

# DETERMINANTS OF NONHEME IRON ABSORPTION IN MAN

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□ ONLY A LIMITED proportion of iron in our diet can be assimilated by the gastrointestinal mucosa. Consequently, it has long been assumed that the availability of dietary iron is a major determinant of body iron stores. While there is little direct evidence for this, the high prevalence of iron deficiency in Third World countries correlates better with the quality of the diet than with total iron intake. During the past two to three decades, a major effort has been made to define factors influencing the assimilation of food iron, and it is this work that is the focus of this review. Before reviewing recent studies in this field, I will briefly outline some basic concepts of iron absorption in humans and some of the earlier observations on which our present methods are based.

## DIETARY IRON

Dietary iron is divided into two distinct compartments of heme and nonheme iron because of their separate pathways from the lumen to the mucosal cell. The largest fraction is nonheme iron which presumably enters the intestinal mucosa in a reduced ionic state. Assimilation of nonheme iron is determined largely by the extent to which it remains soluble within the lumen of the upper intestinal tract. Because foods differ substantially in their content of factors that promote or inhibit iron solubility, the absorption of nonheme iron is greatly influenced by the nature of the meal (Layrisse et al., 1968). In contrast, heme iron gains entry to the mucosal cell as an intact iron porphyrin complex. Once in the cell it is broken down by the enzyme heme oxygenase and then enters the same storage or transit pathways as the nonheme iron fraction. Except for the facilitating effect of meat on absorption of heme iron (Turnbull et al., 1962), the type of meal has little influence on its absorption because the iron remains within the porphyrin complex until absorbed by the mucosal cell.

Because of the overriding importance of heme iron, meat-containing meals and vegetarian meals should be considered separately in regard to bioavailability. Although heme iron constitutes only 5-10% of the iron ingested in a Western diet, it accounts for over one-third of the iron actually absorbed. Because of this nearly eight-fold difference in bioavailability, heme iron is an important determinant of iron stores. Indeed, the content of meat in the diet is the only factor that has been shown to correlate with iron status in population studies (Takkunen, 1976; Bothwell et al., 1979). The high prevalence of iron deficiency in most developing countries is probably due to the lack of heme iron in the diet. However, high meat consumption cannot be advocated in developing countries because of economic restraints. Since the assimilation of heme iron from different meals is relatively constant and because dietary heme iron content is not a factor that can be easily manipulated, the major focus of this review will be on factors affecting the absorption of nonheme iron.

## MEASUREMENTS OF IRON BIOAVAILABILITY

Isotopic studies of food iron absorption in man have been the source of most of our present knowledge of factors affecting food iron absorption. The approach of earlier workers was to prepare radiolabeled foods by growing them in hydroponic media containing  $^{55}\text{Fe}$ . This radioactive tag,

which has been referred to subsequently as the intrinsic label, is therefore incorporated biosynthetically into the food. Animal foods were prepared similarly by repeated injections of radioactive iron. Results of studies performed with biosynthetically labeled foods have been summarized by Bothwell et al. (1979), but the major finding was that food iron of animal origin is much better assimilated than iron in vegetable foods. In normal subjects, absorption from foods such as rice, wheat, and maize averages 1-5% as compared to 10-20% absorption from foods such as meat, fish, or poultry. The usefulness of biosynthetic labeling, however, is diminished by the time and effort required to prepare the test foods. Another important limitation is that food interactions occur which affect iron absorption when two or more single food items are included in the same meal. Because of the complexity of a modern diet, studies performed with biosynthetically labeled foods are of limited value.

Progress in studies of food iron absorption was facilitated when it was demonstrated that results similar to those from biosynthetic tagging can be achieved by adding a small amount of inorganic radioiron to the food at the time it is consumed (Cook et al., 1972; Bjorn-Rasmussen et al., 1974). When this extrinsic tag is added to a food that has been intrinsically labeled with an alternate form of radioiron, absorption of the two tags is nearly identical. A more important observation, however, is that when an extrinsic tag is added to a complete meal containing a small amount of intrinsically labeled food, absorption of the two tags is still identical. This finding indicates that when several food items are included in the same meal, a single pool of nonheme iron is formed within the intestinal lumen and absorption from this pool can be measured by extrinsic labeling. Thus, food absorption depends not on the food source but on the composite effect of factors in a meal that either block or promote iron availability, largely by affecting its solubility.

The appropriate method of expressing iron absorption results depends on the objective of the study. When assessing the nutritive value of a particular food, percentage absorption should be multiplied by the iron content to calculate the actual amount of absorbed iron. On the other hand, when assessing factors affecting bioavailability from the common pool, it is more useful to express absorption as a percentage. Since percentage absorption varies inversely with iron dose, bioavailability studies must be performed with meals containing similar quantities of iron. Because the focus in this work has been on relative bioavailability, iron is added to meals of low iron content to eliminate any dose effect.

The studies reviewed here were all performed with the same basic protocol. Volunteer subjects are usually iron replete males and as a result percentage absorption is low, on the order of 1-2%. Absorption from meals tagged extrinsically with ferric chloride is determined from the radioactivity incorporated into blood drawn two weeks following administration of the test meals. To reduce the

effect of subject-to-subject differences, multiple tests are performed in each subject using double radioiron labels and parallel sequential measurements so that measurements within the same subject may be compared. Because of the highly skewed distribution of percentage absorption, statistical analysis is performed on the logarithms of percentage absorption (Cook et al., 1969). In some of the studies, absorption is also measured from a small reference dose of inorganic iron to permit extrapolations to subjects of varying iron status (Kuhn et al., 1968).

Two types of test meals are employed in most of the studies. The standard meal consists of a hamburger patty, bun, french fries, and a vanilla milkshake. The tandem meal, which is vegetarian, is comprised of semipurified ingredients. This meal was developed to facilitate deletion and substitution studies of various dietary factors; the ingredients can be altered to maintain a constant macronutrient composition (Cook and Monsen, 1975). The protein, carbohydrate, and fat sources in this meal are ovalbumen, dextrimaltose, and corn oil respectively. The content of these macronutrients in the standard and purified meals is matched; the iron content of the purified meal is adjusted to that of the standard meal (4.1 mg) with ferric chloride.

### ABSORPTION ENHANCERS

● **Ascorbic Acid** enhances iron absorption dramatically. The absorption of iron in a meal can be increased three-fold with the addition of 100 ml orange juice (Callender et al., 1970), and up to seven-fold by including fresh fruit such as papaya (Layrisse et al., 1974). When increasing amounts of ascorbic acid are added to the semipurified or standard meal, there is neither a threshold nor a plateau to the enhancing effect up to a dose of 1,000 mg (Cook and Monsen, 1977). At the latter dose, basal absorption of 0.7% from the semipurified meal increases more than ten-fold, to about 8%. With the standard meal, however, there is only a three-fold increase in absorption, from 4% to approximately 13% (Dassenko et al., 1983). Thus, the relative increase with ascorbic acid is greater from a vegetarian meal although the absolute difference in absorbed iron is similar.

● **Animal Foods** are an important dietary constituent not only because of their highly available heme iron content but also because they enhance nonheme iron absorption. Protein-equivalent substitution with foods of animal origin in the semipurified meal produces a two- to four-fold increase in absorption (Cook and Monsen, 1976); 1 g meat is roughly equivalent to 1 mg ascorbic acid in its enhancing effect. In a study by Layrisse et al. (1973), fortification iron as ferric chloride was added in increasing amounts (5–60 mg) to three meals: maize, meat, and a mixture of the two. Absolute absorption from the maize meal increased from 0.10 to 0.34 mg; from the meat meal, 1.11 to 3.27 mg; and from the combined meal, 0.47 to 1.22 mg, reflecting a profound difference in the amount of absorbed fortification iron when a meal contains meat. The efficacy of fortifying the diet with iron obviously depends on the nature of the diet to which the iron is added.

### ABSORPTION INHIBITORS

● **Tea** is the most potent inhibitor of iron absorption yet identified. Disler et al. (Disler et al., 1975) observed a decrease in absorption from 11.5 to 2.5% when a cup of tea was added to a bread meal. This blocking effect is due to the formation of highly insoluble iron tannates. Coffee also has an inhibitory effect on iron absorption although its effect is less potent than that of tea (Morck et al. 1983). A cup of coffee taken with our semi-purified meal reduced absorption from 6% to less than 1%; with the standard meal, a less pronounced reduction of about 50% occurred. The factor in coffee that inhibits iron absorption has not yet been identified but is presumed to be a polyphenolic compound. In countries where tea and coffee are widely consumed, some improvement in iron nutrition might be achieved by eliminating these beverages from the main meals of the day.

● **Protein** is the only macronutrient that has a signifi-

cant effect on iron absorption. When the amount of the protein source ovalbumen was doubled in the semipurified meal, percentage absorption fell significantly, from 2 to 1% (Monsen and Cook, 1979), when ovalbumen was deleted from the meal, percentage absorption rose from 4% to nearly 10%. In contrast, when the carbohydrate or fat sources in the meal were either doubled or deleted, no significant effect on iron absorption was observed.

To determine whether this inhibitory effect is characteristic only of the protein egg albumen, isolated soy protein and sodium caseinate were substituted in protein-equivalent amounts into the semipurified meal (Cook et al., 1981). Percentage absorption with both egg albumen and casein averaged 3%, while absorption from the meal containing soy protein was about 0.5%. A wide range of soy products, including whole soybean, full-fat soy flour, textured soy flour, and highly purified isolated soy protein were also studied, and in all cases a similar inhibitory effect of this food source was observed when meals were adjusted to contain the same amount of iron.

The addition of an enhancing substance such as ascorbic acid to the meal can partially offset the inhibitory effect of a protein. When 100 mg vitamin C was added to the semipurified meal containing either egg albumen or isolated soy protein, the relative increase in iron absorption was much greater in the soy meal, more than five-fold as compared with the two-fold increase with egg albumen. Nevertheless, the inhibitory effect of soy protein could not be completely eliminated with ascorbic acid (Morck et al., 1983). Because of the relatively high iron content of soy, its inhibitory effect is of limited importance in a vegetarian diet. However, if soy protein is used as a meat substitute, a decrease in absorbed iron can be anticipated because of the displacement of heme iron. The importance of soy in iron nutrition therefore relates to its use as a meat substitute.

● **Bran** reduces iron absorption in a dose-dependent fashion. When 12 g of whole bran was added to a meal containing no meat, percentage absorption fell from 4.0 to 0.6%, whereas when meat was added to the meal, less pronounced inhibition occurred, from 3.5 to 1.7% (Simpson et al., 1981). Although earlier studies suggested that the inhibiting effect of bran is due to phytate (McCance et al., 1943), studies by Simpson et al. (1981) have shown this effect to be independent of phytate content. When plain muffins and a milkshake were used as the test meal absorption averaged 2.5% (Simpson et al., 1981). When 12 g bran or its equivalent was added to the muffins a sharp decrease in iron absorption was observed. The degree of this inhibition was similar with whole bran, with lyophilized bran, and finally, with bran in which the phytate had been completely removed by enzymatic digestion.

● **Fiber** has long been assumed to have a negative influence on iron absorption. Absorption has been measured from four muffin meals containing either plain wheat flour muffins or muffins to which bran, pectin, or cellulose was added. Whereas bran inhibited absorption from the meal, no significant inhibitory effect was observed with either pectin or cellulose, suggesting that dietary fiber per se may not be an important inhibitory factor. An additional study was performed to examine the effect of naturally-occurring dietary fiber on iron absorption. Two meals were selected that were matched with respect to macronutrient and mineral composition but differed widely in their content of intrinsic fiber. At the two extremes of fiber content, iron absorption differed only about two-fold. This marked difference in fiber content of two meals would rarely be encountered in a normal diet. It has therefore been concluded that fiber has only a modest inhibitory effect on iron assimilation (Cook et al., 1983).

### SUMMARY

Dietary iron is divided into heme and nonheme iron because of separate entryways into the mucosal cell. Heme iron, because of its high bioavailability, is the most important dietary variable. The absorption of nonheme iron, which varies greatly depending on the meal, is significantly enhanced by ascorbic acid and animal tissue. Our knowledge of factors that inhibit the assimilation of food iron is

less complete, but several have now been identified including tea, coffee, bran, protein, and fiber. Assessment of the quantitative importance of these inhibitors and their interactions with enhancing substances deserves continued study.

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