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A review of the effect of iron supplementation on the gut microbiota of children in developing countries and the impact of prebiotics

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Abstract

Iron is essential for many physiological functions of the body, and it is required for normal growth and development. Iron deficiency (ID) is the most common form of micronutrient malnutrition and is particularly prevalent in infants and young children in developing countries. Iron supplementation is considered the most effective strategy to combat the risk of ID and ID anaemia (IDA) in infants, although iron supplements cause a range of deleterious gut-related problems in malnourished children. The purpose of this review is to assess the available evidence on the effect of iron supplementation on the gut microbiota during childhood ID and to further assess whether prebiotics offer any benefits for iron supplementation. Prebiotics are well known to improve gut-microbial health in children, and recent reports indicate that prebiotics can mitigate the adverse gut-related effects of iron supplementation in children with ID and IDA. Thus, provision of prebiotics alongside iron supplements has the potential for an enhanced strategy for combatting ID and IDA among children in the developing world. However, further understanding is required before the benefit of such combined treatments of ID in nutritionally deprived children across populations can be fully confirmed. Such enhanced understanding is of high relevance in resource-poor countries where ID, poor sanitation and hygiene, alongside inadequate access to good drinking water and poor health systems, are serious public health concerns.

Keywords: **anaemia: iron deficiency: iron supplementation: malnutrition: microbiota: prebiotics**

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Introduction

Iron is a vital micronutrient for most organisms, including humans and microbes. The availability of iron has both direct and indirect impacts on host–microbiota interactions due to its direct role in biochemical processes that are critical to life[1,2]. The most important biological role of iron is as a co-factor for proteins such as haemoglobin and enzymes involved in mitochondrial respiration as well as intermediary and xenobiotic metabolism. Iron also plays an important role in cell growth and differentiation[3]. Despite the importance of iron to life, the prevalence of iron deficiency (ID) in children remains high, especially in recourse-poor areas. Low dietary iron intake or iron overload in the gut caused by malabsorption may alter the immune mucosal response of the host. This has been observed in numerous studies that have reported causal effects between iron and infectious disease, and intestinal inflammatory diseases[4,5]. There is a pool of evidence suggesting a negative impact of iron supplementation on the gut microbiota of infants[6,7]. Thus, both iron deficiency and excess influence gut microbiota composition and function, and the development of disease[7,8]. The gut microbiota plays an important role in metabolic processes that influence vital body functions[7,9,10]. Some gut bacteria, including *Bifidobacterium* and *Lactobacillus*, are beneficial to the host[11,12]. This implies that dietary components that support the growth of such bacteria may be beneficial to gut health and may potentially reduce risk of infection to the host. Prebiotics are notable examples of such supportive dietary components. Prebiotics may be beneficial during iron supplementation in reducing the adverse effects that often occur during iron supplementation (see below).

The aim of this review is to assess the available evidence on iron supplementation and its adverse effects on the gut microbiota in children living in recourse-poor areas where ID and ID anaemia (IDA) are prevalent. Further, this review will consider the evidence on the beneficial effects of prebiotics on the gut microbiota and their potential to mitigate the adverse effects of iron supplementation in children.

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Iron

Iron metabolism, nutrition and absorption

Iron is an essential micronutrient that is involved in numerous metabolic functions including redox-stress resistance, respiration and DNA synthesis[13]. It is best known as a key constituent of haemoglobin (Hb), which is involved in the delivery of oxygen to tissues throughout the body. Iron functions as a co-factor in many metabolic pathways and is required by almost all organisms[13]. There are two main routes through which iron enters the body naturally: via the maternal bloodstream through the placenta, which is relevant during gestation; and from the diet by intestinal absorption, which occurs throughout life after birth. During the first ~6 months of life, dietary iron intake in breastfed infants is relatively low because of the low amount of iron (0.4 mg/l) in breast milk[14] However, newborn infants generally possess significant iron stores (250–300 mg), accumulated from the mother during the last trimester of pregnancy, which largely meet iron requirements for the first 6 months of life[15]. Thus, in infants born at full term to mothers who were iron-sufficient during pregnancy, there is little requirement for dietary iron in the first 6 months[15].

Dietary iron presents in two major forms, haem ('organic') and non-haem ('inorganic') iron. Dietary haem iron is largely derived from haemoglobin and myoglobin in meat; hence, the primary dietary source of haem iron is from the consumption of animal-based food products, including meat and fish, whilst non-haem iron is mainly obtained from plant-based products such as cereals, vegetables, fruits, legumes and pulses. The dietary absorption of haem iron is relatively high (between 15% and 35%); however, it generally accounts for a relatively small proportion (5–10%) of total dietary iron[16]. On the other hand, the absorption rate (1–10%) of non-haem iron is far lower, although it represents the main source of dietary iron in both the developed and developing world[17]. Non-haem iron is absorbed from the diet in the reduced (ferrous) state, mainly in the duodenum and ileum[18,19]. The details of haem absorption remain unclear[20]. One major reason for the relatively high absorption of haem iron is that its uptake is largely unaffected by other dietary components, unlike non-haem iron which is strongly influenced by dietary composition. For instance, ascorbic acid and gastric acid improve the absorption of non-haem iron by reduction and solubilisation[21], whereas phytate, polyphenols (e.g. tannins) and oxalate, all derived from plants, act as iron-chelating agents that reduce iron bioavailability and, thus, restrict iron absorption. An unavoidable consequence of the poor absorption of dietary iron is that it mostly remains unabsorbed and so passes into the lower gut where it is potentially available to the gut microbiota.

Prevalence of iron deficiency

According to the World Health Organization (WHO)[22], IDA affects approximately 2 billion people worldwide, representing almost 30% of the world's population. The WHO[23] also estimates that about 62.3% of preschool children in Africa are anaemic, of which ~50% is attributable to ID[24]. ID is not only a problem of the developing world as it is also the single most prevalent nutrient deficiency in developed countries[15]. ID affects the development and function of key organs, including the brain[25–27]. Children and women are those most susceptible to IDA and its deleterious consequences, especially in developing countries where the situation is further worsened by the high burden of disease and infection in resource-poor areas and communities. In infants and children, IDA impairs cognitive development and function[28]. This has serious consequences for health and is considered to be a major inhibitor of economic development and productivity by reducing the work capacity of the population[29,30].

Prevention and treatment of iron deficiency

There are a number of intervention strategies for the prevention and/or treatment of ID in children. These include the consumption of iron-rich foods, fortification of food with iron, iron supplementation and general health-supporting measures such as deworming programmes. Deworming prevents hookworm infection in children; such infections have been linked to blood loss which drives anaemia[31]. For iron-deficient infants and children in populations where the prevalence of ID is high, iron supplementation (as recommended by the WHO) has been the most effective intervention strategy to prevent and/or treat ID and, hence, reduce anaemia[32,33] as well as ID[34]. Home-based food fortification with 'multiple micronutrient powders' (MNP) is another effective strategy for the prevention/treatment of ID in children below 5 years[35,36]. MNP can reduce anaemia and ID, providing a similar impact to that of iron supplementation, but MNP also carry the added advantage of provision of other critical micronutrients (such as zinc and vitamins) that are beneficial to child growth and development[37]. For instance, in a randomised controlled trial in Ghanaian infants between the ages of 6 and 12 months, the home-based use of MNP resulted in a significantly three-fold lower level of ID in the intervention group[38]. A recent Cochrane systematic review has highlighted the benefits of home-based fortification of food with MNP containing iron as an effective strategy for the prevention and treatment of anaemia and ID in infants below 2 years of age, indicating that the resulting improvement in anaemia and ID are comparable to those achieved using iron supplements[39].

However, the application of iron supplementation and MNP is not without risk. A controversy arose from the publication of a randomised controlled trial of iron supplementation in 24 076 preschool children in Zanzibar where the intervention group (who received a daily iron dose of 12.5 mg) were 11% more likely to be hospitalised and 12% more likely to die from severe illness (including sepsis, measles, pneumonia, meningitis and pertussis) compared with those who did not receive the iron treatment; this led to the termination of the trial[40]. In this study, children were given either: iron (12.5 mg), folic acid (50 μg) and zinc (10 mg); iron and folic acid; zinc alone; or placebo. It was the groups that received the iron and folic acid (with or without zinc) that were withdrawn from the study because of high morbidity and increased mortality rates[40]. The increased morbidity and mortality risk in the children receiving iron supplements could be attributed to the likelihood that most of the iron supplement

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received would remain unabsorbed and thus could favour the proliferation of pathogenic gut bacteria leading to increased risk of illness. Additionally, iron is an important micronutrient for the growth and proliferation of the malaria parasite[41,42], implying that iron supplementation in malaria-endemic areas would most likely increase the prevalence and disease severity within such populations[40]. Another randomised controlled trial in Africa, involving 1956 infants and young children from Ghana, reported increased diarrhoea-related hospital admissions following intake of 12.5 mg of elemental iron per day over 5 months[43], although there was a reduction in the incidence of anaemia in the iron group. Though not investigated, this study implies that iron supplementation in this population might have caused disruption to the gut microbiota, leading to diarrhoea. These findings are similar to the earlier larger population group randomised controlled trial in Zanzibar, as discussed above[40]. Thus, even though iron supplementation may be an effective strategy to combat ID, it may cause adverse effects in populations at increased risk of infection.

Importantly, subsequent studies indicate that iron supplementation does not result in an increased risk of infection or death as long as there is regular infection control and surveillance[41,42,44,45]. Indeed, iron supplementation has been shown to improve immune defence in children by increasing $CD4^+$ cell counts and CD4:CD8 ratios, which is a crucial aspect of cell-mediated immunity[46,47].

Impact of iron on the gut microbiota

Bacterial colonisation of the gut depends on the ability of bacteria to acquire iron and other essential nutrients. It has been shown that almost all gut bacteria, except lactobacilli, are iron requiring, which indicates that iron is a growth-limiting nutrient for the gut microbiota[48]. Thus, the degree of iron availability is expected to influence gut microbiota composition. Indeed, evidence from both animal and human trials has shown that iron supplementation results in alterations in gut microbiota composition[49–51].

In rats, iron depletion significantly decreased *Bacteroides* and *Roseburium* spp., whilst *Lactobacillus*, *Leuconostoc*, *Pediococcus* spp. and Enterobacteriaceae were significantly increased[50,52]. Similarly, in a randomised controlled trial with 147 school children (aged 6–14 years) in Africa (Cote d'Ivoire), the intake of 20 mg doses of electrolytic iron per day for 6 months in iron-fortified biscuits led to a negative alteration in gut microbiota composition where the number of Enterobacteriaceae were increased while lactobacilli decreased. The change in gut microbiota composition was also associated with raised faecal calprotectin, a marker of gut inflammation[49]. Furthermore, there was no improvement in anaemia or iron status compared with the control (non-iron-supplemented group). This is an important study because ID is prevalent in children under 5 years in developing countries and, consequently, such children often receive iron supplements. However, the results indicate that iron supplements can have a negative impact on gut health, whilst not improving iron status, which raises concerns regarding the efficacy of iron fortification programmes for children in developing nations.

As indicated, iron supplementation has also been linked to increased diarrhoea incidence in malnourished children[49]. In a recent study, the effect of high and low iron fortification doses on the gut microbiota was studied in 6-month-old Kenyan children[51]. In this double-blinded randomised controlled trial, 115 children were fed MNP for 4 months with either: no additions; 2.5 mg iron per day, as an ethylenediaminetetraacetic acid (EDTA) complex; or 12.5 mg iron per day, as ferrous fumarate. The results showed a reduction in Bifidobacteriaceae (the predominating bacterial group) after iron supplementation, which shifted the gut microbiome from domination by beneficial bacteria, including *Bifidobacterium* and *Lactobacillus*, to one containing potentially harmful pathogenic bacteria such as Enterobacteriaceae. This effect was more significant with the higher ferrous iron dose. The treatment also resulted in increased intestinal inflammation (as measured by faecal calprotectin) and no decrease in anaemia or ID for children who were iron deficient prior to the intervention. These findings are thus very similar to those described above[49].

In contrast, Dostal *et al.*[53] reported potentially positive effects of iron (ferrous sulphate) supplementation on the gut microbiota, characterised by increases in *Bacteroides* spp., and butyrate producers such as *Faecalibacterium prausnitzii* and *Coprococcus* spp. These effects were observed in a rat model system carrying faecal microbiota from a healthy 6-year-old child. In addition, there were significant increases in caecal propionate, butyrate and acetate concentration in the iron supplemented rats compared with control (iron-deficient group), suggesting that iron promotes a beneficial microbial community and positive metabolite production in a healthy rat model. In addition, a 38-week randomised placebo-controlled iron supplementation trial on ID South African children (6–11 years old) found no effect of iron supplementation on the relative abundance of the gut microbiota population[53a]. It was thus concluded that iron has no clear effect on the gut microbiota and inflammation unless there are associated risk factors such as pathogen infection caused by poor sanitation and hygiene conditions, inadequate access to good drinking water and poor health systems[53a]. Similarly, a recent clinical dose–response study in Nigerian toddlers showed that a multi-nutrient-fortified dairy drink containing iron in three different daily doses (2.24, 4.48 and 6.72 mg) had no adverse effect on the gut microbiota but was effective in treating anaemia[54]. The findings of the above two studies are in contrast to those described earlier in this section[49–51]. This likely reflects the differences in the approaches employed, including the use of healthy children and populations with low risk of infections as compared with populations at high risk of ID and infections. This also highlights the need for further research to fully understand the factors that influence the impact of dietary-iron intervention strategies on gut health in children.

Prebiotics and microbiota in children

The nature of prebiotics

Prebiotics are defined as 'substrates that are selectively utilized by host microorganisms conferring health benefits to the

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host'[55]. Prebiotics have been extensively studied for their beneficial impact on gut microbiota and health. According to Gibson *et al.*, prebiotics must possess the following characteristics to be classified as such: be a selective substrate for specific bacteria; exhibit resistance to low colonic pH, hydrolysis by intestinal enzymes and gastrointestinal absorption; and result in positive effects on host health and wellbeing[55]. Common, well-studied prebiotics include galacto-oligosaccharides (GOS), inulin and fructo-oligosaccharides (FOS)[56]. Inulin and FOS are both fructans consisting of a linear chain of fructose with β(2→1) linkages. The degree of polymerisation of inulin is up to 60, whilst that of FOS is below 10. GOS is classified into two groups: GOS that is derived from lactose through enzymatic *trans*-glycosylation with galactose in β(1→6), β(1→3) and β(1→4) linkages; and GOS with excess galactose at C_3, C_4 or C_6[57,58]. The effect of prebiotics is mediated through their conversion into short-chain fatty acids (SCFA) during fermentation by specific members of the colonic gut microbiota. SCFA serve as a source of energy to gut epithelia cells, lowering the pH of the intestinal lumen thus restricting growth of some pathogens and providing an anti-inflammatory effect to the host[59]. The enhancement of *Bifidobacterium* and *Lactobacillus* spp. in the gut is a common outcome of prebiotic application with subsequent positive effects on host health[60]. These bacteria have been observed to limit the growth of pathogens, improve intestinal barrier function and enhance immune function[61,62] through their production of SCFA.

Health benefits of prebiotics in children

Breast milk is usually the first food taken after birth. A unique characteristic of human milk is the presence of 'human milk oligosaccharides' (HMO), which are an important source of prebiotics for the infant[63]. HMO consist of over 200 distinct structures composed of a range of monomer units including galactose, glucose, *N*-acetylglucosamine, fucose and sialic acid[64]. *In vitro* studies have shown that the utilisation of specific HMO by the infant gut microbiota is species specific with the predominating *Bifidobacterium* spp. acting as the major HMO fermenters[65]. Interestingly, the HMO composition of breast milk is affected by multiple maternal factors including body mass index (BMI), mode of delivery, age, diet, duration of lactation and geographical location[66–68]. These factors are also expected to influence the composition and functional potential of the infant gut microbiota. Breast milk has been reported to contain high amounts of HMO (5–20 g/l)[69]. Indeed, there is an association between the diversity and homogeneity of HMO composition, and the growth and development of healthy children in their first 6 months of life[70]. However, studies in Gambia and Malawi have shown an inverse relationship between HMO and undernutrition in infants[68,71].

Prebiotics are now being considered as a promising therapeutic tool for the improvement of health as well as treatment and prevention of a number of diseases in children[59]. There is increasing evidence suggesting that prebiotics offer a broad spectrum of positive effects on child health, mediated primarily through the gut microbiota[72], as depicted in Fig. 1. In a randomised controlled double-blind clinical trial that assessed the effect of FOS on constipation in infants, Souza *et al.* reported significantly higher relative abundance of *Bifidobacterium* spp. in the faecal microbiota compared to the control[73]. Further, the study reported softer stools in the FOS group compared to the control. The consumption of FOS has been associated with significant increases in the relative abundance of colonic *Bifidobacterium* spp. according to a recent meta-analysis of the impact of FOS consumption on the gut microbiota[74]. As indicated above, the fermentation of these prebiotics by the gut microbiota generates SCFA (in particular, propionate, butyrate and acetate). SCFA exert a range of benefits on child health including provision of about 10% of the total body energy[75,76]. Lactate and succinate are also produced by fermentation of prebiotics. Such fermentation products lower the pH of the gut which helps to prevent the growth of pathogens[77]. Prebiotics also enhance the absorption of micronutrients, especially iron[78–80]. Prebiotics have been proposed to increase iron absorption by the production of SCFA during colonic microbiota fermentation which results in a lower colonic pH. This, in turn, would be expected to raise ferric iron solubility and availability, which would increase the potential for reduction of Fe^{3+} to Fe^{2+}, thus supporting iron absorption[81]. However, Husmann *et al.* did not observe any significant effect of GOS on iron absorption in women, although, in *in vitro*, GOS supported a two-fold increase in iron solubility[82].

There is strong evidence that prebiotics (GOS/FOS) in infant formula support healthy weight gain[83]. In addition, intake of prebiotic supplements among obese and overweight 7–12-year-old children resulted in a significant weight loss compared with the placebo control[84]. Thus, prebiotics can support a healthy body weight in children. However, little is understood concerning the effect of prebiotics on the gut microbiota during undernutrition and how this might affect malnutrition treatment in children. For such reasons, the International Scientific Association for Probiotics and Prebiotics (ISAPP) recommends that more research should be conducted on the mechanism by which the gut microbiota and prebiotics/probiotics influence undernutrition in specific age groups, especially children[85].

Prebiotics can also confer direct antimicrobial effects by adhering to the binding site of bacteria on the enterocyte surface which blocks the attachment of pathogenic bacteria to the intestinal epithelium[86,87]. The beneficial effects of prebiotics during infancy have also been reported in a systematic review that showed a reduced incidence of gastrointestinal infections, an improved stool consistency, reduced frequency of vomiting and reduced regurgitation among infants who received formulae containing FOS/GOS, compared with control formulae[88]. These findings further support the health promoting effects of prebiotics in children.

Effect of prebiotics on gut microbiota in infants and young children receiving iron supplementation

Children in developing countries are at increased risk of ID, and therefore the WHO recommends routine iron supplementation

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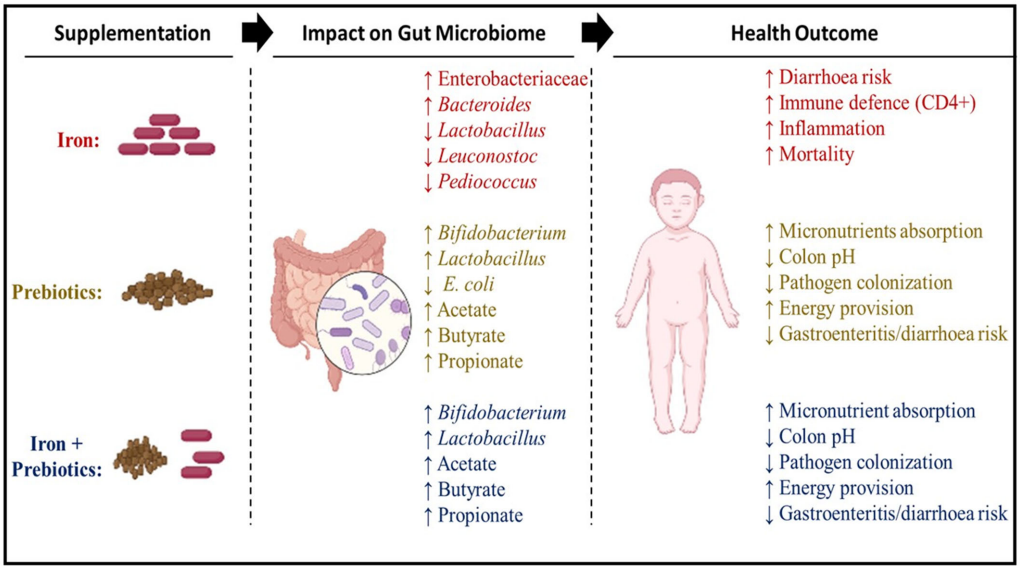

Fig. 1. Effect of prebiotics and iron supplementation on the gut microbiota and health outcomes in children. ↑ and ↓ indicate increase or decrease, respectively, in microbiota factors and health outcomes upon iron (red font), prebiotic (brown font) or iron plus prebiotic (blue font) supplementation.

in areas with high prevalence of ID[23]. With the potential adverse effects of iron supplementation on the gut microbiota in such populations, numerous studies have looked at the mitigating impact of prebiotics during iron supplementation. A recent study in Kenyan infants suggests that provision of prebiotic (GOS) during iron supplementation not only improved anaemia but also offset the adverse effects caused by iron supplementation[89]. In this study, 155 infants of 6.5–9.5 months were enrolled and randomised into three groups. The first (control) group received MNP containing 30 mg of ascorbic acid and other vitamins, and 10.5 g of maltodextrin; the second (Fe group) received the same but with 5 mg of Fe (2.5 mg as sodium iron ethylenediaminetetraacetate and 2.5 mg as ferrous fumarate); and the third (FeGOS) group received the same as the second group but with GOS (7.5 g) instead of maltodextrin (placebo). After 4 months of the intervention, a significant decrease (about 50%) in anaemia was observed in the Fe-only and FeGOS groups compared with the control. In addition, there was a significant increase of bifidobacteria and lactobacilli in the FeGOS group, but these bacteria were less abundant in the Fe-only group. The results indicate that combining a prebiotic (GOS) with Fe supplementation offsets the negative effects of iron on the gut microbiota whilst improving anaemia outcomes[89]. Supplementing iron with prebiotics may also improve iron absorption. For instance, Paganini *et al.* found that GOS supplements resulted in a 62% increase in iron absorption in Kenyan infants consuming ferrous fumarate (FeFum) and ferric sodium EDTA[90]. However, another study reported that a single GOS dose added to the meals of Kenyan infants did not increase iron absorption[91].

The inclusion of prebiotics has now become an important practice in formula milk production due to the growing evidence of the beneficial impact on the gut microbiota in early life and throughout the life cycle. Most formula milk is now fortified with GOS and/or chicory root derived inulin (FOS)[92]. The beneficial impact of these prebiotics (GOS and FOS) in formula milk has been indicated by several studies. For instance, the growth of bifidobacteria and lactobacilli was stimulated by FOS and GOS when incorporated into infant formula milk[93]. Increased abundance of these bacteria (bifidobacteria and lactobacilli) is associated with reduced diarrhoea incidence in children[94–96], culminating in improved nutrient absorption and health. Supplementing formula milk with FOS also resulted in a beneficial impact on the development of the gut microbiota in preterm infants[97]. In this study, healthy preterm infants were randomly fed with infant formula supplemented with FOS or standard infant formula for 14 d after birth. The FOS treatment resulted in significantly higher bifidobacteria and lower *Escherichia coli* levels in the faecal microbiome[97]. Other studies have shown that the addition of prebiotics to infant formula can increase the abundance of faecal bifidobacteria in infants, such that levels resembling those of breastfed infants are achieved. Such effects are associated with increased production of SCFA along with softer and more consistent stools[98–100]. Another study showed significant increases in faecal concentrations of lactate, propionate and butyrate in formula-fed infants who received whey protein with prebiotics, compared with breastfed infants[100]. Other work showed that inulin-type oligosaccharide supplementation of infant formula milk increases bifidobacteria abundance and improves stool consistency (softer stools) and frequency[101]. In addition, GOS supplementation in infants resulted in a reduced incidence of gastroenteritis compared to the non-supplemented group indicating GOS lowers the incidence of intestinal infections in infants[102]. In a double-blind study on formula-fed newborn infants, FOS/GOS prebiotic caused a significant reduction in the levels of faecal clostridia and an increase in bifidobacteria, thus positively impacting the composition of the gut microbiota[103]. In summary, there is

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now considerable evidence supporting the favourable effect of prebiotics on the gut microbiota and gut health of infants.

Furthermore, in a randomised placebo-controlled trial on the influence of prebiotics on the gut health of young children, Soldi *et al.*[104] reported that inulin induces a significant increase in bifidobacteria levels and mitigates the gut microbiota-related adverse effects caused by antibiotics in children (aged 3–6 years). Similarly, a double-blind, randomised controlled trial assessing the effect of prebiotics on body fat and intestinal microbiota in Canadian overweight or obese children (7–12 years) also found that prebiotic treatment significantly increases levels of *Bifidobacterium* spp. and led to a significant reduction in body fat. Similar treatment also resulted in a significant reduction in gut inflammation (as indicated by interleukin 6 levels)[84]. All this evidence suggests that prebiotics can have a beneficial impact on the gut microbiota, gut inflammation and body fat levels of young children, and may also improve iron absorption[59,89].

In summary, ID remains a public health concern, with children and women in developing countries being the most vulnerable. Iron supplementation may be useful for treating and preventing ID in children, but there are concerns of the adverse effects this may have on the health of infants and children. There is a growing body of evidence suggesting that prebiotics provide positive effects on the gut microbiota and gut health of infants and children. Such effects include prevention of childhood infections such as diarrhoea, and the promotion of infant health, growth and development. Furthermore, it is now becoming apparent that prebiotics can also support the application of iron supplementation in the treatment of ID in children even though much evidence is still needed to support its use. Thus, prebiotics have much potential in countering the negative gut health impact of iron supplements in nutritionally deprived children with ID, allowing an improvement both in overall health and in the effectiveness of iron supplementation strategies.

Conclusion and future direction

Iron supplementation and fortification may be considered effective strategies to manage ID and IDA in children in developing countries, but it can also cause adverse effects in populations where risk of malaria and diarrhoea infections is high. Further research is needed to understand the most effective iron supplementation regimens for prevention or treatment of ID whilst avoiding the adverse effects of iron supplementation. Such studies should be prioritised as iron supplementation programmes are on-going in developing countries where there is a high incidence of ID alongside poor sanitation and hygiene, and inadequate access to clean drinking water, especially for children under 5 years of age. Prebiotics have been shown to combat adverse effects caused by iron supplementation and to have an overall health benefit. Prebiotics thus offer an opportunity to overcome the negative effects that iron supplements have on the gut whilst providing other important benefits to the host such as reducing the risk of diarrhoea and malaria.

Manipulation of the gut microbiota represents a clear opportunity for supporting the treatment of ID in children from resource-poor countries. Prebiotics have been reported to improve iron absorption and the potential adverse effects of unabsorbed iron on the gut microbiota in children in developing countries. However, more research is needed in this area to ensure that the best strategies are identified and applied. Therefore, future research should focus on the ability of prebiotics to offset the negative effects of iron supplementation, especially within relevant ID populations, to establish the efficacy and optimum regimen for such intervention strategies. Supporting the gut microbiota in this way would also result in improvement in overall health outcomes in children.

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Competing interests

None.

Authorship

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References

1. Nairz M, Schroll A, Sonnweber T, *et al.* (2010) The struggle for iron – a metal at the host–pathogen interface. *Cell Microbiol* **12**, 1691–1702.
2. Wessling-Resnick M (2010) Iron homeostasis and the inflammatory response. *Ann Rev Nutr* **30**, 105–122.
3. Sheftel AD, Mason AB & Ponka P (2012) The long history of iron in the universe and in health and disease. *BBA* **1820**, 161–187.
4. Ganz T (2018) Iron and infection. *Int J Hematol* **107**, 7–15.
5. Stein J, Hartmann F & Dignass AU (2010) Diagnosis and management of iron deficiency anemia in patients with IBD. *Nat Rev Gastroenterol Hepatol* **7**, 599–610.
6. Yilmaz B & Li H (2018) Gut microbiota and iron: the crucial actors in health and disease. *Pharmaceuticals (Basel)* **11**, 98.
7. Hou K, Wu Z-X, Chen X-Y, *et al.* (2022) Microbiota in health and diseases. *Signal Transduct Target Ther* **7**, 135.
8. Rusu IG, Suharoschi R, Vodnar DC, *et al.* (2020) Iron supplementation influence on the gut microbiota and probiotic intake effect in iron deficiency – a literature-based review. *Nutrients* **12**, 1993.
9. Rowland I, Gibson G, Heinken A, *et al.* (2018) Gut microbiota functions: metabolism of nutrients and other food components. *Eur J Nutr* **57**, 1–24.
10. Oliphant K & Allen-Vercoe E (2019) Macronutrient metabolism by the human gut microbiome: major fermentation by-products and their impact on host health. *Microbiome* **7**, 91.
11. O'Callaghan A & Van Sinderen D (2016) Bifidobacteria and their role as members of the human gut microbiota. *Front Microbiol* **7**, 925.

https://doi.org/10.1017/S0954422424000118 Published online by Cambridge University Press

12. Vlasova AN, Kandasamy S, Chattha KS, *et al.* (2016) Comparison of probiotic lactobacilli and bifidobacteria effects, immune responses and rotavirus vaccines and infection in different host species. *Vet Immunol Immunopathol* **172**, 72–84.
13. Lieu PT, Heiskala M, Peterson PA, *et al.* (2001) The roles of iron in health and disease. *Mol Aspects Med* **22**, 1–87.
14. Cai C, Harding S & Friel J (2015) Breast milk iron concentrations may be lower than previously reported: implications for exclusively breastfed infants. *Matern Pediatr Nutr* **2**, 104
15. Fuqua BK, Vulpe CD & Anderson GJ (2012) Intestinal iron absorption. *J Trace Elem Med Biol* **26**, 115–119.
16. Monsen ER, Hallberg L, Layrisse M, *et al.* (1978) Estimation of available dietary iron. *Am J Clin Nutr* **31**, 134–141.
17. Sharp PA (2010) Intestinal iron absorption: regulation by dietary & systemic factors. *Int J Vitam Nutr Res* **80**, 231–242.
18. Tondeur MC, Schauer CS, Christofides AL, *et al.* (2004) Determination of iron absorption from intrinsically labeled microencapsulated ferrous fumarate (sprinkles) in infants with different iron and hematologic status by using a dual-stable-isotope method. *Am J Clin Nutr* **80**, 1436–1444.
19. Zimmermann MB & Hurrell RF (2007) Nutritional iron deficiency. *Lancet* **370**, 511–520.
20. Geng L, Duan X, Wang Y, *et al.* (2016) Quantum dots-hemin: preparation and application in the absorption of haem iron. *Nanomed Nanotechnol Biol Med* **12**, 1747–1755.
21. Gulec S, Anderson GJ & Collins JF (2014) Mechanistic and regulatory aspects of intestinal iron absorption. *Am J Physiol Gastrointest Liver Physiol* **307**, G397–G409.
22. WHO (2018) Micronutrient deficiencies: Iron deficiency anaemia. Available from: http://www.who.int/nutrition/topics/ida/en/ (accessed 28 February 2018).
23. WHO (2015) *The global prevalence of anaemia in 2011*. Geneva: World Health Organization.
24. Kassebaum NJ, Jasrasaria R, Naghavi M, *et al.* (2014) A systematic analysis of global anemia burden from 1990 to 2010. *Blood* **123**, 615–624.
25. Prado EL & Dewey KG (2014) Nutrition and brain development in early life. *Nutr Rev* **72**, 267–284.
26. Lozoff B (2007) Iron deficiency and child development. *Food Nutr Bull* **28**, S560–S571.
27. Beard J (2007) Recent evidence from human and animal studies regarding iron status and infant development. *J Nutr* **137**, 524S–530S.
28. Lozoff B (2007) Iron deficiency and child development. *Food Nutr Bull* **28**, S560–571.
29. Alderman H, Hoddinott J & Kinsey B (2006) Long term consequences of early childhood malnutrition. *Oxf Econ Pap* **58**, 450–474.
30. Horton S & Ross J (2003) The economics of iron deficiency. *Food Policy* **28**, 51–75.
31. Bauleni A, Tiruneh FN, Mwenyenkulu TE, *et al.* (2022) Effects of deworming medication on anaemia among children aged 6–59 months in sub-Saharan Africa. *Parasites Vectors* **15**, 7.
32. Pasricha SR, Hayes E, Kalumba K, *et al.* (2013) Effect of daily iron supplementation on health in children aged 4–23 months: a systematic review and meta-analysis of randomised controlled trials. *Lancet Global Health* **1**, e77–e86.
33. Brunken GS, Muniz PT & da Silva SM (2004) Weekly iron supplementation reduces anemia prevalence by 1/3 in preschool children. *Rev Bras Epidemiol* **7**, 210–219.
34. Berglund S, Westrup B & Domellöf M (2010) Iron supplements reduce the risk of iron deficiency anemia in marginally low birth weight infants. *Pediatrics* **126**, e874.
35. Salam RA, MacPhail C, Das JK, *et al.* (2013) Effectiveness of micronutrient powders (MNP) in women, children. *BMC Public Health* **13**, S22.
36. Teshome EM, Andang'o PEA, Osoti V, *et al.* (2017) Daily home fortification with iron as ferrous fumarate versus NaFeEDTA: a randomised, placebo-controlled, non-inferiority trial in Kenyan children. *BMC Med* **15**, 89.
37. Zlotkin SH, Schauer C, Christofides A, *et al.* (2005) Micronutrient sprinkles to control childhood anaemia. *PLoS Med* **2**, e1.
38. Adu-Afarwuah S, Lartey A, Brown KH, *et al.* (2008) Home fortification of complementary foods with micronutrient supplements is well accepted and has positive effects on infant iron status in Ghana. *Am J Clin Nutr* **87**, 929–938.
39. Suchdev PS, Jefferds MED, Ota E, *et al.* (2020) Home fortification of foods with multiple micronutrient powders for health and nutrition in children under two years of age. *Cochrane Database Syst Rev* **2**, CD008959.
40. Sazawal S, Black RE, Ramsan M, *et al.* (2006) Effects of routine prophylactic supplementation with iron and folic acid on admission to hospital and mortality in preschool children in a high malaria transmission setting: community-based, randomised, placebo-controlled trial. *Lancet* **367**, 133–143.
41. Neuberger A, Okebe J, Yahav D, *et al.* (2016) Oral iron supplements for children in malaria-endemic areas. *Cochrane Database Syst Rev* **2**, CD006589.
42. Clark MA, Goheen MM & Cerami C (2014) Influence of host iron status on plasmodium falciparum infection. *Front Pharmacol* **5**, 84.
43. Zlotkin S, Newton S, Aimone AM, *et al.* (2013) Effect of iron fortification on malaria incidence in infants and young children in Ghana: a randomized trial. *JAMA* **310**, 938–947.
44. Okebe JU, Yahav D, Shbita R, *et al.* (2011) Oral iron supplements for children in malaria-endemic areas. *Cochrane Database Syst Rev* **2011**, CD006589.
45. Ojukwu JU, Okebe JU, Yahav D, *et al.* (2009) Oral iron supplementation for preventing or treating anaemia among children in malaria-endemic areas. *Cochrane Database Syst Rev* **2009**, CD006589.
46. Das I, Saha K, Mukhopadhyay D, *et al.* (2014) Impact of iron deficiency anemia on cell-mediated and humoral immunity in children: a case control study. *J Nat Sci Biology, Med* **5**, 158–163.
47. Frost JN, Tan TK, Abbas M, *et al.* (2021) Hepcidin-mediated hypoferremia disrupts immune responses to vaccination and infection. *Med* **2**, 164–179.e12.
48. Pandey A, Bringel F & Meyer J-M (1994) Iron requirement and search for siderophores in lactic acid bacteria. *Appl Microbiol Biotechnol* **40**, 735–739.
49. Zimmermann MB, Chassard C, Rohner F, *et al.* (2010) The effects of iron fortification on the gut microbiota in African children: a randomized controlled trial in Cote d'Ivoire. *Am J Clin Nutr* **92**, 1406–1415.
50. Dostal A, Chassard C, Hilty FM, *et al.* (2012) Iron depletion and repletion with ferrous sulfate or electrolytic iron modifies the composition and metabolic activity of the gut microbiota in rats. *J Nutr* **142**, 271–277.
51. Jaeggi T, Kortman GA, Moretti D, *et al.* (2014) Iron fortification adversely affects the gut microbiome, increases pathogen abundance and induces intestinal inflammation in Kenyan infants. *Gut* **64**, 731–742.
52. Werner T, Wagner SJ, Martínez I, *et al.* (2011) Depletion of luminal iron alters the gut microbiota and prevents Crohn's disease-like ileitis. *Gut* **60**, 325–333.
53. Dostal A, Lacroix C, Pham VT, *et al.* (2014) Iron supplementation promotes gut microbiota metabolic activity but not

https://doi.org/10.1017/S0954422424000118 Published online by Cambridge University Press

colitis markers in human gut microbiota-associated rats. *Br J Nutr* **111**, 2135–2145.
53a. Dostal A, Baumgartner J, Riesen N, *et al.* (2014) Effects of iron supplementation on dominant bacterial groups in the gut, faecal SCFA and gut inflammation: a randomised, placebo-controlled intervention trial in South African children. *Br J Nutr* **112**, 547–556.
54. Owolabi AJ, Senbanjo IO, Oshikoya KA, *et al.* (2021) Multi-nutrient fortified dairy-based drink reduces anaemia without observed adverse effects on gut microbiota in anaemic malnourished Nigerian toddlers: a randomised dose–response study. *Nutrients* **13**, 1566.
55. Gibson GR, Hutkins R, Sanders ME, *et al.* (2017) Expert consensus document: the international scientific association for probiotics and prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nat Rev Gastroenterology Hepatol* **14**, 491–502.
56. Roberfroid M, Gibson GR, Hoyles L, *et al.* (2010) Prebiotic effects: metabolic and health benefits. *Br J Nutr* **2**, S1–63.
57. Davani-Davari D, Negahdaripour M, Karimzadeh I, *et al.* (2019) Prebiotics: definition, types, sources, mechanisms, and clinical applications. *Foods* **8**, 92.
58. Gibson GR, Scott KP, Rastall RA, *et al.* (2010) Dietary prebiotics. *Food Sci Technol Bull Funct Foods* **7**, 1–19.
59. Miqdady M, Al Mistarihi J, Azaz A, *et al.* (2020) Prebiotics in the infant microbiome: the past, present, and future. *Nat Rev Gastroenterology Hepatol Pediatr* **23**, 1–14.
60. Adamberg S, Sumeri I, Uusna R, *et al.* (2014) Survival, synergistic growth of mixed cultures of bifidobacteria, lactobacilli combined with prebiotic oligosaccharides in a gastrointestinal tract simulator. *Microb Ecol Health Dis* **25**, 23062.
61. Parnell J & Reimer R (2012) Prebiotic fibres dose-dependently increase satiety hormones and alter Bacteroidetes and Firmicutes in lean and obese JCR:LA-cp rats. *Br J Nutr* **107**, 601–613.
62. Wasilewski A, Zielińska M, Storr M, *et al.* (2015) Beneficial effects of probiotics, prebiotics, synbiotics, and psychobiotics in inflammatory bowel disease. *Inflamm Bowel Dis* **21**, 1674–1682.
63. Bode L, Contractor N, Barile D, *et al.* (2016) Overcoming the limited availability of human milk oligosaccharides: challenges and opportunities for research and application. *Nutr Rev* **74**, 635–644.
64. Ninonuevo MR & Lebrilla CB (2009) Mass spectrometric methods for analysis of oligosaccharides in human milk. *Nutr Rev* **2**, S216–226.
65. Jost T, Lacroix C, Braegger C, *et al.* (2015) Impact of human milk bacteria and oligosaccharides on neonatal gut microbiota establishment and gut health. *Nutr Rev* **73**, 426–437.
66. McGuire MK, Meehan CL, McGuire MA, *et al.* (2017) What's normal? Oligosaccharide concentrations and profiles in milk produced by healthy women vary geographically. *Am J Clin Nutr* **105**, 1086–1100.
67. Hoashi M, Meche L, Mahal LK, *et al.* (2016) Human milk bacterial and glycosylation patterns differ by delivery mode. *Reprod Sci* **23**, 902–907.
68. Davis JC, Lewis ZT, Krishnan S, *et al.* (2017) Growth and morbidity of gambian infants are influenced by maternal milk oligosaccharides and infant gut microbiota. *Sci Rep* **7**, 40466.
69. Uwaezuoke SN, Eneh CI & Ndu IK (2017) Relationship between exclusive breastfeeding and lower risk of childhood obesity: a narrative review of published evidence. *Clin Med Insights Pediatr* **11**, 1179556517690196.
70. Alderete TL, Autran C, Brekke BE, *et al.* (2015) Associations between human milk oligosaccharides and infant body composition in the first 6 months of life. *Am J Clin Nutr* **102**, 1381–1388.
71. Charbonneau MR, O'Donnell D, Blanton LV, *et al.* (2016) Sialylated milk oligosaccharides promote microbiota-dependent growth in models of infant undernutrition. *Cell* **164**, 859–871.
72. Umu ÖCO, Rudi K & Diep DB (2017) Modulation of the gut microbiota by prebiotic fibres and bacteriocins. *Microb Ecol Health Dis* **28**, 1348886.
73. Souza DDS, Tahan S, Weber TK (2018) Randomized, double-blind, placebo-controlled parallel clinical trial assessing the effect of fructooligosaccharides in infants with constipation. *Nutrients* **10**, 1602.
74. Dou Y, Yu X, Luo Y, *et al.* (2022) Effect of fructooligosaccharides supplementation on the gut microbiota in human: a systematic review and meta-analysis. *Nutrients* **14**, 3298.
75. Koh A, De Vadder F, Kovatcheva-Datchary P, *et al.* (2016) From dietary fiber to host physiology: short-chain fatty acids as key bacterial metabolites. *Cell* **165**, 1332–1345.
76. den Besten G, van Eunen K, Groen AK, *et al.* (2013) The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *J Lipid Res* **54**, 2325–2340.
77. Ansell J, Parkar S, Paturi G, *et al.* (2013) Modification of the colonic microbiota. *Adv Food Nutr Res* **68**, 205–217.
78. Rivière A, Selak M, Lantin D, *et al.* (2016) Bifidobacteria and butyrate-producing colon bacteria: importance and strategies for their stimulation in the human gut. *Front Microbiol* **7**, 979.
79. Kuo SM (2013) The interplay between fiber and the intestinal microbiome in the inflammatory response. *Adv Nutr* **4**, 16–28.
80. de Vrese M & Schrezenmeir J (2008) Probiotics, prebiotics, and synbiotics. *Adv Biochem Eng/Biotechnol* **111**, 1–66.
81. Ahmad AMR, Ahmed W, Iqbal S, *et al.* (2021) Iahtisham ul H. prebiotics and iron bioavailability? Unveiling the hidden association – a review. *Trends Food Sci Technol* **110**, 584–590.
82. Husmann FMD, Stierli L, Bräm DS, *et al.* (2022) Kinetics of iron absorption from ferrous fumarate with and without galacto-oligosaccharides determined from stable isotope appearance curves in women. *Am J Clin Nutr* **115**, 949–957.
83. Mugambi MN, Musekiwa A, Lombard M, *et al.* (2012) Synbiotics, probiotics or prebiotics in infant formula for full term infants: a systematic review. *Nutr J* **11**, 81.
84. Nicolucci AC, Hume MP, Martínez I, *et al.* (2017) Prebiotics reduce body fat and alter intestinal microbiota in children who are overweight or with obesity. *Gastroenterology* **153**, 711–722.
85. Sheridan PO, Bindels LB, Saulnier DM, *et al.* (2014) Can prebiotics and probiotics improve therapeutic outcomes for undernourished individuals? *Gut Microbes* **5**, 74–82.
86. Gibson G, McCartney A & Rastall R (2005) Prebiotics and resistance to gastrointestinal infections. *Br J Nutr* **93**, S31–S34.
87. Shoaf K, Mulvey GL, Armstrong GD, *et al.* (2006) Prebiotic galactooligosaccharides reduce adherence of enteropathogenic escherichia coli to tissue culture cells. *Infect Immun* **74**, 6920–6928.
88. Braegger C, Chmielewska A, Decsi T, *et al.* (2011) Supplementation of infant formula with probiotics,/or prebiotics: a systematic review, comment by the ESPGHAN committee on nutrition. *J Pediatr Gastroenterol Nutr* **52**, 238–250.
89. Paganini D, Uyoga MA, Kortman GAM, *et al.* (2017) Prebiotic galacto-oligosaccharides mitigate the adverse effects of iron fortification on the gut microbiome: a randomised controlled study in Kenyan infants. *Gut* **66**, 1956–1967.
90. Paganini D, Uyoga MA, Cercamondi CI, *et al.* (2017) Consumption of galacto-oligosaccharides increases iron

https://doi.org/10.1017/S0954422424000118 Published online by Cambridge University Press

absorption from a micronutrient powder containing ferrous fumarate and sodium iron EDTA: a stable-isotope study in Kenyan infants. *Am J Clin Nutr* **106**, 1020–1031.
91. Mikulic N, Uyoga MA, Paganini D, *et al.* (2021) Consumption of a single dose of prebiotic galacto-oligosaccharides does not enhance iron absorption from micronutrient powders in kenyan infants: a stable iron isotope study. *J Nutr* **151**, 1205–1212.
92. EFSA (2014) Scientific opinion on the essential composition of infant and follow-on formulae. *Eur Food Saf Authority J* **12**, 3760.
93. Civardi E, Garofoli F, Longo S, *et al.* (2017) Safety, growth, and support to healthy gut microbiota by an infant formula enriched with functional compounds. *Clin Nutr* **36**, 238–245.
94. Monira S, Shabnam SA, Alam NH, *et al.* (2012) 16S rRNA gene-targeted TTGE in determining diversity of gut microbiota during acute diarrhoea and convalescence. *J Health Popul Nutr* **30**, 250–256.
95. Ma C, Wu X, Nawaz M, *et al.* (2011) Molecular characterization of faecal microbiota in patients with viral diarrhea. *Curr Microbiol* **63**, 259–266.
96. Solano-Aguilar G, Fernandez KP, Ets H, *et al.* (2013) Characterization of faecal microbiota of children with diarrhea in 2 locations in Colombia. *J Pediatr Gastroenterol Nutr* **56**, 503–511.
97. Kapiki A, Costalos C, Oikonomidou C, *et al.* (2007) The effect of a fructo-oligosaccharide supplemented formula on gut flora of preterm infants. *Early Hum Dev* **83**, 335–339.
98. Vandenplas Y, Zakharova I & Dmitrieva Y (2015) Oligosaccharides in infant formula: more evidence to validate the role of prebiotics. *Br J Nutr* **113**, 1339–1344.
99. Closa-Monasterolo R, Gispert-Llaurado M, Luque V, *et al.* (2013) Safety and efficacy of inulin and oligofructose supplementation in infant formula: results from a randomized clinical trial. *Clin Nutr* **32**, 918–927.
100. Holscher HD, Faust KL, Czerkies LA, *et al.* (2012) Effects of prebiotic-containing infant formula on gastrointestinal tolerance and faecal microbiota in a randomized controlled trial. *J Parenter Enteral Nutr* **36**, 95s–105s.
101. Lohner S, Jakobik V, Mihályi K, *et al.* (2018) Inulin-type fructan supplementation of 3- to 6-year-old children is associated with higher faecal bifidobacterium concentrations and fewer febrile episodes requiring medical attention. *J Nutr* **148**, 1300–1308.
102. Bruzzese E, Volpicelli M, Squeglia V, *et al.* (2009) A formula containing galacto- and fructo-oligosaccharides prevents intestinal and extra-intestinal infections: an observational study. *Clin Nutr* **28**, 156–161.
103. Costalos C, Kapiki A, Apostolou M, *et al.* (2008) The effect of a prebiotic supplemented formula on growth and stool microbiology of term infants. *Early Hum Dev* **84**, 45–49.
104. Soldi S, Vasileiadis S, Lohner S, *et al.* (2019) Prebiotic supplementation over a cold season, during antibiotic treatment specifically modulates the gut microbiota composition of 3–6 year-old children. *Benef Microbes* **10**, 253–263.

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