

Effect of a micronutrient fortificant mixture and 2 amounts of calcium on iron and zinc absorption from a processed food supplement¹⁻³

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ABSTRACT

Background: Iron, zinc, and calcium can interact with each other in a way that inhibits their respective absorption. On the other hand, mineral fortification has been used to improve simultaneous iron and zinc absorption from food supplements.

Objective: We evaluated the effect of a novel fortificant mixture consisting of NaFeEDTA, zinc methionine, ascorbic acid, and citric acid on iron and zinc absorption from a dry food supplement designed for preschool children.

Design: The standard food supplement contained cereal and legume flour, dried milk, and a mixture of micronutrients including ferrous sulfate and zinc sulfate as sources of supplemental iron and zinc, respectively. Standard and novel food products were prepared as porridge with or without the addition of 200 mg Ca as calcium phosphate. Iron absorption and zinc absorption from the food products were evaluated simultaneously in 13 nonpregnant, adult women by extrinsically labeling the products with radioisotopes of iron and zinc and carrying out whole-body counting 7 d after the food products were consumed in random order.

Results: The absorption of iron from the NaFeEDTA-containing (novel) food product was 1.7 times that from the ferrous sulfate-containing (standard) product ($P = 0.015$). There was no significant effect of dietary calcium on iron absorption. Zinc absorption was not associated with the form of zinc consumed, but higher dietary calcium was marginally associated with lower zinc absorption ($P = 0.071$).

Conclusions: A mixture of fortificants containing NaFeEDTA, zinc sulfate or zinc methionine, ascorbic acid, and citric acid, but without calcium, can improve iron and zinc absorption from food products. A cost-benefit analysis of the novel fortificant mixture needs to be performed. *Am J Clin Nutr* 2004;79:244–50.

KEY WORDS Iron, zinc, phytic acid, calcium, iron absorption, zinc absorption, food products

INTRODUCTION

Currently, there is a growing consensus that both iron and zinc should be given to vulnerable groups in developing countries (1–6). Because these 2 elements can interact with each other, with one element inhibiting the absorption of the other, the mode of provision must be considered carefully. Isotope studies showed that iron can inhibit zinc absorption (7) and zinc can inhibit iron absorption in human adults (8), but this appears to occur only when the elements are given in a water

solution, but not when presented in a food product (8, 9). Other studies also showed that the addition of a dietary ligand that increases the solubility of zinc minimizes the interaction between zinc and iron (9–11). Thus, it is likely that the preferred way to present these 2 micronutrients will be as food fortificants, rather than as a liquid supplement.

Few attempts have been made to improve the simultaneous absorption of iron and zinc from food supplements. Moreover, most of the approaches that were attempted were single interventions rather than comprehensive ones. For example, NaFeEDTA was recently used in a study to improve iron absorption from cereals. A modest, positive “spillover” effect on zinc absorption was also noted (10, 12). There have also been attempts to improve zinc absorption from the diet by including various supplemental forms of zinc (13). Again, the attempts were only marginally successful. It is evidently difficult to override the effect of dietary factors that inhibit absorption.

In many developing countries, children’s calcium intakes are below the recommended intakes (14). However, several investigators reported that calcium may interfere with the absorption of iron and zinc (15, 16). Thus, it is not certain whether food products designed for young children in these settings should be fortified with additional calcium or whether such fortification might adversely affect iron and zinc absorption.

The Peruvian government is currently providing preschool children (6–36 mo of age) with a precooked food supplement prepared from a mix of cereals, defatted soybean flour, and dried milk fortified with vitamins and minerals added in amounts equal to 100% of the recommended dietary allowances for vitamin A, vitamin C, iron, zinc, and iodine; 60% of the recommended dietary allowances for thiamin, niacin, folic acid, riboflavin, vitamin B-6, vitamin B-12, and magnesium;

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TABLE 1Declared nutrient content per 90-g (dry weight) portion of the standard food supplement¹

| | |
|---------------------------|-------|
| Energy (kcal) | 405.6 |
| Protein (g) | 14.1 |
| Fat (g) | 14.4 |
| Carbohydrates (g) | 54.9 |
| Iron (mg) ² | 4.7 |
| Zinc (mg) ² | 1.4 |
| Calcium (mg) ² | 192.0 |
| Vitamin and mineral mix | |
| Iodine (μ g) | 70.0 |
| Vitamin A (μ g RE) | 400.0 |
| Folic acid (μ g) | 30.0 |
| Vitamin B-12 (μ g) | 0.5 |
| Thiamine (mg) | 0.5 |
| Riboflavin (mg) | 0.5 |
| Niacin (mg) | 5.0 |
| Vitamin B-6 (mg) | 0.6 |
| Magnesium (mg) | 50.0 |
| Fluoride (mg) | 0.5 |

¹ The food supplement consisted of the following (in % by wt): rice, 24.2%; whole milk powder, 15.0%; defatted soybean flour, 12.0%; vegetable fat, 11.2%; sugar, 11.0%; maltodextrin, 10.0%; amaranth, 6.0%; fructose, 3.0%; peas, 2.5%; fava beans, 2.0%; isolated soybean protein, 1.7%; vitamins and minerals mix, 1.2%; magnesium sulfate, 0.28%; flavoring agents, 0.05%. RE, retinol equivalents.

² From foods.

60% of the recommended amount of fluoride; and 25% of the recommended dietary allowances for calcium and phosphorus. To achieve the desired amounts of iron, zinc, and calcium, 10 mg Fe and 10 mg Zn (as sulfates) and 200 mg Ca (as phosphate) were added per 90-g (dry wt) portion of the food product. The objectives of the present study were to evaluate the effect of the amount of calcium added on the bioavailability of iron and zinc and to examine whether the novel fortificant mixture of iron as NaFeEDTA, zinc as zinc methionine, and ascorbic acid and citric acid as other possible enhancers would improve the availability of these essential minerals.

SUBJECTS AND METHODS

Subjects

Thirteen nonpregnant women aged 20–31 y were recruited from the student population of the University of California, Davis, by placing advertisements on campus. At the time of recruitment, the volunteers were not consuming nutrient supplements containing iron or zinc and were free from gastrointestinal disorders. The protocol was approved by the Human Subjects Committee and the Radiation Use Committee of the University of California, Davis.

Diets

A standard food supplement provided to preschoolers by the Peruvian government was used as the experimental diet. This food supplement contains a mix of cereals, defatted soybean flour, powdered milk, vegetable fat, sugar, fructose, isolated soybean protein, and vitamins and minerals (**Table 1**). Food products for the study were prepared as porridge in 4 forms: standard food product with either low or high calcium and

novel food product with either low or high calcium. The low-calcium food products contained 192 mg Ca, which was the amount intrinsic to the food ingredients; the high-calcium food products were fortified with an additional 200 mg Ca as dicalcium phosphate [total of 392 mg Ca/90-g (dry wt) portion of food product].

The standard food products contained a fortification mixture of 10 mg Fe as FeSO₄, 10 mg Zn as ZnSO₄, and 50 mg ascorbic acid per 90 g of the food. The novel food products contained 10 mg Fe as NaFeEDTA (JT Baker, Phillipsburg, NJ), 10 mg Zn as zinc methionine, 100 mg ascorbic acid, and 1 g citric acid as part of the fortification mixture. The composition of the 4 food products is shown in **Table 2**.

Subsamples of the food products were analyzed for inositol phosphate composition by the method of Sandberg and Ahderinne (17), and iron, zinc, phosphorus, and calcium concentrations were measured by using standard methods of the Association of Official Analytical Chemists (18). Molar ratios of phytic acid:iron, phytic acid:zinc, calcium:phytic acid, and ([calcium] × [phytic acid]):[zinc] in the food products were calculated.

Extrinsic labeling of the food product

All food products were incubated with 1.5 μ Ci (55.5 kBq) ⁵⁹Fe as FeCl₃ and 0.5 μ Ci (18.5 kBq) ⁶⁵Zn as ⁶⁵ZnCl₂ overnight in a refrigerator (4 °C) before administration. Following extrinsic labeling, the food products were offered at breakfast for 1 d each at 7-d intervals.

Iron and zinc absorption

The 4 test food products were evaluated for 1 d each at 7-d intervals. Subjects received the test food products in a randomly assigned order. The first food product was served at breakfast on day 1 after the subjects had fasted overnight, and no additional food or drink was allowed for ≥ 4 h after the food products were consumed. The subjects were asked to eat the entire test food product, rinse the empty plate twice, and drink the rinsed water. Absorption of iron and zinc were determined by using a whole-body counter to count the activity of ⁵⁹Fe and ⁶⁵Zn for 15 min in each subject before and immediately after ingestion of the labeled food product and 7 d later. A fasting blood sample was collected on day 1 and 14 d later to determine iron absorption by using a gamma counter to determine incorporation of ⁵⁹Fe into red blood cells. This procedure was repeated for each test food product.

The counting time (15 min) and the interval of 7 d between consumption of the food products were chosen on the basis of a preliminary experiment that was conducted with one volunteer subject. In the pilot study, whole-body counting was done for 5, 10, and 15 min and at 7, 8, 9, 10, and 13 d after food product consumption. Significant differences ($P < 0.05$) in counts per minute (cpm) were found between the counting times, and in the interests of accuracy, we elected to use a 15-min counting time. No significant differences were found between the readings at 7, 8, 9, 10, and 13 d after food product intake; therefore, the 7-d interval was used for the full study.

Iron absorption was measured by using both the whole-body counting method and the incorporation of ⁵⁹Fe into red blood

TABLE 2
Composition of the experimental diets¹

| Component | Standard food product | | Novel food product | |
|----------------------------------|-----------------------|--------------|--------------------|--------------|
| | Low calcium | High calcium | Low calcium | High calcium |
| Food supplement (g) ² | 90 | 90 | 90 | 90 |
| Iron (mg) | | | | |
| From food supplement | 4.7 | 4.7 | 4.7 | 4.7 |
| From FeSO ₄ | 10 | 10 | — | — |
| From NaFeEDTA | — | — | 10 | 10 |
| Total | 14.7 | 14.7 | 14.7 | 14.7 |
| Zinc (mg) | | | | |
| From food supplement | 1.4 | 1.4 | 1.4 | 1.4 |
| From ZnSO ₄ | 10 | 10 | — | — |
| From zinc methionine | — | — | 10 | 10 |
| Total | 11.4 | 11.4 | 11.4 | 11.4 |
| Calcium (mg) | | | | |
| From food supplement | 192 | 192 | 192 | 192 |
| From dicalcium phosphate | — | 200 | — | 200 |
| Total | 192 | 392 | 192 | 392 |
| Ascorbic acid (mg) | 50 | 50 | 100 | 100 |
| Citric acid (g) | — | — | 1 | 1 |
| PA (mg) | | | | |
| Inositol triphosphate | — | — | — | — |
| Inositol tetraphosphate | 6 | 6 | 6 | 6 |
| Inositol pentaphosphate | 45 | 45 | 45 | 45 |
| Inositol hexaphosphate | 254 | 254 | 254 | 254 |
| Total | 306 | 306 | 306 | 306 |
| PA:Zn molar ratio | 2.7 | 2.7 | 2.7 | 2.7 |
| Ca:PA molar ratio | 10.3 | 21.1 | 10.3 | 21.1 |
| PA:Fe molar ratio | 1.8 | 1.8 | 1.8 | 1.8 |
| ([Ca] × [PA]):[Zn] molar ratio | 0.1 | 0.3 | 0.1 | 0.3 |

¹ PA, phytic acid.

² Without addition of iron, zinc, calcium, ascorbic acid, or citric acid.

cells. Before intake of the labeled test food product, each subject's background radioactivity was determined both by using a whole-body counter and by measuring ⁵⁹Fe in a sample of blood by using the method of Viteri and Kohaut (19). Zinc absorption was estimated by using the method of Arvidsson and Cederblad (20).

Radioactivity was counted before and immediately after each test food product was consumed by using a whole-body counter to determine absorption of ⁵⁹Fe and ⁶⁵Zn. The empty chamber of the whole-body counter was counted as a background measurement. The counts were repeated 7 d later to determine absorption of the previously consumed dose. These measurements also served as the baseline values for the next food product period.

The same procedure was followed for the remaining test food products, which were fed on days 15 and 22 of the study. The last blood draw and whole-body counting took place on day 29 of the study. During the 29 d of the study, daily bowel movements and weekly morbidity records were obtained for each subject to evaluate their possible effect on iron and zinc absorption.

Calculations of iron and zinc absorption

To calculate iron and zinc absorption as a percentage of the ingested radioactive doses, preliminary studies with the whole-body counter were performed by using a cloth dummy and a vial of 0, 0.05, 0.1, 0.2, 0.3, or 0.5 μ Ci ⁶⁵Zn alone; 0, 0.05, 0.10, 0.25, 0.75, 1.00, or 1.50 μ Ci ⁵⁹Fe alone; and the 36

possible combinations of doses of these 2 isotopes. The empty chamber was counted for background radioactivity measurement, and these background values were subtracted from the counts for the various combinations of iron and zinc. Data from this preliminary experiment showed 2 peaks of radioactive counts. One peak, which represented both ⁵⁹Fe and ⁶⁵Zn, was located at \approx 1100 keV, and the other peak, which represented ⁵⁹Fe only, was located at \approx 1300 keV, as shown in **Figure 1**.

Various predictors of the known iron and zinc radioactivity of the test vials were assessed by using the RSQUARE method outlined in the REGRESSION procedure of PC-SAS, release 6.04 (21), with the intercept term suppressed. Possible predictors of iron radioactivity included values at 1280, 1290, and 1300 keV; the maximum value of these 3 (peak 2); and areas around the peak as measured by the peak value plus the j values before and the j values after the peak for $j = 1-10$. Possible predictors for zinc radioactivity included values at 1090, 1100, and 1110 keV; the maximum value of these 3 (peak 1); and areas around the peak as defined above. Additionally, all of the predictors for iron were included as possible predictors for zinc to account for the confounding with a second iron peak, which also occurs at \approx 1100 keV. Final models were selected on the basis of high R^2 and parsimoniousness. The best predictors for iron and zinc radioactivity turned out to be the areas around the peaks as defined by the peak value plus the 5 values before and the 5 values after the peak.

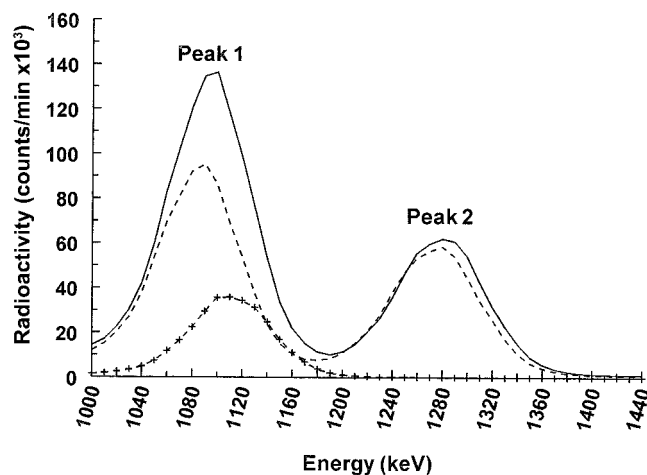


FIGURE 1. Radioactivity counts for ^{59}Fe and ^{65}Zn obtained by using the whole-body counter. Peak 1 (at ≈ 1100 keV) represents both ^{59}Fe and ^{65}Zn , and peak 2 (at ≈ 1300 keV) represents ^{59}Fe only. + + +, maximum ^{59}Fe , no ^{65}Zn ; - - -, no ^{59}Fe , maximum ^{65}Zn ; —, maximum ^{59}Fe and ^{65}Zn .

The following equations were used to predict ^{59}Fe and ^{65}Zn concentrations in the body:

$$\text{Predicted } ^{59}\text{Fe } (\mu\text{Ci}) = 0.002084 \times [\text{area around peak 2 (cpm)}] \quad (1)$$

$$\text{Predicted } ^{65}\text{Zn } (\mu\text{Ci}) = 0.001871 \times [\text{area around peak 1 (cpm)}] - 0.002852 \times [\text{area around peak 2 (cpm)}] \quad (2)$$

The R^2 values for Equations 1 and 2 were 0.997 and 0.999, respectively. These equations were used to calculate the predicted amounts of ^{59}Fe and ^{65}Zn that were ingested with the food product and the predicted amounts of ^{59}Fe and ^{65}Zn that were retained 7 d later. Absorption of ^{65}Zn on day 7 was corrected for endogenous excretion from day 0 to day 7, as estimated from a mean absorption function developed from published measurements of whole-body absorption in a group of healthy subjects who received an intravenous injection of ^{65}Zn (20). Baseline values and isotopic decay were taken into account in the ^{59}Fe and ^{65}Zn calculations.

Statistical analyses

Statistical analyses were carried out by using SAS for WINDOWS, release 8 (SAS Institute Inc, Cary, NC). Variables were transformed as necessary to conform to a Gaussian distribution. Correlation analyses between hematologic measurements, number of bowel movements, and isotope absorption data were performed by using the MIXED procedure to adjust for dependence among observations for the same subjects. The effect of calcium and type of fortificant on iron and zinc absorption was analyzed by using univariate repeated-measures analysis of variance that included type of fortificant, calcium, and sequence as main effects; the interaction between type of fortificant and calcium; and a random effect of subject. Data from 2 subjects were omitted from the analysis because, for 1 of the subjects, data were available from only one period, and

TABLE 3
General characteristics of the subjects¹

| Characteristic | Value |
|------------------------------------|--|
| Body weight (kg) | 67.3 \pm 11.5 (56.73–69.22) ² |
| Hemoglobin (g/L) | 133 \pm 8 (128.4–136.7) |
| Hematocrit (%) | 39.8 \pm 2.2 (38.59–40.98) |
| Serum ferritin ($\mu\text{g/L}$) | 23.8 \pm 2.2 (15.4, 36.7) [15.42–36.69] ³ |
| Bowel movement (times/d) | 1.3 \pm 0.6 (1.02–1.63) |

¹ $n = 13$.

² $\bar{x} \pm \text{SD}$; range in parentheses.

³ Geometric $\bar{x} \pm \text{SD}$; 95% CI in parentheses; range in brackets.

for the other subject, there was an obvious error in radioactivity measurement during the second period, which affected the calculated absorptions for 3 of the 4 periods.

RESULTS

Chemical analysis

The total iron, zinc, and calcium contents, the composition of the inositol phosphates, and the phytic acid:iron, phytic acid:zinc, calcium:phytic acid, and ([calcium] \times [phytic acid]):[zinc] ratios in each experimental food product are shown in Table 2. The food product contained 306 mg total inositol phosphates per portion, 98% of which corresponded to the sum of pentainositol and hexainositol phosphates. Phytic acid:zinc and phytic acid:iron molar ratios were 2.7 and 1.8, respectively, for both amounts of calcium, and the calcium:phytic acid and ([calcium] \times [phytic acid]):[zinc] molar ratios were 10.3 and 0.1, respectively, for the low-calcium experimental food product and 21.1 and 0.3, respectively, for the high-calcium experimental food product.

Iron and zinc absorption

The general characteristics of the subjects are shown in Table 3. The values are expressed as an average for each subject throughout the study period. Hemoglobin and hematocrit values did not differ throughout the study, with mean (\pm SD) values of 133 \pm 8 g/L and 39.8 \pm 2.2%, respectively. The geometric mean ferritin concentration was 23.8 \pm 2.2 $\mu\text{g/L}$. Two subjects were iron deficient (ferritin concentration < 12 $\mu\text{g/L}$) during the study. Iron absorption and zinc absorption from the food products are shown in Table 4. Geometric mean iron absorption values were 1.5% and 1.6% of the intake dose for the food products fortified with ferrous sulfate with low and high amounts of calcium, respectively, and 2.7% and 2.5% for the food products fortified with NaFeEDTA with low and high amounts of calcium, respectively. Arithmetic mean zinc absorption values were 6.7% and 6.6% of the intake dose for the food products fortified with zinc sulfate with low and high amounts of calcium, respectively, and 8.2% and 6.6% for the food products fortified with zinc methionine with low and high amounts of calcium, respectively.

There was a significant effect of the type of fortificant mixture on iron absorption. Iron absorption from the food product fortified with NaFeEDTA was 1.7 times that from the food product fortified with ferrous sulfate ($P = 0.015$). As shown in Figure 2, a significant correlation was found between iron absorption estimated by the whole-body counter and that

TABLE 4Absorption of iron and zinc from the study diets as assessed by whole-body counting¹

| | Iron absorption | | | | Zinc absorption | | | |
|--------|------------------------------------|--------------|---------------------------------|--------------|------------------------------------|--------------|---------------------------------|--------------|
| | Standard food product ² | | Novel food product ³ | | Standard food product ² | | Novel food product ³ | |
| | Low calcium | High calcium | Low calcium | High calcium | Low calcium | High calcium | Low calcium | High calcium |
| | % of dose | | % of dose | | % of dose | | % of dose | |
| Mean | 1.5 | 1.6 | 2.7 | 2.5 | 6.7 | 6.6 | 8.2 | 6.6 |
| 95% CI | 0.9, 2.6 | 1.0, 2.8 | 1.5, 4.6 | 1.5, 4.3 | 4.6, 8.8 | 4.5, 8.7 | 6.1, 10.3 | 4.5, 8.7 |

¹ Geometric \bar{x} for iron absorption and arithmetic \bar{x} for zinc absorption; $n = 13$. The interaction between diet type and calcium was not significant for either variable. The main effect of diet type was significant for iron absorption, $P = 0.015$ (repeated-measures ANOVA). The main effect of calcium was marginally significant for zinc absorption, $P = 0.071$ (repeated-measures ANOVA).

² Food + standard supplement (10 mg Fe as FeSO_4 + 10 mg Zn as ZnSO_4 + 50 mg ascorbic acid) + calcium as dicalcium phosphate at 2 different amounts: low (192 mg Ca/diet) and high (392 mg Ca/diet).

³ Food + novel supplement (10 mg Fe as NaFeEDTA + 10 mg Zn as zinc methionine + 100 mg ascorbic acid) + 1 mg citric acid + calcium as dicalcium phosphate at 2 different amounts: low (192 mg Ca/diet) and high (392 mg Ca/diet).

estimated by incorporation of iron into red blood cells ($r = 0.74$, $P < 0.0001$). Average serum ferritin was negatively correlated with iron absorption both as estimated by the whole-body counter ($r = -0.41$, $P = 0.036$) and as estimated by incorporation of iron into red blood cells ($r = -0.57$, $P = 0.004$).

There was a marginal effect of calcium ($P = 0.071$), with the higher calcium amount resulting in lower zinc absorption. As estimated by the whole-body counter method, zinc absorption and iron absorption were significantly correlated ($r = 0.51$, $P < 0.001$). There were no significant interactions between fortificant and calcium for either zinc absorption or iron absorption. No significant correlation was found between the number of bowel movements and either iron absorption or zinc absorption.

DISCUSSION

In the present study, we used the whole-body counting method to determine the absorption of iron and zinc from 4 food products labeled with both ^{59}Fe and ^{65}Zn . Iron absorption was also determined by measuring the incorporation of radio-labeled iron into red blood cells. Because relatively little zinc would be expected to be incorporated into red blood cells (22) and because the 2 methods for measuring iron absorption were

highly correlated in the present study, the inclusion of radio-labeled zinc in the test food products probably did not interfere with the whole-body counting procedure for measuring iron absorption. As determined by both techniques, iron absorption was also, as expected (23, 24), significantly negatively correlated with serum ferritin concentration, thereby further supporting the validity of the study methods used to determine iron absorption.

Whole-body counting is also considered the most sensitive method for determining the uptake of zinc in human studies (25). Despite the fact that the energy spectra of ^{59}Fe and ^{65}Zn partially overlap, data from the present study suggest that it is possible to simultaneously measure iron absorption and zinc absorption from a food product with the use of the whole-body counter by correcting the portion of the isotopic spectrum in which the 2 sets of data overlap by using the data from the portion of the energy spectrum that is unique to iron.

Absorption of nonheme iron can be affected by the presence of potential inhibitors, such as phytates and calcium, and enhancers, such as animal protein and ascorbic acid (26). Wise (27) claimed that phytate:iron molar ratios > 6 are likely to reduce iron absorption. The phytic acid:iron molar ratio of the present experimental food product was only 1.8, which suggests that the phytic acid amounts probably had little or no effect on iron absorption. This amount of phytic acid is lower than the typical amount in many cereal-legume food products (28). However, milled rice is generally lower in phytic acid than are other cereals, and the processing techniques employed may have reduced the phytic acid content of the soy flour. The low phytic acid:iron molar ratio of the food products was also due to the contribution of iron from ferrous sulfate or NaFeEDTA . Without the additional iron provided by the fortificants, the phytic acid:iron molar ratio of the food products would have been 5.6.

Previous studies found that the effect of calcium on iron absorption from food depends on several factors, such as the total amount of calcium present in the foods themselves, the amount and form of calcium added to the diet, and the presence of animal products in the diet (15, 29, 30). In the present study, there were 2 forms of calcium in the food product, namely, 192 mg Ca that was present in the milk included in the food product and 200 mg additional Ca as calcium phosphate. If the inhibitory effect of calcium is proportional to the total amount

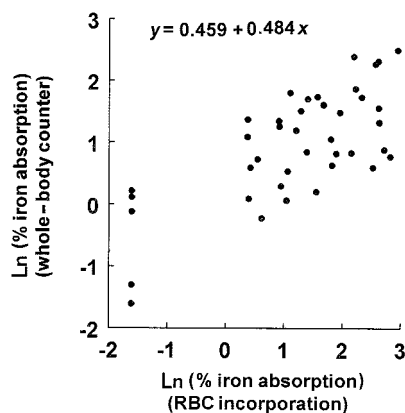


FIGURE 2. Relation between percentage of iron absorption determined by whole-body counting and that determined by incorporation of iron into red blood cells (RBCs) ($n = 12$; $r = 0.68$, $P < 0.0001$ with adjustment for multiple points per person).

contained in the food product and if intrinsic calcium and added calcium are comparable in their effect, a significantly greater inhibition would be expected with the food product with high calcium (392 mg) than with the food product with low calcium (192 mg). However, as reported by Hallberg et al (15), the effect of calcium may not be linear. According to their results, little additional effect of supplemental calcium would be expected beyond the effect due to the calcium intrinsic to the food product. Moreover, in the interpretation of our results, it is necessary to consider that the milk intrinsic to the food product not only contributed a substantial part of the food's dietary calcium but also provided protein. It is possible that any inhibitory effects of the calcium added as calcium phosphate were partially offset by the enhancing effect of the protein from milk, which likely facilitated the absorption of the nonheme iron, as occurs with meat protein (29).

It is important to take into account not only the effect of NaFeEDTA, but also the effect of other components of the novel fortificant mixture, such as ascorbic acid and citric acid as iron-absorption enhancers. Although Siegenberg et al (31) reported only a modest incremental effect on iron absorption when the amount of ascorbic acid was raised from 50 to 150 mg in a diet with similar amounts of phytic acid, in the present study, it was not possible to estimate the independent contribution of each of these factors to the improved iron absorption.

The food product fortified with NaFeEDTA, 100 mg ascorbic acid, and 1 g citric acid resulted in significantly higher iron absorption than did the standard food product containing ferrous sulfate and 50 mg ascorbic acid. These results are consistent with published data, which show that iron from the ferric salt of sodium EDTA is absorbed more efficiently than is that from ferrous sulfate (32). Calculation of absorption ratios showed that iron absorption from the food product fortified with NaFeEDTA, ascorbic acid, and citric acid was 1.7 times that from the food product fortified with ferrous sulfate. The NaFeEDTA:ferrous sulfate iron absorption ratio values in the present study are not different from those reported by Layrisse and Martinez-Torres (33) for milk and a mixed diet of cereals, legumes, and meat. In the present study, it is important to recognize that the study food products had a relatively low phytic acid content and that the enhancing effect of NaFeEDTA on nonheme-iron absorption is greater when there are higher amounts of phytic acid or other inhibitors in the food product (32). As a result, iron absorption ratios between NaFeEDTA and ferrous sulfate would be expected to be lower for this low-phytic acid fortified food product than for a food product with a higher amount of phytic acid.


As is the case for iron, zinc forms insoluble zinc-phytic acid complexes. The food products tested in this study contained ≈ 0.5 mmol (306 mg) phytic acid per food product. The mean zinc absorption values of 6–9% in the present study are not different from those reported by Nävert and Sandström (34), who used the same amounts of phytic acid in their study. The negative effect of phytic acid on zinc absorption may sometimes be predicted by the phytic acid:zinc molar ratios of the diet (35). In the present study, the phytic acid:zinc molar ratios for both study food products were 2.7. This molar ratio is considerably lower than the reported values of 15–20 that have been associated with biochemical and in some cases clinical signs of zinc deficiency in humans (36, 37).

We did not observe an effect of the added ascorbic acid and citric acid as enhancers of zinc absorption. This may be due to the possibility that the amount of zinc absorbed from the food product fortified with zinc sulfate was already at the maximal intestinal absorptive capacity. Sandström (38) has shown that when the amount of zinc in a test meal is increased, the maximum amount of zinc absorbed is ≈ 18 – 20 μmol (1.17–1.30 mg). In our study, 6.6% of 174 μmol (11.4 mg) zinc, ie, 11 μmol (0.75 mg), was absorbed from the food product fortified with zinc sulfate, and 7.4% of 174 μmol (11.4 mg) zinc, ie, 13 μmol (0.84 mg), was absorbed from the food product fortified with zinc methionine, ascorbic acid, and citric acid. Thus, it is conceivable that no further improvement in the amount of zinc absorbed was possible.

In some studies, high amounts of calcium were shown to exacerbate the inhibitory effect of phytic acid on zinc absorption in humans by forming a calcium:zinc:phytic acid complex in the intestine that is even less soluble than are phytic acid and zinc complexes (13, 24). In the present study, zinc absorption from the food product fortified with zinc methionine, ascorbic acid, and citric acid with a low amount of calcium was marginally higher ($P = 0.071$) than that from the same food product with a high amount of calcium.

As with iron absorption, a previous study found that the inclusion of milk having a high protein content in the study food products had a positive effect on zinc absorption (39). It is possible that proteins and peptides formed complexes with zinc that overcame the inhibitory effect of the addition of 200 mg Ca as calcium phosphate to both food products.

With 2 exceptions, all of the volunteers who participated in this study were nonanemic, iron-replete subjects. Determining the zinc status of individual subjects is difficult and was not attempted in our study. However, it is likely that the zinc intake and zinc status of our subjects were satisfactory. Iron absorption would be expected to be high among populations in developing countries with a high prevalence of iron deficiency.

In summary, according to the data from the present study, a fortificant mixture containing NaFeEDTA, zinc methionine, ascorbic acid, and citric acid, but without calcium as calcium phosphate, can improve iron absorption and possibly zinc absorption from food products. However, the differences that were observed may not justify the additional cost of this novel fortificant mixture. 

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