Iron bioavailability and dietary reference values[1–4]

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ABSTRACT

Iron differs from other minerals because iron balance in the human body is regulated by absorption only because there is no physiologic mechanism for excretion. On the basis of intake data and isotope studies, iron bioavailability has been estimated to be in the range of 14–18% for mixed diets and 5–12% for vegetarian diets in subjects with no iron stores, and these values have been used to generate dietary reference values for all population groups. Dietary factors that influence iron absorption, such as phytate, polyphenols, calcium, ascorbic acid, and muscle tissue, have been shown repeatedly to influence iron absorption in single-meal isotope studies, whereas in multimeal studies with a varied diet and multiple inhibitors and enhancers, the effect of single components has been, as expected, more modest. The importance of fortification iron and food additives such as erythorbic acid on iron bioavailability from a mixed diet needs clarification. The influence of vitamin A, carotenoids, and nondigestible carbohydrates on iron absorption and the nature of the "meat factor" remain unresolved. The iron status of the individual and other host factors, such as obesity, play a key role in iron bioavailability, and iron status generally has a greater effect than diet composition. It would therefore be timely to develop a range of iron bioavailability factors based not only on diet composition but also on subject characteristics, such as iron status and prevalence of obesity. *Am J Clin Nutr* 2010;91(suppl):1461S–7S.

INTRODUCTION

Iron metabolism is unusual; it differs from the metabolism of other metals in that there is no physiologic mechanism for iron excretion and close to 90% of daily iron needs are obtained from an endogenous source, namely the breakdown of circulating red cells. There are iron losses, however, which include obligatory losses in all population groups (skin, intestines, urinary tract, and airways) and menstrual blood losses in women of child-bearing age. To maintain iron balance, the sum of these losses plus the iron required for growth in infants, children, and adolescents, and during pregnancy must be provided by the diet. The only reliable quantitative data for obligatory iron losses, however, are derived from a single study that estimated an average iron loss of 0.9–1.0 mg/d (14 μg/kg body weight) in men with normal iron status from the United States, Venezuela, and South Africa (1). Whereas there is some support from Bothwell et al (2) for this estimate of daily iron losses per kilogram of body weight, there is a need to evaluate further the extent to which obligatory iron losses vary with ethnicity, age, and sex groups and with iron status (3).

On the basis of the sum of obligatory and menstrual iron losses and iron needed for growth, the World Health Organization/Food and Agriculture Organization of the United Nations (WHO/FAO), the Institute of Medicine (IOM), and other national organizations have calculated iron requirements for different population groups. To translate these requirements into recommendations for daily dietary iron intakes requires an estimate of iron bioavailability, defined as the extent to which iron is absorbed from the diet and used for normal body functions. This review describes the dietary and host factors reported to influence iron bioavailability, the way in which these factors have been used to establish iron bioavailability factors for the estimation of dietary reference values (DRVs), and the extent to which the bioavailability factors could be refined further.

DIETARY FACTORS THAT INFLUENCE IRON BIOAVAILABILITY

There are 2 types of dietary iron: nonheme iron, which is present in both plant foods and animal tissues, and heme iron, which comes from hemoglobin and myoglobin in animal source foods. Heme iron is estimated to contribute 10–15% of total iron intake in meat-eating populations, but, because of its higher and more uniform absorption (estimated at 15–35%), it could contribute $\geq$40% of total absorbed iron (4, 5). Nonheme iron is usually much less well absorbed than heme iron. All nonheme food iron that enters the common iron pool in the digestive tract is absorbed to the same extent, which depends on the balance between the absorption inhibitors and enhancers and the iron status of the individual. It is important, however, to note that not all fortification iron enters the common pool.

INHIBITORS OF IRON ABSORPTION

Phytate

In plant-based diets, phytate (*myo*-inositol hexakisphosphate) is the main inhibitor of iron absorption. The negative effect of phytate on iron absorption has been shown to be dose dependent and starts at very low concentrations of 2–10 mg/meal (6, 7).

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The molar ratio of phytate to iron can be used to estimate the effect on absorption. The ratio should be <1:1 or preferably <0.4:1 to significantly improve iron absorption in plain cereal or legume-based meals that do not contain any enhancers of iron absorption, or <6:1 in composite meals with certain vegetables that contain ascorbic acid and meat as enhancers (8, 9). Food processing and preparation methods, which include milling, heat treatment, soaking, germination, and fermentation, can be used to remove or degrade phytate to a varying extent (8, 10). The addition of exogenous phytase or its activation during food processing, or the addition to a meal just before human consumption, has been shown to improve iron absorption significantly (7, 11–13).

Polyphenols

Polyphenols occur in various amounts in plant foods and beverages, such as vegetables, fruit, some cereals and legumes, tea, coffee, and wine. The inhibiting effect of polyphenols on iron absorption has been shown with black tea and herb teas (14–16). At comparable amounts, the polyphenols from black tea were shown to be more inhibiting than the polyphenols from herb teas and wine (16, 17). The fact that polyphenol quantity, as well as type, influences iron absorption was also shown in a study with spices. Chili, but not turmeric, inhibited iron absorption in Thai women, although turmeric contained more polyphenols than chili (18). In cereals and legumes, polyphenols add to the inhibitory effect of phytate, as was shown in a study that compared high and low polyphenol sorghum. After complete phytate degradation, iron absorption from low-polyphenol sorghum increased significantly, whereas iron absorption from high-polyphenol sorghum was not improved (19). Further studies should be conducted to investigate the influence of polyphenols in widely consumed legumes and cereals, such as common beans *(Phaseolus vulgaris)* and millet.

Calcium

Calcium has been shown to have negative effects on nonheme and heme iron absorption, which makes it different from other inhibitors that affect nonheme iron absorption only (20–22). Initially, the inhibitory effect was suggested as occurring during the transport of iron across the basolateral membrane from the enterocyte to the plasma because absorption of both forms of iron is equally inhibited, but more recently, it was suggested that the inhibition takes place during the initial uptake into the enterocytes (23, 24). Dose-dependant inhibitory effects were shown at doses of 75–300 mg when calcium was added to bread rolls and at doses of 165 mg calcium from milk products (21). In a recent study the addition of 200 mg calcium to a maize-based test meal had no significant effect on iron absorption from NaFeEDTA (12). It is proposed that single-meal studies show a negative effect of calcium on iron absorption, whereas multiple-meal studies, with a wide variety of foods and various concentrations of other inhibitors and enhancers, indicate that calcium has only a limited effect on iron absorption (25).

Proteins

Whereas animal tissues have an enhancing effect on nonheme iron absorption, animal proteins, such as milk proteins, egg proteins, and albumin, have been shown to inhibit iron absorption (26). The 2 major bovine milk protein fractions, casein and whey, and egg white were shown to inhibit iron absorption in humans (27, 28). Proteins from soybean also decrease iron absorption. Phytate was shown to be the major inhibitor in soy protein isolates, but even after complete phytate degradation iron absorption from soy protein isolates was only half that of the egg-white control (which allows interstudy comparison), which suggests that soy protein itself is inhibiting (7). In another study with soy protein isolates, iron absorption increased 19-fold when the protein was extensively enzyme hydrolyzed and phytate degraded. The authors concluded that phytate and a protein-related moiety contained in the conglycinin fraction were the main inhibitors of iron absorption in soy protein (29).

ENHANCERS OF IRON ABSORPTION

Ascorbic acid

Many single-meal radioisotope studies in human volunteers have shown convincingly the dose-dependent enhancing effect of native or added ascorbic acid on iron absorption (30). The enhancing effect is largely due to its ability to reduce ferric to ferrous iron but is also due to its potential to chelate iron (31). Ascorbic acid will overcome the negative effect on iron absorption of all inhibitors, which include phytate (6), polyphenols (32), and the calcium and proteins in milk products (33), and will increase the absorption of both native and fortification iron. In fruit and vegetables the enhancing effect of ascorbic acid is often cancelled out by the inhibiting effect of polyphenols (34, 35). Ascorbic acid is the only main absorption enhancer in vegetarian diets, and iron absorption from vegetarian and vegan meals can be best optimized by the inclusion of ascorbic acid–containing vegetables (36).

Cooking, industrial processing, and storage degrade ascorbic acid and remove its enhancing effect on iron absorption (37). Several derivatives of ascorbic acid are less sensitive to heat and oxygen. Teucher et al (37) and Pizarro et al (38) recently reported that ascorbyl palmitate retains its enhancing effect on iron absorption after it is baked into iron-fortified bread. Erythorbic acid, an ascorbic acid derivative, is widely used as an antioxidant in processed foods in industrialized countries. In the United States, its intake from processed foods may reach 200 mg/d (39), and erythorbic acid intake could be as high, if not higher, than ascorbic acid intake. Although it has little vitamin C activity, its enhancing effect on iron absorption appears to be almost double that of ascorbic acid (40). The abundance of such compounds in the American diet might help explain why it has not been possible to demonstrate clearly the enhancing effect of vitamin C on iron absorption in multiple-meal studies of self-selected diets (41).

Muscle tissue

Single-meal radioisotope studies have consistently shown an enhancing effect of meat, fish, or poultry on iron absorption from vegetarian meals (42), and 30 g muscle tissue is considered equivalent to 25 mg ascorbic acid (43). Bjorn-Rasmussen and Hallberg (44) reported that the addition of chicken, beef, or fish to a maize meal increased nonheme iron absorption 2–3-fold with no influence of the same quantity of protein added as egg

albumin. More recently, Baech et al (45) reported a dose-response increase in iron absorption when pork meat was added to a high-phytate, low–ascorbic acid meal. As with ascorbic acid, it has been somewhat more difficult to demonstrate the enhancing effect of meat in multiple meals and complete diet studies. Reddy et al (46) reported only a marginal improvement in iron absorption (35%) in self-selected diets over 5 d when daily muscle tissue intake was increased to ≈300 g/d, although, in a similar 5-d study, 60 g pork meat added to a vegetarian diet increased iron absorption by 50% (47).

The nature of the "meat factor" has proved elusive. Most evidence indicates that it is within the protein fraction of muscle tissue; however, it is also possible that other muscle tissue components are involved (48). There is good evidence to support the enhancing effect of cysteine-containing peptides (49, 50), which are rich in digests of myofibrillar proteins and which, like ascorbic acid, could both reduce and chelate iron. Storcksdieck et al (51), however, suggested that the "meat factor" may not be due to a single peptide fraction but more likely to a multitude of small peptides. Unlike other proteins, myofibrillar proteins are digested extensively by pepsin in the stomach and thus could bind iron and prevent its precipitation at the higher pH of the duodenum. Studies with Caco-2 cells have indicated that glycosaminoglycans (52) and L-α-glycerophosphocholine (53) might also contribute to the enhancement of nonheme iron absorption by meat. It is difficult, however, to extrapolate from Caco-2 cells to humans (54), and purified sulfated and unsulfated glycosaminoglycans did not increase iron absorption from a liquid formula meal in young women (55), although it is possible that other glycosaminoglycans that occur naturally may be enhancing. Armah et al (53) reported that purified L-α-glycerophosphocholine increased iron absorption in women who consumed a vegetable lasagna low in inhibitors, although to a lower extent than ascorbic acid. The enhancing effect of L-α-glycerophosphocholine was not confirmed in women who consumed a high-phytate maize meal, although iron absorption from this meal was increased by ascorbic acid (and EDTA) (12).

FORTIFICATION IRON

Bioavailability of fortification iron varies widely with the iron compound used (56), and foods sensitive to color and flavor changes are usually fortified with water-insoluble iron compounds of low bioavailability. Iron compounds recommended for food fortification by the World Health Organization (WHO) (56) include ferrous sulfate, ferrous fumarate, ferric pyrophosphate, and electrolytic iron powder. Many cereal foods, however, are fortified with low-cost elemental iron powders, which are not recommended by WHO (57) and these have even lower bioavailability. Hallberg and Rossander- Hulthén (58) estimated that 25% of the total iron intake in Sweden and the United States comes from fortification iron. When they calculated the bioavailability factors for the complete diet, they assumed the fortification iron was mainly low-bioavailability elemental iron powders and they estimated that it was only 15% as well absorbed as native food iron. Food-fortification practices vary nationally and the need to adjust the dietary iron bioavailability factor for fortification iron will depend on the proportion of fortification iron in the total iron intake and the iron compounds used.

SUBJECT FACTORS

Iron status

The iron status of individuals mainly influences the absorption of nonheme iron, whereas heme iron absorption is generally less affected (59). There is an inverse correlation between iron status and iron absorption, and with the use of ferritin as an indicator of iron status the relation can be described mathematically (60, 61). A study in young women showed that the regulation of iron absorption by ferritin was less pronounced when iron was added as a water-insoluble compound (micronized dispersible ferric pyrophosphate), compared with ferrous sulfate (62). These findings are important for fortification practices because they show that the different compounds are more or less suitable for repletion of iron-deficient subjects. Further studies should be performed in iron-deficient and iron-replete individuals and with different fortification compounds. A study in Indian women investigated the effect of enhancers (ascorbic acid) and inhibitors (tea polyphenols) of iron absorption in an iron-deficient anemic group, compared with a nonanemic iron-replete control group. The difference in iron absorption between the groups was defined by the iron status, but the magnitude of the enhancing and inhibiting effect was shown to be independent of iron status (63).

Nutritional deficiencies

Vitamin A and riboflavin deficiencies have been shown to influence iron metabolism and absorption. Human studies showed that the correction of riboflavin deficiency improved the response to iron supplements (64). An absorption study in Gambian men indicated that the efficiency of iron use is impaired in riboflavin deficiency but that iron absorption is unaffected (65). The effect of vitamin A and vitamin A deficiency on iron absorption is discussed in the following section.

Infection/inflammation

The peptide hepcidin, produced in the liver and adipose tissue, has been identified as a key regulator of iron homeostasis (66, 67). Hepcidin expression is increased in chronic inflammation and obesity (66, 68) and may contribute to the increased prevalence of iron deficiency observed in overweight populations (69, 70). A cross-sectional study in Thai women showed that obesity is associated with decreased iron absorption and increased inflammation, independent of iron status (71). A study in school-aged children showed that overweight children had higher hepcidin concentrations and lower iron status compared with normal-weight children. The iron intake and bioavailability of the 2 groups were not significantly different, which suggests a hepcidin-mediated decreased iron absorption or increased iron sequestration in overweight children (72). Two recent small-scale studies have shown an inverse correlation of hepcidin concentration and iron absorption in iron-replete healthy women and men (73, 74). Further studies in populations with a broad range of iron status are needed to investigate fully the role of hepcidin on iron absorption.

Genetic disorders

Hemochromatosis is a disorder of excessive iron accumulation that affects up to 1 in 150 people in populations of Northern

European origin. The effect of the disorder on iron absorption has been studied in control subjects and in homozygous and heterozygous subjects (75). Homozygous subjects showed increased heme and nonheme iron absorption, whereas the nonheme iron absorption of heterozygous subjects from meals with moderate iron content was not shown to be different from control subjects. However, increased absorption was seen in heterozygous subjects from meals highly fortified with iron. These results were not confirmed in later studies in male C282Y heterozygotes and were suggested to be related to improved methods of genotyping and feeding of test meals (ie, single compared with multiple meals) (76, 77).

The other important group of genetic disorders that leads to iron overload is thalassemias and related hemoglobinopathies, which occur mainly in South and Southeast Asia, the Middle East, and the Mediterranean (78). Thalassemia homozygotes have ineffective erythropoiesis that stimulates iron absorption even if iron stores are adequate, which leads to a risk of iron excess when regular transfusions are given to correct anemia (79, 80). Heterozygotes for α-thalassemia 1, β-thalassemia, and hemoglobin E are usually asymptomatic and have mild anemia but they may be at risk of iron overload if they have some degree of impaired erythropoiesis. To investigate this potential risk, a study was carried out in Thai women heterozygous for α-thalassemia 1, β-thalassemia, hemoglobin E, and compound HbE/β–thalassemia, and control subjects, which measured iron absorption and use from rice meals with the use of stable isotope techniques (81). In subjects with α-thalassemia 1 and β–thalassemia, but not hemoglobin E, iron use was lower and absorption was significantly higher than in control subjects, and absorption was not adequately down-regulated with increased iron stores. In countries with mandatory iron fortification of commonly consumed food products and concurrent high prevalence of thalassemia, the occasional monitoring of iron stores may be useful for early identification of potential iron overload.

UNRESOLVED IRON BIOAVAILABILITY ISSUES

Vitamin A and carotenoids

Vitamin A deficiency, such as iron deficiency, leads to anemia. Vitamin A can affect several stages of iron metabolism (82), which include erythropoiesis and the release of iron from ferritin stores (83). Isotopic studies that investigated the influence of vitamin A on iron absorption have, however, reported contradictory findings. A series of radio-iron studies from Venezuela has consistently shown that vitamin A and β-carotene enhance iron absorption from iron-fortified maize bread, wheat bread, and rice meals (84, 85). The same group (86) reported that 2–4 mg of lycopene, lutein, and zeaxanthin (non–pro-vitamin A carotenoids) likewise increased iron absorption 2–3-fold when added to maize and wheat-bread meals. In contrast, studies from Sweden and Switzerland that used both radio- and stable isotopes reported no influence of vitamin A on iron absorption from similar test meals (87). In the belief that the different findings may be related to the vitamin A status of the subjects, Davidsson et al (88) added vitamin A to iron-fortified maize gruels fed to vitamin A–deficient Ivorian children. In this study, the additional vitamin A significantly decreased iron absorption, although the inhibition disappeared 3 wk after provision of high-dose vitamin A supplements to the children. The Ivorian children in this study were also iron deficient, which might have influenced vitamin A metabolism (89). The interaction of iron and vitamin A metabolism is clearly complex and subject factors or methodologic issues could explain the contradictory findings. The possible influence of carotenoids on iron absorption is important because carotenoids are widely present in fruit and vegetables.

Nondigestible carbohydrates

Nondigestible carbohydrates are widely present in plant foods. They resist digestion in the small intestine but are fermented in the colon to short-chain fatty acids with a variety of reported health benefits, which include increased colonic iron absorption (90). Although most dietary iron is absorbed in the duodenum, the colon mucosa also expresses the iron absorption proteins divalent metal transporter, ferritin, and ferroportin, as shown in pigs (91). Ohkawara et al (92) have reported that infused ferrous iron was absorbed by humans from the colon with ≈30% of the efficiency of the total iron absorption (duodenum and colon). Pectin (93) and inulin (94) have been reported to increase hemoglobin repletion in iron-deficient rats and a mixture of inulin and oligofructose increased hemoglobin repletion in iron-deficient pigs (95). Possible mechanisms for increased colonic iron absorption are the generation of a lower pH, formation of soluble iron complexes, reduction of ferric to ferrous iron by gut microflora, a proliferation of the absorptive area in the colon, and an increase in iron-absorption proteins (90). Human studies have consistently shown that inulin and oligofructose increase colonic calcium absorption (96), but a balance study (97) and a stable isotope study (98) failed to demonstrate an enhancing effect of inulin on iron absorption. The influence of nondigestible carbohydrates on colonic iron absorption merits further investigation.

ESTABLISHMENT OF A "BIOAVAILABILITY FACTOR" FOR DRVs OF IRON

Diet composition and iron status influence iron bioavailability; however, iron status is the overriding determinant (63). The iron bioavailability factor for DRVs thus needs to be practically relevant and for a well-defined iron status (58). It has traditionally been estimated for subjects with no iron stores (serum ferritin < 15 μg/L). The selection of no iron stores as the reference for the bioavailability factor leads to a higher bioavailability factor and lower dietary iron intake recommendation but still ensures that subjects with low or no iron stores will absorb enough iron to meet their demands. Whereas this seems a pragmatic approach, it remains unclear as to how individuals with adequate iron stores, who absorb much less iron, maintain their iron balance.

Long-term dietary iron bioavailability can be measured with the use of isotopic methods, estimated with algorithms, or calculated from iron balance and intake data. Because there are few longer-term isotope studies on whole diets, and because the algorithms for the prediction of iron bioavailability are only precise enough to predict high, medium, and low bioavailability (99), iron bioavailability factors have largely been based on the calculations made by Hallberg and Rossander-Hulthén (58), who measured the amount of absorbed iron needed to maintain iron balance and estimated bioavailability with the use of iron intake.

They concluded that the upper (long-term) bioavailability of iron from Western-type diets is, on average, 15%, with a range of 14% to 17%. They also suggested that iron in diets with little meat (50–100 g/d), only occasionally with fruit or vegetables consumed with the main meals, and more whole-grain cereals, may be 10–12% bioavailable, and that iron bioavailability from Western-type vegetarian diets ranges from 5% to 12%.

These values compare well to the reported iron absorption from typical Latin American diets based on radioisotope studies, which ranged from 7.5% to 13.4% (100). The IOM similarly used the results of 16.8% of a single radio-iron absorption study to estimate iron bioavailability from the American diet in subjects with no iron stores who consumed a self-selected diet over a 2-wk period (60, 101). On the assumption that, conservatively, nonheme iron absorption was 10% and that heme iron absorption was 25%, the IOM estimated that overall iron bioavailability from a mixed American or Canadian diet was 18%, a value similar to the 17% estimated by Hallberg and Rossander-Hulthén (58, 101). The WHO/FAO (102) proposed iron bioavailabilities of 15%, 12%, 10%, or 5%, which depend on dietary composition, the highest bioavailability for diversified diets that contain generous amounts of meat and/or food rich in ascorbic acid, the lowest bioavailability for diets based on cereals and/or tubers with negligible amounts of meat and ascorbic acid–containing foods.

CONCLUSIONS

The iron bioavailability factors for mixed diets in industrialized countries would appear to range from 14% to 18% for subjects with no iron stores. The iron bioavailability factors for vegetarian diets appear to range from 5% to 12%. A high intake of fortification iron would be expected to lower dietary bioavailability because cereal foods are commonly fortified with low-bioavailability elemental iron powders. Because both the consumption of iron-fortified foods and the bioavailability of iron-fortification compounds vary widely, the contribution of fortification iron to the bioavailability factors is difficult to estimate. In addition, it should be remembered that regulation of iron absorption with iron status depends on the solubility of the iron compounds in the gastrointestinal tract.

There are some unresolved iron bioavailability issues. These include the mechanism by which calcium inhibits iron absorption, the nature of the meat factor, and the influence of vitamin A, carotenoids, and nondigestible carbohydrates on iron bioavailability. In addition, the role of widely consumed food additives such as erythorbic acid on iron bioavailability from mixed diets needs clarification. The iron status of the individual is the overriding factor that determines iron bioavailability, and other host-related factors, such as inflammation, may also play an important role. Obesity is an inflammatory disorder and would be predicted to decrease iron bioavailability. Traditionally, in industrialized countries, a mean iron bioavailability factor has been used to generate DRVs for iron for all population groups, irrespective of diet. Now may be the time to consider a range of iron bioavailability factors that depend on the consumption of meat, fruit, vegetables, processed foods, and iron-fortified foods, and the prevalence of obesity.

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