A Complete Diet-Based Algorithm for Predicting Nonheme Iron Absorption in Adults

Seth M. Armah, Alicia Carriquiry, Debra Sullivan, James D. Cook, and Manju B. Reddy

Abstract

Many algorithms have been developed in the past few decades to estimate nonheme iron absorption from the diet based on single meal absorption studies. Yet single meal studies exaggerate the effect of diet and other factors on absorption. Here, we propose a new algorithm based on complete diets for estimating nonheme iron absorption. We used data from 4 complete diet studies each with 12–14 participants for a total of 53 individuals (19 men and 34 women) aged 19–38 y. In each study, each participant was observed during three 1-wk periods during which they consumed different diets. The diets were typical, high, or low in meat, tea, calcium, or vitamin C. The total sample size was 159 (53 × 3) observations. We used multiple linear regression to quantify the effect of different factors on iron absorption. Serum ferritin was the most important factor in explaining differences in nonheme iron absorption, whereas the effect of dietary factors was small. When our algorithm was validated with single meal and complete diet data, the respective $R^2$ values were 0.57 ($P < 0.001$) and 0.84 ($P < 0.0001$). The results also suggest that between-person variations explain a large proportion of the differences in nonheme iron absorption. The algorithm based on complete diets we propose is useful for predicting nonheme iron absorption from the diets of different populations.

Introduction

Iron deficiency anemia is a leading global problem mostly attributed to low intakes of dietary iron and also poor iron bioavailability. Efforts to explore the relationship among iron intake, bioavailability, and status have led to the development of several algorithms to predict iron absorption in the past few decades. These have been reviewed by Reddy (1) and also more recently a new algorithm was published by Rickard et al. (2). A major limitation of the published algorithms is that they were developed based on single meal absorption studies (2–4). It is well known that the effect of dietary factors on iron bioavailability is exaggerated in single meal studies (5). For example, when iron absorption was measured from a 5-d complete diet, the effects of meat, calcium, and ascorbic acid were diminished (6–8). In a human study, Hunt and Roughhead (9) reported a decrease in nonheme iron absorption among men consuming high-bioavailability diets and an increase among those consuming a low-bioavailability diet after 10 wk of feeding, suggesting that individuals adapt to the effect of dietary factors on iron absorption. Although vitamin C is known to increase iron absorption from single meal feeding studies, 2 g/d vitamin C supplementation for 16 wk had no effect on iron stores (10). The above studies suggest that the effect of dietary factors on iron absorption is dampened with longer periods of consumption and that there is the possibility that adaptation may occur over prolonged exposure to iron inhibitors or enhancers. Hence, the lack of agreement between predicted absorption from existing algorithms and iron absorption measured from whole diets and long-term studies is not unexpected (11,12).

Predicting iron absorption from a complete or whole diet thus requires an algorithm that is developed from a complete diet. The complete diet here refers to a person’s total daily intake estimated from one or more days. Studies have been conducted to examine the effect of different dietary factors on iron absorption from a complete diet consumed during a 5-d period (6–8; M. Reddy, R. Hurrell, S. Armah, and J. Cook, unpublished data). Detailed nutrient intakes and absorption measurements have been published from these studies. In each of these studies, participants consumed 3 different diets, each during a period of 5 d. The diets included in the studies were a typical diet and 2 modified diets with low and high amounts of the factor tested in the particular study. The 4 dietary factors that were included in 3 different amounts in the 4 studies were tea, meat, vitamin C, and calcium. By combining data from these studies, we can approximately recreate the heterogeneity of typical diets consumed by various populations. The objective of this study was therefore to develop a new algorithm based on complete dietary data from these studies, which will be useful in assessing iron absorption in populations.

Methods

Data description. We used data from 4 different studies that were designed to measure nonheme iron absorption from a 5-d complete diet...
using an extrinsic radio iron labeling technique (6–8; M. Reddy, R. Hurrell, S. Armah, and J. Cook, unpublished data). Each study included 12–14 participants (19 men and 34 women) aged 19–38 y, with a total of 53 individuals. All participants were healthy with no history of disorders known to affect iron absorption. The studies were designed to determine the effects of ascorbic acid, meat, tea, and calcium on nonheme iron absorption from a complete diet. Each participant consumed diets with low, typical, and high amounts of the dietary factors being studied (ascorbic acid, tea, meat, or calcium). Absorption of nonheme iron was measured for each dietary period using the extrinsic radiolabeling technique by labeling each of the 3 main meals of the day for 5 d and snacking in between meals was not allowed, except for the calcium study, where preliminary data indicated that most participants consumed 2 main meals; thus, only the 2 main meals were labeled (7). Even with the snack consumption, the effect would be very small on iron absorption, because they were not taken together with the meals. Each study reported mean daily nutrient intake during the labeling period, iron status (baseline serum ferritin), and percent iron absorption. Dietary records were kept by participants and nutrient intakes were determined using the NUTRITIONIST IV program (N-squared Computing, First Data Bank Division, Hearst). Phytic acid was not reported in those studies; however, we determined the phytic acid content using the Nutrition Data System for Research (University of Minnesota Nutrition Coordinating Center), because we had access to the complete dietary data. Tea consumption was estimated using black tea equivalents as follows: 2 cups (480 mL) of iced tea and 1.5 cups (360 mL) of herbal tea or coffee were coded as 1 cup (240 mL) of black tea (13). One cup (240 mL) of black tea equals one tea bag (1.9 g of black tea). Other details about data collection procedures were described elsewhere (8). The data collection procedure for the tea study (M. Reddy, R. Hurrell, S. Armah, and J. Cook, unpublished data) was similar to those described above and was approved by the Human Subjects Committee at the Kansas University Medical Center. Because each respondent had 3 data points for high, low, and typical intake of the specified factor, the total number of observations in the combined data was 159.

**Data analysis.** We used R software version 2.12.2 to carry out the data analysis. The factors we considered in the analysis were serum ferritin, calcium, phytic acid, ascorbic acid, tea, nonheme iron, and meat, fish, and poultry (MFP). We transformed all variables using a log transformation to better approximate normality. To develop the algorithm, we fitted a linear multiple regression model using the above factors to predict percent iron absorption. We included a person-level random effect in the model, because each individual contributed 3 different observations and this induces intra-class correlation. We obtained maximum likelihood estimates of fixed effects and variance components (the latter based on a restricted likelihood function) and best linear unbiased predictors of the person-level effects. P values were considered significant if \( P < 0.05 \) and weakly significant if \( 0.05 < P \leq 0.1 \). We reported the median (10th, 90th percentiles) for the dietary factors and the geometric mean (10th, 90th percentile) for serum ferritin and absorption to avoid the influence of extreme values, because the data are not normally distributed.

**Validation of algorithm with human iron absorption data.** We assessed the validity of the algorithm by estimating absorption using dietary intake and serum ferritin data from different published studies (both single meals and complete diets) and comparing results with reported absorption values. The single meal study included mean values for nutrient intakes and absorption from 19 different meals (4), which was designed to measure nonheme iron absorption from single meals fed after an overnight fast. Similarly, we validated our algorithm separately using data from published complete diet studies (9,14–17). The data from these studies were the mean values for dietary intakes, initial serum ferritin concentration, and unadjusted absorption for different arms of each study (\( n = 23 \)). In cases where the mean ferritin values were not given for the different arms, we used the mean for the whole group for each arm. In some of the data from the study by Tetens et al. (15), the quantity of tea consumed was not reported. We therefore assumed one cup of tea was included in the meal and validation was done with and without those data points.

### Results

The serum ferritin concentration ranged from 5 to 162 \( \mu g/L \) (\( n = 53 \)), with a geometric mean of 41.2 \( \mu g/L \) when all studies (6–8), including M. Reddy, R. Hurrell, S. Armah, and J. Cook, unpublished data, were combined. Because each of the 53 participants consumed 3 different diets in the various studies, the dietary intakes presented in Table 1 give the median (10th, 90th percentiles) of the different factors by diet type and for all diets put together.

Results from the mixed effects model (Table 2) suggest that between-person differences explain a large proportion of the variation in nonheme iron absorption. This is reflected by the equal variances for the residual and the grouping variable (identification) for the random effect. Although the dietary factors had minimal contribution in explaining variations in absorption, their inhibitory or enhancing effect was as expected. However, serum ferritin had an effect on absorption (\( P < 0.0001 \)), explaining ~35% of the variance in absorption, as shown under the partial \( R^2 \) column. The residual plot (Fig. 1A) shows no pattern when we plotted the standardized residual against the fitted values and there is good correlation between observed and fitted absorption.

### Table 1

<table>
<thead>
<tr>
<th>Factor/nutrient</th>
<th>Typical</th>
<th>High</th>
<th>Low</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phytate, g/d</td>
<td>0.61 (0.34, 0.98)</td>
<td>0.53 (0.30, 1.02)</td>
<td>0.61 (0.35, 0.98)</td>
<td>0.57 (0.33, 0.96)</td>
</tr>
<tr>
<td>Nonheme iron, mg/d</td>
<td>10.3 (6.9, 19.3)</td>
<td>9.9 (6.5, 18.5)</td>
<td>10.7 (6.4, 17.2)</td>
<td>10.3 (6.5, 18.6)</td>
</tr>
<tr>
<td>Calcium, g/d</td>
<td>0.81 (0.40, 1.23)</td>
<td>0.88 (0.39, 1.40)</td>
<td>0.65 (0.22, 1.26)</td>
<td>0.77 (0.31, 1.35)</td>
</tr>
<tr>
<td>Ascorbic acid, mg/d</td>
<td>85 (32, 196)</td>
<td>78 (27, 256)</td>
<td>54 (28, 125)</td>
<td>73 (28, 236)</td>
</tr>
<tr>
<td>MFP, g/d</td>
<td>94 (39, 228)</td>
<td>108 (43, 242)</td>
<td>82 (0, 162)</td>
<td>91 (11, 215)</td>
</tr>
<tr>
<td>Tea, cups/d</td>
<td>0.0 (0.0, 0.9)</td>
<td>0.1 (0.0, 3.0)</td>
<td>0.0 (0.0, 0.7)</td>
<td>0.0 (0.0, 1.1)</td>
</tr>
<tr>
<td>Serum ferritin, ( \mu g/L )</td>
<td>41 [17, 88]</td>
<td>41 [17, 86]</td>
<td>41 [17, 86]</td>
<td>41 [17, 86]</td>
</tr>
<tr>
<td>Absorption, %</td>
<td>4.3 [1.6, 12.4]</td>
<td>5.3 [1.8, 20.5]</td>
<td>5.7 [1.9, 14.5]</td>
<td>5.0 [1.7, 16.9]</td>
</tr>
</tbody>
</table>

1. Values are median (10th, 90th percentile) or geometric mean (10th, 90th percentile), \( n = 53 \), and are from 4 different studies (6–8; M. Reddy, R. Hurrell, S. Armah, and J. Cook, unpublished data).
2. Diet was high in 1 of 4 dietary factors (meat, ascorbic acid, calcium, or tea).
3. Diet was low in 1 of 4 dietary factors (meat, ascorbic acid, calcium, or tea).
4. 1 cup (240 mL) of black tea = 2 cups of iced tea or 1.5 cups of herbal tea or coffee.
Accurately estimating iron absorption is also essential to set dietary recommendations. Because iron absorption from single meals exaggerates the effect of dietary factors, there is concern about the use of algorithms based on single meals to estimate iron absorption from a whole diet. By overestimating absorption, we risk underestimating the prevalence of inadequate iron intakes. This is one reason why development of an algorithm based on complete diets that can accurately predict iron absorption is critical.

The algorithm we propose was developed from complete diet datasets that include a wide range of intake of the dietary factors known to affect nonheme iron absorption and therefore should reflect the consumption pattern of many populations. Maximum intakes for most dietary factors were extremely high due to the designs of the studies from which the data were obtained. For example, the maximum intake of vitamin C was 584 mg, which far exceeds the usual intake in most populations. Similarly, the very low minimum intakes make the algorithm useful for populations with extremely low intakes of these dietary factors. For instance, the model can be used to estimate iron absorption among vegetarians and also individuals who do not habitually consume tea or coffee. Besides, tea estimation in our study as number of cups (in black tea equivalents) is much easier than estimating the polyphenol content, because inaccuracies and methodological differences exist in estimating different types of polyphenols.

Most of the existing algorithms require estimating iron absorption from each meal separately to obtain total daily iron absorption (3,4,18). When wide ranges of total daily nutrient intakes are used with single meal algorithms, the result may be inaccurate. For instance, when we predicted nonheme iron absorption from the published complete diet datasets (9,14–17) using the Reddy et al. (4) and Hallberg and Hulthen (3) algorithms, some of the predicted absorption values for both algorithms were >100% due to the wide ranges of intakes. A similar observation was made by Rickard et al. (2) and Beard et al. (12). However, with our proposed algorithm, total daily nutrient intakes may be used without the need to calculate bioavailability for individual meals. In addition, this algorithm incorporates iron status (serum ferritin) in the model, unlike previous algorithms. Including serum ferritin in the model avoids any errors that can arise when adjusting for serum ferritin using another equation.

Of the different factors considered in this algorithm, serum ferritin had the strongest effect in the model. It was not surprising to see that the effect of dietary factors was minimal, because even with a single meal study in which dietary factors were accurately measured in the laboratory, only 16% of variation in absorption was explained by dietary factors after adjusting for serum ferritin (4). Cook et al. (5) also showed that

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**FIGURE 1** Diagnostic plots for the complete diet-based nonheme iron absorption model. Residual plot of standardized residuals compared with fitted values (A) and a plot of observed percent absorption in the natural log against the fitted values (B).

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**TABLE 2** Summary for mixed model for predicting nonheme iron absorption (percentage)1

<table>
<thead>
<tr>
<th>Effect and group /factor</th>
<th>Coefficient</th>
<th>SE</th>
<th>t-value</th>
<th>P value</th>
<th>Partial R², %</th>
</tr>
</thead>
<tbody>
<tr>
<td>ID (intercept)</td>
<td>—</td>
<td>0.07</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Residual</td>
<td>—</td>
<td>0.07</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

**Fixed effects**:

- Intercept: 6.294 (t = 1.04, SE = 0.06, P < 0.0001)
- Ferritin, μg/L: -0.709 (t = -6.88, SE = 0.0001, P = 35.30)
- Ascorbic acid, mg: 0.119 (t = 1.62, SE = 0.11, P = 0.79)
- MFP, g: 0.006 (t = 0.28, SE = 0.78, P = 0.03)
- Tea, cups: -0.055 (t = -1.28, SE = 0.20, P = 0.09)
- Phytate, mg: -0.247 (t = -1.70, SE = 0.09, P = 0.84)
- Calcium, mg: -0.137 (t = -1.49, SE = 0.14, P = 0.01)
- Nonheme iron, mg: -0.083 (t = -0.50, SE = 0.62, P = 0.81)

1. ID, participant identification; MFP, meat, fish, and poultry.
2. All fixed-effect variables were log transformed using natural log.

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**Discussion**

The magnitude of the global problem of iron deficiency makes it important to determine means of estimating the percentage of iron absorbed, particularly from nonheme sources, because they provide the main sources of iron in many developing countries. Accurately estimating iron absorption is also essential to set dietary recommendations. Because iron absorption from single meals exaggerates the effect of dietary factors, there is concern about the use of algorithms based on single meals to estimate iron absorption from a whole diet. By overestimating absorption, we risk underestimating the prevalence of inadequate iron intakes. This is one reason why development of an algorithm based on complete diets that can accurately predict iron absorption is critical.

The algorithm we propose was developed from complete diet datasets that include a wide range of intake of the dietary factors known to affect nonheme iron absorption and therefore should reflect the consumption pattern of many populations. Maximum intakes for most dietary factors were extremely high due to the
in the whole diet, the effect of dietary factors is dampened, supporting our findings. Among the dietary factors, only phytic acid was weakly significant in the model ($P = 0.09$). Including higher forms of inositol phosphates (hexa and penta inositols) could have given a better model, because they have a strong iron-binding capacity; however, these are not available in food composition databases. Because the aim of our study was to provide a more convenient model for use in population studies rather than one that requires laboratory analysis, total phytate content was used in developing the model.

Although polyphenols have a strong inhibitory effect on iron absorption, they did not have much effect in our model. Although it would have been more appropriate to use polyphenol content instead of black tea equivalents, doing so would make the use of our model more demanding, because foods and beverages have different forms of polyphenols. Besides, there are different methods for measuring polyphenols and the results differ from method to method. Studies have reported polyphenol content as gallic acid, catechin, or tannin equivalents, which makes it more difficult to compare the results. To avoid difficulties related to type and method of polyphenol analysis, we included in the manuscript a conversion factor for different beverages to black tea equivalents. The conversion factor takes into account the polyphenol contents of the different beverages and is easy to use in the population studies. Based on previous studies showing the effect of different types of beverages on iron absorption (13), it is reasonable to estimate polyphenols using black tea equivalents rather than absolute amounts. Tseng et al. (19) also used tea consumption on weight basis instead of polyphenol content in their model for assessing nonheme iron absorption.

Better prediction of absorption can be obtained if the factors such as tea, calcium, and ascorbic acid are consumed together with meals. For example, Morck et al. (20) and South et al. (21) showed that tea had to be consumed with the meal to inhibit iron absorption. Also, dairy products as sources of calcium may be consumed separately from the meal, thus minimizing their effect on nonheme iron absorption (22). This might explain why phytic acid was the only dietary factor that was significant (albeit weakly), because it is mostly a component of the iron-containing meal and can therefore chelate nonheme iron to reduce its bioavailability. We did not find an effect with MFP in our model, which supports the findings by Reddy et al. (6) that the contribution of heme iron by MFP is more important in improving iron status than in promoting nonheme iron absorption.

When we validated our proposed algorithm with complete diet and single meal studies data, the $R^2$ values were 0.84 and 0.57, respectively. These results suggest that this algorithm can be used to predict absorption of nonheme iron for both complete and single meal studies. The fact that the algorithm was better able to predict for the complete diet than for the single meal datasets was not surprising, because it was developed from complete diet datasets. To compare our algorithm with the 3 most recently developed algorithms (2–4), we predicted nonheme iron absorption using complete diet data (9,14–17) and each of the different algorithms. These algorithms gave lower $R^2$ values [0.64, 0.69, and 0.72 for the Rickard et al. (2), Reddy et al. (4), and Hallberg and Hulthén (3) algorithms, respectively]. Further, some of the predicted absorption values arising from the Reddy et al. (4) and Hallberg and Hulthén (3) algorithms exceeded 100%, as mentioned above. We observed an average overestimation of nonheme iron absorption of 1.87% by our current model, which can be in part attributed to not getting complete information from the published studies, especially ferritin values. If we exclude the data for which mean ferritin for groups were not reported, the average overestimation was reduced to 1.25%. To accurately estimate nonheme iron absorption using our proposed algorithm, we recommend the use of mean daily intake of nutrients estimated from ≥3 d of dietary records and serum ferritin concentration, preferably individual participant values.

Despite the fact that most of the dietary factors did not significantly contribute in explaining variation in absorption, at least based on the data used for this algorithm, we included them all in the model to adjust for any difference in their intakes among participants. Our study also shows that between-person differences contribute to the variations in iron absorption. In a recent review by Hurrell and Egli (23), it was indicated that besides iron status, other host factors such as inflammation and obesity may play a significant role, which supports our observation of high variability in absorption. Further studies are needed to explore other unknown nondietary factors affecting iron absorption.

One limitation of this algorithm is that all 4 of the datasets used were obtained in the United States. However, the diets included wide ranges of intakes with dietary modification, which makes the algorithm applicable to other countries. In conclusion, we have developed a user-friendly model for estimating nonheme iron absorption from the complete diet. This model can predict nonheme iron absorption with high accuracy and will be useful in estimating iron absorption among populations.
Acknowledgments
S.M.A., A.C., and M.B.R. designed the project; S.M.A. performed the statistical analysis; J.D.C. was involved in design of the human studies and is a coauthor of the human study data that we used for developing the algorithm; D.S. was responsible for estimating phytate content; S.M.A., A.C., and M.B.R. wrote the paper; and M.B.R. had primary responsibility for the final content. All authors read and approved the final manuscript.

Literature Cited