Iron Absorption Prediction Equations Lack Agreement and Underestimate Iron Absorption\textsuperscript{1,2}

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Abstract
A number of algorithms have been developed to predict the bioavailability of iron from mixed meals and diets, but their direct validity in predicting change in iron status remains questionable. Throughout the course of conducting a large feeding trial in 10 convents in Manila, we collected weighed food intake data (n = 317) and directly compared the performance of these prediction equations to each other and to the change in serum ferritin (SF). Dietary weighed food intakes were measured on d 3 every 2 wk for each woman and iron status determined at baseline, 4.5 mo, and 9 mo. The Monsen and Balintfy equation predicted higher median absorption efficiency (7.3%) than did the equations of Hallberg and Hulthen (6.1%) and Reddy et al. (5.8%). In contrast, the predictions that used the equations of Bhargava et al. (3.8%), Tseng et al. (2.9%), and Du et al. (2.6%) were significantly lower. The iron absorption efficiencies calculated using the Monsen and Balintfy equation correlated with those using the Hallberg and Hulthen equation ($r = 0.91$, $P < 0.001$). This slope did not differ from unity, whereas all other equations underestimated iron absorption efficiency relative to Monsen and Balintfy’s equation. The median efficiency of absorption, based on change in SF in 114 subjects, was 17.2%, suggesting that these equations underestimate iron absorption. The inhibitory and enhancing factors in the published prediction equations were quantitatively either too large or perhaps too small to correctly predict apparent iron bioavailability over a 9-mo period. The causes of the lack of agreement between change in iron status estimated by SF change and absorption predicted by algorithms are open to discussion and will need to be resolved. J. Nutr. 137: 1741–1746, 2007.

Introduction
Iron deficiency is the most prevalent nutritional deficiency in the world. The Fourth Report of the World Nutrition Situation issued by the United Nations estimates that 2 billion people in developing countries are iron deficient and 1 billion can be defined as iron-deficient anemic (1–4). A recent approach advocated as an alternative to more conventional strategies of intervention is termed “biofortification” of staple foods (5). This approach involves the development of new staple crop varieties that are selectively bred to enhance specific nutritional qualities, such as the levels of biologically available iron. Varieties of such crops as rice, maize, wheat, beans, and cassava have been developed with enhanced levels of specific micronutrients, such as iron, zinc, and β-carotene through the HarvestPlus project and its predecessors (5,6). We recently published the results of a placebo-controlled randomized trial conducted in convents in Manila (7). This study was designed to test the efficacy of biofortified rice consumption under controlled conditions in Filipino women who are at risk of iron deficiency. The trial provided an additional 1.77 mg/d of dietary iron to religious sisters and resulted in a significant gain in body iron in women who were iron deficient. Part of the control exerted in the study was the collection of weighed food intakes for all sisters during the trial.

Prediction equations for bioavailability of iron exist in the literature (8–13). A primary application of these equations is to predict the efficiency of iron absorption when dietary change is instituted. Three of the published algorithms were generated from research groups with a primary interest in the identification of factors in the diet that alter iron absorption and utilized radioisotope absorption studies to determine those effects (8,9,13). Three other equations resulted from field trials in which food recalls and dietary record analysis were analyzed relative to iron status biomarkers in a population sample (10–12). The first such algorithm of Monsen and Balintfy (8) utilized only enhancing factors of meat-fish-poultry (MFP)\textsuperscript{6} and vitamin C in a summative combination with a variable that accounted for the powerful inverse relation between body iron stores and absorption efficiency to predict bioavailability of dietary iron. Tseng

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\textsuperscript{6} Abbreviations used: Hb, hemoglobin; MFP, meat-fish-poultry; SF, serum ferritin.
et al. (10), Bhargava et al. (11) and Du et al. (12) modified the Monsen and Balintfy algorithm by adding the inhibitory factors of tea and phytates. The Tseng et al. (10) algorithm was based on a field study in Russia and added a fixed effect for tea consumption and a log variable for phytate content. Bhargava et al. (11) used data from a Bangladeshi study and argued that formula of Tseng et al. for calculating the inhibitory effects of phytates contained a mathematical error. Du et al. (12) attempted to utilize the Tseng et al. or Bhargava et al. equations in a large food consumption survey in China and again requantified the influence of phytates and polyphenols on iron absorption when they surmised those equations did not work well with a Chinese diet. This decision to modify the existing equations was based on a lack of agreement between what was predicted and what was observed in terms of iron status gain as assessed by a change in hemoglobin (Hb) distribution over time. Thus, the comparison between prediction and reality was based on Hb and not the more complex and accurate measures of iron status that utilize serum ferritin (SF), serum transferrin receptor, and other biomarkers. The most complex of the algorithms is that of Hallberg and Hulthen (9), which includes 9 different variables (alcohol, ascorbic acid, calcium, coffee/tea, eggs, MFP, phytate, polyphenols, and soy protein), 4 of which are exponential variables (ascorbic acid, calcium, phytates, and polyphenols). The internal validity was shown in a sample of 31 human subjects given test meals labeled with radioactive iron and data were adjusted to the reference dose absorption of 40%. The most recent equation is from Reddy et al. (13) who re-adjusted the MFP variable to improve the algorithm’s prediction based on radioisotope absorption studies in 86 subjects provided ~25 different western-style meals.

We took the opportunity provided by our unique data set to compare the performance of these 6 equations in predicting the gain in iron status of religious sisters during 9 mo using the quantitatively more satisfactory weighed food intake method (14) and markers of iron status that are more sensitive than Hb. In addition, we performed direct comparisons between these 6 equations, which has not been undertaken previously in a field setting in which change in iron status was directly and sensitively measured.

Subjects and Methods

The study was conducted in young women training to become religious sisters of the Roman Catholic Church and subject screening and protocols are described elsewhere (7). Subjects were chosen from convents located near Manila, the Philippines. This study was a prospective, randomized, controlled, double-blind, longitudinal (9 mo), intervention trial involving 317 women assigned to either a high-iron or low-iron variety of rice. Randomization was done according to 2 strata based on SF and Hb concentrations. Sisters included in the published efficacy trial data (n = 192) had a SF concentration of <20 μg/L and/or Hb concentration <120 g/L after exclusion of those with severe anemia, poor health, advanced age, high α-glycosylated protein, used supplements, or were transferred out of the convents (7). There were 34 subjects who were anemic (Hb <120 g/L) and had a low SF concentration. The details of the randomization and design are provided elsewhere (7).

Because all religious sisters in each convent were fed in the trial (regardless of eligibility for the efficacy study), we collected intake data on all 317 women for the 9-mo trial. The dietary analysis performed on the entire data set (n = 317), on subjects with SF <20 μg/L (n = 138), and only those subjects that had an increase in SF (n = 114) over the duration of the feeding trial.

The procedures were reviewed and approved by the Institutional Review Boards for use of human subjects in research at The Pennsylvania State University, Cornell University, and the University of the Philippines and were in accordance with the Helsinki Declaration of 1975 as revised in 1983. Informed consent was obtained from all participants.

Study protocol. The details of the protocol have been previously described (7).

Weighed food intake. Weighed intakes of the entire diet were collected from each of the study participants on 3 random days (including 1 weekend day) every 2 wk for a total of 54 daily food intake measurements from each woman. Conversion of weighed food items to nutrients was made using Philippine food composition tables, the ASEAN food composition tables, and comparison to the WorldFood2 database system for moisture content (15). Phytate contents of cooked rice were measured (16), but levels of phytate, polyphenols, etc. in other food compounds were obtained from food composition data bases (15).

Calculation of predicted iron absorption. Each of the 6 published algorithms mentioned above were used to predict absorption of iron from the diet on a meal-by-meal basis. We chose an iron store level of 250 mg (SF of ~28–30 μg/L) to compare performance of algorithms at the same level of iron status. This level corresponded with the mean ferritin concentrations of the total sample (n = 317; SF = 25 μg/L) and the 114 women (SF = 34 μg/L) whose ferritin levels increased throughout the course of the study and in whom we calculated actual efficiency of absorption.

Algorithm comparisons. The predicted efficiency of iron absorption was first calculated based on mean dietary intake values and then various components of the diet were manipulated (±1 SD for MFP, ascorbic acid, and phytates). We then compared the results with those obtained using the mean values.

Blood samples. Blood samples were collected at baseline, midpoint (4.5 mo), and endpoint (9 mo) using blood from an antecubital vein and the following were measured/calculated: ferritin, transferrin saturation, transferrin receptor, body iron, Hb, and hematocrit (7).

Statistics. The data were analyzed using linear modeling procedures (17) as implemented in R-2.2.1 (R Foundation for Statistical Computing). ANOVA was utilized with control for convent, season of the year, and rice type as potential confounders. As noted above, we computed predicted iron absorption for each meal for each subject on each of the 54 weighed food intake days during the 9 mo that data were collected. Iron absorption was computed for each meal, summed for the day, and daily efficiency of absorption was calculated for each subject. Daily within-subject variations in dietary patterns are reported elsewhere (18). We used diagnostic plots and statistics to examine conformance between modeling assumptions and model residuals. Where necessary, log transformation was used to remedy violations of assumptions (19). Tolerance for Type I error was fixed at α = 0.05.

Results

The dietary intake data based on 54 d of weighed food intakes during 9 mo in >300 religious sisters and the results of the efficacy trial were presented elsewhere and will not be addressed in this report (7,18). Briefly, on average, 43% of daily food energy was consumed from rice, 18% from cereals, 3% from starchy tubers, and ~18% from MFP. The cooked rice intake for women who consumed the control variety of rice was 623 ± 133 g/d, whereas that of the women who consumed the high iron variety of rice was 553 ± 120 g/d. Among the macronutrients, the groups differed in only carbohydrate intake, which was explained by the additional 79 g of cooked rice consumed daily by the control group (P < 0.001). The 2 rice varieties differed in iron concentration (P < 0.001). The high-iron rice contained 3.21 mg/kg of cooked rice, whereas the control rice contained 0.57 mg/kg (P < 0.001). Dietary iron intake of this population
was low, with about 8.0 mg/d obtained from sources other than rice, representing ~44% of frequently recommended (U.S.) dietary intakes of 18 mg/d for this age group.

The predicted median daily dietary iron absorption (milligrams) and median percentage efficiency of absorption for each of the 6 published algorithms were calculated using equations assuming 250 mg of storage iron in the individuals (Table 1). This corresponds to a SF of ~30 μg/L, which was very close to our mean SF at baseline for the total sample of sisters (n = 315; SF = 25 μg/L) and the sisters who actually gained iron over the 9-mo trial (n = 114; SF = 34 μg/L). Based on the prediction equations, median percentage efficiency of absorption and median dietary iron absorption did not differ between those women in the high-iron rice group and those in the low-iron rice group, with the exception of the iron absorption predicted using the Reddy et al. (13) algorithm. This was because the Reddy et al. algorithm predicted >100% efficiency of absorption for ~20% of our data. Comparing between the algorithms, median efficiency of absorption was highest for Monsen and Balintfy (Eq. 1) (8) followed by Hallberg and Hulthen (Eq. 2) (9) and Reddy et al. (Eq. 3) (13). The Tseng et al. (Eq. 5) (10) and Du et al. (12) algorithms (Eq. 6) predicted the lowest median efficiency of absorption, whereas predictions from the Bhargava et al. (11) algorithm (Eq. 4) were between the other algorithms (Table 1). All of these predictions differed due to our large number of observations (~16,000).

The distribution of efficiency of absorption for each of the algorithms was non-normal (Fig. 1). These data were log transformed before comparing the prediction equations by ANOVA. Convent and season of the year did not affect the efficiency of iron absorption. The prediction equation utilized was the only significant main effect factor (P < 10⁻¹², R² = 0.29). There were no significant interaction terms between the 3 main effect variables considered (rice type, convent, and season; P = 0.345-0.455).

The actual overall median efficiency of absorption was calculated for the subset of sisters who gained iron (e.g. increased SF) during the study (n = 114) and was 17.2%. This was calculated by taking the median gain in iron stores (change in ferritin multiplied by 8 mg storage iron·μg⁻¹·L⁻¹·1⁻¹ ferritin) added to the median iron requirement for women of this body size and dividing by the median total iron consumed over the 9-mo study. These subjects, who increased body iron during 9 mo, had a mean ferritin concentration of 34 μg/L and were not necessarily those women with the lowest iron status. Fifty-five of these subjects had an initial ferritin >20 μg/L (43 of these subjects had an initial SF >30 μg/L and 59 women had an initial SF <20 μg/L. Comparison of the actual efficiency of absorption (17.2%) to the predicted efficiencies of the 6 algorithms shows that all equations were dramatic underestimates of the efficiency of absorption approximated by the gain in SF and body iron (7). Eq. 1, 2, and 3 provided estimates that were closer to what is indicated by the change in iron stores than the other 3 equations.

Table 1 shows that predictions from the Bhargava et al. (Eq. 4) were between the other algorithms and slopes differed significantly from unity. The comparison of Eq. 4 to Eq. 5 had a high correlation (r = 0.94, P < 0.001), but both were much lower than in Eq. 1 (data not shown). Subsections of data for sisters with an initial ferritin <20 μg/L or >20 μg/L did not modify the interrelations of any of these equations.

These algorithms include different variables in their equations and all provide different weightings to these variables, yet several of the equations gave nearly identical predicted iron absorption. To evaluate the impact of an increase or decrease in the size of some of these components (e.g. MFP or phytates), we computed the SD of each of the principal components in the entire sample of >16,000 d (>48,000 meals; 317 subjects) and

### Table 1: Predicted amounts of absorbed iron per day and iron absorption efficiency from 6 prediction equations applied to women that consumed high-iron or low-iron varieties of rice for 9 mo

<table>
<thead>
<tr>
<th>Rice</th>
<th>Eq. 1 Monsen and Balintfy (8)</th>
<th>Hallberg and Hulthen (9)</th>
<th>Reddy et al.² (13)</th>
<th>Bhargava et al. (11)</th>
<th>Tseng et al. (10)</th>
<th>Du et al. (12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dietary iron absorption, mg/d</td>
<td>0.75 ± 0.16</td>
<td>0.64 ± 0.13</td>
<td>0.67 ± 0.11</td>
<td>0.35 ± 0.07</td>
<td>0.27 ± 0.05</td>
<td>0.26 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>0.60 ± 0.15</td>
<td>0.49 ± 0.10</td>
<td>0.39 ± 0.17</td>
<td>0.35 ± 0.08</td>
<td>0.26 ± 0.06</td>
<td>0.22 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>0.68 ± 0.15</td>
<td>0.67 ± 0.1</td>
<td>0.53 ± 0.1</td>
<td>0.40 ± 0.08</td>
<td>0.26 ± 0.05</td>
<td>0.28 ± 0.08</td>
</tr>
<tr>
<td>Efficiency of absorption, %</td>
<td>7.5 ± 1.10</td>
<td>6.4 ± 0.78</td>
<td>8.1 ± 0.80</td>
<td>2.5 ± 0.60</td>
<td>2.7 ± 0.44</td>
<td>2.6 ± 0.71</td>
</tr>
<tr>
<td></td>
<td>7.2 ± 1.10</td>
<td>5.9 ± 0.54</td>
<td>3.9 ± 1.3</td>
<td>4.2 ± 0.70</td>
<td>3.1 ± 0.54</td>
<td>2.7 ± 0.70</td>
</tr>
<tr>
<td></td>
<td>7.3 ± 1.11</td>
<td>6.1 ± 0.69</td>
<td>5.8 ± 1.06</td>
<td>3.8 ± 0.68³</td>
<td>2.9 ± 0.50³</td>
<td>2.6 ± 0.70³</td>
</tr>
<tr>
<td>Ferritin, &lt;20 μg/L</td>
<td>7.6 ± 0.2 (11.2 ± 0.3)³</td>
<td>6.8 ± 0.2</td>
<td>6.1 ± 0.2</td>
<td>3.9 ± 0.1 (4.8 ± 0.2)³</td>
<td>2.9 ± 0.1 (5.0 ± 0.2)³</td>
<td>2.7 ± 0.2</td>
</tr>
</tbody>
</table>

¹ Values are medians ± SEM, n = 317.
² A significant effect of rice type on absorbed iron existed only for the Reddy et al. equation, P < 0.001. ANOVA for mean iron intake and mean efficiency showed a highly significant effect of prediction equation, P < 10⁻¹², but not rice variety, convent, or season of the year. Means in a row with superscripts without a common letter differ.
³ The number in parentheses is the efficiency of absorption based on individual ferritin values of subjects with initial SF <20 μg/L, n = 109. The individual’s ferritin could not be easily used in the Reddy et al., Hallberg and Hulthen, or Du et al. equations, because these equations impede a rounded-off iron store.
then recomputed the predicted efficiency of absorption when the dietary intake “shifted” by 1 SD in a single component (Table 2). For example, the mean percentage absorption predicted from Eq. 1 was 5.6%; when the intake of MFP increased by 1 SD, the mean absorption increased to 8.4%. This was a 50% improvement. Because an increase in MFP also increased the amount of heme and nonheme iron, however, we further adjusted the efficiency of absorption for a resulting absorption efficiency of 9.0%, an improvement of 61%. MFP intake had a substantial impact on predicted iron absorption in Eq. 1, 2, and 3 but a smaller effect in the other 3 algorithms. In contrast, decreased MFP intake had a much more powerful negative influence in Eq. 2, 4, 5, and 6 than in the other 2 equations. In this Asian diet, the underweighting of high intakes of MFP, or overweighting of lower intakes of MFP, may have contributed to the large underestimation of true iron absorption in Eq. 4, 5, and 6. Ascorbic acid had a strong influence in Eq. 1 and 2 but was minimal in other equations. Increased phytate intake by 1 SD really only affected absorption in Eq. 3, where the absorption decreased by nearly 50%. In contrast, decreased phytate intakes increased efficiency of absorption by 173–461% across most equations with the exception of Eq. 1 and 6.

**Discussion**

The original study was designed to test the biological effects of consuming additional dietary iron from biofortified rice. The study was conducted in convents where the research team could exercise considerable control over the research environment. We showed that an additional 1.42 mg/d iron led to a significant gain in iron status (7). Our report has a somewhat different focus and has 3 central questions: 1) was there any difference in apparent bioavailability between the diets consumed by sisters eating the high-iron vs. low-iron varieties of rice? 2) what was the extent of agreement between the 6 published algorithms that predict bioavailability when applied to a large quantitatively accurate data set? and 3) which of the published algorithms agrees with the gain in iron experienced by some sisters during a 9-mo period?

These questions could be addressed because we utilized weighed food intake measurements many times during the 9 mo, thus providing a level of accuracy of food intake not available in other studies (10–12,20,21). In addition, this feeding trial used multiple indicators of iron status, a feature not utilized in some of the other studies from which iron absorption was predicted. Zimmermann et al. (20) used multiple measures of iron status in a study assessing bioavailability but did not have a strong quantitative measure of iron intakes. Weighed food intake approaches are superior to 24-h recalls, food records, or food inventories in indexing dietary intake but are usually only done in highly controlled clinical settings (22).
The setting of this study, convents, allowed us to provide quantitative estimates of iron intakes for a large sample of subjects. There is the reasonable concern that the dietary pattern of the religious sisters in these 10 convents was different than habitual intakes of Filipinos. This does not appear to be the case, as the macronutrient and food group consumption patterns are quite similar to a recent report on dietary intakes in a Filipino community setting (2). That the convent or rice type apparently did not affect bioavailability is consistent with our previous report on the lack of effect of convent on iron gain (7). Importantly, there was no interaction between convent and rice type on predicted amount of iron absorbed, supporting the analysis that the compositions of the diets did not differ between convents.

Perhaps the most surprising finding was that the simplest algorithm of all 6, Eq. 1 (8), was in very strong agreement with the most complicated algorithm, Eq. 6 (9). There are several important differences in the 2 algorithms: Eq. 1 contains only fixed values for baseline iron status (0, 250, 500, or 1000 mg of storage iron) and a simple summation of 2 enhancing factors, MFP and vitamin C. In contrast, Eq. 6 from Hallberg and Hulthen (9) contains 4 exponential variables to account for the influence of the well-described inhibitors of iron absorption in addition to an exponential variable to account for the influence of iron status. One possibility for the considerable agreement between these 2 equations is that inhibitory factors are not very powerful in this Filipino diet. The levels of phytates in our database are comprised of both actually measured levels in the rice and computed values from food composition tables (15). The high-iron rice had a mean phytate concentration of 2.97 mg/g rice and the low-iron rice only 0.46 mg/g for control rice, for an iron molar ratio of ~50:1. Because rice contributed ~50% of dietary intake, it was not a low-phytate diet compared with other Asian diets (23,24). In contrast, the amount of tea and coffee consumed was quite low, contributing few polyphenols and tannins. They are well below the levels that Bhargava et al. (11) observed in Bangladesh and Tseng et al. (10) observed in the Russian data set. Calcium intakes were not extreme and unlikely to exert a strong influence on iron absorption in this data set (9). That the major inhibitors of iron absorption were not particularly low in this diet is not a likely explanation for the strong agreement between Eq. 1 and 2. A second related hypothesis is that the MFP factor and ascorbic acid factor were very powerful in this diet. Hallberg et al. (23) and others (24–26) reported iron bioavailability as high as 20% from rice consumed in Asian diets, far higher than those computed in our study.

The large underestimation of efficiency of iron absorption by Eq. 4 (12), 5 (10), and 6 (11) was quite surprising considering all of these equations manipulated the previously existing Eq. 1 or 2 to obtain a better fit. Although it is tempting to suggest that each diet type examined in those studies (Chinese, Russian, European, Bangladeshi, etc.) needs their own special prediction equation, this is an unsatisfactory explanation to the field of iron absorption, where we would like to have a universal tool for predicting bioavailable iron. Our current data set measures “real change” in iron status in a cohort of iron-deficient women over a lengthy period of time to quantitatively measure iron intake. We computed a median iron requirement for iron (based on body size) and then assumed that the incremental gain in SF during 9 mo represented the extra iron that went into iron stores. The ratio of absorbed iron divided by consumed iron yields a median estimate of 17.2% for those 114 women who gained iron. Clearly, the 6 equations do not approximate that number. This may suggest that the careful isotope studies conducted (8,9,13) are not in error but cannot be used for purposes of future prediction because they are point estimates, usually from a single meal, and do not consider iron requirements over a long period of time. The error in estimation around that point estimate is not really known, although in some selected cases, the prediction equation and reality of iron absorption from a single meal were quite close (9). When that same experiment was repeated by another research group, the results were far less convincing, so the equation was modified (13). The field studies in Bangladesh, Russia, and China (10–12) did not have strong, direct measures of iron status and thus their modifications were tied to an insensitive biomarker and would not necessarily be expected to be quantitatively accurate.

The implications of this analysis are substantial given the large sample size, extensive quantitative dietary information, and the careful assessment of iron status. The study subjects were healthy, free of intestinal parasites, consumed a generally balanced diet without experiencing periods of food insecurity, and lived in a relatively protected situation. Well-developed prediction equations offer the opportunity to provide an estimate of whether a dietary pattern can result in a change in iron status in a population. This includes biofortification and other forms of fortification and supplementation implemented in a thoughtful fashion. The current analysis suggests that most of these equations will not accomplish that task in their current formulation. There are new approaches to predicting adequacy of iron intake based on dietary data and prevalence of iron deficiency in the populations that will be applied to this data set.
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Literature Cited