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Algorithms to Assess non-Heme Iron Bioavailability

Manju B. Reddy

Department of Food Science and Human Nutrition, Center for Designing Foods to Improve Nutrition, Iowa State University, Ames, IA 50011

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Abstract: While sufficient information exists on the effect of individual factors on iron absorption, their net effect in a mixed meal is less well characterized, being dependent on the combination and quantity of the factors present in the meal. Over a period of more than 25 years, several models have been developed to estimate non-heme iron bioavailability, either to assess iron absorption from a meal or iron sufficiency in populations. Initially, a model was developed to calculate iron absorption in individuals with varying iron status that included only enhancers. This model was useful in classifying the diets but has limited value for accurale assessment. Later models were modified and improved by including inhibitors in the calculations. However, some included either phytate or tea but not in combination. The models that included all the factors in calculations assumed their effect was independent and additive rather than interactive, which is an important issue in addressing iron bioavailability. Although some of the models correlated estimated bioavailability with iron status of the population, the accuracy of the estimations is of concern due to lack of quantitative measurements of bioavailability modifiers, inability to consider interactive effects, and the use of non-iron status measurements. Recent research has led to the development of refined models to assess iron bioavailability of complex meals by comprehensively taking into consideration the interactive effect among enhancers and inhibitors. However, the models are based on single-meal studies and their application to whole diets at a population level is not clear. Accurate measurements of dietary factors and independent validation are needed before using these models. To date, no single model is applicable to all diets and additional studies are needed to develop new models to predict bioavailability of whole diets accurately, in addition to addressing dietary adequacy in all populations.

Key words: Iron, absorption, bioavailability, algorithm, human

Introduction

Iron absorption is dependent not only on the amount of the iron in the diet but also on its bioavailability. The majority of iron in the diet exists as non-heme iron in populations where iron deficiency is prevalent [1]. The amount of non-heme iron absorbed depends greatly on the composition of the meal with which it is consumed [2]. Non-heme iron absorption is mainly enhanced by animal tissue [3, 4] and ascorbic acid [4–7]; and is inhibited by phytate [7, 8], polyphenols in tea and coffee [9, 10], and calcium [11, 12]. There is insufficient evidence to demonstrate that iron absorption can be improved by increasing the quantity of enhancers alone in the diet. For example, if the diet has high levels of inhibitors, the enhancing effect of ascorbic acid on iron absorption may be minimal. It is important to consider the interaction of all the above bioavailability modifiers in order to assess their net effect on non-heme iron bioavailability.

Considerable data have accumulated over the years since the extrinsic tag method was introduced [13] on the effect of individual factors on non-heme iron absorption in humans. Because of the many interactions that may occur simultaneously, the net effect of the various combined factors in a meal is not equal to the sum of the individual factors [14]. However, a limited number of combinations

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have been tried in human studies to study the interaction of those factors. Because of cost and complexity, it is impractical to depend on human absorption studies to screen a number of food combinations. *In vitro* methods, using either dialyzability [15] or the Caco-2 cell model [16, 17], offer rapid methods to screen bioavailability. However, direct extrapolation of the *in vitro* results to the *in vivo* situation is not always possible.

Based on accumulated data, many researchers have attempted to predict iron bioavailability from the meal composition and relate it to the absorption or the population iron status (Table I). Earlier models [18–20] qualitatively classified the diets and later models were developed to quantitatively estimate iron bioavailability. If consideration is given to interactive effects, these bioavailability models are very useful for assessing diets and providing dietary recommendations without the need for directly measuring iron absorption. Since bioavailability is not a concern for heme iron, this review deals primarily with the algorithms that are available for estimating non-heme iron bioavailability. The discussion begins with general models, followed by more specific models for iron bioavailability calculations.

Models with Enhancers

More than two decades ago, based on the available information on the enhancing effect of ascorbic acid and meat as well as on the inverse relationship of iron absorption and body iron stores, experts in the iron nutrition field collaborated to publish the classic model to estimate heme and non-heme iron absorption [18]. Since heme forms a separate pool and is affected by iron status but largely not by dietary factors, its absorption estimations were based only on iron stores. Heme and non-heme iron absorption was estimated in individuals with iron stores of 0, 250, 500, and 1000 mg, respectively. For the non-heme iron bioavailability model, meals were classified into three levels based on the additive effect of meat, fish, and poultry (MFP), and ascorbic acid. Low (3%), medium (5%), and high (8%) meals represent < 30 g MFP or < 25 mg ascorbic acid, 30 g MFP or 25–75 mg ascorbic acid, and > 90 g MFP or 75 mg ascorbic acid, or 30–90 g MFP plus 25–75 mg ascorbic acid, respectively. These absorption values were based on individuals with 500 mg body iron, reflecting a normal iron status of 50 µg/L of serum ferritin.

Later, Monsen and Balintfy [19] refined this model for computerized calculations to estimate iron absorption in individuals with normal iron stores. Thus, based on the concept of 1 mg of ascorbic acid equivalent to 1 g of cooked MFP, the calculations are provided for estimating non-heme iron absorption with 1–75 units of enhancing factors (EF = MFP + ascorbic acid). If EF factors were 0 or > 75, absorption was assumed to be 0 or 8%, respectively.

Similarly, FAO/WHO [20] also categorized the diets as low, medium, and high bioavailable diets, with absorption of 5, 10, and 15% respectively in individuals with no iron stores but with normal iron transport, based on the consumption of meat and ascorbic acid-rich foods. This model is useful for classifying diets qualitatively but no data are available to validate the results in relation to iron status of populations.

The enhancer models were developed using the limited data available at that time on the effect of factors and have several limitations. For example, the inhibiting factors such as phytic acid and polyphenols were not considered in calculations and may therefore cause an overestimation of absorption in a meal containing these inhibiting factors. Heme absorption values were well accepted previously, but the study showing the inhibiting effect of calcium on heme absorption raises some concerns [12]. Hence, this model is not applicable for many populations that consume a very low meat- or ascorbic acid-containing diet. Several researchers modified models by including inhibitors to estimate iron bioavailability in the diets of many countries [21–23].

Model with Enhancers and Tea

Based on the limitation of using only enhancers in the algorithms, later studies qualitatively incorporated the effect of tea. Using NHANES I data collected during 1971–1974, Singer *et al* [21] attempted to calculate bioavailable iron using a combination of Monsen and FAO/WHO [18, 20] methods and related it to iron status of the US population. Bioavailable iron was calculated based on the amount of MFP protein, ascorbic acid, and tea consumed that was obtained by a 24-hour recall method. Initially, iron bioavailability was classified as low (25 mg ascorbic acid or 6 g MFP protein), medium (25–75 mg ascorbic acid or 6–18 g MFP protein), or high (> 75 mg ascorbic acid or > 18 g MFP protein). If more than 225 g tea was consumed with the meal, the bioavailability classification was dropped one category unless it had been previously classified as low. Adjusting total iron intake for bioavailability did not provide any additional advantage to explain low hemoglobin concentrations. No association was found between iron status (hemoglobin and transferrin saturation) and total or bioavailable iron intake in women (12–54 years) as well as in older persons (65–74 years) after accounting for socioeconomic and demo-

Table I: Published studies on algorithms to calculate nonheme iron bioavailability

Study	Dietary Factors Considered	Objective	Limitations
Monsen *et al*, 1978 Monsen and Balintfy, 1982[18, 19] (published information)	Meat, fish, poultry (MFP) and ascorbic acid	Classified meals low, medium, and high bioavailability	MFP effect was considered additive; inhibitors' effect was not included
FAO/WHO, 1988[20] (qualitative assessment)	Enhancers (MFP and ascorbic acid) and tea/coffee	Classified meals low, medium, and high bioavailability	Qualitative classification based on type of food intake
Singer *et al,* 1982[21] (24-h recalls, U.S. diets)	Animal tissue protein, ascorbic acid, and tea	Classified meals low, medium, and high bioavailability; related bioavailability to iron status (transferrin saturation and hemoglobin)	Phytate and coffee's effect and the type of tea consumption was not considered; Specific indicators for iron status were not used
Cook *et al,* 1991[24] (diet records, U.S. diets)	MFP, ascorbic acid, coffee, tea, bran, and eggs	Diets were scored; bioavailability scoring was related to measured iron absorption	Lacks quantitative data; Interaction of bioavailability modifiers was not considered
Tseng *et al,* 1997[22] (24-hour recalls, Russian diets)	MFP, ascorbic acid, phytate, and tea	Iron bioavailability was compared in rural and Urban areas	Interactions of bioavailability modifiers were not considered; Bioavailability was not validated with iron status
Du *et al,* 2000[26] (24-hour recalls, Chinese diets)	Ascorbic acid, animal source foods, vegetables, fruits, rice, beans, and tea	Bioavailability was compared with various algorithms and related to hemoglobin	Interactions of bioavailability modifiers were not considered; Lacks quantitative data on the bioavailability modifiers and specifications on the type of foods
Hallberg *et al,* 2000[27] (quantitative measurements)	MFP, ascorbic acid, phytate, polyphenols, calcium, soy protein, alcohol, and eggs	Continuous effect of factors on iron bioavailability was calculated and compared with measured single meal and absorption (published own data)	Developed based on single-meal absorption studies; Accurate and specific methodology are needed for measuring bioavailability modifiers; Needs validation with independent data
Reddy *et al,* 2000[28] (quantitative, US diets)	MFP, ascorbic acid, calcium, polyphenols, nonheme iron and phytate	Multiple regression for the interactive affect	Developed based on single meal absorption studies; Accurate measurements of the bioavailability modifiers are needed; Not applicable to vegetarian diets; Needs validation
Bhargava *et al,* 2001[23] (24-hour recalls, Bangladeshi diets)	MFP, ascorbic acid and phytate	Bioavailability with enhancers alone and with phytate; related hemoglobin	Interactions of bioavailability modifiers were not considered; Not applicable to tea consuming populations

Numbers in the superscripts represent the references.

graphical variables. Health-related variables had a more significant effect on iron status than dietary intake, suggesting that bioavailability may play a minor role in explaining the differences in iron status in this population. However, this study examined only hemoglobin and transferrin saturation as iron status measurements and if other measurements such as ferritin and transferrin receptor had been included, different results might have been expected. Overall, the limitations of this study include non-specific indicators of iron status, lack of reliability of 24-hour recalls for meal composition, failing to account for the effect of type or strength of tea, and, most importantly, not accounting for the effect of phytic acid, a major inhibitor of iron absorption.

Model with Enhancers and Phytic Acid

An encouraging relationship was found between iron status and bioavailability when algorithms were modified taking into account individuals with no iron stores and including phytic acid with the enhancers. Bhargava *et al* [23] refined the existing models [18, 19, 22] for assessing iron bioavailability and related the bioavailability to iron status in Bangladeshi women. This model used calculations for individuals with no iron stores for calculating iron bioavailability and introduced a correcting factor in Tseng's model [22] to avoid an overestimation with low phytate intakes. In repeated observations with 24-hour dietary recalls in 514 women, bioavailable iron and supplemental iron use were found to be significant contributors of hemoglobin status. The results of this study suggest the importance of considering body iron stores, and the need to develop suitable algorithms for subjects living in developing countries. The bioavailable iron contribution in explaining iron status was higher when phytate was included in the algorithms compared to enhancers alone [18, 19]. The calculations estimated that doubling bioavailable iron (from 0.2 to 0.4 mg) would increase hemoglobin by 3.6% in the population studied, indicating the importance of bioavailability in determining iron status. The results from a previous study [21], showing no relationship between bioavailability and iron status, do not agree with this study. This may be due to differences in the use of inhibitors in calculations. Although the results of this study are encouraging and show the importance of bioavailability on iron status, it has limited advantages in calculating bioavailability in tea/coffee-consuming populations. The reliability of phytate estimations from the databases is also questionable.

Model with Enhancers and Phytate Plus Tea

By utilizing existing models [18, 19], a more recent study developed algorithms to estimate iron absorption by including the independent effect of tea and phytic acid. Using the data obtained from the Russian Longitudinal Monitoring Survey with 9,890 women and children, computerized calculations were developed by Tseng *et al* [22] to assess bioavailable iron in rural and urban Russian diets. From dietary intakes estimated from the 24-hour recalls, non-heme iron bioavailability was adjusted for MFP, ascorbic acid, phytate, and tea intakes. In this model, non-heme iron absorption was adjusted for the amount of enhancers [18, 19], which ranged from 3–8%. The amount of bioavailable iron was further adjusted for phytate using the dose-response relationship described by Hallberg *et al* [7]. Further adjustment was made based on the amount of tea consumed (40% reduction with 225 g tea). Iron bioavailability, estimated after adjusting for enhancers and inhibitors, was lower than the 25th percentile of the requirements across all the age groups. The authors compared the bioavailability of the rural and urban Russian diets, but no evidence was provided on the relationship of bioavailability with iron status. In addition, the model appears to correct for phytate and tea individually, however the computerized program included in the paper suggests that tea correction was made after phytate adjustment. Although it is not as strong as tea, coffee is also known to inhibit iron absorption [24] but was not included in the study. A major limitation of the study is the assumption of an additive effect of phytate and polyphenols and the order of the correction. For example, if the meal had a high polyphenol content, additional phytate may not have exerted additional inhibition. This model would have been useful if the bioavailability had been validated with iron status.

Models with Enhancers and Inhibitors

Food Composition Data

Two studies have reported iron bioavailability estimations based on the food composition data, either from diet records [25] or from 24-hour recalls [26]. Both studies utilized individual foods that are known to contain inhibitors in their models rather than the absolute amount of individual components such as phytic acid and polyphenols. A simple qualitative scoring system was developed to rank

the foods for bioavailability and was compared with the measured iron absorption in a group of human subjects [25]. Utilizing a score of 5 for a neutral bioavailability meal, points were either added or subtracted based on enhancers (ascorbic acid and MFP) and inhibitors (tea, coffee, and phytate-rich foods) in the meal. The bioavailability scores, ranging from 1–10 for 28 meals, corresponded well with the measured dietary absorption in the same subjects. The degree of enhancement or inhibition with an enhancing or inhibiting meal was similar when assessed with a scoring system or with single-meal iron absorption measured with radiolabeled iron. Although this study incorporated many inhibitors and has a practical application, it does not take into account the interactive effect of enhancers and inhibitors or the variation in inhibitor concentrations in prepared/processed foods.

Another practical approach was taken to calculate iron bioavailability based on the types of foods consumed that are known to contain inhibiting factors and related it to the prevalence of anemia. By using the 3rd Chinese National Nutrition Survey consumption data containing information from 24-hour recalls plus food inventory with a large number of subjects, Du *et al* [26] developed a model for calculating iron bioavailability from Chinese diets. Despite the prevalence figure of 18% anemia in the Chinese population, a total iron intake of 177% of Chinese recommended daily allowance (RDA) was observed, which clearly underscored the importance of bioavailability rather than total iron intake. Non-heme iron absorption was calculated based on enhancing factors (ascorbic acid and animal tissue, vegetables, and fruits) and inhibitors (rice, beans, and tea). When compared with other methods for examining the bioavailability relationship with hemoglobin status, it was reported that this method was more sensitive and specific in predicting anemia in Chinese population. Although the method is simple and the bioavailability shows a significant relationship with hemoglobin due to a large sample size, caution should be taken for the use of food consumption data, which does not reflect accurate intakes. For example, nutrient content, especially ascorbic acid content, may vary between cooked versus raw vegetables. The amount of rice included in the model is not specified (brown or white), which may have varied phytate content. Similarly, the type of beans and tea were not specified in the calculations. As in the previous study [23], this algorithm has a practical value in relating iron status with bioavailability in the Chinese population but may not be applicable to other populations.

Biochemical Composition Data

Studies that use food composition data, including the comprehensive list of inhibitors and enhancers, seem to be appealing for practical use. Since the effect of enhancers and inhibitors were considered independent but additive when present together, the accuracy of absorption estimations is questionable. The effect of enhancers and inhibitors may vary with food preparation methods or is dependent on their combinations present in the same food. For instance, there is enough evidence to show that polyphenols in tea affect iron absorption but the preparation and type of tea are also important factors in dictating the amount of absorbable iron [10]. In a human study, it has been shown that 5% strength (3 g tea in 300 mL of boiling water is 100% strength) black tea significantly inhibits iron absorption by 79%, compared to a 94% reduction with 100% strength tea. With identical polyphenol concentrations, black tea was more inhibitory than herbal teas or cocoa, suggesting the importance of type of polyphenols to be considered for iron bioavailability estimations. These findings strongly suggest the importance of preparation of tea and type of tea in iron absorption. Although previous models [22, 23] have used the dose-response inhibition effect of phytate, which was developed based on a simple bread roll meal [7], we cannot expect a similar relationship in mixed meals containing both enhancers and inhibitors. Equal amounts of phytate (300 mg) have shown different degrees of inhibition in human absorption when the protein source was varied. In contrast to egg albumin and soy protein, meat was shown to have a partial counteractive effect on phytate [8]. Similarly, ascorbic acid was also shown to counteract the phytate's inhibition [7]. The food matrix also may have an effect on iron absorptionsince the enhancement of meat varied from 1.1- to 3.2-fold when tested in human subjects fed with either corn meal, baladi bread, or a regular standard meal [3, 4]. Ascorbic acid also was shown to have a pronounced effect in vegetarian diets compared with non-vegetarian diets. Studies conducted in two independent labs have shown that ascorbic acid can enhance iron absorption 3- to 4-fold in a vegetarian diet compared to a < 2-fold increase in a meat-containing diet [5, 6]. Hence, it is important to consider quantitative amounts of enhancing and inhibitory factors and their interactive effect, which was considered by two research groups [27, 28] in developing algorithms for accurately calculating bioavailability. These two studies had used single-meal human absorption data to develop the algorithms. Based on the exaggerating effect of inhibitors and enhancers [29] seen in single meals compared to whole diets, caution should be used when employing these models for assessing iron bioavailability of whole diets and addressing dietary adequacies at a population level.

By including all of the enhancers and inhibitors, and their interactions that are known to date, Hallberg *et al* [27] developed a comprehensive algorithm to assess iron

bioavailability. These researchers utilized the wealth of information, collected over many years, on iron absorption from single-meal studies (from their own research as well as from other published studies) to develop a model to calculate iron bioavailability. Dietary absorption from a meal was determined based on iron status, heme and non-heme iron contents, and the quantity of various factors that influence iron absorption. The algorithm was based on the basal absorption from a wheat roll made with low-extraction flour containing no known inhibitors or enhancers, and was adjusted to a reference dose absorption of 40%, which corresponds to an individual with a serum ferritin value of 23 μg/L. Initially, equations were developed to determine the dose effect and interactive effect of individual factors that were considered necessary based on the available information. An absorption ratio was obtained for the effect of each factor and every value was multiplied with the basal absorption of 22%. When the effect of ascorbic acid was determined, they took into consideration the interaction between phytate and ascorbic acid. Similarly, the effect of polyphenols was determined by the individual dose-response effect as well as the interactive effect with ascorbic acid.

The enhancing effect of MFP was determined alone as well as in the presence of phytate. However, the interaction of polyphenols or ascorbic acid with MFP was not considered. It is not clear whether MFP can counteract the strong inhibiting effect of polyphenols. In an ascorbic acid-rich or MFP-rich meal, the effect of MFP and ascorbic acid may be exaggerated in these calculations. Although the evidence is not clear on the inhibiting effect of calcium, its effect was included in the algorithms along with soy protein, alcohol, and eggs. The authors attempted to validate the algorithms using their own data on single-meal absorption studies conducted 15 years ago but food analysis was performed at the time of calculations with the new ingredients. Despite these limitations, a very significant correlation was obtained between estimated and measured absorption. A similar agreement was also found between iron absorption measured in four different meals in a 5-day period. However, these agreements were made with their own data, some of which was used to develop algorithms. To be able to use these algorithms we need accurate measurements of the factors that affect iron absorption, and this creates difficulties with its practical application. Simplifying the calculations and independent validation from other researchers' data may strengthen the wider application of this model.

Using a regression model to account for all possible interactions, Reddy *et al* [28] published a study to estimate iron bioavailability based on single-meal absorption and the meal composition. Twenty-five meals were fed to groups of subjects and iron absorption was measured using a standard extrinsic radiolabeling technique [13]. The factors known to affect iron absorption (MFP, ascorbic acid, calcium, polyphenols, non-heme iron, and phytate) were measured in the laboratory or obtained from the food composition tables. Individual absorption values were adjusted to a common ferritin value to account for the confounding effect of iron status. Multiple regression analysis was used to determine the significant determinants of adjusted iron absorption. After taking into account for inter-correlations, 16.4% of the variability in absorption was explained by three factors: MFP, phytic acid, and ascorbic acid. The following equation was reported for estimating iron absorption:

ln absorption % = 1.9786 + (0.123 × animal tissue, g) – (0.0034 x phytic acid, mg) + (0.0065 ×x ascorbic acid, mg).

ln absorption = Absorption adjusted to a serum ferritin of 30 μg/L based on Cook *et al* [25] correction.

Although this study utilized meals that have a varied content of enhancers and inhibitors, it should be noted that they are representative of the US diets. This regression model was not developed for vegetarian meals. It is possible that ascorbic acid may show a higher contribution in explaining iron absorption in a meal containing no meat. This method can have a practical application as long as MFP, ascorbic acid, and phytic acid are all estimated accurately from the food tables. Because the model is also developed on absorption data from single meals and not validated with independent studies, it is difficult to assess the accuracy of the predicted absorption values.

Summary

Although many models have been developed for estimating iron bioavailability that are useful for addressing iron adequacy issues in various regions or age groups [21–23], their application is limited because they will be appropriate only to the populations from which the algorithms were developed. Accuracy in bioavailability estimations is also of concern since the interactions of the bioavailability modifiers have not been taken into consideration. Although validation is of concern, two recent models [27, 28] may be useful for estimating iron bioavailability from single meals, provided accurate information is available and the same methods are used for measuring the dietary factors. In an attempt to validate the Hallberg *et al* model [27] with independent data, meal composition information was used from the study of Reddy *et al* [28] to calculate bioavailable iron. It should be pointed out that the

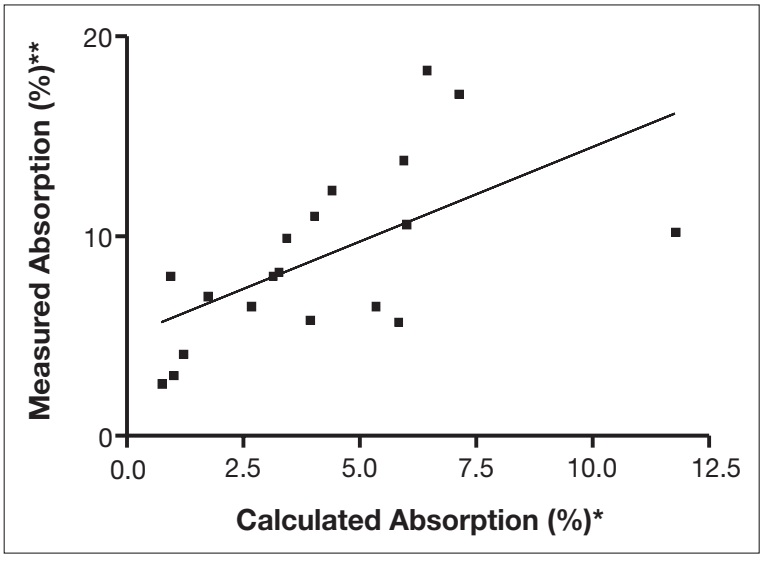

Figure 1: Correlation between measured [28] and calculated non-heme iron absorption [27]. Data represent the mean values for 19 meals. $R^2 = 0.36$; $p < 0.01$.

* Adjusted to 40% reference dose (reflects serum ferritin of 23 ug/L)
** Adjusted to serum ferritin of 30 µg/L

methods used were not the same for the determination of phytic acid and polyphenols, nor were the corrections for iron status. Despite these limitations, a significant correlation was found between measured and calculated absorption (Figure 1, $R^2 = 0.36$; $p < 0.01$) for the 19 meals for which there was information on the factors required for calculations. However, for a specific measured absorption value, there was a 2- to 9-fold variation in the calculated absorption, which makes it difficult to interpret the results. Overall, the calculated absorbed iron is generally lower than measured absorption. It is possible that the calculated absorption was over-corrected for inhibitors. For example, calcium may inhibit iron absorption in isolation, as was shown with a bread meal (12), but does calcium have the same level of inhibition in the presence or absence of polyphenols and/or phytate? Since polyphenols and phytate are potent inhibitors of iron absorption, calcium may not inhibit absorption further if these inhibitors are present in the same meal. Another limitation of these models is for their usefulness for single-meal iron absorption estimations but not for dietary availability estimations, the latter of which ultimately dictates iron status. Based on the variation found in calculated bioavailability even with accurate estimations of bioavailability modifiers, this model can be of less practical value when used with dietary 24-hour recalls that are commonly used at a population level. Additional studies may be warranted for developing algorithms for estimating dietary iron bioavailability and validating these algorithms with the iron status of populations.

In conclusion, to date, the information available on algorithms for calculating iron bioavailability from complex meals or from the whole diet is limited. The refined models that have used human absorption data will be useful for estimating single-meal iron absorption, as long as accurate measurements of dietary factors are available. These models however, have limited use for whole diets or for addressing the adequacy of dietary iron in large population groups. On the other hand, the available algorithms addressing iron adequacy in diets are useful for specific population groups, but no evidence exists thus far on how accurately they can estimate bioavailable iron. Future studies are needed to validate more recent models, either with absorption or with iron status data, to develop a universal algorithm applicable to all populations.

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Manju B. Reddy

Department of Food Science and Human Nutrition,
Center for Designing Foods to Improve Nutrition,
Iowa State University, Ames, IA 50011
Phone: (515) 294-2024
Fax: (515) 294-5390
E-mail: mbreddy@iastate.edu.