

# Prediction of dietary iron absorption: an algorithm for calculating absorption and bioavailability of dietary iron<sup>1-3</sup>

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## ABSTRACT

**Background:** Dietary iron absorption from a meal is determined by iron status, heme- and nonheme-iron contents, and amounts of various dietary factors that influence iron absorption. Limited information is available about the net effect of these factors.

**Objective:** The objective was to develop an algorithm for predicting the effects of factors known to influence heme- and nonheme-iron absorption from meals and diets.

**Design:** The basis for the algorithm was the absorption of iron from a wheat roll ( $22.1 \pm 0.18\%$ ) containing no known inhibitors or enhancers of iron absorption and adjusted to a reference dose absorption of 40%. This basal absorption was multiplied by the expected effect of different amounts of dietary factors known to influence iron absorption: phytate, polyphenols, ascorbic acid, meat, fish and seafood, calcium, egg, soy protein, and alcohol. For each factor, an equation describing the dose-effect relation was developed. Special considerations were made for interactions between individual factors.

**Results:** Good agreement was seen when measurements of iron absorption from 24 complete meals were compared with results from use of the algorithm ( $r^2 = 0.987$ ) and when mean iron absorption in 31 subjects served a varied whole diet labeled with heme- and nonheme-iron tracers over a period of 5 d was compared with the mean total iron absorption calculated by using the algorithm ( $P = 0.958$ ).

**Conclusions:** This algorithm has several applications. It can be used to predict iron absorption from various diets, to estimate the effects expected by dietary modification, and to translate physiologic into dietary iron requirements from different types of diets. *Am J Clin Nutr* 2000;71:1147-60.

**KEY WORDS** Humans, iron absorption, heme iron, nonheme iron, algorithm, diet, meals, bioavailability, iron status, iron requirements, phytate, polyphenols, ascorbic acid, meat, soy protein, alcohol, eggs, calcium

## INTRODUCTION

Knowledge about the absorption of iron from the diet and about factors influencing absorption has increased considerably since the extrinsic tag was introduced to label dietary iron in meals (1, 2). The amount of iron absorbed from a meal is determined by iron status, the content of heme and nonheme iron, and the bioavailability of the 2 kinds of iron, which in turn is deter-

mined by the balance between dietary factors enhancing and inhibiting the absorption of iron, especially nonheme iron (3). It is well known that the variation in dietary iron absorption from meals is due more to differences in the bioavailability of the iron, which can lead to a >10-fold variation in iron absorption, than to a variation in iron content.

Therefore, several attempts have been made to devise algorithms to estimate the bioavailability of the dietary iron content of meals. The aim of the first attempt was to illustrate the fact that the composition of meals greatly influences the absorption of dietary nonheme iron (4). Later, attempts were made to improve the algorithm (5, 6). A simpler method using a score system to estimate the expected bioavailability of dietary nonheme iron was also suggested (7). In this model, factors inhibiting iron absorption were also considered.

Several dietary factors (eg, ascorbic acid, meat, fish, and poultry) enhance iron absorption, whereas other factors [eg, inositol phosphates (phytate), calcium, and certain structures in polyphenols] inhibit iron absorption. In the present study, we analyzed the dose-response relation between amounts of these factors and their effects on nonheme-iron absorption. All of these factors must be considered in an algorithm to predict the amount of iron absorbed from a meal. For almost all of the factors, it has been possible to develop continuous functions related to the amounts of each in the meal. Moreover, interactions between different factors have been examined and considered.

The hypothesis tested in the present algorithm was that the bioavailability of iron in a meal is a product of all factors present in the meal that inhibit or enhance iron absorption. A starting point for the present work was to find a food or meal that contained no known inhibiting or enhancing components and then use this food

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as a basis for evaluating the effects of different factors added in different amounts. For many years we used, as a control, wheat rolls made of low-extraction wheat flour and fermented to such an extent that no inositol phosphates could be detected. Various factors to be tested were added in different amounts to such rolls and iron absorption was measured from the rolls, when served with or without a specific factor in known and various amounts, after the rolls were labeled with 2 different radioiron isotopes. Iron status in each fasted subject was measured by using the absorption from a standard reference dose of ferrous iron to describe the iron status of the individuals studied. The reference dose was introduced by Layrisse et al (8) and the entire procedure was described in detail (9). Iron absorption can also be related to log serum ferritin as suggested by Cook et al (10).

Numerous studies on factors influencing the bioavailability of dietary iron have been published by several research groups (discussed below), in addition to the studies by our group. It has only been possible, however, to use some of the data from their studies. This is true also for some of the older data from our laboratory. The reason is simply that there is a lack of information about the content of phytate and sometimes that of polyphenols in the meals studied.

## METHODS

The method used to predict dietary iron absorption is based on an algorithm containing the value for iron absorption (relative to 40% of the absorption of the reference dose of iron) from a single basal meal ([low-extraction (40%) wheat flour] that contained no components known to inhibit or enhance iron absorption. This basal value was then multiplied by factors expressing the effect of different dietary components present in the meal known to influence iron absorption: phytate, polyphenols, soy protein, calcium, eggs, ascorbic acid, meat (including fish and seafood), and alcohol. For each factor, an equation was derived that also considered interactions between components in the meal.

### Iron absorption from a basal meal

The basal meal was composed of wheat rolls served with margarine and water on 2 mornings while subjects were in a fasting state. The rolls were made of a special low-extraction (40%) wheat flour and the dough was fermented for 2 periods (30 + 10 min) to ensure that no inositol phosphates could be detected with a sensitive method (11). The iron content of the rolls was adjusted to 4.1 mg by adding ferrous sulfate to the dough. The rolls were labeled with an extrinsic radioiron tracer. Iron absorption was measured as described previously (9, 12).

The rolls were included in different studies of factors influencing iron absorption. Rolls were served with and without a factor to be studied in specific amounts and were labeled with 2 different radioiron isotopes (13–15). Iron absorption from these rolls was measured in 310 subjects (194 female and 116 male volunteers). In each subject, iron absorption from a reference dose containing 3 mg Fe as ferrous sulfate, given while subjects were in a fasting state on 2 consecutive mornings, was also measured. All absorption values were adjusted to correspond to an absorption of 40% from the reference dose. Thus, absorption measurements from the same meal could be pooled from different groups of subjects with different iron statuses. The mean ( $\pm$ SEM) absorption of iron from the rolls in all studies, adjusted to a 40% reference dose absorption, was  $22.1 \pm 0.18\%$ .

### Effect of phytate and other inositol phosphates

The effect of different amounts of phytate on iron absorption was examined when wheat rolls were served with and without different amounts of added sodium phytate. Seven groups of subjects ( $n = 63$ ) were studied and the added phosphorus as phytate (phytate-P) varied from 2 to 250 mg (14). A similar study was performed in another laboratory in which the basal wheat rolls contained 10 mg phytate-P ( $n = 57$ ). Four different amounts of phytate-P (14–58 mg) were added (16). Because the effect of 10 mg phytate-P was examined in the previous study, it was possible to recalculate the effect of the added phytate-P. The effect of phytate was similar in the 2 studies. When the data from the 2 studies were pooled, the following relation was found:

$$\text{Log absorption ratio} \\ (\text{with/without phytate}) = -0.30 \times \log (1 + \text{phytate-P}) \quad (1)$$

where phytate-P is in milligrams. The correlation coefficient was  $r^2 = 0.926$  ( $n = 120$ ). Antilog of the log absorption ratio thus constitutes the phytate factor.

When the content of phytate-P in bread is determined, some of the inositol phosphates are present in forms with a fewer number of phosphate groups than the 6 groups present in phytate. In a previous study we found that the total number of phosphate groups bound to inositol, present in a bread, determines the degree of inhibition (11). This implies that the total inhibitory effect of inositol phosphates is better expressed as the number of phosphate groups bound to inositol than as moles of inositol. (Conversion factor: 1 mg phytate-P = 3.53 mg phytic acid = 5.56  $\mu$ mol phytic acid.)

### Effect of ascorbic acid

Ascorbic acid is a strong promoter of iron absorption, as shown in several studies (see reference 16 for a review). In an extensive study by Cook and Monsen (17) in 1977 in which 6 different amounts of ascorbic acid (25–1000 mg) were added to a semisynthetic meal, a strong relation was seen between log amounts of ascorbic acid and the log absorption ratio ( $r^2 = 0.958$ ;  $n = 25$ ). The counteracting effect of ascorbic acid on phytate and polyphenols was also reported by other groups (18). In the study by Cook and Monsen, it was not mentioned whether an inhibitor was present in the control meals, which showed a very low absorption of iron ( $\approx 0.75\%$ ). The enhancing effect of ascorbic acid is more marked in the presence of phytate or iron-binding polyphenols. In subsequent studies from the same group using the same liquid formula, it was noted that vanilla extract had been added to the formula, probably to improve the taste (19, 20). Recent analyses in our laboratory indicate that vanilla extracts contain appreciable amounts of iron binding polyphenols (**Appendix A**). This fact might explain the marked effect of ascorbic acid in Cook and Monsen's (17) comprehensive study. Addition of 100 mg ascorbic acid to the semisynthetic liquid formula increased iron absorption 4.14 times, whereas addition of the same amount of ascorbic acid to a so-called standard meal containing meat, potatoes, and milk increased iron absorption by only 67%. In another study from the same group, addition of 100 mg ascorbic acid to another but similar liquid formula containing 85 mg phytate-P increased iron absorption 3.14 times (20).

These findings suggest that the ability of ascorbic acid to reduce iron and thus to prevent the formation of less-soluble ferric compounds is probably an important mechanism of action for the absorption-promoting effect of ascorbic acid. An

enhancing effect of ascorbic acid on iron absorption, however, was also seen in the absence of phytate and polyphenols. Addition of 50 mg ascorbic acid, for example, to wheat rolls with no detectable phytate increased mean iron absorption from 22.4% to 37.6% (14).

These facts are taken into account in an algorithm describing the expected effect of ascorbic acid in the presence of phytate. The algorithm was calculated as follows:

- 1) Even in the absence of inhibitors, ascorbic acid increases iron absorption in a dose-dependent way: absorption ratio =  $(1 + 0.01) \times$  ascorbic acid (in mg).
- 2) The more phytate that is present, the greater the effect of ascorbic acid. Linear relations were seen between the absorption ratio (with and without ascorbic acid) and log phytate-P. Five regression lines describing this relation had different linear slopes for different log amounts of ascorbic acid (5–500 mg). The squared correlation coefficients for the 5 lines varied from 0.837 to 0.877. The content of phytate varied from 0 to 250 mg. The 5 slopes of the regression lines were related to log amounts of ascorbic acid and showed a best fit in an exponential equation with an  $r^2$  value of 0.995. The following general equation was thus derived:

$$\text{Absorption ratio} = [1 + 0.01 \text{ AA (in mg)} + \log \text{ phytate-P (in mg)} + 1] \times 0.01 \times 10^{0.8875 \times \log(\text{AA}+1)} \quad (2)$$

where AA is mg ascorbic acid and is mg in the meal. This equation is based on studies in 240 subjects in 24 studies. The enhancing effect of ascorbic acid was the same in meals with and without calcium and the same in meals with and without meat. These observations suggest that the mechanisms of action on iron absorption are different for ascorbic acid, meat, and calcium.

### Effect of polyphenols

In earlier studies it was shown that tea inhibits the absorption of non-heme iron (21–23). Similarly, coffee (22–24) and red wines (25, 26) were reported to inhibit iron absorption. This inhibition was considered to be due to polyphenols present in these beverages. Addition of tannic acid to a meal was shown to reduce iron absorption (27). Further studies showed that gallic acid and tannic acid had identical inhibitory effects on iron absorption and identical iron binding properties (13). Galloyl groups with their 3 adjacent hydroxyl groups were found to be the main, common structure in polyphenols binding iron, probably by a direct chemical binding, especially of ferric iron, and presumably through the formation of chelates.

The strong binding of ferric iron to galloyl groups explains the counteracting effect of ascorbic acid on the inhibition of iron absorption by phenolic compounds. Iron binding polyphenols are widespread in foods because they occur naturally in a variety of cereals, vegetables, and spices, and in many beverages such as wine, coffee, and tea (13, 28). A chemical method for specifically determining galloyl groups has been designed (29).

The inhibition of iron absorption by coffee is explained mainly by its content of chlorogenic acid. The binding of iron to this compound is less strong than the binding of iron to galloyl groups. The relative inhibition by equimolar amounts of gallic acid and chlorogenic acid was found to be 1.6:1 (13). In Appendix A, tannic acid equivalents, chlorogenic acid, and total tannic acid equivalents (the sum of tannic acid and the amount of chlorogenic acid divided by 1.6) in various foods are reported.

### Effect on iron absorption of the contents of polyphenols, ascorbic acid, and meat in meals

In calculating the effect of tannic acid on iron absorption it was necessary to consider both the amount of galloyl groups and the amount of ascorbic acid present in a meal. In studies in which different known amounts of tannic acid were added to a wheat roll (range: 5–200 mg), a linear relation was observed when the log absorption ratio was plotted (absorption with/without tannic acid) against the log amounts of tannic acid added to the rolls (13). The following equation ( $r^2 = 0.978$  for the mean values) was based on measurements in 59 subjects:

$$\text{Log absorption ratio} = 0.4515 - 0.715 \times \log \text{ tannic acid (in mg)} \quad (3)$$

The slope of this regression line ( $-0.715$ ) changed when different amounts of ascorbic acid were added. The regression lines for different amounts of ascorbic acid converged to the point log absorption ratio (0.4515) and log tannic acid (0).

The effect of ascorbic acid on the inhibition of iron absorption by tannins was reported in 2 studies (18, 30). Moreover, in developing the equations we also used recent unpublished data from our laboratory on the effect of ascorbic acid on the inhibition by phenolic compounds. Two studies of the effect of meat on the inhibition by tannic acid were conducted ( $n = 20$  each). Other studies also showed that  $\approx 100$  g meat reduced the inhibition by tannic acid by half (24). The effect of meat on the inhibition by polyphenols is also included in the equation below.

The effect of polyphenols on iron absorption is expressed in the following equation, in which the amounts of tannic acid equivalents (TA; in mg), ascorbic acid (AA; in mg), and meat or fish ( $M$ ; in g) in the meal are considered:

$$\text{Absorption ratio} = (1 + 0.01M) \times 10^{0.4515 - [0.715 - 0.1825 \times \log(1 + AA)] \times \log(1 + TA)} \quad (4)$$

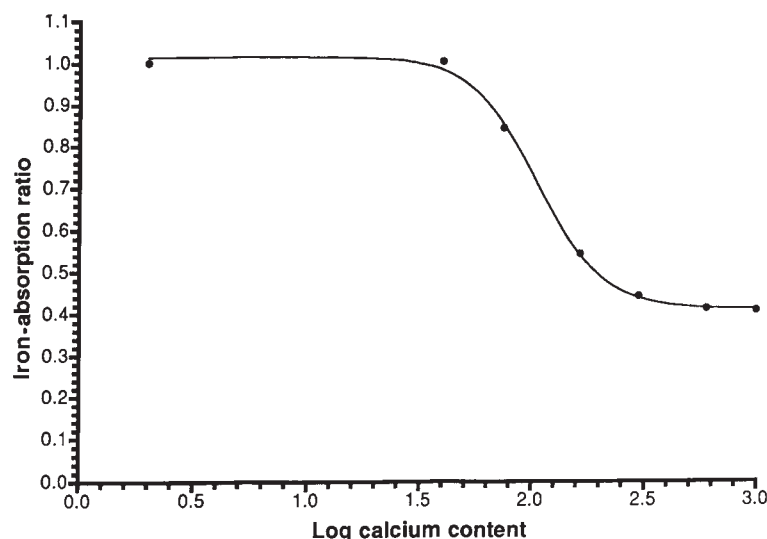
The absorption ratio should be  $\leq 1$  and corrected to 1 if it is not.

### Effect of coffee and tea

Coffee and tea are widely consumed as beverages with meals or directly after meals. These beverages have a high content of phenolic compounds and have been shown to strongly inhibit the absorption of nonheme iron (13, 21, 22, 24). A cup of tea ( $\approx 200$  mL) reduces iron absorption by  $\approx 75$ –80%. Variations in the results of different studies are probably related to the different amounts of phenolic compounds in the tea resulting from differences in the amounts, brands, and steeping time of teas used. A cup of coffee ( $\approx 150$  mL) reduces iron absorption by  $\approx 60$ %. When tea or coffee was served with a meal containing  $\approx 100$  g meat, the inhibition of iron absorption was reduced by 50% (24). This agrees with the first part of equation 4 above (eg, when  $M = 100$  g,  $1 + 0.01M = 2.0$ ).

On the basis of its content of phenolic compounds, coffee is expected to reduce iron absorption even more than was observed. It is well known that coffee stimulates the gastric secretion of hydrochloric acid, which may explain the lower than expected effect. We tested this possibility by measuring the inhibition of iron absorption by coffee in patients with pentagastrin-proven achlorhydria and found that in these patients the inhibitory effect was twice as high (absorption ratio: 0.19 compared with 0.39) as that in healthy subjects and corresponded to the content of phenolic compounds in coffee (L Hulthén, L Hallberg, A Killander, unpublished observations, 1995).





**FIGURE 1.** Relation between the log calcium content in a meal and the ratio of iron absorption from a meal served with or without different amounts of calcium. The equation describing the relation is given in the text.

To circumvent the problem encountered when the algorithm was applied to coffee and tea, because of variations in the content of iron binding polyphenols and different extraction times of the beverages, we used a factor of 15 mg tannic acid equivalents for one cup of regular coffee and 30 mg tannic acid equivalents for one cup of tea. These values apply to beverages consumed with a meal or up to a few hours after a meal (24). We are aware that strong coffee may reduce iron absorption even further (eg, 50 mg tannic acid equivalents gives a tannic acid factor of 0.17) and that other strengths of tea or other kinds of tea may reduce iron absorption even more. We found for a common green tea, for example, a tannic acid factor of 0.17 (Appendix A)—a reduction in iron absorption of 83%.

#### Effect of calcium

A strong dose-effect relation between the amount of calcium in a meal and the reduction in nonheme-iron absorption has been observed (15). The relative reduction of iron absorption was the same for the same amount of calcium given as a calcium salt, milk, or cheese. No inhibition was seen when the amount of calcium in a meal was <50 mg (10 mg native and 40 mg added Ca) and the inhibition was maximal at a content of  $\approx 300$ –600 mg. Moreover, calcium also inhibited the absorption of heme iron similarly (31), suggesting a common step in the transport of these 2 kinds of iron; therefore, the effect was not located in the intestinal lumen but within the mucosal cell. The observed relation between the absorption ratio (absorption with/without calcium) and the amount of calcium in a meal had a clear sigmoid curve, suggesting one-site competitive binding at a receptor (**Figure 1**). Such a step may be located in the active transport pathway for calcium (32). An equation was tested describing such a relation for the present data [ $n = 7$  (mean values);  $r^2 = 0.9984$ ].

$$\text{Absorption ratio} = 0.4081 + \frac{[0.6059/1 + 10^{-[2.022 - \log(\text{Ca} + 1)] \times 2.919}]}{\quad} \quad (5)$$

where Ca is the calcium content in the meal (in mg). The calculations are based on the computer program GRAPHPAD

PRISM (version 2.0; Intuitive Software for Science, San Diego). Iron absorption increased after the addition of ascorbic acid to a meal containing calcium (33); however, the relative increase was the same as would have occurred had no calcium been present.

#### Effect of meat, poultry, and fish and seafood

Several studies have shown that meat, poultry, and fish and other seafood increase the absorption of nonheme iron. It was first noted by Layrisse et al (34). For a review, see reference 3. Despite numerous studies of the effect of meat on iron absorption by several groups, there is still insufficient information about the magnitude of the effect of meat in different types of meals and the possible mechanisms for the absorption-promoting effect of meat and fish.

In developing an algorithm for the effect of meat and fish on iron absorption, results from several absorption studies were pooled. The effect was measured as the absorption ratio when meals were served with and without meat or fish (19, 20, 35–37). The effect of meat was calculated in the following steps. In the first step, the effect of meat was measured in meals not containing phytate. The relation between the amount of meat and the absorption ratio ( $r^2 = 0.899$ ) was examined in 135 subjects from 15 studies.

$$\text{Absorption ratio} = 1 + 0.00628 \times \text{amount of meat and fish (in g)} \quad (6)$$

In the second step, we analyzed the effect of phytate on the slope of this relation. In 10 studies in which meat with different amounts of phytate was served, we found that the factor influencing the slope in the first relation could be expressed as  $(1 + 0.006) \times \text{amount of phytate-P (in mg)}$ ;  $r^2 = 0.877$ . The final meat factor obtained was thus as follows:

$$\text{Absorption ratio (with/without meat)} = 1 + 0.00628 \times M \times [1 + 0.006 \text{ phytate-P (in mg)}] \quad (7)$$

where  $M$  is meat, fish, and seafood expressed as grams of uncooked food. According to the previous model of Monsen and



Balintfy (5), 1.3 g raw weight is equivalent to 1.0 g cooked weight of meat, poultry, and fish.

Several investigators examined the effect of both meat and fish but direct comparisons have not been reported. The balance of evidence suggests that meat and fish are interchangeable in this equation. Meat has the clear nutritional advantage because it also provides considerable amounts of heme iron.

### Effect of soy protein

In several studies it was observed that soy protein reduced the fraction of iron absorbed from a meal (38, 39). The high content of phytate in soy products led the researchers to suspect that the inhibition by soy might be related to phytate. Reduction of the phytate content by repeated washings with acidic solutions, however, did not totally abolish the inhibition (39, 40).

In a recent comprehensive study in which almost all of the phytate in soy was removed by enzymatic degradation with a phytase, however, the inhibition by soy proteins was markedly reduced (41). Four groups of subjects were studied ( $n = 32$ ). Iron absorption was measured from semisynthetic meals, each containing 30 g protein as soybean-protein isolates or egg white as a control. A significant inhibitory effect on iron absorption by soy protein remained. The egg white contained 96 mg Ca/meal compared with 19.2, 27.4, and 44 mg Ca/meal in the soybean-protein isolates. It can be estimated from Equation 5 that the higher calcium content in the control meals would reduce iron absorption by 25%. The average absorption ratio of iron from the soy meals and the control meals was 0.33 after correction for the higher calcium content in the control meals. The inhibitory effect on iron absorption per gram of soy protein ( $x$ ) would thus be as follows:  $1 - 30x = 0.33$ . Solving the equation gives  $x = 0.022$  and the soy-factor absorption ratio would thus be as follows:

$$\text{Absorption ratio} = 1 - 0.022 \times \text{soy protein (in g)} \quad (8)$$

This equation is valid up to  $\approx 20$  g soy protein. For a hamburger that might contain a commercial soy-protein isolate containing phytate, it is necessary to consider in the algorithm the amounts of pure meat, soy protein, and phytate-P present in the hamburger.

### Effect of eggs

In an early study of the effect of eggs on iron absorption in 28 humans, white-wheat bread was given with eggs together with coffee or tea (42). A reference dose (5 mg Fe) was also given in this study. It is possible to estimate iron absorption from this meal corresponding to a reference dose absorption of 40%. About 16% could be estimated to have been absorbed had tea or coffee not been fed. Relating this absorption to 22.1% absorption from the basal wheat-roll meal (*see above*) at the same iron status, the egg factor would be  $16/22 = 0.73$ , ie, a reduction in iron absorption of 27%.

In our studies of iron absorption from different breakfast meals, the introduction of a boiled egg reduced the absorption by 28%, from 9.3% to 7.6% in 12 subjects (43). In one of the studies by Cook and Monsen (35), powdered eggs were fed to 10 subjects in an amount corresponding to 2.9 eggs, each weighing 60 g. When eggs are substituted for other proteins in a standard meal, the absorption ratio with or without eggs was reduced to 0.22 (a reduction of 78%). For one egg this corresponds to a reduction of 27% ( $0.78/2.9 = 0.27$ ). The results on the inhibiting effects of eggs by different groups are thus aston-

ishingly consistent. The effect of eggs has been studied in a total of 50 subjects.

When the data were pooled assuming a proportional inhibition of iron absorption to the amount of eggs included in a meal, the following equation was derived:

$$\text{Absorption ratio} = 1 - 0.27 \times \text{number of eggs} \quad (9)$$

The number of eggs can also be expressed as grams (one egg = 60 g). Equation 9 is valid only for  $\leq 3$  eggs/meal. Check that the absorption ratio for eggs is not  $< 0.2$  (equivalent to the inhibition by 3 eggs).

### Effect of alcohol

Studies in humans have shown that alcohol increases the absorption of ferric but not of ferrous iron (44). This increase has been attributed to an enhancement of gastric acid secretion. In a study serving a hamburger meal with or without 23.8 g alcohol (as a 40% solution), a statistically significant 23% increase in iron absorption was seen when alcohol was given with the meals (23). When the same meal was served with red wine, no significant increase was seen, possibly because of the simultaneous inhibiting effect of iron binding polyphenols present in red wine. In a study of the effect of different wines on iron absorption, a dinner roll was served with or without different wines, some of which had a markedly reduced alcohol content because of vacuum distillation (26). Adjustment for differences in iron status made it possible to make 4 pairwise comparisons of iron absorption from meals served with the same type of wine but with different alcohol contents (low or high). The mean absorption ratio between the meal with the low-alcohol compared with the high-alcohol content was  $1.33 \pm 0.14$  ( $P = 0.039$ ). The amount of alcohol served with the rolls was 12.6 g, which was about half the amount served with the hamburger meals mentioned above (23). Assuming that the effect of alcohol is related to stimulation of gastric acid secretion, it is possible that with a meal containing meat, more acid is formed than when a bread roll is served. The further stimulation of gastric secretion by alcohol may thus be lower from a full meal than from the meal containing only a roll. The studies strongly indicate, however, that alcohol also enhances iron absorption from composite meals (23). After careful consideration, we provisionally decided to use a single factor of 1.25 for the stimulation of iron absorption by alcohol. We also provisionally decided to use this factor for meals consumed together with, for example, 1–2 glasses of wine or 1–2 alcoholic beverages. The inhibitory effect of red wine on iron absorption, related to the content of iron binding polyphenols, should be considered separately in the calculation of the tannic acid factor.

### Effect of other factors

It is reasonable to assume that there are other factors in meals influencing iron absorption that have not been considered in the present algorithm. For example, some soy sauces may enhance iron absorption (45), whereas some flavonoids, especially myricetin, may inhibit iron absorption. Myricetin has a molecular structure similar to that of the galloyl group in polyphenols, which we know inhibits iron absorption via chelation with ferric iron. In our food analyses, we based the inhibiting effect of polyphenols on the content of such groups. Flavonoids with a similar structure may therefore be expected to have the same inhibitory effect on iron absorption (13, 29).



### Formula for log heme-iron absorption and the computer program

#### *Iron absorption from single meals*

The formula is the product of the basal factor 22.1 multiplied by one or more of the 8 dietary factors present in each meal: the phytate factor, the ascorbic acid factor, the polyphenol factor (or, tannic acid factor), the calcium factor, the meat factor, the soy-protein factor, the egg factor, and the alcohol factor. The value obtained is thus the percentage absorption of the nonheme iron present in a meal at an iron status corresponding to a reference dose absorption of 40%. The percentage absorption of heme iron was adjusted to the same iron status by using a formula presented in a previous study (46).

$$\text{Log heme-iron absorption (\%)} = 1.9897 - 0.3092 \times \text{log serum ferritin} \quad (10)$$

Heme-iron absorption is then corrected for the content of calcium in the meal by using the same calcium factor as used for nonheme-iron absorption (*see above*) (31).

To obtain the amount of iron absorbed from a meal, the percentage absorption of nonheme and heme iron have to be multiplied by the amounts of the 2 kinds of iron present in the meal. For nonheme iron it is important to consider any fortification iron present in the meal and to what extent this iron is potentially bioavailable. Similarly, if food components are contaminated with iron (eg, from soil), the fraction of such iron that is potentially absorbable (or, exchangeable with an extrinsic radioiron tracer) should be considered. A method is available to quantify this fraction (47). In Appendix A, the fraction of heme iron present in different kinds of meat and meat products is provided.

#### *Iron absorption from the whole diet*

The amount of iron absorbed from the whole diet is obtained by summing the amounts of iron absorbed from all the single meals and snacks for a certain period of time, eg, a single day or several days. Potential interference between meals is discussed below.

#### *Use of computer programs for the calculations*

We used Microsoft's EXCEL program (Redmond, WA) for the calculations. To avoid problems with calculating some of the factors based on logarithmic functions, we used a value of 1 in equations 1, 3, 4, and 7. In equation 4, an absorption ratio > 1 had to be changed to 1.

### Validation studies

The validity of the present algorithm was examined in 2 ways. In study 1, the observed absorption of nonheme iron from 24 single meals in 3 previous studies (43, 48, 49) was compared with the absorption values estimated by using the algorithm. These 3 studies were performed > 15 y ago. At that time, no sufficiently sensitive method for measuring small amounts of phytate and no specific method for measuring iron binding polyphenols was available. At the time of the studies, for example, we were not aware of the rather high contribution of phytate from potatoes in many meals (200 g potato contains 14 mg phytate-P) or of the fact that commercial products for making mashed potatoes also contained appreciable amounts of calcium from dried milk powder. Similarly, the content of polyphenols in different vegetables, spices, and beverages was not known, nor were the effects of polyphenols on iron absorption. New analyses had to be per-

formed to estimate the probable contents of phytate, polyphenols, ascorbic acid, and calcium in the meals. The variation in contents of iron and energy and amounts of nonheme iron absorbed from the 24 meals are provided in **Table 1**.

In study 2, a comparison was made between the estimated total amount of iron absorbed by using the algorithm in 31 men served 4 different meals for 5 d and the actual total iron absorption measured in these men by using 2 radioiron tracers. One tracer was given as intrinsically labeled radioiron to label hemoglobin and the other as inorganic iron to label nonheme iron. All meals were labeled with the 2 tracers to ensure a homogenous specific activity of both nonheme and heme iron in all meals. The total absorption of heme and nonheme iron was determined by using a whole-body counter to determine <sup>59</sup>Fe and a blood sample to analyze the ratio of <sup>55</sup>Fe to <sup>59</sup>Fe. The method used and the menus given were described in detail previously (46, 50, 51).

### RESULTS

#### *Study 1*

The result of the comparison of observed and calculated nonheme-iron absorption is shown in **Figure 2**. The main finding was the remarkably good agreement between observed and estimated nonheme absorption. The observed mean ( $\pm$ SEM) percentage absorption of nonheme iron in the 24 meals was  $12.91 \pm 1.84\%$  and the corresponding value for the absorption calculated by using the algorithm was  $13.33 \pm 1.95\%$ . There was no significant difference between the mean values. The correlation coefficient was high and the slope of the regression line was not significantly different from the line of equality. We also made the interesting observation that minor food components, such as a correct amount of calcium (cheese, vegetables, and milk) or a correct value for phytate content had a marked effect on the absorption calculated. The same was true for the amount of ascorbic acid served. Detailed knowledge of meal composition is thus essential to achieve reasonably good estimates of the iron absorption by using the algorithm. The average compositions of the 24 meals in studies 1 and 2 are shown in **Table 2**.

#### *Study 2*

The calculated iron absorption from the 20 different meals varied considerably between meals and days as shown in **Table 3**. The composition of these meals was also described in detail previously (51). Among the 31 men, there were 4 patterns in the choice of beverages (coffee, tea, and water) with the breakfast meals and the evening snacks; therefore, analyses were conducted separately in these 4 groups.

With the algorithm, the absorption of heme and nonheme iron from each meal was calculated separately and summed for the whole period. Nonheme-iron absorption in each subject was that expected at an iron status corresponding to a reference dose of 40%. For each individual, nonheme-iron absorption was then adjusted to the individual serum ferritin concentration and body weight according to equation 11 (*see below*). The individual amounts of heme-iron absorption expected were calculated by using equation 10. The calculated total amounts of iron absorbed in the 31 subjects were then obtained by adding the amounts of heme- and nonheme-iron absorption calculated in all meals. These figures were then compared with the actual absorption obtained when total iron absorption was measured directly. It was thus possible to compare the observed amounts of iron absorbed with the



**TABLE 1**

Study 1: observed absorption of nonheme iron before and after adjustment to a reference dose absorption of 40%, and the calculated absorption with the algorithm<sup>1</sup>

Type of meal and reference <sup>2</sup>	Nonheme iron	Energy	Iron absorption		
			Observed	Adjusted to 40% of reference dose absorption	Algorithm calculation
			mg (%)	mg (%)	%
Galician meat soup (48)	7.2	980 (4100)	0.96 (13.4)	1.16 (16.2)	17.2
Spaghetti with cheese (48)	4.9	1020 (4268)	0.54 (11.0)	0.59 (12.1)	11.6
Hamburger meal (48)	3.9	1030 (4310)	0.54 (13.8)	0.48 (12.2)	13.0
Soup, steak, and kidney pie (48)	5.7	1010 (4226)	1.09 (19.2)	1.08 (18.9)	18.9
Pizza (48)	4.2	1040 (4351)	0.38 (9.0)	0.33 (7.9)	9.6
Vegetable soup (48)	7.0	1010 (4226)	0.50 (7.2)	0.55 (7.9)	8.0
Pancake and jam (49)	5.1	630 (2636)	0.09 (1.7)	0.18 (3.3)	3.2
Breakfast basal (43)	2.8	320 (1339)	0.21 (7.6)	0.16 (5.7)	5.0
Breakfast + orange juice (43)	3.1	390 (1632)	0.25 (8.0)	0.40 (12.9)	13.1
Breakfast + egg (43)	4.1	405 (1695)	0.31 (7.6)	0.19 (4.6)	3.6
Breakfast + egg + bacon (43)	4.2	410 (1715)	0.30 (7.1)	0.25 (6.0)	5.9
Breakfast + corn flakes (43)	3.6	555 (2322)	0.19 (5.4)	0.16 (4.4)	3.1
Sauerkraut + sausage (43)	2.0	470 (1966)	0.91 (45.8)	0.90 (45.0)	41.4
Beetroot soup + meat (51)	2.8	300 (1235)	0.85 (30.3)	0.81 (29.1)	26.4
Sole au gratin (49)	2.1	330 (1381)	0.39 (18.7)	0.38 (18.0)	18.2
Brown beans + pork (49)	5.4	750 (3138)	0.22 (4.0)	0.43 (7.8)	5.5
Roast beef meal (49)	3.1	480 (2008)	0.36 (11.6)	0.58 (18.7)	17.7
Spaghetti with meat sauce (51)	2.7	600 (2510)	0.31 (11.3)	0.31 (11.1)	12.5
Cod (48)	7.8	1050 (4393)	0.63 (8.1)	0.80 (10.4)	10.9
Gazpacho and chicken (48)	7.6	1040 (4351)	1.10 (14.5)	1.35 (17.6)	17.1
Meatballs (49)	2.6	600 (2510)	0.19 (5.4)	0.29 (11.1)	10.6
Shrimp and beef (48)	6.2	980 (4100)	0.95 (15.3)	0.94 (15.1)	14.0
Antipasti misti and meat (48)	7.8	1150 (4812)	1.55 (18.0)	1.80 (23.1)	23.0
Vegetarian meal "low" (48)	5.8	730 (3054)	0.14 (2.5)	0.13 (2.3)	0.4

<sup>1</sup>The study included 24 meals. Details of meals are described in individual references.

<sup>2</sup>"Breakfast" encompasses coffee, white-wheat bread, margarine, cheese, and marmalade.

amounts estimated by using the algorithm (Table 4). The mean total iron absorption obtained with the 2 methods was not statistically different on the basis of a *t* test; the difference between the means with both methods was only 0.06 mg (or 3.4%) and was not statistically significant ( $t = -0.588$ ,  $P = 0.561$ ).

#### Application of the algorithm for different levels of iron status

The present calculations are based on absorption values adjusted to a reference dose absorption of 40%. Because the relation between reference dose absorption and log serum ferritin is known, it is possible to convert the algorithm to any iron status (Appendix A).

$$\text{Iron absorption (mg)} = \text{iron absorption (alg mg)} \times 23^{0.94/\text{SF}} (\mu\text{g/L}) \quad (11)$$

where iron absorption (alg mg) is the absorption calculated (mg) by using the algorithm, ie, at a reference dose absorption of 40%, and SF is serum ferritin.

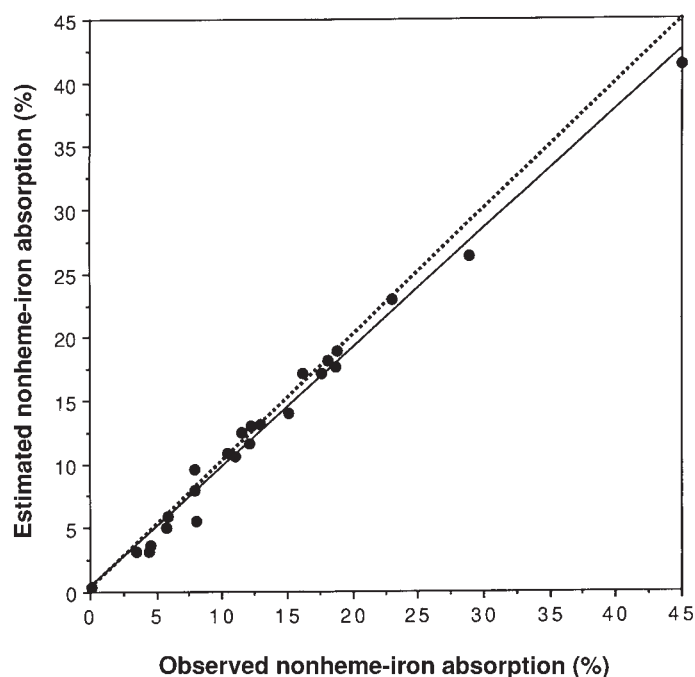
#### DISCUSSION

It has been nearly 20 y since the first simple algorithm for estimating iron absorption was published (4). Since then, much new knowledge has accumulated about dietary iron absorption, as emphasized in a recent review (52). It is thus probable that new information will lead to modifications of the present algorithm. Instead of waiting for the "final version," we developed an algo-

gorithm based on as much present knowledge as possible and we think the present algorithm has many practical applications.

Note that the method of measuring iron absorption from the whole diet with tracers has been validated. In each subject, a comparison was made between the absorption measured and iron requirements. In men, requirements were calculated from body weight and in women from body weight and measured menstrual losses of iron (53). The comparison in study 1 clearly showed that iron absorption estimated with the algorithm agreed well with measured iron absorption.

Nonheme-iron absorption was estimated for the 24 meals in study 1 by using the 2 previously published algorithms, in which effects of both enhancers and inhibitors were included. In the earliest study (7), there was a significant relation between observed and estimated absorption ( $r^2 = 0.192$ ,  $P = 0.032$ ). There was also a significant relation between observed and estimated nonheme-iron absorption ( $r^2 = 0.256$ ,  $P = 0.0116$ ) when a more recent algorithm was used (6). These correlation coefficients are thus considerably lower than that obtained with the present algorithm ( $r^2 = 0.987$ ) for estimated and observed nonheme-iron absorption. Probable reasons are that, in contrast with the 2 previous algorithms mentioned, the present algorithm 1) is based on continuous variables for content of enhancers and inhibitors, 2) takes into consideration interactions between factors, and 3) includes more factors. In study 2, the same mean heme- and nonheme-iron absorption values were seen despite the expected markedly varying bioavailability of iron in the 20 different meals included (Table 3).



**FIGURE 2.** Relation between observed and estimated nonheme-iron absorption in study 1 with use of the algorithm. Data reflect the mean values from 24 studies in 243 subjects.  $y = 0.43 + 0.94x$ ;  $r^2 = 0.987$

An important difference between the 2 validation studies was that each absorption value in study 1 was the mean of 10 subjects (observed and calculated by using the algorithm; Table 1), whereas each absorption value in study 2 was the mean of 31 subjects measured over 5 d (Table 4). In study 1 the slope of the regression line did not differ from the identity line and there were no statistically significant differences between observed absorption and absorption estimated by using the algorithm at the same iron status. In study 2, the total amounts of observed and calculated (algorithm) iron absorbed from the whole diet were not significantly different after adjustment to the same iron status (Table 4).

#### Effect of meal size and iron content of meals

It may seem obvious that the size of a meal should be taken into account in an algorithm for estimating iron absorption. A cer-

tain amount of ascorbic acid, for example, should be expected to have a greater effect in a small meal than in a large meal because the concentration would be higher in the small meal. Meal size, however, is an ambiguous concept because it can be interpreted in terms of volume, weight, or content of energy or iron. The concentration of a nutrient may also be influenced by the amount of beverage consumed with the meal. Another factor that can influence absorption is the rate of gastric emptying and, in turn, the volume of the meal and its fat content. Meal size as well as body size can influence the absorption of iron from a specific meal; however, we did not observe either in our adult volunteers.

There was almost a 4-fold variation in the content of both energy and iron and a 3-fold variation in nutrient density (nonheme iron/energy) in the meals in study 1 (Table 1). Despite these variations, the relation between calculated and observed

**TABLE 2**

Descriptive characteristics of the 24 different meals included in study 1 and the 20 different meals included in study 2

	Study 1			Study 2		
	$\bar{x} \pm SD$	Median	Range	$\bar{x} \pm SD$	Median	Range
Energy (MJ)	$3.03 \pm 1.21$	2.85	1.26–4.81	$2.52 \pm 0.46$	2.51	1.63–3.26
Nonheme iron (mg)	$4.74 \pm 1.92$	4.2	2–7.8	$2.75 \pm 1.32$	2.2	1.2–5.1
Heme iron (mg)	$0.38 \pm 0.44$	0.1	0–1.2	$0.73 \pm 0.83$	0.2	0.04–2.7
Total iron (mg)	$5.07 \pm 1.93$	4.6	2.2–8.4	$3.48 \pm 1.95$	2.63	1.25–6.8
Ascorbic acid (mg)	$15.9 \pm 23.8$	4	0–85	$23.2 \pm 29.0$	5	0–95
Meat and fish (g)	$59.6 \pm 53.3$	60	0–175	$68 \pm 71.2$	55	0–175
Calcium (mg)	$171.8 \pm 160.5$	130	0–600	$226 \pm 183$	229.5	30–409
Phytate phosphorus (mg)	$26.8 \pm 56.3$	8	0–271	$18.3 \pm 10.5$	12.8	11.4–52
Tannic acid equivalents (mg)	$13.8 \pm 23.1$	4	0–100	$28.9 \pm 33.1$	19.5	0–80
Eggs (n)	$0.21 \pm 0.388$	0	0–1	0	—	—
Soy	0	—	—	0	0	—
Alcohol factor	$1.03 \pm 0.08$	1	0.73–1	1.0	—	—



**TABLE 3**

Total amounts of heme and nonheme iron absorbed from different meals on different days in Study 2 in 11 of the 31 subjects having an identical pattern in the consumption of beverages with the meals

Day	Heme- + nonheme-iron absorption (mg)				
	Breakfast	Lunch	Dinner	Evening	Whole day
	<i>mg</i>				
1	0.082	1.87	2.66	0.083	4.70
2	0.045	1.32	1.11	0.082	2.56
3	0.076	1.51	1.11	0.058	2.75
4	0.055	2.01	2.09	0.081	4.24
5	0.076	1.39	1.84	0.098	3.40
$\bar{x}$	0.067	1.62	1.76	0.080	3.53
Median	0.076	1.51	1.84	0.082	3.40
SD	0.0159	0.304	0.665	0.014	0.925
CV	23.8	18.8	37.8	17.8	26.2

absorption was more similar than we had expected. Thus, the balance of evidence indicates that meal size per se had no major systematic effect on the validity of the algorithm. The algorithm may need modification when used in infants and small children. In a recent study, however, direct comparison of iron absorption from a formula given to adults and infants showed no difference in absorption (54). Moreover, a 3-fold increase in meal size (and iron content) in adults did not change fractional iron absorption (54). It is thus reasonable to assume that the algorithm will also be useful in infants.

A linear relation was observed between log amounts of iron administered and log amounts of iron absorbed (*see* references 55 and 56 for a review). Most of these studies used therapeutic doses of iron or pure iron solutions; iron given with food seems to behave differently. In one of our early studies, we found that the percentage iron absorption from a meal was the same despite an almost 5-fold difference in iron content (57). This result is thus compatible with the results mentioned above (54), probably because the concentration of iron in the gastrointestinal lumen is many times lower when a certain amount of iron is present in a meal than when the iron is provided as a salt without food.

#### Iron absorption from single meals compared with that from the whole diet

To estimate iron absorption from the whole diet, absorption measurements from all the single meals consumed over a certain time period are summed. Almost all studies of factors influencing iron absorption are based on single meals served in a fasting state, with and without a factor to be studied given in different amounts. Note that direct measurements have shown that a preceding meal has no effect on the absorption of iron from a subsequent meal. In studies of 4 diets, it was shown that iron absorption from a meal served in the morning after an overnight fast was the same as that from a meal eaten during the day at lunch or supper (58). Similarly, we found that iron absorption was the same from a hamburger meal served in the morning or after breakfast (with or without added calcium) 2 or 4 h earlier (59).

It has been suggested that the variation in iron absorption from single meals under laboratory conditions would exaggerate the variation in iron absorption from the whole diet (7). The variation in iron absorption between single meals of different compositions may be much greater than the variation in iron absorp-

tion from whole diets composed of several single meals. The iron content and bioavailability of single meals varies markedly, whereas iron absorption from whole diets is the mean absorption of several single meals. The expected lower variation in iron absorption from the whole diet than from single meals was documented previously (7) and in the 3 studies of iron absorption from whole diets in our laboratory (46, 50, 51).

Some investigators seem to have misinterpreted these results and assumed that the absorption of iron from single meals per se, for some unknown reason, would be falsely high or low. The present result that the sum of the calculated iron absorption from 4 different meals served for 5 d (ie, 20 meals in 31 men for a total of 620 meals) did not differ significantly from that obtained from meals in which heme and nonheme iron were homogeneously labeled with 2 different tracers, clearly indicates the validity of basing total dietary iron absorption on the sum of iron absorption from single meals. This issue was also discussed in our previous review (53).

#### Some applications of the algorithm

The algorithm can be used to evaluate the nutritional value of meals with respect to iron, for example, in school-lunch programs, in catering programs for the elderly, and for military services. The algorithm may be used to translate data from dietary surveys into amounts of iron expected to be absorbed. The main requirement for such calculations is that detailed information is available about the meal composition and its variation over a representative and sufficiently long period of time. A 7-d record, for example, may not represent the iron absorption from the habitual diet.

The algorithm can be used to estimate the expected effects of different dietary modifications that can be considered realistic in both developed and developing countries. In developed countries, the main concerns are low energy expenditure and, thus, low energy intakes. To adequately provide for high iron needs, especially in infants, adolescents, and menstruating women, a high nutrient density and a high bioavailability is required. The algorithm can also be used to examine the overall effects of a higher extraction of flour (increasing the intake of both intrinsic phytate and iron) on bioavailability and iron content. It can be used to estimate the expected effects of iron

**TABLE 4**

Iron absorption from the whole diet<sup>1</sup>

	Calculated absorption from the algorithm			Observed absorption <sup>3</sup>
	Iron intake	At 40% of the	Adjusted	
		reference dose absorption <sup>2</sup>	to actual iron status <sup>3</sup>	
	<i>mg</i>	<i>mg</i>	<i>mg</i>	
Nonheme iron	11.0	2.61	1.24 ± 0.22	1.23 ± 0.19
Heme iron	2.94	1.05	0.52 ± 0.02	0.52 ± 0.03
Total	13.94	3.65	1.77 ± 0.19	1.76 ± 0.21

<sup>1</sup>*n* = 31 healthy men. Absorption was calculated by using the algorithm. The original calculation is based on an iron status corresponding to a reference dose absorption of 40%. For each subject, this absorption was then adjusted to the individual iron status based on the serum ferritin concentration and was compared with the actual observed absorption by using whole-body counting (*see text*).

<sup>2</sup> $\bar{x}$ .

<sup>3</sup> $\bar{x} \pm \text{SEM}$ .


fortification or increased intakes of fruit, vegetables, and meat in the diet. In developing countries, the problems are similar but the knowledge about the chemical composition of foods and its variation is even more limited; for example, knowledge is limited about the contents of phytate and iron binding polyphenols in common foods, including spices and condiments. Evaluation of the expected effects on iron absorption and iron balance resulting from modification of food-preparation methods may also be required.

An important use of the algorithm would be to translate physiologic iron requirements into dietary requirements under different dietary conditions known to prevail in a certain population. In the Food and Agriculture Organization/World Health Organization recommendations, 3 levels of bioavailability (5%, 10%, and 15%) were used arbitrarily for this translation (60). The validity of choices of representative bioavailability values can be tested by using the algorithm. It is obvious from the present results that there is marked variation in the bioavailability of different types of diets in developed countries. The recommended dietary allowances (61) for different groups of subjects with different physiologic iron requirements should, therefore, not be given as single values, but rather as 3–4 values adjusted for different types of diets (eg, vegan or vegetarian, low-meat, and high meat). The algorithm can then be used to make rough estimates of the bioavailability of diets in some groups in the population with different dietary habits. The algorithm may be useful in the future search for realistic recommendations to be used in food-based strategies to improve iron nutrition in developing countries. However, more knowledge about the composition and properties of diets in developing countries is needed.

In the screening for unknown dietary factors influencing iron absorption, new starting points can be obtained by comparing actual absorption values from a certain meal with absorption values estimated from the content of presently known factors. A significant discrepancy would indicate that some unknown nutritionally important factor is present.

#### Importance of correct values for the factors included in the algorithm

One problem with the application of the algorithm is limited knowledge about the content of factors such as phytate and iron binding polyphenols in different foods. An extensive report on the phytate content in foods was published previously (62). Note that even low phytate contents play an important role in the bioavailability of iron, but are often not detectable with the current method used by the Association of Official Analytical Chemists, which was used in that report. A simple modification of the current method of the Association of Official Analytical Chemists was made to determine low phytate contents in foods and was calibrated against HPLC (11).

Another practical problem in applying the algorithm is the difficulty in estimating the ascorbic acid content in a meal at the time of consumption because cooking times and food-preparation methods markedly influence the final phytate content. In Appendix A, we provide data for some common foods. Appendix A also contains data from our laboratory about the content of total iron and heme iron in different kinds of meat. More detailed food-composition tables are needed. The lack of knowledge of the presence of different factors in different foods is even more obvious when the algorithm is applied to diets in developing countries. 

#### REFERENCES

- Hallberg L, Björn-Rasmussen E. Determination of iron absorption from whole diet. A new two-pool model using two radioiron isotopes given as haem and non-haem iron. *Scand J Haematol* 1972;9:193–7.
- Cook JD, Layrisse M, Martinez-Torres C, Walker R, Monsen E, Finch CA. Food iron absorption measured by an extrinsic tag. *J Clin Invest* 1972;51:805–15.
- Hallberg L. Bioavailability of dietary iron in man. *Annu Rev Nutr* 1981;1:123–47.
- Monsen ER, Hallberg L, Layrisse M, et al. Estimation of available dietary iron. *Am J Clin Nutr* 1978;31:134–41.
- Monsen ER, Balintfy JL. Calculating dietary iron bioavailability, refinement and computerization. *J Am Diet Assoc* 1982;80:307–11.
- Tseng M, Chakraborty H, Robinson DT, Mendez M, Kohlmeyer L. Adjustment for iron intake for dietary enhancers and inhibitors in population studies: bioavailable iron in rural and urban residing Russian women and children. *J Nutr* 1997;127:1456–68.
- Cook JD, Dassenko SA, Lynch SR. Assessment of the role of non heme-iron availability in iron balance. *Am J Clin Nutr* 1991; 54:717–22.
- Layrisse M, Cook JD, Martinez-Torres C, Roche M, Kuhn IN, Walker RB. Food iron absorption: a comparison of vegetable and animal foods. *Blood* 1969;33:430–43.
- Magnusson B, Björn-Rasmussen E, Rossander L, Hallberg L. Iron absorption in relation to iron status. Model proposed to express results of food iron absorption measurements. *Scand J Haematol* 1981;27:201–8.
- Cook JD, Lipschitz DA, Miles LEM, Finch CA. Serum ferritin as a measure of iron stores in normal subjects. *Am J Clin Nutr* 1974; 27:681–7.
- Brune M, Rossander-Hultén L, Hallberg L, Gleeurup A, Sandberg A-S. Iron absorption from bread in humans: inhibiting effects of cereal fiber, phytate and inositol phosphates with different numbers of phosphate groups. *J Nutr* 1992;122:442–9.
- Hallberg L. Food iron absorption. In: Cook JD, ed. *Methods in hematology*. Vol 1. London: Churchill, 1980:116–33.
- Brune M, Rossander L, Hallberg L. Iron absorption and phenolic compounds: importance of different phenolic structures. *Eur J Clin Nutr* 1989;43:547–58.
- Hallberg L, Brune M, Rossander L. Iron absorption in man: ascorbic acid and dose-dependent inhibition by phytate. *Am J Clin Nutr* 1989;49:140–4.
- Hallberg L, Brune M, Erlandsson M, Sandberg AS, Rossander-Hultén L. Calcium: effect of different amounts on nonheme- and heme-iron absorption in man. *Am J Clin Nutr* 1991;53:112–9.
- Hallberg L, Brune M, Rossander L. Effect of ascorbic acid on iron absorption from different types of meals. Studies with ascorbic-acid-rich foods and synthetic ascorbic acid given in different amounts with different meals. *Hum Nutr Appl Nutr* 1986;40A:97–113.
- Cook JD, Monsen ER. Vitamin C, the common cold, and iron absorption. *Am J Clin Nutr* 1977;30:235–41.
- Siegenberg D, Baynes RD, Bothwell TH, et al. Ascorbic acid prevents the dose-dependent inhibitory effects of polyphenols and phytates on nonheme-iron absorption. *Am J Clin Nutr* 1991;53:537–41.
- Hurrell RF, Lynch SR, Trinidad TP, Dassenko SA, Cook JD. Iron absorption in humans: bovine serum albumin compared with beef muscle and egg white. *Am J Clin Nutr* 1988;47:102–7.
- Reddy MB, Hurrell RF, Juillerat MA, Cook JD. The influence of different protein sources on phytate inhibition of nonheme-iron absorption in humans. *Am J Clin Nutr* 1996;63:203–7.
- Disler PB, Lynch SR, Charlton RW, et al. The effect of tea on iron absorption. *Gut* 1975;16:193–200.
- Derman D, Sayers M, Lynch SR, Charlton RW, Bothwell TH. Iron absorption from a cereal-based meal containing cane sugar fortified with ascorbic acid. *Br J Nutr* 1977;38:261–9.
- Hallberg L, Rossander L. Effect of different drinks on the absorption of non-heme iron from composite meals. *Hum Nutr Appl Nutr* 1982;36A:116–23.

24. Morck TA, Lynch SR, Cook JD. Inhibition of food iron absorption by coffee. *Am J Clin Nutr* 1983;37:416–20.
25. Bezwoda WR, Torrance JD, Bothwell TH, Macphail AP, Graham B, Mills W. Iron absorption from red and white wines. *Scand J Haematol* 1985;34:121–7.
26. Cook JD, Reddy MR, Hurrell RF. The effect of red and white wines on nonheme-iron absorption in humans. *Am J Clin Nutr* 1995;61:800–4.
27. Gillooly M, Bothwell TH, Torrance JD, et al. The effect of organic acids, phytates and polyphenols on absorption of iron from vegetables. *Br J Nutr* 1983;49:331–42.
28. Harborne JB. Plant phenolics. *Encycl Plant Physiol* 1980;8:329–402.
29. Brune M, Hallberg L, Skånberg A-B. Determination of iron-binding phenolic groups in foods. *J Food Sci* 1991;56:131–67.
30. Tuntawiroon M, Sritongkul N, Brune M, et al. Dose-dependent inhibitory effect of phenolic compounds in foods on nonheme-iron absorption in man. *Am J Clin Nutr* 1991;53:554–7.
31. Hallberg L, Rossander-Hulthén L, Brune M, Gleerup A. Inhibition of haem-iron absorption in man by calcium. *Br J Nutr* 1992;69:533–40.
32. Bronner F. Intestinal calcium absorption: mechanisms and applications. *J Nutr* 1987;117:1347–52.
33. Hallberg L, Rossander-Hulthén L, Brune M, Gleerup A. Calcium and iron absorption: mechanism of action and nutritional importance. *Eur J Clin Nutr* 1992;46:317–27.
34. Layrisse M, Martinez-Torres C, Roch M. Effect of interaction of various foods on iron absorption. *Am J Clin Nutr* 1968;21:1175–83.
35. Cook JD, Monsen ER. Food iron absorption in human subjects. III. Comparison of the effect of animal proteins on nonheme iron absorption. *Am J Clin Nutr* 1976;29:859–67.
36. Björn-Rasmussen E, Hallberg E. Effect of animal proteins on the absorption of food iron in man. *Nutr Metab* 1979;23:192–202.
37. Hallberg L, Rossander L. Improvement of iron nutrition in developing countries: comparison of adding meat, soy protein, ascorbic acid, citric acid, and ferrous sulphate on iron absorption from a simple Latin American-type of meal. *Am J Clin Nutr* 1984;39:577–83.
38. Cook JD, Morck TA, Lynch SR. The inhibitory effect of soy products on nonheme iron absorption in man. *Am J Clin Nutr* 1981;34:2622–9.
39. Hallberg L, Rossander L. Effect of soy protein on nonheme iron absorption in man. *Am J Clin Nutr* 1982;36:514–20.
40. Morck TA, Lynch SR, Cook JD. Reduction of the soy-induced inhibition of nonheme iron absorption. *Am J Clin Nutr* 1982;36:219–28.
41. Hurrell RF, Juillerat MA, Reddy MB, Lynch SR, Dassenko SA, Cook JD. Soy protein, phytate, and iron absorption in humans. *Am J Clin Nutr* 1992;56:573–8.
42. Callender ST, Marney SR Jr, Warner GT. Eggs and iron absorption. *Br J Haematol* 1970;19:657–65.
43. Rossander L, Hallberg L, Björn-Rasmussen E. Absorption of iron from breakfast meals. *Am J Clin Nutr* 1979;32:2484–9.
44. Charlton RW, Jacobs P, Seftel HC, Bothwell TH. Effect of alcohol on iron absorption. *Br J Med* 1964;2:1427–9.
45. Baynes RD, MacFarlane BJ, Bothwell T. The promotive effect of soy sauce on iron absorption in human subjects. *Eur J Clin Nutr* 1990;44:419–24.
46. Hallberg L, Hulthén L, Gramatkovski E. Iron absorption from the whole diet in men: how effective is the regulation of iron absorption? *Am J Clin Nutr* 1997;66:347–56.
47. Hallberg L, Björn-Rasmussen E. Measurement of iron absorption from meals contaminated with iron. *Am J Clin Nutr* 1981;34:2808–15.
48. Hallberg L, Rossander L. Bioavailability of iron from Western-type whole meals. *Scand J Gastroenterol* 1982;17:151–60.
49. Hallberg L, Rossander L. Absorption of iron from Western-type lunch and dinner meals. *Am J Clin Nutr* 1982;35:502–9.
50. Gleerup A, Rossander-Hulthén L, Gramatkovski E, Hallberg L. Iron absorption from the whole diet: comparison of the effect of two different distributions of daily calcium intake. *Am J Clin Nutr* 1995;61:97–104.
51. Hulthén L, Gramatkovski E, Gleerup A, Hallberg L. Iron absorption from the whole diet. Relation to meal composition, iron requirements and iron stores. *Eur J Clin Nutr* 1995;49:794–808.
52. Hunt JR. Bioavailability algorithms in setting recommended dietary allowances: lessons from iron, applications to zinc. *J Nutr* 1996;126:2345S–53S.
53. Hallberg L, Hulthén L. Methods to study dietary iron absorption in man—an overview. In: Hallberg L, Asp N-G, eds. *Iron nutrition in health and disease*. London: John Libbey & Company Ltd, 1996: 81–95.
54. Hurrell RF, Davidsson L, Reddy M, Kastenmayer P, Cook JD. A comparison of iron absorption in adults and infants consuming identical infant formulas. *Br J Nutr* 1998;79:31–6.
55. Bothwell TH, Finch CA. *Iron metabolism*. Boston: Little, Brown and Company, 1962.
56. Bothwell TH, Charlton RW, Cook JD, Finch CA. *Iron metabolism in man*. London: Blackwell Scientific Publications, 1979.
57. Björn-Rasmussen E, Hallberg L, Rossander L. Absorption of fortification iron. Bioavailability in man of different samples of reduced Fe, and prediction of the effects of Fe fortification. *Br J Nutr* 1977;37:375–88.
58. Taylor PG, Méndez-Castellanos H, Jaffe W, et al. Iron bioavailability from diets consumed by different socioeconomic strata of the Venezuelan population. *J Nutr* 1995;125:1860–8.
59. Gleerup A, Rossander-Hulthén L, Hallberg L. Duration of the inhibitory effect of calcium on non-haem iron absorption in man. *Eur J Clin Nutr* 1993;47:875–9.
60. Report of a joint FAO/WHO Expert Consultation. Requirements of vitamin A, iron, folate and vitamin B12. Rome: FAO, 1988. (Food and Nutrition Series 23.)
61. National Research Council. Recommended dietary allowances. 10th ed. Washington, DC: National Academy Press, 1989.
62. Harland BF, Oberlees D. Phytate in foods. *World Rev Nutr Diet* 1987;52:235–59.

## APPENDIX A

### Observed iron absorption at a certain iron status adjusted to the absorption expected at another iron status

The relation between log serum ferritin and the reference dose absorption was examined in 1066 subjects in whom an adequate serum ferritin standard had been used in the calibration. In these studies we found that a reference dose of 40% corresponded to a serum ferritin concentration of 23  $\mu\text{g/L}$ . Data from a recent study of iron absorption from whole diets in men ( $n = 31$ ) showed that there is a linear relation between log iron absorption and log serum ferritin (SF) ( $r^2 = 0.720$ ):

$$\begin{aligned} \text{Log iron absorption} \\ (\mu\text{g Fe} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}) = (2.9251 - 0.94049) \\ \times \log \text{SF } (\mu\text{g/L}) \end{aligned} \quad (A1)$$

The constant in the equation (2.9251) is valid for a diet with a high bioavailability (1). The slope of this regression line (0.94) was the same for different diets but the intercept on the y axis varied, implying that different diets are represented by different parallel regression lines. A general formula for such regression equations is as follows:

$$\begin{aligned} \text{Log iron absorption} = (C \text{ for a certain diet} \\ - 0.94049) \times \log \text{SF} \end{aligned} \quad (A2)$$



where *C* is a constant. This formula assumes that the iron absorption from this diet was as calculated in equation A3 below at an iron status expressed as the SF<sub>1</sub> corresponding to a reference dose absorption of 40%. We know that this reference dose absorption corresponds to a serum ferritin concentration of 23 µg/L. Another data pair from the same diet would be used to calculate iron absorption:

$$\text{Log absorption 1} = (C - 0.94049) \times \log SF_1 \quad (A3)$$

To calculate iron absorption from the same diet but at a different SF concentration (SF<sub>2</sub>) than used in equation A3, the following equation would be used:

$$\text{Log absorption 2} = (C - 0.94049) \times \log SF_2 \quad (A4)$$

In equations A3 and A4, iron absorption can be expressed as µg Fe/kg body wt or as mg. *C* is eliminated by subtracting equation A3 from equation A4 and the following equation is obtained:

$$\begin{aligned} \text{Log absorption 2} \\ - \text{log absorption 1} &= (-0.94 \times \log SF_2) \\ &+ (0.94 \times \log SF_1) \end{aligned} \quad (A5)$$

Log absorption 2 can then be calculated. The equation can be written in a simpler way by an antilog-transformation, which gives the following equation:

$$\begin{aligned} \text{Log absorption 2} &= \text{log absorption 1} \\ &\times [SF_1 \times (0.94/SF_2)] \end{aligned} \quad (A6)$$

To adjust the iron absorption from a reference dose absorption of 40% to a certain SF concentration, SF<sub>1</sub> is set at 23 µg/L. Log absorption 1 is the observed absorption at this iron status and log absorption 2 is the calculated absorption at the corresponding known SF<sub>2</sub> concentration.

**TABLE A1**  
Phytate and iron binding polyphenols in vegetables, legumes, fruit, berries, beverages, spices, nuts, seeds, soy products, and cereal and cereal products

	Phytate phosphorus <sup>1</sup>	Tannin equivalents	Chlorogenic acid	Total tannin equivalents
<i>mg/100 g dry matter</i>				
Root, leaf, and stem vegetables, and legumes				
Aubergine, whole	3	7	51	31
Asparagus				
Green	2	—	—	—
White	3	—	—	—
Beans				
Black	262	0	—	0
Brown	195	0	—	0
Green	15	—	—	—
Mung	188	140	—	140
Red	271	1	—	1
White	269	0	—	0
Beetroot	2	3	—	3
Broccoli	10	1	40	20
Brussels sprouts	11	0	—	0
Cabbage				
Chinese	2	—	—	—
White	1	0	—	0

(Continued)

**TABLE A1** (Continued)

	Phytate phosphorus <sup>1</sup>	Tannin equivalents	Chlorogenic acid	Total tannin equivalents
<i>mg/100 g dry matter</i>				
Carrot	4	0	28	13
Cauliflower	3	0	—	0
Celeriac	5	0	—	0
Chicory	2	0	—	0
Corn	24	—	—	—
Cucumber	1	0	0	0
Garden cress	7	—	—	—
Garlic	4	0	7	3
Horseradish	13	—	—	—
Kohlrabi	2	—	—	—
Leek	4	0	11	5
Lentils				
Brown	142	190	—	190
Red	122	0	—	0
Lettuce, iceberg	0.5	—	—	—
Mushrooms	13	1	—	1
Olives, black				
Onion	3	—	—	—
Red	5	10	—	10
Yellow	16	6	—	6
Parsley leaves	8	—	—	—
Parsnips	9	0	20	9.5
Peas				
Chickpeas	140	0	—	0
Green peas	175	0	0	0
Yellow peas	270	—	—	—
Peppers				
Sweet green	2	0	—	0
Sweet red	0.5	0	—	0
Sweet yellow	1	0	—	0
Potato	7	0	0	0
Radish				
White	4	0	—	0
Black	1	—	—	—
Rutabaga	1	0	—	0
Sauerkraut	1	0	—	0
Skorzonera (black salsify)	—	0	—	0
Spinach	2	20	12	26
Squash, summer	3	—	—	—
Tomato	2	0	—	0
Fruit and berries				
Apple	0.1	160	—	160
Apricot	—	0	—	0
Avocado	1	0	—	0
Banana	0.4	40	—	40
Blackberry	4	390	—	390
Blueberry	6	80	—	80
Currant				
Black	78	—	—	—
Red	55	—	—	—
Dates	—	5	—	5
Figs	—	0	—	0
Kiwi	10	0	0	0
Cowberry	5	3	250	122
Mango	1	—	—	—
Melon, honey	0.6	—	—	—
Orange	2	0	—	0
Pears	0.2	4	70	37
Raspberry	4	70	61	99

(Continued)



TABLE A1 (Continued)

	Phytate phosphorus <sup>1</sup>	Tannin equivalents	Chlorogenic acid	Total tannin equivalents
<i>mg/100 g dry matter</i>				
Rhubarb	0.2	0	16	8
Strawberry	4	—	—	—
Beverages				
Coffee, brewed <sup>2</sup>	—	21	71	55
Tea				
English breakfast <sup>3</sup>	—	53	14	60
Green <sup>4</sup>	—	26	17	35
Herb	—	18	—	18
Peppermint <sup>4</sup>	—	20	23	31
Cacao powder				
Marabou	504	4400	520	4648
De Zaan <sup>5</sup>	513	—	—	—
De Zaan low fat	342	—	—	—
Fazer <sup>6</sup>	481	—	—	—
With sugar	93	380	69	413
Beer				
Light lager	—	0.4	—	0.4
Strong	—	0.1	—	0.1
Whiskey, Cutty Sark <sup>7</sup>	—	2.9	—	2.9
Wine				
White	—	0	4	2
Red <sup>5,8</sup>	—	0.2–2.3	20–40	10–21
Fruit syrup, sloe	—	6.2	—	6.2
Spices <sup>9</sup>				
Allspice	—	0	—	0
Basil	—	2.7	7.9	6.5
Black pepper	—	2	—	2
Caraway	—	2.8	6.4	5.8
Cardamom	—	0.3	—	0.3
Chervil	—	0.4	2	1.4
Chili pepper	—	0.4	0.8	0.8
Cinnamon	—	43	14.3	50
Clove	—	95	—	95
Cumin	—	2.8	6.4	5.8
Curry	—	6.2	9.9	10.9
Fennel	—	0.3	—	0.3
Ginger	—	0.2	—	0.2
Green pepper	—	1.4	—	1.4
Marjoram	—	6.4	9.9	11.1
Oregano	0.2	21	6	24
Thyme	—	12	4.4	14.1
Turmeric	—	34	0.7	34
Vanilla	—	0.7	—	0.7
White pepper	—	0.4	—	0.4
Nuts, seeds, and soy products				
Brazil nut	—	10	—	10
Cashew nut	—	0	—	0
Hazel nut	—	256	—	256
Peanut	—	0	—	0
Walnut	303	1400	—	1400
Sweet almond	296	43	—	43
Linseeds	296	14	—	14
Sesame seeds	576	—	—	—
Sunflower seeds	393	120	—	120
Soy sauce				
Kikkoman <sup>10</sup>	4	—	—	—
Tamari <sup>11</sup>	15	—	—	—
Chinese mushroom <sup>12</sup>	5	—	—	—
Cereals and cereal products				
Bran				

(Continued)

TABLE A1 (Continued)

	Phytate phosphorus <sup>1</sup>	Tannin equivalents	Chlorogenic acid	Total tannin equivalents
<i>mg/100 g dry matter</i>				
Barley	185	—	—	—
Oat	399–628	0	—	0
Wheat	680–1189	0	58	28
Wheat, coarse	1338	3	—	3
Crisp bread				
Rye, thin	72–86	—	—	—
Rye, fiber	114–193	—	—	—
Graham	192	—	—	—
Oat	166	—	—	—
Rice cakes	113	—	—	—
Rice flour	27	—	—	—
Long grain	53–64	—	—	—
Parboiled	71	—	—	—
Wild, brown	181–215	0	—	0
Starch				
Lentil	3	—	—	—
Maize	3	—	—	—
Rice	1–37	—	—	—
Wheat	0	—	—	—
Corn flakes	12	—	—	—
Millet	217	—	—	—
Oats, rolled	282	0	—	0
Semolina	19	—	—	—
Sorghum				
Red	279	480	—	480
White	389	15	—	15
Spaghetti				
Buitoni <sup>13</sup>	6	—	—	—
Barilla <sup>14</sup>	71	—	—	—
Wheat germ	467	0	—	0

<sup>1</sup> See reference 2. 1 mg phytate phosphorus = 3.5 mg phytic acid = 5.56 μmol phytic acid.

<sup>2</sup> 3.3 g coffee/100 mL water.

<sup>3</sup> 1 g tea/100 mL water.

<sup>4</sup> 1.4 g tea/100 mL water.

<sup>5</sup> Droste, Harlem, Netherlands.

<sup>6</sup> Fazer AB, Solna, Sweden.

<sup>7</sup> Berry Brothers and Rudd, Edinburgh.

<sup>8</sup> Ranges given.

<sup>9</sup> Values are per 1 g.

<sup>10</sup> Kikkoman (s) PTE, Ltd, Singapore.

<sup>11</sup> Kung Markatta AB, Örebro, Sweden.

<sup>12</sup> CHE-BE Trading AB, Stockholm.

<sup>13</sup> Milano, Italy.

<sup>14</sup> Parma, Italy.



**TABLE A2**

Phytate phosphorus, ash, and total iron contents of flour and bread

	Ash content % of dry matter	Total iron mg/100 g dry matter	Phytate phosphorus		
			In flour	In bread baked with water	
				In dry substance mg/100 g dry matter	In bread as eaten
Corn flour	—	—	70–265 <sup>1</sup>	—	—
Rye flour <sup>2</sup>	1.41	2.8	175–244 <sup>1</sup>	86 [44]	50 [26]
Wheat flour	0.38	0.4	13	—	—
Wheat flour	0.45	0.6	22	9	5
Wheat flour	0.63	1.1	61	10	6
Wheat flour	0.72	—	65	—	—
Wheat flour <sup>3</sup>	0.76	1.6	86	19 [81]	11 [48]
Wheat flour	1.38	3.4	181	98	58
Wheat flour	1.60	3.6	227	154	90
Wheat flour	2.34	5.9	364	289	170
Wheat flour	2.50	6.3	348	—	—
Wheat flour	2.74	7.3	404	350	205

<sup>1</sup>Ranges.<sup>2</sup>Values in brackets reflect sourdough fermentation.<sup>3</sup>Values in brackets reflect milk as an ingredient.**TABLE A3**Phytate and iron binding phenols in cooked meat and meat products<sup>1</sup>

	Total iron	Heme iron	Heme iron
	mg/100 g dry matter	% of total iron	mg/100 g dry matter
<b>Beef</b>			
Rump steak	2.9	52	1.5
Sirloin steak	2.5	52	1.3
Round steak	3.2	50	1.6
Topside, round	2.5	48	1.2
Ground	2.5	40	1.0
Corned beef (brisket)	2.0	25	0.5
Roast, sliced	1.7	35	0.6
Pork tenderloin	1.3	23	0.3
Chicken	0.6	0	0
Lamb, leg	3.1	55	1.7
Deer meat	4.5	51	2.3
Moose loin	2.7	41	1.1
<b>Processed meat products</b>			
Sausage (Falú)	0.8	0	0
Sausage, veal	0.7	0	0
Ham, boiled and sliced	0.7	0	0
Liver paste and paté	5.0	16	0.8
<b>Fish</b>			
Cod	0.2	0	0
Mackerel	0.7	0	0
Salmon	0.6	17	0.1
Mussels	4.6	48	2.2

**REFERENCES**

- Hallberg L, Hulthén L, Gramatkovski E. Iron absorption from the whole diet in men: how effective is the regulation of iron absorption? *Am J Clin Nutr* 1997;66:347–56.
- Brune M, Rossander-Hultén L, Hallberg L, Gleerup A, Sandberg A-S. Iron absorption from bread in humans: inhibiting effects of cereal fiber, phytate and inositol phosphates with different numbers of phosphate groups. *J Nutr* 1992;122:442–9.
- Brune M, Hallberg L, Skånberg A-B. Determination of iron-binding phenolic groups in foods. *J Food Sci* 1991;56:131–67.
- Hallberg L. Food iron absorption. In: Cook JD, ed. *Methods in hematology*. Vol 1. London: Churchill, 1980:116–33.

### Erratum

Maine D. Role of nutrition in the prevention of toxemia. *Am J Clin Nutr* 2000;72(suppl):298S–300S. In Belizan's second comment in the first column of page 300S, the amount taken should be 2 g Ca/d, not 5 mg/d.

### Erratum

Hallberg L, Hulthén L. Prediction of dietary iron adsorption: an algorithm for calculating absorption and bioavailability of dietary iron. *Am J Clin Nutr* 2000;71:1147–60. On page 1149, Equation 2 should be as follows:

$$\text{Absorption ratio} = 1 + 0.01 \text{ AA (in mg) + log (phytate P + 1) (in mg)} \\ \times 0.01 \times 10^{0.8875 \times \log (\text{AA} + 1)}$$

The brackets that appeared in the equation were added in error. The Production Office regrets the mistake.

### Mild hyperhomocysteinemia: vitamin supplementation or not?

Dear Sir:

We read with interest the articles by Brattström and Wilcken (1) and Ueland et al (2), which raise several important questions about homocysteine and cardiovascular risk.

We studied 62 adults aged <45 y with vascular disease [20 with cerebrovascular disease (stroke or TIA; group 1), 20 with deep-vein thrombosis (group 2), and 22 with retinal vein occlusion (group 3)] and 25 healthy control subjects (3–6). We measured glucose, total cholesterol, triacylglycerol, creatinine, red blood cell folate, serum vitamin B-12, and plasma total homocysteine (tHcy) concentrations in blood samples collected at 0 h. An additional blood sample for measurement of plasma tHcy was collected 4 h after administration of oral methionine (100 mg L-methionine/g diluted in orange juice).

tHcy was determined by HPLC with fluorescence detection. In all patients, we tested for a mutation in the coding region of the *MTHFR* gene—a C-to-T substitution at nucleotide 677 (*MTHFR* 677C→T); 9.5% and 9.8% of groups 1 and 2, respectively, were homozygous for the *MTHFR* 677C→T polymorphism (NS compared with the control group). In group 3, 18% of the patients were homozygous for the mutation, which was significantly different from the value (10%) in control subjects ( $P < 0.05$ ).

tHcy concentrations before and after the methionine load were higher in 15% of the patients in groups 1 and 2 than in the control subjects (fasting values:  $12.5 \pm 6.5$   $\mu\text{mol/L}$  compared with  $7.6 \pm 4.1$   $\mu\text{mol/L}$ ; after the methionine load:  $28 \pm 10.1$  compared with  $17.4 \pm 6.5$   $\mu\text{mol/L}$ ); there was no significant increase in group 3. Values  $\geq 12$   $\mu\text{mol/L}$  were considered to indicate fasting hyperhomocysteinemia.

Our studies raised 2 questions: 1) Should we treat patients with mild hyperhomocysteinemia but not homozygous for the *MTHFR* 677C→T polymorphism or patients homozygous for the mutation but with normal tHcy concentrations? 2) Should decisions about treating mild hyperhomocysteinemia be based on absolute tHcy concentrations or on differences between tHcy concentrations measured after fasting and those measured after a methionine load?

Because homozygosity for the *MTHFR* 677C→T mutation is expected to increase cardiovascular risk only when tHcy concentrations are mildly elevated, we suggest that only patients with mild hyperhomocysteinemia receive folate supplementation. Finally, we believe that other risk factors that may provoke vascular lesions in patients with mild hyperhomocysteinemia must be considered before any decisions regarding folate supplementation be made.

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### REFERENCES

1. Brattström L, Wilcken DEL. Homocysteine and cardiovascular disease: cause or effect? *Am J Clin Nutr* 2000;72:315–23.
2. Ueland PM, Refsum H, Beresford SAA, Vollset SE. The controversy over homocysteine and cardiovascular risk. *Am J Clin Nutr* 2000;72:324–32.
3. Minniti G, Piana A, Armani U, Cerone R. Determination of plasma homocysteine by high performance liquid chromatography with fluorescence detection. *J Chromatogr A* 1998;828:401–5.
4. Minniti G, Cerone R, Piana A, Armani U, Lorini R. Plasma and serum total homocysteine concentrations in paediatric patients, evaluated by high-performance liquid chromatography with fluorescence. *Clin Chem Lab Med* 2000;38:675–76.
5. Piana A, Verrastro G, Minniti G, et al. Homocysteine and *MTHFR* genotype in young adults with retinal vein occlusion. *Haematologica* 2000;85:55–6.
6. Cerone R, Minniti G, Piana A, Armani U, Susco G, Bertolini S. Screening for hyperhomocysteinemia in young adults with premature coronary artery disease. *J Southeast Asian Trop Med Public Health* 1999;30:145–9.

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### Reply to R Cerone et al

Dear Sir:

We thank Cerone et al for their comments about our article. In relation to the 2 questions they posed, the first relates to the question of whether to treat patients with mild hyperhomocysteinemia but no 677C→T mutation (C-to-T substitution at nucleotide 677) of the *MTHFR* gene or patients homozygous for the mutation but with normal total homocysteine concentrations. We attempted to answer this question in a meta-analysis of 23 case-control studies involving 5869 patients with established atherosclerotic vascular disease and in 6644



control subjects. We found no significant difference in the prevalence of the mutant gene in the vascular disease patients regardless of whether it was associated with a mild elevation in homocysteine (1). Thus, the presence of the gene variant does not itself provide an indication for folate treatment. Furthermore, in our recent article that included a meta-analysis of 2683 patients with venous thromboembolism and 3306 control subjects, the prevalence of homozygotes for the mutant *TT* genotype was the same in patients with venous thrombosis and control subjects (2). Thus, all of these results are consistent with our view that the presence of the *MTHFR* gene mutation is benign and does not itself enhance cardiovascular risk.

With regard to the second question about homocysteine measurement, it should be remembered that an abnormal increase in homocysteine after a methionine load reflects impaired transsulfuration, which is pyridoxine-dependent, and not the remethylation of homocysteine to methionine, which is predominantly folate-dependent but also requires vitamin B-12. Thus, a practical approach is simply to measure total homocysteine concentrations after an overnight fast because elevated concentrations reflect abnormalities in either of these 2 pathways. Total free homocysteine concentrations may be a more accurate indicator of homocysteine-related endothelial dysfunction (3).

The most important and as yet unresolved question is whether therapy that lowers modestly elevated circulating homocysteine concentrations also reduces cardiovascular risk. This will be determined within the next few years when the results of ongoing trials designed specifically to answer this question are available (4). In the meantime, it is reasonable to consider treatment in patients with established vascular disease and a paucity or absence of conventional risk factors other than a modest elevation in homocysteine.

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## REFERENCES

1. Brattström L, Wilcken DEL, Öhrvik J, Brudin L. Common methyl-ene-tetrahydrofolate reductase gene mutation leads to hyperhomocysteinemia but not to vascular disease. *Circulation* 1998;98:2520–6.
2. Brattström L, Wilcken DEL. Homocysteine and cardiovascular disease: cause or effect? *Am J Clin Nutr* 2000;72:315–23.
3. Chambers JC, Ueland PM, Obeid OA, Wrigley J, Refsum H, Kooner JS. Improved vascular endothelial function after oral B vitamins: an effect mediated through reduced concentrations of free plasma homocysteine. *Circulation* 2000;102:2479–83.
4. Clarke R, Collins R. Can dietary supplements with folic acid or vitamin B<sub>6</sub> reduce cardiovascular risk? Design of clinical trials to test the homocysteine hypothesis of vascular disease. *J Cardiovasc Risk* 1998;5:249–55.

## Fat emulsion infusions and prolongation of the Q-T interval

Dear Sir:

The article by Marfella et al (1) showing that elevated plasma fatty acid concentrations prolong cardiac repolarization has important implications for hospitalized patients. In that study, healthy volunteers were given an infusion of a 10%-fat emulsion (Intralipid; Pharmacia, Uppsala, Sweden) or a saline solution. Infusion of the fat emulsion resulted in a significant prolongation of the Q-T interval. Increases in plasma fatty acids and epinephrine resulting from the fat infusion were each independently associated with the prolongation of the Q-T interval.

Infusions of fat emulsions are routinely used as a source of energy and essential fatty acids in critically ill patients requiring parenteral nutrition (2). The same patients may be at risk of acquired Q-T interval prolongation from the use of medications such as antiarrhythmics, phenothiazines, tricyclic antidepressants, and erythromycin or electrolyte disturbances such as hypokalemia and hypomagnesemia (3). Because prolongation of the Q-T interval is associated with arrhythmias and sudden death (4), infusion of fat emulsions in patients receiving drugs or with conditions that can also prolong the Q-T interval should be monitored closely and, perhaps, contraindicated if the Q-T interval is prolonged.

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## REFERENCES

1. Marfella R, De Angelis L, Nappo F, et al. Elevated plasma fatty acid concentrations prolong cardiac repolarization in healthy subjects. *Am J Clin Nutr* 2001;73:27–30.
2. McMahon MM. Parenteral nutrition. In: Goldman L, Bennett JC, eds. *Cecil textbook of medicine*. Philadelphia: WB Saunders, 2000: 1166–70.
3. Zipes DP. Specific arrhythmias: diagnosis and treatment. In: Braunwald E, ed. *Heart disease. A textbook of cardiovascular medicine*. Philadelphia: WB Saunders, 1997:640–704.
4. Zipes DP. The long QT interval syndrome: a Rosetta stone for sympathetic related ventricular tachyarrhythmias. *Circulation* 1991;84: 1414–9.

## Reply to MR Goldstein

Dear Sir:

We are grateful to Goldstein for his interesting comment regarding the potential risk associated with infusion of fat emulsions in critically ill patients. As he correctly stated, patients requiring parenteral nutrition may be at increased risk of acquired prolongation of the Q-T interval as the result of either drug administration or electrolyte disturbances. In theory, an increased susceptibility to pro-

longation of the Q-T interval with infusion of fat emulsions may augment the risk of cardiac arrhythmias and sudden death; in practice, there is a surprising lack of information about this association. We agree that the Q-T interval should be monitored in patients receiving parenteral nutrition, particularly in those with additional risk factors for acquired prolongation of the Q-T interval. Additional studies are needed to characterize in full the risk of Q-T interval prolongation during parenteral nutrition in hospitalized patients.

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prevalence in the general population. Theoretically, a lower cancer prevalence in health professionals may not correspond to the same reduction in cancer risk in the general population with a similar intake of fruit and vegetables.

Because the media coverage of the results from the NHS and the HPFS is so extensive, it would be helpful to the nutrition community if the discrepancy between the low vegetable consumption and the calculated high carotenoid intake and the other raised questions were resolved before we draw final conclusions from these studies regarding diet and cancer prevention.

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## Validity of dietary assessment

Dear Sir:

In light of recently published data from the Nurses' Health Study (NHS) and the Health Professionals Follow-Up Study (HPFS) on fruit and vegetable consumption and cancer risk (1–3), we would like to comment on the validity of the dietary assessment and on the conclusions that were based on these results. Over the past decade, these 2 cohorts have been highly valuable study groups, generating important data on the relation between diet and disease risk. However, the recent analyses of the combined study groups (a total of  $\leq 136089$  participants) have raised several questions (1–3).

Data on the frequency of fruit and vegetable intake among participants of the HPFS show that 89% of all men consumed  $\leq 2$  servings of vegetables/d and 62% consumed  $\leq 2$  servings of fruit/d (3). Without discussing the problems of overreporting on food-frequency questionnaires for foods perceived as healthy and the potential measurement error of repeated dietary assessments (2, 4), such a low intake of fruit and vegetables should result in low carotenoid intakes (5). However, as reported by Michaud et al (1), men with lung cancer in the lowest quintile of the HPFS had a total carotenoid intake of 7.8 mg/d and men in the highest quintile had an intake of 33.2 mg/d. Because vegetables are the major carotenoid sources in the diet, we wonder how the reported low intake of vegetables could result in the relatively high carotenoid intakes. In comparison, German men consume  $\approx 2.5$  servings of vegetables daily (6), which in terms of total carotenoids results in a daily average intake of 5.6 mg/d (7). At the time of the dietary assessments in the HPFS, no carotenoid supplements were available except for  $\beta$ -carotene. Therefore, supplements could not have contributed to the high carotenoid intake. We will not comment on the observations that increased consumption of fruit and vegetables is related to increased energy intake, and on the small variability in the intake of fruit and vegetables (3), because others have already pointed out these issues (8, 9).

A further point is the question of how representative the prevalence of diet-related cancer in nurses and physicians is of the

## REFERENCES

1. Michaud DS, Feskanich D, Rimm EB, et al. Intake of specific carotenoids and risk of lung cancer in 2 prospective US cohorts. *Am J Clin Nutr* 2000;72:990–7.
2. Feskanich D, Ziegler RG, Michaud DS, et al. Prospective study of fruit and vegetable consumption and risk of lung cancer among men and women. *J Natl Cancer Inst* 2000;92:1812–23.
3. Michels KB, Giovannucci E, Josphipura KJ, et al. Prospective study of fruit and vegetable consumption and incidence of colon and rectal cancers. *J Natl Cancer Inst* 2000;92:1740–52.
4. Flood A, Schatzkin A. Colorectal cancer: does it matter if you eat your fruits and vegetables? *J Natl Cancer Inst* 2000;92:1706–7.
5. Broekmans WMR, Klopping-Ketelaars IAA, Schuuman CRWC, et al. Fruits and vegetables increase plasma carotenoids and vitamins and decrease homocysteine in humans. *J Nutr* 2000;130:1578–83.
6. Mensink GMB, Thamm M, Haas K. Die Ernährung in Deutschland 1998. (Dietary intake in Germany 1988.) *Gesundheitswesen* 1999;61: S200–6 (in German).
7. Pelz R, Schmidt-Faber B, Hesecker H. Die Carotinoidzufuhr in der Nationalen verzehrsstudie. (Carotenoid intake in the German National Food Consumption Survey.) *Z Ernährungswiss* 1998;37: 319–27 (in German).
8. American Institute for Cancer Research. Re: recent findings of Nurses and Health Professionals Study on fruits, vegetables and colon cancer. Press release, October 31, 2000. World Wide Web: <http://www.aicr.org/r103100b.htm> (accessed 4 June 2001).
9. American Institute for Cancer Research. Cancer researchers to Harvard Nurses Study: not so fast! Press release, October 31, 2000. World Wide Web: <http://www.aicr.org/r103100a.htm> (accessed 4 June 2001).

## Reply to B Watzl and G Rechkemmer

Dear Sir:

We are grateful for the opportunity to inform readers of an error in our publication in the *Journal of the National Cancer Institute* in November 2000 [(1); erratum in press]. The error, which does not affect the conclusions, pertains to the distribution

of men (in the Health Professionals Follow-Up Study cohort) across categories of servings of vegetables per day [Table 1 of the article by Michels et al (1)]. It is shown in Table 1 that 89% of men in the Health Professionals Follow-Up Study cohort ate  $\leq 2$  servings of vegetables daily; however, this is incorrect. The median intake of total vegetables in this cohort was 3.1 servings/d; the distribution is presented in Table 1 of the article by Michaud et al (2). At baseline, almost 20% of men consumed  $\geq 5$  servings of vegetables daily.

We would like to raise a few additional points. In our population, similar to most populations in the United States, lycopene contributed most to the total carotenoid values (3). The main sources of lycopene are tomato-based products, such as tomato sauce and tomato soup. These items may not be commonly consumed in some other non-US populations, and are often not included in the calculations of total fruit and vegetable intake. Thus, fruit and vegetable and carotenoid intakes across populations are not directly comparable. Additionally, the median values for total carotenoid intake from Table 1 of the publication in the *American Journal of Clinical Nutrition* (3) were based on categories and not quintiles. As detailed in the footnote of the table and in the Methods, high intake corresponded to participants consuming high amounts (fifth quintile) of  $\geq 3$  of the individual carotenoids, whereas individuals consuming low amounts (first quintile) of  $\geq 3$  of the individual carotenoids were placed in the bottom category. The more restrictive categorization based on simultaneous high consumption of multiple carotenoids created more extreme comparisons than quintiles of total intake.

Watzl and Rechkemmer were also concerned about the low prevalence of diet-related cancers in these 2 healthy cohorts. Although the incidence of certain cancers in these 2 cohorts is lower than that in the average US population, the range of fruit and vegetable intake is quite large. Thus, the relation between

fruit and vegetable intake and cancer risk can be determined over a wide range of intakes. The associations observed over comparable ranges of intakes in these populations should be similar in other, maybe less educated, populations. Although overall rates of lung cancer were lower in these populations largely because of lower smoking prevalences, we did examine a wide range of fruit and vegetable intakes over strata of current smokers, past smokers, and never smokers.

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#### REFERENCES

1. Michels KB, Giovannucci E, Joshipura KJ, et al. Prospective study of fruit and vegetable consumption and incidence of colon and rectal cancers. *J Natl Cancer Inst* 2000;92:1740–52.
2. Michaud D, Spiegelman D, Clinton S, Rimm E, Willett W, Giovannucci E. Fruit and vegetable intake and incidence of bladder cancer in a male prospective cohort. *J Natl Cancer Inst* 1999;91:605–13.
3. Michaud DS, Feskanich D, Rimm EB, et al. Intake of specific carotenoids and risk of lung cancer in 2 prospective US cohorts. *Am J Clin Nutr* 2000;72:990–7.

### Erratum

Hallberg L, Hulthén L. Prediction of dietary iron absorption: an algorithm for calculating absorption and bioavailability of dietary iron. *Am J Clin Nutr* 2000;71:1147–60.

In tables A1, A2, and A3, the unit mg/100 g dry matter should be mg/100 g.

**Erratum**

Reaven GM. The metabolic syndrome: is this diagnosis necessary? *Am J Clin Nutr* 2006;83:1237–47.

In Table 6 (page 1243), the references cited should read from 83 through 101, rather than from 82 through 100.

**Erratum**

Hallberg L, Hulthen L. Prediction of dietary iron absorption: an algorithm for calculating absorption and bioavailability of dietary iron. *Am J Clin Nutr* 2000;71:1147–60.

The correct equation for adjusting for iron status (Equation 11) should be as follows:

$$\text{Iron absorption (mg)} = \text{iron absorption (alg mg)} \times (23/\text{SF})^{0.94049} \quad (1)$$

Additionally, the correct equation for calculating the expected iron absorption ration set when calcium is present (Equation 5) should read as follows:

$$0.4081 + (0.5919/1 + 10^{-[2.022 - \log(\text{Ca}+1)] \times 2.919}) \quad (2)$$

**Erratum**

Bischoff-Ferrari HA, Giovannucci E, Willett WC, Dietrich T, Dawson-Hughes B. Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes. *Am J Clin Nutr* 2006;84:18–28.

A sentence in the abstract begins “An intake for all adults of  $\geq 1000$  IU (40  $\mu\text{g}$ ) vitamin D...” The value for vitamin D should be 25  $\mu\text{g}$ , not 40  $\mu\text{g}$ .