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Dietary and Physiological Factors That Affect the Absorption and Bioavailability of Iron

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Abstract: Iron deficiency, a global health problem, impairs reproductive performance, cognitive development, and work capacity. One proposed strategy to address this problem is the improvement of dietary iron bioavailability. Knowledge of the molecular mechanisms of iron absorption is growing rapidly, with identification of mucosal iron transport and regulatory proteins. Both body iron status and dietary characteristics substantially influence iron absorption, with minimal interaction between these two factors. Iron availability can be regarded mainly as a characteristic of the diet, but comparisons between human studies of iron availability for absorption require normalization for the iron status of the subjects. The dietary characteristics that enhance or inhibit iron absorption from foods have been sensitively and quantitatively determined in human studies employing iron isotopes. People with low iron status can substantially increase their iron absorption from diets with moderate to high availability. But while iron supplementation and fortification trials can effectively increase blood indices of iron status, improvements in dietary availability alone have had minimal influence on such indices within several weeks or months. Plentiful, varied diets are the ultimate resolution to iron deficiency. Without these, more modest food-based approaches to human iron deficiency likely will need to be augmented by dietary iron fortification.

Key words: Iron, absorption, human, dietary factors, physiological factors

Introduction

As described by Mielczarek and McGrayne [1], life on earth evolved in an early sea environment with ready access to the ferrous form of iron, the 4th most abundant element on the planet. Resulting photosynthesis flooded the atmosphere with reactive oxygen, requiring the surviving organisms to modify their iron-based energy metabolism to utilize oxygen, but with complex controls to prevent excess oxidation. Oxygen rusted earth's iron, ferrous iron became much less abundant, and problems with iron bioavailability had begun.

At the beginning of the 21st century, the World Health Organization recognizes iron deficiency as one of the ten greatest global health risks, ranked according to the number of lost healthy life years [2]. Iron deficiency impairs reproductive performance, cognitive development, and work capacity. Effectively resolving this problem with preventative nutritional strategies is a challenge that requires improving the bioavailable iron content of human diets through supplementation, fortification (including biofortification), and ultimately, dietary diversification.

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Mechanisms of iron absorption

Iron absorption is defined as the physiological movement of iron into the enterocytes that line the luminal surface of the gastrointestinal tract and then into the bloodstream. Iron absorption is influenced by both endogenous factors, including the physiological characteristics of the organism, and exogenous factors such as characteristics of the ingested form of iron and the dietary matrix. The molecular process of iron absorption and its control is an active field of research, with information rapidly accumulating (for a recent review, see reference 3).

The mechanisms of absorption differ for heme and inorganic (non-heme) forms of iron, although both are absorbed primarily in the upper portion of the duodenum, the portion of the small intestine with the lowest luminal pH. Heme iron absorption is not as well understood as that of non-heme iron, and no receptors have been identified for mucosal uptake of heme iron. Hemoglobin iron is better absorbed than iron from isolated heme because the peptide remnants resulting from proteolytic digestion of the globin proteins prevent heme polymerization. The heme molecule is absorbed as an intact porphyrin structure, possibly involving endocytosis. In the mucosal cell, heme iron can be catabolized into ferrous iron and bilirubin by heme oxygenase, adding iron to a common cellular pool for subsequent transport into plasma or intracellular storage for release as the cells exfoliate. The recent description of a heme export protein that is expressed in the intestine suggests that heme iron may also be absorbed intact [4].

Non-heme iron is best absorbed if presented to the intestinal villi as soluble ions (preferably in the reduced, ferrous form) or as low affinity, low-molecular-weight iron ligands. Stomach acid facilitates these conditions. Ascorbic acid concurrently ingested with iron helps to maintain the iron in a soluble, reduced, low-molecular ligand form in the intestinal lumen.

Within the past few years, several proteins have been identified that facilitate and regulate mucosal uptake and transfer of non-heme iron (Figure 1). Ferric iron is converted to the ferrous form at the duodenal apical surface by the ferrireductase duodenal cytochrome b (Dcytb) [5]. A divalent metal transporter (DMT1) then facilitates transfer of the ferrous iron across the brush border membrane into the mucosal cell [6, 7]. Iron taken up into the enterocyte may be further transported to the blood through the basolateral membrane, completing absorption, or it may be held and returned to the intestinal lumen with cellular desquamation. IREG1, also called ferroportin, is involved in the efflux of iron from the mucosal cell at the basolateral membrane [8–10]. In addition, the transfer of iron out of the enterocyte into the circulation involves hephaestin, a ferroxidase that is homologous to ceruloplasmin, a copper-containing ferroxidase in plasma [11] that oxidizes iron to the ferric form, enabling its transfer to transferrin in the plasma. Dcytb, DMT1, and IREG1 are preferentially expressed in the duodenum, the main site of iron absorption, and are up-regulated in iron deficiency. Although DMT1 has the highest affinity for iron, other divalent ions, such as manganese, lead, cadmium, zinc, and copper also bind to this transporter [7]. Such an affinity for multiple minerals at the binding sites of transport proteins suggests a potential mechanism for nutritional interactions that affect iron absorption.

Iron absorption is responsive to recent iron intake, iron stores, erythropoiesis, hypoxia, pregnancy, and inflam-

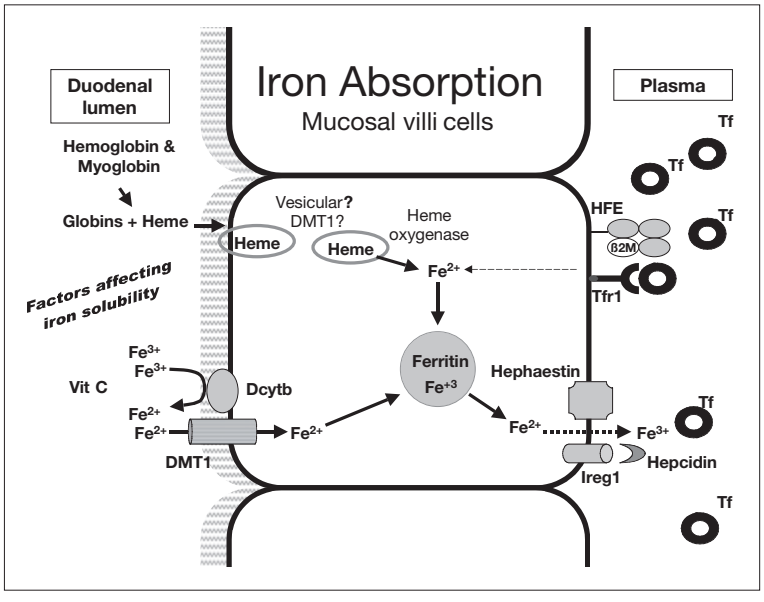

Figure 1: Absorption of heme and non-heme iron in the intestinal mucosal cells, involving various transport and regulatory proteins (see text).

mation. Hepcidin, an antimicrobial peptide released from the liver into the circulation, reduces iron absorption and is likely a primary point of control that is affected by several of these stimuli [12, 13]. Hepcidin reduces the basolateral transport of iron from the enterocyte by binding to and facilitating the internalization of IREG1/ferroportin [14]. Control of absorption also involves the HFE protein located in the basolateral membrane. A mutation in this protein is associated with the excessive iron absorption observed in the iron storage disorder hemochromatosis [15]. Although the mechanism of the HFE influence on iron absorption is incompletely understood, HFE appears to interact with membrane transferrin receptors in the basolateral membrane, possibly enabling the enterocyte to sense body iron stores from transferrin saturation levels and influencing expression of transporters such as DMT1 and IREG1/ferroportin [16]. The principle influence of the HFE protein may be in the liver, where interaction with transferrin receptor to sense transferrin saturation levels could affect hepcidin expression and release.

Absorption as affected by iron status, dietary bioavailability and content

Both heme and non-heme iron are absorbed in inverse proportion to body iron stores. Figure 2 demonstrates how iron absorption by healthy men and women is influenced by their body iron stores and by high and low bioavailability diets [17, 18]. The high bioavailability diet of this figure was rich in meat, ascorbic acid, and refined grains, while the low bioavailability diet contained only small amounts of poultry, fish, and ascorbic acid, but plenty of whole grains, legumes, and tea beverage (enhancers and inhibitors of iron absorption are further discussed below). Heme iron, contributing 0–2 mg to total daily iron intake (depending on meat consumption), is absorbed at 20 to 50% efficiency (Figure 2). Non-heme iron, accounting for the remaining 12–18 mg of dietary iron consumed daily, is absorbed at 0.1 to > 35% efficiency, depending on body iron status and dietary bioavailability. These absorptive efficiencies enable greater control of non-heme iron absorption, compared to that of heme iron. When iron stores are high, absorption of non-heme iron can be minimized more completely and when iron stores are low, non-heme iron is absorbed nearly as efficiently as heme iron [19]. Because ~85–100% of dietary iron is in the non-heme form, this form accounts for most of the physiological control of iron absorption in relation to iron needs.

Nutrient bioavailability can be defined as the dietary characteristics that influence the fraction of the nutrient that can be absorbed and used for normal physiological functions and storage. This nutrient absorption, utilization, and storage is influenced by endogenous or host factors, such as nutritional status, gastric acidity, maturation, and physiological requirements, and by extrinsic dietary characteristics including nutrient content, form, and other factors that enhance or inhibit bioavailability. Body iron is mainly controlled at the point of intestinal absorption rather than excretion [20], and iron bioavailability is largely determined by the factors that affect absorption. Dietary factors that affect iron absorption include the form of iron

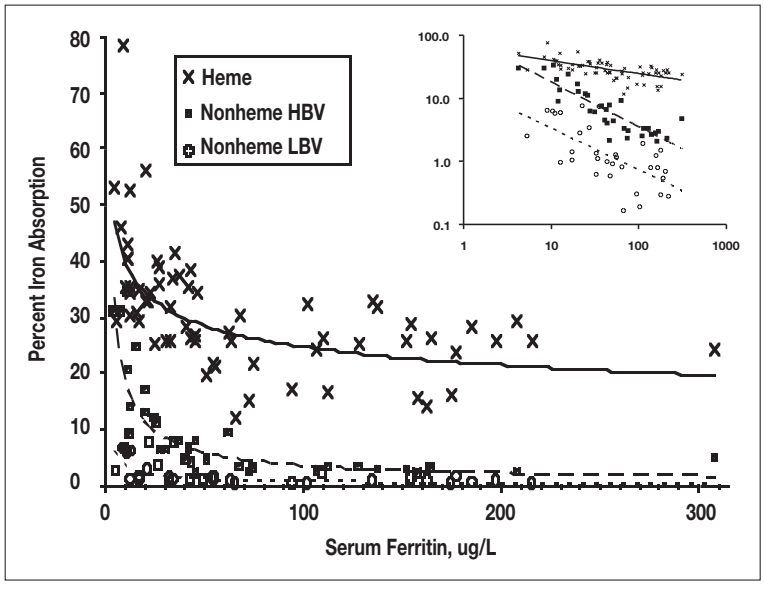

Figure 2: Iron absorption as affected by body iron status (indicated by serum ferritin) and dietary bioavailability. Absorption of heme iron (top, solid line) from meat is most efficient and is generally unaffected by other dietary factors. Absorption of non-heme iron is substantially influenced by dietary enhancers and inhibitors (see text). Despite similar iron content (16 mg iron/10 MJ in these diets), the high bioavailability diet enables substantially increased iron absorption by women with low iron stores (middle, dashed line), while a low bioavailability substantially suppresses absorption of non-heme iron (bottom, dotted line). On a logarithmic scale, dietary bioavailability affects the intercept, but not the slope of the relationship between non-heme iron absorption and body iron stores (inset, also showing individual data), so the ratio of non-heme iron absorption from diets differing in bioavailability is unaffected by body iron status. Data from similar studies with men and women [17,18] have been combined.

(heme or non-heme), as well as dietary constituents consumed simultaneously that influence the fraction of soluble, reduced iron in the intestinal lumen.

Although iron absorption is strongly influenced by both body iron status and dietary availability (Figure 2), there is little evidence that these interact. Dietary characteristics especially influence the absorption of the non-heme form of iron, but this influence alters the intercept, not the slope of the relationship between fractional non-heme iron absorption and iron status (note the parallel lines in the logarithmic inset of Figure 2). Accordingly, the relative ratio of non-heme iron absorption from two diets with different iron availability is independent of the subject's iron status (see also [21]). It follows that the availability of dietary iron can be determined by testing absorption in subjects with varying iron status. From a practical standpoint, the relative variability in absorption measurements may be reduced by not testing subjects (men) with high iron stores, but iron-deficient subjects are not required to measure the availability of dietary iron.

In prosperous countries with varied diets, iron absorption depends mainly on the subjects' iron status and on factors affecting dietary availability, independent of iron content. However, with severely limited diets based on grains such as rice or de-germed corn, dietary iron content also may be an important limiting factor in determining the total iron absorbed.

Factors affecting the bioavailability of dietary iron

The bioavailability of dietary iron is affected mainly by the chemical form, by dietary enhancers and inhibitors that affect luminal iron solubility, and to a lesser extent by other cations that may compete for mucosal transport, and by the amount ingested.

Chemical Form

For food iron, the main forms affecting absorption by humans are heme and non-heme iron. Heme iron accounts for ~40% of the iron in meat, poultry, and fish flesh. Non-heme iron, for practical purposes, comprises the rest of iron in foods, which is almost completely exchangeable with extrinsically added radio-iron ionic salts. In humans, absorption of non-heme iron from foods intrinsically radio-labeled during the growth of the food, or extrinsically radio-labeled in the final stages of food preparation results in extrinsic to intrinsic absorption ratios of approximately 1.1 [22]. This validation of an extrinsic labeling technique to study non-heme iron absorption from food has been extensively tested by using foods such as maize, soybeans, wheat, wheat bran, white wheat flour, black beans, and eggs [22, 23]. All non-heme iron consumed simultaneously (in a meal) appears to be absorbed to a similar degree from an exchangeable, non-heme iron pool in the intestinal lumen. Absorption from this pool is affected by other dietary constituents consumed with that meal, but not those consumed several hours before [24].

An exception to this exchangeable non-heme iron pool is food iron in the ferritin form. Liver and spleen are rich in ferritin iron, which occurs in much lower but generally not well-quantified amounts in other animal and as well as plant foods. Ferritin iron is less efficiently absorbed than non-heme iron when tested by using animal ferritin intrinsically radio-labeled [25–27], a difference which is not detected when the animal ferritin has been isolated and extrinsically radio-labeled [27, 28]. Although increased expression of ferritin in plant foods has been proposed as a means of increasing food iron content [29], the bioavailability of iron from ferritin remains unresolved.

The possible contamination of iron with soil residues, dust, or water minerals provides another exception to the concept of a completely exchangeable pool of non-heme iron from a meal. Such contamination is minimal (2–3%) in most Western diets, but low exchangeability (~50%) has been observed in a Chinese meal of soy bean cake, red pepper, fried bamboo, and chicken, and in a Tanzanian meal of cassava leaves [30].

Bioavailability also varies for the forms of iron used in food fortification or supplementation. A ranking of the bioavailability to humans of chemical salts (ferrous sulfate, ferrous succinate, ferrous lactate, ferrous fumarate, ferrous glycine sulfate, ferrous glutamate, ferrous gluconate, > ferrous citrate, ferrous tartrate, ferrous pyrophosphate > ferric sulfate, ferric citrate [31]), is likely determined by their iron valence and solubility. Bioavailability has been enhanced by chelation of iron in forms such as NaFeEDTA or ferrous bis-glycinate, which apparently improve luminal solubility and moderate the influence of inhibitors such as phytic acid [32, 33]. Iron fortification sources such as ferric pyrophosphate, ferric orthophosphate, and elemental iron powders are relatively inert in dry foods; this provides the advantage of minimizing adverse chemical reactions that may impair food color, taste, and shelf-life, but is also associated with lower iron absorption relative to salts such as ferrous sulfate. Some micronization and emulsification technologies appear to improve the bioavailability of ferric pyrophosphate [34]. The bioavailability of elemental iron powders, composed of relatively pure iron metal with a zero valence state, is related to particle size, surface area, and solubility and differs according to specific production processes; bioavailability, as determined with anemic rats, is

greatest for carbonyl, followed by electrolytic, and then the various reduced iron powders [35]. However, the bioavailability of elemental iron powders is difficult to determine sensitively in humans because the commercial powders cannot be isotopically labeled; carbonyl iron that was radio-labeled by neutron activation was poorly absorbed by humans [36].

Dietary enhancers and inhibitors of iron absorption

When consumed concurrently in the same meal, dietary factors can substantially enhance or inhibit iron absorption. Heme iron absorption is enhanced by unidentified factor(s) in meat, poultry and fish [37, 38] and inhibited by calcium [39]. These two and several other dietary factors influence absorption of the non-heme iron form (Table 1). The enhancing effects of meat, poultry, and fish [37, 38], and of ascorbic acid [24, 40], increase in a dose-dependent manner, and are especially effective in the presence of inhibitors such as phytic acid and polyphenols [41, 42]. Alcohol enhances non-heme iron absorption, possibly by enhancing gastric acid secretion which promotes the reduced valence state [42]. Carotenes have been reported as enhancers of non-heme iron absorption [43, 44]. Reports of enhancement by retinoids are inconsistent [43, 45]. Citric, malic, and tartaric acids also appear to enhance non-heme iron absorption, and while this enhancement requires a 100-fold molar ratio, it has been suggested to be of practical relevance when diets include citric acid from citrus fruits, malic acid from deciduous fruits such as plums, peaches, and apples, or tartaric acid from white wines [46].

Inhibitors of non-heme iron absorption include phytic acid (inositol hexaphosphate) in whole grains, legumes, lentils, and nuts [41, 46]; iron-binding polyphenols, such as flavonoids, phenolic acids, and their polymerization products in tea, coffee, red wines, and a variety of other cereals, vegetables, and spices [42, 46, 47]; soy protein (apparently independent of the phytic acid in soy) [48]; and eggs [42, 49]. Inositol hexaphosphate is the main form of inositol phosphates in food and it is primarily the hexa- and pentaphosphates that inhibit non-heme iron absorption [50]. The dose-dependent inhibition by phytic acid is nearly maximal at phytate-to-iron molar ratios greater than one, and partial reductions in phytic acid may not substantially improve iron absorption [51]. Phytic acid content can be reduced by germination, fermentation, and soaking of cereal flours [52], including the yeast leavening of bread, but calcium sources in bread dough will inhibit phytic acid degradation by yeast phytase [39].

Table I: Food components that enhance or inhibit absorption of non-heme iron, if consumed concurrently.

Enhancers	Inhibitors
Meat, poultry, fish (unidentified factor)[1]	Phytic acid
Ascorbic acid[2]	Polyphenols/ tannins (tea & coffee)
Alcohol	Soy protein
Retinols and carotenes??	Egg
Other organic acids??	Calcium[1]

[1] Absorption of the heme form of iron is also enhanced by a factor in meat, poultry, and fish, and inhibited by calcium.
[2] Ascorbic acid interacts with inhibitors such as phytic acid and iron-binding polyphenols, resulting in an even greater enhancing effect when such inhibitors are present.

Influence of other minerals and of the amount of iron

As indicated above, other cations in addition to ferrous iron may bind to iron transport proteins such as DMT-1, potentially inhibiting iron absorption. Calcium inhibits the absorption of both heme and non-heme iron [39, 53]. The inhibition of non-heme iron occurs when the calcium content of a meal exceeds 50 mg, and is maximal at about 300 mg [42]. Zinc in supplemental quantities may inhibit iron absorption [54, 55]. Increasing the dose of iron ingested reduces the efficiency of (fractional) absorption, but increases the absolute amount absorbed [56].

Assessing iron bioavailability from human diets

Intrinsic vs. extrinsic isotopic labeling

As discussed above *(see Factors affecting iron bioavailability; Chemical form),* it is well established that non-heme iron from foods consumed in the same meal is absorbed from a common, exchangeable iron pool. The use of extrinsic isotopic labels to measure absorption of the non-heme iron in a meal has been extensively validated [22, 23, 57].

Testing with single meals

Meals with similar iron content can differ by up to 10-fold in iron bioavailability when tested by using a well-accepted test meal protocol. With this frequently applied protocol, iron absorption is tested with iron isotopes extrinsically added to weighed meals that are consumed in the morning by fasted subjects. Retention of the isotopes is determined after two weeks, either by whole body counting of gamma-emitting ^{59}Fe, or more commonly by erythrocyte retention of the isotopic tracers. The erythrocyte

retention method requires estimation of the subject's blood volume and of the fraction of absorbed isotope that is incorporated into red cells within two weeks. The whole body counting and erythrocyte retention methods are highly correlated under conditions assuming 80% erythrocyte incorporation of absorbed iron isotope [17, 58]. Although absorption and erythrocyte incorporation are both inversely related to body iron stores [17, 58, 61], this does not interfere with sensitive estimations of food iron bioavailability in studies designed with subjects serving as their own controls. As described in the discussion of Figure 2 above, the relative ratios of iron bioavailability between diets are independent of the iron status of the subjects tested, making it unnecessary to test only iron-deficient subjects to evaluate dietary bioavailability. However, to make comparisons between experiments, it is necessary to normalize data for differences in iron status of the subjects, either by expressing absorption relative to that from a reference dose (3 mg iron with 20 mg ascorbic acid), a reference meal, or by adjusting the absorption to that expected with a specific concentration of serum ferritin [62].

Single meals vs. diets fed throughout the day

The practical impact of the large bioavailability differences observed with single meal studies has been challenged, mainly because of a relatively minimal impact of dietary interventions on iron status after several weeks or months (discussed further below). Investigations from the laboratory of Cook *et al* [62–64] reported that single, controlled meals served in the laboratory exaggerated the differences in bioavailability that were observed when the volunteers were given radio-labeled rolls to consume with whole diets that were self-selected, away from the laboratory, according to the investigators' instructions. However, the greater differences in bioavailability observed with single meals (high/low bioavailability ratio of ~6 for non-heme iron) than with diets consumed through the day (ratio of ~2.5) was likely attributable to the poorer experimental control of the self-selected diets [62–64]. When weighed, two-day diets were ingested under laboratory conditions [17, 18, 65], bioavailability ratios were more similar to those observed with single meals (ratios of ~5–6). Tidehag *et al* [66] reported lower non-heme iron absorption from a combination of foods with amounts distributed as 1/7, 2/7, and 4/7 for three meals, compared with absorption from a single morning meal with a 1/7 portion of the same foods. However, because the second and third meals of the day each contained higher amounts of iron that may reduce absorptive efficiency, this difference could not be attributed just to single vs. multiple meals. Taylor *et al* [67] found no difference in iron absorption from identical foods and amounts in two studies of Venezuelan meals, consumed either at breakfast after an overnight fast, or at lunch time after just a four-hour fast. The evidence suggests that the iron absorbed from a diet consumed throughout the day is the sum of iron absorbed from the single meals of that diet. At the same time, it must be recognized that enthusiastic investigators are likely to test more extreme food combinations with single meals than with complete diets.

Adaptation to differences in dietary iron bioavailability

Whether absorption is measured from single meals or a few days of complete diets, dietary factors that affect iron bioavailability are only important to the extent that they influence iron absorption from a sustained diet. Extensive exposure does not seem to modify the degree of enhancement or inhibition by dietary factors that influence non-heme iron absorption. In single meal comparisons, dietary phytate inhibited non-heme iron absorption to a similar degree in long-term vegetarians and controls [68]. Ascorbic acid enhanced non-heme iron absorption to a similar degree before and after 16 weeks of ascorbic acid supplementation; however, this supplementation tended to reduce the general efficiency of non-heme iron absorption (nonsignificantly) by about 25%, both with and without added ascorbic acid [69]. Adult men partially adapted within 10 weeks to significantly increase iron absorption from a low bioavailability diet from 0.12 to 0.17 mg/day, and decrease iron absorption from a high bioavailability diet from 0.96 to 0.69 mg/day [17]. However, in a similar experiment with premenopausal women, adaptation was less pronounced, and was not quantitatively important [18]. It can be concluded that dietary equilibration is not necessary to evaluate relative iron bioavailability from foods.

Although short-term adaptation to differences in dietary bioavailability is limited, the cross-sectional inverse relationship between iron absorption and body iron stores (estimated from serum ferritin; Figure 2) suggests a long-term adaptive response to regulate body iron. Such adaptation is a physiological adjustment related to the change in iron status, rather than to specific dietary enhancers or inhibitors of iron absorption. However, as indicated in Figure 2, the bioavailability of the diet can restrict the impact of the greater absorptive efficiency of people with low iron stores.

Short-term absorption vs. long-term changes in body iron status

Despite considerable differences in iron absorption with dietary changes, there has been little demonstration of the efficacy of changes in dietary bioavailability on human iron status. In controlled trials of weeks or months duration, serum ferritin was unresponsive to changes in ascorbic acid [69–73] calcium [74, 75], or meat [76] intake. Women consuming controlled diets differing in meat and phytic acid for 8 weeks each had no change in serum ferritin despite a 6-fold difference in iron absorbed [77]. Similarly, 10 weeks of controlled diets high or low in bioavailability (based on differences in meat, ascorbic acid, phytic acid, and tannins) resulted in 4- to-6-fold differences in total iron absorbed, but did not influence serum ferritin of U.S. adult men or women [17, 18]. Such unresponsiveness to dietary iron bioavailability was also observed in iron-deficient rural Mexican women, some with anemia, whose iron status was unresponsive to the addition of a food source of ~50 mg ascorbic acid consumed with meals for 8 months [73, 78]. A commonly used guideline derived from studies of human iron depletion by phlebotomy, indicating that 1 μg/L difference in serum ferritin corresponds to 8–10 mg stored iron or 120 μg storage iron/kg body weight [79], has not been predictive of the minimal changes in serum ferritin observed with differences in dietary iron absorption for weeks or months. The reasons for this apparent discrepancy between phlebotomy and absorption results are not clear, but it should be expected that dietary changes affecting iron absorption would only gradually influence body iron, likely requiring months or years.

Cross-sectional studies provide some evidence of such long-term effects of dietary iron bioavailability. In comparisons of vegetarians and omnivores, vegetarians consistently have lower iron stores, although without any apparent increase in iron deficiency anemia [80]. In addition, a detailed dietary assessment of elderly subjects found that serum ferritin was positively associated with ingestion of heme iron, supplemental iron, dietary vitamin C, and alcohol and negatively associated with coffee drinking [81].

Although most experimental interventions with dietary bioavailability have not changed body iron stores, interventions with supplemental or fortification sources of iron are efficacious in improving body iron [58, 82, 83]. Sustained interventions are required, as recently documented in Moroccan children who, despite their iron-replete status after a successful iron fortification trial, rapidly developed deficits in tissue iron and reduced hemoglobin concentrations when their low-iron bioavailability, legume- and cereal-based diets were no longer fortified [84].

Conclusions

Our knowledge of the molecular basis of iron absorption is growing rapidly. Both body iron status and dietary characteristics substantially influence iron absorption, with little interaction between these factors. When assessing the dietary characteristics that affect iron availability, absorption data must be normalized for the iron status of the subjects. Human studies employing iron isotopes have helped to quantitatively define the dietary characteristics that enhance or inhibit iron absorption from foods. High bioavailability diets can enable those with low iron status to substantially increase dietary iron absorption. However, in contrast to iron supplementation and fortification, dietary bioavailability improvements have had minimal effect on indices of human iron status within several weeks or months. Iron deficiency can be best resolved with plentiful, varied diets. Otherwise, iron fortification will likely be needed to augment more limited dietary availability approaches to human iron deficiency.

References

1. Mielczarek, E. V. and McGrayne, S. B. Iron, (2000) Nature's Universal Element: Why People Need Iron and Animals Make Magnets. Rutgers University Press, New Brunswick.
2. World Health Organization. (2002) The World Health Report 2002; Reducing Risks, Promoting Healthy Life. p. 248, WHO, Geneva, Switzerland.
3. Fleming, R. E. (2005) Advances in understanding the molecular basis for the regulation of dietary iron absorption. Curr. Opin. Gastroenterol. 21, 201–206.
4. Quigley, J. G., Yang, Z., Worthington, M. T. *et al* (2004) Identification of a human heme exporter that is essential for erythropoiesis. Cell 118, 757–766.
5. Frazer, D.M., Wilkins, S.J., Becker, E.M. *et al* (2003) A rapid decrease in the expression of DMT1 and Dcytb but not Ireg1 or hephaestin explains the mucosal block phenomenon of iron absorption. Gut 52, 340–346.
6. Fleming, M. D., Trenor, C. C., 3rd, Su, M. A. *et al* (1997) Microcytic anaemia mice have a mutation in Nramp2, a candidate iron transporter gene. Nat. Genet. 16, 383–386.
7. Gunshin, H., Mackenzie, B., Berger, U. V. *et al* (1997) Cloning and characterization of a mammalian proton-coupled metal-ion transporter. Nature 388, 482–488.
8. Donovan, A., Brownlie, A., Zhou, Y. *et al* (2000) Positional cloning of zebrafish ferroportin1 identifies a conserved vertebrate iron exporter. Nature 403, 776–781.
9. McKie, A. T., Marciani, P., Rolfs, A. *et al*. (2000) A novel duodenal iron-regulated transporter, IREG1, implicated in the basolateral transfer of iron to the circulation. Mol. Cell 5, 299–309.
10. Abboud, S. and Haile, D.J. (2000) A novel mammalian iron-regulated protein involved in intracellular iron metabolism. J. Biol. Chem. 275,19906–19912.

11. Vulpe, C. D., Kuo, Y. M., Murphy, T. L. *et al* (1999) Hephaestin, a ceruloplasmin homologue implicated in intestinal iron transport, is defective in the sla mouse. Nat. Genet. 21, 195–199.
12. Wessling-Resnick, M. (2002) A possible link between hepcidin and regulation of dietary iron absorption. Nutr. Rev. 60, 371–374.
13. Leong, W. I. and Lonnerdal, B. (2004) Hepcidin, the recently identified peptide that appears to regulate iron absorption. J. Nutr. 134, 1–4.
14. Nemeth, E., Tuttle, M. S., Powelson, J. *et al* (2004) Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. Science 306, 2090–2093.
15. Feder, J. N., Gnirke, A., Thomas, W. *et al* (1996) A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis [see comments]. Nat. Genet. 13, 399–408.
16. Waheed, A., Grubb, J. H., Zhou, X. Y. *et al* (2002) Regulation of transferrin-mediated iron uptake by HFE, the protein defective in hereditary hemochromatosis. Proc. Natl. Acad. Sci. USA 99, 3117–3122.
17. Hunt, J. R. and Roughead, Z. K. (2000) Adaptation of iron absorption in men consuming diets with high or low iron bioavailability. Am. J. Clin. Nutr. 71, 94–102.
18. Hunt, J. R. (2003) High-, but not low-bioavailability diets enable substantial control of women's iron absorption in relation to body iron stores, with minimal adaptation within several weeks. Am. J. Clin. Nutr. 78, 1168–1177.
19. Cook, J. D. (1990) Adaptation in iron metabolism. Am. J. Clin. Nutr. 51, 301–308.
20. McCance, R. A. and Widdowson, E. M. (1937) Absorption and excretion of iron. Lancet 2, 680–684.
21. Hallberg, L. (2001) Perspectives on nutritional iron deficiency. Annu. Rev. Nutr. 21, 1–21.
22. Cook, J. D., Layrisse, M., Martinez-Torres, C., Walker, R., Monsen, E. and Finch, C. A. (1972) Food iron absorption measured by an extrinsic tag. J. Clin. Invest. 51, 805–815.
23. Hallberg, L. and Björn-Rasmussen, E. (1972) 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. 9, 193–197.
24. Cook, J. D. and Monsen, E. R. (1977) Vitamin C, the common cold, and iron absorption. Am. J. Clin. Nutr. 30, 235–241.
25. Layrisse, M., Martinez-Torres, C., Renzy, M. and Leets, I. (1975) Ferritin iron absorption in man. Blood 45, 689–698.
26. Derman, D. P., Bothwell, T. H., Torrance, J. D. *et al* (1982) Iron absorption from ferritin and ferric hydroxide. Scand. J. Haematol. 29, 18–24.
27. Skikne, B., Fonzo, D., Lynch, S. R. and Cook, J. D. (1997) Bovine ferritin iron bioavailability in man. Eur. J. Clin. Invest. 27, 228–233.
28. Davila-Hicks, P., Theil, E.C. and Lonnerdal, B. (2004) Iron in ferritin or in salts (ferrous sulfate) is equally bioavailable in nonanemic women. Am. J. Clin. Nutr. 80, 936-40.
29. Lucca, P., Hurrell, R. and Potrykus, I. (2001) Approaches to improving the bioavailability and level of iron in rice seeds. Journal of the Science of Food and Agriculture 81, 828–834.
30. Hallberg, L. and Björn-Rasmussen, E. (1982) Measurement of iron absorption from meals contaminated with iron. Am. J. Clin. Nutr. 34, 2808–2815.
31. Brise, H. and Hallberg, L. (1962) Absorbability of different iron compounds. Acta Med. Scand.171,Suppl. 23–38.
32. Hurrell, R.F. (2002) Fortification: overcoming technical and practical barriers. J. Nutr. 132, 806S–812S.
33. Bovell-Benjamin, A. C., Viteri, F. E. and Allen, L. H. (2000) Iron absorption from ferrous bisglycinate and ferric trisglycinate in whole maize is regulated by iron status. Am. J. Clin. Nutr. 71, 1563–1569.
34. Fidler, M. C., Walczyk, T., Davidsson, L. *et al* (2004) A micronised, dispersible ferric pyrophosphate with high relative bioavailability in man. Br. J. Nutr. 91, 107–112.
35. Swain, J. H., Newman, S. M. and Hunt, J. R. (2003) Bioavailability of elemental iron powders to rats is less than bakery-grade ferrous sulfate and predicted by iron solubility and particle surface area. J. Nutr. 133, 3546–3552.
36. Hallberg, L., Brune, M. and Rossander, L. (1986) Low bioavailability of carbonyl iron in man: studies on iron fortification of wheat flour. Am. J. Clin. Nutr. 43, 59–67.
37. Layrisse, M., Martinez-Torres, C. and Roche, M. (1968) Effect of interaction of various foods on iron absorption. Am. J. Clin. Nutr. 21, 1175–1183.
38. Martinez-Torres, C. and Layrisse, M. (1971) Iron absorption from veal muscle. Am. J. Clin. Nutr. 24, 531–540.
39. Hallberg, L., Brune, M., Erlandsson, M., Sandberg, A. S. and Rossander-Hulten, L. (1991) Calcium: effect of different amounts on nonheme- and heme-iron absorption in humans. Am. J. Clin. Nutr. 53, 112–119.
40. Hallberg, L., Brune, M. and Rossander, L. (1986) Effect of ascorbic acid on iron absorption from different types of meals. Studies with ascorbate rich foods and synthetic ascorbic acid given in different amounts with different meals. Human Nutr. Appl. Nutr. 40A, 97–113.
41. Hallberg, L., Brune, M. and Rossander, L. (1989) Iron absorption in man: ascorbic acid and dose-dependent inhibition by phytate. Am. J. Clin. Nutr. 49, 140–144.
42. Hallberg, L. and Hulthen, L. (2000) Prediction of dietary iron absorption: an algorithm for calculating absorption and bioavailability of dietary iron (See erratum Am. J. Clin. Nutr. 2000; 72, 1242). Am. J. Clin. Nutr. 71, 1147–1160.
43. Garcia-Casal, M. N., Layrisse, M., Solano, L. *et al* (1998) Vitamin A and beta-carotene can improve nonheme iron absorption from rice, wheat, and corn by humans. J. Nutr. 128, 646–650.
44. Layrisse, M., Garcia-Casal, M. N., Solano, L. *et al* (2000) New property of vitamin A and beta-carotene on human iron absorption: effect on phytate and polyphenols as inhibitors of iron absorption. Arch. Latinoam. Nutr. 50, 243–248.
45. Walczyk, T., Davidsson, L., Rossander-Hulthen, L., Hallberg, L. and Hurrell, R. F. (2003) No enhancing effect of vitamin A on iron absorption in humans. Am. J. Clin. Nutr. 77, 144–149.
46. Gillooly, M., Bothwell, T. H., Torrance, J. D. *et al* (1983) The effects of organic acids, phytates and polyphenols on the absorption of iron from vegetables. Br. J. Nutr. 49, 331–342.
47. Brune, M., Rossander, L. and Hallberg, L. (1989) Iron ab-

sorption and phenolic compounds: importance of different phenolic structures. Eur. J. Clin. Nutr. 43, 547–557.
48. Hurrell, R.F., Juillerat, M.A., Reddy, M.B., Lynch, S.R., Dassenko, S.A. and Cook, J.D. (1992) Soy protein, phytate, and iron absorption in humans. Am. J. Clin. Nutr. 56, 573–578.
49. Callender, S.T., Marney, S.R., Jr. and Warner, G.T. (1970) Eggs and iron absorption. Br. J. Haematol. 19, 657–665.
50. Sandberg, A.S., Brune, M., Carlsson, N.G., Hallberg, L., Skoglund, E. and Rossander-Hulthen, L. (1999) Inositol phosphates with different numbers of phosphate groups influence iron absorption in humans. Am. J. Clin. Nutr. 70, 240–246.
51. Hurrell, R.F. (2003) Influence of vegetable protein sources on trace element and mineral bioavailability. J. Nutr. 133, 2973S–2977S.
52. Gibson, R.S. (2004) Strategies for preventing micronutrient deficiencies in developing countries. Asia Pac. J. Clin. Nutr. 13, S23.
53. Cook, J.D., Dassenko, S.A. and Whittaker, P. (1991) Calcium supplementation: effect on iron absorption. Am. J. Clin. Nutr. 53, 106–111.
54. Herman, S., Griffin, I.J., Suwarti, S. *et al* (2002) Cofortification of iron-fortified flour with zinc sulfate, but not zinc oxide, decreases iron absorption in Indonesian children. Am. J. Clin. Nutr. 76, 813–817.
55. Donangelo, C.M., Woodhouse, L.R., King, S.M., Viteri, F.E. and King, J.C. (2002) Supplemental zinc lowers measures of iron status in young women with low iron reserves. J. Nutr. 132, 1860–1864.
56. Werner, E., Roth, P. and Kaltwasser, J.P. (1982) Relationship between the dose administered and the intestinal absorption of iron from ferrous sulphate in humans. In: The Biochemistry and Physiology of Iron. (Satlman, P. and Hegenauer, J., eds.) pp. 821–823, Elsevier North Holland, Inc.
57. Layrisse, M., Martinez-Torres, C., Cook, J.D., Walker, R. and Finch, C.A. (1973) Iron fortification of food: its measurement by the extrinsic tag method. Blood 41, 333–352.
58. Roughead, Z.K. and Hunt, J.R. (2000) Adaptation in iron absorption: iron supplementation reduces nonheme- iron but not heme-iron absorption from food. Am. J. Clin. Nutr. 72, 982–989.
59. Cook J.D., Lipschitz, D.A., Miles, L.E.M. and Finch, C.A. (1974) Serum ferritin as a measure of iron stores in normal subjects. Am. J. Clin. Nutr. 27, 681–687.
60. Taylor, P., Martinez-Torres, C., Leets, I., Ramirez, J., Garcia-Casal, M.N. and Layrisse, M. (1988) Relationships among iron absorption, percent saturation of plasma transferrin and serum ferritin concentration in humans. J. Nutr. 118, 1110–1115.
61. Lynch, S.R., Skikne, B.S. and Cook, J.D. (1989) Food iron absorption in idiopathic hemochromatosis. Blood 74, 2187–2193.
62. Cook, J.D., Dassenko, S.A. and Lynch, S.R. (1991) Assessment of the role of nonheme-iron availability in iron balance. Am. J. Clin. Nutr. 54, 717–722.
63. Reddy, M.B. and Cook, J.D. (1997) Effect of calcium intake on nonheme-iron absorption from a complete diet. Am. J. Clin. Nutr. 65, 1820–1825.
64. Cook, J.D. and Reddy, M.B. (2001) Effect of ascorbic acid intake on nonheme-iron absorption from a complete diet. Am. J. Clin. Nutr. 73, 93–98.
65. Hunt, J.R. and Roughead, Z.K. (1999) Nonheme iron absorption, fecal ferritin excretion, and blood indexes of iron status in women consuming controlled lactoovovegetarian diets for 8 wk. Am. J. Clin. Nutr. 69, 944–952.
66. Tidehag, P., Hallmans, G., Wing, K. *et al* (1996) A comparison of iron absorption from single meals and daily diets using radioFe (^{55}Fe, ^{59}Fe). Br. J. Nutr. 75, 281–289.
67. Taylor, P.G., Méndez-Castellanos, H., Martínez-Torres, C. *et al* (1995) Iron bioavailability from diets consumed by different socioeconomic strata of the Venezuelan population. J. Nutr. 125, 1860–1868.
68. Brune, M., Rossander, L. and Hallberg, L. (1989) Iron absorption: no intestinal adaptation to a high-phytate diet. Am. J. Clin. Nutr. 49, 542–545.
69. Cook, J.D., Watson, S.S., Simpson, K.M., Lipschitz, D.A. and Skikne, B.S. (1984) The effect of high ascorbic acid supplementation on body iron stores. Blood 64, 721–726.
70. Malone, H.E., Kevany, J.P., Scott, J.M., O'Broin S.D. and O'Connor, G. (1986) Ascorbic acid supplementation: Its effects on body iron stores and white blood cells. Irish J. Med. Sci. 155, 74–79.
71. Monsen, E.R., Labbe, R.F., Lee, W. and Finch, C.A. (1991) Iron balance in healthy menstruating women: Effect of diet and ascorbate supplementation. In: Trace Elements in Man and Animals (TEMA-7). (Momcilovic, B., ed.) pp. 6.2–6.3, Institute for Medical Research and Occupational Health, University of Zagreb, Dubrovnic, Yugoslavia.
72. Hunt, J.R., Gallagher, S.K. and Johnson, L.K. (1994) Effect of ascorbic acid on apparent iron absorption by women with low iron stores. Am. J. Clin. Nutr. 59, 1381–1385.
73. Garcia, O.P., Diaz, M., Rosado, J.L. and Allen, L.H. (2003) Ascorbic acid from lime juice does not improve the iron status of iron-deficient women in rural Mexico. Am. J. Clin. Nutr. 78, 267–273.
74. Sokoll, L.J. and Dawson-Hughes, B. (1992) Calcium supplementation and plasma ferritin concentrations in premenopausal women. Am. J. Clin. Nutr. 56, 1045–1048.
75. Minihane, A.M. and Fairweather-Tait, S.J. (1998) Effect of calcium supplementation on daily nonheme-iron absorption and long-term iron status. Am. J. Clin. Nutr. 68, 96–102.
76. Hunt, J.R., Gallagher, S.K., Johnson, L.K. and Lykken, G.I. (1995) High- versus low-meat diets: effects on zinc absorption, iron status, and calcium, copper, iron, magnesium, manganese, nitrogen, phosphorus, and zinc balance in postmenopausal women. Am. J. Clin. Nutr. 62, 621–632.
77. Hunt, J.R., Matthys, L.A. and Johnson, L.K. (1998) Zinc absorption, mineral balance, and blood lipids in women consuming controlled lactoovovegetarian and omnivorous diets for 8 wk. Am. J. Clin. Nutr. 67, 421–430.
78. Diaz, M., Rosado, J.L., Allen, L.H., Abrams, S. and Garcia, O.P. (2003) The efficacy of a local ascorbic acid-rich food in improving iron absorption from Mexican diets: a field study using stable isotopes. Am. J. Clin. Nutr. 78, 436–440.

79. Finch, C. (1994) Regulators of iron balance in humans. Blood 84, 1697–1702.
80. Hunt, J. R. (2003) Bioavailability of iron, zinc, and other trace minerals from vegetarian diets. Am. J. Clin. Nutr. 78, 633S–639S.
81. Fleming, D. J., Jacques, P. F., Dallal, G. E., Tucker, K. L., Wilson, P. W. F., and Wood, R. J. (1998) Dietary determinants of iron stores in a free-living elderly population: The Framingham Heart Study. Am. S. Clin. Nutr. 67, 722–733.
82. Hinton, P. S., Giordano, C., Brownlie, T. and Haas, J. D. (2000) Iron supplementation improves endurance after training in iron-depleted, nonanemic women. J. Appl. Physiol. 88, 1103–1111.
83. Thuy, P. V., Berger, J., Davidsson, L. *et al* (2003) Regular consumption of NaFeEDTA-fortified fish sauce improves iron status and reduces the prevalence of anemia in anemic Vietnamese women. Am. J. Clin. Nutr. 78, 284–290.
84. Zimmermann, M. B., Chaouki, N. and Hurrell, R. F. (2005) Iron deficiency due to consumption of a habitual diet low in bioavailable iron: a longitudinal cohort study in Moroccan children. Am. J. Clin. Nutr. 81, 115–121.

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