Inulin modifies the bifidobacteria population, fecal lactate concentration, and fecal pH but does not influence iron absorption in women with low iron status

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ABSTRACT

Background: Bioavailability of nonheme iron is influenced by the concentration of inhibitors and enhancers in the diet. The fructans inulin and oligofructose have been shown to improve iron absorption in animals through colonic uptake, but this has not been confirmed in humans.

Objective: The aim of the intervention study was to evaluate the influence of inulin on iron absorption, bifidobacteria, total bacteria, short-chain fatty acids (SCFAs), and fecal pH in women with low iron status (plasma ferritin <25 µg/L).

Design: The subjects (n = 32) consumed inulin or placebo 3 times/d (~20 g/d) for 4 wk, separated by a 2-wk washout period. Iron absorption was measured after 3 wk of inulin and placebo consumption from a standard test meal by using stable-iron-isotope techniques. Fecal bacteria were measured by quantitative polymerase chain reaction, and fecal acids by HPLC.

Results: Mean fractional iron absorption in the inulin (15.2%; 95% CI: 8.0%, 28.9%) and placebo (13.3%; 95% CI: 8.1%, 24.3%) periods did not differ significantly (P = 0.10). Inulin decreased fecal pH (P < 0.001) and increased fecal bifidobacteria (P < 0.001) and fecal lactate (P < 0.001) but had no effect on fecal SCFAs and total bacteria. Changes in lactate and acetate concentrations were positively correlated with changes in propionate (P < 0.001) and butyrate (P < 0.02) concentrations, respectively. Iron absorption correlated with fecal pH in the placebo period (P < 0.01) but not in the inulin period (P = 0.37).

Conclusion: Although inulin showed prebiotic activity, we were unable to show an increase in iron absorption in women with low iron status. This trial was registered at clinicaltrials.gov as NCT0148309. Am J Clin Nutr 2012;96:325–31.

INTRODUCTION

Iron deficiency (ID) is the most prevalent micronutrient deficiency worldwide, affecting nearly 2 billion people, mainly women and children in both developing and industrialized countries (1). A major cause of ID is the regular consumption of diets based on plant foods and consequently low in bioavailable iron (2). The bioavailability of nonheme iron, which provides most of the iron in a typical diet in developing countries, is strongly influenced by the concentration of enhancers and inhibitors (3). Phytic acid is the most important iron-absorption inhibitor (4), but polyphenol compounds can be as potent as phytate in inhibiting iron absorption and do occur at high concentrations in beverages such as tea, coffee, and red wine and in certain legumes, fruit, and vegetables (5, 6). The main enhancer of nonheme-iron absorption is ascorbic acid (7), which exerts its effect by reducing ferric iron to the ferrous form and by binding ferrous iron in a soluble chelate (8). The only other widely accepted enhancer of iron absorption is muscle tissue, whose enhancing effect is commonly known as the “meat factor.” The effect has been shown in humans (9), but until today the identification of its structure and mechanism remains unclear (10).

Other potential enhancers of iron absorption are the nondigestible carbohydrates inulin and oligofructose, which are considered prebiotics because they selectively stimulate the growth and metabolic activity of specific bacteria beneficial for the human host (11). Studies in rats (12) and pigs (13, 14) have reported enhanced iron absorption in combination with inulin and have proposed that the fermentation of inulin in the colon and the associated changes in the gut microbiota increase colonic iron absorption (15). Although iron in humans is mainly absorbed in the duodenum, a radioiron study has shown the ability of the human colon to absorb iron (16). Investigation of the iron-absorption pattern with radioisotopes further strengthened the evidence that humans can absorb iron from the colon. In these studies, the larger iron fraction (60–80%) of total iron absorption was absorbed within the first 2–4 h, whereas the remaining iron was absorbed at a much slower rate over the next 22–48 h and might be attributed to colonic iron absorption (17, 18). Colonic iron absorption, however, might be a physiologic mechanism developed by the human host to protect itself against potentially pathogenic bacteria, which might use iron present in the colon (19). Nevertheless, previous human studies have failed to show an effect of inulin on iron absorption (20, 21). However, a recent long-term feeding study has reported

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4 Abbreviations used: CRP, C-reactive protein; DMT-1, divalent metal transporter 1; ID, iron deficiency; PF, plasma ferritin; SCFA, short-chain fatty acid.

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39% less iron deficiency anemia in children fed iron-fortified milk enriched with oligosaccharides and *Bifidobacterium lactis* than in children fed the control milk without oligosaccharides and *B. lactis* (22). In the context of biofortification of staple foods, the concentration of inulin in wheat (1–4%) (23) could be increased by plant breeding, and it has been suggested that inulin-enriched wheat might improve iron nutrition in targeted populations and reduce the number of people with ID (13).

The following study used a stable-iron-isotope technique to investigate the influence of inulin on iron absorption from test meals consumed by women with low iron status. To monitor the effect of inulin on gut microbiota and metabolic activity, the bifidobacteria population, total bacteria, fecal pH, and fecal short-chain fatty acid (SCFA) profile were measured in the feces of the subjects.

### Subjects AND METHODS

#### Subjects

One hundred thirty-two women from the student and staff population of ETH Zurich and the University Hospital Zurich were screened for iron status [hemoglobin, plasma ferritin (PF), and C-reactive protein (CRP)], body weight, and height. Thirty-six apparently healthy, nonpregnant, nonlactating women with low iron status (PF <25 µg/L), aged between 18 and 40 y and with a body weight <65 kg and a normal BMI (in kg/m²; 19–24), were included in the study. Women with known metabolic, chronic, and gastrointestinal disease and women taking medications long term were excluded. Intake of vitamin and mineral supplements and intake of probiotics or other prebiotics were not allowed 2 wk before or during the study period. No women were recruited who had donated blood or experienced significant blood loss within 6 mo of the beginning of the study. The experimental procedures were approved by the ethical committee of ETH Zurich, and written informed consent was obtained from all study subjects before the investigation began.

#### Study design

A randomized, double-blind, crossover design was used, in which each subject acted as her own control. Participants were randomly assigned to receive either inulin (Fibruline Instant; Cosucra Group Warcoing; a concentration of 90% inulin in the product with an average degree of polymerization of ~10) or an identical-appearing placebo (Maltodextrin; Blattmann Schweiz AG) for 4 wk followed by a 2-wk washout period (Figure 1). After the washout period, each subject received the alternative treatment for 4 wk. Subjects received a container of inulin or placebo for each treatment period. They were instructed to consume 3 measuring spoons (6–7 g each) of inulin or placebo daily. Inulin or placebo was dissolved in water and consumed with breakfast, lunch, and dinner. Total inulin and placebo intakes were calculated by reweighing the container at the end of each period.

On day 22 of each treatment period (days 22 and 66), the test meals were administered to the subjects (Figure 1). To reduce the effect of the intrasubject meal to meal variation in iron absorption, each subject received 2 identical test meals per treatment period, one in the morning and the second 3 h later. Each test meal was isotopically labeled with 2 mg Fe (total: 4 mg Fe isotopes per treatment period). The iron isotopes $^{58}$Fe and $^{57}$Fe were randomly assigned to the test meals. The subjects received $^{58}$Fe during the placebo period and $^{57}$Fe during the inulin period or vice versa. Test meals were of moderate iron bioavailability and consisted of cooked rice (50 g dry weight) and a pureed, boiled vegetable sauce (25 g fresh weight) containing Chinese cabbage, carrots, zucchini, onions, oil, and salt, which was boiled for 4 h to reduce the concentration of compounds influencing iron absorption, such as ascorbic acid. The labeled meals were administered in the morning between 0700 and 0900 after an overnight fast and a second identical meal ~3 h later. The subjects were not allowed to eat and drink between the test meals and 3 h after the second meal. Test meals plus water (300 mL) were consumed completely in the presence of the investigators. On the days of test meal administration, the subjects consumed their inulin or placebo doses without food 1 h before the test meals to optimize the effect of inulin on iron absorption by ruling out the possibility of a direct, possibly negative interaction of inulin and iron in the duodenum. After subjects fasted overnight, blood was collected for iron isotopic analysis 14 d after test meal administration on day 36 and day 80 (Figure 1). Iron absorption was calculated based on erythrocyte incorporation of iron stable-isotope labels 14 d after intake of the labeled test meals (24). A total of 5 fecal samples were collected: 1 baseline sample and 2 samples in each period. Feces were collected on days 0, 21, 29, 65, and 73 (Figure 1) to measure fecal microbiota, pH, and the SCFA profile. The 2 fecal

![FIGURE 1. Study design. d, day.](https://academic.oup.com/ajcn/article-abstract/96/2/325/4576860/fig1)
samples taken in the same period (days 21 and 29, days 65 and 73) were pooled for analysis.

Dietary intake was assessed by using a 3-d weighed food record in each of the 2 treatment periods. Dietary data from the 2 records were evaluated by using a software program (EBIISPRO for Windows 6.0; J Erhardt, University of Hohenheim, Germany).

Stable-isotope labels
Isotopically labeled $^{58}$FeSO$_4$ and $^{57}$FeSO$_4$ were prepared from isotopically enriched elemental iron ($^{57}$Fe-metal: 97.8% enriched; $^{58}$Fe-metal: 99.5% enriched; both from Chemgas) by dissolution in 0.1 mol sulfuric acid/L. The solutions were flushed with argon to keep the iron in the +II oxidation state. They were analyzed before the start of the study for iron isotopic composition and tracer iron concentration by reversed isotope dilution mass spectrometry by using the experimental techniques outlined below.

Analytic methods

Blood samples
Venous blood samples were drawn into EDTA-treated tubes to determine iron status, including hemoglobin and PF. Hemoglobin was measured by using a Coulter Counter. PF and CRP were measured on an IMMULITE automatic system (DPC Bühlmann GmbH).

Whole blood samples collected on days 22 and 66 were mineralized by using an HNO$_3$/H$_2$O$_2$ mixture and microwave digestion followed by separation of the iron from the blood matrix by anion-exchange chromatography and a solvent/solvent extraction step into diethyl ether (25). All isotopic analyses were performed by negative thermal ionization mass spectrometry with a magnetic sector field mass spectrometer (MAT 262; Finnigan MAT) equipped with a multicollector system for simultaneous ion beam detection (24, 25).

Fecal samples
For the analysis of microbiota, pH and SCFA profile fecal samples were freshly transferred by the subjects themselves into a tube containing a carbon dioxide generator system so as to create an anaerobic atmosphere (Microbiology Anaerocult A mini; Merck). The fecal sample was stored in anaerobiosis at 4°C for a maximum of 12 h until delivery to the laboratory. Immediately on reception, fecal samples were portioned into aliquots and either stored at −20°C until the day of analysis or centrifuged at 14,000 rpm for 120 min at 4°C. The fecal water was carefully removed and stored at −20°C until analyzed. The pH of fecal water was measured by using a digital pH meter (Metrohm; Zofingen). HPLC (Hitachi LaChrome; Merck) measurements for SCFAs (acetate, propionate, butyrate, and formate), iso-acids (iso-butyrate and iso-valerate), and lactate were performed as described previously (26).

Nucleic acid was extracted from 100 mg feces by using a FastDNA Spin Kit for Soil (Qbiogene AG). Quantification was carried out with a Nanodrop ND-1000 Spectrophotometer (Witec AG) at 260 nm.

DNA amplification and detection by quantitative polymerase chain reaction was done with a 7500 Fast Real-Time PCR System (Applied Biosystems Europe BV) by using optical-grade 96-well plates. The samples were analyzed in a total volume of 25 µL by using an SYBR Green PCR Master Mix (Applied Biosystems) containing 200 nmoi/L of the appropriate primers and 1 µL template DNA diluted 10- or 100-fold, depending on the targeted bacteria group. For the quantification of total bacteria, the primers Eub338F (5’ ACT CCT ACG GGA GGC AGC AG 3’) and Eub518R (5’ ATT ACC GCG GCT GCT GG 3’) were used. To detect Bifidobacterium spp., we used the primers xfp-fw (5’ ATC TTC GGA CCB GAY GAG AC 3’) and xfp-rv (5’ CGA TVA GTG GTA CGA AGC 3’). Real-time quantitative polymerase chain reaction conditions were kept at the presettings of the ABI PRISM 7500-PCR, as described earlier (27).

Calculation of iron absorption
The amounts of $^{57}$Fe and $^{58}$Fe isotopic labels in blood 14 d after administration of the test meals were calculated on the basis of the shift in iron isotope ratios and on the estimated amount of iron circulating in the body. Circulating iron was calculated on the basis of the blood volume estimated from height and weight according to Brown and Hopper (28) and measured hemoglobin concentrations. The calculations were based on the principles of isotope dilution and took into account that iron isotopic labels were not monoisotopic (24). For the calculation of fractional absorption, 80% incorporation of the absorbed iron into red blood cells was assumed.

Statistical analysis
The analyses were conducted with SPSS statistical software (SPSS 19.0; SPSS Inc) and Microsoft Office Excel 2003. Iron absorption and PF values were log transformed for statistical analysis and reconverted for reporting. Iron absorption values from the 2 different test treatments within the same participant were compared by a paired Student’s t test. Differences were considered significant at $P < 0.05$. The study was powered to resolve a 25% difference in iron absorption between test meals by using each volunteer as her own control.

For variables that were not normally distributed (SCFAs, pH, total bacteria, and bifidobacteria), multiple comparisons of values from the 3 experimental periods (baseline, inulin, and placebo) within the same participants were done by using Friedman followed by a Wilcoxon signed-rank test with Bonferroni adjustment. Differences were considered significant at $P < 0.017$ (Bonferroni-adjusted $P$ value).

A 1-factor ANOVA followed by a post hoc Bonferroni test was used for comparisons of normally distributed values (CRP and hemoglobin). Differences were considered significant at $P < 0.05$. Pearson’s correlations and multiple linear regression models were used to study associations between different variables. $P$ values <0.05 were considered significant.

RESULTS

Subjects
Two subjects dropped out of the study because of health issues, and another 2 subjects were excluded from the evaluation because they consumed prebiotics and probiotics; 32 subjects completed the study. At baseline, 2 of the 32 study subjects had a hemoglobin concentration <120 g/L. The mean PF concentration was
12.5 μg/L (7.1, 22.8), and 17 subjects had a PF concentration <15 μg/L; none had a concentration >25 μg/L. Eight women showed a slightly elevated CRP concentration of >3 mg/L at baseline, but none had a CRP concentration >10 mg/L (Table 1). The mean BMI was 21.5 ± 2.2. Hemoglobin, PF, and CRP concentrations and BMI did not change during the study.

**Inulin and iron intakes**

The average daily inulin (Fibruline Instant) and placebo intakes were 20.2 ± 2 and 20.8 ± 3.2 g, respectively, and did not differ significantly. On the basis of the 3-d weighed records, inulin intake from meal components ranged between 0.4 and 4.6 g/d, mainly from wheat. Daily iron consumption was estimated at 10.9 ± 3 mg. Both inulin and iron intakes from the food components did not differ between the inulin and placebo periods.

**Iron absorption**

Mean fractional iron absorption from the test meal given during the inulin period was 14% higher than that from the test meal administered in the placebo period (Table 2); this difference was not statistically significant (P = 0.10).

**Gut microbiota**

The total median (range) bacteria concentration at baseline was 11.2 (9.8, 12.3) log copies/g feces and did not vary between stools collected at baseline and in the placebo and inulin periods. In contrast, stool bifidobacteria concentrations increased during the inulin administration from 9.6 (8.3, 10.6) to 10.5 (8.8, 11.3) log copies/g feces (P < 0.001). The bifidobacteria concentration did not differ between baseline (9.6 log copies/g feces) and placebo (9.9 log copies/g feces; Figure 2).

Total SCFA concentrations in fecal water and the concentrations of acetate, butyrate, propionate, and formate did not differ between stools collected at baseline or in the inulin and placebo periods. Fecal concentrations of iso-acids were also concentrations of acetate, butyrate, propionate, and formate did not differ between the inulin and placebo periods. Lactate concentrations were higher in stools of women who consumed inulin or placebo (n = 32).

**TABLE 2**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>Inulin period²</th>
<th>Placebo period²</th>
<th>Ratio³</th>
<th>P⁴</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (g/L)</td>
<td>133 ± 11</td>
<td>133 ± 12</td>
<td>133 ± 11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>1.5 ± 1.9</td>
<td>1.5 ± 2.4</td>
<td>1.1 ± 1.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PF (µg/L)</td>
<td>12.5 (7.1, 22.8)</td>
<td>11.4 (5.2, 24.9)</td>
<td>12.2 (6.3, 23.6)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹ CRP, C-reactive protein; PF, plasma ferritin.
² Values were obtained from blood samples collected at 36 and 80 d.
³ None of the values are significantly different between periods (1-factor ANOVA, Bonferroni test).
⁴ Mean ± SD (all such values).
⁵ None of the values are significantly different between periods (1-factor ANOVA on logarithmically transformed data, Bonferroni test).
⁶ Geometric mean; 95% CI in parentheses (all such values).

DISCUSSION

The consumption of inulin increased the numbers of bifidobacteria in the stool of study participants, which indicates that the inulin dose and study duration were sufficient for inulin to change the colonic environment in a way that could potentially increase...
FIGURE 2. Median bifidobacteria and total bacteria contents by real-time polymerase chain reaction in fecal DNA collected from subjects (n = 32) at baseline and in the placebo (2 time points) and inulin (2 time points) periods. Bars with different lowercase letters are significantly different, P < 0.017 (Friedman test, Wilcoxon signed-rank test, and Bonferroni test).

TABLE 3
Total SCFAs, selected SCFAs (acetate, propionate, butyrate, and formate), lactate, iso-butyrate, and iso-valerate concentrations in the feces of study participants (n = 32) at baseline and in the inulin (2 time points) and placebo (2 time points) periods.

<table>
<thead>
<tr>
<th></th>
<th>Total SCFAs</th>
<th>Acetate</th>
<th>Propionate</th>
<th>Butyrate</th>
<th>Formate</th>
<th>Lactate</th>
<th>Iso-butyrate</th>
<th>Iso-valerate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>120 (39, 207)</td>
<td>65 (15, 113)</td>
<td>20 (12, 90)</td>
<td>19 (5, 66)</td>
<td>0 (0, 18.7)</td>
<td>0 (0, 1.9)</td>
<td>3.0 (0.9, 8.3)</td>
<td>3.0 (0.9, 8.3)</td>
</tr>
<tr>
<td>Inulin (2 time points)</td>
<td>115 (57, 175)</td>
<td>76 (18, 107)</td>
<td>19 (0, 52)</td>
<td>22 (5, 56)</td>
<td>0 (0, 29.5)</td>
<td>0 (0, 74)</td>
<td>2 (0, 5.1)</td>
<td>2.0 (0, 5.1)</td>
</tr>
<tr>
<td>Placebo (2 time points)</td>
<td>112 (35, 230)</td>
<td>72 (16, 121)</td>
<td>21 (10, 72)</td>
<td>21 (9, 48)</td>
<td>0 (0, 17)</td>
<td>0 (0, 5)</td>
<td>3.3 (1, 7.7)</td>
<td>3.3 (1, 7.7)</td>
</tr>
</tbody>
</table>

1 All values are medians; ranges in parentheses (in mmol/L). Values in a column with different superscript letters are significantly different, P < 0.017 (Friedman test, Wilcoxon test, and Bonferroni test). SCFAs, short-chain fatty acids.
Animal studies indicate that low stool pH and low iron status favor colonic iron absorption (43), and, because of the latter, we selected subjects of low iron status for the current study. Campos et al (48) reported that colonic iron absorption was upregulated in iron-deficient rats, because of an increase in both passive and active iron absorption. In the iron-deficient rats, passive colonic absorption of calcium, zinc, and copper was also increased, and the authors suggested that this was the result of an increased permeability of the colonic membrane due to ID.

Further evidence that ID upregulated colonic iron absorption, to an even greater extent than duodenal iron absorption, came from the work of Chernelc et al (43) in dogs. After the dogs were phlebotomized, colonic iron absorption increased from 2.5% to 15% and duodenal iron absorption increased from 8.6% to 24%. The same workers also reported a doubling of iron absorption from an iron dose directly injected into the colon of dogs at pH 2 compared with pH 6.

A decrease in colonic pH after the consumption of inulin has been reported in rats and is one of the proposed mechanisms by which inulin and oligofructose might enhance colonic iron absorption (12). Linear regression analysis in our study showed no significant treatment effect and no treatment $\times$ pH interaction but indicated that a lower pH favors iron absorption. However, Yasuda et al (13) in 2006 found an increase in iron solubility in the stools of iron-deficient pigs after they had been fed a diet containing 4% of a mixture of oligofructose and inulin, but with no changes in colonic pH. The same workers also reported an inulin-induced decrease in the expression of inflammatory genes in a subsequent study with anemic piglets (49). It is possible, therefore, that inulin consumption may reduce inflammatory cytokines and improve iron absorption by decreasing hepcidin transcription (50).

Tako et al (14) reported an increased expression of DMT-1 and ferroportin in the duodenum of pigs after inulin supplementation and increased DMT-1, ferritin, and transferrin receptor expression in the colon. In a subsequent review, the same group suggested the following ways by which inulin could increase colonic iron absorption: decreasing colonic pH, reducing ferric to ferrous state, stimulating the expression of gene coding for iron-regulatory proteins, and stimulating the proliferation of epithelial cells, thus providing a greater surface for iron absorption (15).

Despite these consistent demonstrations in rat and pig models, showing that inulin increases colonic iron absorption, human studies have failed to confirm inulin as an enhancer of iron absorption. Coudray et al (21) investigated the effect of inulin on human iron absorption in a metabolic balance study in 9 healthy men receiving up to 40 g inulin/d. Apparent iron absorption from the control meal was 21.8% ± 12.3% and did not increase further with the inulin diet. One reason may be that iron bioavailability from the control diet without inulin was already very high in a group of iron-replete men and therefore was unlikely to be further increased. A second study by van den Heuvel et al (20) used stable-iron isotopes; 15 g inulin was consumed with a basal diet for 21 d. They also reported no influence of inulin on erythrocyte incorporation of stable-iron isotopes. However, the protocol was not ideal because ascorbic acid consumption was >400 mg/d and the study subjects were iron-replete men with a PF >80 μg/L.

Our study was the third human study to report no effect of inulin on iron absorption. Whereas it seems probable that iron is absorbed in the colon in humans, colonic absorption is likely to be a minor component of total iron absorption compared with duodenal absorption and is thus difficult to quantify via the erythrocyte incorporation of isotopes. Our study included 32 subjects and was powered to detect a 25% difference in iron absorption. Although iron absorption in the inulin period increased by 14%, this difference was not statistically significant. However, an inulin-induced increase in iron absorption would probably be too small to recommend plant breeders to increase the inulin content of staple foods by biofortification with the aim of usefully improving iron absorption and iron status.

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The authors’ responsibilities were as follows—NP, IE, CC, CL, and RH: designed the research; NP: conducted the research and analyzed the data; NP, IE, CC, and RH: wrote the manuscript; and NP, IE, and RH: had primary responsibility for the final content. All authors read and approved the final version of the manuscript. None of the authors had a conflict of interest.

REFERENCES
INULIN’S EFFECT ON HUMAN GUT AND IRON ABSORPTION


