

Review

Oral Iron Supplementation—Gastrointestinal Side Effects and the Impact on the Gut Microbiota

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Abstract: Iron deficiency anaemia (IDA) is a worldwide healthcare problem affecting approximately 25% of the global population. The most common IDA treatment is oral iron supplementation, which has been associated with gastrointestinal (GI) side effects such as constipation and bloating. These can result in treatment non-adherence and the persistence of IDA. Intravenous iron does not cause GI side effects, which may be due to the lack of exposure to the intestinal lumen. Luminal iron can cause changes to the gut microbiota, aiding the promotion of pathogenic species and decreasing beneficial protective species. Iron is vital for methanogenic archaea, which rely on iron for growth and metabolism. Increased intestinal methane has been associated with slowing of intestinal transit, constipation, and bloating. Here we explore the literature to understand a potential link between iron and methanogenesis as a novel way to understand the mechanism of oral iron supplementation induced GI side effects.

Keywords: iron; constipation; bloating; methane; methanogens; gut microbiota



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1. Introduction

Iron deficiency anaemia (IDA) affects up to 25% of the worldwide population [1], or approximately 2 billion people [2]. Annually, in the U.K., IDA costs the NHS £55.48 million and causes 57,000 hospital admissions [3]. Some of the main causes of iron deficiency include dietary insufficiency, blood loss, malabsorption, pregnancy, infections, inflammation, and inflammatory bowel disease (IBD). It is estimated that IDA will occur in 60–80% of IBD patients [3], with it being the most common extraintestinal complication of IBD [4]. The high prevalence of IDA in the IBD community is likely to be multifactorial, including inflammation, poor iron absorption, intestinal bleeding and a restricted diet [3].

Iron supplementation is heavily prescribed to a wide range of patients to prevent and treat iron deficiency (ID) and IDA. Intravenous and oral iron therapy restores iron levels. However, an important unexplained clinical limitation of oral iron therapy is that it often causes significant gastrointestinal (GI) side effects such as constipation, abdominal pain, nausea, and bloating [5–7]. Oral iron is the most common treatment for ID and IDA due to its low cost, high bioavailability and effectiveness [5,6]. There are many different types of oral iron supplements available (Table 1), but the most commonly prescribed oral iron is ferrous sulphate [7]. Ferrous iron supplements are effective; however, they have a high frequency of side effects in comparison to ferric iron sources [8]. First-line treatment is recommended to be ferrous sulphate two or three times per day, totalling a daily maximum elemental iron dosage of 195 mg [9,10].

Intravenous (IV) iron is administered directly into the bloodstream and therefore bypasses the GI lumen. When first developed, IV iron was toxic and not well tolerated causing anaphylaxis and hypersensitivity reactions [11]. Subsequently, IV iron preparations have gone through numerous iterations to develop a safer and better-tolerated formulation.

An example of this is ferric carboxymaltose, which is non-dextran and deemed to be safe and significantly better than oral iron at replenishing haemoglobin levels with a single dose of 750 mg. Very few adverse effects have been found with ferric carboxymaltose with minor side effects and no discontinuation of treatment needed [12].

Table 1. The three most common oral iron supplements used for the treatment of iron deficiency anaemia.

Oral Iron Supplement	Dose (mg)	Elemental Iron Dose (mg)
Ferrous sulphate	200	65
Ferrous fumarate	200	65
Ferrous gluconate	300	35

Intravenous iron does seem to have several benefits over oral iron. Gastrointestinal side effects are not as frequently reported [9], and compliance is much greater, resulting in the quicker restoration of haemoglobin and resolution of iron deficiency [10]. In addition, in adult IBD patients, IV iron is more effective and well tolerated [4]. Many studies also report quicker replenishment of iron stores in the body [13] due to the high cellular uptake of intravenous iron in comparison to oral supplements, where unabsorbed iron is lost in the faeces [14,15]. However, IV iron is significantly more costly, being over 60 times more expensive than oral iron [16]. Despite this, cost-effectiveness analysis for the treatment of IDA in IBD patients identified ferric carboxymaltose as the most cost-effective treatment due to suitable adherence to treatment, the high number of patients that respond to the treatment, improvements in hospitalisation rates, and patient quality of life [17].

Up to 60% of people taking oral iron supplements report gastrointestinal side effects [10]. These GI complaints cause up to 50% of oral iron receiving patients to not follow their treatment plan, meaning their IDA persists [9]. However, patients that receive IV iron infusions report a lower occurrence of these side effects [10] such as nausea (1.6% vs. 4.9%), vomiting (1.0% vs. 6.8%), abdominal pain (1.3% vs. 7.9%), and diarrhoea (0.9% vs. 8.3%) [4]. As IV iron bypasses the gastrointestinal lumen, it is thought that GI side effects observed are mainly driven by the direct interaction of iron with the gastrointestinal milieu from oral iron administration.

Despite millions of patients taking oral iron, the mechanisms by which GI side effects are mediated are poorly understood. Numerous theories as to the cause of iron-induced side effects have been proposed, including via hydroxyl radicals, lipid peroxidation, cellular damage and microbiota changes [18]. Understanding the mechanism to improve the side effect profile could have significant benefits in terms of patient outcome and healthcare economics.

2. Iron Absorption and Dosing of Iron Therapy

Iron is absorbed in the duodenum and proximal jejunum. Heme and non-heme iron have different mechanisms of absorption (Figure 1). For heme iron, heme carrier protein 1 (HCP-1) transports iron into the enterocyte lumen, where the ferrous (Fe^{2+}) iron is released from the protoporphyrin XI ring by heme oxygenase [19]. Non-heme iron is first converted from ferric (Fe^{3+}) to Fe^{2+} iron by the reductase enzyme duodenal cytochrome B (DCYTB). Then divalent metal transporter-1 (DMT-1) on the apical surface of duodenal enterocytes actively transports the reduced iron into the enterocyte cytoplasm [19]. Iron is regulated at the level of intestinal absorption to prevent iron overload as there is a lack of an iron excretory pathway. Hepcidin is the main regulator for the accumulation of iron in the body. When iron stores and circulating iron in transferrin are at saturation point, liver hepatocytes produce hepcidin. This triggers the degradation of ferroportin, a transmembrane protein involved in iron efflux, preventing further iron absorption [20].

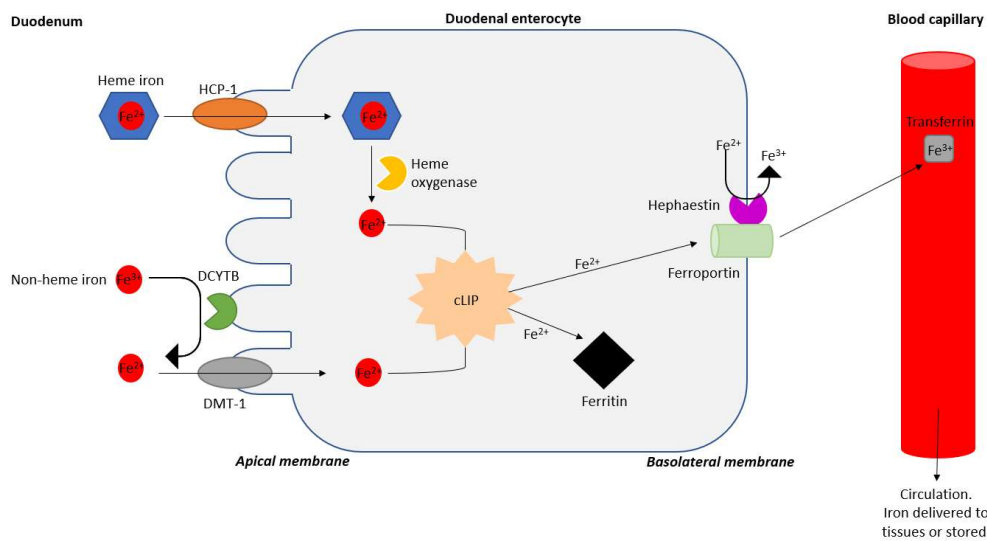


Figure 1. Iron absorption in the body. This can be in the form of heme or non-heme iron. Heme iron is transported into the duodenal enterocyte cytoplasm via HCP-1 (heme carrier protein 1), where the heme iron is then removed from the protoporphyrin X1 ring by heme oxygenase. The Fe²⁺ iron then forms part of the cLIP (cytosolic labile iron pool). Non-heme iron must be in the ferrous state before entering the enterocyte lumen. DCYTB (duodenal cytochrome B) reduces Fe³⁺ iron to Fe²⁺ iron, and then DMT-1 (divalent metal transporter 1) transports Fe²⁺ into the enterocyte, where it forms part of the cLIP. Iron in the cLIP can either bind to ferritin for storage or be transported out of the enterocyte via ferroportin and hephaestin, which oxidises the iron to its ferric state and can bind to transferrin in the blood for transport around the body.

The typical adult diet should contain between 8 and 15 mg of iron per day, with an average of 1–2 mg absorbed daily to balance losses from the body [21,22]. However, a maximum of 25 mg elemental iron per day can be absorbed, but this amount is only under extreme iron deficiency conditions [23]. Despite this, clinical guidance recommends iron deficiency anaemia is treated with oral ferrous sulphate 200 mg tablet, with two or three tablets taken daily to replenish iron levels [24,25]. Each ferrous sulphate tablet contains 65 mg of elemental iron; therefore, patients could be receiving up to 195 mg of elemental iron daily, which is significantly more than is capable of being absorbed. This unabsorbed iron will pass through the gastrointestinal tract interacting with the milieu before being excreted in faeces.

Over the years, many iron supplements have been developed with the aim of decreasing GI side effects. This has been trialled by iron in a modified-release tablet or liquid form. In vitro model experiments of conventional release tablets of ferrous sulphate, ferrous fumarate and ferrous gluconate were compared to controlled-release ferrous sulphate with ascorbic acid tablets and sustained-release capsules of ferrous fumarate. Absorption of iron was significantly greater from conventional release ferrous sulphate, whilst both modified-release tablets showed low absorption. Therefore, modified-release tablets have been contraindicated due to the low absorption as a result of the slower iron release rate [26]. Liquid formulations of iron are commonly mixed with fruit juices with a high polyphenol concentration, known to prevent absorption of iron (Table 2), so also not advised [26].

Table 2. Foods and medications that either enhance or inhibit the absorption of iron heme and non-heme iron [27,28].

Increase Absorption	Decrease Absorption
Ascorbic acid (Vitamin C) Meat Fish	Antacid medications (e.g., proton pump inhibitors) Phytates and polyphenols Calcium

Spatone[®] iron-Plus water has been found to be a highly bio-available source of iron. However, one sachet of Spatone Iron-Plus water contains just 5 mg of iron to ensure that no gastrointestinal side effects occur. Whilst 28–34% of this is absorbed, this form of iron has only been recommended in a healthy pregnancy to maintain iron levels as lower amounts of supplemental iron are required in comparison with those suffering from iron deficiency anaemia [29–31]. Therefore, Spatone[®] should not be used to treat ID or IDA.

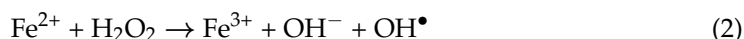
Another oral iron supplement aimed at reducing gastrointestinal side effects is sucrosomial iron. In sucrosomial iron, a phospholipid bilayer and sucrosome protect ferric pyrophosphate until it reaches the intestines. Pre-clinical data indicate that it may be more tolerable than other oral iron formulations whilst having similar effectiveness at restoring iron levels [32].

Experimental evidence suggests that taking iron two to three times a day may not be the best way to take iron supplements despite it being the current guidelines. Moretti et al. 2015 [33] found that high doses of oral iron supplements cause an increase in hepcidin, which inhibits iron absorption for up to 24 h. They found that dosing 48 h apart enhanced iron absorption compared to three daily doses as it allows sufficient time for hepcidin levels to decrease and remove the mucosal block on absorption [34,35]. Other groups have also confirmed enhanced iron absorption with single dosing once daily or alternate day dosing [33,34,36].

Overall, changing the formulation of iron supplementation reduces side effects by lowering the dosage and, in turn, reduces the amount of unabsorbed iron in the GI tract. It appears the best way to restore iron levels with the least number of side effects is with fewer doses of iron (even just two doses per week), which would also be more cost effective [35].

3. Iron and Inflammation

Both iron deficiency and iron overload can cause oxidative stress. Due to its ability to accept or donate electrons, iron can be harmful to the human body. Iron contains unsaturated electrons [37] and is a pro-oxidant [38], favouring the Fenton reaction (Equation (1)) and Haber–Weiss reaction (Equation (2)).



In the Fenton reaction, reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2) are reduced by electrons to produce free radical species such as hydroxyl ions (OH^-) [37,39]. In the GI tract, this can cause oxidative stress to the intestinal cells causing damage to membrane proteins and lipid peroxidation [39]. Therefore, when there is excess iron in the gastrointestinal tract, it can cause damage to intestinal villi resulting in a decrease in height, shape, and stability, along with damage to tight junction proteins between the mucosal enterocytes [39]. This causes inflammation of intestinal mucosa [37]. Inflammation in turn decreases iron absorption by stimulating hepcidin production and secretion and decreasing DMT-1 protein levels [39].

4. Iron and the Gut Microbiota

The gut microbiota are the microorganisms present in the GI tract. To survive, these microorganisms use nutrients from the human diet, and thus dietary changes, nutrient deficiencies, and oral medications can have profound effects on the microbiota. One nutrient the gut microbiota heavily relies on is iron, and its deficiency is often growth and virulence limiting for many bacteria.

As earlier discussed, iron supplements contain significantly more iron than can be absorbed by the body. This means that large amounts of unabsorbed iron are left in the lumen of the GI tract. Increased levels of luminal iron in the GI tract affects the composition of the gut microbiota, with an elevated level of enteropathogens and a decrease in the protective species *Lactobacilli* [40]. This can be caused by oral iron supplementation and

the consumption of fortified food. Iron fortification (~8 mg/day) of African children's diet resulted in increased growth of pathogenic bacteria such as *Salmonella*, *Shigella* and *Escherichia coli*, and a decrease in *Lactobacillus*, which is responsible for inhibiting pathogen colonisation [41]. Studies investigating the effect of iron supplementation in Kenyan infants also found increased levels of *Enterobacteriaceae* and decreased levels of *Lactobacillus* and *Bifidobacterium* [42]. However, the effects of iron supplementation and fortification on the gut microbiota in the long term are unknown [43].

Overall, iron has many impacts on the microbiota (Figure 2), but most commonly, it results in enhanced growth of pathogenic species and a decrease in beneficial bacterial species. Iron availability for pathogenic bacteria is so essential that the mammalian immune system has developed pathways known as nutritional immunity—the host's ability to manipulate metals availability mediated by the expression of metal-binding proteins (lipocalin-2, lactoferrin, transferrin and intracellular ferritin) that can withhold iron [44,45]. This precise mechanism, however, has little chance to mediate balance in ID combined with iron overload. Transferrin and lactoferrin have been implicated in nutritional immunity by iron sequestration from invading pathogens, with evidence that lactoferrin can increase iron absorption by 56% [46–48]. Data to determine if lactoferrin can reduce iron-induced GI side effects is ambiguous, but lactoferrin can modulate GI inflammation by helping resolve the infection and prevent tissue damage along with reducing the growth of pathogens [49].

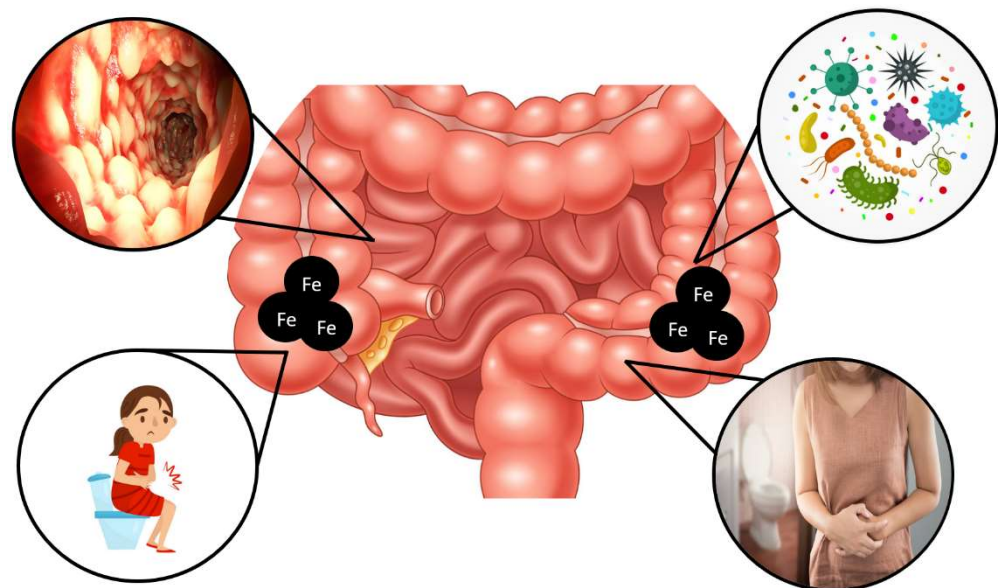


Figure 2. Summary of the impact of iron on the GI tract. Oral iron supplementation causes up to 60% of patients to report gastrointestinal side effects such as constipation, nausea, and bloating. Iron is known to cause intestinal inflammation via the production of ROS. Iron also causes changes to the gut microbiota by increasing the level of enteropathogens and decreasing protective species and may cause changes to archaeal species.

Coupling iron supplementation with prebiotics and probiotics could allow for mitigations against iron-induced microbiota changes. *Lactobacillus fermentum* increases iron absorption at DMT-1 by converting Fe^{3+} to Fe^{2+} via its ferric reducing activity; therefore, it could help reduce the GI side effects of iron [50]. In addition, studies investigating supplementing Kenyan infants with an iron-containing micronutrient powder with and without the prebiotic galacto-oligosaccharides (GOS) found GOS mitigated adverse effects of iron on the gut microbiota [51]. This is because GOS and probiotics decrease the pH of the intestines, which aids iron absorption [52] in addition to enhancing the commensal bacteria to protect against enteropathogens, which prevents colonisation and overgrowth [51]. Therefore, iron supplementation with GOS and lactoferrin may help reduce the impact on the gut microbiota by increasing the absorption of iron [53].

Archaeal species could also be impacted by iron, and it has been hypothesised that ferrous sulphate, a more soluble form of iron, could augment methanogenic species growth [41]. Numerous experiments have found that constipated individuals have a higher abundance of methanogenic species in their intestinal microbiota, along with slower intestinal transit times and higher faecal pH [54–58]. In addition, it is well known that taking oral iron supplements associates with constipation [9]. However, the mechanistic explanation of this relationship is unknown.

5. Methanogens, Methanogenesis, and GI Symptoms

Methanogens are ancient single-celled microorganisms that are part of the kingdom Archaea. They are believed to be present in small numbers in at least 30–50% of the population [57]. Numerous species are known to be able to colonise the human body, the most common being *Methanobrevibacter smithii* and *Methanosphaera stadtmanae* in the GI tract and *Methanobrevibacter oralis* in the oral cavity [57]. There are seven orders of methanogens belonging to the phylum Euryarchaeota, with the seventh order, *Methanomassiliicoccales*, only recently discovered [59].

Methanogens can produce methane (CH₄) via a range of methanogenesis reactions (Table 3) [59]. Methanogenic species within the gastrointestinal tract predominantly use a hydrogenotrophic methanogenesis reaction to produce methane as they use hydrogen produced by the bacterial fermentation of carbohydrates [60]. Very few other species in the microbiota are capable of producing methane, with the exception of *Clostridium* and *Bacteroides* species [60–62].

Table 3. Methods of methanogenesis by different methanogens.

Type of Methanogenesis	Substrates	Mechanism
Hydrogenotrophic	Hydrogen or formate	The substrates can reduce carbon dioxide to produce methane
Methylotrophic	Methanol, methyl-sulphides, methylamines	Methyl group of the substrate is converted to methane using specific methyltransferases
Acetotrophic	Acetate	Fermentation of acetate or decarboxylation to carbon dioxide followed by reduction

The *Christensenellaceae* family coexist with the *Methanobacteriaceae* family, and in particular, the *Christensenellaceae* support *M. smithii* metabolism via hydrogen production that is then consumed by *M. smithii* for methane production. In addition, the presence of *M. smithii* causes an alteration in the short-chain fatty acid production of *Christensenella minuta* towards acetate over butyrate [63], which can then be used for acetotrophic methanogenesis.

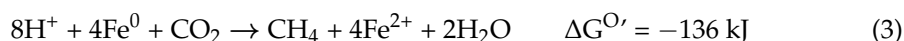
Methane has been linked with the slowing of intestinal transit [56]. Oro-caecal and whole gut transit time significantly decrease in methane producers compared to non-methane producers [56] and methane attenuates peristaltic movement in the intestines by promoting the contraction of non-propagating circular muscles [57,64]. Whilst there is an association between methane and constipation, it is uncertain whether methane is a cause or consequence of constipation [58].

Theories as to how methane impacts gut transit have been proposed. Methane has been suggested to be a gaseous transmitter, such as nitric oxide, with the ability to permeate through the intestinal wall to mediate neuronal and smooth muscle activities [56]. How methane slows intestinal transit may be mechanistically similar to the jejunal and ileal brake following the ingestion of fat [56]. Serotonin may also have a role in transit time control, with 95% of the serotonin in the body found in the GI tract [65]. Methane producers have a decreased serotonin level after meals, and methane gas was found to inhibit serotonin uptake in circulation [57,65].

6. Methanogenesis and Iron

In the Earth's history, the main source of methane was via hydrogenotrophic methanogenesis in anaerobic ferruginous oceans. A modern-day example of this is Lake Matano in Indonesia, where iron oxides and methane are heavily abundant, and there is supportive evidence for methanogenesis in the presence of low reactive Fe^{3+} [61]. Biogas production experiments are also in favour of iron supported methanogenesis. The addition of zero-valent iron to algal sludge or wastewater fermenters has been found to enhance methane production by up to 17% [62,66].

Iron, along with other metals, is essential trace elements for methanogen growth, metabolism and enzymatic activity [67]. This is due to the large number of key proteins in methanogens using the iron-sulfur (Fe-S) clusters [68], on which methanogenesis is dependent [69]. However, iron may be important for methanogenesis in other ways, including as a source of electrons in the reduction in carbon dioxide to methane (Equation (3)). Metal corrosion experiments found that iron can be oxidised via the methanogenesis reaction, producing methane gas and creating energy to assist methanogenic species growth [70]. The methanogenesis reaction is the only way methanogens gain energy for growth [71]. However, some evidence suggests that pure cultures of hydrogenotrophic methanogens can have a decrease in methane production when iron is present [72].



Sulphate-reducing bacteria (SRB) and methanogens live in similar environments and, as a result, commonly compete for the same substrates [73], which in turn can limit methanogenesis. Iron can help methanogens outcompete SRB as iron can precipitate sulphides to ferrous sulphide (FeS) [74]. This assists with methanogenesis by preventing the formation of hydrogen sulphide (H_2S), which is toxic to methanogens and SRB [75].

7. Modulating Methane and Methanogens

Whilst few drugs and supplements have been found to have an impact on methane production, little is known on how to effectively reduce methane production or eradicate methanogens completely.

An increase in methane is a direct biomarker that correlates with slow transit, and a decrease in methane has a clinical benefit; therefore, it is important to understand ways to reduce methane. Whilst most antibiotics are not suitable for treating excessive methane production, a combination of rifaximin and neomycin have been effective in reducing methanogens with concurrent improvement in constipation symptoms [76–78]. Taking these antibiotics in combination with a pro-motility agent can help prevent the reestablishment of methanogens [79].

The mevalonate pathway targeted by cholesterol-lowering drugs (statins) is involved in the formation of cholesterol for archaeal cell walls. Therefore, the archaeal mevalonate pathway could act as a suitable target for the reduction in methane. There is evidence that lovastatin can inhibit archaeal cell wall biosynthesis when converted to its hydroxyacid form without impacting other bacteria, therefore, selectively targeting methanogens [80,81]. Inhibition occurs at the first step in cholesterol biosynthesis to 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (HMG CoA reductase). Red yeast rice extract contains a chemically identical compound to lovastatin, monacolin K, and oyster mushroom extract naturally contains lovastatin, so these are also a potential candidate for a reduction in methane gas within the GI tract [82,83].

The supplement Atrantil could also be explored for use in methane eradication. Evidence suggests a benefit of methane-positive patients with a response rate of 88% of constipation-predominant IBS patients and marked improvements in both constipation and abdominal pain [84]. However, this has not been tested alongside breath testing to investigate whether the decrease in symptoms is accompanied by a decrease in methane levels.

Seaweed could be a natural anti-methanogenic compound [85]. Sheep that have been confined to a Scottish Orkney island beach shore surviving on a diet of seaweed do not

produce any methane [86] in comparison to the 70–120 kg a year of methane produced by cattle [87]. Seaweed species such as *Asparagopsis taxiformis* and *Asparagopsis armata* contain bromoforms and dibromochloromethane, which can prevent methane production during digestion [85,88]. Just 1–2% of *Asparagopsis* per day of a cattle feed may reduce methane production by up to 70% [89], and 5% of *A. taxiformis* can reduce methane by 95% [90]. There is currently no evidence for seaweed reducing methane in humans and could be explored further.

Other methane treatment options could be with faecal microbiota transplantation (FMT). Previously this has worked in the eradication of *Clostridium difficile* infection; however, it has only been recently postulated as a treatment for methanogen overgrowth [91]. Probiotics containing *Lactobacillus reuteri* (DSM 17938) could also be beneficial as studies in infants with chronic constipation indicate *L. reuteri* could increase the frequency of bowel movements [92]. In addition, *L. reuteri* taken over a 4-week period is known to reduce methane production [58]. Multistrain probiotics used in adults with functional constipation reduced whole gut transit time by 13.75 h and increased the number of weekly bowel movements [93]. In the same meta-analysis, the use of multistrain formulations also significantly reduced bloating; however, methane production was not assessed.

Increasing iron absorption would reduce the availability of methanogens. The probiotic strain *Lactiplantibacillus plantarum* 299v has been shown to increase iron absorption. A study with pregnant women taking a capsule containing *L. plantarum* 299v along with 4.2 mg iron and low dose folic acid and ascorbic acid from the first trimester found improved iron status in comparison to the placebo [94]. Therefore, investigating probiotics that could enhance iron absorption could be a way of modulating methane production.

8. Conclusions and Future Directions

Oral iron supplementation is known to cause microbiota changes that could potentially include the increase in methanogenic species. Future experiments are needed to further understand the mechanism(s) at work to develop effective treatments resulting in better adherence to iron and an increased cost benefit for healthcare services. This will require assessment of biomarkers of bacterial fermentation and microbiota composition before and after administration of oral iron supplementation and capturing information on provoked symptoms.

There are several potential treatments that could target methanogens; however, besides antibiotics, there remains little evidence of their ability to reduce methane production and limit methanogen growth. Important future directions for this field need to evaluate the effectiveness of the variety of treatments explored in this review.

We have highlighted the current clinical issues with oral iron supplements. Whilst they are effective at restoring iron levels in ID and IDA patients, they are poorly tolerated by a large proportion of patients, including the IBD patient community. Their frequent GI side effects can lead to treatment non-adherence and therefore delays in restoration of iron levels resulting in GI inflammation. However, the mechanism by which oral iron supplements cause these adverse GI effects is unknown.

We hypothesize that potential causes of GI side effects provoked by oral iron supplementation include changes to the gut microbiota. Methanogenic archaea, methanogenesis, and methane gas up-regulation, could be a likely mechanism. The evidence suggests that iron is essential to support methanogenic species growth and methane production. This, taken together with the growing clinical evidence of the effect of methanogens on a range of GI symptoms and conditions, make it an attractive target. Future steps to investigate the mechanism of oral iron-induced GI side effects have been laid out.

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Conflicts of Interest: Anthony Hobson is the cofounder of Functional Gut Diagnostics and has an equity interest in the company.

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