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IRON, FERRITIN, AND NUTRITION

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■ **Abstract** Ferritin, a major form of endogenous iron in food legumes such as soybeans, is a novel and natural alternative for iron supplementation strategies where effectiveness is limited by acceptability, cost, or undesirable side effects. A member of the nonheme iron group of dietary iron sources, ferritin is a complex with Fe^{3+} iron in a mineral (thousands of iron atoms inside a protein cage) protected from complexation. Ferritin illustrates the wide range of chemical and biological properties among nonheme iron sources. The wide range of nonheme iron receptors matched to the structure of the iron complexes that occurs in microorganisms may, by analogy, exist in humans. An understanding of the chemistry and biology of each type of dietary iron source (ferritin, heme, Fe^{2+} ion, etc.), and of the interactions dependent on food sources, genes, and gender, is required to design diets that will eradicate global iron deficiency in the twenty-first century.

CONTENTS

INTRODUCTION

Nutritional iron is usually divided into two types: heme, where iron is absorbed as the stable porphyrin complex unaffected by other food components, and nonheme iron, which is envisioned as "free" or in weak complexes. Food components such

as phytate or tannins can trap iron from weak complexes in other foods during digestion, altering the bioavailability of food iron. Thus it is not the total amount of food iron ingested, but the distribution among different chemical forms of iron and the reactivity among the different chemical forms of iron ingested that will determine the bioavailability of iron in any one meal. New information emphasizes the complexity of nonheme iron in food, and the apparent stability of some nonheme iron complexes such as ferritin, where the iron is absorbed even from phytate-rich soybeans.

New approaches to solving iron deficiency are critical because the problem remains one of great significance in early childhood as well as in menstruating and pregnant women, where average frequencies of iron deficiency are estimated at 43% and reach 85% in some populations (47). Iron depletion affects not only overall health but also cognitive development (78). The problem is not restricted to those in poverty or to underdeveloped countries. In Japan, for example, ~15% of blood donors are rejected because of iron deficiency and estimates of iron deficits in young women are as high as 25% (Y. Kohgo, personal communication). In the United States, moreover, approximately 75% of college-aged women report low iron intake (74), and suboptimal dietary intake of iron occurs in 90% of pregnant Americans (84). Current iron supplementation regimens, some known for centuries, can have negative consequences and side effects from the oxidative damage of oxygen and iron chemistry (22, 61). New forms of iron supplementation are needed, particularly for vulnerable groups such as children and menstruating and pregnant women (9, 14, 22). Recent studies (68) confirm a 1973 study (79) showing that soybeans (and possibly other, yet-to-be-discovered ferritin-rich foods) have the potential to be novel, natural iron supplements that minimize dietary iron deficiency (91). Recent reviews on iron absorption itself and general features of iron nutrition include References 8, 11, 38, 66, and 100. The theme of the sections that follow is the properties of ferritin and its chemistry and biology that distinguish it from other forms of iron in the diet.

CONSEQUENCES OF THE CHEMICAL FORM OF IRON

Molecular Absorption Mechanisms for Iron

Absorption of iron from food requires recognition of the chemical form of the iron by gut receptors. Both shape and charge are important in the recognition process. The shape and chemistry of the Fe^{3+} iron is much more dependent on pH compared to Fe^{2+} ions. At the low pH of the stomach, both Fe^{3+} and Fe^{2+} ions in solution will be single ions surrounded by water, much like Na^{+}, Ca^{2+}, Zn^{2+}, Mn^{2+}, Cr^{3+}, etc., unless the Fe^{3+} ion is chelated (e.g., citrate, phytate, and heme), or encased as a multi-ion form in a protein such as ferritin (Figure 1); ferritin is extremely stable (23, 88, 90) and survives in vitro digestion conditions. At the pH of the intestine, and in other neutral parts of the body, Fe^{3+} ions form large

insoluble complexes of iron and oxygen, whereas most of the other ions, including ferrous, are stable as solitary ions surrounded by water. Absorption of the larger and/or complexed forms of Fe^{3+} ions in food appears to depend on recognition by the gut cells and, based on recognition genes in bacteria (5, 6, 16, 105), may be specific for each Fe^{3+} complex. Fe^{2+}, Ca, Zn, and Mn ions are recognized by the gut receptor DMT1, which also functions in other tissues (4, 30, 37). The iron-lactoferrin complex appears to be recognized by a specific gut cell molecule for absorption, at least at certain stages of development (83), and may have a more restricted role in iron trafficking than does DMT1. Heme is a stable iron complex readily recognized by gut cells, based on absorption properties, but the gene product that recognizes heme is yet to be identified (66). Finally, Fe^{3+} phytate is an Fe^{3+} complex for which there may not be a gut recognition molecule because it is poorly absorbed (26, 39), except in the monophytate form (56). The recent studies on iron availability of ferritin iron in both animals and humans (10, 67, 68, 79) (Table 1 and Figure 2), the absorption of pure ferritin by humans, and the resistance of ferritin to digestion under conditions similar to those during human digestion (S.L. Kelleher, E.C. Theil, & B. Lonnerdal, unpublished observations) indicate the possible existence of yet another gene product required for recognition and absorption of intact ferritin. Support for the idea that intact ferritin can enter enterocytes comes from the fact that viruses can cross membranes (101).

TABLE 1 Comparison of soy iron availability in humans in three studies

	1973[a]	**1984**[a]	**2003**[a]
Number of subjects	5	10	18
Gender	Female	Male	Female
Labeling	Intrinsic	Extrinsic	Intrinsic
Food iron form	Bean protein (ferritin)	$FeCl_3$	Bean protein (ferritin)[b]
Test iron form	$FeSO_4$	$FeSO_4$	$FeSO_4$
Form of meal	Biscuit	Soup	Soup/muffin
% Uptake (soy meal)	19.8	1.6	25.9
% Uptake (reference dose)	72.8	16	58.9
% Uptake (meal/reference dose)	27.5	10.0	44.0
Hct[c]	—	0.47	0.40
Hb (g/L)[c]	108	—	131
Serum ferritin (μg/L)[c]	—	61	11.2

[a]References for data are: 1973 (79); 1984 (59); and 2003 (68), which is also the source of the table.

[b]About 50% of the iron in the soybeans was in the ferritin form.

[c]Values on day of soybean meal consumption.

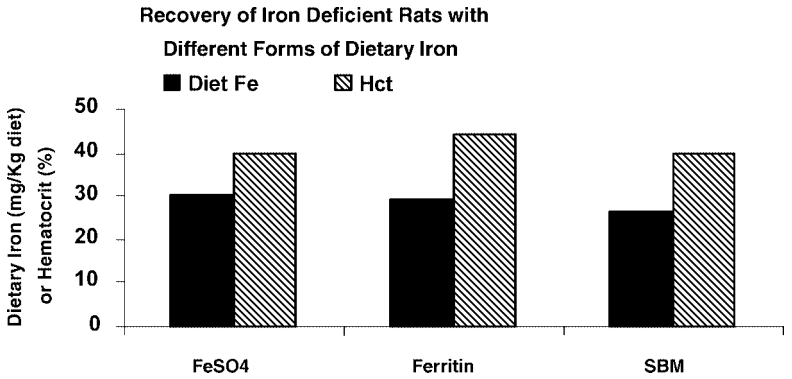

Figure 2 Different forms of dietary iron, at equal Fe content (mg/kg food), are equally effective in correcting iron-deficiency anemia. Fe-deficient rats, 28 days after Fe repletion with diets containing equal amounts of iron in different chemical forms: ferrous sulfate ($Fe^{2+}SO_4$), iron in the Fe^{3+} mineral of horse spleen ferritin (HSF), and soybean (SBM). The majority of the iron in SBM is the Fe^{3+} in the ferritin iron mineral. Data are from Reference 10.

At the pH of the intestine, Fe^{3+} and Fe^{2+} ions have very different chemistry and effective sizes because of the properties of the water molecules bound or coordinated to each ion. The size and chemical differences emphasize the need for different molecular pathways in absorption. (See the next paragraph for a discussion of the hypothesis that all absorbed nonheme iron is reduced to Fe^{2+}.) For Fe^{2+} ions, the coordinated water has a pKa ~9, similar to that of other metal ions such as Cu^{2+}, Zn^{2+}, Mn^{2+}, and will be in the form of a small ion surrounded by four to six water molecules. The water coordinated to the Fe^{3+} ion has a pKa ~3, and at neutral pH, will behave as a weak acid, but still stronger than acetic acid. When the protons dissociate from the waters bound to Fe^{3+} ions, a reactive species forms that produces complexes with multiple iron atoms linked by oxygen bridges ("rust").

Reduction of Fe^{3+} to Fe^{2+} is often proposed as a solution to the problems of nutrition created by Fe^{3+} chemistry. The recent discovery of a ferrireductase related to gut iron absorption (32, 63, 66) supports such an idea. However, other solutions to the chemical problems of iron nutrition have evolved in microorganisms for different Fe^{3+} complexes such as Fe(III)-citrate and heme (5, 6, 46, 82, 105). The "parsimony of nature" suggests that in humans the multiple receptors evolved for selective absorption of different forms of iron in microorganisms is conserved. Support for such an idea comes from the recent identification of three nonheme iron uptake proteins expressed by cells on the internal side of the gut: DMT1, transferrin receptor 1, and transferrin receptor 2 (4, 31, 37, 48). In addition to evolutionary arguments, and cell biology, the hypothesis that reduction of iron in the gut from Fe^{3+} to Fe^{2+} is the sole mechanism for iron absorption has other drawbacks, which

include substrate variability, and electron transfer that can increase free radical production. An example is the use of ascorbate to convert Fe^{3+} to Fe^{2+} in foods. When Fe^{3+} is reduced to Fe^{2+} by ascorbate, the mixture of oxidized ascorbate, Fe^{2+}, and oxygen or oxidants produces a chain of free radicals, altered enterocyte function, and gut irritability (24, 44).

Extrinsic and Intrinsic Labeling in Studies of Dietary Iron Bioavailability

The absorption of added iron will depend on the amount of absorbable iron in the diet, a value that can vary considerably. In the case of soybeans, for example, the amount of phytate and the distribution between ferritin and phytate can vary; compare References 3, 19, and 68. Moreover, variations in the phytate-to-iron ratio will also affect absorbability because the absorbability of iron in monoferric phytate is much higher than in the other Fe^{3+} phytates (56). Results of iron bioavailability studies with the same foods that appear to conflict with each other may reflect differences in the distribution of iron in the food among the different forms: Fe^{2+}, absorbable Fe^{3+} salts, heme, ferritin, and nonabsorbable Fe^{3+} salts.

EXTRINSIC Fe^{3+} or Fe^{2+} ions have been used as extrinsic labels. The addition of iron salts to foods can have different outcomes depending on the food composition and the form of the iron added, such as Fe^{3+} citrate, Fe^{3+} EDTA, $Fe^{3+}(Cl_3^-)$, or Fe^{2+} sulfate/gluconate.

Iron forms used as labels include $Fe^{3+}(Cl^-)_3$ and $Fe^{3+}(citrate)^{-3}$, a commercially available 1:1 salt, which will dissolve in solutions or in the stomach, with some of the six Fe^{3+} binding sites available to form new complexes with chelators in foods or to form "rust" at the pH of the intestine.

Agents in foods, such as phytates or oxalates, are known to influence iron absorption (8). When the phytate-to-iron ratio is high during digestion, monophytate complexes can form, which have a higher absorption based on studies in dogs (56). Food composition, which will depend on growth conditions of plants, therefore can influence the outcome of dietary studies with extrinsic labels (59, 79). Iron availability can also be influenced by mixing foods with varying amounts and forms of phytate and iron (19).

Fe(III)-EDTA and Fe(III)-citrate are nonheme iron complexes in which all Fe^{3+} ion binding sites are stably filled by the complexing agent. The efficacy of Fe-EDTA supplements, even though the complex is an unnatural one, is well established (13, 29, 102), and relates to the stability of the iron complex. In contrast, Fe(III)-citrate, a more stable iron complex, has been less studied as a dietary source, even though bacteria have specific receptor recognition and uptake systems for Fe(III)-citrate (16, 17, 29, 105), and mechanisms for absorption of Fe(III)-citrate may have been conserved during evolution.

Fe^{2+} salts, in solution at the pH of outside the gut enterocyte, react with oxygen and initiate radical chain reactions. Unless rapidly incorporated into cells, via

DMT1 (64, 66) and/or into stable complexes, the reaction products of free Fe^{2+} ions and oxidants can be toxic (24, 44). Nevertheless, Fe^{2+} sulfate will continue to remain a reasonable, if not optimal, standard for absorption because of past use, until other, more selective standards are developed.

INTRINSIC Studies of iron absorption using intrinsic labels have led to results that appear to conflict (52, 59, 68, 79, 99). In some seeds such as cereals, the iron content is relatively low; ferritin is low, and most of the iron is complexed in phytates or polyphenols (62). On the other hand, in seeds such as soybeans, a major portion of the seed iron is a solid mineral inside the ferritin protein (3, 20), which isolates the iron from phytates. In addition, plant ferritin is inside of plastids (81), which further separates ferritin and iron from phytate. The higher distribution of ferritin in the hulls (2) may provide a further barrier between ferritin iron and phytic acid. In the case of ferritin uptake, it needs to be remembered that complexes as large as viruses can cross cell membranes (101), although the mechanisms are little understood. Similarly, the mechanism by which ferritin enters the cells in the gut is not understood at this time, and is a subject for future exploration. Variations in seed development and composition also appear to influence the distribution of iron within the seed between phytate and ferritin.

A number of variables are known to influence the iron availability in diets with added ferritin that was intrinsically labeled (see discussions in References 10, 92). In the case of pure animal ferritin fed to animals, inflammation, often used to induce increased ferritin accumulation (52), can produce a type of ferritin with the iron "locked in" (65). In the case of soybeans, the distribution of labeled iron in the seed is influenced by the time during plant development when the label is added (20, 99), and whether or not the plant has formed iron-rich nodules that donate iron to the seed (20).

The amount of seed phytate in soybeans does not appear to alter the availability of *endogenous* soy iron Fe in ferritin (68, 99). However, the amount of seed phytate can influence the availability of extrinsic iron added to foods from such seeds (see the discussion of extrinsic labeling above and Reference 56). Moreover, developmental changes in the seed can affect the form of the intrinsically labeled iron in the seed (99). A possible reason for the developmental effect on intrinsic labeling is the fact that iron continues to accumulate in soybeans (20) as the seed ripens. Adding labeled iron in a single dose early in plant development and using nodulating plants appears to produce soybeans with the highest percent of the labeled iron in ferritin (70% to 90%) (2, 20, 68).

FERRITIN IN NATURE

Ferritin is a very large, unique, and conserved protein encoded in genomes from Archea to humans and expressed in most cell types. Apparently, the ferritin structural motif is nature's only solution to the problem of concentrating iron. Deletion

of a ferritin gene is embryonic lethal in mice (28). The conserved features of the ferritin protein reside in quaternary and secondary structure: a spherical protein cage around the solid iron mineral "core" (or around solvent in empty ferritin protein), assembled from 12 or 24 polypeptides, folded into four helix bundles. Primary sequence and tertiary structure vary, depending on the main function of ferritin (concentrating iron or detoxifying hydrogen peroxide or oxygen). In contrast to most metalloproteins, where the metal is bound to a specific site on the protein, in ferritin, iron is a substrate for the catalytic oxidase sites, binding two iron atoms together to form the mineral (see References 23 and 90 for reviews), in a transient state involving only a few (<50) iron atoms. Most of the iron in ferritin is Fe^{3+} (usually thousands of iron atoms) and is not bound to the ferritin protein itself, but is in the solid mineral bound through oxygen atoms to other Fe^{3+} atoms.

Animals

Ferritin is mainly a cytoplasmic protein in animals. In some cells, the large amounts of iron used by ferrochelatase to make heme appear to be provided by a mitochondrial ferritin (54) encoded in the nuclear genome and transported into the mitochondria. Ferritin gene expression is tightly coupled to iron status in cells and to cell differentiation. Transcription regulation controls both the total amount of ferritin mRNA and the relative amount of the two types of ferritin mRNA (H and L), which are varied at the time of cell differentiation (95).

Translational regulation is a second major level of control for ferritin expression that is superimposed on the transcriptional regulation. The effect of iron status, for example, is so dramatic that translational regulation was discovered long before the genes were cloned, and made ferritin mRNA an early model for translational mRNA regulation in higher animals. In the noncoding region of ferritin mRNA, near the cap structure, is a stem-loop structure folded into such a specific 3D shape that only two proteins bind, IRP1 and IRP2, to repress ribosome binding. The IRPs, coincidentally, are aconitase homologues and the amounts can vary depending on the cell type, iron status, oxygen status, etc. Similarly, IRE structures, but with specific mRNA features and IRP2 binding properties, occur in a number of other mRNAs involved in iron homeostasis to create a combinatorial array of interactions (93) that coordinately regulate synthesis of the proteins encoded in IRE-mRNAs (21, 27, 40, 77, 80, 89, 93). The mRNA-specific differences in IRE structure and IRP2 binding lead to a range or hierarchy of responses to iron or to anoxia (93).

Cellular mechanisms for retrieving the iron concentrated and stored in ferritin is currently the subject of active discussion and investigation. Two current hypotheses, which may function in parallel or selectively in different cell types are: (*a*) protein degradation after transport to lysosomes (34, 71), which requires identification of mineral-dissolving mechanisms and iron carriers; and/or (*b*) protein unfolding at the evolutionary conserved ferritin pores, which are exquisitely sensitive to changes in solvent, temperature, and amino acid substitution, to increase

access of reductants and chelators to the mineral (43, 57, 85), which requires identification of the physiological protein unfolders/chelators. Both hypotheses could occur in parallel, in the same cell, if ferritin in lysosomes is damaged protein with the iron mineral turning over, and ferritin in the cytoplasm is undamaged protein with gated pores that control access of reductant and intracellular transporters to the iron mineral.

Plants

Iron in plants needs to be concentrated in cells for the same functions as in animals. Ferritin is found in all plant cells at some time during cell development (18), usually in plastids (chloroplasts in leaves, amyloplasts in tubers and seeds, etc.) (81, 94). Encoded in the nuclear genome, as in animals, ferritin in plants is targeted to plastids by an extension peptide, N-terminal to the sequences common to animal and plant ferritins (72). The ferritin iron-phosphate mineral characteristic of plants appears to form in plastids after protein transport to the plastid (97). How the iron in ferritin is retrieved from plastid ferritin for heme synthesis, and synthesized in cell compartments other than the plastid, is not known. However, in contrast to animals, where the main use of iron is in heme, photosynthesis in green plants requires large amounts of nonheme iron in a type of reverse Krebs cycle (33). The need for plastid iron appears to be dominant over cytoplasmic need in plants and has influenced regulation and gene structure profoundly, when compared to animal ferritin genes and regulation (18, 49, 53, 70, 72, 98).

Plant ferritin mRNA is regulated by iron during transcription. There is no IRE structure in plant ferritin mRNA. In fact, when the animal ferritin mRNA IRE is linked to plant ferritin mRNA in a chimera, IRE function, even in animal cell extracts, is inhibited (49). The iron responsive element that regulates transcription of ferritin mRNA in soybeans, for example, is a bipartite promoter that binds a protein *trans* receptor factor to repress transcription when iron is at low levels in the cell (98), and has no sequence homology to other promoters known at this time. In plant ferritin genes, the intron number is twice that in animals and appears to be unrelated to the structure of the exon-encoded proteins (70), suggesting that in plants regulation of ferritin transcription, perhaps coordinated to plastid development, dominated the evolution of gene structure.

In soybeans, and possibly in other legumes, the majority of the iron is in ferritin (3, 20). The high ferritin and iron content of legumes depends, at least in part, on the large amounts of iron used for nitrogen fixation by the nodules (12, 45, 50, 86, 87). Accumulation of nodule ferritin is developmentally regulated (69, 73). Iron is recovered by nodule ferritin during nodule senescence and the iron is recycled to the seed (20) by a mechanism yet to be identified.

Microorganisms

Most microorganisms, archea, algae, protozoa, bacteria, and fungi have ferritin. *Saccharomyces cerevesiae*, which has lost many genes (7), has no recognizable

ferritin gene in available genome sequences. In such an exceptional circumstance, an organism could concentrate and store iron in acidic vacuoles.

The function of ferritin in microorganisms appears to be more variable than in higher plants and animals. For example, *Escherichia coli* has three different ferritin genes (Bfr, FtnA, and *dps*) (1, 36, 41). FtnA appears to be important in trapping iron from protein degradation in cells of stationary cultures (1), whereas FtnA and Bfr together protect cells from oxygen toxicity (96). Bfr, a heme containing ferritin, also appears to modulate catalase (katA) and peroxide resistance (60). The ferritin active sites in bacterial ferritins vary from those in ferritins of higher plants and animals. Differences include a variety of oxy substrates (oxygen, hydrogen peroxide) and several different oxygen products (hydrogen peroxide/water). Anaerobes have ferritins as well (76). Sorting out the various reaction pathways of oxygen in ferritin reactions is an active area of research (e.g., 42, 55, 103, 104).

DNA in bacteria is protected from hydrogen peroxide damage by *dps*(DNA protection in starvation) proteins, which are mini-ferritins with the characteristic quaternary and secondary structure constructed with 12 rather than 24 polypeptide subunits (6, 15, 36, 41). Based on crystal structures, the active site (ferroxidase site) in *dps*, which oxidizes iron and reduces an oxy substrate, is in a different part of the molecule than in the ferritin of plants and animals (41), and may relate to whether hydrogen peroxide is a substrate or a product (15, 42, 104). *Dps* represents the use of ferritin by the pathogen to defeat the release of toxic oxygen species produced by the host, while the host uses ferritin to decrease pathogen access to iron. The amino acid sequence of *dps* proteins is very different from the other ferritins, but the subunit folding and protein cage around a solvent or mineral-filled core are conserved.

DIETARY FERRITIN

Iron in food ferritin is a "slow-release" form, a solid mineral inside the hollow protein. In foods, the ferritin iron is chemically and physically different from the inorganic or organic iron salts or complexes often used as iron supplements, and different from the natural chelated forms of iron such as Fe(III)-phytate. In ferritin then, the protein cage protects the mineralized iron from complexing food agents such as phytates, oxalates, and tannins. Ferritin protein, which is resistant to proteolysis (25) and further protected in seeds by concentration in the hull, is likely to survive digestion in the stomach. Because the bioavailability of ferritin iron and $FeSO_4$ are similar (11, 67), and the DMT1 mechanism for iron uptake from $FeSO_4$ is restricted to single, divalent cations (reviewed in Reference 66), novel mechanisms, yet to be identified, are indicated for ferritin interactions with gut cells.

Currently, the only human genes known to participate in gut iron absorption are DMT1 and Dcytb, which transport Fe^{2+} ions into the enterocyte from the gut, or reduce iron in the gut lumen to Fe^{2+}, respectively (63, 64). In addition, the

lactoferrin receptor will contribute to iron absorption at certain stages of life (58, 83). The small number of genes identified for iron absorption in humans contrasts with the more than 20 genes known to participate in iron absorption in bacteria (5, 6, 16, 17), and as they are specific for different iron forms such as heme, Fe^{2+}, Fe(III)-citrate, FE(III)-enterobactin, transferrin, etc., it is entirely possible that a gut enterocyte receptor for ferritin occurs in humans.

Absorption Studies in Rats

The use of labeled iron in ferritin or soybean absorption studies in the past has led to varying results (e.g., 52, 59, 79). However, recent studies emphasize that nature uses ferritin at critical periods of human, animal, plant, and microbial development (5, 6, 18, 88, 94), which suggests it might be a good dietary source. To reexamine the availability of ferritin and soy iron, diets with unlabelled, endogenous iron in different chemical forms were given to iron-deficient rats. The response of the erythron (hematocrit) was determined for iron-deficient rats given iron-replete diets where the amount of iron in each diet was matched, but the chemical form of the iron was varied: $Fe^{2+}SO_4^{-2}$ or horse spleen ferritin or soybean seeds (~80% iron in ferritin). Iron-deficient rats were divided into three groups and each group received one of the diets for 14 days. In all three groups the hematocrits had returned to normal, indicating that in iron deficiency, dietary $Fe^{2+}SO_4^{-2}$, pure ferritin, and soybean (ferritin) iron were equally able to provide iron to the erythron (10, 92).

The availability of iron in rice with increased ferritin, introduced as the soybean ferritin transgene targeted to the seed (35), has also been demonstrated in iron-deficient rats. The erythron of iron-deficient rats used the iron in rice transgenic for soybean ferritin and soybeans with comparable efficiency (10, 67), which indicates the potential to solving problems of iron nutrition by modifying the ferritin and iron composition of seeds through engineering or breeding.

ABSORPTION STUDIES IN HUMANS Iron absorption studies using unlabeled ferritin aren't feasible in humans, as they were in rats (9), because of the large amount of ferritin required. In a recent study, intrinsically labeled soybeans were used (68). The combination of three features distinguished the study from earlier studies (Table 1) in which conflicting results were obtained about the availability in humans of iron from soybeans (59, 79): (*a*) subjects were borderline iron deficient to increase overall iron uptake and increase the sensitivity of the measurements; (*b*) dietary uniformity was maximized through the use of a clinical nutrition research center; and (*c*) developmental studies on iron incorporation and distribution during growth of the soybean plant (20) were used as a guide for intrinsic labeling of the iron in soybeans.

Soybean iron, measured as red blood cell ^{55}Fe (68), was efficiently absorbed by the American women (Table 1). In the women who had blood values for hemoglobin 131 g/L, hematocrits 40.0%, and serum ferritin mean 7.4 μg/L, absorption was 25.9% (68). The results confirm those obtained with a group of Indian

and African women with low hemoglobin (10.8 g/100 ml), analyzed 30 years ago, where absorption was 19.8% of the ^{55}Fe in intrinsically labeled soy in biscuits and 21.2% of ^{59}Fe, added as ferric ammonium citrate (a nonstoichiometric complex of Fe^{3+}, NH_4, and citric acid) in the cooking water (79). Differences with subsequent experiments such as those in American men (59) may relate to iron status, gender, and the form of the extrinsic label [$Fe^{3+}(Cl^{-})_3$], for which the solution chemistry will lead to some trapping either by phytate or as "rust."

Direct studies on ferritin iron availability in humans, using purified ferritin from which iron was removed and replaced in vitro by ^{59}Fe, under conditions known to give normal iron mineral (75), again showed availabilities of 21% to 27%, indistinguishable from $Fe^{2+}SO_4^{-2}$, for both red blood cell incorporation and whole body retention. Ferritin was given in apple juice as part of a normal breakfast (P. Davila-Hicks, S.L. Kelleher, E.C. Theil, & B. Lonnerdal, submitted). In foods such as legumes the majority of iron is in ferritin, with a significant amount in the hulls (2) that will provide additional protection to digestion. Combined with the stability of the isolated protein to denaturation and proteolytic digestion (25, 51), soybean ferritin is likely to survive digestion intact and to arrive at the intestinal villi as the protein-coated mineral. The known mechanisms for iron uptake during digestion have lagged behind those for iron by cells inside the body. Even for the more intensely studied case of cells in the body, iron, a number of new mechanisms of iron uptake have been revealed in the last few years, such as a second transferrin receptor and the use of the enterocyte DMT1 protein in cells such as the liver. Endogenous ferritin is a natural alternative dietary iron source for humans that has been underutilized in supplementation studies and plans. Questions for the future include: How much ferritin is in nonsoy food legumes? How available is iron in the ferritin of nonsoy, food legumes? Can ferritin-rich foods be consumed in quantities sufficient to minimize dietary iron deficiency? Are there enough variations in the types of ferritin-rich foods with high acceptability to have a major impact on global iron deficiency?

PERSPECTIVE

Treatment of iron deficiency anemia with iron supplements has been known for centuries, yet the problem of iron deficits and anemia still afflicts 30% of the world's population. Acceptance of known supplements is a significant issue. Problems of eradicating nutritional iron deficits include (*a*) acceptance of known supplements; (*b*) confusion when similar experimental designs to test potential iron supplements have apparently conflicting outcomes; and (*c*) knowledge of the complexity of molecular mechanisms of iron uptake in humans lags behind that in other organisms, although the rate of change is promising.

To solve the problem of dietary iron deficits more knowledge is required of iron absorption mechanisms, the contributions of genetic ethnicities, and the contributions of gender to iron absorption. In addition, expansion and use of knowledge

about the chemistry and biochemistry of different nonheme iron forms in foods (chemistry of different iron complexes, natural variability of ratios of iron, complexing agents in plant foods, and alterations during digestion and absorption) are crucial. Alternative iron supplements and diets will need to be developed to solve the problem of dietary iron deficiency. A novel, alternative dietary iron source, with an enormous potential contribution to the eradication of global iron deficiency in the twenty-first century is ferritin.

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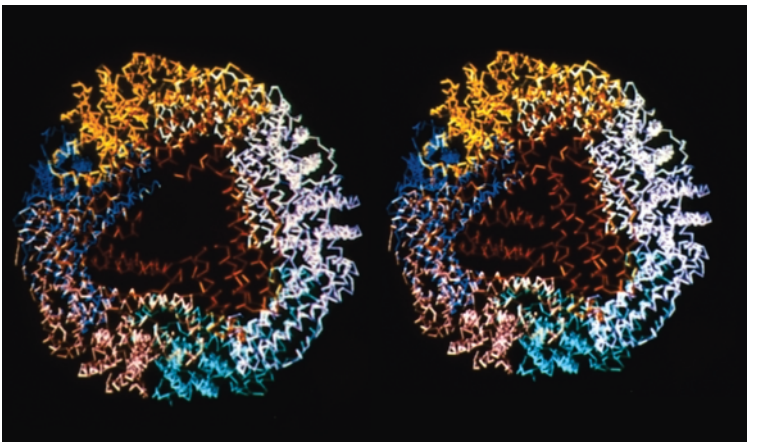

Figure 1 Ferritin pores opened/unfolded or closed/folded. Backbone structure (24 subunits) of a ferritin protein, looking down an axis at the junction of three subunits that form the gated pore leading from the cavity where the iron mineral forms. There are eight such pores per ferritin molecule. Photo reproduced from Reference 57.

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CONTENTS