Iron Bioavailability from a Lipid-Based Complementary Food Fortificant Mixed with Millet Porridge Can Be Optimized by Adding Phytase and Ascorbic Acid but Not by Using a Mixture of Ferrous Sulfate and Sodium Iron EDTA1–3

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Abstract
Home fortification with lipid-based nutrient supplements (LNSs) is a promising approach to improve bioavailable iron and energy intake of young children in developing countries. To optimize iron bioavailability from an LNS named complementary food fortificant (CFF), 3 stable isotope studies were conducted in 52 young Beninese children. Test meals consisted of millet porridge mixed with CFF and ascorbic acid (AA). Study 1 compared iron absorption from FeSO4-fortified meals with meals fortified with a mixture of FeSO4 and NaFeEDTA. Study 2 compared iron absorption from FeSO4-fortified meals without or with extra AA. Study 3 compared iron absorption from FeSO4-fortified meals with meals containing phytase added prior to consumption, once without or once with extra AA. Iron absorption was measured as erythrocyte incorporation of stable isotopes. In study 1, iron absorption from FeSO4 (8.4%) was higher than that from the mixture of NaFeEDTA and FeSO4 (5.9%; P < 0.05). In study 2, the extra AA increased absorption (11.6%) compared with the standard AA concentration (7.3%; P < 0.001). In study 3, absorption from meals containing phytase without or with extra AA (15.8 and 19.9%, respectively) increased compared with meals without phytase (8.0%; P < 0.001). The addition of extra AA to meals containing phytase increased absorption compared with the test meals containing phytase without extra AA (P < 0.05). These findings suggest that phytase and AA, and especially a combination of the two, but not a mixture of FeSO4 and NaFeEDTA would be useful strategies to increase iron bioavailability from a CFF mixed with cereal porridge. J. Nutr. 143: 1233–1239, 2013.

Introduction
Iron deficiency (ID)7 with or without anemia is common in sub-Saharan Africa, particularly in children <5 y of age with high iron requirements during growth (1). The etiology of ID in children living in developing countries is multifactorial, but major contributors are low dietary iron bioavailability and intake from complementary foods mainly based on cereals (2). Complementary foods in developing countries are often bulky, cereal-based porridges that are not only low in bioavailable micronutrients such as iron but also low in energy density. Therefore, young children in developing countries often have difficulties meeting their daily energy requirements (3).

The use of fortified complementary foods to prevent ID is recommended in guidelines for complementary feeding (4). Complementary fortified foods can be divided into commercially fortified blended foods (FBFs) and complementary food supplements such as lipid-based nutrient supplements (LNSs) or micronutrient powders (MNPs) (5). Complementary food supplements (also called in-home fortification of complementary foods) could be a promising approach to improve micronutrient status in younger children when parents cannot afford commercial FBFs

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3 Supplemental Figure 1 available from the “Online Supporting Material” link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.
4 To whom correspondence should be addressed. E-mail: ccolin@ethz.ch.
7 Abbreviations used: AA, ascorbic acid; ADI, acceptable daily intake; CFF, complementary food fortificant; CRP, C-reactive protein; DM, dry matter; FBF, fortified blended food; FM, fresh matter; FTU, phytase unit; GAE, gallic acid equivalent; Hb, hemoglobin; ID, iron deficiency; LNS, lipid-based nutrient supplement; MNP, micronutrient powder; PF, plasma ferritin.

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(6). They can provide the necessary amounts of micronutrients needed by each age subgroup (e.g., 6–12 or 12–36 mo) irrespective of how much food the children eat and without major changes in food habits. LNSs have the advantage that they provide energy, essential fatty acids, and often protein. In a 6-mo intervention trial, LNSs, MNP s, and iron tablets significantly increased iron status compared with a control group, but only LNSs improved the growth of young children (7,8). Initially, LNSs, also called ready-to-use foods, were designed as therapeutic foods to treat severe malnutrition (9–11). The use to prevent malnutrition and promote growth came into focus over recent years (12).

For the present study, an LNS type named complementary food fortificant (CFF) was developed. The CFF is in the form of a paste and contains a mixture of micro- and macronutrients that should be added as a fortificant to cereal-based porridges commonly consumed by children in developing countries. The CFF was designed as a preventive food with the capability to treat mild to moderate malnutrition. The recommended portion of CFF (30 g) will thus provide lower amounts of fat than previous therapeutic LNSs and the amounts of iron and other micronutrients are adjusted to prevent deficiencies and treat mild to moderate malnutrition. Regarding iron fortification, 10% iron bioavailability from the cereal-based porridge mixed with the CFF was assumed, so for the CFF containing 6 mg iron, children 12–36 mo old would be able to meet their daily iron requirements of 0.6 mg (1) by consuming one recommended CFF portion per day.

To test and optimize iron absorption from an iron and ascorbic acid (AA)-fortified CFF blended with millet porridge, we carried out 3 iron absorption studies in young Beninese children using a stable isotope technique. In the first study, we compared iron absorption from FeSO₄ to a 1:1 mixture of NaFeEDTA and FeSO₄. In the second study, we investigated the effect of extra AA on iron absorption from FeSO₄. In study 3, we investigated the effect of adding phytase just before consumption on iron absorption, once with extra AA, once without.

**Subjects and Methods**

**Subjects.** Sixty apparently healthy children, 19–36 mo of age, of both sexes were recruited in Natitingou, Atacora Department, Benin. Children and their caregivers were registered during meetings in different districts of Natitingou and invited for the screening of exclusion criteria at the local state hospital. Exclusion criteria included fever (ear temperature >37.5°C), symptomatic malaria (sexual P. falciparum parasitemia in blood smears + fever), body weight <8.3 kg, weight-for-age Z-score <−3, sickle cell trait [hemoglobin (Hb) S], severe hematuria, regular intake of medications, and known gastrointestinal or metabolic disorders. No children were recruited who had received a blood transfusion or experienced substantial blood loss within 6 mo of the beginning of the study. None of the children consumed vitamin or mineral supplements and fortified foods 2 wk before and during the entire study. Ethical approval for the study was given by the ethical review committee at the Ministry of Health in Benin and ETH Zurich (Zurich, Switzerland). The caregivers of the children were informed about the aims and procedures of the study, and oral and written consent was obtained.

**Study design.** The recruited children were randomly allocated to the 3 studies. In all studies, a randomized cross-over design was used and each child served as his own control. Two different test meals (A and B) on 2 consecutive days were fed to each child in studies 1 and 2. Three different test meals (A, B, and C) on 3 consecutive days were fed to each child in study 3. The order of the different test meals was randomized in all studies. Test meals were fed in the morning after a fast of at least 2 h. No intake of food and fluids was allowed during the 3 h following the intake of the labeled test meals. During recruitment (baseline measurements), body weight and height of the children were measured and a first blood sample was drawn for iron status determination [Hb, plasma ferritin (PF), C-reactive protein (CRP)], detection of Hb S, and malaria parasitemia. Stool and urine samples were taken for the detection of soil-transmitted helminthes. Fourteen days after the last test meal (endpoint measurements), body weight and height were measured again and a second blood sample was drawn for iron status determination and iron isotopic analysis. Stool and urine samples were taken again for confirmation of soil-transmitted helminthe analyses. Ear temperature was measured at screening and endpoint and daily before administration of test meals. Iron absorption was determined by using a stable isotope technique in which the incorporation into erythrocytes of isotopic iron labels was measured 14 d after the administration of the last test meal (13).

**Tested enhancers of iron absorption.** The principle of a CFF is to add a micronutrient mixture to the CFF to meet the requirements of young children for multiple micronutrients. In the current test phase of our CFF, such a micronutrient mixture was not yet used. However, AA would almost certainly be part of the micronutrient mixture to cover the child’s requirements and also enhance iron absorption. In all 3 studies, 40 mg AA [L(+)-ascorbic acid puriss; Sigma-Aldrich] diluted in 1 mL distilled water was added to all test meals shortly before consumption. In studies 2 and 3, meals 2B and 3C contained 40 mg extra AA (total AA = 80 mg).

In study 1, test meal 1B was fortified with NaFeEDTA according to the acceptable daily intake (ADI) of 1.9 mg EDTA/kg body weight per day (14). To meet ADI regulations, a 1:1 mixture of FeSO₄ and NaFeEDTA was used and children with a body weight <8.3 kg who would consume EDTA in excess were excluded.

In study 2, DSM phytase Tolerase 20000G provided by DSM Nutritional Products was added to test meals 2A and 3B and 3C at room temperature just before consumption. The quantity of phytase added to the meals was calculated assuming the following: a phytate concentration of 200 mg/portion and a residence time in the stomach of 60 min. Phytase activity is measured as the amount of enzyme that liberates 1 μmol inorganic phosphorus/min and is called a phytase unit (FTU). Because the phytase activity at gastric pH is expected to be 50–60%, 8 FTU was necessary to adequately degrade 1 μmol phytate (~0.7 mg phytate), leading to ~40 FTU/serving to completely degrade phytate in the stomach. To ensure complete degradation, we used a factor of 10 and added 400 FTU phytase, which is in the same range as in a previous efficacy study (15).

**Stable isotope labels.** Isotopically labeled FeSO₄, FeSO₄, and FeSO₄ were prepared from isotopically enriched elemental iron (16Fe-metal: enriched >99.8%; 15Fe-metal: enriched >97.8%; 15Fe-metal: enriched >99.8%; all Chemgas) by dissolution in 0.1 mol/L sulfuric acid. The solutions were flushed with argon to keep the iron in the +II oxidation state. NaFeEDTA was prepared in solution from 15Fe-enriched elemental iron (15Fe-metal: 99.9% enriched; Chemgas). The metal was dissolved in 2 mL HCl and diluted with water. The resulting FeCl₃ solution was mixed immediately before use with an aqueous Na₂EDTA solution (Na₂EDTA·H₂O₂ Sigma Chemical) at a molar ratio of 1:1 (Fe: EDTA). The resulting NaFeEDTA solution was added to the test meal.

All test meals were fortified with 6 mg iron and labeled as follows: in study 1, meal 1A, 3 mg Fe as FeSO₄ plus 3 mg iron as FeSO₄ (normal isotopic composition); and meal 1B, 3 mg Fe as FeSO₄ plus 3 mg Fe as NaFeEDTA. Study 2 was: meal 2A, 3 mg Fe as FeSO₄ plus 3 mg iron as FeSO₄ and meal 2B, 3 mg Fe as NaFeEDTA plus 3 mg iron as NaFeEDTA. In study 3, labels were: meal 3A, 3 mg Fe as NaFeEDTA plus 3 mg iron as FeSO₄ and meal 3B, 3 mg Fe as NaFeEDTA plus 3 mg iron as FeSO₄ and meal 3C, 3 mg Fe as FeSO₄ plus 3 mg iron as FeSO₄.

**Test meals.** The test meal consisted of 80 g millet porridge blended with 30 g CFF. Pearl millet (Pennisetum glaucum) bought from the local market in Natitingou was manually cleaned and washed and then sun-dried. Dried millet was milled using a portable household mill (Novum, Hawos Kornmühlen). To obtain a fine flour, a sieving step was added after milling. The flour was produced in bulk and used for the entire
study. The millet porridge was freshly prepared on each study day by weighing and mixing millet flour and water at a ratio of 1:2.2. This blend was then added to boiling water (blend: boiling water ~1:4:1) and cooked for 1 min. After cooling, the porridge was blended with the CFF. The CFF was based on 24% canola oil (Florin), 23% peanut paste (Central African Seed Services), 23% soybean flour from milled, extruded soybean flakes (Morga), 22% icing sugar (Zuckermühlen Rupperswil), and 8% palm stearin (Florin). Ingredients were blended at ETH Zurich, Switzerland using a food mixer (Kenwood Swiss). The CFF was produced in bulk and used for the entire study.

Weighed amounts of labeled and unlabeled iron as FeSO₄ and NaFeEDTA in dilute acid and AA and phytase were blended with the millet porridge and the CFF shortly before feeding. The children consumed the test meals with the assistance of their caregivers under close supervision of investigators in a hospital facility in Nattitingo. Complete intake of isotopically labeled meals was assured by rinsing the bowl 2 times with 10 mL distilled water after consumption of the test meal and letting the children consume the rinsing.

Test meal analysis. Sample analyses were done in triplicate at ETH Zurich, Switzerland. Iron concentrations of millet porridge and CFF were measured by graphite-furnace atomic absorption spectrophotometry (AA240Z; Varian) after mineralization by microwave digestion (MLS ETHOSplus, MLS). The phytate concentration was measured using a modification of the Makower method (16), in which iron was replaced by cerium in the precipitation step. After the mineralization of the precipitate, inorganic phosphate was determined according to Van Veldhoven and Manns (17) and converted to phytate concentrations. The total polyphenol concentration in the test meal was determined using a modified Folin-Ciocalteau method, as suggested by Singleton et al. (18) and was expressed as gallic acid equivalents (GAEs).

Blood analysis. Blood smears were screened for HB S by using the bilisulte method (19) at the hospital in Nattitingou, Benin. HB was measured in whole blood on the day of collection by using HemoCue hemoglobin 201+ (HemoCue); anemia was defined as HB <11 g/dL (1). PF and CRP were measured with an IMMULITE automatic system (Siemens) at ETH Zurich, Switzerland. ID was defined as PF <12 µg/L, and ID anemia was defined as HB <11 g/dL and PF <12 µg/L (1). Expected high-sensitivity CRP concentrations for healthy individuals were <5 mg/L.

Each isotopically enriched blood sample was analyzed in duplicate for its isotopic composition. Whole blood was mineralized by microwave digestion and iron was separated by anion-exchange chromatography and a subsequent precipitation step with ammonium hydroxide (20). Iron isotope ratios were determined by an MC-ICP-MS instrument (NEPTUNE, Thermo Finnigan) at ETH Zurich, Switzerland.

Parasite diagnosis. Thick and thin blood smears were stained in duplicate by using the Giemsa coloration technique and were examined independently by 2 experienced microscopists (21). Rapid malaria diagnostic tests (ICT Mlo1 Malaria Pf kit; ICT Diagnostics) were used to support the results of the blood smears. For the detection of soil-transmitted helminthes, the KatoKatz method and syringe filtration technique were used to examine stool and urine samples (21). Hematuria was detected using test strips for urinalysis (Hemastix, Siemens).

Calculation of iron absorption. The amounts of ⁵⁴Fe, ⁵⁷Fe, and ⁵⁸Fe labels in the blood were calculated based on the shift in iron isotope ratios and the estimated amount of iron circulating in the body. Circulating iron was calculated based on the blood volume estimated from height and weight and measured Hb concentration (means from recruitment and endpoint measurements) (22). The calculations were based on the principles of isotope dilution, taking into account that iron isotopic labels were not monoisotopic, using the methods described by Walczyk et al. (13) in study 1 and 2 and Turnlund et al. (23) in study 3. Calculation of iron absorption in study 3 is shown in detail in Supplemental Figure 1. For calculation of fractional absorption, 90% incorporation of the absorbed iron into RBCs was assumed (24).

FIGURE 1  Between-study comparison of fractional iron absorption from different test meals within the same child was compared by a linear mixed model with repeated-measure followed by a post hoc Bonferroni test for multiple comparisons. In the linear mixed model, types of test meal and study day were the fixed effects and individual child was the random effect. The dependent variable was iron absorption and study day was treated as the repeated measure assuming an unstructured covariance type. For the between-study comparison, the same linear mixed model with repeated measure was applied, but study as a fixed effect was added and PF as covariate. Between-study comparisons of children (age, anthropometric features, HB, PF, CRP) were done by 1-way ANOVA followed by a Bonferroni test. Differences were considered significant at P < 0.05. A sample size of 16 children/group is sufficient to detect an intra-subject difference of 30% in iron absorption with an α level of 0.05. To compensate for possible dropouts, ~20 children were recruited for each study.

Results

Participant characteristics. Fifty-two children (34 boys and 18 girls) completed the study, 18 in study 1 and 3 and 16 in study 2. Four children were excluded because they did not completely consume the test meals and 3 children because they developed fever, malaria, or a wound infection requiring medication during the feeding days. Furthermore, 1 child was excluded, because it was not fully clear when she received a blood transfusion. Age, anthropometric features, HB, PF, and CRP concentrations of the children at baseline are summarized in Table 1. Stunting (height-for-age Z-score < −2) was observed in 18 children (~35%); 3 (~6%) of them were severely stunted. Eight of the children (~15%) were underweight (weight-for-age Z-score <
–2 > –3) and 3 (–6%) were anemic (weight-for-height Z-score < –2 > –3). Twenty-two children (–42%) were anemic. ID and ID anemia were found in 9 (–17%) and 5 (–10%) children, respectively. Ten children (–19%) had CRP concentrations >0 mg/L at baseline. Eight of the children (–15%) had a positive stool smear for Schistosoma mansoni and 3 children (–6%) had detected hookworms at baseline and/or endpoint measurements. According to the infection intensities defined by WHO (25), 1 child had a moderate-intensity infection of S. mansoni while all the other children had low-intensity-infections of S. mansoni or hookworms. The infected children were treated against soil-transmitted helminthes after the endpoint measurements. Twelve (–23%) and 6 (–12%) children had asymptomatic malaria at baseline and endpoint measurements, respectively. These children were treated against malaria parasitemia after the endpoint measurements. Test meal composition. The native iron concentration in the millet porridge was 4.5 ± 0.2 mg/100 g dry matter (DM) and in the CFF was 1.4 ± 0.0 mg/100 g fresh matter (FM). The phytate and polyphenol concentrations in the millet porridge were 376 ± 1 mg/100 g DM and 8 ± 0 mg GAEs/100 g FM, respectively. The phytate and polyphenol concentrations in the CFF were 568 ± 16 mg/100 g FM and 80 ± 1 mg GAEs/100 g FM, respectively. The meal based on 80 g millet porridge (5.5% DM) blended with 30 g CFF therefore contained 0.7 mg native iron, 6 mg fortificant iron, 191 mg phytate, and 32 mg polyphenols. The phytate:iron molar ratio in the iron-fortified test meals was 2.5:1. The molar ratio of AA:iron in the test meals with extra AA was 3.8:1 compared with 1.9:1 with standard but no extra AA.

Iron absorption measurements. As shown in Table 2, the fractional mean iron absorption in study 1 from the meals fortified with FeSO₄ was ~30% higher than that from the test meals fortified with a mixture of NaFeEDTA and FeSO₄ (P < 0.01). In study 2, the addition of extra AA (40 mg) increased the fractional iron absorption from FeSO₄ (P < 0.001). In study 3, adding phytase almost doubled the fractional iron absorption from FeSO₄-fortified test meals (P < 0.001). The addition of extra AA to the test meals containing phytase increased fractional iron absorption compared with the test meals containing phytase without extra AA (P < 0.05). Adding AA and/or phytase to the meals increased the geometric mean of total absorbed iron per meal to >0.6 mg, which is the needed amount of daily iron in children 12–36 mo of age. When adding phytase and extra AA, the total absorbed iron more than doubled. A between-study comparison (Fig. 1) showed no difference in fractional absorption when phytase was added compared with adding extra AA (P > 0.05). Iron absorption when adding phytase plus extra AA was higher than adding only extra AA without phytase (P < 0.05). Fractional absorption from FeSO₄-fortified test meals containing phytase with or without extra AA was higher than that from test meals fortified with a mixture of FeSO₄ and NaFeEDTA (P < 0.05).

Discussion

In study 2, the addition of extra AA increased the fractional iron absorption by ~60%. The enhancing effect of AA on human nonheme iron absorption has been reported in several previous studies (26,27) and is related to the reducing and chelating properties of AA during digestion of the food (28). The 40 mg AA chosen as the standard concentration to meet AA requirements in this study is between the 30 mg proposed by FAO/WHO for healthy children aged 1–5 y old (29) and the 70–90 mg proposed in a recent publication for moderate, acute, malnourished children of the same age group (30). We chose 40 mg AA primarily because the CFF should be a preventive rather than a therapeutic complementary food and also because 40 mg resulted in an AA:iron molar ratio of ~2:1, which is recommended to enhance iron absorption from foods with normal phytate concentrations (31). Our results, however, indicate that a higher AA:iron molar ratio of 4:1 in the CFF would further

### Table 1

<table>
<thead>
<tr>
<th>Variable</th>
<th>Study 1</th>
<th>Study 2</th>
<th>Study 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participants, n</td>
<td>18</td>
<td>16</td>
<td>18</td>
</tr>
<tr>
<td>Age, mo</td>
<td>28.2 ± 5.3</td>
<td>30.8 ± 4.8</td>
<td>28.3 ± 3.9</td>
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<tr>
<td>Weight, kg</td>
<td>10.8 ± 1.1</td>
<td>11.7 ± 1.3</td>
<td>10.9 ± 1.2</td>
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<tr>
<td>Height, cm</td>
<td>84.2 ± 4.8</td>
<td>85.3 ± 4.6</td>
<td>86.2 ± 4.8</td>
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<td>Height-for-age Z-score</td>
<td>–1.85 ± 0.65&lt;sup&gt;a&lt;/sup&gt;</td>
<td>–1.95 ± 0.89&lt;sup&gt;b&lt;/sup&gt;</td>
<td>–0.31 ± 1.35&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>Weight-for-height Z-score</td>
<td>–0.56 ± 0.66&lt;sup&gt;a&lt;/sup&gt;</td>
<td>–0.09 ± 0.71&lt;sup&gt;a&lt;/sup&gt;</td>
<td>–1.06 ± 0.79&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Weight-for-age Z-score</td>
<td>–1.38 ± 0.66</td>
<td>–1.16 ± 0.62</td>
<td>–1.23 ± 0.89</td>
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<td>Hb, g/L</td>
<td>109 ± 10</td>
<td>110 ± 8</td>
<td>109 ± 8</td>
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<td>Plasma CRP, mg/L</td>
<td>21.8 (14.8, 32.3)</td>
<td>21.9 (15.6, 30.7)</td>
<td>28.6 (21.1, 38.8)</td>
</tr>
</tbody>
</table>

<sup>1</sup> Values are means ± SDs or geometric means (95% CIs) analyzed as log-transformed data. Labeled means in a row without a common letter differ, *P < 0.05. CRP, C-reactive protein; Hb, hemoglobin; PF, plasma ferritin.

### Table 2

<table>
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<tr>
<th>Study</th>
<th>n</th>
<th>Meal</th>
<th>Iron fortification (label)</th>
<th>Meal additives</th>
<th>Fractional iron absorption</th>
<th>Total absorbed iron per meal</th>
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<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>% of dose</td>
<td>mg</td>
</tr>
<tr>
<td>1</td>
<td>18</td>
<td>1A</td>
<td>3 mg &lt;sup&gt;57&lt;/sup&gt;FeSO₄ + 3 mg &lt;sup&gt;56&lt;/sup&gt;FeSO₄</td>
<td>—</td>
<td>8.4 (5.6, 12.6)</td>
<td>0.56 (0.38, 0.84)</td>
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<tr>
<td></td>
<td>1B</td>
<td></td>
<td>3 mg &lt;sup&gt;57&lt;/sup&gt;FeSO₄ + 3 mg Na&lt;sup&gt;57&lt;/sup&gt;FeEDTA</td>
<td>—</td>
<td>5.9 (3.8, 9.0)&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.39 (0.25, 0.60)&lt;sup&gt;*&lt;/sup&gt;</td>
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<td>2</td>
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<td>3 mg &lt;sup&gt;57&lt;/sup&gt;FeSO₄ + 3 mg &lt;sup&gt;56&lt;/sup&gt;FeSO₄</td>
<td>—</td>
<td>7.3 (4.8, 11.1)</td>
<td>0.49 (0.32, 0.74)</td>
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<tr>
<td></td>
<td>2B</td>
<td></td>
<td>3 mg &lt;sup&gt;57&lt;/sup&gt;FeSO₄ + 3 mg &lt;sup&gt;56&lt;/sup&gt;FeSO₄</td>
<td>AA</td>
<td>11.6 (7.8, 17.2)&lt;sup&gt;**&lt;/sup&gt;</td>
<td>0.77 (0.52, 1.14)&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>18</td>
<td>3A</td>
<td>3 mg &lt;sup&gt;57&lt;/sup&gt;FeSO₄ + 3 mg &lt;sup&gt;56&lt;/sup&gt;FeSO₄</td>
<td>—</td>
<td>8.0 (5.5, 11.5)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.53 (0.37, 0.77)&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>3B</td>
<td></td>
<td>3 mg &lt;sup&gt;57&lt;/sup&gt;FeSO₄ + 3 mg &lt;sup&gt;56&lt;/sup&gt;FeSO₄</td>
<td>Phytase</td>
<td>15.8 (11.1, 22.6)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.05 (0.74, 1.50)&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>3C</td>
<td></td>
<td>3 mg &lt;sup&gt;57&lt;/sup&gt;FeSO₄ + 3 mg &lt;sup&gt;56&lt;/sup&gt;FeSO₄</td>
<td>Phytase, AA</td>
<td>19.9 (14.4, 27.6)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.33 (0.96, 1.83)&lt;sup&gt;b&lt;/sup&gt;</td>
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</tbody>
</table>

<sup>1</sup> Values are geometric means (95% CIs) analyzed as log-transformed data. Labeled means in a row without a common letter differ, *P < 0.01; **P < 0.001. Labeled means within a study without a common letter differ, P < 0.05. All meals consisted of 80 g millet porridge blended with 30 g CFF and 40 mg AA. Meals 2B and 3C contained 40 mg extra AA. AA, ascorbic acid; CFF, complementary food fortificant.
enhance iron absorption. AA is easily oxidized to inactive compounds by exposure to air and to some degree by heat treatment (28); therefore, further studies are needed to ensure adequate AA in CFF after storage.

It was rather surprising that fractional iron absorption from test meals fortified with the mixture of NaFeEDTA and FeSO₄ was significantly lower than from test meals fortified with only FeSO₄. A potential explanation for this effect is that the standard concentration of AA added in our study increased the absorption of FeSO₄ but not that from NaFeEDTA. Previous studies reported no benefit to nonheme iron absorption when AA was added to NaFeEDTA-fortified test meals at AA:iron molar ratios of 5:1 (32) and 3:1 (33); thus, it is possible that the relatively high amount of AA added in our study enhanced absorption from FeSO₄ but not from NaFeEDTA, resulting in a lower overall absorption from NaFeEDTA.

EDTA is an iron chelator and binds iron strongly at the pH of gastric juice. After pH rises in the duodenum, EDTA exchanges ferric iron for other metals, thus releasing iron for absorption. EDTA is thus reported to prevent iron from forming insoluble, nonabsorbable complexes in the stomach with food components such as phytate and to release iron from the iron-EDTA complex in the duodenum for absorption (31). NaFeEDTA or the EDTA moiety alone (added as Na₂EDTA) have repeatedly been reported to enhance iron absorption from phytate-containing meals in adults (34,35) and also children (36,37). However, in less inhibitory meals, the use of NaFeEDTA or Na₂EDTA did not enhance iron absorption (38,39). In sugar cane syrup, which contained no phytate or other known iron absorption inhibitors, iron absorption from NaFeEDTA was 67% lower than from FeSO₄ (40) and earlier work indicated that increasing the molar ratio of EDTA:iron with Na₂EDTA progressively decreased iron absorption (41). Cook and Monsen (41) investigated the effect of EDTA on iron absorption from a composite American meal and concluded that the inhibitory effect of EDTA on iron absorption can only be excluded at EDTA:iron molar ratios <0.5 (41). Additionally, results in infants have not always been conclusive (42).

In the present study, the total amount of native and fortificant iron in the test meals was 6.7 mg (3 mg iron as NaFeEDTA), resulting in an EDTA:iron molar ratio of ~0.5:1. This molar ratio is in the range that has been reported to increase iron absorption in adults from phytate-containing cereal meals (35,39). Our test meal was only partly based on cereals and contained high amounts of fat, low to moderate amounts of phytate, and a high amount of AA. In such a meal, the potential benefit of NaFeEDTA may be differentially affected by the EDTA:iron molar ratios. Similar to our study, Chang et al. (43) investigated a NaFeEDTA-fortified complementary food partly based on cereals. Compared with FeSO₄-fortified meals, they reported no change in absorption in infants at a molar ratio of EDTA:iron of 0.7:1 but a significant increase in iron absorption at a molar ratio of 0.4:1.

Another factor that could have decreased iron absorption from NaFeEDTA in the present study is Helicobacter pylori. The moderately malnourished children who participated in the study live in an area where *H. pylori* infections are widely prevalent (44,45). *H. pylori* infections are associated with an increased postprandial duodenal acid load and a prolongation of low pH in the duodenal bulb (46,47). This could possibly impair iron release from EDTA, because there is no rise in pH that weakens the bond between EDTA and iron.

Based on our findings, we cannot recommend NaFeEDTA as an iron fortificant for an LNS or CFF. It is more expensive than ferrous sulfate (48) and, in the presence of recommended AA concentrations (29,31), offers no improvement in absorption. Another disadvantage is the limited quantity of NaFeEDTA that can be added because of the ADI (14). Further studies are needed to investigate the reasons for the lower iron absorption from NaFeEDTA in our study.

The addition of microbial phytase just before consumption in study 3 almost doubled iron absorption compared with the control meal. This increase is similar to 2 previous human studies using a phytase active at gastric pH in highly inhibitory meals based on whole maize (32) or wheat bran (49). The increase in our study was slightly higher than that in the previous study using the same phytase in whole-maize meals (32). This was probably due to the lower amount of phytate in our test meal and the addition of more FTUs. Adding extra AA together with phytase had an additive effect and further increased iron absorption compared with standard AA concentrations. This is comparable with earlier findings where iron absorption from dephytinized, AA-fortified, wheat-soy porridges was higher than from the dephytinized porridges without AA (50).

LNSs have been widely used in the field of child malnutrition and are well accepted by children and caregivers (12,51,52). Current LNSs are typically made from vegetable oil, peanut paste, milk powder, sugar, and a micronutrient premix. The use of milk powder and canola oil contributes to the reasonably high costs of LNs at the present time. High costs might limit the long-term use of LNSs among the most vulnerable population groups. Replacing milk powder and canola oil with domestic products such as defatted soybean flour or soybean oil would make LNSs cheaper and they could be locally manufactured (12). On the down side, soybean flour will introduce more phytate into the LNSs. In our study, we used canola oil, but it could be easily replaced by soybean oil, which is not expected to differ from canola oil in terms of affecting iron absorption. Soybean oil is readily available in many African countries and the essential fatty acid composition is only slightly inferior to canola oil (12).

To our knowledge, the present study is the first study in young children investigating iron absorption from an LNS type using phytase. Our study provides important insights for the optimization of iron absorption from LNSs designed to prevent ID in young children. Our findings suggest that adding phytase to a CFF based on locally available products and blended with cereal porridge would be a very promising food-based approach to simultaneously improve bioavailable iron and energy intake of young children. However, several questions have to be solved. In our study, the phytase was added just before consumption. For a commercial CFF, the phytase optimally would be mixed and packaged with the other ingredients and stability for storage must be confirmed. In a similar way to AA, phytase is heat sensitive; thus, mothers would need to be instructed when to add the phytase-containing CFF to the porridge. As an important step toward regulatory approval, the Joint WHO/FAO expert committee on Food Additives allocated an ADI “not specified” for use of *A. niger* phytase in applications such as LNSs, FBFs, and MNPs (53). An alternative to phytase would be extra AA or, even better, a mixture of phytase and extra AA. Our study was a single-meal study and may not accurately predict long-term absorption. However, we think that a CFF optimized for iron absorption is a promising approach to increase bioavailable iron and energy intake in children living in a malaria-endemic area.

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