The Effects of Iron Supplementation and Fortification on the Gut Microbiota: A Review

Emma CL Finlayson-Trick 1, Jordie AJ Fischer 2,3,*, David M Goldfarb 1,3,4 and Crystal D Karakochuk 2,3,*

1 Faculty of Medicine, University of British Columbia, Vancouver, BC V6T 1Z3, Canada; efinlaysontrick@alumni.ubc.ca (E.C.F.-T); david.goldfarb@cw.bc.ca (D.M.G.)
2 Department of Food, Nutrition and Health, University of British Columbia, Vancouver, BC V6T 1Z4, Canada; jordie.fischer@ubc.ca
3 British Columbia Children’s Hospital Research Institute, Vancouver, BC V5Z 4H4, Canada
4 Department of Pathology and Laboratory Medicine, BC Children’s and Women’s Hospital and University of British Columbia, Vancouver, BC V6T 1Z7, Canada
* Correspondence: crystal.karakochuk@ubc.ca

Received: 30 August 2020; Accepted: 24 September 2020; Published: 26 September 2020

Abstract: Iron supplementation and fortification are used to treat iron deficiency, which is often associated with gastrointestinal conditions, such as inflammatory bowel disease and colorectal cancer. Within the gut, commensal bacteria contribute to maintaining systemic iron homeostasis. Disturbances that lead to excess iron promote the replication and virulence of enteric pathogens. Consequently, research has been interested in better understanding the effects of iron supplementation and fortification on gut bacterial composition and overall gut health. While animal and human trials have shown seemingly conflicting results, these studies emphasize how numerous factors influence gut microbial composition. Understanding how different iron formulations and doses impact specific bacteria will improve the outcomes of iron supplementation and fortification in humans. Furthermore, discerning the nuances of iron supplementation and fortification will benefit subpopulations that currently do not respond well to treatment.

Keywords: iron supplementation; gut microbiome; iron metabolism; gastrointestinal homeostasis

1. Introduction

The majority of living organisms require iron for survival. Iron can exist in one of two oxidation states, and due to this redox potential, can function in several fundamental processes, such as respiration, DNA replication, energy production, and cellular proliferation [1]. Humans absorb iron from their diet in a dynamic, tightly regulated process within the intestine [2]. In addition to controlling the amount of iron absorbed, this process dictates iron availability for the complex community of bacteria living in the intestine, hereafter referred to as the gut microbiota. As such, many bacteria have developed sophisticated systems to obtain, store, and regulate iron. Iron deficiency and excess both impact gut microbial health and lead to diseases, such as iron deficiency anemia and iron overload, respectively. Iron deficiency is highly prevalent worldwide and is commonly treated with oral iron supplements and fortificants [3]. In this review, we cover the effects of oral iron supplementation and fortification on gut health and disease. We begin with an overview of how the body acquires and utilizes iron. Then, we discuss the complex relationship between iron homeostasis and the gut microbiome. Finally, we summarize the microbial changes that occur following iron supplementation and fortification in animal and human trials, and we identify areas in need of continued research. In this literature review, we used PubMed and MEDLINE databases to search for articles related to...
“human iron metabolism”, “bacterial iron metabolism”, “iron and gut flora”, and “the effects of iron on the gut microbiota/microbiome in animals and/or humans”.

2. Overview of Iron Absorption

Humans lose approximately 0.5–2 mg of iron every day from skin cell desquamation, intestinal epithelial cell (IEC) sloughing, and urine and sweat production [4]. Additional iron may also be lost during specific physiological processes, such as menstruation and lactation [5]. To balance this loss, the human duodenum and proximal jejunum absorb approximately 2 mg of dietary iron daily, a small proportion of the total daily dietary intake [6,7]. Iron from the diet is found primarily as heme, derived from myoglobin and hemoglobin, or nonheme iron, derived from plants and iron-fortified foods [6]. Nonheme iron exists in two forms as reduced ferrous iron or oxidized ferric iron. IECs, known as enterocytes, can absorb only ferrous iron [Figure 1]. As such, ferric iron is reduced to ferrous iron by the membrane-bound ferric reductase duodenal cytochrome B (Dcytb) that is expressed on the apical brush border membrane of IECs [8]. Once in the ferrous form, iron is transported across the apical membrane of enterocytes by the 12 transmembrane domain protein, divalent metal transporter 1 (DMT1, also known as Nramp2) [9]. Within enterocytes, iron is stored in ferritin, used in a variety of cellular processes, or transported into systemic circulation by crossing the basolateral membrane through the 12 transmembrane domain protein, ferroportin [10]. Ferroportin is also expressed on macrophages and hepatocytes [10]. On the basolateral membrane, hephaestin oxidizes ferrous iron to ferric iron, enabling the transportation of iron in the blood by transferrin [5]. In comparison to nonheme iron, heme absorption remains enigmatic [11]. There are two current hypotheses for intestinal heme absorption: Either heme is endocytosed from the apical membrane or transported through a specific receptor into the cytosol [12].

![Figure 1. Absorption of nonheme iron by intestinal epithelial cells (IECs). Ferric iron is first reduced to ferrous iron by duodenal cytochrome B (Dcytb) on the apical membrane. Then, ferrous iron is transported across the apical membrane by divalent metal transporter 1 (DMT1). Once inside the cell, iron is stored in ferritin, transported across the basolateral membrane by ferroportin, or used in a variety of cellular processes. After transport across the basolateral membrane, ferrous iron is oxidized to ferric iron by hephaestin. Ferric iron is then transported by transferrin in circulation. Iron absorption is reduced when hepcidin binds to ferroportin because hepcidin causes the internalization and degradation of ferroportin. The figure created with www.BioRender.com.](image-url)
3. Maintenance of Systemic Iron Homeostasis

Humans have no active iron excretory mechanism; therefore, systemic iron homeostasis is primarily regulated at the point of absorption. Hepcidin, a peptide hormone produced by the liver, is considered the master regulator of systemic iron homeostasis [13]. Hepcidin binds to and degrades ferroportin, which consequently impacts how iron is recycled by macrophages, absorbed by IECs, and stored by hepatocytes [14,15]. Hepcidin expression is upregulated when iron stores are adequate or high, or in response to inflammation, infection, or injury. Conversely, hepcidin expression is downregulated to improve iron absorption when iron stores are low, as in the case of iron deficiency or instances of certain genetic hemoglobinopathies, such as β-thalassemia [6,16,17]. As an aside, low hepcidin levels, as seen in some thalassemias and hereditary hemochromatosis, increase the risk of iron overload, due to increased intestinal iron absorption [18,19]. In hereditary hemochromatosis, a disease related to mutations in iron metabolism genes, excess iron is deposited throughout the body in the heart, pancreas, and liver, as well as the skin and joints [20]. Regardless of etiology, iron overload can result in several different diseases related to specific organ damage from oxidative stress. Iron overload also is known to increase the risk of infection.

Altered hepcidin levels are also associated with a variety of gastrointestinal conditions, including colorectal cancer (CRC) and inflammatory bowel disease (IBD). In CRC, hepcidin production is increased, which enables the tumor to retain more iron as tumoral ferroportin expression is decreased [21]. Intratumoral iron promotes oncogene activation, inflammation, and tumor growth [21]. In comparison, hepcidin levels in IBD do not follow a clear pattern, despite many patients with active disease experiencing reduced iron absorption [22–25]. Nevertheless, when examined using a dextran sulfate sodium (DSS) induced colitis mouse model, hepcidin levels were found to be reduced [26]. For both CRC and IBD, current research is focused on developing treatments and management strategies that reduce hepcidin levels. Vitamin D administration and anti-TNF-α monoclonal antibody therapy, for example, have shown promising results for the treatment and management of anemia in IBD [23,27].

4. Role of the Gut Microbiota in Maintaining Iron Homeostasis

Along the length of the intestine, there are physiological gradients (e.g., pH, oxygen, nutrient, etc.) that produce not only distinct bacterial habitats, but also influence the solubility and availability of iron [2]. The iron that is not absorbed by the duodenum passes into the colon, where it is thought to be metabolized by gut bacteria, as well as other microorganisms, such as parasites and fungi [1,28]. Iron is known to be a growth-limiting nutrient for both human cells and bacteria alike [29]. Accordingly, bacteria have developed two main strategies to obtain iron from their environment. The most prevalent mechanism involves the synthesis and secretion of siderophores, which are high-affinity ferric iron chelators [30]. *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Mycobacterium tuberculosis* are just some of the bacteria that use siderophores to acquire iron [31,32]. In addition to benefiting the bacterium, siderophores also appear to assist the host. As an example, Qi and Han (2018) observed in their study that enterobactin, an archetypical siderophore produced by many Gram-negative bacteria, promoted mitochondrial iron uptake and homeostasis by binding to the ATP synthase α subunit [33]. The results from this study emphasize that the battle for iron between microbes and host is far more complicated than currently understood, and opens the field for continued exploration of cooperative iron-mediated host-microbe interactions. The second strategy involves specific receptors that enable bacteria to acquire iron directly from host proteins, such as heme, transferrin, and lactoferrin [34]. Finally, some potentially beneficial gut bacteria, such as *Lactobacillus plantarum*, do not require iron at all but instead depend on manganese [35]. Therefore, in the presence of iron, iron-independent bacteria do not increase at a rate proportional to iron-dependent (and possibly pathogenic) bacteria [36].

Commensal gut bacteria play a vital role in maintaining iron homeostasis. In a recent study by Das et al. (2020), gut microbial metabolites were shown to suppress intestinal hypoxia-inducible factor 2-α activity (a master transcription factor of intestinal iron absorption) and upregulate ferritin
expression [37]. Additionally, Das et al. (2020) identified *Lactobacillus* species as the key sensors of intestinal iron [37]. Several studies have so far attempted to characterize the relationship between lactobacilli and iron. Using a mouse knockout model for iron regulatory protein 2, which increases fecal iron concentration, Buknik-Rosenblau et al. (2012) observed an increase in fecal *Lactobacillus* species abundance [38]. It remains to be seen whether the increase in lactobacilli was due to greater iron bioavailability or due to changes in the bacterial community that allowed lactobacilli to proliferate. Nevertheless, the work by Buknik-Rosenblau et al. (2012) demonstrates that deletions or mutations of iron metabolism genes affect the intestinal bacterial composition, which has clinically important implications. In human trials, a study by Balamurugan et al. (2010) found that young women in South India with iron deficiency had a low abundance of gut lactobacilli [39]. In comparison, the study by Kalipatnapu et al. (2017) observed an inverse relationship between fecal iron concentration and *Lactobacillus* species in rural children in India [40]. Regardless of the exact mechanism, several groups are testing whether specific *Lactobacillus* species improve iron absorption and status. In a meta-analysis of eight studies, Vonderheld et al. (2019) observed that *Lactobacillus plantarum* 299v significantly improved nonheme dietary iron absorption in humans [41]. Improvement in iron absorption may be due to an increase in Dcytb activity as Sandberg et al. (2018) observed activation of this axis following treatment with an *L. plantarum* 299v supplement in their human intestinal co-culture model of enterocytes and goblet cells [42]. In contrast, Rosen et al. found that *L. plantarum* 299v did not enhance iron absorption in iron-deficient pediatric patients treated with ferrous sulfate [43]. To improve the efficacy of *L. plantarum* 299v as a probiotic, future studies need to further assess the effects of dose, formulation, the timing of administration, and diet. In addition to *Lactobacillus*, other bacteria have been examined for their probiotic properties, and those studies are summarized in the recent review by Rusu et al. (2020) [44]. The use of prebiotics and synbiotics for the treatment of iron deficiency is also summarized in the same Rusu et al. (2020) review.

When the gut bacterial composition is altered or when gut bacteria are absent, iron homeostasis is disturbed. In experiments using germ-free mice [45] and rats [46], iron uptake and storage was reduced within IECs. In their study, Deschemin et al. (2016) also reported a decrease in ferroportin expression by IECs in germ-free mice, providing a mechanism for the observed reduction in iron absorption [45]. Similarly, iron absorption was reduced in rats [47] and rabbits [48] treated with antibiotics. These results, however, seem to conflict with a more recent study in mice that found iron absorption increased following antibiotic treatment [37]. These findings suggest that antibiotic administration may improve iron absorption in patients with iron deficiency.

While gut bacteria are important for maintaining systemic iron homeostasis, iron can also promote the replication and virulence of enteric pathogens, such as *Salmonella* spp., *Shigella* spp., and *Campylobacter* spp. [49,50]. When iron is abundant, bacteria proliferate, and form biofilms readily, which is hypothesized to be one of the reasons why individuals with iron overload are more susceptible to infection [7]. It has been shown that humans with iron overload syndromes, including hemochromatosis and refractory anemias are more susceptible to bacterial infections, including *Yersinia* spp., *Listeria monocytogenes*, and *Vibrio vulnificus* [51–53]. Iron limitation, therefore, serves as an innate immune defence mechanism termed “nutritional immunity” [54]. Mediated by hepcidin, iron withholding strategies, such as hypoferraemia, denies iron to invading pathogens [55]. As an aside, iron retention within cells, such as macrophages, promotes the virulence of intracellular pathogens like *Salmonella enterica* [56]. Parmanand et al. (2019) observed in their in vitro colonic fermentation study that iron chelation resulted in a lower relative abundance of potentially pathogenic bacteria [57]. Similarly, Kortman et al. (2015) observed using a mouse model that dietary iron limitation reduced disease pathology upon oral challenge with *Citrobacter rodentium*, a well-established model for infectious gastroenteritis [58]. Finally, some of the pathogens that benefit from increased iron availability are procarcinogenic [59]. Specifically, *Streptococcus bovis*, *Bacteroides*, *Enterococcus faecalis*, and *Clostridia* are all implicated in carcinogenesis as these bacteria promote inflammation through the production of genotoxic metabolites [60]. These gut microbiota can contribute to the start and/or the
progression of colorectal cancer. With this knowledge in mind, there needs to be a reassessment as to whether providing oral iron therapy to colorectal cancer patients with iron deficiency is the best route of administration.

5. Effect of Iron Supplementation and Fortification on the Gut Microbiota

Iron supplementation and fortification are two different methods used to address iron deficiency. Iron supplementation is considered the more effective method, while iron fortification is often considered the safer method (as iron is delivered in smaller doses and is more amenable to physiological uptake when combined with food) [61]. While supplementation is a population-specific approach, iron-fortified foods, such as cereal products, milk, meal replacements, and infant foods, is a public health strategy to enhance the nutritional quality of diets in a population.

Iron salts are widely used in oral iron supplementation programs. As an inexpensive iron supplement, ferrous sulfate (FeSO₄) is one of the most commonly used iron salts. Unfortunately, ferrous sulfate is well known to irritate the stomach lining, causing gastrointestinal side effects, including stomach pain, nausea, diarrhea, and constipation, which makes supplementation adherence challenging [62,63]. Ferrous gluconate, another type of iron salt, appears to have fewer side-effects than ferrous sulfate [64]. Iron absorption from oral supplements is typically low, with less than 20% absorbed in the duodenum and the remainder passing unabsorbed into the colon [65]. Of the commonly used iron supplements, ferrous fumarate has the most iron per gram [64]. Emerging iron preparations, such as ferrous bisglycinate are marketed as having greater gastrointestinal tolerance, bioavailability, and protection against dietary iron inhibitors (e.g., phytates), as iron amino acid chelate does not form insoluble compounds with substances containing high content of iron absorption inhibitors, commonly found in cereal-based foods [66,67]. Nagpal and Choudhury (2004) previously conducted a comprehensive review of ferrous salts, ferric salts, iron amino acid chelates, iron polymaltose complex, and carbonyl iron; therefore, this review will not discuss the characteristics of these iron forms [68]. In comparison to iron supplementation, there are three primary forms of iron fortification: The fortificant can be added during food processing (e.g., flour) or during food preparation (e.g., multiple micronutrient powders—MNPs), or food can be genetically engineered to contain more iron (e.g., biofortified cereals) [17,69–71].

5.1. Animal Studies

In animal studies, microbial composition and metabolite production are altered by varying colonic iron availability. Mice weaned onto iron-deficient diets for eight weeks experienced a decrease in microbial richness compared to their baseline measurements and the control diet group [72]. Furthermore, iron repletion could not fully restore microbial richness [72]. In rats, iron deprivation increased Lactobacillus species while concomitantly reducing Bacteroides species and Roseburia species/Eubacterium rectale [73]. These compositional changes were associated with decreased levels of fecal propionate and butyrate (two types of short-chain fatty acids [SCFAs] used to fuel the gut) and were partially restored following ferrous iron repletion [73]. Roseburia species are found in a high abundance within the gut microbiota and are significant butyrate producers [74]. Similar findings were reported by Dostal et al. (2012) in their in vitro colonic fermentation models inoculated with immobilized fecal microbiota from a child [75]. Under very low iron conditions (0.9 mg Fe/L), Roseburia species/E. rectale decreased, as did butyrate levels, while Lactobacillus species increased [75]. The results from this experiment were subsequently confirmed in another Dostal et al. (2015) study that additionally showed that Roseburia intestinalis grown in low iron conditions preferentially produced lactate over butyrate [76]. Under high iron conditions, R. intestinalis increased expression of genes involved in butyrate production compared to results from the normal iron condition [76]. Another study in iron deprived rats also observed an increase in lactobacilli, in addition to total fecal anaerobes and Enterococcus species [77]. In comparison to iron deprivation, iron supplementation enabled bacterial taxa within the Clostridia class to proliferate in mice [78]. It should be noted that while iron
metabolism in mice is similar in many ways to humans, mice do not absorb iron, as well as humans; mice lose more iron relative to what they store, and thus, derive ~50% of their daily iron turnover from their diet, and mice have a more active iron excretory system that humans [78,79]. The fact that bacteria respond to iron bioavailability has led some researchers to pursue whether changes in fecal bacterial composition could provide unintrusive hints towards host iron status. Liu et al. (2020) showed in mice that five key bacterial taxa (Porphyromonadaceae Parabacteroides, Clostridiales Peptostreptococcaceae, Akkermansia muciniphila, Clostridium perfringens, and Clostridia Clostridiales) could be used as biomarkers to predict tissue iron levels in the small intestine and the liver ($R^2 = 99.7\%$ and 99.6\%, respectively) [80]. Still, to be validated in humans, this method potentially offers a non-invasive way to diagnose iron-related diseases and monitor nutritional intervention. Not all studies, however, have observed changes to bacterial composition following changes to the availability of iron. Work by Alexeev et al. (2017) found that iron supplementation had no profound impact on individual rat pup bacterial operational taxonomic units [81].

Several studies have also examined the impact of iron supplementation on gut bacteria in animal models of IBD. Mahalhal et al. (2018) observed in their DSS-treated mouse model that any changes to dietary iron concentration (either an increase or decrease from standard) exacerbated the severity of colitis [82]. Interestingly, DSS-treated mice fed high iron diets did not lose as much weight as mice fed low iron diets, but had worse intestinal inflammation as measured by fecal calprotectin [82]. These same mice fed high iron diets experienced an increase in Proteobacteria, and a concomitant decrease in Firmicutes and Bacteroidetes [82]. Within the gut, Firmicutes are the most predominant producers of SCFAs, which have been shown to have anti-inflammatory properties [83]. In their DSS mouse model, Constante et al. (2017) observed worsened colitis in mice fed the iron preparation known as FEDTA (ferrous sulfate, ferrous bisglycinate, and ferric ethylenediaminetetraacetic acid) in comparison to the mice fed ferrous bisglycinate [84]. These mice had a reduced abundance of Roseburia species, which are known butyrate producers (a type of SCFA) [84]. Constante et al. (2017) also observed that iron supplementation with iron sulfate had a modest yet significant protective effect in mice treated with DSS by increasing survival [84]. These results are supported by research that shows that the severity of trinitrobenzene sulfonic acid-induced colitis can be reduced with oral ferric iron oral [85]. Still, these results conflict with research showing that ileitis in mice can be prevented by depleting luminal iron [86]. The discrepancy between studies may be a result of the different animal models as TNF-alpha ΔARE mice are susceptible to ileitis [86]; whereas, DSS- and trinitrobenzene sulfonic acid-treatment in mice results in colitis [84,85]. Werner et al. (2011) also observed that iron repletion by way of injection maintained the protective effect of the iron sulfate free diet, suggesting a role for luminal iron in the pathogenesis of chronic ileitis [86].

5.2. Human Studies

Iron supplementation and fortification have varying effects on human gut bacterial composition. Dostal et al. (2014) observed that South African children with iron deficiency who received high-dose iron supplements for 38 weeks (50 mg iron as FeSO$_4$ for four days/week) did not have significantly different concentrations of dominant gut bacterial groups or fecal SCFAs compared to children with no iron deficiency [87]. Similarly, Tang et al. (2016) observed in their study with American infants that iron supplementation did not create a more pathogenic microbial profile, rather, the abundance of Escherichia species decreased [88]. Likewise, Nitert et al. (2018) found no significant difference in the fecal microbiome at any taxonomic level between pregnant women receiving low-dose (0–10 mg Fe/day) or high-dose (>60 mg Fe/day) iron supplements [89]. Contrasting these studies, Jaeggi et al. (2015) and Tang et al. (2017) noted increased pathogen abundance in Kenyan infants receiving iron-containing micronutrient powder (12.5 mg/day) [90,91]. In healthy, non-anemic Swedish infants, consumption of high-iron formula (6.6 mg Fe/day) for 45 days did not increase the growth of pathogenic bacteria; however, the relative abundance of Bifidobacterium decreased [92]. In the same study, infants who received iron drops (6.6 mg Fe/day) had a lower relative abundance of Lactobacillus species than
infants who received high-iron formula. Despite the comparable doses, this study suggests that form of administration (i.e., as formula vs. drop) differentially influences gut microbial composition. Furthermore, as iron drops lead to a decrease in lactobacilli, which are important commensal bacteria, iron drops may increase susceptibility to infection. The varying microbial responses to iron reinforce the idea that multiple factors influence the gut bacterial composition. Future studies need to analyze how varying concentrations of iron influence fecal bacteria diversity and abundance. In addition, ethnicity and geography can influence gut microbial composition; therefore, future studies also need to investigate more disparate human populations [93]. These investigations will contribute to the developing field of microbiome-based personalized medicine [94]. Finally, another avenue for future research is examining the association between iron supplementation and exacerbation of infections such as malaria. In the infamous Pemba trial, iron supplementation in a malaria-endemic area was shown to increase the incidence of severe adverse events, including hospitalizations, due to malaria and other infections [95]. The potential mechanism for the worsening of malaria infection is thought to be excessive iron suppressing ferroportin, an iron exporter preventing iron excess in red blood cells, protecting against infection [96].

Human studies have also focused on characterizing the effects of iron supplementation and fortification on gut health as measured by inflammatory markers and incidence of diarrhea. Dostal et al. (2014) observed that the iron-deficient children on iron supplementation (study described in the previous paragraph) did not experience an increase in gut inflammation [87]. In contrast, Zimmermann et al. (2010) found that anemic children in Côte d’Ivoire who were iron-fortified biscuits (2 biscuits containing 20 mg Fe 4 times/week) presented with increased levels of fecal calprotectin, indicating increased levels of gastrointestinal inflammation. Several other studies in infants and toddlers from around the world also demonstrated increased intestinal inflammation following iron supplementation or fortification [88,90,97,98]. In their systematic review, Chanchi et al. (2019) examined 19 studies to evaluate the impact of oral iron supplementation and fortification on diarrhea incidence among children aged 4–59 months [99]. In 12 of the 19 studies, they found iron not to affect diarrheal incidence [90,100–110]. In the remaining studies, four recorded a significant increase in diarrheal incidence [111–114], and three recorded an increase within a specific subpopulation [115–117]. While most studies suggest iron supplementation and fortification do not induce diarrhea, there are two leading hypotheses to explain the sometimes-observed effect. Firstly, iron can produce reactive oxygen species within the intestine (through Haber-Weiss and Fenton reactions), which can cause intestinal damage and lead to inflammatory diarrhea [118]. This hypothesis is supported by in vitro experiments where intestinal epithelial cell lose their integrity following iron exposure [119,120]. Secondly (as previously discussed), iron can alter gut bacterial composition creating a more inflammatory environment [90,121]. In their review, Lönnerdal (2017) describes the other effects excess iron can have on children, such as impairing cognitive and motor development [29].

6. Conclusions

Iron supplementation and fortification studies have demonstrated that there are several factors, including diet, hygiene, inflammation status, disease burden, and genetics, that influence the complex interplay between iron and the gut. As the need for effective treatments for iron deficiency is indisputable, there are three main areas to explore related iron supplementation and fortification. Firstly, due to the previously mentioned complex interplay, future studies could benefit from a more thorough assessment and description of the study population to identify patterns and compare populations. Secondly, as newer iron preparations have been shown to have higher bioavailability and have been associated with fewer gastrointestinal side effects, there is a need to investigate the effects of varying doses, periodicity, and forms of iron supplementation on the gut microbiome. Similarly, as iron fortification within complex dietary matrices needs to overcome iron inhibitors, there is a need to examine the potential effects of iron-fortified foods on the gut microbiota. Finally, while this review has focused on oral iron supplementation and fortification, future reviews should assess how gut
microbial health is impacted by other iron therapies, including iron delivered intravenously and through biofortification.

**Author Contributions:** E.C.F.-T. developed the concept for this review; E.C.F.-T. and J.A.F. drafted the manuscript; E.C.F.-T. developed the figures; D.M.G. and C.D.K. provided revisions until the final version; All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.

**Conflicts of Interest:** The authors declare no conflict of interest.

**References**

1. Cassat, J.E.; Skaar, E.P. Iron in Infection and Immunity. *Cell Host Microbe* 2013, 13, 509–519. [CrossRef] [PubMed]
2. Yilmaz, B.; Li, H. Gut Microbiota and Iron: The Crucial Actors in Health and Disease. *Pharmaceuticals* 2018, 11, 98. [CrossRef] [PubMed]
3. James, S.L.; Abate, D.; Abate, K.H.; Abay, S.M.; Abbafati, C.; Abbasi, N.; Abbastabar, H.; Abd-Allah, F.; Abdela, J.; Abdelalim, A.; et al. Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries in 195 countries and territories, 1990–2017: A systematic analysis for the Global Burden of Disease Study 2017. *Lancet* 2018, 392, 1789–1858. [CrossRef]
4. Abbaspour, N.; Hurrell, R.; Kelishadi, R. Review on iron and its importance for human health. *J. Res. Med. Sci.* 2014, 19, 164–174. [PubMed]
5. Winder, W.E.; Bazydlo, L.A.L.; Harris, N.S. The Molecular Biology of Human Iron Metabolism. *Lab. Med.* 2014, 45, 92–102. [CrossRef]
6. Gulec, S.; Anderson, G.J.; Collins, J.F. Mechanistic and regulatory aspects of intestinal iron absorption. *Am. J. Physiol. Gastrointest. Liver Physiol.* 2014, 307, G397–G409. [CrossRef]
7. Ganz, T. Hepcidin, a key regulator of iron metabolism and mediator of anemia of inflammation. *Blood* 2003, 102, 783–788. [CrossRef]
8. McKie, A.T. An Iron-Regulated Ferric Reductase Associated with the Absorption of Dietary Iron. *Science* 2001, 291, 1755–1759. [CrossRef]
9. Garrick, M.D.; Dolan, K.G.; Horbinski, C.; Ghio, A.J.; Higgins, D.; Porubcin, M.; Moore, E.G.; Hainsworth, L.N.; Umbreit, J.N.; Conrad, M.E.; et al. DMT1: A mammalian transporter for multiple metals. *BioMetals* 2003, 16, 41–54. [CrossRef]
10. Drakesmith, H.; Nemeth, E.; Ganz, T. Ironing out Ferroportin. *Cell Metab.* 2015, 22, 777–787. [CrossRef]
11. Anderson, G.J.; Frazer, D.M. Current understanding of iron homeostasis. *Am. J. Clin. Nutr.* 2017, 106, 1559S–1566S. [CrossRef] [PubMed]
12. West, A.R.; Oates, P.S. Mechanisms of heme iron absorption: Current questions and controversies. *World J. Gastroenterol.* 2008, 14, 4101. [CrossRef] [PubMed]
13. Wallace, D.F. The Regulation of Iron Absorption and Homeostasis. *Clin. Biochem. Rev.* 2016, 37, 51–62.
14. Conway, D.; Henderson, M.A. Iron metabolism. *Anaesth. Intensive Care Med.* 2019, 20, 175–177. [CrossRef]
15. Aschemeyer, S.; Qiao, B.; Stefanova, D.; Valore, E.V.; Sek, A.C.; Ruwe, T.A.; Vieth, K.R.; Jung, G.; Casu, C.; Rivella, S.; et al. Structure-function analysis of ferroportin defines the binding site and an alternative mechanism of action of hepcidin. *Blood* 2018, 131, 899–910. [CrossRef]
16. Jones, E.; Pasricha, S.-R.; Allen, A.; Evans, P.; Fisher, C.A.; Wray, K.; Premawardhena, A.; Bandara, D.; Perera, A.; Webster, C.; et al. Hepcidin is suppressed by erythropoiesis in hemoglobin E β-thalassemia and β-thalassemia trait. *Blood* 2015, 125, 873–880. [CrossRef] [PubMed]
17. Paganini, D.; Zimmermann, M.B. The effects of iron fortification and supplementation on the gut microbiome and diarrhea in infants and children: A review. *Am. J. Clin. Nutr.* 2017, 106, 1688S–1693S. [CrossRef]
18. Taher, A.T.; Saliba, A.N. Iron overload in thalassemia: Different organs at different rates. *Hematology* 2017, 2017, 265–271. [CrossRef]
19. Papanikolaou, G.; Tzilianos, M.; Christakis, J.I.; Bogdanos, D.; Tsimirika, K.; MacFarlane, J.; Goldberg, Y.P.; Sakellaropoulos, N.; Ganz, T.; Nemeth, E. Hepcidin in iron overload disorders. *Blood* 2005, 105, 4103–4105. [CrossRef]
20. Porter, J.L.; Rawla, P. Hemochromatosis. In *StatPearls; StatPearls Publishing*: Treasure Island, FL, USA, 2020.
21. Xue, X.; Shah, Y. Intestinal Iron Homeostasis and Colon Tumorigenesis. *Nutrients* 2013, 5, 2333–2351. [CrossRef]

22. Nielsen, O.H.; Ainsworth, M.; Coskun, M.; Weiss, G. Management of Iron-Deficiency Anemia in Inflammatory Bowel Disease: A Systematic Review. *Medicine* 2015, 94, e963. [CrossRef] [PubMed]

23. Moran-Lev, H.; Galai, T.; Yerushalmy-Feler, A.; Weisman, Y.; Anafy, A.; Deutsch, V.; Cipok, M.; Lubetzky, R.; Cohen, S. Vitamin D Decreases Hepcidin and Inflammatory Markers in Newly Diagnosed Inflammatory Bowel Disease Paediatric Patients: A Prospective Study. *J. Crohns Colitis* 2019, 13, 1287–1291. [CrossRef] [PubMed]

24. Krawiec, P.; Mroczkowska-Juchkiewicz, A.; Pac-Kozuchowska, E. Serum Hepcidin in Children with Inflammatory Bowel Disease. *Inflamm. Bowel Dis.* 2017, 23, 2165–2171. [CrossRef] [PubMed]

25. Paköz, Z.B.; Çekiç, C.; Arabul, M.; Sarıtaş Yüksel, E.; İpek, S.; Vatansever, S.; Ünsal, B. An Evaluation of the Correlation between Hepcidin Serum Levels and Disease Activity in Inflammatory Bowel Disease. *Gastroenterol. Res. Pract.* 2015, 2015, 1–4. [CrossRef] [PubMed]

26. Shanmugam, N.K.N.; Trebicka, E.; Shi, H.N.; Cherayil, B.J. Intestinal Inflammation Modulates Expression of the Iron-Regulating Hormone Hepcidin Depending on Erythropoietic Activity and the Commensal Microbiota. *J. Immunol.* 2014, 193, 1398–1407. [CrossRef]

27. Shu, W.; Pang, Z.; Xu, C.; Lin, J.; Li, G.; Wu, W.; Sun, S.; Li, J.; Li, X.; Liu, Z. Anti-TNF-α Monoclonal Antibody Therapy Improves Anemia through Downregulating Hepatocyte Hepcidin Expression in Inflammatory Bowel Disease. *Mediat. Inflamm.* 2019, 2019, 1–13. [CrossRef]

28. Caza, M.; Kronstad, J.W. Shared and distinct mechanisms of iron acquisition by bacterial and fungal pathogens of humans. *Front. Cell. Infect. Microbiol.* 2013, 3. [CrossRef]

29. Lönnerdal, B. Excess iron intake as a factor in growth, infections, and development of infants and young children. *Am. J. Clin. Nutr.* 2017, 106, 1681S–1687S. [CrossRef]

30. Page, M.G.P. The Role of Iron and Siderophores in Infection, and the Development of Siderophore Antibiotics. *Clin. Infect. Dis.* 2019, 69, S529–S537. [CrossRef]

31. Wilson, B.R.; Bogdan, A.R.; Miyazawa, M.; Hashimoto, K.; Tsuji, Y. Siderophores in Iron Metabolism: From Mechanism to Therapy Potential. *Trends Mol. Med.* 2016, 22, 1077–1090. [CrossRef]

32. Golonka, R.; Yeoh, B.S.; Vijay-Kumar, M. The Iron Tug-of-War between Bacterial Siderophores and Innate Immunity. *J. Innate Immun.* 2019, 1–13. [CrossRef]

33. Qi, B.; Han, M. Microbial Siderophore Enterobactin Promotes Mitochondrial Iron Uptake and Development of the Host via Interaction with ATP Synthase. *Cell* 2018, 175, 571–582.e11. [CrossRef] [PubMed]

34. Buhnik-Rosenblau, K.; Moshe-Belizowski, S.; Danin-Poleg, Y.; Meyron-Holtz, E.G. Genetic modification of the Host via Interaction with ATP Synthase. *Cell* 2018, 175, 571–582.e11. [CrossRef] [PubMed]

35. Mosbahi, K.; Wojnowska, M.; Albalat, A.; Walker, D. Bacterial iron acquisition mediated by outer membrane translocation and cleavage of a host protein. *Proc. Natl. Acad. Sci. USA* 2018, 115, 6840–6845. [CrossRef] [PubMed]

36. Weinberg, E.D. The Lactobacillus Anomaly: Total Iron Abstinence. *Archibald, F. Lactobacillus plantarum, an organism not requiring iron.* *Nutrients* 2019, 11, 2938. [CrossRef]

37. Das, N.K.; Schwartz, A.J.; Barthel, G.; Inohara, N.; Liu, Q.; Sankar, A.; Hill, D.R.; Ma, X.; Lamberg, O.; Schnizlein, M.K.; et al. Microbial Metabolite Signaling Is Required for Systemic Iron Homeostasis. *Cell Metab.* 2020, 31, 115–130.e6. [CrossRef]

38. Buhnik-Rosenblau, K.; Moshe-Belizowski, S.; Danin-Poleg, Y.; Meyron-Holtz, E.G. Genetic modification of iron metabolism in mice affects the gut microbiota. *BioMetals* 2012, 25, 883–892. [CrossRef]

39. Balamurugan, R.; Mary, R.R.; Chittaranjan, S.; Jancy, H.; Shobana Devi, R.; Ramakrishna, B.S. Low levels of faecal lactobacilli in women with iron-deficiency anaemia in south India. *Br. J. Nutr.* 2010, 104, 931–934. [CrossRef]

40. Cohen, S. Vitamin D Decreases Hepcidin and Inflammatory Markers in Newly Diagnosed Inflammatory Bowel Disease Paediatric Patients: A Prospective Study. *J. Crohns Colitis* 2019, 13, 1287–1291. [CrossRef] [PubMed]

41. Kalipatnapu, S.; Kuppuswamy, S.; Venugopal, G.; Kaliaperumal, V.; Ramadass, B. Fecal total iron concentration is inversely associated with fecal Lactobacillus in preschool children: Fecal iron is inversely associated with Fecal Lactobacillus. *J. Gastroenterol. Hepatol.* 2017, 32, 1475–1479. [CrossRef] [PubMed]
42. Sandberg, A.-S.; Önning, G.; Engström, N.; Scheers, N. Iron Supplements Containing Lactobacillus plantarum 299v Increase Ferric Iron and Up-regulate the Ferric Reductase DCYTB in Human Caco-2/HT29 MTX Co-Cultures. *Nutrients* **2018**, *10*, 1949. [CrossRef] [PubMed]

43. Rosen, G.M.; Morissette, S.; Larson, A.; Stading, P.; Griffin, K.H.; Barnes, T.L. Use of a Probiotic to Enhance Iron Absorption in a Randomized Trial of Pediatric Patients Presenting with Iron Deficiency. *J. Pediatr.* **2019**, *207*, 192–197.e1. [CrossRef] [PubMed]

44. Rusu, I.G.; Suharoschi, R.; Vodnar, D.C.; Pop, C.R.; Socaci, S.A.; Vultur, R.; Istrati, M.; Moroșan, I.; Fărcaș, A.C.; Kerezi, A.D.; et al. Iron Supplementation Influence on the Gut Microbiota and Probiotic Intake Effect in Iron Deficiency—A Literature-Based Review. *Nutrients* **2020**, *12*, 93. [CrossRef]

45. Deschemin, J.; Noordine, M.; Remot, A.; Willemetz, A.; Afif, C.; Canonne-Hergaux, F.; Langella, P.; Karim, Z.; Vaulont, S.; Thomas, M.; et al. The microbiota shifts the iron sensing of intestinal cells. *FASEB J.* **2016**, *30*, 252–261. [CrossRef] [PubMed]

46. Reddy, B.S.; Pleasants, J.R.; Westmann, B.S. Effect of Intestinal Microflora on Iron and Zinc Metabolism, and on Activities of Metalloenzymes in Rats. *J. Nutr.* **1972**, *102*, 101–107. [CrossRef]

47. Forrester, R.H.; Conrad, M.E.; Crosby, W.H. Measurement of total body iron in animals using whole-body liquid scintillation detectors. *Exp. Biol. Med.* **1962**, *111*, 115–119. [CrossRef]

48. Stern, P.; Košak, R.; Misirlija, A. The problem of iron resorption. *Experientia* **1954**, *10*, 227. [CrossRef]

49. Sanchez, K.K.; Chen, G.Y.; Schieber, A.M.P.; Redford, S.E.; Shokhirev, M.N.; Leblanc, M.; Lee, Y.M.; Ayres, J.S. Cooperative Metabolic Adaptations in the Host Can Favor Asymptomatic Infection and Select for Attenuated Virulence in an Enteric Pathogen. *Cell* **2018**, *175*, 146–158.e15. [CrossRef]

50. Kortman, G.A.M.; Boleij, A.; Swinkels, D.W.; Tjalsma, H. Iron Availability Increases the Pathogenic Potential of Salmonella Typhimurium and Other Enteric Pathogens at the Intestinal Epithelial Interface. *PLoS ONE* **2012**, *7*, e29968. [CrossRef] [PubMed]

51. Abbott, M.; Galloway, A.; Cunningham, J.L. Haemochromatosis presenting with a double yersinia infection. *J. Infect.* **1986**, *13*, 143–145. [CrossRef]

52. Van Asbeck, B.S.; Verbrugh, H.A.; van Oost, B.A.; Marx, J.J.; Imhof, H.W.; Verhoef, J. *Listeria monocytogenes* meningitis and decreased phagocytosis associated with iron overload. *BMJ* **1982**, *284*, 542–544. [CrossRef] [PubMed]

53. Mora; Verheul; Marx A functional defect in hereditary haemochromatosis monocytes and monocyte-derived macrophages. *Eur. J. Clin. Invest.* **1998**, *28*, 164–173. [CrossRef] [PubMed]

54. Núñez, G.; Sakamoto, K.; Soares, M.P. Innate Nutritional Immunity. *J. Immunol.* **2018**, *201*, 11–18. [CrossRef]

55. Barton, J.C.; Acton, R.T. Hepcidin, iron, and bacterial infection. In *Vitamins and Hormones*; Elsevier: Amsterdam, The Netherlands, 2019; Volume 110, pp. 223–242. ISBN 978-0-12-817842-3.

56. Abbott, M.; Galloway, A.; Cunningham, J.L. Haemochromatosis presenting with a double yersinia infection. *J. Infect.* **1986**, *13*, 143–145. [CrossRef]

57. Van Asbeck, B.S.; Verbrugh, H.A.; van Oost, B.A.; Marx, J.J.; Imhof, H.W.; Verhoef, J. *Listeria monocytogenes* meningitis and decreased phagocytosis associated with iron overload. *BMJ* **1982**, *284*, 542–544. [CrossRef] [PubMed]

58. Kortman, G.A.M.; Mulder, M.L.M.; Richters, T.J.W.; Shanmugam, N.K.N.; Trebicka, E.; Boekhorst, J.; Timmerman, H.M.; Roelofs, R.; Wiegerinck, E.T.; Laarakkers, C.M.; et al. Low dietary iron intake restrains colonic fermentation study. *J. Nutr. Biochem.* **2019**, *67*, 20–27. [CrossRef]

59. Phipps, O.; Al-Hassi, H.O.; Quraishi, M.N.; Kumar, A.; Brookes, M.J. Influence of Iron on the Gut Microbiota in Colorectal Cancer. *Nutrients* **2020**, *12*, 2512. [CrossRef] [PubMed]

60. Ng, O. Iron, microbiota and colorectal cancer. *Wien. Med. Wochenschr.* **2016**, *166*, 431–436. [CrossRef]

61. Baltussen, R.; Knai, C.; Sharan, M. Iron Fortification and Iron Supplementation are Cost-Effective Interventions to Reduce Iron Deficiency in Four Subregions of the World. *J. Nutr.* **2004**, *134*, 2678–2684. [CrossRef]
64.  Canelo-Hidalgo, M.J.; Castelo-Branco, C.; Palacios, S.; Haya-Palazuelos, J.; Ciria-Recasens, M.; Manasanch, J.; Pérez-Edo, L. Tolerability of different oral iron supplements: A systematic review. *Curr. Med. Res. Opin.* 2013, 29, 291–303. [CrossRef] [PubMed]

65.  Tondeur, M.C.; Schauer, C.S.; Christofides, A.L.; Asante, K.P.; Newton, S.; Serfass, R.E.; Zlotkin, S.H. 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.* 2004, 80, 1436–1444. [CrossRef] [PubMed]

66.  Bagna, R.; Spada, E.; Mazzone, R.; Saracco, P.; Boetti, T.; Cester, E.A.; Bertino, E.; Coscia, A. Efficacy of Supplementation with Iron Sulfate Compared to Iron Bisglycinate Chelate in Preterm Infants. *Curr. Pediatr. Rev.* 2018, 14, 123–129. [CrossRef] [PubMed]

67.  Milman, N.; Jønsson, L.; Dyre, P.; Pedersen, P.L.; Larsen, L.G. Ferrous bisglycinate 25 mg iron is as effective as ferrous sulfate 50 mg iron in the prophylaxis of iron deficiency and anemia during pregnancy in a randomized trial. *J. Perinat. Med.* 2014, 42. [CrossRef] [PubMed]

68.  Nagpal, J.; Choudhury, P. Iron formulations in pediatric practice. *Indian Pediatr.* 2004, 41, 807–815. [PubMed]

69.  Ramsay, L.C.; Charles, C.V. Review of Iron Supplementation and Fortification. In *Topics in Public Health*; Claborn, D., Ed.; InTech: London, UK, 2015; ISBN 978-953-51-2132-9.

70.  De-Regil, L.M.; Suchdev, P.S.; Vist, G.E.; Walleser, S.; Peña-Rosas, J.P. Home fortification of foods with multiple micronutrient powders for health and nutrition in children under two years of age. *Cochrane Database Syst. Rev.* 2011. [CrossRef]

71.  Pachón, H.; Spohrer, R.; Mei, Z.; Serdula, M.K. Evidence of the effectiveness of flour fortification programs on iron status and anemia: A systematic review. *Nutr. Rev.* 2015, 73, 780–795. [CrossRef]

72.  Pereira, D.I.A.; Aslam, M.F.; Frazer, D.M.; Schmidt, A.; Newton, S.; Serfass, R.E.; Zlotkin, S.H. 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.* 2004, 80, 1436–1444. [CrossRef] [PubMed]

73.  Dostal, A.; Chassard, C.; Hilty, F.M.; Zimmermann, M.B.; Jaeggi, T.; Rossi, S.; Lacroix, C. Iron Depletion and Repletion with Ferrous Sulfate or Electrolytic Iron Modifies the Composition and Metabolic Activity of the Gut Microbiota in Rats. *J. Nutr.* 2012, 142, 271–277. [CrossRef]

74.  Louis, P.; Flint, H.J. Diversity, metabolism and microbial ecology of butyrate-producing bacteria from the human large intestine. *FEMS Microbiol. Lett.* 2009, 294, 1–8. [CrossRef]

75.  Dostal, A.; Fehlbaurm, S.; Chassard, C.; Zimmermann, M.B.; Lacroix, C. Low iron availability in continuous in vitro colonic fermentations induces strong dysbiosis of the child gut microbial consortium and a decrease in main metabolites. *FEMS Microbiol. Ecol.* 2013, 83, 161–175. [CrossRef] [PubMed]

76.  Dostal, A.; Lacroix, C.; Bircher, L.; Pham, V.T.; Follador, R.; Zimmermann, M.B.; Chassard, C. Iron Modulates Butyrate Production by a Child Gut Microbiota In Vitro. *mBio* 2015, 6, e01453-15. [CrossRef] [PubMed]

77.  Tompkins, G.R.; O’Dell, N.L.; Bryson, I.T.; Pennington, C.B. The Effects of Dietary Ferric Iron and Iron Deprivation on the Bacterial Composition of the Mouse Intestine. *Curr. Microbiol.* 2001, 43, 38–42. [CrossRef]

78.  La Carpia, F.; Wojczyk, B.S.; Annavajhala, M.K.; Rebbaa, A.; Culp-Hill, R.; D’Alessandro, A.; Freedberg, D.E.; Uhlemann, A.-C.; Hod, E.A. Transfusional iron overload and intravenous iron infusions modify the mouse gut microbiota similarly to dietary iron. *NPJ Biofilms Microbiomes* 2019, 5, 26. [CrossRef] [PubMed]

79.  Coffey, R.; Ganz, T. Iron homeostasis: An anthropocentric perspective. *J. Biol. Chem.* 2017, 292, 12727–12734. [CrossRef]

80.  Liu, B.; Pan, X.; Liu, Z.; Han, M.; Xu, G.; Dai, X.; Wang, W.; Zhang, H.; Xie, L. Fecal microbiota as a noninvasive biomarker to predict the tissue iron accumulation in intestine epithelial cells and liver. *FASEB J.* 2020, 34, 3006–2020. [CrossRef]

81.  Alexeev, E.E.; He, X.; Slupsky, C.M.; Lönnerdal, B. Effects of iron supplementation on growth, gut microbiota, metabolomics and cognitive development of rat pups. *PLoS ONE* 2017, 12, e0179713. [CrossRef]

82.  Mahalhal, A.; Williams, J.M.; Johnson, S.; Ellaby, N.; Duckworth, C.A.; Burkitt, M.D.; Liu, X.; Hold, G.L.; Campbell, B.I.; Pritchard, D.M.; et al. Oral iron exacerbates colitis and influences the intestinal microbiome. *PLoS ONE* 2018, 13, e0202460. [CrossRef]

83.  Lucas López, R.; Grande Burgos, M.J.; Gálvez, A.; Pérez Pulido, R. The human gastrointestinal tract and oral microbiota in inflammatory bowel disease: A state of the science review. *APMIS* 2017, 125, 3–10. [CrossRef]
84. Constante, M.; Fragoso, G.; Lupien-Meilleur, J.; Calvé, A.; Santos, M.M. Iron Supplements Modulate Colon Microbiota Composition and Potentiate the Protective Effects of Probiotics in Dextran Sodium Sulfate-induced Colitis. *Inflamm. Bowel Dis.* 2017, 23, 753–766. [CrossRef]

85. Ettreiki, C. Juvenile ferric iron prevents microbiota dysbiosis and colitis in adult rodents. *World J. Gastroenterol.* 2012, 18, 2619. [CrossRef] [PubMed]

86. Werner, T.; Wagner, S.J.; Martinez, I.; Walter, J.; Chang, J.-S.; Clavel, T.; Kisling, S.; Schuemann, K.; Haller, D. Depletion of luminal iron alters the gut microbiota and prevents Clohn’s disease-like ileitis. *Gut* 2011, 60, 325–333. [CrossRef]

87. Dostal, A.; Baumgartner, J.; Riesen, N.; Chassard, C.; Smuts, C.M.; Zimmermann, M.B.; Lacroix, C. 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.* 2014, 112, 547–556. [CrossRef]

88. Tang, M.; Frank, D.N.; Sherlock, L.; Ir, D.; Robertson, C.E.; Krebs, N.F. Effect of Vitamin E with Therapeutic Iron Supplementation on Iron Repletion and Gut Microbiome in US Iron Deficient Infants and Toddlers. *J. Pediatr. Gastroenterol. Nutr.* 2016, 63, 379–385. [CrossRef] [PubMed]

89. Nitert, M.D.; Gomez-Arango, L.F.; Barrett, H.L.; McIntyre, H.D.; Anderson, G.J.; Frazer, D.M.; Callaway, L.K. Iron supplementation has minor effects on gut microbiota composition in overweight and obese women in early pregnancy. *Br. J. Nutr.* 2018, 120, 283–289. [CrossRef] [PubMed]

90. Jaeggi, T.; Kortman, G.A.M.; Moretti, D.; Chassard, C.; Holding, P.; Dostal, A.; Boekhorst, J.; Timmerman, H.M.; Swinkels, D.W.; Tjalsma, H.; et al. Iron fortification adversely affects the gut microbiome, increases pathogen abundance and induces intestinal inflammation in Kenyan infants. *Gut* 2015, 64, 731–742. [CrossRef]

91. Tang, M.; Frank, D.; Hendricks, A.; Ir, D.; Esamai, F.; Liechty, E.; Hambidge, K.; Krebs, N. Iron in Micronutrient Powder Promotes an Unfavorable Gut Microbiota in Kenyan Infants. *Nutrients* 2017, 9, 776. [CrossRef]

92. Sjödin, K.S.; Domellöf, M.; Lagerqvist, C.; Hernell, O.; Lönnerdal, B.; Szymlek-Gay, E.A.; Sjödin, A.; West, C.E.; Lind, T. Administration of ferrous sulfate drops has significant effects on the gut microbiota of iron-deficient infants: A randomised controlled study. *Gut* 2019, 68, 2095–2097. [CrossRef]

93. Gupta, V.K.; Paul, S.; Dutta, C. Geography, Ethnicity or Subsistence-Specific Variations in Human Microbiome Composition and Diversity. *Front. Microbiol.* 2017, 8, 1162. [CrossRef]

94. Behrouzi, A.; Nafari, A.H.; Siadat, S.D. The significance of microbiome in personalized medicine. *Clin. Transl. Med.* 2019, 8, 16. [CrossRef]

95. Sazawal, S.; Black, R.E.; Ramsan, M.; Chwaya, H.M.; Stoltzfus, R.J.; Dutta, A.; Dhingra, U.; Kabole, I.; Deb, S.; Othman, M.K.; et al. 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* 2006, 367, 133–143. [CrossRef]

96. Zhang, D.-L.; Wu, J.; Shah, B.N.; Greut, M.C.; Ghosh, M.C.; Ollivierre, H.; Su, X.; Thuma, P.E.; Bedu-Addo, G.; Mockenhaupt, F.P.; et al. Erythrocytic ferroportin reduces intracellular iron accumulation, hemolysis, and malaria risk. *Science* 2018, 359, 1520–1523. [CrossRef] [PubMed]

97. Ma, J.; Sun, Q.; Liu, J.; Hu, Y.; Liu, S.; Zhang, J.; Sheng, X.; Hambidge, K.M. The Effect of Iron Fortification on Iron (Fe) Status and Inflammation: A Randomized Controlled Trial. *PLoS ONE* 2016, 11, e0167458. [CrossRef] [PubMed]

98. Qasem, W.; Azad, M.B.; Hossain, Z.; Azad, E.; Jorgensen, S.; Castillo San Juan, S.; Cai, C.; Khabipour, E.; Beta, T.; Roberts, L.J.; et al. Assessment of complementary feeding of Canadian infants: Effects on microbiome & oxidative stress, a randomized controlled trial. *BMC Pediatr.* 2017, 17, 54. [CrossRef]

99. Ghanchi, A.; James, P.T.; Cerami, C. Guts, Germs, and Iron: A Systematic Review on Iron Supplementation, Iron Fortification, and Diarrhea in Children Aged 4–59 Months. *Curr. Dev. Nutr.* 2019, 3. [CrossRef]

100. Chen, K.; Zhang, X.; Li, T.; Chen, L.; Wei, X.; Qu, P.; Liu, Y. Effect of vitamin A, vitamin A plus iron and multiple micronutrient-fortified seasoning powder on infectious morbidity of preschool children. *Nutrition* 2011, 27, 428–434. [CrossRef]

101. Javaid, N.; Haschke, F.; Pitschning, B.; Schuster, E.; Huemer, C.; Shebaz, A.; Ganesh, P.; Steffan, I.; Hurrel, R.; Secretin, M.C. Interactions between infections, malnutrition and iron nutritional status in Pakistani infants. A longitudinal study. *Acta Paediatr. Scand. Suppl.* 1991, 374, 141–150. [CrossRef]
102. Barth-Jaeggi, T.; Moretti, D.; Kvalsvig, J.; Holding, P.A.; Njenga, J.; Mwangi, A.; Chhagan, M.K.; Lacroix, C.; Zimmermann, M.B. In-home fortification with 2.5 mg iron as NaFeEDTA does not reduce anaemia but increases weight gain: A randomised controlled trial in Kenyan infants. *Matern. Child. Nutr.* 2015, 11, 151–162. [CrossRef]

103. Christofides, A.; Schauer, C.; Sharief, W.; Zlotkin, S.H. Acceptability of micronutrient sprinkles: A new food-based approach for delivering iron to First Nations and Inuit children in Northern Canada. *Chronic Dis. Can.* 2005, 26, 114–120.

104. Lemaire, M.; Islam, Q.S.; Shen, H.; Khan, M.A.; Parveen, M.; Abedin, F.; Haseen, F.; Hyder, Z.; Cook, R.J.; Zlotkin, S.H. Iron-containing micronutrient powder provided to children with moderate-to-severe malnutrition increases hemoglobin concentrations but not the risk of infectious morbidity: A randomized, double-blind, placebo-controlled, noninferiority safety trial. *Am. J. Clin. Nutr.* 2011, 94, 585–593. [CrossRef]

105. Paganini, D.; Uyoga, M.A.; Kortman, G.A.M.; Cercamondi, C.I.; Moretti, D.; Barth-Jaeggi, T.; Schwab, C.; Boekhorst, J.; Timmerman, H.M.; Lacroix, C.; et al. Prebiotic galacto-oligosaccharides mitigate the adverse effects of iron fortification on the gut microbiome: A randomised controlled study in Kenyan infants. *Gut* 2017, 66, 1956–1967. [CrossRef]

106. Giovannini, M.; Sala, D.; Usuelli, M.; Livio, L.; Francescato, G.; Braga, M.; Radaelli, G.; Riva, E. Double-Blind, Placebo-Controlled Trial Comparing Effects of Supplementation with Two Different Combinations of Micronutrients Delivered as Sprinkles on Growth, Anemia, and Iron Deficiency in Cambodian Infants. *J. Pediatr. Gastroenterol. Nutr.* 2006, 42, 306–312. [CrossRef] [PubMed]

107. Witzig, R.S.; Black, R.E.; Caulfield, L.E.; Zavaleta, N.; Shankar, A.H.; Richard, S.A. Zinc and Iron Supplementation and Malaria, Diarrhea, and Respiratory Infections in Children in the Peruvian Amazon. *Am. J. Trop. Med. Hyg.* 2006, 75, 126–132. [CrossRef]

108. Luabeya, K.-K.A.; Mpontshane, N.; Mackay, M.; Ward, H.; Elson, I.; Chhagan, M.; Tomkis, A.; den Broeck, J.V.; Bennish, M.L. Zinc or Multiple Micronutrient Supplementation to Reduce Diarrhea and Respiratory Disease in South African Children: A Randomized Controlled Trial. *PLoS ONE* 2007, 2, e541. [CrossRef] [PubMed]

109. Chen, K.; Chen, X.; Zhang, L.; Luo, H.; Gao, N.; Wang, J.; Fu, G.-Y.; Mao, M. Effect of simultaneous supplementation of vitamin A and iron on diarrheal and respiratory tract infection in preschool children in Chengdu City, China. *Nutrition* 2013, 29, 1197–1203. [CrossRef] [PubMed]

110. Rosado, J.L.; López, P.; Muñoz, E.; Martínez, H.; Allen, L.H. Zinc supplementation reduced morbidity, but neither zinc nor iron supplementation affected growth or body composition of Mexican preschooleers. *Am. J. Clin. Nutr.* 1997, 65, 13–19. [CrossRef] [PubMed]

111. Abdelrazik, N.; Al-Haggar, M.; Al-Marsafawy, H.; Abdel-Hadi, H.; Al-Baz, R.; Mostafa, A.-H. Impact of long-term oral iron supplementation in breast-fed infants. *Indian J. Pediatr.* 2007, 74, 739–745. [CrossRef] [PubMed]

112. Dewey, K.G.; Domellöf, M.; Cohen, R.J.; Landa Rivera, L.; Hernell, O.; Lönnerdal, B. Iron supplementation affects growth and morbidity of breast-fed infants: Results of a randomized trial in Sweden and Honduras. *J. Nutr.* 2002, 132, 3249–3255. [CrossRef]

113. Baqui, A.H.; Zaman, K.; Persson, L.A.; El Arifeen, S.; Yunus, M.; Begum, N.; Black, R.E. Simultaneous weekly supplementation of iron and zinc is associated with lower morbidity due to diarrhea and acute lower respiratory infection in Bangladeshi infants. *J. Nutr.* 2003, 133, 4150–4157. [CrossRef]

114. Chang, S.; El Arifeen, S.; Bari, S.; Wahed, M.A.; Rahman, K.M.; Rahman, M.T.; Mahmud, A.B.A.; Begum, N.; Zaman, K.; Baqui, A.H.; et al. Supplementing iron and zinc: Double blind, randomized evaluation of separate or combined delivery. *Eur. J. Clin. Nutr.* 2010, 64, 153–160. [CrossRef]

115. Menon, P.; Ruel, M.T.; Loechli, C.U.; Armond, M.; Habicht, J.-P.; Pelto, G.; Michaud, L. Micronutrient Sprinkles reduce anemia among 9- to 24-mo-old children when delivered through an integrated health and nutrition program in rural Haiti. *J. Nutr.* 2007, 137, 1023–1030. [CrossRef] [PubMed]

116. Soofi, S.; Cousens, S.; Iqbal, S.P.; Akhund, T.; Khan, J.; Ahmed, I.; Zaidi, A.K.M.; Bhatta, Z.A. Effect of provision of daily zinc and iron with several micronutrients on growth and morbidity among young children in Pakistan: A cluster-randomised trial. *Lancet.* 2013, 382, 29–40. [CrossRef]

117. Mitra, A.K.; Akramuzzaman, S.M.; Fuchs, G.J.; Rahman, M.M.; Mahalanabis, D. Long-term oral supplementation with iron is not harmful for young children in a poor community of Bangladesh. *J. Nutr.* 1997, 127, 1451–1455. [CrossRef]
118. Bhattacharyya, A.; Chattopadhyay, R.; Mitra, S.; Crowe, S.E. Oxidative Stress: An Essential Factor in the Pathogenesis of Gastrointestinal Mucosal Diseases. *Physiol. Rev.* **2014**, *94*, 329–354. [CrossRef] [PubMed]

119. Natoli, M.; Felsani, A.; Ferruzza, S.; Sambuy, Y.; Canali, R.; Scarino, M.L. Mechanisms of defence from Fe(II) toxicity in human intestinal Caco-2 cells. *Toxicol. In Vitro* **2009**, *23*, 1510–1515. [CrossRef]

120. Ferruzza, S.; Scarino, M.L.; Gambling, L.; Natella, F.; Sambuy, Y. Biphasic effect of iron on human intestinal Caco-2 cells: Early effect on tight junction permeability with delayed onset of oxidative cytotoxic damage. *Cell Mol. Biol. Noisy Gd. Fr.* **2003**, *49*, 89–99.

121. Zimmermann, M.B.; Chassard, C.; Rohner, F.; N’Goran, E.K.; Nindjin, C.; Dostal, A.; Utzinger, J.; Ghattas, H.; Lacroix, C.; Hurrell, R.F. The effects of iron fortification on the gut microbiota in African children: A randomized controlled trial in Côte d’Ivoire. *Am. J. Clin. Nutr.* **2010**, *92*, 1406–1415. [CrossRef]