Prebiotics and Iron Bioavailability—Is There a Connection?

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ABSTRACT: Poor bioavailability of dietary iron, especially from diets rich in cereals and legumes, is a major factor contributing to the high prevalence of nutritional iron deficiency in developing countries. Dietary modification to increase intake of components that promote iron absorption from low-bioavailability meals is an effective strategy for combating nutritional iron deficiency. Prebiotics are nondigestible oligosaccharides that selectively stimulate the growth and activity of specific species of bacteria in the colon with benefits to human health. Common prebiotics such as inulin and fructooligosaccharides occur naturally in a wide variety of plant-based foods and have recently been suggested to have an enhancing effect on iron absorption. The hypothesis that prebiotics enhance iron absorption is biologically plausible because fermentation of prebiotics by natural microflora present in the colon may decrease the pH of the luminal content, promote reduction of Fe(III) to Fe(II), stimulate proliferation of epithelial cells to expand the absorptive surface area, and potentially stimulate expression of mineral-transport proteins in epithelial cells. However, data available in the literature characterizing the enhancing properties of prebiotics on iron absorption are inconsistent, and mechanisms of actions involved are poorly understood. The notion that the colon can function as a significant site of iron absorption in response to stimulation by prebiotics, and the effect of long-term exposure to prebiotics on the iron status of iron-deficient subjects remain to be clarified. This review discusses the functional properties of prebiotics as a promising dietary factor that enhances iron absorption.

Keywords: prebiotics, iron, colon, oligosaccharides, inulin

Introduction

Poor bioavailability of dietary iron is a major factor contributing to the widespread problems of nutritional iron deficiency (ID) and iron deficiency anemia (IDA) in the world today. It has been estimated that about 1/3 of the global population is iron-deficient (Gillespie 1998). Even in a developed country such as the United States, the prevalence of iron deficiency was 9% and 11% for growing children and women of childbearing age, respectively (Looker and others 1997). Also, vegetarianism is growing in popularity, and vegetarian diets may negatively affect nonheme iron absorption (Hunt and Roughhead 1999) and could further compromise the iron status of individuals at risk in developed countries (Obed and others 2002). One effective, but often neglected, strategy for alleviating iron deficiency is dietary modification to increase intake of dietary components that promote iron absorption from low-bioavailability meals (Heath and others 2001). Iron absorption is heavily influenced by meal composition (Hulten and others 1995). Meat and ascorbic acid enhance, whereas phytate, phenolic compounds, calcium, caseins, and soy proteins inhibit iron absorption (Fair-weather-Tait 1992; Hallberg 2001). In addition, some naturally occurring nondigestible oligosaccharides known as prebiotics, and their products of fermentation by intestinal microflora, have been suggested to have an enhancing effect on iron absorption (Delzenne and others 1995; Ohta and others 1995, 1998b). Although it is widely accepted that prebiotics help balance intestinal microflora, their enhancing effects on iron absorption and the mechanisms involved are poorly understood. A greater knowledge of these effects and mechanisms would be useful for designing diets with enhanced iron bioavailability, as well as for developing food products that appeal to health-conscious consumers.

Definitions and Functions of Prebiotics, Probiotics, and Synbiotics

Prebiotics

Prebiotics are defined as nondigestible food ingredients that selectively stimulate the growth and activity of specific species of bacteria in the gut, usually Bifidobacteria and Lactobacillus, with benefits to health (Cummins and others 2001). Common prebiotics include nondigestible oligosaccharides (NDOs) such as inulin and its partial hydrolysate fructooligosaccharides (Figure 1), which are short-chain carbohydrate polymers found naturally in more than 36000 plants including Jerusalem artichoke, asparagus, garlic, leeks, onions, and chicory roots (Gorski 1997; Tungland and Meyer 2002). Mean daily intakes of inulin and fructooligosaccharides in diets of Americans were estimated to be more than 5 g, with approximately 70% of this amount contributed by wheat products (Moshfegh and others 1999). Other identified prebiotics include galactooligosaccharides and lactulose (Van Loo 2004).

Also known as “colonic foods,” prebiotics resist digestion by gastric acid and pancreatic enzymes in vivo, but are preferentially fermented by beneficial intestinal bacteria once they reach the colon (Andersson and others 1999). Supplementation of diets with inulin increased surface counts of Bifidobacteria and Lactobacilli in biopsy samples taken from the cecum, transverse and descending colon, and rectum of human subjects during colonoscopy (Langlands and others 2004). This proliferation of beneficial bacteria in the colon has been associated with numerous health benefits. Some reported benefits include strengthening the intestinal flora’s ability to defend against invasion by potentially pathogenic bacteria, reduction of...
toxic metabolites and detrimental enzymes secreted by other bacteria, and prevention of constipation (Spiegel and others 1994).

It should be noted that both inulin and fructooligosaccharides are “generally-regarded-as-safe” and their acceptable intake levels depend on the sensitivity of the consumer, ranging from <10 g/d to >30 g/d, as reported by Coussement (1999).

Probiotics

The definition of probiotics revised by Schrezenmeir and de Vrese (2001) states that probiotics are “a preparation of, or a product containing, viable, defined microorganisms in sufficient numbers, which alter the microflora (by implantation or colonization) in a compartment of the host and by that exert beneficial health effects in this host.” The presumed beneficial effects of probiotics are achieved through modulation of gut flora populations or activities, provided that the ingested probiotic bacteria survive passage through the stomach and small intestine in sufficient numbers to be metabolically active (Bezkorovainy 2001; Dunne and others 2001).

Synbiotics

The term “synbiotics” is used sometimes to describe products that contain both probiotics and prebiotics (Schrezenmeir and de Vrese 2001). Combinations of probiotics and prebiotics may exhibit synergistic effects because prebiotics not only promote the growth of existing strains of beneficial bacteria in the colon, but also act to improve the survival, implantation, and growth of probiotic strains ingested with the prebiotics (Niness 1999; Schrezenmeir and de Vrese 2001). The concept of synbiotics is popular among dairy manufacturers in Europe based on the premise that some dairy products, such as yogurts, are good vehicles for both viable probiotic bacteria and prebiotics (Niness 1999).

Biological Plausibility of an Iron Absorption-Enhancing Effect of Prebiotics

Although there is some evidence supporting the hypothesis that prebiotics enhance iron absorption, data from the limited number of human studies published on the topic are conflicting. It is important, therefore, to consider the biological plausibility of this putative enhancing effect. This section discusses the plausibility of the hypothesis.

Iron solubility in the gastrointestinal tract

Presumably, iron must be in a soluble form in the lumen of the gastrointestinal tract before it can be taken up by the enterocytes. Iron solubility in aqueous systems is influenced by many factors including pH, redox potential, concentrations and chemical forms of ligands that may form complexes or chelates with the metal iron, and concentrations of other cations (Miller and Berner 1989).

Both Fe(III) and Fe(II) are hexacoordinate and are capable of forming soluble complexes with 6 water molecules in aqueous solution. However, the acidity of water molecules increases when they are complexed with iron, and dissociation occurs at pH values as low as 2. This process is called hydrolysis, and Fe(III) has a much stronger tendency to hydrolyze in aqueous environments than Fe(II). Hydrolysis leads to polymeric iron hydroxides that eventually precipitate (Sylva 1972):

$$\text{Fe(H}_2\text{O)}_{6}^{3+} \rightarrow \text{Fe(OH)}_{3}^{2+} + \text{H}^+$$

or, more generally:

$$x\text{Fe}^{3+} + y\text{H}_2\text{O} \rightarrow [\text{Fe}_y(\text{OH})_{3x-y}]^{3x-y} + y\text{H}^+$$

The solubilities of Fe(II) and Fe(III) were estimated to be $10^{-1}$ mol/L and $10^{-18}$ mol/L, respectively, at pH 7 (Spiro and Saltman 1974). It is important to note, however, that these hydrolysis reactions occur slowly, and it may take days for precipitates to form, depending on pH, the presence of ligands that may complex with iron thereby displacing complexed water molecules, temperature, and other factors (Spiro and Saltman 1974). Given the complexity of the aqueous environment in the lumen of the colon, it is extremely difficult to predict how iron solubility will be influenced by changes in pH and redox potential as a result of microbial fermentation of prebiotics. However, the fact that Fe(II) is more soluble than Fe(III) by a factor of $10^{17}$ suggests that any increase in reducing equivalents in the colon could enhance iron absorption, unless the ferrous iron is tightly bound and thus not available for absorption.

There remains a concern that an increase in soluble iron content could increase free radical production in the colon to a level that could cause mucosal cell damage (Lund and others 1999). Lee and others (2004) hypothesized that iron supplements could increase the risk of colon cancer in populations with high intakes of dietary fiber and resistant starch because fermentation of these substrates would increase concentrations of soluble ferrous iron in the colon. However, studies conducted in iron-overloaded mice showed that ingestion of probiotic strains of *Bifidobacteria* and lactic acid bacteria ($2 \times 10^{8}$ colony-forming units [CFU] per animal per day) exerts an antioxidant effect on the colonic mucosa of iron-overloaded mice without reducing the soluble iron concentration of the cecal contents (Ito and others 2001, 2003). These results suggest that probiotics (or proliferation of beneficial intestinal microflora in the colon due to consumption of prebiotics) protect the colonic mucosa from oxidative damage, and do so in a way that does not significantly decrease the amount of available iron in the colon.

Iron absorption in the colon

As mentioned previously, the beneficial effects of prebiotics are mediated primarily through fermentation in the colon by intestinal microflora. It follows that the colon must have the potential for sig-

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**Figure 1—Structure of (a) inulin and (b) fructooligosaccharides, where n represents the number of repeating units in each molecule**

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Significant iron absorption if prebiotics are to show any enhancing effect. Conventional dogma is that iron is absorbed predominantly in the duodenum and that insignificant amounts are absorbed in the colon. This concept is based, in part, on the fact that the lower pH in the duodenum, resulting from the entry of acid chyme from the stomach, promotes iron solubility and thereby enhances absorption (Cook and others 1964). The pH environment in the duodenum may range from as low as 2 at the proximal section to 6 at the distal section (Conrad and others 1987). Tyssandier and others (2003) reported that the pH of duodenal fluid obtained from the junction of the 2nd and 3rd portions of the duodenum in 10 healthy male volunteers was about 5 in the fasting state, increased to about 6.1 to 6.6 after intake of vegetable meals, and remained constant during digestion. It is thus reasonable to expect that iron absorption beyond the proximal small intestine would be diminished due to increases in the pH of intestinal contents as they move along the gastrointestinal tract (Schumann and others 1990). The absorption rate in the duodenum in rats was reported to be 1.7, 1.3, and 1.9 times higher than in the jejunum, ileum, and cecum, respectively (Bougle and others 2002).

Very few direct measurements of iron absorption in the colon have been reported, but the studies that are available show substantial absorption. In 1 study, radiolabeled ferric and ferrous chloride solutions were infused directly into the colons of human subjects through a sigmoidoscope (Ohkawara and others 1963). Mean absorption of the infused iron was 9.3% in the case of ferrous chloride but only 0.5% for ferric chloride. The subjects were not anemic, but no further information on iron status was given. In another study, radiolabeled iron solutions were infused directly into the duodenum or cecum of dogs, and iron absorption was estimated by whole-body counting 2 wk after dosing (Chernelch and others 1970). When the iron was administered as a pH 2 ferrous sulfate solution, iron-replete dogs absorbed 11.3% of the duodenal dose and 2.5% of the cecal dose. The dogs were then rendered iron-deficient by phlebotomy and retested. The iron-deficient dogs absorbed 30.8% and 15.7% of the administered iron from the duodenum and cecum, respectively. Phlebotomy caused a 3-fold increase in iron absorption from the dose delivered to the duodenum and a 6-fold increase when directly infused into the cecum, suggesting that the colon could be a significant site for iron absorption in iron deficiency, but not in iron adequacy. Iron absorption from the colon is admittedly less efficient than from the duodenum, but nevertheless significant, especially when the subjects are iron-deficient. Studies of gastrectomized rats showed that dietary fructooligosaccharides prevented anemia, but this effect was diminished by cecectomy (Sakai and others 2000), suggesting that the enhancing effect of fructooligosaccharides on iron absorption takes place to some extent in the proximal colon.

**Plausible mechanisms**

**Role of pH in luminal contents.** Products of the fermentation of prebiotics include short-chain fatty acids such as acetic, propionic, and butyric acids (Wang and Gibson 1993). It has been hypothesized that these acids enhance mineral absorption by decreasing the pH of colon contents (Coudray and others 1997). This decrease in pH will presumably promote the release of iron bound to proteins, thereby increasing its bioavailability (Cook and others 1964). Ohta and others (1995) reported that feeding iron-deficient rats diets supplemented with fructooligosaccharides increased apparent iron absorption (determined by fecal balance), hemoglobin concentration, and hematocrit, compared with a control group fed the diet without the supplemental fructooligosaccharides. Feeding fructooligosaccharides decreased the pH of the cecal contents while increasing the organic acid pool (Afsana and others 2003), suggesting that 1 mechanism for the enhanced iron absorption was the reduction of pH in the cecum and colon environment. Shiga and others (2003) showed that gastrectomy-induced anemia in rats could be prevented by adding water-soluble soybean fiber to a basal diet and that the addition of the readily fermentable fiber significantly decreased the pH of cecal contents in rats. In addition to the effect of lower pH, propionate may form soluble complex with iron, thereby maintaining the solubility of iron in the lumen of the colon, as well as facilitating the transfer across endosome membranes of the enterocytes (Bougle and others 2002).

**Reduction of Fe(III) to Fe(II).** A 2nd plausible mechanism for an enhancing effect of prebiotics is the creation of an environment in the colon that promotes the reduction of Fe(III) to Fe(II). To our knowledge, the speciation of iron in the colon relative to its valence has never been examined quantitatively. However, Ohkawara and others (1963) were able to detect ferrous iron, using o-phenanthroline, in an aqueous extract of rabbit cecal contents. They also observed a positive reaction with o-phenanthroline in human feces in 8 of 10 cases. There is extensive literature on the effects of “natural organic matter” from various sources in the environment on ferric iron reduction in both abiotic systems and in the presence of bacteria. For example, soil humic acid reduced about 10% of the iron in a ferric chloride solution at pH 3 but less than 3% at pH values above 5 (Chen and others 2003). In the presence of *Shewanella putrefaciens*, however, soil humic acid converted nearly 100% of Fe(III) to Fe(II) in 1 h under anaerobic conditions at pH 6.8. It is also believed that microbial fermentation of organic matter in soil microclimates near plant roots produces reducing equivalents and acids. This increases iron solubility by lowering the pH and reducing Fe(III) to Fe(II), thereby increasing the bioavailability of soil iron (Lindsay 1991). Thus it is plausible that in the anaerobic environment of the colon, unabsorbed organic matter, such as prebiotics, and intestinal microflora could combine to convert Fe(III) to Fe(II). Presumably, the superior solubility of Fe(II) in the near neutral pH environment renders it more bioavailable than Fe(III).

There is, however, an important caveat to the hypothesis that reducing Fe(III) to Fe(II) will increase iron absorption in the colon. The microflora in the large intestine generate hydrogen sulfide from sulfate and sulfur amino acids (Magee and others 2000). Hydrogen sulfide may react with ferrous iron to form insoluble ferrous sulfide (Rickard 1995):

\[
Fe^{2+} + H_2S \rightarrow FeS + 2H^+
\]

The amount of H$_2$S generated in the colon lumen is unknown, and it appears that more than 95% of the sulfide released is quickly absorbed by the mucosa (Levitt and others 2002). Therefore, levels of H$_2$S may not be sufficient to cause appreciable iron precipitation. Also, other metal cations present in the colon contents may release iron from FeS (Simpson 2001):

\[
Me^{2+} + FeS \rightarrow MeS + Fe^{2+}
\]

where Me could be Zn, Cu, Pb, Ni, or Cd.

**Proliferation of epithelial cells.** Petkevicius and others (2003) showed that feeding pigs a diet containing nondigestible but fermentable carbohydrates (inulin and sugar beet fiber) resulted in increases in the tissue weights of cecum and colon by 28% and 42%, respectively, compared with pigs fed a diet containing oat hull meal instead. The authors attributed these increases to the production of short-chain fatty acids by microbial fermentation of the nondigestible carbohydrates, as short-chain fatty acids have been shown to stimulate epithelial cell proliferation in the colon of rats (Frankel and others 1994). Interestingly, Brunsgaard (1997, 1998) observed...
lower proliferative activity (decreases in mitotic count in the crypts) in mucosal cells in the distal compared with the proximal colon of pigs fed barley diets. Presumably, fermentable carbohydrates are depleted by rapid fermentation in the cecum and proximal colon, leaving less substrate for fermentation in the distal colon. A similar decline in proliferative activity along the large intestine was observed in pigs fed rye-bread diets (Glitso and others 1998). Taken together, these studies support a 3rd plausible mechanism where-by prebiotics may have an enhancing effect on iron absorption in the long term because products of their intestinal microbial fermentation (that is, short-chain fatty acids) stimulate epithelial cell proliferation, thus increasing the absorptive area of the colon, particularly in the proximal section.

**Role of iron transport proteins.** A 4th plausible mechanism could involve effects of prebiotics and/or products of their fermentation on iron transport proteins at the brush border of intestinal epithelial cells or other cellular proteins involved in the trafficking of iron across the mucosal barrier. At the cellular level, dietary fructooligosaccharides increased levels of calbindin-D9k, a cholecalciferol-induced calcium-binding protein associated with intestinal calcium absorption in the cecum and colorectum in rats (Ohta and others 1998a). This suggests that prebiotics may affect the expression of mineral transport proteins at the brush border, but it is unclear whether fermentation is required for this effect.

There is currently no information on the effects of prebiotics on the expression of iron regulatory genes such as DMT-1 (Gunshin and others 1997; Zoller and others 2001), HFE (Zhou and others 1998), Dcytb (McKie and others 2001), and Ireg-1 (Frazer and others 2001), all of which are involved in regulating iron uptake at the intestinal mucosa, particularly for ferrous iron. Therefore, effects of dietary constituents on the expression of any of these genes are essential to an understanding of mechanisms involved in their effects on iron absorption.

Conrad and Umbreit (1993) also described a separate pathway for ferric iron absorption involving mobil ferrin and mucin, but uptake of ferric iron by intestinal epithelial cells appears to be much lower than ferrous iron, and not as tightly regulated as ferrous iron (Gangloff and others 1970; Yeung and others 2005). Mucin expression was not altered by inulin supplementation in humans, as determined in biopsy specimens of ileoanal pouch mucosa of 20 subjects receiving 2 g of inulin daily for 3 wk (Meijer and others 2000). Nevertheless, it is possible that the lack of an effect is due to the low amount of inulin that was administered; a larger amount (for example, at the acceptable intake level of 10 g/d) may be needed to promote mucin expression.

**Data from Human Studies**

Ellegard and others (1997) showed that feeding 16 to 17 g/d of inulin or fructooligosaccharides had no effect on the excretion of calcium, magnesium, zinc, or iron in ileostomy subjects. Their study also confirmed that inulin and fructooligosaccharides are not digested in the stomach or the small intestine, as recoveries in the ileostomy effluent were 88% and 89%, respectively. Using a chemical balance technique, Coudray and others (1997) showed that the addition of 40 g/d of inulin to a control diet did not increase iron absorption in 9 healthy young men, but increased calcium absorption by about 50%. The control diet (the diet without inulin) contained some legumes, meat or fish, starchy foods or vegetable, cheese, fruit, and biscuits and constituted a dietary fiber intake of 18 g/d. Iron absorption from the control diet was 21.8%, much higher than expected for iron-replete men. In another study of 12 healthy young men using a stable-isotope technique, inulin, fructooligosaccharides, and galactooligosaccharides, when administered at 15 g/d, showed no effects on either calcium or iron absorption (Van den Heuvel and others 1998).

The effect of prebiotics on iron absorption, or lack thereof, shown in Van den Heuvel’s study could be due to the vitamin C intake of the subjects (100 mg/meal and 3 meals per d for 19 d). Such a high intake may overwhelm the effect of prebiotics as vitamin C is widely considered an iron absorption enhancer, and the effect of prebiotics may be apparent only when added to a meal low in bioavailable iron, but not to a meal high in bioavailable iron. In both of the aforementioned studies, the subjects were young men of adequate iron status. Therefore, if the control diets used in the studies provided sufficient bioavailable iron to meet the subjects’ requirements, an increase in bioavailability may not produce a detectable increase in absorption. As mentioned previously, iron absorption from the colon was much greater in iron-deficient dogs than in iron-replete dogs (Chernichel and others 1970). Nonetheless, there is currently no information about the effects of prebiotics on iron absorption in iron-deficient or anemic human subjects, whereas prebiotics have been shown to prevent anemia in gastrectomized rats (Ohta and others 1998b). It is possible that the appropriate environment for enhancement of iron uptake is generated only after a suitable period of growth for the microflora in the colon, perhaps with maintenance of optimal conditions such as a high inulin diet.

Recently, a 12-mo efficacy study involving 634 children aged 1 to 3 y in India evaluating the impact of milk fortified with prebiotics and probiotics in prevention of diarrhea and on iron status showed that fortification of milk formulation with *Bifidobacterium lactis* (107 to 109 CFU/100 g) and galactooligosaccharides (2.5 g/100 g) resulted in a 35% reduction in the proportion of iron-deficient children when compared with a control group given the same milk without fortification (Sazawal and others 2004). It was, nevertheless, unclear whether this improvement was due to enhanced iron absorption or due to the reduction of bloody diarrhea as a result of healthier intestinal microflora.

**Summary and Research Needs**

Despite some inconsistencies between human studies and in vitro/animal studies, no human studies so far have shown any adverse effects of prebiotics on iron absorption (Van Loo and others 1999). Apart from the benefits on human health that have been classically associated with their consumption, prebiotics may enhance iron absorption in several plausible ways: (1) organic acids produced by the fermentation of prebiotics in the colon may facilitate iron absorption by lowering luminal pH and improving iron solubility; (2) prebiotics or products of their fermentation may create an environment in the colon that promotes the reduction of Fe(III) to Fe(II); (3) short-chain fatty acids produced by the fermentation of prebiotics in the colon may stimulate the proliferation of epithelial cells, thereby increasing absorptive surface area in the colon; and (4) prebiotics or products of their fermentation may stimulate the expression of iron regulatory genes, thereby increasing iron absorption. Many questions remain unanswered though: How efficient is the colon as a site of iron absorption in response to stimulation by prebiotics? What are the effects of prebiotics on anemic subjects and are these effects dependent on the bioavailability of the iron in the meal? Is the expression of iron regulatory genes affected by prebiotics, in either unfermented or fermented form? Do prebiotics have to reach the colon before they exert an enhancing effect on iron uptake? Further research is warranted because prebiotics occur in a wide variety of plant-based foods, which are the primary components of diets in developing countries where IDA is usually more prevalent. A better understanding of prebiotics’ potential to enhance iron absorption and mechanisms of actions involved could lead to additional strategies for combating nutritional iron deficiency.

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