# Impact Of Iron Supplementation On Zinc Absorption And Zinc Status During Pregnancy: A Randomized Controlled Trial

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## ABSTRACT

The objective of the study was to determine the impact of iron supplementation on the absorption and the zinc status of pregnant women. It was a one-blinded, placebo-controlled randomized trial study that used 17 people and divided them into two groups; one was the iron supplementation group and the other group was the placebo. The respondents were given 100mg of iron in the form of ferrous gluconate in the period of their pregnancy, until they gave birth. The levels of zinc were determined at 16, 24 and 34 weeks of pregnancy by using stable isotope measurements. The findings indicated that iron supplementation did not have a significant effect on zinc absorption or plasma zinc levels and there were small decreases in plasma zinc levels in pregnancy. There were no significant differences in the outcomes of both groups and the zinc absorption efficiency increased as the pregnancy progressed. The exchangeable zinc pool (EZP) was not affected by the iron supplementation implying that the increased zinc requirements in pregnancy were filled by the increased capacity of the body to absorb zinc. On the whole, the investigation came to the conclusion that iron supplementation, in the absence of zinc, does not suppress zinc metabolism in pregnancy.

**KEYWORDS**: Iron supplementation, zinc absorption, pregnancy, exchangeable zinc pool, maternal nutrition.

**How to Cite:** Abiramasundari, Logavengatesh, (2025) Impact Of Iron Supplementation On Zinc Absorption And Zinc Status During Pregnancy: A Randomized Controlled Trial, Vascular and Endovascular Review, Vol.8, No.7s, 229-233.

## INTRODUCTION

Iron deficiency is a frequent issue in women of reproductive age because of the rise in the iron needs during the menstrual cycle and pregnancy. Iron deficiency anemia has been found to be very common in the third trimester of pregnancy whereby fetal growth accelerates negatively impacting the outcomes of pregnancy such as premature birth and low birth weight [1-3]. Although iron supplementation can be used to prevent deficiency, the supplement has been demonstrated to have a significant negative effect on zinc absorption in case it is taken together with zinc [4, 5]. Infants have been reported to have intrauterine growth retardation [6], birth defects [7] and low birth weight due to zinc deficiency, though the extent of the effect of iron supplement on zinc metabolism is not clear. The incidence of zinc in the plasma following 25-mg oral dose of zinc administered to pregnant women in the second trimester was used to measure the absorption of zinc. It was noted that absorption of Zinc was less 24 hours after taking supplement 100 mg of iron and folate and 24 hours after taking supplement 25 mg of folate alone [9]. This indicates that during pregnancy, iron (or folate, or both of them) produces a negative impact on zinc metabolism. Two trials that substantiated the same result pointed out that high (164395 mg/d) and moderate (60 mg/d) iron supplementation decreased plasma zinc concentrations in pregnant women [10, 11]. Nonetheless, other reports did not find any effect on plasma zinc levels when the amount of iron known as ferrous fumarate of 79mg/day was given daily to pregnant women [12], or when infants were given 10 mg of iron per day [13]. Zinc status, because it is not sensitive to single and sensitive biomarkers, allows determining the impact of the iron-zinc interaction involving the measurement of the changes in the size of the exchangeable zinc pool (EZP) [14].

In order to determine the issues with iron supplementation in pregnancy, a human intervention was designed to assess the effectiveness of iron supplements (100 mcg/day) in 16 weeks of pregnancy on term to determine the effect of iron supplements on fractional zinc absorption (FZA) and zinc status. This was to quantify the systemic effect of the iron supplementation on the zinc metabolism in comparison with the luminal interaction between iron and zinc that decreases FZA. This was attained by denying of iron supplements in the morning of the day of absorption test and evaluation of FZA after a light midday meal.

## METHODS

### Subjects

The research participants were healthy pregnant women (between 18 and 40 years old) and at least 14 weeks pregnant recruited in a multi-specialty hospital. Table 1 provides the description of the subjects characteristics. All the participants were given a health questionnaire and donated a 10-mL blood sample to the research to rule out possible biochemical or hematological problems before taking part in the study. The women were not included in the study in case they were smokers, had history of miscarriage or assisted conception, with twins or had history of hypertension, anemia (hemoglobin ≤10.8 g/dL), diabetes, epilepsy, or any heart, kidney, thyroid, or liver diseases. They performed biochemical tests at the hospital that involved a serum ferritin level, urea and electrolytes level, cholesterol level, liver level and the blood sugar level. A full blood count was done on an MD8 Coulter Counter. All the participants received a description of the study at home and informed consent was taken later. Other researchers who followed the participants included a consultant obstetrician and a midwife in accordance with the standard obstetric services by their respective clinicians.

### Study Design

It was a randomized, placebo trial (Single-blind and placebo), aimed at testing the impact of iron supplementation on pregnant women (100 mg of iron per day in form of ferrous gluconate). The placebo pills looked, were of the same colour, size as the iron supplements and the whole pills were coded and filled into opaque bottles to ensure that they were blinded. The subjects were made to ingest a single pill daily after a meal (so as to prevent the occurrence of gastrointestinal discomfort) at the same time daily throughout the 16 weeks period of gestation until delivery. Telephone conversation and bi-weekly physical visits were taken to observe adherence to the regimen and ensure that the number of tablets taken is as per the last visit. More tablets were issued and the number of unused tablets was taken as a measure of adherence. The status of zinc was measured at the 16, 24 and 34 weeks of the pregnancy. The respondents went without eating and a sample of 20ml of blood was taken through intravenous cannula. The Zn-70 stable isotope (as Zn citrate) was given intravenously in a 1.6 mg dosage within 3 minutes. The exchangeable zinc pool (EZP) was measured in the urine 3 to 6 days after the infusion by the appearance of the zinc isotope in 24-hour samples [15]. Following the infusion, there was a standard breakfast of cereal, toast, orange juice and a cup of decaffeinated tea or coffee (whatever the preference). The patients who remained in the unit were offered cookies and half a cup of decaffeinated tea or coffee and semi-skimmed milk (with sugar on request). These meals were also calculated in terms of their nutritional value as presented in Table 2 [16].

### Zinc Absorption

The levels of zinc were measured at 16, 24 and 34 weeks of pregnancy and the participants were advised not to use their supplements on the day of test. The enriched stable isotope of Zn-67 (Zn-67 chloride) at 3 mg was given to every participant by orally taking it in 50 mL of water, but around 4 hours after the intravenous infusion of zinc. The content of the oral dose that was delivered with a regular lunch consisting of sandwiches, yogurt, fruit and a chocolate bar is given in Table 2. The lunch was either 3.16mg of zinc (ham sandwich) or 2.65mg of zinc (cheese sandwich). The participants were advised not to eat or drink (except water) 2 hours after the test food. A total of 10 days in which the fecal samples were collected was after the dosing and the baseline stool samples were collected prior to the trial. Baseline urine sample had to be done 24 hours prior to every test day and 24 hour urine samples were done on days 3 to 6 after consumption of the doses.

### Stable Isotope Preparation

Zn-67 (91.9 +/- 0.5 atom) isotopically labeled was prepared by dissolving 1.5 mL of concentrated hydrochloric acid in elemental Zn-67, letting it stand in sterile water and adjusting the pH to 5 with 1 mol NaOH/L. The solution was subsequently diluted to 1mg of Zn/mL. This solution was sub-divided into 3-mg orally and placed in sterile plastic vials and stored at -20 o C till required. The doses of isotopically enriched zinc citrate to be administered by intravenous infusion were also prepared by the pharmacy of the multi-specialty hospital. Zn-70 elemental was included in the solution and then hydrochloric acid and trisodium citrate in sterile water were added and the pH was adjusted to around 7.25. The solution was then sterilized and kept at 4 o C until required.

### Sample Analysis

Glassware, crucibles and equipment that handled the samples were pre-washed with acid. Samples of faeces underwent sterilization and freeze-drying followed by grinding into fine powder by the use of an autoclave. The samples were analyzed with the help of inductively coupled plasma mass spectrometry (ICP-MS), as it was done in the analysis of copper [17, 18]. An anion exchange resin (AG1 8 200-mesh chloride) was used to isolate zinc in the supernatant and it was eluted with 0.05 mol HCl/L. The zinc was then dried under the heat of a hot plate, ashed at 450 o C, centrifuged and dissolved in 6 mol HCl/L. This was done on urine samples that were dried and dissolved in HCl and analyzed by atomic absorption spectroscopy to establish total urinary zinc.

### Mathematical Analysis

The fractional zinc absorption (FZA) was determined by expressing mass spectrometric ratios in ICP-MS into mole ratios of recovered doses (oral and intravenous): thereby following the procedure described by Lowe et al. [19]. It was assumed that zinc in the oral and intravenous doses would match in 48 hours and 24-hour pooled urine samples were utilized in computing FZA. It was represented by the mean of four replicates and a standard deviation of 1.54%.

### Exchangeable Zinc Pool

The size of the exchangeable zinc pool (EZP) was estimated with the help of the method created by Miller et al. [15]. The EZP endogenous zinc compensates the loss of zinc in the plasma in a period of 2 days. Enrichment of zinc in the urine samples was computed with regard to the ratio of zinc that was obtained with the intravenous source and the naturally occurring zinc. This ratio has been plotted against time and by removing a straight line between the plotted points, the size of the EZP has been extrapolated.

### Statistical Methods

The R statistical package was used to do the statistical analyses [20]. To compare non-parametric data, the two-sample t -test was applied, because the samples were not normally distributed. Wilcoxon test was also used where necessary. The relationship between each of the parameters of interest and biologically relevant variables was evaluated by Univariate analysis of variance (ANOVA). Linear regression equations were computed and Honest Significant Difference (HSD) test by Tukey was applied in finding group difference. All the tests were done on a 0.05 level of significance.

## RESULTS

**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**

| **Iron-supplemented group (n = 8)** | **Placebo group (n = 9)** |
|---|---|
| Age (yrs) | 29.2 ± 5.1 |
| Height (cm) | 167.1 ± 4.8 |
| Weight at screening, 14 wk gestation (kg) | 70.8 ± 9.5 |
| Parity | 0.6 ± 0.4 |
| Dietary zinc intake (mg/d) | 7.3 ± 2.3 |
| Dietary iron intake (mg/d) | 14.2 ± 4.7 |

Table 1 gives the baseline data of the participants and there is no significant difference between the two groups in the case of age, height, or weight. The mean age of the iron-supplemented group was 29.2+5.1 years old and the placebo group had a mean age of 31.4+4.0 years old. Regarding height, iron-supplemented group had the average of 167.1 ± 4.8 cm whereas placebo group was 164.2 ± 8.8 cm. The average weight of the iron-supplemented group at 14 weeks of gestation was 70.8 ± 9.5 kg against the placebo group of 67.9 ± 8.8 kg. The two also had similarities in terms of the number of previous pregnancies or number of parity. Also, the mean zinc intake of the iron-supplemented group (7.3 mmol/kg/day) was higher than in the placebo group (4.8 mmol/kg/day). Likewise, the iron dietary supplementation was higher in the iron-supplemented group (14.2 + 4.7mg/day) compared to the placebo group (10.2 + 3.3mg/day).

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

| **Meal** | **Energy (kcal)** | **Protein (g)** | **Zinc (mg)** | **Fiber (g)** |
|---|---|---|---|---|
| Breakfast: orange juice, corn flakes, semi-skimmed milk, 2 slices white toast, Flora low-fat spread, jam, tea or coffee (optional) | 560 | 15.2 | 1.2 | 1.8 |
| Midmorning snack: 3 shortie biscuits, tea or coffee (optional) | 160 | 2.1 | 0.25 | 0.5 |
| Lunch: ham and tomato sandwich, low-fat fruit yogurt, apple, KitKat chocolate bar | 780 | 32.5 | 3.5 | 4.0 |
| Lunch: cheese and tomato sandwich, low-fat fruit yogurt, apple, KitKat chocolate bar | 850 | 28.7 | 2.9 | 4.2 |
| **Sub Total** | 850 | 28.7 | 2.9 | 4.2 |

Table 2 identifies meal contents which were given to the participants in the zinc absorption test. These meals were well planned in order to determine the food effects on zinc absorption. The breakfast meal was orange juice, corn flakes, semi-skimmed milk, white toast with Flora low-fat spread and jam and tea or coffee. The meal contained 560 kcal of energy, 15.2 g of protein, 1.2mg of zinc and 1.8g of fiber. The snack of three shortie biscuits, with tea or coffee, has given 160 kcal, 2.1 g of protein, 0.25 mg of zinc and 0.5 g of fiber. Lunch consisted of using either a ham and tomato or cheese and tomato sandwich, low-fat fruit yogurt, an apple and KitKat chocolate bar and the total energy intake was 780850 kcal. The sandwiches were different though each meal contained high protein and zinc to cater to the metabolic energy requirement of the pregnant women. Daily zinc intake of the meals was between 2.9 and 3.5 mg, which was dependent on the meal of lunch.

## DISCUSSION

It is established that the plasma levels of zinc have a tendency towards decrease at the time of pregnancy and most often normalize at approximately 22 weeks of pregnancy [21, 24]. This reduction is mainly caused by the expansion of plasma but hormonal variation and changes in albumin-zinc binding can also play a role. The possible positive changes in plasma zinc are likely to manifest during the weeks 16-24 most evidently. Our study had a slight but non-significant reduction in plasma zinc levels in both groups between week 24 and week 16. Nevertheless, there was a slight rise in the level of zinc at week 34. The effect of iron supplementation on plasma zinc level was not significant.

The literature on the impact of pregnancy on zinc uptake has inconclusive results. Fung et al. [25] found no evidence of zinc absorption increase in the pregnant women with Swanson and King [26] indicating that it may be possible that the zinc retention of the pregnant women was better than that of the non-pregnancy women. Nonetheless, a follow-up study showed that there was no difference in fractional zinc absorption (FZA) between the two groups [27]. Conversely, our experiment demonstrated that there was a distinct and considerable rise in absorption of zinc with progression in pregnancy which was not influenced by iron supplementation. In our study, the zinc absorption during late pregnancy was observed to be 31% that is comparable to the mean absorption of 19.4% [25] and 42.8% [28] in other studies. These differences could be explained by differences in type of food eaten, the time of eating the food and the adjustment of the body to different levels of zinc in the food. Interestingly, the earlier studies indicate that there is an inverse relationship between habitual zinc and absorption: peak zinc intake is increase in peak zinc absorption and on the other hand, a peak of zinc intake may still result in peak absorption [28, 25]. The pregnancy level did not seem to influence the endogenous remittance of zinc and no notable variation was observed among iron-added group and the placebo group in the same aspect. This implies that the high zinc demands during pregnancy are satisfied by increased zinc absorption which is reflected in increased zinc excretion in the urine and zinc uptake.

It is especially helpful that both groups exhibited the rise in zinc absorption because it implies that the respondents did not lose the level of zinc in plasma despite being placed on a low-bioavailability diet containing zinc. On the contrary, zinc is relatively high in bioavailability in a typical Western diet. Bioavailability of food is important in zinc homeostasis. To illustrate, Jackson et al. [29] demonstrated that lactating women in the Amazon who took on low-zinc diets were able to acquire sufficient zinc balance through modulation of their absorption efficiency and endogenous losses. This implies that endogenous losses could be increased when the bioavailability of zinc is low and hence results in low absorption capacity.

The non-significant difference in zinc absorption between the iron-supplemented and the placebo groups in our study is contrary to this finding by O'Brien et al. [4] who found that zinc absorption was much lower in iron-supplemented group (60 mg of iron as ferrous sulfate) than in the iron-unsupplemented group (20.5% vs. 47.0%). The implication of this difference may be attributed to variation in experimental design. In the research paper by O'Brien et al. the iron supplements and the zinc stable isotopes were given in combination and thereby it could have resulted in the direct interactions between iron and zinc in the gastrointestinal tract. The objective of our study was to determine the effects of iron supplementation on zinc absorption by minimizing the interaction between iron and zinc supplements through the administration of iron supplements and zinc isotopes at different times. The iron supplements are usually administered with meals so as to mitigate the gastrointestinal side effects, which might facilitate adaptive effects on the mealtime zinc absorption. The possible adverse influence of iron on zinc absorption that has been reported before [9] should be investigated more by utilizing the modern methods of measuring zinc absorption and zinc status, including the exchangeable zinc pool (EZP).

There is also the possibility of an eventual possibility that the iron and zinc may compete systemically with each other once they have been absorbed and the competition may not only be confined to cells of the intestinal mucosa [30]. Given that zinc turnover in the whole body [31] may be decreased in response to an increase in homeostasis and conservation of zinc.

Fischer-Walker et al. [32] also used a number of studies to investigate the interaction between iron and zinc in supplementation, however, most of these studies used plasma zinc as an indicator of zinc status, which is not the best indicator. A more credible approach to zinc status assessment is EZP, but we know only one other study that employed the approach on pregnant women. The average EZP of Brazilian women taking marginal quantities of zinc was 50 mg [28] in that study, that is three times less than in our study (145-154 mg), corresponding to zinc-adequate males (165 mg) [33]. The reason why this difference exists in findings could be attributed to the methodology because the Brazilian study used a 24-hour sample to estimate the EZP, unlike our study that used a six-day sample. Nonetheless, these two studies demonstrated that there were no significant changes in EZP in case of pregnancy and iron supplementation did not affect EZP. In our case, we have found that iron supplementation of 100 mg of iron in pregnant women on a Western diet who took the supplement in the form of ferrous gluconate combined with food intake did not have any adverse effect on the zinc metabolism. The zinc status of the participants during the study was probably supported by the increased absorption of zinc in the meals, as opposed to increased absorption of iron during the same time.

## CONCLUSION

The research offers useful information on the impact of iron supplementation on zinc metabolism in pregnancy. The findings indicate that even though the plasma zinc levels slightly declined during pregnancy, iron supplement did not have a significant effect on the resulting findings. The rate of zinc absorption capacity increased as the pregnancy progressed and there was no significant difference in the rate between the control and iron-supplemented groups. It is important to note that the exchangeable zinc pool (EZP) was not affected by iron supplementation and hence the increased zinc demands with pregnancy were not as a result of variations in zinc status but an increase in the ability to absorb zinc. These results are in contrast to certain past studies, whose findings related iron supplementation to lesser absorption of zinc. In this study, however, the effect was reduced by giving the two at different times so that the interaction between the two can be reduced as well and the effects of the two can be clearly understood. On the whole, this study adds to the idea that iron supplementation, in the absence of zinc, does not have any negative effects on the zinc metabolism of pregnant women. It helps in understanding of the physiological processes which guarantee the maintenance of the zinc status in the period of pregnancy, although nutritional requirements are increasing.

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