REVIEW

The Effect of Prebiotics on Human Iron Absorption: A Review

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ABSTRACT

Iron deficiency remains the most common nutritional deficiency. Oral iron supplementation is the recommended first-line treatment and used as a preventive measure as well. Enhancers of iron absorption are highly sought after to improve supplementation outcomes. Evidence from animal and human studies exists that prebiotics can enhance iron absorption. The purpose of this present narrative review of the literature is to summarize the existing evidence on the effects of prebiotics on human iron absorption. Relevant articles were identified from PUBMED, Scopus, and Web of Science from inception to November 2021. Only human trials investigating the effect of prebiotics on iron absorption were included. Eleven articles were identified and included for review. There are promising findings supporting an enhancing effect of certain prebiotics, but inconsistencies between the studies and results exist. The most convincing evidence exists for the prebiotics galacto-oligosaccharides and fructo-oligosaccharides combined with the commonly used iron compound ferrous fumarate, from studies in adult women with low iron stores and in anemic infants. Many factors seem to play a role in the enhancing effect of prebiotics on iron absorption such as type of prebiotic, dose, acute (single-dose) or chronic (long-term) prebiotic consumption, iron compound, iron status, inflammatory status, and age of the population studied. More research investigating the optimal combination of prebiotic, iron compound, and dose as well as the effect of long-term application on iron status outcomes is needed. *Adv Nutr* 2022;13:2296–2304.

Statement of Significance: Whether prebiotics can enhance iron bioavailability has been discussed previously, but several recent human studies using stable iron isotopes to quantify iron absorption have provided the first clear evidence of this effect. This is the first narrative review to summarize the current literature including those recent stable isotope studies on the effects of prebiotics on human iron absorption.

Keywords: iron absorption, prebiotic, iron deficiency, anemia, human trial

Introduction

Iron deficiency (ID) is the most common global nutritional deficiency, thought to affect >2 billion people (1, 2). It is estimated that ID contributes to half of all anemia cases in women and to 42% of anemia cases in children (<5 y) (3). According to the Global Burden of Disease Study 2016 (4), iron deficiency anemia (IDA) is one of the main causes of global disability burden, and the first cause in women. Most at risk are infants, children (<5 y), adolescents, pregnant women, and women of reproductive age (5, 6). Common risk factors are increased iron requirements during phases of rapid growth, decreased iron intake, poor iron absorption, blood loss, and chronic infections and inflammatory disorders (7). Signs and symptoms of ID are anemia, fatigue, and muscle weakness, as well as impairments in cognition, immune function and physical performance (8, 9).

Common strategies to improve iron status on a population level are dietary diversification, food fortification, and oral iron supplementation, as well as prevention and treatment of chronic infections (e.g., malaria, hookworm, and tuberculosis). Oral iron supplementation is used to prevent ID and IDA in some settings and is also the recommended first-line treatment of ID and IDA in most cases (2, 10–13). However, oral iron therapy has its limitations. Absorption of iron from

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Abbreviations used: DMT-1, divalent metal transporter 1; FeFum, ferrous fumarate; FePP, ferric pyrophosphate; FOS, fructo-oligosaccharide; GOS, galacto-oligosaccharide; ID, iron deficiency; IDA, iron deficiency anemia; NaFeEDTA, sodium iron ethylenediaminetetraacetate; SIAC, stable isotope appearance curve.

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oral iron supplements is highly variable, typically between 5% and 50% (14), due to poor solubility of commonly used iron salts and, if supplements are taken with meals, due to food components that chelate iron and prevent absorption (15, 16). Furthermore, adverse gastrointestinal side effects during supplementation, such as abdominal pain, constipation, and diarrhea, are common, resulting in poor compliance (17, 18). Unabsorbed iron in the gut can cause irritation and bleeding, increase free radical production in enterocytes (19), cause gut inflammation (20), and favor growth of enteropathogens over beneficial bacteria (21). Therefore, enhancers of iron absorption from supplements are highly sought after, but only a few are available, including ascorbic acid (22) and EDTA (23). Prebiotics have previously been shown to enhance absorption of minerals such as calcium and magnesium (24). However, new evidence indicates that prebiotics can increase iron absorption as well (25, 26), and this is the focus of this review.

Prebiotics

The original definition of a prebiotic by Gibson and Roberfroid in 1995 stated that "a prebiotic is a nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health" (27, 28). A more recent definition was given by the International Scientific Association for Probiotics and Prebiotics, which states that it is "a substrate that is selectively utilized by host microorganisms conferring a health benefit" (28). Prebiotics can be fibers, but are not limited to fibers, and not all fibers are prebiotics. In general, fibers and prebiotic fibers differ in that a fiber promotes the growth of many microorganisms in the digestive tract, whereas a prebiotic stimulates the growth of certain beneficial microorganisms selectively. An example is prebiotic galacto-oligosaccharides (GOS), which stimulate the growth of lactobacilli and *Bifidobacterium* (29). Common prebiotics include inulin, human milk oligosaccharides, fructo-oligosaccharides (FOS), and GOS (29), of which the last 2 are the most studied (28). With the new definition, other prebiotics now include conjugated linoleic acids, PUFAs, certain phenolics and phytochemicals, mannan-oligosaccharides, xylo-oligosaccharides, lactulose, and polydextrose (28). Purported health benefits of prebiotics include positive effects on the gastrointestinal tract, blood cholesterol, the immune system, and mental and bone health (28, 30).

It is proposed that prebiotics can enhance the colonic absorption of minerals such as calcium and magnesium (24) by increasing production of SCFAs in the proximal colon, decreasing gut luminal pH and increasing their dissolution (31), but the exact mechanisms involved remain uncertain (32). Whether prebiotics can enhance iron absorption has been discussed previously (26, 33, 34), but several recent human studies using stable iron isotopes to quantify iron absorption have provided the first clear evidence of this effect. The aim of this narrative review is to summarize the current literature on the effects of prebiotics on human iron absorption.

Literature Search Strategy

In PUBMED, Scopus, and Web of Science we searched all literature to date in November 2021 using the following search term: prebiotic AND iron, prebiotics AND iron, prebiotic AND anemia, prebiotics AND anemia, prebiotic AND iron deficiency, prebiotics AND iron deficiency, prebiotic AND iron absorption, prebiotics AND iron absorption. The search yielded 31 publications. Once filtered to remove duplicates and only include trials in humans investigating the effect of prebiotics on iron absorption explicitly (excluding reviews and meta-analyses), 6 publications were available for review (35–40). Additionally, reference lists from the available publications and 3 reviews (26, 33, 34) were consulted to ensure all relevant studies were captured, and 4 more studies were included for review (25, 41–43), leading to a total of 10 publications. One human study from our research group, which was published in early 2022, was further included (44). We therefore included a final total of 11 human studies. An overview of the included studies is given in **Table 1**.

Metabolic Mineral Balance Studies

Coudrey et al. (25) gave healthy young men ($n = 9$) a control diet or a diet supplemented with ≤40 g/d inulin or sugar beet fiber for 28 d in a metabolic mineral balance study. Iron was measured in diets and in an 8-d urine and fecal composite to assess mineral absorption and balance. Apparent absorption and balance of iron was not significantly changed by the ingestion of the prebiotic. In another balance study (41), healthy men ($n = 11$) consumed a control diet alone or supplemented with 7.5 g/1000 kcal Na-carboxymethylcellulose, locust bean gum, or karya gum for 4 wk each. During the last 8 d of each feeding block, food as well as urine and fecal composites were collected to determine apparent mineral balance. A mean positive iron balance was found with karaya gum. The addition of the other fibers did not affect apparent balance of iron. A major limitation of these studies is that assessing iron absorption through balance studies is difficult and inexact, and is no longer recommended (45). This might have contributed to the mostly negative findings.

Radioactive Iron Isotope Studies

In a radioactive iron absorption study, Weinborn et al. (42) randomly assigned healthy women ($n = 24$) to consumption of a yogurt with a prebiotic for 12 d (a mixture containing inulin, polydextrose, arabic gum, and guar gum, a total of 2 g per yoghurt) (treatment group) or without the mixture (control group). Consumption of the prebiotic increased heme iron absorption by 56% ($P < 0.007$), but did not significantly affect absorption from ferrous sulfate ($FeSO_4$).

Stable Iron Isotope Studies

In a randomized crossover stable isotope study in nonanemic adult men ($n = 12$), van den Heuvel and colleagues (43)

TABLE 1 Overview of studies included in the review[1]

Author/year	Country/subjects	Baseline iron status (ferritin)	Design/blinding	Iron compound	Prebiotic, dose, frequency, duration, and delivery method	Effects on iron absorption[6]
Coudray et al., 1997 (25)	France $n = 9$ Healthy young men Age: not reported	Not reported	Metabolic mineral balance study, crossover design, blinding not reported	Dietary iron	Inulin or sugar beet fiber, 40 g/d, 28 d, incorporated into bread (60%) and liquid foods (40%)	↔
Behall et al., 1987 (41)	USA $n = 11$ Healthy men Age: 23–62 y	49 ± 14 μg/L[2]	Metabolic mineral balance study, crossover design, blinding not reported	Dietary iron	Na-carboxymethylcellulose, locust bean gum, or karaya gum, 7.5 g fiber per 1000 cal/d, 4 wk each, in baked muffins or fruit gel	↔
Weinborn et al., 2017 (42)	Chile $n = 24$ Healthy women Age: 35–45 y	Prebiotic group: 20.1 ± 8.4 μg/L[2] Control group: 16.9 ± 9.8 μg/L[2]	Radioactive Fe absorption study, subjects randomly assigned in 2 groups: prebiotic and control, blinding not reported	3 mg Fe as heme Fe 3 mg nonheme Fe as $FeSO_4$	Mixture containing inulin, polydextrose, arabic gum, and guar gum, 2 g/d, 12 d, in a yogurt	Heme iron ↑ Nonheme iron ↔
van den Heuvel et al., 1998 (43)	The Netherlands $n = 12$ Healthy men Age: 20–30 y	Not reported	Double stable-isotope Technique (oral and intravenous), randomized crossover, double-blind	Orally: 37 mg Fe as $FeSO_4$ divided in 21 meals, consumed with prebiotics Intravenous: 1 mg Fe as $FeSO_4$	Inulin, FOS, or GOS, 15 g/d, 5 g per meal, 21 d each, with 100 mL orange juice	↔
Petry et al., 2012 (36)	Switzerland $n = 32$ Nonanemic iron-depleted women Age: 18–40 y	12.5 (7.1, 22.8) μg/L[3]	Stable isotope study, randomized crossover, double-blind	4 mg Fe as $FeSO_4$, consumed 1 h after inulin or maltodextrin (control) consumption	Inulin or control (maltodextrin), 3 times per day (20 g/d total), 4 wk, dissolved in water and consumed with main meals	↔
Paganini et al., 2017 (38)	Kenya $n = 50$ Mostly anemic infants Age: 6–14 mo	Prebiotic group: 19.5 (13.0–39.1) μg/L[4] Control group: 15.1 (9.8–25.5) μg/L[4]	Stable isotope study, randomized, single-blind	5 mg Fe as FeFum + NaFeEDTA 5 mg Fe as $FeSO_4$; consumed with prebiotic in prebiotic group	GOS, 7.5 g/d, 3 wk, in a maize porridge fortified with a micronutrient powder	FeFum + NaFeEDTA ↑ $FeSO_4$ ↔
Mikulic et al., 2021 (39)	Kenya $n = 23$ Partly anemic infants Age: 6–14 mo	22.5 (9.7–52.1) μg/L[5]	Stable isotope study, randomized, crossover, single-blind	5 mg Fe as FeFum + NaFeEDTA 5 mg Fe as $FeSO_4$, consumed with and without GOS	GOS, 7.5 g/d, single dose, in a maize porridge fortified with a micronutrient powder	FeFum + NaFeEDTA ↔ $FeSO_4$ ↔

(*Continued*)

TABLE 1 (*Continued*)

Author/year	Country/subjects	Baseline iron status (ferritin)	Design/blinding	Iron compound	Prebiotic, dose, frequency, duration, and delivery method	Effects on iron absorption[6]
Jeroense et al., 2019 (part 1) (35)	Switzerland $n = 34$ Nonanemic, iron-depleted women Age: 18–45 y	16.4 (11.3–30.8 μg/L[4]	Stable isotope study, randomized, crossover, single-blind	14 mg Fe as FeFum	GOS, 15 g, as single dose, given fasted with 200 mL water only or with a bread-based meal	↑
Jeroense et al., 2019 (part 2) (35)	Switzerland $n = 34$ Nonanemic, iron-depleted women Age: 18–45 y	16.4 (11.3–30.8) μg/L[4]	Stable isotope study, randomized, crossover, single-blind	14 mg Fe as FeFum, consumed with or without GOS	GOS, 15 g/d, 4 wk daily consumption, on day of isotope administration given fasted with 200 mL water and a bread-based meal	↔
Jeroense et al., 2019 (part 3) (35)	Switzerland $n = 34$ Nonanemic, iron-depleted women Age: 18–45 y	16.4 (11.3–30.8) μg/L[4]	Stable isotope study, randomized, crossover, single-blind	14 mg Fe as $FeSO_4$, without GOS before and with GOS after the intervention	GOS, 15 g/d, 4 wk daily consumption + single dose, given fasted with 200 mL water in a bread-based meal	↔
Jeroense et al., 2020 (37)	Switzerland $n = 46$ Nonanemic, iron-depleted women Age: 18–45 y	17.1 (11.4–25.3) μg/L[4]	Stable isotope study, randomized, crossover, single-blind	14 mg Fe as FeFum 14 mg Fe as $FeSO_4$ 14 mg Fe as FePP	GOS, 3.5, 7, or 15 g, single dose, given fasted with 200 mL water	3.5 g GOS FeFum ↔ 7 g GOS FeFum ↑ 15 g GOS $FeSO_4$ ↔ 15 g GOS FePP ↔
Giorgetti et al., 2022 (44)	Switzerland $n = 30$ Nonanemic, iron-depleted women Age: 18–45 y	12.9 (10.0–19.9 μg/L[4]	Stable isotope study, randomized, crossover, single blind	100 mg Fe as FeFum	GOS, FOS, and acacia gum, 15 g, single dose, given fasted with 200 mL water	GOS ↑ FOS ↑ Acacia gum ↔
Husmann et al., 2022 (40)	Switzerland $n = 11$ Nonanemic, iron-depleted women Age: 18–45 y	15.2 (12.6–21.2) μg/L[4]	Stable isotope study, randomized, crossover, single blind	14 mg Fe as FeFum	GOS, 15 g, single dose, given fasted with 200 mL water	↔

[1] FeFum, ferrous fumarate; FePP, ferric pyrophosphate; $FeSO_4$, ferrous sulfate; FOS, fructo-oligosaccharides; GOS, galacto-oligosaccharides; NaFeEDTA, sodium iron ethylenediaminetetraacetate.
[2] Mean ± SD.
[3] Geometric mean (95% CI).
[4] Median (IQR).
[5] Geometric means (−SD, +SD).
[6] ↑ = statistically significant increase, ↔ = no statistically significant difference.

did not find a significant effect on iron absorption from $FeSO_4$ from a diet supplemented with 15 g/d (5 g in each meal) inulin, FOS, or GOS for 21 d, compared with a control diet without a prebiotic. In another randomized, double-blind, crossover stable iron isotope study, Petry et al. (36) assessed the effect of 4-wk consumption of 20 g/d inulin (given in 3 doses each day) on iron absorption from $FeSO_4$ in nonanemic iron-depleted adult women ($n = 32$; plasma ferritin <25 μg/L) compared with placebo (maltodextrin). After the first block of consumption, there was a 2-wk washout period before the subjects crossed over to the other block. On the last day of each block, iron absorption was measured. On the measurement days, the prebiotic or placebo doses were consumed 1 h before the test meal, which contained the iron stable isotopes. Inulin decreased fecal pH, increased fecal bifidobacteria and fecal lactate, but had no significant effect on iron absorption, fecal SCFAs, or total bacteria count.

In a stable isotope study in Kenya (38), mostly anemic, 6–14-mo-old infants ($n = 50$) consumed a maize porridge fortified with a micronutrient powder with and without the addition of 7.5 g GOS daily for 3 wk. Iron absorption from 5 mg ferrous fumarate (FeFum) + sodium iron ethylenediaminetetraacetate (NaFeEDTA) in a test meal given with a single dose of GOS in the group receiving the GOS intervention (18.8%; IQR: 8.3–37.5%) was significantly increased by 62% ($P = 0.047$) compared with the group who received the test meal and intervention without GOS (11.6%; IQR: 6.9–19.9%). The intervention did not have a significant effect on iron absorption from $FeSO_4$ in the test meal. In contrast, in a recent randomized prospective crossover stable isotope study in 6–14-mo-old, iron-deficient, partly anemic Kenyan infants ($n = 23$) (39), a single dose of 7.5 g GOS given with a maize porridge meal containing 5 mg iron as FeFum + NaFeEDTA, or as $FeSO_4$, did not significantly affect iron absorption.

In a stable isotope study in young women in Switzerland with depleted iron stores ($n = 34$; plasma ferritin <30 μg/L) (35) at baseline, compared with FeFum alone, a single dose of 15 g GOS significantly increased iron absorption from 14 mg FeFum when given with water only (+61%; $P < 0.001$) and in a bread-based meal (+28%; $P = 0.002$). After 4 wk of daily at-home consumption of 15 g GOS, a single dose of 15 g GOS again significantly increased iron absorption from FeFum in the meal (+29%; $P = 0.044$) compared with FeFum alone. However, iron absorption from $FeSO_4$ given with a single dose of 15 g GOS in a meal after 4 wk of daily GOS consumption was not significantly greater than iron absorption from $FeSO_4$ in a meal without GOS at baseline. Furthermore, in another stable isotope study in iron-depleted young women ($n = 46$; plasma ferritin <30 μg/L) by Jeroense et al. (37), compared with FeFum alone, a single dose of 7 g GOS significantly increased iron absorption from 14 mg FeFum (+26%; $P = 0.039$), whereas 3.5 g GOS did not. GOS did not significantly enhance iron absorption from 14 mg $FeSO_4$ or ferric pyrophosphate (FePP) at a single dose of 15 g compared with the respective iron compound alone.

Giorgetti et al. (44) investigated the effect of coadministration of single oral doses of GOS, FOS, or acacia gum (each 15 g) on iron absorption from a 100-mg oral iron dose as FeFum in Swiss women with depleted iron stores ($n = 30$; plasma ferritin <25 μg/L). GOS and FOS increased iron absorption from the iron supplement by +45% and +51% respectively ($P < 0.001$ for both), whereas acacia gum had no effect.

A recent stable isotope study investigated potential mechanisms of the enhancing effect of GOS on iron absorption (40). In iron-depleted young women in Switzerland ($n = 11$; plasma ferritin 15–30 μg/L), the absorption kinetics of iron from FeFum combined with a single dose of 15 g GOS were determined using the stable isotope appearance curve (SIAC) technique. In a randomized order, the subjects received 2 labeled 14-mg iron doses as FeFum with or without a single dose of GOS (15 g). Several blood samples were collected over the following 24 h to determine serum stable isotope appearance as well as a single venipuncture 14 d later to determine erythrocyte incorporation of the labels. The area under the SIAC and overall fractional iron absorption were not significantly greater ($P = 0.064$; $P = 0.080$), possibly due to the small sample size, when iron was given with GOS. Furthermore, the mean time of peak serum stable isotope concentration was not different in the 2 conditions ($P = 0.096$), and there was no delayed peak of absorption that would suggest iron absorption from the distal gut.

Discussion

Overall, several prebiotics appear to be promising enhancers of iron absorption, but data remain limited and there are inconsistencies between studies. The absorption-enhancing property of prebiotics seems to depend on the type of prebiotic and its dose, the iron compound, duration of intake, population group, and on whether the prebiotic was taken together with the iron compound or was previously administered. Because the number of studies available is limited and they vary widely in the above-mentioned factors, it was not possible to conduct a meaningful meta-analysis to generate more conclusive results. Nevertheless, the individual points are discussed in the following sections.

Prebiotic type

Most of the studies looked at the effect of GOS, FOS, or inulin on iron absorption. The studies with inulin failed to show a significant enhancing effect on iron absorption (25, 36, 43), apart from 1 study that found an enhancing effect on heme iron absorption from a mixture containing inulin, polydextrose, arabic gum, and guar gum at a dose of 2 g/d (42). Nevertheless, a 10% high-performance inulin and oligofructose diet in anemic growing rats ($n = 21$) increased expression of the divalent metal transporter 1 (DMT1) in the cecum and expression of duodenal cytochrome b reductase in the colon (46). In the same study, the expression of ferroportin in the duodenum was decreased by oligofructose, and oligofructose decreased concentrations of IL-10, IL-6, and TNF in the cecum as well as concentrations of

hepcidin in urine. The authors concluded that prebiotics might influence systemic factors that regulate intestinal iron absorption as well as the expression of intestinal proteins involved in iron absorption.

In contrast to inulin, several human studies using GOS have shown an enhancing effect on iron absorption from FeFum (with increases in iron absorption of 28% to 62%) (35, 37, 44), and the 1 study investigating the effect of FOS also showed an enhancing effect on absorption from FeFum (+51%). In the 2 studies that did not find an enhancing effect of GOS on iron absorption from FeFum, this could have been due to a small sample size in the adult study (only 11 participants) (40) or, in the infant study, due to the fact that the GOS was only given as a single dose instead of GOS consumption for several weeks (compare later discussion) (39). Several animal studies also showed a positive effect of GOS or FOS on iron metabolism. The combination of GOS with NaFeEDTA or $FeSO_4$ fed over 12 wk improved iron status in anemic rats compared with a control without GOS (47). Similarly, in rats, GOS (at 0.5% w/w daily consumption of diet, for 15 d) derived from lactulose and kojibiose reduced circulating hepcidin and improved hemoglobin concentrations compared with no prebiotic (48). In another rat study ($n = 24$), a novel impure GOS mixture containing mono- and disaccharides was fed to the animals for 28 d (5% of the diet) and compared with a control group. The prebiotic improved apparent absorption of calcium, magnesium, and iron, but had no impact on zinc absorption (49). In rats, FOS supplementation (0.8 g/100 mL) for 1 wk in milk or soy-based beverages had a beneficial effect on hemoglobin and DMT-1 protein expression in the duodenum, and improved iron absorption (50). Thus, the available animal and human studies suggest that GOS and FOS have a stronger enhancing effect on iron absorption and/or its regulation, than inulin or acacia gum. However, this is only true for FeFum and not for other iron compounds (see Iron source, below).

Iron source

Several studies have shown that GOS and FOS enhance iron absorption from FeFum, but none found an enhancing effect for $FeSO_4$ or FePP. Also, none of the studies providing inulin found a significant effect on iron absorption from $FeSO_4$. However, it is possible that inulin might enhance iron absorption from FeFum, because FOS and inulin share strong structural similarities; however, this has not yet been tested. In addition, 2 metabolic mineral balance studies did not find an effect of prebiotics on absorption of native dietary iron (25, 41).

It is unclear why GOS and FOS enhance iron absorption from FeFum and not from $FeSO_4$, or, for GOS, from FePP. It is possible that when GOS or FOS and the iron compound are given together, the addition of the prebiotics could increase gastric residence time allowing for greater iron dissolution. This would likely have a stronger effect on FeFum than on $FeSO_4$, because FeFum needs a pH ~2 for complete dissolution, whereas $FeSO_4$ is water soluble (51). FePP is poorly soluble regardless of pH, and could benefit less than FeFum from a longer gastric residence time (12). It is also possible that, compared with $FeSO_4$, the more limited solubility of iron from FeFum at proximal gut pH could be increased by the reducing effect of the prebiotics, allowing more dissolved iron to reach DMT-1 on the enterocyte membrane. This hypothesis is supported by in vitro findings showing a ~50% increase of solubility of FeFum at pH 4 and 6 when GOS was added (40).

Thus, the available data suggest that iron source matters because in human studies only FeFum showed effects. However, more studies are needed using other iron sources because many commonly used compounds have to our knowledge not been tested yet.

Effect of single dose compared with long-term administration

Study designs vary as to whether the effect of a single dose of prebiotic on iron absorption was tested compared with longer term prefeeding for several days, weeks, or even months. A study in iron-depleted women found that a single dose of 15 g GOS enhanced iron absorption from FeFum, whereas an additional 4-wk consumption of GOS did not further boost this effect, despite the fact that the 4-wk GOS intervention significantly decreased fecal pH and increased counts of *Bifidobacterium* spp. and *Lactobacillus/Pediococcus/Leuconostoc* spp. The authors concluded that in healthy women without inflammation, GOS given acutely together with the iron supplement enhances absorption and that long-term consumption, and thus a change in the microbiota, does not (35). Two later studies confirmed the acute enhancing effect of a single dose of 7 g and 15 g GOS on iron absorption from FeFum in young women (37, 44).

The study in Kenyan infants (38), which first reported an enhancing effect of 7.5 GOS on iron absorption from FeFum, could not distinguish between the acute effect of a single dose of GOS or a chronic effect of daily consumption of GOS, because the iron stable isotope was administered only with the GOS after 3 wk of prefeeding. A second study in Kenyan infants (39) tried to clarify this, but did not observe an effect of a single dose of 7.5 g GOS on iron absorption from FeFum or $FeSO_4$. The difference between the adult studies, where an acute single dose of GOS enhanced iron absorption (35, 37), and the infant studies, where the effect of GOS on iron absorption was apparent only after long-term prefeeding (38, 39), could be due to several factors. Infants have higher postprandial gastric pH than adults and therefore might absorb iron from FeFum less well compared with adults because FeFum requires a low pH (~2) for dissolution (52). Infants also have a different, less diverse gut microbiota composition, with typically greater abundances of bifidobacteria than adults (53). GOS could have a stronger bifidogenic effect in infants and/or production of SCFAs in response to GOS feeding might be greater in infants. Another difference between the infant and adult studies is that the Kenyan infants were both more iron deficient and anemic than the Swiss women, and it has been suggested

that GOS had a stronger enhancing effect in more iron-deficient subjects (40). Both studies in which inulin was administered to adults (36, 43) only looked at a long-term effect of the prebiotic on iron absorption, so did not test whether coadministration of inulin might acutely enhance iron absorption.

Thus, there is evidence that in adults, a single dose of prebiotic can improve iron absorption, whereas the current available data suggest there is no effect of long-term administration in this population group. In contrast, in infant studies there only seems to be an effect of long-term administration. However, only limited studies have been conducted so far investigating an acute compared with long-term effect and more studies in different populations using different iron compounds and types of prebiotic are needed.

Prebiotic dose

The effect of GOS on iron absorption from FeFum seems to be dose dependent, because 3.5 g GOS was not enough to enhance iron absorption in iron-depleted women, whereas 7 g and 15 g were (35, 37). Christides et al. (54) assessed in vitro iron bioavailability from a commercial infant formula supplemented with the prebiotics FOS and GOS using the Caco-2 cell model. They measured iron bioavailability from 4 commercially available infant formulas with equal amounts of iron but with different amounts of ascorbic acid, FOS, and GOS and used Caco-2 cell ferritin formation as reference for iron bioavailability. They showed that the formulas with the highest amount of ascorbic acid, FOS, and GOS had the highest iron bioavailability whereas those with the lowest amounts had the lowest iron bioavailability. Pérez-Conesa et al. (55) investigated the effect of 30-d administration of probiotic (*Bifidobacterium bifidum* and *Bifidobacterium longum*), prebiotic (GOS at 1.2%, 5%, and 10% of the diet), and synbiotic (bifidobacteria and GOS at 1.2%, 5%, and 10% of the diet) follow-up infant formulas on iron absorption in rats ($n = 54$) measured by iron balance. All formulas increased apparent iron absorption or retention, but only the 10% prebiotic and symbiotic diets did so significantly. These findings support the idea that there is a dose dependent effect of GOS on iron absorption and that there is a minimum dose at which an effect can be observed. This could explain why some of the studies that used a lower dose of prebiotic might not have found an enhancing effect.

Population group

Most of the studies investigated iron-deficient, mostly anemic infants or adult women with low iron stores but without anemia. The few studies in healthy individuals did not find an effect of prebiotics on iron absorption. This could be because very low iron absorption in iron-replete subjects, who are downregulating their iron absorption, obscured potential small differences in absorption. All studies that found an effect of the prebiotic on iron absorption studied either iron-depleted or anemic subjects. Husmann et al. (40) also reported that iron absorption of FeFum significantly increased with decreasing serum ferritin concentrations and that this effect was significantly enhanced by the prebiotic GOS, suggesting iron status plays a role in the enhancing effect. As mentioned before, some animal studies found that prebiotics upregulate the expression of DMT-1. If this occurs in humans, prebiotics might augment the upregulation of DMT-1 that is already present in iron-deficient subjects.

Furthermore, in healthy women without inflammation a single dose of GOS increased iron absorption (35), whereas in African infants with inflammation a single dose of GOS did not significantly increase iron absorption (38, 39). This suggests that the presence of inflammation can reduce the acute effect of prebiotics.

Prebiotic effects on the gut

Three studies assessed the effect of prolonged administration of a prebiotic on gut microbiota composition and metabolism, while assessing the prebiotic effect on iron absorption. Petry et al. (36) observed that 4-wk inulin administration in women had no significant effect on iron absorption, but it decreased fecal pH, increased fecal bifidobacteria and fecal lactate, and had no significant effect on fecal SCFAs and total bacteria. Similarly, Jeroense et al. (35) showed that 4-wk GOS administration in women significantly decreased fecal pH and increased counts of *Bifidobacterium* spp. and *Lactobacillus/Pediococcus/Leuconostoc* spp., but also found no effects of chronic prefeeding on iron absorption. On the other hand, Paganini and Zimmermann (20) found that 3-wk consumption of GOS increased *Bifidobacterium* spp. and maintained higher abundances of *Lactobacillus/Pediococcus/Leuconostoc* spp. in mostly anemic infants. They did observe an enhancement of iron absorption from FeFum, but the study design could not distinguish between a single-dose effect or an effect of long-term GOS administration. These findings suggest that in adults the prebiotic effect on the gut microbiome itself is not responsible for the enhancement in iron absorption, but that long-term consumption, and changes in gut microbiota composition or metabolism, might play a role in infants.

Mode of action

Several mechanisms might explain the enhancing effect of prebiotics on iron absorption (26), including: *1*) by increasing gastric residence time allowing more time for iron to solubilize; *2*) by stimulation of gene expression of proteins involved in iron absorption in the enterocytes; *3*) by stimulation of the proliferation of enterocytes creating a greater surface for iron absorption; *4*) by stimulation of the production of SCFAs by gut commensal bacteria, thereby decreasing distal gut luminal pH and increasing iron dissolution in this part of the gut; *5*) by increasing iron solubility via chelation or via iron reduction; and *6*) by reduction of gut and/or systemic inflammation (56–59).

Only 1 study investigated the mechanism or site of action of the enhancing effect of GOS on iron absorption from FeFum by using SIAC in nonanemic iron-depleted female subjects. Unfortunately, the sample size was likely

too small ($n = 11$) to detect a significant difference in iron absorption (40). As mentioned before, prolongation of gastric residence time has been proposed as a putative mechanism for how GOS enhances iron absorption from FeFum. The shape of the SIAC and time of peak serum stable isotope concentration were not different when GOS was given with FeFum compared with without. This suggests that GOS does not increase gastric residence time and hence this is not responsible for the enhancing effect. Based on the shape of the curves, the authors concluded that their findings were consistent with effects on iron absorption in the proximal rather than distal gastrointestinal tract, and that the effect was not due to higher absorption from the colon as previously hypothesized. They also performed in vitro studies in which they found that iron dialyzability from FeFum was 75% greater and iron solubility at pH 4 and pH 6 were doubled with GOS compared with control. These data thus suggested that GOS maintains iron dissolved from FeFum in the alkaline duodenum, favoring higher intestinal iron absorption in women with relatively low iron stores who are expected to have upregulated DMT-1 expression and can thus absorb more of the available iron. On the other hand, at least in adult women without inflammation, the production of SCFAs and decrease in luminal pH of the distal gut, even though observed, does not seem to play a significant role (35). Contrarily, several animal studies have investigated the effect of prebiotic supplementation on gene expression for intestinal proteins involved in iron absorption, and some have reported significant effects. However, due to the variations in study design and prebiotics used no clear conclusions can be drawn from those studies as yet (46, 50, 60–62).

Conclusion

To conclude, many factors seem to play a role in a possible enhancing effect of prebiotics on iron absorption such as type of prebiotic, dose, acute or chronic prebiotic consumption, iron compound, and iron status and age of the population studied, which makes it difficult to draw a generalizable conclusion. Nevertheless, so far, the prebiotics GOS and FOS seem the most promising enhancers in combination with iron from FeFum at doses >3.5 g of the prebiotic.

Future research should first focus on the mechanism by which prebiotics enhance iron absorption, which will then also help define the optimal combinations and doses. Ultimately, the long-term efficacy of a combined iron-prebiotic supplement should be investigated in populations at risk of iron deficiency and taking into consideration other outcomes besides iron status, such as gut health, inflammatory status, as well as a potential reduction of iron-induced gastrointestinal side effects.

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