The effects of iron fortification and supplementation on the gut microbiome and diarrhea in infants and children: a review

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ABSTRACT

In infants and young children in Sub-Saharan Africa, iron-deficiency anemia (IDA) is common, and many complementary foods are low in bioavailable iron. In-home fortification of complementary foods using iron-containing micronutrient powders (MNPs) and oral iron supplementation are both effective strategies to increase iron intakes and reduce IDA at this age. However, these interventions produce large increases in colonic iron because the absorption of their high iron dose ($\geq$12.5 mg) is typically $<$20%. We reviewed studies in infants and young children on the effects of iron supplements and iron fortification with MNPs on the gut microbiome and diarrhea. Iron-containing MNPs and iron supplements can modestly increase diarrhea risk, and in vitro and in vivo studies have suggested that this occurs because increases in colonic iron adversely affect the gut microbiome in that they decrease abundances of beneficial barrier commensal gut bacteria (e.g., bifidobacteria and lactobacilli) and increase the abundance of enterobacteria including entropathogenic *Escherichia coli*. These changes are associated with increased gut inflammation. Therefore, safer formulations of iron-containing supplements and MNPs are needed. To improve MNP safety, the iron dose of these formulations should be reduced while maximizing absorption to retain efficacy. Also, the addition of prebiotics to MNPs is a promising approach to mitigate the adverse effects of iron on the infant gut.
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Keywords: calprotectin, childhood, diarrhea, enterobacteria, gut inflammation, gut microbiome, infancy, iron fortification, iron supplementation, micronutrient powders

BACKGROUND

In Africa, 62.3% of preschool children are anemic (1), and it has been estimated that ~50% of anemia in Africa is due to iron deficiency (ID) (2). Infants have particularly high rates of anemia during the weaning period because requirements for growth and erythropoiesis are often not covered by the low amounts of bioavailable iron that are typically present in complementary foods (3). Iron-deficiency anemia (IDA) in infants and young children may impair cognitive development, and this impairment may be irreversible or only partially reversible by iron repletion (4, 5). Because IDA is common during infancy and childhood in Africa and can lower the intelligence quotient, it has serious health and economic costs and may be a brake on national development (6, 7). Thus, effective and safe interventions are urgently needed for this age group.

Complementary foods for infants in Africa are typically cereal or legume based (or both) and are often high in phytic acid and polyphenols that inhibit iron absorption (3). To increase dietary iron intakes without changing the traditional diet, micronutrient powders (MNPs) that contain iron and other vitamins and minerals can be added to complementary foods that are prepared at home (8). Iron-containing MNPs have been shown to reduce IDA in African infants even in areas with a high burden of infection and inflammation (8–10), although not all studies agree (11). Sprinkles are the most widely used MNP and contain a high dose of iron (12.5 mg Fe as ferrous fumarate) (8–10). The iron dose is set high to try to deliver adequate absorbed iron even when added to inhibitory complementary foods and when given to infants with inflammation-mediated increases in serum hepcidin, which is a peptide that reduces iron absorption from the gut (12). In settings with high rates of infant and children anemia, the WHO has recommended that MNPs containing $\geq$12 mg Fe be used in "home fortification" to reduce IDA in infants and children aged 6–23 mo and concluded that the MNPs can be as efficacious as iron supplements (9). Oral iron supplements that contain approximately the same daily dose of iron (10–12.5 mg/d) given to infants and children aged 4–23 mo can reduce anemia, and the WHO has recommended the use of these supplements (13).

However, the safety of iron-containing MNPs and supplements during infancy and childhood is uncertain. A controlled trial in Tanzanian preschool children showed that oral iron supplements (12.5 mg/d) increased the risk of serious adverse events, hospitalizations, and mortality (14), and a controlled trial in Ghanian infants showed that iron-containing MNPs (12.5 mg Fe/d) increased

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Abbreviations used: GOS, galacto-oligosaccharides; ID, iron deficiency; IDA, iron-deficiency anemia; MNP, micronutrient powder; NaFeEDTA, sodium iron EDTA; qPCR, quantitative polymerase chain reaction; SCFA, short-chain fatty acid.

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hospitalizations (11). A WHO consultation did not recommend the use of iron-containing supplements and MNPs in malaria-endemic areas because of concerns about potential increases in infection (15).

As a potentially safer alternative to MNPs that contain 12.5 mg Fe/d, a low–iron-dose MNP (MixMe; DSM Nutritional Products, Basel, Switzerland) has been developed that contains 2.5 mg Fe as sodium iron EDTA (NaFeEDTA) (16). NaFeEDTA is a chelated form of iron that has high bioavailability in foods that contain phytic acid or polyphenol and is a WHO-recommended iron fortificant for inhibitory foodstuffs (17). The acceptable daily intake for EDTA limits the iron dose that can be provided by NaFeEDTA to infants to 2–3 mg Fe/d (18). In the MixMe MNP, NaFeEDTA is combined with ascorbic acid and an exogenous phytase, both of which are enhancers of iron absorption, and this combination results in higher iron absorption from maize porridge (16). This MNP has shown to have weak efficacy in preschool children in Kenya (19) and school-age children in South Africa (20); however, in a 1-y controlled trial in Kenyan infants, the MNP did not reduce IDA (21), possibly because the absorbed iron dose was insufficient as a result of the low dose and the high prevalence of infections increasing serum hepcidin (12). Thus, it is uncertain how far the iron dose in MNPs can be lowered while maintaining efficacy against anemia. A lower dose would be advantageous because it may reduce the adverse impact of iron on the gut by decreasing the amount of unabsorbed iron that enters the infant colon as is discussed in subsequent sections of this review.

EFFECTS OF VARYING IRON SUPPLY AND THE GUT MICROBIOTA

Fractional iron absorption from iron fortificants and oral iron supplements is low; typically <20% of the iron is absorbed in the duodenum (3, 22), and most of the iron passes unabsorbed into the colon. The provision of iron fortificants or supplements to infants who have previously been mainly breastfeeding sharply increases colonic iron; iron concentrations in mature human milk are only ~0.47 mg/L (23), but the introduction of an MNP with 12.5 mg Fe will typically result in >10 mg unabsorbed Fe entering the infant colon each day. Iron is an essential and often growth-limiting nutrient for many gut bacteria (24). For many enteric gram-negative bacteria (e.g., *Salmonella*, *Shigella*, and pathogenic *Escherichia coli*), iron acquisition is critical for virulence and colonization (24–26). In contrast, beneficial commensal gut bacteria from the genera *Lactobacillus* and *Bifidobacterium* require little or no iron (27) and reduce growth and colonization by enteric pathogens (28, 29). A major *Bifidobacterium* species in breastfed infants is *Bifidobacterium breve*, which can sequester luminal iron via a metal permease (30), but the majority of *Bifidobacterium* species do not produce siderophores or other iron carriers. Thus, an increase in colonic iron through fortification or supplementation may shift the colonic microbiota equilibrium and favor the growth of pathogenic strains over healthy barrier strains.

Animal and in vitro studies

In animal models, varying colonic iron leads to major shifts in microbiota abundances in the gut and modifies metabolic activity (31). In adult rodents, iron deprivation increased fecal total anaerobes, *Enterococcus* spp. as well as lactobacilli (32). ID in rats increased *Enterobacteriaceae* and *Lactobacillus*/*Leuconostoc*/*Pediococcus* spp. but reduced *Bacteroides* spp. and *Roseburia* spp./*Eubacterium rectale*; these effects were associated with decreased fecal short-chain fatty acids (SCFAs), propionate, and butyrate (31). Iron supplementation partly restored the original gut microbiota composition and resulted in a recovery of metabolic activity (31). In vitro, continuous colonic fermentation models that use human infant gut microbiota provide high cell density, diversity, and stability because of bacterial immobilization on gel beads, which allows for the investigation of the gut microbiota and dietary factors via highly controlled variables independent of the host (33). Fermentation models can be used to study the impact of low– and high–colonic iron conditions on immobilized infant fecal microbiota (34). In these systems, low iron conditions decreased *Roseburia* spp./*E. rectale*, *Clostridium* cluster IV members, and *Bacteroides* spp. whereas *Lactobacillus* spp. and *Enterobacteriaceae* increased; there was a decrease of butyrate and propionate (34). Compared with normal iron conditions, high-iron conditions had no discernible impact on the gut microbiota composition or metabolic activity (34). A limitation of in vitro systems is that they cannot take into account host factors such as the mucosal response to iron exposure or systemic effects on iron status. However, the findings generally agree with animal studies indicating that chronically low intakes of dietary iron cause a shift in the gut microbiota and decrease SCFA production (31), which could potentially reduce the gut barrier to enteric pathogens, thereby underlining the importance of adequate dietary iron.

Controlled studies of iron fortification and supplementation in African children

In a randomized controlled trial in Côte d'Ivoire (35), we assessed the effect of iron fortification of wheat flour on iron status, hookworm prevalence, and the gut microbiota and inflammation. For 6 mo, 6- to 14-y-old children (n = 139) received iron-fortified biscuits that provided 20 mg Fe/d 4 times/wk as electrolytic iron or nonfortified control biscuits. At baseline, as measured via a real-time quantitative polymerase chain reaction (qPCR), there were greater numbers of fecal enterobacteria than of lactobacilli and bifidobacteria—a different ratio than is typically seen in children in industrialized countries where enterobacteria do not predominate (35). In a study that compared the gut microbiota in young children from the United States, Venezuela, and Malawi, a common pattern was the dominance of bifidobacteria through the first year after birth; thereafter, bifidobacteria numbers fell steadily leading to the establishment of an adult-like gut microbiota at ~3 y of age (36). In the Ivorian trial (35), iron fortification was ineffective; after 6 mo, there were no differences in iron status or anemia and no differences in hookworm prevalence or intensity. However, in the iron group compared with the control group, there was an increase in the number of enterobacteria, a decrease in lactobacilli, and an increase in fecal calprotectin, which is a marker of gut inflammation (37), that was correlated with the increase in fecal enterobacteria. To our knowledge, the study (35) was the first to show that anemic African children carry an unfavorable ratio of fecal enterobacteria to bifidobacteria and lactobacilli and that iron fortification causes a dysbiosis that is associated with gut inflammation. In contrast, a randomized

controlled trial (38) of iron supplements (50 mg/d for 4 d/wk) in South African schoolchildren from an area with an improved water supply and better hygiene showed no significant effects on gut inflammation (as measured by fecal calprotectin) or on the gut microbiota as measured via qPCR. In this study, unlike in the Ivorian study (35), at baseline, there were not greater numbers of fecal enterobacteria than of lactobacilli or bifidobacteria (38). The contrasting findings from these studies (35, 38) suggest that the local context is critical; risk of adverse effects from iron on the gut microbiome is likely to be high where hygiene standards are low and the microbiome is higher in opportunistic pathogens.

Controlled studies of iron-containing MNPs in African infants

We recently assessed the effects of iron-containing MNPs on the gut microbiome and inflammation in Kenyan infants who consumed home-fortified maize porridge (39). Two 4-mo randomized controlled trials were done in 6-mo-old infants ($n = 115$); in the first trial, infants received daily an MNP that contained 2.5 mg Fe as NaFeEDTA or the MNP without iron; in the second trial, infants received a different MNP that contained 12.5 mg Fe as ferrous fumarate or the MNP without iron (39). The primary outcome was the gut microbiome composition, which was analyzed via 16S sequencing and qPCR, and secondary outcomes were iron status and hemoglobin, fecal calprotectin, and incidence of diarrhea. The trials were analyzed both separately and combined. There was a treatment effect of the MNP that contained 12.5 mg Fe on body iron, serum ferritin, soluble transferrin receptor, and zinc protoporphyrin. In contrast, there was no treatment effect of the MNP that contained 2.5 mg Fe on any of the iron-status indicators, thereby suggesting that this iron dose was too low for efficacy (21). In accordance with previous studies on the human gut microbiota (40–43), the 4 dominant phyla in the infants at baseline were Actinobacteria (63%, mainly *Bifidobacteriaceae*), Firmicutes (22%), Bacteroidetes (9%), and Proteobacteria (4%). However, there were high prevalences of the following potential enteropathogens: *Bacillus cereus* in 39.5% of infants, *Staphylococcus aureus* in 65.4% of infants, *Clostridium difficile* in 56.5% of infants, members of the *Clostridium perfringens* group in 89.7% of infants, and *Salmonella* in 22.4% of infants. Enteropathogenic *E. coli* was detected in 65.0% of infants, enterotoxigenic *E. coli* producing heat-labile toxin was detected in 49.2% of infants, enteropathogenic *E. coli* producing heat-stable toxin was detected in 7.0% of infants, enterohemorrhagic *E. coli* producing shiga-like toxin 1 was detected in in 9.6% of infants, and enterohemorrhagic *E. coli* producing shiga-like toxin 2 was detected in in 8.5% of infants (39). Compared with the control group, there were increases in enterobacteria, *Escherichia/Shigella*, the enterobacteria:bifidobacteria ratio, and *Clostridium* spp. in the iron groups. The sum of the pathogenic *E. coli* (mean ± SD) at endpoint was greater in the iron groups (6.0 ± 0.5 log gene copy number/g feces) compared with the no iron groups (4.5 ± 0.5) ($P = 0.029$) (39). In the gut microbiota, abundances of closely related species predict the susceptibility to colonization by enteropathogenic bacteria (44). Thus, the iron-stimulated increase in enterobacteria may have encouraged the growth of pathogenic members of the genus *Escherichia/Shigella* spp. as indicated by the greater abundances of this genus in the iron groups at the endpoint and, in particular, of pathogenic *E. coli*. Gut inflammation, as measured by fecal calprotectin, was significantly increased by the 12.5-mg dose of iron compared with the control but not by the 2.5-mg dose of iron (39). There were no differences in fecal SCFA concentrations between the iron and no-iron groups during the intervention. During the intervention, 27.3% of infants in the iron groups, compared with 8.3% of infants in the control groups, required treatment of diarrhea (39). To our knowledge, these controlled studies were the first to suggest that iron-containing MNPs that were given to African infants in an area of poor hygiene produced adverse shifts in the gut microbiome and increased the numbers of potential enteropathogens and gut inflammation, which may have increased risk of diarrhea. In contrast, in 6-mo-old Malawian infants who were randomly assigned to no intervention or to received lipid-based nutrient supplements or a micronutrient-fortified corn-soya blend (both of which provided 5.5–6 mg Fe/d), there were no differences in the gut microbiota after 12 mo; overall, abundances of *Prevotella* and *Faecalibacterium* increased, whereas *Bifidobacteriaceae* and *Enterobacteriaceae* decreased (45). The varied findings from these infant studies in Africa may have been due to age-related differences in the gut microbiota, differences in methods that were used to characterize the gut microbiome, differences in complementary feeding patterns, and differences in the iron compound or dose given.

Increasing iron intakes and risk of diarrhea

The provision of iron supplements and iron-containing MNPs to infants and children in low-resource settings may increase risk of diarrhea (46). An early systematic review of controlled trials of iron supplementation and fortification in children concluded that the provision of iron was associated with a significant 11% increased risk of diarrhea (47); the review included 4 fortification studies that reported diarrheal outcomes, 3 studies of which provided iron-fortified infant formula, and 1 study of which provided an iron-fortified infant food; only 1 study reported a significantly higher diarrhea incidence (48). Since that review, several controlled iron-supplementation trials have reported a link between supplemental iron and diarrhea (49–52). In 6- to 9-mo-old Honduran and Swedish infants who were given iron supplements, iron increased risk of diarrhea in the nonanemic infants (49). There was a significant increase in diarrhea in 2 controlled iron-supplementation trials that gave 12.5–15 mg Fe/d in Peru (50) and Bangladesh (51). In Nepal (52) and Tanzania (14), large controlled intervention trials of folic acid and iron supplementation (12- to 35-mo-old children received 12.5 mg Fe/d; 1- to 11-mo-old infants received 6.25 mg Fe/d) reported on cause-specific mortality and diarrhea incidence as secondary outcomes. In Nepal, there was no significant difference in the incidence of diarrhea (RR: 0.94; 95% CI: 0.84, 1.05), but there was a nonsignificant increase in mortality from diarrhea with iron supplementation (RR: 1.21; 95% CI: 0.66, 2.11) (52). In Tanzania, iron supplementation did not significantly increase the incidence of diarrhea (RR: 0.92; 95% CI: 0.68, 1.25) but did increase risks of serious adverse events, hospitalizations, and mortality (14). Although the increased morbidity and mortality in Tanzania were thought to have been mainly due to increases in severe malaria, it is possible that sepsis from an enteric source may have contributed because it is difficult to differentiate severe malaria from sepsis in low-resource settings (53). A systematic review of randomized controlled trials of daily oral iron

supplementation in children 4–23 mo of age concluded that iron significantly increased fever (RR: 1.16; 95% CI: 1.02, 1.31) and vomiting (RR: 1.38; 95% CI: 1.10, 1.73), but in the 6 trials that assessed diarrhea, iron did not increase the prevalence or incidence of diarrhea [RR: 1.03 (95% CI: 0.86, 1.23); rate ratio: 0.98 (95% CI: 0.88, 1.09), respectively] (13). In Tanzanian infants and young children, a 45-wk intervention study of multiple micronutrient supplementation containing 18 mg Fe as ferrous fumarate reported an increase in diarrhea (HR: 1.19; 95% CI: 0.94, 1.50) (54).

Most early studies that show the efficacy of iron-containing MNPs in young children did not systematically evaluate morbidity (9, 10). However, 2 large trials have shown that the provision of MNPs containing 12.5 mg Fe was not without risk. In a large controlled trial in 6- to 35-mo-old Ghanaian children (n = 1958) that was conducted over 6 mo (5 mo of intervention followed by 1 mo of further monitoring), children received either an MNP containing 12.5 mg Fe/d or the MNP without iron (11). During the intervention period, there were significantly more hospital admissions in the iron group (RR: 1.23; 95% CI: 1.02, 1.49), and on the basis of outpatient records, 83% of the additional hospitalizations in the iron group were due to diarrhea (RR: 1.12; 95% CI: 0.86, 1.46) (11). The largest safety study of MNPs to date, to our knowledge, was a 12-mo cluster randomized trial in Pakistani infants (n = 2746) aged 6–18 mo who were assigned to a control group who did not receive MNPs or to a group who received an MNP containing 12.5 mg Fe with or without 10 mg Zn (55). The incidence risk ratios for the comparison of the control group to the group who received the MNP with zinc were as follows: for bloody diarrhea: 1.63 (95% CI: 1.12, 2.39); for severe diarrhea (≥6 stools/d): 1.28 (95% CI: 1.03, 1.57); and for admission to hospital with diarrhea: 1.30 (95% CI: 0.71, 2.38). For the comparison of the MNP and control groups, the difference in the incidence of bloody diarrhea was ∼0.08/child year, which would correspond to one additional episode of bloody diarrhea per year for every 12–13 children who were given iron-containing MNPs (55). The distribution of the MNPs was stopped when the children were 18 mo of age, and in the following 6-mo observation period, there was no difference in bloody diarrhea or severe diarrhea when the groups were compared, thereby suggesting that the effect was due to the intervention (55). A systematic review on the provision of MNPs in children (56) included 17 studies that evaluated the impact of MNP compared with that of no intervention or a control; most of the studies were done in children aged 6 mo to 6 y of age in developing countries, and most of the studies were effectiveness trials that evaluated the impact of MNPs in community settings. MNPs were shown to be associated with a significant increase in diarrhea (RR: 1.04; 95% CI: 1.01, 1.06) (56). A more recent systematic review (57) of studies that was done only in malaria-endemic areas included trials of both iron supplementation and fortification if they provided ≥80% of the Recommended Dietary Allowance by age in infants and children. For the comparison of iron treatment with placebo or no treatment (10 trials; ∼24,000 children), there was a significant 15% increase in diarrheal episodes per patient-month (risk ratio: 1.15; 95% CI: 1.06, 1.26) (57). Taken together, the available data suggest that oral iron supplements and iron-containing MNPs that are given to young children in low-resource settings modestly increase risk of diarrhea. This suggestion is concerning because diarrhea continues to contribute to the death of ∼1 in 9 children <5 y of age in Sub-Saharan Africa (58).

Strategies to balance need for iron and risk of adverse effects

Because MNP-fortification programs are planned or in place in many low-resource countries with poor hygiene conditions, including 15 programs in Sub-Saharan Africa (59), there is an urgent need to find safer MNP formulations. To reduce the amount of unabsorbed iron entering the colon from MNPs, the iron dose in MNPs should be lowered as much as possible while maintaining efficacy against anemia. To do this, iron absorption should be maximized by including components in the MNP sachet that enhance absorption, such as iron in the form of NaFeEDTA, ascorbic acid, or an exogenous phytase (16). In addition, the MNP could include constituents that reduce the adverse impact of iron on the gut microbiome and maintain abundances of the beneficial barrier bacteria. During breastfeeding, human milk oligosaccharides in breast milk (60) function as endogenous prebiotics that support the growth of beneficial commensal gut bacteria. Because breast milk is gradually replaced by complementary foods during weaning, intakes of human milk oligosaccharides fall; therefore, the addition of an exogenous prebiotic to MNPs, such as galacto-oligosaccharides (GOSs), could be beneficial (29, 61). GOS is a well-established prebiotic that is commonly added to infant formula and can selectively enhance the growth of bifidobacteria and lactobacilli, increase SCFA production, and decrease gut pH (29, 61). In the infant colon, these effects may provide a less-favorable growth environment for enteric pathogens and enhance the commensal barrier against colonization by pathogens (28, 29). A recently completed intervention trial in Kenyan infants has demonstrated that addition of GOSs to an MNP containing 5 mg Fe (2.5 mg as ferrous fumarate and 2.5 mg as NaFeEDTA) effectively reduces anemia and mitigates many of the adverse effects of the iron on the gut microbiome (62).

IMPLICATIONS FOR IRON-REPLETE INFANTS AND YOUNG CHILDREN IN HIGH-RESOURCE SETTINGS

The potential effects of iron supplementation and fortification on the gut microbiome of iron-replete infants in high-income countries are uncertain because controlled studies are not available to our knowledge. The extrapolation of results from low-income countries with poor hygiene where infants carry high numbers of potential enteropathogens (39) to settings with high hygienic standards is challenging because the impact of iron supplementation may be context specific. However, a small trial in United States infants showed that complementary foods that vary substantially in iron may have differing effects on the development of the infant gut microbiota (63). Also, in a controlled study in breastfeeding Swedish infants (49), iron supplementation was associated with lower gains in length and head circumference and increases in diarrhea in nonanemic infants. Thus, the potential untoward effects of increased dietary iron for iron-replete infants and young children in high-income countries remain unclear.

SUMMARY

In conclusion, more research is needed to better understand the effects of varying dietary iron on the gut microbiota of infants and young children from low- and high-income countries. In low-income countries, more studies are needed to define the lowest iron dose in MNPs that remains effective to prevent ID and IDA and to determine

whether the provision of prebiotics, or other substances such as probiotics or lactoferrin, can mitigate the adverse effects of iron on the infant gut. In high-income countries, because little is known about how dietary iron intakes in infants and young children affect the gut microbiome, more research is needed, particularly in the context of the high-iron fortification of infant formula such as in the United States.

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