Estimation of nonheme-iron bioavailability from meal composition[1–3]

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ABSTRACT

Background: Considerable data are available on the individual effects of dietary factors on nonheme-iron absorption, but their combined effect when they are present in the same meal is not known.

Objective: Our objective was to predict the bioavailability of iron from complex meals that are consumed commonly in the United States on the basis of the contents of factors that are known to promote or inhibit food iron absorption.

Design: Radioisotopic measurements of nonheme-iron absorption from 25 meals were made in 86 volunteer subjects by using extrinsic radioiron labeling. The meal contents of nonheme iron, calcium, ascorbic acid, polyphenols, and phytic acid were determined by biochemical analysis; energy and protein contents were estimated from food-composition tables. Animal tissue content was based on weight or was obtained from the manufacturer.

Results: After adjusting iron absorption for differences in iron status, the significant biochemical predictors of iron absorption as determined by multiple regression analysis were the contents of animal tissue ($P = 0.0001$), phytic acid ($P = 0.0001$), and ascorbic acid ($P = 0.0441$). Collectively, these 3 variables accounted for 16.4% of the variation in absorption. On the basis of the multiple regression analysis, we developed the following equation to estimate iron absorption: Ln absorption, % (adjusted to serum ferritin concentration of 30 μg/L) = 1.9786 + (0.0123 × animal tissue in g) − (0.0034 × phytic acid in mg) + (0.0065 × ascorbic acid in mg).

Conclusion: For the 25 meals evaluated, only the contents of animal tissue, phytic acid, and ascorbic acid were useful for estimating nonheme-iron absorption. *Am J Clin Nutr* 2000; 71:937–43.

KEY WORDS Iron, bioavailability, absorption, biochemical composition, nonheme iron, phytic acid, phytate, ascorbic acid, vitamin C, animal tissue content

INTRODUCTION

The large and expanding list of dietary factors that affect the bioavailability of food iron has made it increasingly difficult to predict iron absorption from complex meals (1–3). Because of their cost and complexity, it is impractical to rely on radioisotopic measurements of iron absorption in humans to evaluate the unlimited number of possible food combinations in meals. One approach to addressing this problem has been the development of in vitro measurements of food iron bioavailability, such as dialyzable iron (4) or iron uptake in the Caco-2 cell culture model (5). However, these techniques have been more useful for assessing the effects of isolated determinants than for evaluating the overall iron bioavailability of a complex meal.

Considerable data have accumulated over the years from studies that reported the effects of individual dietary factors on iron absorption. The facilitating effects of animal tissues and ascorbic acid have been known for several decades and form the basis for a widely used model for estimating nonheme-iron absorption (6). Recently, vitamin A and β-carotene have also been shown to enhance nonheme-iron absorption in humans (7). A variety of factors that inhibit food iron absorption have been identified; these include phytic acid (8–11), polyphenols (12, 13), phosphorus (14), and calcium (14–16). Earlier studies indicated that dietary fiber has an inhibiting effect on iron absorption (17, 18), but this finding was later attributed to its phytate content (19). Most of the studies concerning the effects of these inhibitors have been performed with single food items and there is scant information about their influence in complex meals or their relative potency in the presence of other facilitating or inhibiting factors.

The purpose of the present investigation was to determine whether analyses of the primary determinants of food iron availability could be used as a practical guide for estimating the amount of nonheme iron absorbed from meals containing several food items. In human subjects, we measured nonheme-iron absorption from 25 widely consumed convenience meals that contained a wide spectrum of ingredients. The results were correlated with 6 meal constituents measured in fresh or lyophilized portions of each meal.

SUBJECTS AND METHODS

Subjects

Nine separate studies were performed with a total of 86 subjects. Each study group included 8–10 subjects, with the exception of one study that involved 15 subjects. The composite group

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included 52 men and 34 women ranging in age from 19 to 40 y, with an overall mean age of 26 y. The geometric mean serum ferritin concentrations in the study groups ranged from 28 to 77 μg/L, with a composite mean of 41 μg/L. Eleven of the subjects had iron deficiency, as defined by a serum ferritin value < 12 μg/L, but none were anemic. All subjects were healthy and denied a history of disorders known to influence the gastrointestinal absorption of iron. Written, informed consent was obtained from each volunteer before the study. All experimental procedures were approved by the Human Subjects Committee of the University of Kansas Medical Center.

Absorption measurements

Four separate iron absorption tests were performed in each subject by using dual ^{59}Fe and ^{55}Fe radioisotopes sequentially. Because iron absorption tests were performed for unrelated investigations in some studies, absorption data reported here were obtained from 1–4 meals in any particular group of subjects. All meals were consumed between 0700 and 0900 after subjects had fasted for 10 h. Subjects were permitted to drink only water for 3 h after consuming the test meal, after which they were allowed to resume their regular diets. On the day before they consumed the first test meal, we obtained 30 mL blood for the measurement of background blood radioactivity, packed cell volume, and serum ferritin concentration. On the next 2 d, test meals tagged with either 74 kBq ^{55}Fe or 37 kBq ^{59}Fe were consumed. Fourteen days after the second test dose, the subjects returned to the laboratory and a fasting blood sample was obtained for measurement of incorporated red cell radioactivity. The third and fourth test meals, tagged with ^{59}Fe or ^{55}Fe, were consumed on 2 successive days. Subjects returned 14 d after the final test meal so we could obtain blood to measure the increase in red cell radioactivity.

All test meals were labeled extrinsically by adding 1 mL 0.01 mol HCl/L containing 0.1 mg Fe as $FeCl_3$ plus the required amount of radioactive iron. The extrinsic label was mixed with the entire meal when studying frozen meals and was added to the bread for meals that included a sandwich. All measurements of blood radioactivity were performed on duplicate 10-mL samples of whole blood by using a modification of the method of Eakins and Brown (20). Absorption was calculated by using blood volume estimated from the height and weight of each subject (21, 22). Incorporation of absorbed radioactivity was assumed to be 80% in all subjects (23).

Because of the marked influence of iron stores on iron absorption and the appreciable differences in iron status among the subjects, a method was required to adjust absorption data obtained for each subject to a common amount of iron stores. Dietary iron absorption by each subject was adjusted to a value corresponding to a serum ferritin concentration of 30 μg/L by using the following equation, which was reported by Cook et al (24):

$$\text{Ln [Adj Abs (\%)]} = \ln [A_o\ (\%)] + \ln [F_o\ (\mu g/L)] - \ln [30\ (\mu g/L)] \quad (1)$$

where Adj Abs is adjusted dietary nonheme-iron absorption, A_o is measured nonheme-iron absorption, and F_o is observed serum ferritin. Mean absorption values for each meal were calculated for both measured and adjusted values.

Test meals

Twenty-five meals were selected to represent a wide spectrum of meal ingredients. To facilitate preparation and serving of meals to an entire study group, 17 meals were chosen as frozen dinners that were purchased from the supermarket. The dinners were prepared in a microwave oven according to the manufacturers' instructions. Absorption measurements were repeated with added tea or coffee for 4 of these meals (meals 11, 12, 16, and 17). Meals 18–21 were purchased from fast food restaurants on the mornings of the days when they were consumed and were reheated in a microwave oven before serving. Meal 18 contained an egg and muffin (115 g), cereal (28 g 40% Bran Flakes; Kellogg's, Battle Creek, MI), milk with 2% fat content (240 mL), and brewed coffee (180 mL). Meal 19 contained a biscuit (94 g), sausage (49 g), egg (57 g), hash browns (71 g), orange juice (160 mL), and coffee (240 mL). Meal 20 contained a hamburger with bun (160 g), vanilla milkshake (140 g), and French fries (80 g). Meal 21 contained a fish sandwich (255 g), French fries (120 g), and a soft drink (360 mL). The final 4 meals were prepared in the laboratory. Meal 22 was a breakfast that included cereal (28 g bran flakes; Kellogg's), milk with 2% fat content (180 mL), one slice of enriched whole-wheat bread (20 g; Interstate Brands Corp, Kansas City, MO), butter (5 g), orange juice (180 mL), and coffee (240 mL). Meal 23 was a rice-based dinner prepared by cooking cabbage (20 g), cauliflower (20 g), green peas (85 g), green beans (80 g), soy sauce (30 mL), and vegetable oil (14 g) for 5 min in a microwave oven and then mixing it with cooked, unfortified rice (uncooked weight, 60 g; Riviana Foods Inc, Houston). Meal 24 contained a sandwich prepared with 2 slices of white enriched bread (40 g; Interstate Brands Corp, Kansas City, KS), mayonnaise (5 g), ham (21 g), lettuce (15 g), and tomato (40 g). Meal 25 was the same as meal 24 except that it also contained milk with 2% fat content (240 mL).

Meal composition

The energy and protein contents of all the meals were obtained from computerized food-composition tables (N-squared Computing, Hearst Corporation, San Bruno, CA). These tables provided meal composition for the frozen dinners (meals 1–17) as composite meals. The composition of the remaining meals (nos. 18–25) was determined from the individual components. Animal tissue content (cooked weight) for the frozen dinners (meals 1–17) was obtained from the manufacturers. For meals 18–25, animal tissue (beef, fish, and poultry) content was determined by weighing the components individually.

Meal content of the remaining components (nonheme iron, calcium, ascorbic acid, phytic acid, and polyphenols) was determined by biochemical analysis. All meals were homogenized separately to a creamy consistency in a blender and lyophilized. After it was ground to a fine powder, the freeze-dried sample was placed in an airtight plastic bag and stored at −20°C until analyzed. Ascorbic acid was analyzed in freshly cooked and blended meals that were mixed with 10% metaphosphoric acid to a final concentration of 2%, protected from light, and kept frozen until analyzed.

The nonheme-iron content of each meal was determined by using the modification of the method of Torrance and Bothwell (25). Calcium content was analyzed by atomic absorption spectroscopy for all the meals except nos. 24 and 25, for which calcium content was obtained from the food-composition tables. Phytic acid content was determined by using an anion-exchange method as described by Harland and Oberleas (26). Polyphenol content was measured colorimetrically as gallic acid equivalents by using the Folin-Ciocalteau method (27). First, 300 mg dried sample was extracted 3 times with 10 mL

TABLE 1
Meal composition

Meal number and description		Energy[1]	Protein[1]	Animal tissue[2]	Ascorbic acid	Calcium	Polyphenols	Phytic acid	Nonheme iron
		kJ	*g*	*g*	*mg*	*mg*	*mg*	*mg*	*mg*
1	Potato, broccoli, and cheese	1213	14	0	NA[3]	354	147	168	1.1
2	Macaroni and cheese	1213	15	0	4.4	271	81	190	0.7
3	Chicken and vegetables	1087	20	44	3.8	58	51	123	1.3
4	Turkey, rice, and vegetables	1004	23	54	6.7	27	43	90	0.6
5	Beef enchilada	1171	15	18	67	86	128	102	1.9
6	Steak and potato	1046	21	61	NA	111	78	134	1.1
7	Zucchini lasagne	1087	20	0	9.9	208	157	84	1.8
8	Swedish meatballs and noodles	1213	26	70	4.4	48	58	143	2.1
9	Chicken chow mein	1046	14	42	7.4	22	67	45	0.7
10	Chicken fiesta	1046	21	57	25.5	26	59	108	1.0
11	Beef enchilada and coffee	1171	15	18	67	75	304	102	1.9
12	Chicken chow mein and tea	1046	14	42	7.4	23	224	45	0.7
13	Vegetable lasagne	1757	23	0	NA	589	112	110	1.2
14	Green pepper, beef, and rice	1883	20	37	29.1	59	152	80	2.7
15	Tuna and noodles	837	10	33	3.7	163	25	95	0.3
16	Green pepper, beef, rice, and tea	1883	20	37	29.1	52	362	80	2.7
17	Tuna, noodles, and coffee	837	10	33	3.0	163	224	95	0.3
18	Breakfast no. 1[4]	1933	25	0	NA	512	361	541	11.2
19	Breakfast no. 2[5]	3238	19	49	56.9	101	368	291	3.2
20	Hamburger meal	3243	32	70	24.8	273	167	171	4.0
21	Fish sandwich meal	4992	29	50	19	151	156	317	2.7
22	Breakfast no. 3[6]	1548	13	0	44.3	286	450	282	20.8
23	Rice and vegetables	1929	13	0	13.5	82	137	181	3.0
24	Ham sandwich	841	8	20	4.8	68[1]	NA	15	2.1
25	Ham sandwich and milk	1469	16	20	9.1	355[1]	NA	15	2.1
	$\bar{x}$ ± SD	1635 ± 960	19 ± 6	31 ± 24	21 ± 20	163 ± 115	170 ± 122	145 ± 114	2.9 ± 4.2

[1] Obtained from food-composition tables.
[2] Animal tissue includes beef, poultry, and seafood.
[3] NA: data not available because not enough sample was available for analysis.
[4] Egg, muffin, bran flakes, milk, and coffee.
[5] Biscuit, sausage, egg, hash browns, and orange juice.
[6] Bran flakes, milk, toast, butter, orange juice, and coffee.

acetone:water (4:1) by shaking for 15 min on a rotating shaker. The combined extract was centrifuged at 1125 × *g* for 5 min at room temperature. The acetone was evaporated and the aqueous extract was diluted to a volume of 10 mL with water. For the color reaction, 0.5 mL extract was mixed with 2.5 mL of Folin-Ciocalteau reagent diluted 10-fold and 2 mL of 0.5 mol NaOH/L. The color was read at 760 nm after a reaction time of 18 min at room temperature against a blank containing no reagent. Gallic acid (5–60 mg/L) was used as a standard. Ascorbic acid content was measured by HPLC (Merck-Hitachi System; Hitachi Ltd, Tokyo) according to the method of Sapers (28) and Parvianen and Nyssonen (29) by using a reversed-phase column and photometric detection. Dehydroascorbic acid was reduced to ascorbic acid before analysis by addition of dithiothreitol (Fluka, Buchs, Switzerland).

Statistical methods

The percentage-iron-absorption values were converted to logarithms for statistical analysis and the results were transformed to recover the original units (30). All nutrient values were expressed as the total amounts in each meal. The correlations between nutrients in each meal and their relations to the adjusted percentage iron absorption were determined with Pearson's product-moment correlation coefficients. Multiple regression analysis was used to test the combined influence of meal constituents (animal tissue, ascorbic acid, calcium, polyphenols, nonheme iron, and phytic acid) on log-adjusted iron absorption. To determine the multicollinearity among variables, the variance factor option in regression analysis was used; it measures inflation in variances of the parameter estimates due to multicollinearities that exist among the regressor variables. The variance inflation factor was found to be <1.2 for the variables included in the model, which is considered acceptable (31). Statistical analysis was performed by using the SAS PC program (version 6.1; SAS Institute, Cary, NC).

RESULTS

The 25 meals studied varied widely in composition (**Table 1**). The energy and protein contents ranged from 837 to 4992 kJ ($\bar{x}$: 1635 kJ) and from 8 to 32 g ($\bar{x}$: 18 g), respectively. Ascorbic acid content measured in 21 of 25 meals averaged 21 mg (range: 3–67 mg); meals with higher ascorbic acid contents contained tomato sauce or orange juice. The mean animal tissue content (cooked lean weight) was 31 g; this calculation included 7 vegetarian meals. The polyphenol content averaged 170 mg (range: 25–450 mg). Phytic acid content averaged 145 mg and was particularly high in the 3 breakfast meals, which included cereal

TABLE 2
Intercorrelations of meal constituents[1]

	Ascorbic acid	Calcium	Polyphenols	Phytic acid	Nonheme iron
Animal tissue	−0.12 [21]	−0.57[2] [25]	−0.31 [23]	−0.18 [25]	−0.32 [25]
Ascorbic acid		−0.04 [21]	0.60[2] [19]	0.33 [21]	0.35 [21]
Calcium			0.22 [23]	0.44[3] [25]	0.34 [25]
Polyphenols				0.49[3] [23]	0.68[4] [23]
Phytic acid					0.60[2] [25]

[1] Values in brackets are the numbers of observations included in the analysis. Correlations are Pearson's product-moment correlation coefficients.
[2] $P \leq 0.01$.
[3] $P \leq 0.05$.
[4] $P \leq 0.001$.

with high bran content. The calcium content varied widely, from 27 to 589 mg, with a mean of 163 mg. The mean nonheme-iron content was 2.8 mg, with high amounts in meals 18 and 22 because they contained iron-fortified cereal.

Intercorrelations of meal constituents are shown in **Table 2**. The highest positive correlations were observed between nonheme-iron and both phytic acid and polyphenol contents and between polyphenol and ascorbic acid contents. In contrast, the highest negative correlation was observed between animal tissue and calcium contents. Less significant correlations were observed between phytic acid and both calcium and polyphenol contents.

There was striking variation in measured mean iron absorption, from low values of 1.2% and 2.8% for 2 large breakfast meals containing >15 mg Fe to a high value of 19.7% for meal 14 (**Table 3**). The overall mean absorption for the 25 meals was 5.4%. After correcting for differences in iron status, the adjusted mean absorption values were similar to the measured mean absorption values because the iron status of the subjects did not differ appreciably. The one exception was that the mean serum ferritin of 77 μg/L in study V resulted in a 2-fold increase in adjusted absorption. After adjusting for iron status, the composite mean absorption for all 25 meals increased from 5.4% to 7.4%. The correlations between meal constituents and adjusted iron absorption are shown in **Table 4**. A highly significant, positive correlation with iron absorption was observed for animal tissue content, whereas negative correlations were seen with phytic acid and calcium contents and, to a lesser extent, with polyphenol and nonheme iron contents. Ascorbic acid content was not significantly related to iron absorption.

In view of the significant intercorrelations between various meal constituents, a more meaningful assessment of their influence on iron absorption was obtained by performing multiple regression analysis (**Table 5**). A backward variable-elimination procedure was performed by including all the variables (listed in Table 4) at first and then eliminating the least significant variable, one at a time, until only significant variables ($P \leq 0.05$) were left in the model. After the regression analysis was completed, 16.4% ($P = 0.0001$) of the variance in iron absorption was accounted for by animal tissue ($P = 0.0001$), phytic acid ($P = 0.0001$), and ascorbic acid ($P = 0.0441$) contents. A major portion of the total variance in iron absorption was explained by the animal tissue and phytic acid contents of the meals. Ascorbic acid made a much smaller, although significant, contribution than did animal tissue and phytic acid. In contrast, polyphenols, calcium, and nonheme iron were not significant in predicting iron absorption. Based on the regression analysis (Table 5), the following model was developed to estimate iron absorption:

$$\text{Ln [Adj Abs (\%)]} = 1.9786 + (0.0123 \times \text{AT}) - (0.0034 \times \text{PA}) + (0.0065 \times \text{AA}) \quad (2)$$

where Adj Abs is nonheme-iron absorption adjusted to a serum ferritin concentration of 30 μg/L, AT is animal tissue (g), PA is phytic acid (mg), and AA is ascorbic acid (mg).

DISCUSSION

In recent years, several dietary factors that influence the absorption of nonheme iron from foods have been identified. Most of the data were obtained in human subjects by measuring the absorption of nonheme iron from single meals tagged extrinsically with radioactive iron (30). Certain obstacles interfere with attempts to translate this information into practical dietary recommendations for increasing or reducing the absorption of dietary iron. Many previous studies were performed by adding purified ingredients to test meals rather than by attempting to define the influence of the native dietary components; this has been particularly true for phytic acid (8–11). Other food constituents, such as polyphenols, occur in numerous biochemical forms or complexes in foods, and different assay methodologies were used in each study. Difficulties arise even when the biochemical nature of the dietary compound is well defined. For example, in most studies of ascorbic acid, purified vitamin was added to the meal because the native dietary form is often partially degraded by cooking or allowing the food to stand before serving (32). Ascorbic acid may be partially degraded to dehydroascorbic acid, which is detected by some methods but not others. A similar problem exists in studies with meat, because of the variable degradation of heme depending on the method of preparation (33).

Another important limitation in applying our current knowledge of factors that affect the absorption of food iron is the interactions that occur when ≥2 active compounds are contained in the same meal. For example, the promoting effects of ascorbic acid and animal tissue on iron absorption are not additive when they are contained in the same meal (34, 35) and the same is undoubtedly true for a large number of dietary inhibitors of iron absorption. Ascorbic acid was shown to be more effective in increasing iron absorption from low-bioavailability foods than from those containing meat (34, 36). Another example of the unpredictable effects of multiple dietary factors is the studies with NaFeEDTA, which is a promising form of iron for fortification. When added to a meal of low bioavailability, this iron chelate enhances iron absorption, but iron absorption is unaffected when it is added to a meal of high bioavailability (37). The model that was developed many years ago to estimate iron bioavailability does not take into consideration either the amounts of the absorption inhibitors or the combined effect of the absorption enhancers that are present in the same meal (6). Recently, Tseng et al (38) refined this model by including inhibitors found in Russian diets, but the authors did not account for the combined effect of enhancers and inhibitors. Because the inhibitory or enhancing effects of various factors depend on

TABLE 3
Measurement of iron status and iron absorption in humans

Meal number	Study number	Serum ferritin[1]	Iron absorption[1]	
			Measured	Adjusted[2]
		μg/L	*%*	
1 (*n* = 10)	I	34 (24–49)	5.9 (4.0–8.6)	6.7 (5.2–8.6)
2 (*n* = 10)	I	34 (24–39)	2.3 (1.6–3.4)	2.7 (1.9–3.8)
3 (*n* = 10)	I	34 (24–39)	9.8 (7.2–13.3)	11.1 (9.4–13.2)
4 (*n* = 8)	II	37 (27–49)	14.0 (10.4–19.0)	17.2 (13.8–21.4)
5 (*n* = 8)	II	37 (27–49)	11.3 (7.8–16.5)	13.9 (11.6–16.5)
6 (*n* = 8)	II	37 (27–49)	6.9 (4.5–10.6)	8.4 (6.2–11.5)
7 (*n* = 10)	III	28 (22–36)	3.4 (2.7–4.3)	3.12 (2.5–4.0)
8 (*n* = 10)	III	28 (22–36)	7.0 (4.9–10.1)	6.6 (4.9–8.8)
9 (*n* = 10)	III	28 (22–36)	11.5 (9.6–15.2)	10.7 (8.2–14.0)
10 (*n* = 9)	IV	45 (32–64)	6.9 (5.3–9.0)	10.3 (7.9–13.5)
11 (*n* = 9)	V	77 (60–98)	2.3 (1.7–3.1)	5.9 (4.39–7.81)
12 (*n* = 9)	V	77 (60–98)	3.2 (2.1–4.8)	8.1 (5.9–11.0)
13 (*n* = 10)	I	34 (24–39)	2.6 (2.0–3.5)	3.0 (2.1–4.1)
14 (*n* = 10)	III	28 (22–36)	19.7 (13.5–28.7)	18.4 (13.7–24.7)
15 (*n* = 8)	II	37 (27–49)	6.8 (4.5–10.1)	8.3 (6.0–11.4)
16 (*n* = 9)	V	77 (60–98)	4.9 (3.8–6.1)	12.4 (10.5–14.8)
17 (*n* = 9)	V	77 (60–98)	3.2 (2.3–4.3)	8.1 (6.2–10.5)
18 (*n* = 9)	VI	66 (49–111)	1.2 (0.8–1.7)	2.6 (2.0–4.2)
19 (*n* = 9)	IV	45 (32–64)	3.9 (3.21–4.7)	5.8 (4.7–7.2)
20 (*n* = 15)	VII	32 (24–41)	9.4 (7.3–12.2)	10.0 (9.0–11.6)
21 (*n* = 9)	IV	45 (32–64)	4.4 (3.6–5.3)	6.6 (4.9–8.8)
22 (*n* = 9)	IV	45 (32–64)	2.8 (2.0–3.9)	4.2 (3.1–5.5)
23 (*n* = 8)	VIII	55 (38–79)	3.9 (2.8–5.3)	7.1 (5.1–9.9)
24 (*n* = 8)	IX	34 (26–43)	10.4 (8.1–13.3)	11.7 (9.0–15.3)
25 (*n* = 8)	IX	34 (26–43)	8.3 (6.9–10.0)	9.4 (7.8–11.8)
$\bar{x}$ (*n* = 86)		41 (30–44)	5.4 (5.0–5.8)	7.4 (6.9–7.9)

[1] Geometric mean (±1 SE).
[2] Absorption for each subject was adjusted to a serum ferritin concentration of 30 μg/L before the mean was calculated for each meal.

other factors in the meal, it is important to examine the combined effect on iron absorption of all the factors.

The difficulty in predicting iron absorption from complex meals was approached in the present study by basing the estimates of bioavailability on the contents of factors known to influence nonheme-iron absorption. We attempted to include the main reported determinants of nonheme-iron absorption and it is unlikely that other major factors will be found. There are several potential limitations in using this approach. Selection of the type of meals studied could be important. Many of our meals were frozen dinners because this facilitated preparation for several participants at one time. These meals have a well-defined composition but are widely consumed only in the United States and may not represent the diets in other parts of the world. We attempted to select these meals in such a way that they contained a wide spectrum of factors that are known to influence iron absorption. The choice of the analytic methodology may also play a major role in developing a model for predicting iron absorption. We attempted to select the most widely used methods of food analysis.

Finally, variation in iron status, and therefore iron absorption, among different groups of subjects is a well-known methodologic problem in human radioisotopic studies. In the preliminary evaluation of our data, we used serum ferritin concentration as one of the independent variables and observed that it accounted for 32% of the overall variability in iron absorption. In the regression model reported here, we eliminated the effects of differences in iron status by adjusting each individual absorption value to a constant serum ferritin concentration of 30 μg/L. We used this approach rather than reference-dose absorption because a previous study showed that the ferritin-correction method is more reliable (24).

One of the surprising findings of the current study was that even after we adjusted each individual's absorption to their iron status, only 16% of the variation in absorption was explained by dietary factors and that animal tissue and phytic acid were the main factors that had a major influence. Inclusion of serum ferritin concentration as one of the independent variables along with the dietary factors improved the variability of unadjusted absorption to 50%. Our results indicate that dietary factors make a small contribution to determining the amount of iron absorbed and that some unknown physiologic factors contribute the majority of variation in iron absorption. Variation in iron absorption data in human studies is not uncommon even after adjustment for reference-dose absorption or iron status.

Certain dietary factors were correlated with iron absorption when examined individually (Table 4), but their influence largely disappeared in multiple regression analysis. The most notable example was calcium, which correlated negatively with iron absorption when tested separately but had no effect in multiple regression analysis, perhaps reflecting its relations with phytic acid content, animal tissue content, or both. A similar finding was the significant correlation between iron absorption and polyphenol content when examined in isolation; this correlation also disappeared with multiple correlation analysis, perhaps because of

TABLE 4
Correlations of meal constituents with iron absorption (%)[1]

Variable	Correlation coefficient[2]
Animal tissue	0.34 [232][3]
Phytic acid	−0.30 [232][3]
Calcium	−0.38 [232][3]
Nonheme iron	−0.19 [232][4]
Polyphenols	−0.16 [216][5]
Ascorbic acid	0.01 [195]

[1] Iron absorption was adjusted to a serum ferritin concentration of 30 μg/L. Values in brackets are the numbers of observations included in the analysis.
[2] Pearson's product-moment correlation coefficients.
[3] $P \leq 0.0001$.
[4] $P \leq 0.005$.
[5] $P \leq 0.05$.

the relation between polyphenols and phytic acid. Ascorbic acid had no effect on iron absorption when tested alone, but showed a borderline significant effect in multiple regression analysis ($P \leq 0.05$). The influence of ascorbic acid on iron absorption was less pronounced than that of animal tissue and phytic acid; the reason might be that most of our test meals contained animal tissue, which may have obscured the influence of ascorbic acid.

One important issue not addressed in this investigation is the relevance of using single meals to estimate food iron bioavailability. There is increasing evidence of large differences in nonheme-iron absorption with extrinsic labeling of single meals, and these differences were greatly reduced when iron absorption was measured from a complete diet (24, 39–41). For example, calcium has been shown to reduce iron absorption in single-meal studies, but the effect was dampened when absorption was measured from a 5-d varied diet (41) or from a complete diet (39). In one study, the difference in absorption between high- and low-bioavailability meals was 6-fold when the foods were consumed as single meals, but was only 2.5-fold when the total diet was evaluated (24). These differences can be attributed to differences in meals because single meals were controlled by the investigator whereas the total diet was self-selected by the subjects. However, these results parallel to some extent the findings of the present study in that a 10-fold difference in iron absorption from individual meals was observed, but only 3 meal components were found to be relevant when all meals were analyzed together. Although it is clear that the extent of enhancement or inhibition is less pronounced when iron absorption is measured over several days than when it is measured from single meals, single-meal studies are still useful for identifying the relative effects of enhancers and inhibitors of iron absorption.

TABLE 5
Regression analysis: predictors of nonheme-iron absorption[1]

Independent variable	Parameter estimate	Percentage variance[2]	*P* value
Intercept	1.9786		
Animal tissue	0.0123	9.17	0.0001
Phytic acid	−0.0034	8.65	0.0001
Ascorbic acid	0.0065	1.80	0.0441

[1] Percentage ln absorption adjusted to a serum ferritin concentration of 30 μg/L. Model $R^2 = 0.1642$; adjusted $R^2 = 0.1510$; $F = 12.50$, $P = 0.0001$.
[2] Squared semipartial correlation.

In conclusion, our regression equation based on animal tissue, phytic acid, and ascorbic acid contents is useful for predicting nonheme-iron absorption from meals typical of Western diets. To use our model, one has to estimate or measure the animal tissue content of the meals. Although cooked, lean, animal tissue content is needed to predict iron absorption, a reasonable estimate can be made from the raw tissue content (1.3 g raw tissue = 1 g cooked tissue) as suggested by Monsen (42). Ascorbic acid content can be obtained easily from food-composition tables. Phytic acid content is difficult to obtain, unless it is measured in the laboratory, because of the lack of information in food-composition tables; however, reasonable estimates can be made from published values (43).

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