Iron status and food matrix strongly affect the relative bioavailability of ferric pyrophosphate in humans

Diego Moretti, Michael B Zimmermann, Rita Wegmüller, Thomas Walczyk, Christophe Zeder, and Richard F Hurrell

ABSTRACT

Background: Although ferric pyrophosphate is a promising compound for iron fortification of foods, few data are available on the effect of food matrices, processing, and ascorbic acid on its bioavailability.

Objective: We compared the relative bioavailability (RBV) of ferrous sulfate in an experimental form of micronized dispersible ferric pyrophosphate (MDFP) in a wheat-milk infant cereal given with and without ascorbic acid with the RBV of MDFP from a processed and unprocessed rice meal.

Design: A crossover design was used to measure iron absorption in young women (n = 26) from test meals fortified with isotopically labeled [57Fe]-MDFP and [58Fe]-ferrous sulfate, based on erythrocyte incorporation of stable isotope labels 14 d later.

Results: Geometric mean iron absorption from the wheat-based meal fortified with MDFP was 2.0% and that from the meal fortified with ferrous sulfate was 3.2% (RBV = 62). The addition of ascorbic acid at a molar ratio of 4:1 to iron increased iron absorption from MDFP to 5.8% and that from ferrous sulfate to 14.8% (RBV = 39). In the rice meals, mean iron absorption from MDFP added to the rice at the time of feeding was 1.7%, and that from ferrous sulfate was 11.6% (RBV = 15). The mean iron absorption from MDFP extruded into artificial rice grains was 3.0% and that from ferrous sulfate to 14.8% (RBV = 39). In the rice meals, mean iron absorption from MDFP added to the rice at the time of feeding was 1.7%, and that from ferrous sulfate was 11.6% (RBV = 15). The mean iron absorption from MDFP extruded into artificial rice grains was 3.0% and that from ferrous sulfate to 14.8% (RBV = 39).

Conclusion: RBV of the experimental MDFP varied markedly with food matrix and iron status. Assigning a single RBV value to poorly soluble compounds may be of limited value in evaluating their suitability for food fortification.

KEY WORDS: Iron absorption, ferric pyrophosphate, food processing, ascorbic acid, relative bioavailability, elemental iron, iron stores, food matrix, iron fortification, rice

INTRODUCTION

Iron fortification of foods is challenging because poorly water-soluble iron compounds cause fewer sensory problems when added to foods than does soluble iron (1). A potential strategy for overcoming this problem is the reduction of the particle size of poorly water-soluble iron compounds to increase their dissolution rate and thereby improve their bioavailability. Reducing the particle size of elemental iron powders increases the relative bioavailability (RBV) of ferrous sulfate in humans (2, 3) and animals (4). A micronized ferric pyrophosphate with a mean particle size (MPS) of 0.5 μm, coated in monoglycerides and diglycerides to minimize aggregation (SunActive Fe; Taiyo Kagaku Ltd, Yokkaichi, Japan), was developed (5) and reported to have an RBV in humans of 82% and 92% from a wheat-milk infant cereal and a yogurt drink, respectively (6).

Micronized dispersible ferric pyrophosphate (MDFP) was developed for addition to liquid products, but its high bioavailability makes it potentially useful in other food vehicles that readily undergo adverse sensory changes when fortified with soluble iron, such as rice, infant cereals, and salt. MDFP has excellent sensory qualities when extruded into artificial rice grains (7). A ground form of micronized ferric pyrophosphate, with an MPS of ≈2.5 μm, was shown in North Africa to be efficacious in fortified salt (8).

Although MDFP is a promising iron fortificant, the potential influences of food matrix, food processing, and absorption enhancers on its bioavailability are uncertain. Hallberg et al (9) reported that the food matrix influenced the bioavailability of poorly soluble carbonyl iron, presumably through effects on gastric pH and gastric emptying. Food processing may also influence the RBV of poorly soluble iron compounds, although the effects have not been consistent. In a human study, the RBV of ferric pyrophosphate fell from 75% to 21% when it was processed into a vacuum-dried chocolate drink (10), whereas, in a rat study, the RBV increased when ferric pyrophosphate was added to canned liquid infant formula (11). One concern of feeding MDFP to humans is that its small MPS (0.3 μm) could result in bypassing of the normal absorptive control mechanisms for dietary iron. Therefore, the aims of our study were 1) to ascertain the influence of the food matrix on the RBV of MDFP by measuring iron absorption of MDFP and ferrous sulfate from wheat-based and rice-based meals; 2) to investigate whether MDFP enters the common iron pool by measuring iron absorption from the same
meals with added ascorbic acid; and 3) to measure the effect of rice extrusion processing on the bioavailability of MDFP.

SUBJECTS AND METHODS

Subjects

Twenty-six apparently healthy young women (aged 20–40 y; body weight <60 kg) were recruited from the student and staff populations at the Swiss Federal Institute of Technology and the University of Zurich. Exclusion criteria were pregnancy or lactation and known gastrointestinal or metabolic disorders. No medication (except oral contraceptives) or vitamin and mineral supplements were allowed during the study. Subjects who regularly consumed vitamin and mineral supplements were asked to discontinue supplementation 2 wk before the start of the study. None of the subjects had donated blood <4 mo before the start of the study.

Written informed consent was obtained from all subjects. The study protocol was approved by the ethics committee at the Swiss Federal Institute of Technology (Zurich, Switzerland).

Preparation of stable isotope labels

The [57Fe]-MDFP used for the absorption study was an experimental form of MDFP prepared in the laboratory according to the method of Nambu et al (5). The experimental MDFP differed in 2 ways from the commercial form of Sunactive Fe, which is manufactured by the same procedure but on an industrial scale. First, the particle size was >2-fold the specified particle size of the commercial compound. The MPS of the [57Fe]-MDFP, expressed as mean Sauter diameter, was 0.77 μm, whereas the manufacturer had specified an MPS of 0.3 μm (5). Second, the iron content of the [57Fe]-MDFP was 14.6 mg Fe/g, whereas that specified for the commercial product was 12.0 mg Fe/g. Isotopically labeled [58Fe]-FeSO4 was prepared from isotopically enriched elemental iron by dissolution in diluted sulfuric acid. The solution was stored in polytetrafluoroethylene containers and flushed with argon to keep the iron in the +II oxidation state.

[57Fe]-labeled MDFP was prepared from isotopically enriched elemental iron (Chemgas, Boulogne, France) by first dissolving the elemental iron in concentrated hydrochloric acid and then oxidizing ferrous to ferric iron by using H2O2. For purification, [57FeCl3]− was extracted into diethyl ether, and that process was followed by reextraction into water. The aqueous [57Fe]-FeCl3 solution was evaporated under vacuum at 80 °C by using rotary evaporation (Rotavapor; Buechi, Flawil, Switzerland). The dark red paste obtained was crystallized to bright yellow [57Fe]-FeCl3 · 6H2O crystals by scratching the glass surface. From this base compound, micronized dispersible [57Fe]-ferric pyrophosphate was produced in collaboration with Taiyo Kagaku by mixing [57Fe]-FeCl3 · 6H2O, emulsifiers (enzymatically hydrolyzed lecithin and polyglycerol fatty acid ester), and sodium pyrophosphate (5). Particle size distribution of [57Fe]-MDFP was measured with a laser-diffraction particle sizer (Mastersizer 2000; Malvern Instruments Ltd, Malvern, United Kingdom).

Study design

Two iron-absorption studies were conducted. In study 1, 10 subjects were fed a wheat-milk infant cereal fortified with MDFP or ferrous sulfate, with or without added ascorbic acid. In study 2, 16 subjects were fed a rice meal with MDFP added either at the time of feeding or extruded into artificial rice grains (7), and ferrous sulfate was added at the time of feeding. In both studies 1 and 2, each subject consumed 4 test meals in a crossover design. In study 1, the wheat-milk infant cereal fortified with [58Fe]-FeSO4 and the same test meal fortified with [57Fe]-MDFP were fed on 2 consecutive days (days 1 and 2). The isotopically labeled iron compounds were added directly to the prepared test meal at the time of feeding. After 14 d (days 16 and 17), the same test meals were served with the addition of 63 mg ascorbic acid. In study 2, the rice and vegetable meal fortified with [58Fe]-FeSO4 or with [58Fe]-MDFP, both added directly to the prepared test meal at the time of serving, was given on days 1 and 2. On days 30 and 31, rice and vegetable meals with [58Fe]-FeSO4 were again compared with meals with [57Fe]-MDFP, but the labeled MDFP was first extruded into artificially fortified rice grains (7). All test meals were served between 0700 and 0900 after an overnight fast, under standardized conditions, and with close supervision. No food or drink was allowed for 3 h after consumption of the meals. Fasting venous blood was drawn into EDTA-treated tubes at baseline (day 0), on day 16, and on day 32 for study 1 and on days 14, 29, and 45 for study 2. Calculation of iron absorption was based on the shift in the isotopic ratio of 58Fe to 57Fe after a 14-d incorporation period (12).

Production of extruded rice fortified with [57Fe]-MDFP

Long-grain rice flour (350 g) was mixed in a Hobart mixer (Hobart Corporation, Troy, OH), and 194.1 mg [57Fe] as [57Fe]-MDFP was added. To reach a water content of 25%, distilled water was added to the mixture and mixed for 30 min to ensure water absorption of the flour particles and to obtain a free-flowing powder. To obtain maximum homogeneity, the liquid [57Fe]-MDFP formulation was slowly added to the rice flour during mixing with the use of a manual spray vaporizer. Extrusion was performed with the use of a Brabender single-screw extruder (Model 2003; CW Brabender Instruments Inc, South Hackensack, NJ) with a 20:1 length-to-diameter ratio by using a screw with a 3:1 compression ratio, as described previously (7). Extruded rice grains underwent an extrusion processing step at 90–95 °C for ≈30 s.

Homogeneity of iron distribution in the rice flour

The homogeneity of the rice flour-MDFP mixture after mixing was tested before extrusion. Rice flour (350 g) and nonlabeled MDFP (194.1 mg) were mixed in a food mixer for 30 min, and distilled water was added to reach a moisture content of 25%. Aliquots (n = 11) of ≈0.5 g were taken from the mixed rice flour, and the iron content was measured (n = 3). The fortified rice flour was mineralized by using a mixture of HNO3 and H2O2 and microwave digestion. The iron content of the solution was analyzed with atomic absorption spectrometry by using a commercial iron standard (Titrisol; Merck, Darmstadt, Germany) and a standard addition technique to minimize matrix effects.

Concentration of iron isotopic labels in solution and in the labeled rice grains

Isotope dilution–mass spectrometry was used to measure the concentration of the isotopic labels in solution and in the rice. The iron standard used was prepared gravimetrically from an isotopic
reference material (IRM-014; EU Institute of Reference Materials, Geel, Belgium). Isotopic analysis was done with negative thermal ionization–mass spectrometry by using a magnetic sector field mass spectrometer (MAT 262; Finnigan MAT, Bremen, Germany) equipped with a multicollector system for simultaneous ion beam detection (13). Iron absorption was calculated on the basis of the shift in the iron isotope ratios, the determined isotope ratios of the pure isotopic labels, and the iron isotopic composition of the iron isotopic standard used.

Measurements of iron status and isotopic composition in blood

Hemoglobin was measured by using the cyanomethemoglobin method, and serum ferritin (SF) was measured by using an immunoassay (Ramco Laboratories, Houston, TX) with quality-control materials for hemoglobin (Digitana, Horgen, Switzerland) and SF (Ramco). Anemia was defined as a hemoglobin concentration <120 g/L, and iron deficiency was defined as an SF concentration <12 μg/L. Each isotopically enriched blood sample was analyzed in duplicate for its iron isotopic composition under chemical blank monitoring. Whole-blood samples were mineralized by using a mixture of HNO₃ and H₂O₂ and microwave digestion, which was followed by separation of the sample iron from the matrix by anion-exchange chromatography and a solvent-solvent extraction step into diethyl ether (14). All isotopic analyses were performed by negative thermal ionization–mass spectrometry.

Calculation of iron absorption

Cirulating iron was calculated on the basis of the blood volume, which was estimated from height and weight according to Brown et al (15). For calculation of fractional absorption, 80% incorporation of the absorbed iron into red blood cells was assumed (14). Corrections for enriched baseline values were made when iron absorption from the third and fourth test meal was calculated.

Test meals

The test meal for study 1 was a roller-dried, wheat-based infant cereal (Nestlé PTC, Orbe, Switzerland) prepared with reconstituted milk (50 g cereal plus 8 g milk powder and 75 mL water). Each test meal provided 5 mg added iron, either as 4 mg [⁵⁷Fe]-FeSO₄ plus 1 mg FeSO₄ of natural iron isotopic composition (test meal 1) or as 5 mg [⁵⁷Fe]-MDFP (test meal 2). To the test meals 3 and 4, ascorbic acid (Haenseler AG, Herisau, Switzerland) was added in a molar ratio of ascorbic acid to iron of 4:1 (63 mg ascorbic acid). In study 2, test meals were composed of 41 g natural cooked rice and 9 g extruded rice. Each test meal provided 5 mg added iron as 4 mg [⁵⁷Fe]-FeSO₄ plus 1 mg FeSO₄ of natural isotopic composition (test meals 1 and 3), as 5 mg [⁵⁷Fe]-MDFP added in solution immediately before consumption (test meal 2), or as extruded rice grains fortified with [⁵⁷Fe]-MDFP (test meal 4). A mixed-vegetable sauce (8 g; 44% Chinese cabbage, 21% carrots, 21% zucchini, 12% onions, 2% oil, and salt) was added to the rice meal. The sauce was prepared in bulk and boiled for 2 h in excess water, so as to have a negligible amount of ascorbic acid. After boiling, the vegetables were blended in a kitchen blender and stored in portions at −18 °C. Rice was steamed in food-grade, heat-resistant plastic containers by using nanopure water at a water-to-rice ratio of 2:1 (wt:wt). The extruded grains were boiled at 100 °C for 15 min.

Individual rice portions were prepared the evening before and stored at 4 °C. Just before meal administration, the sauce was added and the test meals were heated in a microwave oven. Nanopure water (200 g) was served as a drink with the test meals. To minimize aggregation, [⁵⁷Fe]-MDFP was treated in an ultrasound bath for 10 min before rice extrusion and before direct administration.

Statistical analysis

Statistical analyses were done by using SPSS software (version 13.0; SPSS Inc, Chicago, IL) and SYSTAT software (version 10; Systat Software Inc, Richmond, CA). Non-normally distributed data were log transformed for statistical analysis. Iron absorption was presented as geometric means and compared by using paired or unpaired t tests. P values <0.05 were considered significant. Stepwise linear regression was used with iron absorption from ferrous sulfate, iron absorption from MDFP, and RBV as dependent variables and with SF, food matrix, MDFP processing, and presence of ascorbic acid as independent variables. Data were standardized before analysis to avoid collinearity according to the following formula:

\[ Z_x = \frac{x - \text{mean}(x)}{\text{SD}(x)} \]

where \( x \) is the log-transformed nonstandardized variable and \( Z_x \) is the standardized variable. A parameter was included in the model at the significance level of 0.05.

RESULTS

Iron status of the test subjects

In study 1, 2 of the 10 test subjects had iron deficiency anemia (hemoglobin: <120 g/L; SF: <12 μg/L), and 5 had iron deficiency (SF: <12 μg/L). Of the 16 subjects enrolled in study 2, 3 had iron deficiency anemia and 11 had iron deficiency.

Composition of the test meals

The mean iron concentration of iron-fortified rice flour for extrusion was 0.49 mg/g (range: 0.48–0.52 mg/g), whereas the expected iron concentration, considering water content, was 0.484 mg Fe/g; intermeasurement and intrameasurement CVs were 4.0% (n = 11) and 3.6% (n = 3), respectively. The wheat-milk infant cereal provided 0.6 ± 0.05 mg native iron, 158 ± 15 mg calcium (148 mg calcium/100 g cereal and 1055 mg calcium/100 g milk powder), and 84 ± 8 mg phytic acid (168 mg phytic acid/100 g cereal). The ascorbic acid concentration of the cereal and milk powder was not measured because it was assumed to be negligible. The rice meal with vegetable sauce had mean contents of 0.4 ± 0.05 mg native iron, 52 ± 5 mg phytate, and 1.5 ± 0.1 mg ascorbic acid. The phytate:iron of the rice meal was ≈0.8:1, whereas the phytate:iron for the wheat-milk infant cereal was ≈1.3:1.

Iron absorption from wheat-milk infant cereal

Mean [⁵⁷Fe]-MDFP absorption from wheat-milk infant cereal was 5.8% (range: 3.2–10.5%) and 2.0% (range: 1.3–3.0%) with and without ascorbic acid, respectively. Mean [⁵⁷Fe]-FeSO₄ absorption increased from 3.2% (range: 1.4–7.2%) to 14.8% (range: 7.1–30.9%) when the cereal was given with ascorbic acid.
The RBV of MDFP to FeSO₄ when the cereal was given with and without ascorbic acid was 39% and 62%, respectively (Fortified with MDFP at the time of feeding). The RBV of MDFP to FeSO₄ when the cereal was given with and without ascorbic acid was 1.7% (range: 1.0–2.9%), whereas that from the same meal with added [57Fe]-FeSO₄ was 3% (range: 1.3–6.6%), which is significantly higher than that of unprocessed [57Fe]-MDFP (11.6% (range: 5.5–24.7%) (Table 2). When the [57Fe]-MDFP compound was extruded into iron-fortified rice, iron absorption was 3% (range: 1.3–6.6%), which is significantly higher than that of unprocessed [57Fe]-MDFP (P < 0.05). RBV of the MDFP compared with FeSO₄ was 15 for the unprocessed-rice meal and 24 for the processed-rice meal (P < 0.05). Iron absorption in the FeSO₄-fortified meals (meals 1 and 3) that were given twice to calculate RBV did not differ significantly.

**Factors influencing RBV and absorption from FeSO₄ and MDFP**

The presence of ascorbic acid (P < 0.001), the SF concentration (P < 0.01), and meal type (P < 0.01) were significant predictors of iron absorption from FeSO₄. In contrast, the presence of ascorbic acid (P < 0.001) was the only significant predictor of iron absorption from MDFP, and processing (P = 0.023), type of meal (P = 0.133), and SF concentration (P = 0.225) were not significant predictors. In the regression with RBV of MDFP as the dependent variable, meal type (P < 0.001), SF concentration (P < 0.01), and processing of the MDFP compound (P < 0.01) were all significant predictors, whereas the presence of ascorbic acid (P = 0.22), hemoglobin concentration (P = 0.77), and subject (P = 0.52) were not significant predictors. The regression formula was as follows:

\[ Z(_{SF}) \times 0.390 + Z(_{food\ matrix}) \times (-0.666) + Z(_{processing}) \times 0.556 + 0.343 \]

### Table 1

<table>
<thead>
<tr>
<th>Test meal</th>
<th>Hemoglobin</th>
<th>Serum ferritin</th>
<th>Iron absorption (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(g/L)</td>
<td>(μg/L)</td>
<td>MDFP²,³,⁴</td>
</tr>
<tr>
<td>Without ascorbic acid</td>
<td>128 ± 9²</td>
<td>13.1 (3.3, 35.6)³</td>
<td>2.0 (1.3, 3.0)</td>
</tr>
<tr>
<td>With ascorbic acid</td>
<td>—</td>
<td>—</td>
<td>5.8 (3.2, 10.5)⁷</td>
</tr>
</tbody>
</table>

¹ RBV, relative bioavailability.
² Compared by using paired t tests.
³ Significant interaction between ascorbic acid and iron compound, P < 0.05.
⁴ Geometric mean ± 1 SD.
⁵ Significantly different between compounds, P < 0.0001.
⁶ Significantly different between compounds: ⁶P < 0.05, ⁶P < 0.0001.
⁷ Significant interaction between ascorbic acid and iron compound, P < 0.05.

### Table 2

<table>
<thead>
<tr>
<th>Test meals</th>
<th>Hemoglobin</th>
<th>Serum ferritin</th>
<th>Iron absorption</th>
<th>RBV²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fortified with MDFP at the time of feeding²</td>
<td>—</td>
<td>—</td>
<td>1.7 (1.0, 2.9)</td>
<td>15 (8, 30)</td>
</tr>
<tr>
<td>Fortified with FeSO₄ at the time of feeding²</td>
<td>126 ± 9³</td>
<td>7.7 (2.7, 20.4)⁴</td>
<td>11.6 (5.5, 24.7)³</td>
<td>24 (13, 44)⁶</td>
</tr>
<tr>
<td>Fortified with MDFP extruded into rice grains²</td>
<td>—</td>
<td>—</td>
<td>3.0 (1.3, 6.6)⁵</td>
<td>—</td>
</tr>
<tr>
<td>Fortified with FeSO₄ at the time of feeding²</td>
<td>—</td>
<td>—</td>
<td>12.6 (4.9, 32.7)³</td>
<td>—</td>
</tr>
</tbody>
</table>

¹ RBV, relative bioavailability.
² Compared by using paired t tests.
³ Geometric mean ± 1 SD.
⁴ Significantly different between compounds, P < 0.0001.
⁵ Significantly different with processing of MDFP, P < 0.05.
The inverse relation between iron stores (represented by SF) and iron absorption from both ferrous sulfate and MDFP is shown in Figure 1. In study 1, a negative correlation was observed between SF and iron absorption from FeSO₄ ($r = -0.83, P = 0.003$ with ascorbic acid; $r = -0.79, P = 0.006$ without ascorbic acid). Absorption of iron from MDFP was also negatively correlated with SF, both with and without ascorbic acid ($r = -0.77, P = 0.010; r = -0.64, P = 0.046$, respectively). In study 2, iron absorption from FeSO₄ and SF were negatively correlated ($r = -0.71, P = 0.002$), whereas for MDFP the inverse relation was significant only for the processed form ($r = -0.49, P = 0.053$). The greater absorption of FeSO₄ at low SF concentrations results in a positive correlation between SF and RBV ($r = -0.56, P < 0.001$) (Figure 2).

**DISCUSSION**

One of the important findings from this study is that the RBV of MDFP (particle size: 0.77 μm) varied according to the food vehicle: it was 62% in a wheat-milk infant cereal and only 15–25% in a rice meal. This finding compares with previously reported RBV values for an MDFP (particle size: 0.3 μm) of 95% from a yogurt drink and 83% in a wheat-milk cereal (6). In the current study, although the RBV of MDFP in the 2 rice meals was ≈50% of that in the wheat-based meal without ascorbic acid, the mean absorption of the MDFP from the 2 meals was similar. Thus, the effect of the food matrix on RBV was primarily due to the much higher iron absorption from FeSO₄ in the rice meal (11.6% and 12.6%) than in the wheat-based meal (3.2%). Although these results were obtained with ferric pyrophosphate, 2 previous studies also suggested that food matrix influences iron absorption from FeSO₄ more than it influences that from poorly water-soluble iron compounds.

In a study by Hallberg et al (9), absolute absorption from carbonyl iron in the presence of meat increased from 1% to 1.7%, whereas common pool iron absorption increased from 5.6% to 12.7%, which resulted in a reduction in RBV of the carbonyl iron from 20% to 11%. In a similar study with a complex ferric orthophosphate, RBV was 64% in an infant cereal; 37% in fortified bread rolls served with margarine, corn flakes, sour milk,

**FIGURE 1.** Iron absorption plotted against serum ferritin concentration from a wheat-based infant cereal with ($n = 10$) and without ($n = 10$) the addition of ascorbic acid (4:1 ascorbic acid-to-iron molar ratio) fortified with micronized dispersible ferric pyrophosphate (MDFP; ◊) or ferrous sulfate (×). Linear regression on MDFP is represented by solid lines; linear regression on ferrous sulfate is represented by broken lines. Statistical analysis on log$_{10}$-transformed data. The iron compound × ascorbic acid and iron compound × serum ferritin interactions were significant ($P < 0.001$ and $< 0.05$, respectively) with univariate general linear model. Slopes were significantly different, $P < 0.05$ (paired $t$ test).

**FIGURE 2.** Relation between serum ferritin and the average relative bioavailability (RBV) obtained in the study subjects ($n = 26$). $R = 0.64 (P < 0.001$, Pearson correlation coefficient).
cheese, and coffee; and 30% in bread rolls served with a meat broth (16). However, in both studies, the addition of ascorbic acid at different dose amounts did not affect RBV (9, 16).

In previous studies that used radioisotope labels, we reported the RBV of nonmicronized ferric pyrophosphate to be 15% from a purely wheat infant cereal (17), 39% from a wheat-milk infant cereal (18), and 75% from a chocolate drink (10), all of which were added to the test meal just before consumption. Therefore, at least for poorly water-soluble iron compounds, the use of a single RBV value to set a fortification level and predict potential efficacy in all food vehicles may be of limited value.

The reason for the higher iron absorption from FeSO₄ in the rice meal than from that in the wheat-based meal in the current study may be due both to differences in meal composition and to the iron status of the test subjects. The calcium in the milk given with the wheat-based cereal, as well as the higher phytic acid:iron ratio in the wheat meal (1.3:1 compared with 0.8:1 in the rice meal) likely reduced the iron absorption from FeSO₄ (19, 20). It is possible that these compounds are less inhibitory for poorly water-soluble iron compounds, such as MDFP, because the iron is dissolved in the common pool at a slower rate. In addition, meal composition may affect gastric motility, stomach emptying, and gut pH. These data suggest that, depending on the iron compound, the digestion and release of nonheme iron into the common pool or its subsequent absorption can be strongly influenced by the effects of the food matrix (9).

A significant inverse relation was observed between iron status (as defined by SF concentration) and iron absorption from both FeSO₄ and MDFP (Figure 1). Increased iron absorption with decreasing iron stores is a central mechanism of iron homeostasis in humans (21, 22). Our data suggest that this adaptive up-regulation of iron absorption is more effective for FeSO₄. The greater absorption of iron from FeSO₄ than from MDFP at low SF concentrations produced RBV values for MDFP that varied inversely with SF. These data suggest that the RBV of a poorly water-soluble iron compound may vary according to the iron status of the person (Figure 2).

We previously reported that the RBV of MDFP in the same wheat-milk infant cereal used in the current study was 82% of that of FeSO₄. The lower RBV in this study (62%) was probably due to the higher MPS of the labeled MDFP batch used in this study (0.77 μm) compared with that used in the previous study, which more closely matched the commercial specification (0.3 μm). Our values for iron absorption of FeSO₄ from a rice-based meal (=12%) are similar to the range of values (6–13%) reported in previous human studies (23–25).

From the inverse relation between SF concentration of the subjects and iron absorption (Figure 1), as well as the ≈3-fold increase in iron absorption on the addition of ascorbic acid to the wheat-based meal, it would appear that MDFP enters the common iron pool, where its absorption is regulated by normal mechanisms. The addition of ascorbic acid was previously shown to enhance iron bioavailability from other poorly soluble iron compounds (9, 26–28). However, with the addition of ascorbic acid, the RBV of MDFP decreased from 62% to 39%. A similar decrease in RBV with the addition of ascorbic acid was previously reported for ferric pyrophosphate with larger MPSs (28). Similarly, EDTA increases the absorption of iron from ferrous sulfate but not that from ferrous fumarate, ferric pyrophosphate, or elemental iron (17). In contrast, studies with ferric orthophosphate and elemental iron found an identical RBV before and after the addition of ascorbic acid (16, 27). Differences in chemical and crystalline properties of ferric pyrophosphate and orthophosphates may influence their rates of solubility and bioavailability (16).

In this study, heated extrusion of MDFP to produce artificial rice grains followed by boiling was associated with a small but significant increase in the RBV that mainly resulted from a greater absorption of iron from the MDFP. It is possible that extrusion into the rice grain fixes the micronized ferric pyrophosphate particles and minimizes their potential aggregation. In a previous study in rats, heat sterilization (121 °C, 20 min) of an infant formula containing ferric pyrophosphate increased its RBV from 75% to 125% (29), likely because of solubilization of the ferric pyrophosphate in the liquid formula. In contrast, in a human study that used chocolate milk powder, vacuum drying (90 °C for 3 h) decreased iron absorption from ferric pyrophosphate (2.1% and 0.6%, before and after processing, respectively) but did not affect ferrous sulfate absorption (2.8% and 2.6%), which resulted in a decrease in the RBV of ferric pyrophosphate from 75% to 21% (10). The effects of processing on RBV are therefore variable and difficult to predict.

RBV values from animal and human studies have proven valuable in the choice of iron compounds to fortify foods. They were used to divide iron compounds into 3 groups: water-soluble compounds with an RBV close to that of ferrous sulfate, compounds that dissolve more or less completely in the gastric juice and have an RBV similar to that of ferrous sulfate in adults (eg, ferrous fumarate), and compounds that are only partly soluble in the gastric juice. The latter group includes ferric pyrophosphate, whose bioavailability has been reported to vary from 15% to 75% compared with ferrous sulfate (10, 17, 18), other phosphate compounds, and the different forms of elemental iron. Our findings suggest some explanation for this wide variability. In the case of poorly water-soluble iron compounds, RBV alone should not be used to judge the potential of an iron compound for fortification. The absolute iron absorption in the fortified food is likely to be a better predictor of efficacy.

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