Bioavailability of Elemental Iron Powders to Rats Is Less than Bakery-Grade Ferrous Sulfate and Predicted by Iron Solubility and Particle Surface Area¹

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ABSTRACT Foods are fortified with elemental forms of iron to reduce iron deficiency. However, the nutritional efficacy of current, commercially produced elemental iron powders has not been verified. We determined the bioavailability of six commercial elemental iron powders and examined how physicochemistry influences bioavailability. Relative biological value (RBV) of the iron powders was determined using a hemoglobin repletion/slope ratio method, treating iron-deficient rats with repletion diets fortified with graded quantities of iron powders, bakery-grade ferrous sulfate or no added iron. Iron powders were assessed physicochemically by measuring iron solubility in hydrochloric acid at pH 1.0 and 1.7, surface area by nitrogen gas adsorption and surface microstructure by electron microscopy. Bioavailability from the iron powders, based on absolute iron intake, was significantly less than from FeSO₄ (100%; P < 0.05) with the following rank order: Carbonyl (64%; Ferronyl, U.S.) > Electrolytic (54%; A-131, U.S.) > Electrolytic (46%; Electrolytic Iron, India) > H-Reduced (42%; AC-325, U.S.) > Reduced (24%; ATOMET 95SP, Canada) > CO-Reduced (21%; RSI-325, Sweden). Solubility testing of the iron powders resulted in different relative rankings and better RBV predictability with increasing time at pH 1.7 (R² = 0.65 at 150 min). The prediction was improved with less time and lower pH (R² = 0.82; pH 1.0 at 30 min). Surface area, ranging from 90 to 370 m²/kg, was also highly predictive of RBV (R² = 0.80). Bioavailability of iron powders is less than bakery-grade ferrous sulfate and varies up to three times among different commercial forms. Solubility at pH 1.0 and surface area were predictive of iron bioavailability in rats. J. Nutr. 133: 3546–3552, 2003.

KEY WORDS: • iron absorption • elemental iron powder • food fortification • iron bioavailability
• hemoglobin repletion • rats

Iron fortification of staple foods is considered to be a cost-effective, long-term strategy for reducing iron deficiency and its associated anemia, the most common nutrient deficiency in the world (1). Previously, elemental iron powders were thought to be advantageous because they do not cause unacceptable sensory changes in the fortified food and are relatively low cost. However, there has been little or no verification concerning the efficacy of elemental iron powders used widely as commercial food fortificants (2). Because production methods differ among suppliers, elemental iron powders differ physicochemically (3) and evidence suggests that these physicochemical differences substantially influence their bioavailability (4,5). More information is required on the nutritional efficacy of these powders and how efficacy relates to measurable physicochemical properties of the different elemental iron powders currently used to fortify foods (2,6).

On the basis of a review of animal (4–12) and human studies (6,13–21), an expert panel recently concluded that incomplete and inconsistent information exists on the food-grade elemental iron powders previously investigated and on iron powders currently used by the food industry (22). In most cases, the inconsistent findings may reflect the following: 1) the practice of testing elemental iron powders that were prepared experimentally and thus, are different from their commercially produced counterparts; 2) nonspecific identification of the elemental iron powders that were investigated; and 3) methodological differences (22). By the 1980s, elemental iron powders accounted for most of the iron used for food fortification in the United States (23), and the pattern of use was changing rapidly as the total iron content of the U.S. food supply continued to increase dramatically through the 1990s (24). To make specific qualitative and quantitative recommendations, more information is required on the bioavailability and physicochemistry of the current, commercially available elemental iron powders (2,22).

The specific objectives of the present investigation were as follows: 1) to determine the bioavailability of six different elemental iron powders, relative to ferrous sulfate, assessed by measuring change in hemoglobin concentration and total hemoglobin iron in rats; 2) to assess each elemental iron powder physicochemically; and 3) to determine the extent that physicochemistry predicts the bioavailability of the elemental iron powders. In addition to evaluating products recently on the market, this assessment uniquely compares an established elec-

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trolytic iron powder produced in the United States to one produced in India and includes a newer reduced iron powder from Canada that has been commonly used in cereal fortification in North America.

MATERIALS AND METHODS

Iron powders. The six elemental iron powders [Carbonyl (Ferronyl, U.S.), Electrolytic (A-131, U.S.), Electrolytic (Electrolytic Iron, India), H-reduced (AC-725, U.S.), Reduced (ATOMET 955P, Canada), and CO-reduced (RSI-325, Sweden)] and bakery-grade ferrous sulfate (FeSO$_4$·H$_2$O) used in the present study were obtained from commercial suppliers in 2001 by SUSTAIN. The iron powders were chosen because they are currently used extensively for food fortification throughout the world. Because of proprietary constraints, specific statistics on the use of elemental iron powders found in the food supply are difficult to obtain. However, carbonyl iron powder is generally used in vitamins and pharmaceuticals, but less in foods except previously by some Northern European countries and the U.S. military. Electrolytic iron powder is used commonly to fortify infant cereals. Reduced forms of elemental iron powder are prominent in cereal flours and ready-to-eat breakfast cereals.

Descriptions of the specific elemental iron powders (e.g., carbonyl, electrolytic, reduced) generally reflect the method of production rather than the final chemical form or compound (3), which is relatively pure elemental iron (>98% iron; zero oxidation state) with >97% of the particle sizes <45 μm diameter. Once received, the elemental iron powders and ferrous sulfate were stored in a desiccator under vacuum at room temperature until use.

Hemoglobin repletion tests in rats. The relative biological value (RBV) of the six elemental iron powders was determined by a RBV repletion/slope ratio method, based on the rat hemoglobin repletion/slope ratio method, based on the rat hemoglobin concentration and change in total hemoglobin iron (26) to receive the powders onto the exposed adhesive surface, distributed uniformly with a stainless steel spatula. Compressed tetrafluoroethylene (Dust-Pro, Peca Products, Janesville, WI) was directed at the sample surface to remove loose particles, taking care to avoid contamination. The stubs were coated with ~10 nm of gold/palladium (50/50 electrode) using a sputter coater (Hummer V; Anatech, Alexandria, VA), before examining in a field emission electron microscope (Model S-4700; Hitachi Scientific Instruments, Pleasanton, CA) operated at 15.0 kV and recording images (Type 55 P/N film; Polaroid, Cambridge, MA).

Physicochemical determinations. Nanopure, analytical grade, 18.0 Ω·cm water (Barnstead/Thermolyne, Dubuque, IA) was used for the physicochemical determinations. Solubility was measured (n = 3) using a method similar to that described by Forbes et al. (6) by placing 20 mg iron powder in 250 mL dilute 0.1 and 0.02 mol/L hydrochloric acid (calculated pH of 1.0 and 1.7, respectively) with constant orbital shaking (ORS 200; Boekel Scientific, Feasterville, PA) at 150 rpm and 37°C. No glass beads or magnetic stir devices were used. Iron in solution was assayed at 15, 30, 45, 60, 90, 120, and 150 min time points. Each time, shaking was stopped and 1 mL of solution was quickly taken and centrifuged (BiofugePico; Heraeus Instruments, South Plainfield, NJ) at 11,600 × g for 5 min. Immediately after centrifugation, 500 μL of the supernatant was diluted to 5 mL with 0.02 mol/L hydrochloric acid and then analyzed by ICP-AES. Solubility values are expressed as the mean ± SEM of triplicate samples determined on different days in randomized order.

Surface area was determined (Flowsorb 2300; Micromeritics Instrument, Norcross, GA) by nitrogen gas adsorption (30:70 nitrogen to helium molar ratio), based on the method of Brunauer et al. (28). Surface area values were expressed as the mean ± SEM of six adsorption measures.

Slope-ratio modeling and statistical analyses. Data on repletion of hemoglobin concentration and change in total hemoglobin iron relative to dietary iron concentration (analyzed; mg/kg) from each iron source, as well as absolute iron intake (μg/d), were analyzed by the slope-ratio assay method, expressing bioavailability relative to ferrous sulfate (29). Linearity of the regression curves was ascertained for each source of iron separately. Then, a single multiple regression
model was derived to determine the slope of the responses for each of the six dietary iron sources, with the “no added iron” group serving as the blank (30). Tests were conducted to determine whether the mean of the blank differed significantly from the common intercept for the six iron powder sources. CI for relative bioavailability were obtained using Fieller’s method (29). Solubility and surface area data were analyzed using one-way ANOVA; differences among the means were tested using Tukey’s multiple comparison tests with significance set at \( P \leq 0.05 \) and values expressed as means ± SEM (31).

RESULTS

Relative bioavailability was calculated on the basis of absolute iron intake (mg/d) as well as diet iron concentration (mg/kg) and on change in hemoglobin concentration (g/L) as well as change in total body hemoglobin iron (mg/rat). For the two models based on diet iron concentration, the mean of the blank was different (\( P = 0.001 \)) from the common intercept, probably because it did not account for the lower food intake (data not shown) by rats fed the lowest iron concentrations. The two models based on absolute iron intake accounted for these differences in food intake, and the mean of the blank was not different from the common intercept for these models. All four models produced similar RBV values for each iron powder. The \( R^2 \) values ranged from 0.53 to 0.97 for individual iron sources and 0.87 to 0.91 for each of the four general models. The relative bioavailability of the iron powders did not differ using the criteria for any of the four models (Table 1). The model relating hemoglobin concentration to diet iron concentration (Fig. 1) was subsequently used for all comparisons.

Iron bioavailability of each of the six iron powders differed (\( P < 0.05 \)) from that of ferrous sulfate, ranging from 21 to 64% that of ferrous sulfate. Iron bioavailability between several powders also differed (\( P < 0.05 \)) (Table 1). Carbonyl iron powder was more bioavailable than the other elemental iron powders. The RBV of the electrolytic iron powder from India did not differ (\( P < 0.05 \)) from its U.S. counterpart in any of the models except that based on diet iron (mg/kg), using change in total hemoglobin iron as a criterion. The two electrolytic iron powders were intermediate in bioavailability between the carbonyl and the three reduced iron powders.

Based on the variation in subsequent diet iron analyses, it was more difficult to mix the H- and CO-reduced iron powders homogeneously into the low iron diet. Thus, the 24-mg iron/kg H-reduced diet and the 12- and 36-mg iron/kg CO-reduced diets were remixed for an additional 15 min to obtain more homogeneous diets. Although the final variability of iron in these diets was similar to that of the other iron sources, the H-reduced diet, which was designed to contain 36 mg iron/kg, was found by analysis to contain only 27.2 mg iron/kg; if this value was undermeasured, the RBV for H-reduced iron powder may somewhat overestimate true bioavailability.

![Figure 1](https://example.com/fig1.png)

**FIGURE 1** Linear regression model used for slope-ratio assay, showing change in hemoglobin of rats consuming graded concentrations of bakery-grade ferrous sulfate (FeSO₄·H₂O) and the six different elemental iron powders. Values are means ± SEM, \( n = 9–10 \).

### TABLE 1

Relative biological value (RBV) of elemental iron powders to rats

<table>
<thead>
<tr>
<th>Iron powder3</th>
<th>RBV2 criterion</th>
<th>Hemoglobin, g/L</th>
<th>Hemoglobin iron, mg/rat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diet iron</td>
<td>Iron intake</td>
<td>Diet iron</td>
</tr>
<tr>
<td>FeSO₄·H₂O (bakery-grade ferrous sulfate, U.S.)</td>
<td>100</td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>Carbonyl (Ferronyl, U.S.)</td>
<td>64 (62, 67)’a</td>
<td>66 (61, 71)’a</td>
<td>65 (62, 67)’a</td>
</tr>
<tr>
<td>Electrolytic (A-131, U.S.)</td>
<td>54 (50, 58)’b</td>
<td>55 (50, 60)’b</td>
<td>54 (52, 56)’b</td>
</tr>
<tr>
<td>Electrolytic (Electrolytic Fe, India)</td>
<td>43 (43, 50)’b</td>
<td>50 (46, 54)’b</td>
<td>44 (44, 48)’c</td>
</tr>
<tr>
<td>H-reduced (AC-325, U.S.)</td>
<td>42 (37, 46)’c</td>
<td>43 (37, 48)’b</td>
<td>41 (39, 44)’c</td>
</tr>
<tr>
<td>Reduced (ATOMET 95SP, Canada)</td>
<td>24 (20, 28)’d</td>
<td>24 (19, 28)’d</td>
<td>24 (22, 29)’d</td>
</tr>
<tr>
<td>CO-reduced (RSI-325, Sweden)</td>
<td>21 (17, 25)’d</td>
<td>24 (15, 25)’d</td>
<td>21 (19, 23)’d</td>
</tr>
</tbody>
</table>

1 Values are means (95% CI). Means in a column without a common letter differ, \( P < 0.05 \).

2 Bioavailability (RBV) relative to FeSO₄·H₂O (−100), measured by the slope-ratio assay method, using either repletion in hemoglobin concentration or in total hemoglobin iron (y-axis) vs. either diet iron concentration or absolute iron intake (x-axis) to determine slopes. All simple linear regressions for each iron source were significant (\( P < 0.0001, n = 26–49 \) rats per iron source) for all the models.

3 Method by which iron was produced (product name or grade, country produced).
Scanning electron microscopy showed that particle surface microstructure differed among the iron powders (Fig. 2a–f). The carbonyl iron powder particles appeared smaller and spherical with a smooth surface and were more homogeneous than the other iron powders. Particles of both electrolytic and the three reduced iron powders were highly irregular. The electrolytic and H-reduced and Reduced (ATOMET 95SP) iron powders had a coarse, flake-like appearance with apparent fissures, whereas particles of the CO-reduced iron powder were less coarse with grooved surfaces.

At both pH 1.0 and 1.7, differences (P < 0.05) in iron solubility between the iron powders were apparent at each time point measured, but with a different rank order of the iron powders at different time points (Fig. 3a, b). At both pH 1.0 and 1.7 at 150 min, Carbonyl iron powder was most and the Reduced (ATOMET 95SP) iron powder least soluble. At pH 1.7, iron solubility accounted for 36–65% of the variation in RBV, with improved predictability as the dissolution time increased, and with 150 min as the most predictive (R² = 0.65; P < 0.05). The iron powders were more soluble at the lower pH, which improved the prediction of in vivo RBV. At pH 1.0, iron solubility at different time points accounted for 38–82% of the variation in in vivo RBV, with 30 min as the most predictive (R² = 0.82; P < 0.05) (Fig. 4a). Additional time beyond 30 min at pH 1.0 provided less differentiation predictive of RBV.

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**FIGURE 2** Differences in particle size distribution and surface texture are apparent from scanning electron micrographs of the six elemental iron powders (panels a–f): a) Carbonyl (Ferronyl, U.S.); b) Electrolytic (A-131, U.S.); c) Electrolytic (Electrolytic Iron, India); d) H-reduced (AC-325, U.S.); e) Reduced (ATOMET 95SP, Canada); and f) CO-reduced (RSI-325, Sweden). Two magnifications are shown in each panel, with reference bars of 100 μm on the left and 1.0 μm on the right.

**FIGURE 3** Iron solubility and the resulting rank order of the six elemental iron powders differed with time and at pH 1.0 (panel a) and pH 1.7 (panel b). Values are means ± SEM (n = 3).

**DISCUSSION**

It is challenging to select appropriate methods for evaluating the bioavailability of elemental iron powders. Because commercial elemental iron powders cannot be readily reproduced with radioactive tracers, human studies of these powders are currently limited to less sensitive methods, such as efficacy studies, which require greater time and resources and which test fewer products at one time. Rats have generally proven to be poor models for human iron absorption as influenced by dietary enhancers and inhibitors (32). However, in the only published comparison using the same (laboratory produced) electrolytic iron powder, Forbes et al. (6) reported that relative bioavailability results agreed well between the rat hemoglobin...
elemental iron powders varies up to three times among different commercial forms, are generally consistent with previous reports (4–12,33–38). For each type of iron powder studied previously, our RBV values were within the range of those reported for powders of a similar mesh (sieve size) specification, but higher than those with a greater proportion of larger particles.

Others (4,5) have also found highly correlated positive associations ($R^2 = 0.89$ at 30 min and 0.90 at 10 min, both at pH 1.2) between solubility and in vivo RBV in rats. Although solubility of the powders in the present study was reproducible with low batch-to-batch variation, the relative solubilities between different powders differed consistently with pH and time. Possible explanations for the differences in this rank order based on solubility may include the differences in internal and surface microstructure among forms. The present results demonstrate that both the pH and time at which solubility is tested influence comparative results and thus, how well solubility predicts iron bioavailability.

In this study, surface area was also positively associated with in vivo RBV ($R^2 = 0.80$; Fig. 4b), but an additional related observation suggests that there may be an upper plateau in the relation between surface area and bioavailability. In addition to the electrolytic iron powder supplied by SUSTAIN for the present study, we measured the surface area of an electrolytic iron powder of the same description from the same supplier, but produced ~6 y earlier (1994–1995). Although an irradiated sample of the earlier lot had 55% RBV in piglets (38), similar to the RBV observed with rats in the present study, the nonirradiated sample from the earlier lot had twice the surface area ($784 \pm 3$ vs. $370 \pm 3$ m$^2$/kg). Extrapolation of the relation described in Figure 4b to predict bioavailability from this greater surface area would suggest an RBV greater than that of ferrous sulfate, which seems unlikely on the basis of the piglet data (38) and the high solubility and bioavailability of the latter iron salt.

On the basis of information provided by the manufacturers for each of the elemental iron powders used in this study, >97% of the iron powder particles were $<45 \mu$m in diameter. The electron micrographs of the present study (Fig. 2) demonstrate differences in particle size, especially the smaller particle size of the carbonyl iron powder, which may explain in part the increased bioavailability of this product. A reduction in the mean particle size of an elemental iron powder has generally been shown to increase RBV (4,8,39,40). However, elemental iron powders of similar particle size, manufactured by different methods, have different in vivo RBV values (40).

**TABLE 2**

<table>
<thead>
<tr>
<th>Elemental iron powder</th>
<th>Surface area</th>
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<tbody>
<tr>
<td></td>
<td>m$^2$/kg</td>
</tr>
<tr>
<td>Carbonyl (Ferrenyl, U.S.)</td>
<td>362 ± 4$^a$</td>
</tr>
<tr>
<td>Electrolytic (A-131, U.S.)</td>
<td>370 ± 3$^a$</td>
</tr>
<tr>
<td>H-reduced (AC-325, U.S.)</td>
<td>260 ± 3$^a$</td>
</tr>
<tr>
<td>Electrolytic (Electrolytic Iron, India)</td>
<td>245 ± 4$^b$</td>
</tr>
<tr>
<td>Reduced (ATOMET 95SP, Canada)</td>
<td>225 ± 6$^c$</td>
</tr>
<tr>
<td>CO-reduced (RSI-325, Sweden)</td>
<td>90 ± 2$^d$</td>
</tr>
</tbody>
</table>

$^a$ Values are means ± SEM. Means in a column without a common letter differ, $P < 0.05$.

$^b$ Method by which iron was produced (product name or grade, country produced).

repletion method and a human radiotracer method. The strong correlations between the in vivo bioavailability and both the solubility and surface area measurements in the present study suggest that the hemoglobin repletion assay with rats can provide a meaningful in vivo comparison of multiple elemental iron powders. The observation that time and pH are important in simulating the conditions predictive of in vivo bioavailability is also likely to affect the predictive value of complex in vitro systems, such as measuring iron uptake by human Caco-2 cells after simulated gastrointestinal digestion. Although tested here in the matrix of a rat diet, we found no evidence that differences in the dietary matrix influenced the relative bioavailability of these powders. Baking the iron into bread before inclusion in the animal diets did not affect the relative bioavailability of H-reduced (33,34), electrolytic, carbonyl or ferrous sulfate sources of iron (34). Furthermore, the bioavailability of an irradiated carbonyl iron powder relative to a radiolabeled ferrous sulfate tracer was unchanged by fermentation and baking into wheat rolls, when tested in humans (19). Until the bioavailability of the elemental iron products (as commercially produced) can be measured sensitively in humans, animal hemoglobin repletion results offer a useful comparative measure of these iron sources.

These results, demonstrating that the bioavailability of
suggesting that particle size alone does not fully explain differences in bioavailability of the iron powders.

The present results indicate that as much as 4–5 times more of the reduced iron fortificant may be required to achieve the same iron availability as bakery-grade ferrous sulfate. During the past decade, iron intake in the United States increased from 19.4 to 23.1 mg/d per person (24). A substantial proportion of this increase is due to greater consumption of iron from enriched grains and fortified ready-to-eat breakfast cereals (24). Because much of this iron is from reduced iron powders (23), which are not as bioavailable as other forms of nonheme iron, this expansion in intake to 23.1 mg/d likely does not reflect a corresponding increase in bioavailable iron. Algorithms used to estimate the bioavailability of nonheme iron from meal composition (41) may overestimate bioavailable iron if some of the nonheme iron is from an elemental iron powder.

The cost of different elemental iron powders also varies, typically as much as sixfold among the forms of elemental iron powder tested in this study, with carbonyl iron powder being the most and the reduced forms the least expensive (42,43). Although differences in RBV exist among elemental iron powders, when cost is considered, it may still be reasonable to fortify foods using greater quantities of a less expensive estimate of iron bioavailability. Current guidelines (44) for iron fortification of infant cereals: a proposal for the use of ferrous fumarate or ferrous succinate. Am. J. Clin. Nutr. 49: 1274–1277.

ACKNOWLEDGMENTS

We thank Denice Schafer and vivarium personnel for care and handling of the animals, Jim Lindlauf and Karin Tweten for preparation of the diets, and LuAnn Johnson and Sheila Bichler for statistical analyses. We are grateful to Liz Turner (SUSTAIN) for the iron powders, Janet Lucht (Research Specialist, Energy and Environmental Research Center, Grand Forks, ND) for use of the gas adsorption-meter and Edward Carlson (Chair, Department of Anatomy, University of North Dakota) for use of the electron microscope.

LITERATURE CITED