and 24, and urinary zinc excretion was higher at week 34 than at week 16 ($P = 0.02$). The size of the EZP remained unchanged throughout pregnancy and was unaffected by iron supplementation. The iron status of iron-supplemented women was higher than that of the placebo group.

Conclusions: In iron-replete pregnant women who consumed a Western diet, no detectable adverse effects on zinc metabolism were observed after ingestion of 100 mg Fe/d. An increase in the efficiency of zinc absorption was observed during late pregnancy. Am J Clin Nutr 2007;85:131–6.

KEY WORDS Pregnancy, iron supplements, stable isotopes, zinc, women, iron status, absorption

INTRODUCTION

Women in their reproductive years are at increased risk of iron deficiency because of the higher iron requirements associated with menses and pregnancy. Iron deficient anemia is more likely to develop as fetal growth accelerates during the third trimester of pregnancy and can adversely affect pregnancy outcome, including causing premature delivery (1, 2) and low birth weight (3).

Iron deficiency can be prevented by iron supplementation, but, when taken with zinc, there is a significant reduction in zinc absorption (4, 5). Zinc deficiency is associated with intruterine growth retardation (6), congenital malformations (7), and low birth weight (8), but the effect of iron supplements on systemic zinc metabolism is unclear. Zinc absorption was measured from plasma appearance after a 25-mg dose of oral zinc was given to pregnant women taking 100 mg Fe plus folate or folate alone during the second trimester and was shown to be reduced 24 h after taking either iron plus folate or folate supplement (9), which suggests that iron (or folate or both) may have a deleterious effect on zinc metabolism during pregnancy. This is supported by the results of 2 studies that report a reduction in plasma zinc concentrations in pregnant women given high (164–395 mg/d) or moderate (60 mg/d) doses of iron (10, 11). However, in contrast, no effect on serum zinc concentration was observed in pregnant women given 2 daily supplements of ferrous fumarate containing 79 mg iron (12) or in plasma zinc concentration in infants given 10 mg Fe/d (13). Changes in zinc status are difficult to measure because of the lack of sensitive and specific biomarkers, but the effect of an iron-zinc interaction can be evaluated by measuring changes in the size of exchangeable zinc pool (EZP) (14).

To address concerns about iron supplementation during pregnancy, a human intervention study was undertaken to examine the consequence of consuming daily iron supplements (100 mg/d) from 16 wk gestation until delivery on fractional zinc absorption (FZA) and zinc status. The aim was to measure the systemic effect of iron supplementation on zinc metabolism rather than investigating the luminal interaction between iron and zinc that reduces FZA. This was achieved by avoiding iron supplements on the morning of the absorption test day and measuring FZA from a light midday meal.

SUBJECTS AND METHODS

Subjects

Thirteen apparently healthy, pregnant women aged 18–40 y and <14 wk gestation were recruited to the study through local medical practitioners and the Maternity Department of the Norfolk and Norwich University Hospital. The subject characteristics are given in Table 1. Before being accepted onto the study, subjects gave informed consent and the study was approved by the local Ethics Committee.

<table>
<thead>
<tr>
<th>Subject Characteristics</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td></td>
</tr>
<tr>
<td>Gestation wk</td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td></td>
</tr>
<tr>
<td>Height (cm)</td>
<td></td>
</tr>
</tbody>
</table>

1 From the Institute of Food Research, Norwich Research Park, Colney, Norwich, United Kingdom (LJH, JRD, WHJ, VJB, JAH, RJF, and SJF-T), and the University of Ulster, Coleraine, Northern Ireland (LM and JJS).
2 Supported by the European Commission (FeMMES Project contract no. QLK1-1999-00337) and the Biotechnology and Biological Sciences Research Council.
3 Reprints not available. Address correspondence to SJ Fairweather-Tait, Institute of Food Research, Norwich Research Park, Colney, Norwich, NR4 7UA, United Kingdom. E-mail: sue.fairweather-tait@bbsrc.ac.uk. Received June 16, 2006. Accepted for publication September 1, 2006.
TABLE 1
Baseline characteristics and mean dietary zinc and iron intakes of pregnant women given either 100 mg Fe/d (as ferrous gluconate) or a placebo from 16 wk gestation until term

<table>
<thead>
<tr>
<th></th>
<th>Iron-supplemented group (n = 6)</th>
<th>Placebo group (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (ys)</td>
<td>28.0 ± 5.6</td>
<td>31.4 ± 4.0</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>166.4 ± 5.2</td>
<td>164.2 ± 4.9</td>
</tr>
<tr>
<td>Weight at screening, &lt; 14 wk gestation (kg)</td>
<td>69.5 ± 10.7</td>
<td>67.9 ± 8.8</td>
</tr>
<tr>
<td>Parity</td>
<td>0.5 ± 0.5</td>
<td>0.7 ± 0.8</td>
</tr>
<tr>
<td>Dietary zinc intake (mg/d)</td>
<td>6.8 ± 2.0</td>
<td>4.8 ± 2.8</td>
</tr>
<tr>
<td>Dietary iron intake (mg/d)</td>
<td>13.7 ± 5.3</td>
<td>10.2 ± 3.3</td>
</tr>
</tbody>
</table>

1 All values are x ± SD. Zinc and iron intakes were determined from 7-d duplicate diets collected on a rolling week basis between weeks 18 and 25 of pregnancy. No significant differences between the groups for any of the variables presented were observed (two sample t test, Mann-Whitney U test, or chi-square test as appropriate).

TABLE 2
Composition of meals provided on the day of the zinc absorption test, calculated from food-composition tables

<table>
<thead>
<tr>
<th></th>
<th>Energy (kcal)</th>
<th>Protein (g)</th>
<th>Zinc (mg)</th>
<th>Fiber (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breakfast: orange juice, corn flakes, semiskim milk, 2 slices white toast, Flora low-fat spread, jam, tea or coffee (optional)</td>
<td>531</td>
<td>14.31</td>
<td>1.04</td>
<td>1.66</td>
</tr>
<tr>
<td>Midmorning snack: 3 shortie biscuits, tea or coffee (optional)</td>
<td>147</td>
<td>1.95</td>
<td>0.18</td>
<td>0.47</td>
</tr>
<tr>
<td>Lunch: ham and tomato sandwich, low-fat fruit yogurt, apple, Kit Kat chocolate bar</td>
<td>744</td>
<td>30.2</td>
<td>3.16</td>
<td>3.68</td>
</tr>
<tr>
<td>Lunch: cheese and tomato sandwich, low-fat fruit yogurt, apple, Kit Kat chocolate bar</td>
<td>823</td>
<td>26.1</td>
<td>2.65</td>
<td>3.68</td>
</tr>
<tr>
<td>Sub Total</td>
<td>823</td>
<td>26.1</td>
<td>2.65</td>
<td>3.68</td>
</tr>
</tbody>
</table>

1 Calculated by using the Englyst method.
2 Flora low-fat spread manufactured by Unilever UK, London, United Kingdom.
3 Kit Kat chocolate bar is manufactured by Nestlé, York, United Kingdom.
4 Kit Kat chocolate bar is manufactured by Nestlé, York, United Kingdom.

Human Nutrition Unit at the Institute of Food Research and a 20-mL blood sample was taken via a cannula from a vein in the forearm for biochemical and hematological measurements. An infusion of 1.6 mg Zn-70 stable isotope, as zinc citrate, was administered intravenously over a period of 3 min. The size of the EZP was measured from the appearance of the zinc stable isotope in complete 24-h urine collections made on days 3–6 postdosing (15). After the intravenous infusion, the volunteers were given a standard breakfast of cereal, toast, orange juice, and a cup of decaffeinated tea or coffee (optional). The volunteers remained in the Human Nutrition Unit during the morning, and ≥2 h after the intravenous infusion they were offered a midmorning snack of cookies and a cup of decaffeinated tea or coffee with semi-skimmed milk (with sugar as required). The components of the meal and nutritional composition, calculated from food tables (16), are given in Table 2.

FZA was measured at 16, 24, and 34 wk of pregnancy, and the volunteers were instructed not to take their supplement on these test days. Approximately 4 h after the intravenous zinc infusion each volunteer received a 3-mg oral dose of the highly enriched stable isotope Zn-67 (as zinc chloride) in 50 mL water. The oral dose was administered with a standard lunch of sandwiches, yogurt, fruit, and a chocolate bar containing 3.16 mg Zn (ham sandwich) or 2.65 mg Zn (cheese sandwich), as shown in Table 2. The volunteers refrained from eating and drinking (except water) for 2 h after the test meal. Complete fecal collections were made for a period of 10 d postdosing, and, in addition, each volunteer collected a baseline stool sample before each test day. A 24-h baseline urine collection was made before each test day, and complete 24-h urine collections were made on days 3–6 postdosing.

Stable isotope preparation

Oral doses of isotopically enriched (91.9 ± 0.5 atom%) zinc chloride were prepared from elemental Zn-67 (Techsnab Export,
Moscow, Russia) by dissolving the metal in 1.5 mL concentrated hydrochloric acid. Sterile water was added, and the solution adjusted to pH 5 by the addition of 1 mol NaOH/L. The solution was made up to an appropriate volume with sterile water to give a final concentration of ≈1 mg Zn/mL. The solution was divided into individual 3-mg oral doses and stored in sterile plastic vials at −20 °C until required.

Isotopically enriched zinc citrate doses for intravenous infusion were prepared at Ipswich Hospital NHS Trust Pharmacy. Elemental zinc (Zn-70) was dissolved in a minimum volume of 6 mol hydrochloric acid/L, an equimolar quantity of trisodium citrate in sterile water was added, and the solution was adjusted to approximately pH 7.25 with 0.5 mol sodium bicarbonate/L and to the final volume with sterile water. The solution was divided into individual 1-mL doses in glass ampoules and checked for sterility by the Ipswich Hospital Pharmacy Quality Control Department. Ampoules were stored at 4 °C until required. The zinc concentration of all doses (oral and intravenous) was accurately determined by inductively coupled plasma–mass spectrometry (ICP-MS).

Sample analysis

All glassware, crucibles, and other equipment used during sample processing were acid washed before use. Autoclaved fecal samples were freeze-dried and subsequently ground to a fine powder. Samples were prepared for ICP-MS analysis as previously described for copper (17). Zinc was subsequently extracted from the supernatant by ion-exchange chromatography by using an analytic grade anion exchange resin (AG1 × 8 200-mesh chloride; BioRad Ltd, Hemel Hempstead, United Kingdom). Zinc was eluted in 15-mL fractions into polytetrafluoroethylene vials with 0.05 mol HCl/L, dried under a hot lamp, and reconstituted for multicollector ICP-MS analysis in 2% (by vol) ultrapure HNO₃. The zinc content of the samples was measured by atomic absorption spectrophotometry.

Urine aliquots (≈80 mL) were accurately weighed into 150-mL beakers, evaporated to dryness on a hot plate, covered with a porcelain lid, and ashed at 450 °C for 48 h. The ash was subsequently dissolved in 2 mL of 6 mol hydrochloric acid/L and left to stand overnight before centrifugation at 2500 × g for 10 min at room temperature. The samples were then processed in a similar manner to the fecal samples before ICP-MS analysis. Total urinary zinc was measured by evaporating urine (10 mL) to dryness on a hotplate, dissolving in 6 mol HCl/L, adding water (MilliQ; Millipore Ltd, Watford, United Kingdom) to 5 mL, and analyzed by atomic absorption spectrophotometry.

Zinc stable-isotope ratios were determined by using a multicollector ICP-MS (Micromass Isoprobe multicollector inductively coupled plasma mass spectrometer; GVi, Manchester, United Kingdom). SpectrosoL (BDH, Poole, United Kingdom) was used as an internal reference for zinc in the absence of a certified isotopic standard, and the zinc isotopic composition was assumed to be the average natural composition (18). The internal reproducibility of the measurement was 0.1%, 0.1%, 0.2%, and 0.3% for ⁶⁶Zn, ⁶⁷Zn, ⁶⁸Zn, and ⁷⁰Zn, respectively. The external reproducibility of the calculated zinc isotopic enrichment for ⁶⁷Zn and ⁶⁸Zn was 0.22% and 0.27% for the fecal samples and 0.43% and 1.03% for the urine samples, respectively.

The dietary zinc intake of each subject was assessed from a 7-d duplicate diet collected 1 day per week, on a rolling week basis, between weeks 18–25 of pregnancy. An exact duplicate of all food and drink consumed in each 24-h period was collected, homogenized (Ultra-Turrax T-50 homogenizer; Janke & Kunkel IKA-Labortechnic, Staufen, Germany), subsampled into acid-washed plastic vials, freeze-dried, and analyzed by atomic absorption spectroscopy. Plasma zinc concentration was measured by using an assay kit (Randox Kit ZN 2341; Randox, crumlin, N Ireland) automated on a Hitachi 912 autoanalyzer (Roche Diagnostics, Lewes, United Kingdom).

Mathematical analysis

FZA was measured from urinary monitoring, which involved converting the mass spectrometric ratios measured by the ICP-MS into mole fractions of recovered dose (oral and intravenous) with the use of the equations described by Lowe et al (19). It was assumed that 48 h after dose administration, the zinc from the oral and intravenous doses had equilibrated and that thereafter any 24-h pooled sample was adequate to calculate FZA of the oral dose. In this case, 24-h samples were collected for days 3–6. Thus, for the third 24-h sample (t = 72 h), FZA is given by the following equation:

$$FZA (t = 72 h) = \frac{mass_{oral}}{mass_{IV}} \times \frac{dose_{IV}}{dose_{oral}}$$

where mass_{oral} and mass_{IV} are the masses of zinc in the urine sample derived from the oral and intravenous doses, respectively, from a single 24-h pooled urine sample collected ≥72 h after the dose, and dose_{oral} and dose_{IV} are the quantities of labeled zinc given as oral and intravenous doses, respectively. This was then repeated for all subsequent 24-h samples (t = 96, 120, and 144 h). The final FZA value was then calculated as the mean of the 4 values; for all volunteers, the average SD of the 4 replicate percentage zinc absorption measurements was 1.54%.

Exchangeable zinc pool

Calculation of the EZP was based on the technique developed by Miller et al (15). Endogenous zinc in the pool is assumed to exchange with any newly absorbed zinc in the plasma within 2 d. Enrichment in any urine sample is defined as the ratio of the mass of zinc from the intravenous source (mass of zinc_{IV}) to the mass of zinc from the naturally abundant source (mass of zinc_{NA}), and by plotting the natural logarithm of this ratio against time (for t >72 h), fitting a straight line through the data, and extrapolating back to t = 0, the size of the EZP is obtained. The entire intravenous dose is assumed to equilibrate with the EZP instantaneously, and extrapolation back to t = 0 is assumed to compensate for any loss of intravenous dose from the EZP.

Statistical methods

The statistical package R (20) was used to carry out all statistical analyses. Evidence for a difference between groups was tested by using the two-sample t test where appropriate (ie, normally distributed data); otherwise, the Wilcoxon’s test (a non-parametric alternative to the t test) was used.

The relation between each parameter of interest and a range of biologically relevant variables was examined by using univariate analysis of variance. The initial linear regression models included all biologically relevant variables as main effects only.
TABLE 3
Indexes of iron and zinc status in women at 16, 24, and 34 wk of pregnancy given either 100 mg Fe/d (as ferrous gluconate) or a placebo

| Plasma zinc (μmol/L) | 16  | 24  | 34  | P for interaction
|----------------------|-----|-----|-----|------------------|
| Iron supplement      | 13.1 ± 2.6 \(^1\) | 12.0 ± 2.2 | 12.7 ± 2.5 | NS
| Placebo              | 13.4 ± 1.5  | 13.1 ± 1.8 | 14.3 ± 2.7 | NS
| Exchangeable zinc pool (mg) |     |     |     |     |
| Iron supplement      | 152 ± 33   | 154 ± 37 | 147 ± 31 |     |
| Placebo              | 145 ± 22   | 149 ± 11 | 146 ± 32 |     |
| Serum ferritin (μg/L) |     |     |     |     |
| Iron supplement      | 34.4 (56.0, 21.1) \(^a\) | 21.5 (34.2, 13.5) \(^a,b\) | 19.8 (33.8, 11.5) \(^b\) | 0.0128 |
| Placebo              | 35.4 (61.1, 20.5) \(^a\) | 14.0 (31.4, 6.2) \(^b\) | 7.3 (11.2, 4.8) \(^b\) |     |
| Hemoglobin (g/L)     |     |     |     |     |
| Iron supplement      | 123 ± 7   | 124 ± 9 | 126 ± 8 | 0.0498 |
| Placebo              | 123 ± 8\(^a\) | 118 ± 10\(^b\) | 117 ± 5\(^b\) |     |
| Transferrin receptor (nmol/L) |     |     |     | 0.0001 |
| Iron supplement      | 16.5 ± 5.5 | 18.5 ± 6.1 | 17.9 ± 5.0 |     |
| Placebo              | 18.3 ± 4.1\(^a\) | 21.3 ± 2.8\(^b\) | 32.7 ± 6.9\(^b\) |     |

\(^1\) n = 6 for the iron-supplemented group, 7 for the placebo group (unless otherwise stated). Values in a row with different superscript letters are significantly different based on Tukey’s honestly significant difference test when the interaction term was significant. No significant differences were observed at week 16 (ANCOVA linear regression model).

\(^2\) Interaction between supplement group and stage of gestation.

\(^3\) \(\bar{x} ± SD\) (all such values).

\(^4\) Geometric \(\bar{x}\); \(± SD\) in parentheses (all such values).

\(^5\) n = 6.

Subsequently, a combination of forwards and backwards selection including up to 2-way interaction terms of interest was used for achieving the final model. Once all nonsignificant terms were omitted from the models, Tukey’s honest significant difference test was used to identify differences between groups. A power transformation, usually the log or square root transformation, was applied to variables when necessary to improve regression diagnostics, in particular the normality of errors assumption. A significance level of 0.05 was used for all statistical tests.

RESULTS

A total of thirteen volunteers completed the study (7 placebo, 6 supplement). No significant differences in maternal anthropometric measures were observed between the groups, namely, age \((P = 0.224)\), screening weight \((P = 0.776)\), height \((P = 0.447)\) and parity (Table 1). Each volunteer gave birth to an apparently healthy, full-term baby (7 females, 6 males) with a birth weight \(\geq 2700g\). No significant differences were observed between the placebo and iron supplement groups in dietary intakes of zinc \((P = 0.159)\) or iron \((P = 0.172)\) assessed from duplicate diet collections made on a rolling week basis between weeks 18 and 25 of pregnancy (Table 1).

Mean values for iron and zinc status are presented in Table 3. The regression model generated for each iron status indicator showed that the supplement group-by-stage of gestation interaction was significant in all cases. Neither group nor stage of gestation was a significant term in the regression models with either plasma zinc or EZP size as the response variable. However, body weight at screening (\(<14\ wk \text{ gestation}\) was positively associated with the size of the EZP \((P = 0.002, \text{ partial correlation} = 0.52)\). The group-by-stage of gestation interaction was highly significant for serum ferritin \((P = 0.0128)\), but there was no significant difference in ferritin concentration between the placebo and supplement group at week 16. To examine the significant interactions between group and stage of gestation, the data were split according to group and separate linear regression models fitted to each. Tukey’s honest significant differences were then used on each of these models so that the pairwise comparisons of week of gestation could be compared between the supplement group and the placebo group. For serum ferritin, the placebo group concentrations were significantly less in weeks 24 and 34 than in week 16. There was also weak \((0.05 < P < 0.1)\) evidence that serum ferritin concentrations were less in week 34 than in week 24. For the supplement group, these differences were less marked and only the comparison between week 34 and week 16 was significant \((P = 0.0539)\) that serum ferritin concentrations were less in week 24 than in week 16. This implies that for the supplement group, nearly the entire drop in serum ferritin occurred between weeks 16 and 24.

The group-by-stage of gestation interaction was significant for hemoglobin \((P = 0.0498)\). In the placebo group, week 24 concentrations were significantly less than those in week 16, but there were no significant changes in hemoglobin levels over time for the supplement group. The interaction term was also highly significant for transferrin receptors \((P = 0.0001)\); in the placebo group week 34 levels were significantly greater than both weeks 16 and 24 whereas in the supplement group there were no significant changes.

Zinc absorption data are presented in Table 4. The regression model revealed no significant effect of iron supplementation on FZA estimated from urinary monitoring, but stage of gestation
had a significant effect \( (P < 0.001) \), with zinc absorption at week 34 being significantly higher than at weeks 16 and 24. An increase in FZA during pregnancy was observed, which was independent of iron supplementation. Urinary zinc excretion was significantly higher \( (P = 0.02) \) at week 34 than at week 16, but there was no significant effect of iron supplementation on urinary zinc.

**DISCUSSION**

The decline in plasma zinc concentration throughout gestation, which generally reaches a plateau at week 22, is well documented \( (21–24) \). The primary reason for the fall in concentration is plasma expansion, but hormonal changes and changes in albumin–zinc affinity may also play a part. Any observed decrease in plasma zinc concentration in the present study would be expected to have occurred between weeks 16 and 24 of pregnancy, and indeed both groups showed a slight, although not significant, decrease in concentration at week 24 compared with week 16. Subsequently, zinc concentration increased slightly by week 34. No significant effect of iron supplementation on plasma zinc concentration was observed.

Reports in the literature describing the effect of pregnancy on zinc absorption are inconsistent. Fung et al \( (25) \) showed no increase in zinc absorption in pregnancy, but Swanson and King \( (26) \) reported a tendency for zinc retention to be higher in pregnant women than in nonpregnant women, although a follow-up study showed no difference in FZA between pregnant and nonpregnant women \( (27) \). Our data, however, clearly shows a significant increase in zinc absorption as pregnancy progresses, which was not affected by iron supplementation. The efficiency of absorption from a standard sandwich lunch at 34 wk gestation was 31%, which is midway between the mean of 19.4% \( (25) \) and 42.8% \( (28) \) reported from breakfast meals given in late pregnancy. The differences may be related to the type of meal consumed, time of day, and adaptive responses to different levels of dietary zinc. There appears to be an inverse relation between habitual zinc intake and efficiency of absorption, such that the study with the lowest zinc intake recorded the highest absorption \( (28) \) and that with the highest zinc intake recorded the lowest absorption \( (25) \). Endogenous losses of zinc isotope were, on the other hand, unaffected by stage of pregnancy, but similar to the zinc absorption data, there was no difference between the iron-supplemented and placebo groups. Thus it would appear that the increased requirements for zinc during pregnancy are met by an increase in the efficiency of absorption of dietary zinc; the higher urinary zinc excretion is consistent with the observed changes in zinc absorption.

In the present study, the up-regulation in absorption in both groups may have contributed toward the maintenance of plasma zinc concentrations, especially as the habitual dietary zinc intake of the volunteers was low, although the bioavailability of dietary zinc from a Western-style diet is relatively high. Dietary bioavailability is an important determinant of the mechanisms initiated to maintain zinc homeostasis. Jackson et al \( (29) \) observed that slum-dwelling lactating women in the Amazon who consumed a low-zinc diet were able to maintain a positive zinc balance through regulating the efficiency of absorption as well as endogenous losses of zinc. It is likely that the latter becomes a more important pathway when the capacity to up-regulate absorption is limited by low dietary zinc bioavailability.

Our finding that zinc absorption did not differ significantly between the women taking iron supplements and those receiving placebo contrasts with an earlier stable-isotope study in which zinc absorption during the third trimester was reported to be significantly lower in women receiving daily iron supplements \( (60 \text{ mg of iron as ferrous sulfate}) \) \( (20.5\%) \) than in unsupplemented controls \( (47.0\%) \) \( (4) \). The apparent disparity can be explained by examining the experimental design. In the study by O’Brien et al \( (4) \), the iron supplement and zinc stable isotope were given together. The aim of our study was to determine the effect of taking iron supplements on zinc absorption, rather than measuring the well-described luminal interaction between iron and zinc. Iron supplements are generally taken once a day with a meal, to reduce gastrointestinal side-effects, therefore, there is scope for adaptive up-regulation of zinc absorption from meals consumed at a different time to the iron supplement if the iron supplement significantly impairs zinc absorption. In addition, the earlier observations of a detrimental carry-over effect of iron on zinc absorption \( (9) \) require further investigation by using the most up-to-date methods for measuring zinc absorption and assessing zinc status \( \text{EZP} \). For these reasons, the iron supplement was not given with the zinc stable-isotope test meals. It is also
possible that postabsorptive systemic competition between iron and zinc may occur if interactions are not limited to the intestinal mucosal cells (30); a reduction in whole-body zinc turnover is another adaptive mechanism whereby zinc may be conserved to maintain homeostasis (31).

Fischer Walker et al (32) reviewed a number of studies investigating the interactive effects of iron and zinc in supplementation trials, all of which used plasma zinc as an index of status, which is far from ideal. The EZP is an accepted technique for assessing zinc status, but, as far as we are aware, it has only been used in one other study conducted in pregnant women. Brazilian women who consumed marginal zinc diets had a mean EZP of 50 mg (28). The EZP estimate in the present study was 3 times larger (145–154 mg) and was similar to that measured in zinc-replete men (165 mg) (33). This is probably a reflection of differences in the methods used to estimate EZP: in the Brazilian study, the EZP was estimated from samples collected for only 24 h, whereas the present study was undertaken over a 6-d period. Nevertheless, both studies showed that the EZP was similar in early and late pregnancy. Iron supplementation had no effect on EZP.

The data obtained from our study indicate that in iron-replete pregnant women who consume a Western diet, there were no adverse effects associated with taking 100 mg iron (as ferrous gluconate) with meals on zinc metabolism. Zinc status appeared to be maintained by an increased efficiency in absorption of zinc from meals consumed without an iron supplement.

We thank all the mothers and babies who took part in the study, Tim Overton the consultant obstetrician, the midwives at the Norfolk and Norwich University Hospital who supported the study, the IFR mass spectrometry team for sample analysis, and the nursing and catering staff at the IFR Human Nutrition Unit for providing technical support. We also thank Treasa nic Sualbhe, Fiona O’Neill, and Katherine Patrick from the University of Coderaine for their assistance in contributing to the practical aspects of the study.

SIF-T was responsible for the study design, manuscript preparation and the conception and funding of the study in collaboration with JJS. LJH supervised the study and assisted with design and manuscript preparation. WHJ and VJB carried out the clinical study, most of the biochemical analyses, and sample preparation for mass spectrometry. JAH undertook the mass spectrometry analysis. JRD undertook the stable-isotope calculations and the estimates of EZP. RJF performed the statistical analysis. LM performed the serum zinc analysis. None of the authors had a personal or financial conflict of interest.

REFERENCES