

Improving iron supplements: cooking with GOS

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Commentary on 'Prebiotic galacto-oligosaccharides mitigate the adverse effects of iron fortification on the gut microbiome: a randomized controlled study in Kenyan infants' by Paganini *et al*

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Abbreviations: MNPs: multiple micronutrient powders
 GOS: galacto-oligosaccharides
 GRAS: Generally Regarded as Safe
 Fe-MNPs: MNPs with 5mg Fe
 FeGOS: MNPs with 5mg Fe and with 7.5g GOS
 RTI: respiratory tract infection

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Iron is essential for oxygen transport, generation of energy, synthesis of DNA and multiple enzymatic systems. Iron deficiency impairs these functions and a familiar and important manifestation of advanced iron deficiency is anaemia. Around a quarter of a billion children worldwide are anaemic, and at least half of childhood anaemia is caused in part by a lack of iron; a heavy burden of anaemia is especially present in sub-Saharan Africa and South Asia[1]. Iron replenishments, including iron-containing multiple micronutrient powders (MNPs) can be effective treatments to increase haemoglobin. However, despite the use of such agents for many years, the estimated prevalence of anaemia worldwide in pre-school children only decreased from 47% to 43% between 1993 and 2011[2]. Furthermore, iron replenishments have been associated with adverse events, including infections, intestinal inflammation and diarrhea, in some (but not all) trials. There is a need to make iron treatments both safer and more effective especially in the developing world, and the study in Kenya by Zimmermann and colleagues[3] in this issue of *Gut* addresses these issues.

Iron is needed for growth not only by humans, but also by microbes that colonize and infect us. In particular, pathogenic bacteria in the gut require iron for their virulence, while conversely beneficial microbes include *Lactobacilli* that are a rare example of life that does not use iron to grow[4]. Previous work by the Zimmermann group has shown that iron-containing MNPs can favour the outgrowth of harmful enteropathogens at the expense of protective *Bifidobacteriaceae* and *Lactobacillaceae*, and increase intestinal inflammation in some children[5]; and a trial of MNPs containing iron in Pakistan found an increased level of diarrhea (including bloody-diarrhea)[6]. Additionally, there is strong evidence that iron can exacerbate the risk of malaria in children[7], so that in malarial-endemic areas, anti-malarial measures must be implemented alongside iron supplementation[8].

The Zimmermann group engineered a new formulation of iron-containing MNPs in an attempt to reduce intestinal morbidities while sustaining the beneficial effects of iron. First, they lowered the dose of iron from 12.5mg to 5mg; second, half of this iron was given in a chelated highly-bioavailable form (NaFeEDTA); third, they included a prebiotic of galacto-oligosaccharides (GOS) that can selectively enhance growth of *Bifidobacteriaceae* and *Lactobacillaceae*[9]. GOS has GRAS status (Generally Recognized as Safe – FDA designation) and is frequently added to infant formula milk in industrialized countries.

To test the new formulation, 155 infants aged 6.5–9.5 months were recruited, all of whom had had no vitamin and mineral supplements in the previous 8 weeks, no antibiotic treatments in the previous 10 weeks, and had anticipated residence in the study area, in southern coastal Kenya, for the four-month protocol[3]. Children were randomly assigned to one of three groups; children within each group received daily either MNPs without iron (control), or MNPs with 5mg Fe (Fe-MNPs), or MNPs with 5mg iron and with 7.5g GOS (FeGOS; Trial Registration Number: NCT02118402). At the end of four months, anaemia decreased by about half in both of the groups receiving iron compared to control, indicating the GOS had not interfered with the ability to MNPs containing iron to increase haemoglobin levels. In terms of the microbiota, fecal material was used and ~23000 bacterial 16S rDNA sequences per sample were analysed, along with qPCR measurement of specific genes from selected enteropathogenic bacterial species. The results showed that compared to both the control and the FeGOS groups, children receiving Fe-MNPs had lower abundances of protective *Bifidobacterium* and *Lactobacillus*, higher abundances of *Clostridiales* and of virulence and toxin genes of pathogens, plus higher plasma levels of intestinal fatty acid binding protein, indicating increased damage to enterocytes. Notably, there were no significant differences in these measures between FeGOS and control groups.

Intriguingly, a higher incidence of treated respiratory tract infections (RTIs) occurred in the Fe-MNP group compared to either the control or FeGOS groups. Neither the increase in RTIs with iron nor the ameliorative effect of GOS are straightforward to explain, but the former result is consistent with a previous study suggesting increased evidence of RTIs in children receiving iron-MNPs[6]. Interestingly, the fecal microbiota has also been linked to important respiratory infections in pigs[10]. The protective effects of GOS on the gut microbiota may have wider positive implications for systemic immunity that could decrease RTIs. This raises two further issues: firstly, what is the effect of GOS on iron-associated susceptibility to other infections in children, especially malaria? Second, oral iron replenishment can be ineffective due to blockade of iron absorption by the master iron regulatory hormone hepcidin, which is upregulated by infection and inflammation[11]; so what is the effect of GOS on hepcidin and on the efficiency of dietary iron uptake?

Overall, these important results indicate that GOS may be able to counteract the adverse effect of iron on the infant gut microbiota observed in this and previous studies, while maintaining efficacy against anaemia at a relatively low dose of iron. As such the data deserve significant attention. Future studies are needed to understand and further evaluate the effects and utility of GOS. For example, gut microbiota composition appears to be particularly labile and vulnerable in early life[12], so are the beneficial effects of GOS restricted to infants or would there also be benefit to their use in older children? What are the minimum doses of iron and GOS that together have both haematological and microbiota benefits? Would children in other developing countries or regions, for example South Asia, also experience beneficial effects of GOS? How does the gut microbiota develop over longer time periods after infant exposure to GOS, and are there wider effects of GOS on uptake of other nutrients besides iron?

A broader point is this study illustrates that nutritional interventions for specific indications (here, iron for anaemia) may cause unexpected collateral damage (here, stimulation of gut pathogen growth, increased RTIs) that require countermeasures. This highlights the need for awareness of effects beyond the main outcomes in intervention studies – while this need is demonstrably important in the context of iron, it may also be relevant for other nutrients.

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