Balancing Benefits and Risks of Iron Fortification in Resource-Rich Countries

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For the last 25 years, the American Academy of Pediatrics (AAP) has endorsed the use of iron-fortified infant formulas, noting "no role for the use of low-iron formulas." The rationale for these policies was the recognition that the increase in the use of iron-fortified formulas, accounting for 80% of all formula sold in 1985, was responsible for the declining prevalence of iron-deficiency anemia in US infants.[1] These recommendations were also based on the absence of evidence of discernible adverse effects. Controlled trials had reported no differences in gastrointestinal symptoms, such as colic, constipation, diarrhea, regurgitation, and fussiness, among infants receiving low-iron vs iron-fortified formulas.[2,3] Likewise, evidence was lacking to support another theoretical concern of clinically significant interactions with other micronutrients, specifically zinc and copper. In 1999, the AAP took an even stronger stand and recommended that low iron formulas be removed from the market entirely,[4] for reasons similar to those of the 1989 policy. Further, it was recommended that the minimum iron content for all term infant formulas be at least 4 mg/L.[4] Currently, standard, term infant formulas on the market are all iron-fortified and contain 4-12 mg/L of iron, even though there are some regional differences. In the US, the AAP recommends that infant formulas have an iron content of 10-12 mg/L[5]; in Europe, the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition recommends 4-8 mg/L.[6]

In the recommendations, the contrast in the iron exposure of formula-fed infants vs breastfed infants has primarily focused on the better bioavailability of the iron in breast milk. Although an absorption efficiency of approximately 50% is often quoted, some studies have actually reported absorption in the range of 12%-16%,[7,8] making that bioavailability distinction much less potent. This also suggests that absorption of substantial amounts of dietary iron simply is not critical during the early months of life in healthy infants of normal birth weight. Among all the compositional differences between human milk and formula, the differences in iron content are the most extreme. Virtually all mammalian milks are low in iron, with the exception of rodents, in which postnatal growth is extremely rapid. It seems implausible that this conserved biological pattern is without purpose. It is also clear that iron deficiency occurs in breastfed infants only after the very early months of life. The practical challenge is to identify when the birth iron endowment is exhausted, at which point the infant needs a source of iron from the diet.

This article will discuss the potential advantages of a low iron intake for the infant and the potential adverse effects of drastically altering this, especially in the first 6 months of life. From the outset, two realities must be acknowledged. First, iron deficiency (especially without anemia) in infants remains common, particularly in high-risk groups, including older normal breastfed infants and premature and/or in low birth weight infants. Whether this mild iron deficiency has adverse effects on development is not known. Second, research on potential adverse effects of early and excessive iron exposure is limited, and the evidence base for caution is suggestive but not yet demonstrated by rigorously designed trials. Thus, this represents an emerging area of consideration, and, given the advances in understanding of iron metabolism, the interaction between iron and inflammation, and the importance of early influences on the immature gut and immune system, it is an area that warrants much stronger scientific investigation.

Mineral Concentrations in Human Milk vs Formula

The iron concentration in early human milk is ~0.5 mg/L and declines slightly to ~0.2-0.4 mg/L in mature milk.[9] Thus, even with a relatively low level of fortification in infant formula (eg, 4 mg/L), the amount is ~10-fold higher. For the high end of fortification levels (eg, 12 mg/L), the difference is up to 60-fold greater. Thus, typical intakes from formula by the young infant represent a distinctly unnatural exposure. Another distortion of the balance of micronutrients resulting from fortification of formula is the ratio of zinc to iron. In contrast to iron, zinc is very high in early human milk (2-3 mg/L) and remains >1 mg/L until ~6 months postpartum.[10] Therefore, during the 0- to 6-month period, the Zn:Fe in human milk is ~3-8, depending on the stage postpartum (or Fe:Zn ~0.25). In contrast, current levels

AAP	American Academy of Pediatrics
T1DM	Type 1 diabetes mellitus

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of iron and zinc fortification in formulas are the opposite; the iron concentration is typically about twice that of zinc, which is at least 5-7 mg/L, resulting in Zn:Fe of only 0.5. The effects of this difference are unknown but may be relevant to the effects discussed below.

Potential Adverse Effects of High Iron Exposure in Early Life

Inflammatory and Oxidative Stress Responses

Iron is recognized as a reactive element; its easy redox cycling properties contribute to its utility as a biocatalyst in proteins and as an electron carrier in energy metabolism. It is, however, a potent pro-oxidant. Under anoxic or anaerobic conditions, free iron can be toxic by the formation of reactive oxygen species, including superoxide and other free radicals. Thus, given the "double-edged sword" features of iron—its essentiality as a nutrient and its potential toxicity—very little free iron is present in the circulation under normal circumstances. The majority of iron is bound as part of the heme molecule in hemoglobin; the other major pool is storage iron in the form of ferritin. During transport in the circulation, iron is tightly bound to transferrin.

Growth

Additional iron given to iron-replete infants has been suggested to impair growth. This has been shown in several randomized, controlled studies where iron supplementation was given to infants after 4 months of age.[11-14] However, this possible adverse effect has not been confirmed in meta-analyses.[15] Only a few studies have compared growth of infants <4 months of age receiving formulas with different levels of iron fortification. One small study compared 2 vs 4 mg/L and found no difference in growth between the 2 iron levels or any difference between the formula-fed and breastfed infants from 1-6 months of age.[16] An earlier randomized trial compared two relatively high iron concentrations (7.4 vs 12.7 mg/L formula) from 1-6 months and found no difference between the two groups, but both groups were longer and heavier than a concurrent group of breastfed infants.[17]

Mineral-Mineral Interactions

Potential interactions among trace minerals were noted in the 1989 AAP policy statement and are often raised as a concern for adverse effects of iron fortification and supplementation.[18-20] Several investigations have been undertaken to evaluate this, and, overall, the evidence does not support a potent adverse effect of iron fortification on either zinc or copper absorption.[21-23] Several investigators have shown that iron supplements decrease serum or plasma zinc concentrations.[19,20,24,25] However, plasma zinc, as an index of zinc status, has low sensitivity, is susceptible to many confounding factors including inflammation, and is not a direct reflection of absorption. Data on the effects of different levels of iron fortification in formula have been somewhat conflicting, with very high iron fortification (~14-19 mg/L vs 1.4-2.4 mg/L) having a depressing effect on plasma zinc concentrations in 3- to 4-month-old infants.[26]

Infections and Gastrointestinal Problems

A frequently cited systematic review of oral iron supplementation trials reported a modest but statistically significant increase in the risk of developing diarrhea with oral iron administration, but this review was not specific for or limited to fortification of formula nor to young infants.[27] Recent trials in Pakistan, Ghana, and Kenya[28-30] relating iron-containing micronutrient powders to increased diarrhea, including severe and bloody diarrhea, also are not within the scope of this chapter, which concerns infants in resource-rich countries.

As noted above, the early recommendations for the safety of iron-fortified formulas, including from birth, were based on trials undertaken with different levels of fortification.[2,3] Gastrointestinal complaints such as constipation, spitting-up, vomiting, fussiness, or cramping were not different among infants randomized at birth and continued on fortified (12 mg/L) or unfortified (1.5 mg/L) formulas for approximately 6-12 weeks.[2,3] Both studies concluded that, in the absence of gastrointestinal signs or symptoms, "there are few indications for feeding commercially prepared formulas that are not fortified with iron."[2]

Relevant to the topic of this article, the gastrointestinal tract of the young infant is particularly vulnerable to any imbalances that can alter the mucosal barrier function, the maturation of the intraepithelial tight junctions and intestinal permeability, and the development of the innate immune system and a favorable intestinal microbial community. In recent years, there has been a growing appreciation of the critical interrelationships of the enteric microbiome with the host immune system and metabolism and of the influence of diet on both the compositional and functional features. In particular, early postnatal life is a time for intestinal maturation and colonization by the commensal microbiota and the establishment of immunologic and metabolic programming that may have long-term consequences. Differences in the enteric microbiome between breastfed and formula-fed infants have been clearly documented.[31-33] Generally recognized patterns include breastfed infants as having higher counts of *Bifidobacteria* and *Lactobacillus* and lower counts of *Bacteroides*, *Clostridium coccoides* group, *Staphylococcus*, and *Enterobacteriaceae* than formula-fed infants.[34] Breast milk is an important source of the specific colonization pattern of the infant that resembles closely the maternal genotypes. Prebiotics, especially human milk oligosaccharides, are thought to favorably shape the commensal bacteria of the newborn's intestinal tract. In addition to feeding type, mode of delivery, antibiotic exposure, and environmental factors have been found to influence the enteric microbiome.[34] However, primary determinants of its composition within different nutritional sources are not yet clear.

The potential impact of iron exposure on young infants' microbiota has not been investigated in controlled interventional studies. Interest is emerging specifically on the

influence of iron exposure on the gut (specifically colonic) microbiota, and this raises particular theoretical concern for the potential impact for the young infant when colonization is rapidly established after virtual "sterility" at birth. A low iron environment, such as that resulting from exclusive feeding of human milk, influences intestinal bacterial growth (ie, fosters growth of *Lactobacillus*), which is distinctly not dependent on iron. The initial colonization pattern following vaginal delivery shows a predominance of facultative anaerobes *E coli* and *Enterobacteriaceae*, changing in the low oxygen environment in the gut to a predominance of strict anaerobes, especially *Bifidobacterium, Clostridium*, and *Bacteroides*. In contrast, a high intraluminal iron enhances both bacterial replication and the production of virulence factors in pathogenic bacteria.[35] The iron in formula that is not absorbed may, in effect, interfere with the typical progression of colonization.

The low iron content of human milk fosters a "simple" microbiota and the predominance of commensal bacteria. Illustrative of this, in a study reported nearly 3 decades ago, newborn nonbreastfed infants were randomized to receive cow milk based preparations with (5 mg/L) and without (0.5 mg/L) iron fortification, and both groups were compared with exclusively breastfed infants. Using culture techniques, the gut flora of the breastfed infants at 3 months of age comprised predominantly *Bifidobacteria*, low counts of *E coli*, and virtually no other bacteria. Similar patterns were reported for the unfortified cow milk preparation, except for a greater frequency of *E coli*. In contrast, infants receiving the iron-fortified cow milk preparation had low counts of *Bifidobacteriaceae*, high counts of *Bacteroides* spp and *E coli*, and frequent counts of other bacteria.[36] Notably, results of cultures were significantly different among the 3 groups as early as 1 week of age.[37]

As noted above, human milk contains several prebiotic factors, including a range of oligosaccharides. Lactoferrin, which binds and facilitates the uptake of iron by the enterocyte,[38] is also a prebiotic with important functional characteristics that foster a healthy intestinal microbiome. Lactoferrin binds iron avidly and contributes to the low iron environment in the gut lumen of breastfed infants. It is also resistant to proteolysis and present in the stool of breastfed infants, thus, potentially offering a colonic "mechanism" to deprive pathogenic microbes of iron and facilitate growth of commensals. Lactoferrin also has immunomodulatory activity by means of induction of T-helper cells that protect the young infant against infection.[39] Thus, in addition to the striking difference in iron content of standard infant formulas compared with human milk, the absence of lactoferrin and its iron binding effects is another potential risk factor for gastrointestinal effects, especially in the young healthy term infant for whom iron status is likely to be adequate and the drive for absorption of dietary iron is minimal.

Compared with iron, even less is known about the effects of other micronutrients on the developing microbiome, including the role of zinc. In contrast to the low iron content of human milk and the iron binding capacity of lactoferrin, the young exclusively breastfed infant is naturally exposed to a very high zinc intake, of which only about 50% is absorbed. Besides its effects on intestinal maturation,[40] the high ratio of zinc to iron may be another factor that promotes development of a healthy microbial profile for the young infant and/or protects against invasion by pathogenic organisms. Inhibition of several virulence factors of enteropathogens has been associated with high intraluminal zinc content, as would be expected in the young breastfed infant.[41] This poses a potential adverse effect of the reversal of the balance of zinc to iron described above resulting from the use of iron-fortified formula for young infants.

Metabolic and Developmental Impact

One retrospective case control analysis examined the relationship between feeding mode from birth to 4 months and the development of type 1 diabetes mellitus (T1DM) by 1-6 years of age.[42] Children who developed T1DM had significantly higher total iron intake, reflecting consumption of high iron fortified formula. For each SD of increase in iron intake, the OR for T1DM was 2.0 among all children and 2.26 when affected children were compared with a sibling control. Several limitations of this study were noted, including use of retrospective self-reported dietary intake data and potential bias for feeding choice (breast milk vs low- and high-iron formula). However, the findings raise important questions that merit prospective large-scale studies to investigate the potential causal relationship between early iron exposure and T1DM, as well as other autoimmune-based conditions.

The long-term developmental effects of iron exposure during infancy were assessed in a 10-year follow-up report of infants randomized at 6 months of age to either a high iron (12.7 mg/L) or low iron (2.3 mg/L) infant formula.[43] Infants assigned to the high iron formula scored lower on all developmental outcomes tested.[44] The findings illustrate the potential adverse effects of unduly high iron exposure, especially to those who seemed to be iron replete as would be the case for the majority of healthy term infants in the first few postnatal months.

Preterm and Low Birth Weight Infants in Immediate Postnatal Period

This discussion concerns iron needs primarily of very low birth weight infants during their hospital stay.[45] The dilemma is that the iron needs of the preterm infant, which are high, need to be met while at the same time avoiding unduly high intakes of iron that could potentially overwhelm the diminished antioxidant defenses of the premature infant.[46] Preterm infants are born with lower iron stores than term infants. In the neonatal period, premature infants typically incur substantial losses of hemoglobin iron because of phlebotomy but also often receive red blood cell transfusions that provide substantial intakes of iron. For these reasons, the amount of storage iron available to the infant at any given

time is not known. Determination of serum ferritin could provide this information.[47]

The amount of iron needed for growth can be estimated by the factorial method. At birth, the total body iron of the premature infant is assumed to be 75 mg/kg body weight, the same as that of the term infant. The physiologic increase in body iron of the growing premature infant was estimated by Griffin and Cooke[48] to be approximately 0.29 mg/kg/d at around 32 weeks, rising to 0.37 mg/kg/d close to term, and declining gradually after term. These estimates serve as basis for estimating necessary intakes of iron. Absorption of iron determined by isotope balance in premature infants averaged 31.5%,[49] 41.6%,[50] and 30%-34%.[51] The fact that the percentage of iron absorption is high despite the documented or presumed presence of storage iron and that iron absorption (mg/d) was found to be a linear function of iron intake[52] strongly suggest that the usual mechanism(s) controlling iron absorption are not (yet) operating in preterm infants. It should be mentioned that methods using red cell incorporation of labels yield substantially lower values than balance studies, presumably because the initial dilution of the label into the large body iron pool does not represent absorption. Assuming an accretion rate of 0.37 mg/kg/d[48] and absorption of 30%, the necessary intake of ion may be calculated as 1.23 $mg \cdot kg^{-1} \cdot day^{-1}$. A number of supplementation trials have been performed over the years[45] have shown that iron supplementation improves hematologic and other indicators of iron status. In the few studies that evaluated growth, no effect of iron supplementation was noted. Cognitive outcome was not evaluated in the majority of studies.

Adverse Effects

Adverse effects of iron supplementation have been noted infrequently, although only a handful of studies have specifically looked for adverse effects. Long et al[53] found that no adverse effects of any kind were consistently reported. In a study by Barclay et al,[54] lower erythrocyte superoxide dismutase was noted in the group receiving high iron supplementation, possibly indicating altered copper metabolism. Braekke et al[55] gave a very high dose (18 mg/d) of iron in the form of ferrous fumarate to infants with birth weight less than 1500 g. After 1 week of treatment, there was no change in urinary 8-isoprostane excretion, plasma total hydroperoxides, and most plasma antioxidants. Although the period of exposure was short in this study, the dose of iron was relatively high.

Age at Start of Supplementation

Three studies have compared earlier with later initiation of iron supplementation. Franz et al[56] enrolled 204 infants with birth weight less than 1300 g and assigned them randomly to receive iron supplementation starting at 14 days (early iron) or at 61 days (late iron) in a dose of 2 $mg \cdot kg^{-1} \cdot day^{-1}$. The iron dose was increased to 4 $mg \cdot kg^{-1} \cdot day^{-1}$ if the hematocrit dropped to less than 30%. Infants in the late iron group needed significantly more blood transfusions, and a significantly greater percentage of infants had iron deficiency. At 61 days, iron status variables did not differ significantly between groups, probably because the late iron group received a greater number of blood transfusions. When followed up at 5.3 years of age, a significantly greater percentage (35%) of children in the late iron group had neurologic abnormalities than those in the early iron group (19%).[57] Arnon et al[58] provided supplemental iron in a dose of 5 $mg \cdot kg^{-1} \cdot day^{-1}$ in the form of iron polymaltose complex and compared initiation of supplementation at 2 and 4 weeks. At 8 weeks of age, infants initiated at 2 weeks had significantly higher hemoglobin and better iron status than those started at 4 weeks. Infants started at 2 weeks also needed significantly fewer red blood cell transfusions than those started at 4 weeks. In a recent study, Joy et al[59] compared initiation of iron supplementation at 2 weeks and at 6 weeks. At 12 weeks of age, infants started on iron early showed significantly higher serum ferritin and better hematologic indicators of iron status. No differences in growth and clinical outcomes were observed. In each of the 3 above studies, outcomes were better if iron was started at 2 weeks than at some later age.

Anemia of Prematurity

It is common practice to increase the dose of supplemental iron if the hematocrit is low and/or falling. Anecdotal reports indicate that such increasing of iron supplements in the face of a falling hematocrit is widely practiced despite the absence of any evidence that the anemia of prematurity responds to iron intakes greater than 2 $mg \cdot kg^{-1} \cdot day^{-1}$. On the contrary, there is good evidence from a randomized controlled trial that giving iron above what were presumed to be adequate iron intakes from the diet did not improve hematologic status at all.[60] It is, of course, possible that the occasional premature infant with low or falling hematocrit is iron deficient, most likely because of low birth iron endowment. Such infants clearly need iron supplementation, whereas the great majority of premature infants with anemia do not. Increased iron supplementation (greater than 2 $mg \cdot kg^{-1} \cdot day^{-1}$) should be reserved for those infants who show evidence of diminished iron stores. Domellöf[61] has suggested that the cut-off should be a plasma ferritin of 35 $\mu g/mL$.

In the aggregate, these trials have shown that without iron supplementation a high percentage of premature infants develop iron deficiency. The available evidence leads to the conclusions that 2 $mg \cdot kg^{-1} \cdot day^{-1}$ of iron is adequate to prevent iron deficiency in most infants and iron deficiency anemia and that supplementation should start at 2 weeks of age in very low birth weight infants. The paucity of documented adverse effects of iron supplements in the face of high risk of iron deficiency supports routine iron supplementation in preterm infants during the hospital stay.

Conclusions

For healthy term infants, especially those who had the benefit of delayed cord clamping, several arguments can be made

against the use of iron-fortified formula for the first 3-4 months of postnatal life. First, the dietary iron requirement is minimal during this period, and very little exogenous iron is likely to be absorbed. Second, the unabsorbed iron in the gut fosters a proinflammatory response in the intestine, possibly with systemic effects. Third, emerging evidence supports an adverse effect of iron exposure on the enteric microbiome during a very formative stage of development of the complex relationship between intestinal defenses and host immune responses. There is growing appreciation of the role of early immunologic effects, including the enteric microbiome, on long-term health. This, along with the reality that a very large percentage of normal infants are formula-fed and, thus, face iron exposure that diverges markedly from that of breastfed infants, justifies a more expansive investigation of potential adverse effects of current iron fortification practices. ■

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References

1. American Academy of Pediatrics Committee on Nutrition. Iron-fortified infant formulas. Pediatrics 1989;84:1114-5.
2. Oski FA, Bennett R, Campbell J, Charles W, Cirincione FJ, Corwin R, et al. Iron-fortified formulas and gastrointestinal symptoms in infants: a controlled study. Pediatrics 1980;66:168-70.
3. Nelson SE, Ziegler EE, Copeland AM, Edwards BB, Fomon SJ. Lack of adverse reactions to iron-fortified formula. Pediatrics 1988;81:360-4.
4. American Academy of Pediatrics Committee on Nutrition. Iron fortification of infant formulas. Pediatrics 1999;104:119-23.
5. Baker RD, Greer FR, American Academy of Pediatrics Committee on Nutrition. Diagnosis and prevention of iron deficiency and iron-deficiency anemia in infants and young children (0-3 years of age). Pediatrics 2010;126:1040-50.
6. Domellöf M, Braegger C, Campoy C, Colomb V, Decsi T, Fewtrell M, et al. Iron requirements of infants and toddlers. J Pediatr Gastroenterol Nutr 2014;58:119-29.
7. Davidsson L, Kastenmayer P, Yuen M, Lönnerdal B, Hurrell RF. Influence of lactoferrin on iron absorption from human milk in infants. Pediatr Res 1994;35:117-24.
8. Domellöf M, Lönnerdal B, Abrams SA, Hernell O. Iron absorption in breast-fed infants: effects of age, iron status, iron supplements, and complementary foods. Am J Clin Nutr 2002;76:198-204.
9. Domellöf M, Lönnerdal B, Dewey KG, Cohen RJ, Hernell O. Iron, zinc, and copper concentrations in breast milk are independent of maternal mineral status. Am J Clin Nutr 2004;79:111-5.
10. Krebs NF, Reidinger CJ, Hartley S, Robertson AD, Hambidge KM. Zinc supplementation during lactation: effects on maternal status and milk zinc concentrations. Am J Clin Nutr 1995;61:1030-6.
11. Idjradinata P, Watkins WE, Pollitt E. Adverse effect of iron supplementation on weight gain of iron-replete young children. Lancet 1994;343:1252-4.
12. Lind T, Seswandhana R, Persson LA, Lönnerdal B. Iron supplementation of iron-replete Indonesian infants is associated with reduced weight-for-age. Acta Paediatr 2008;97:770-5.
13. Majumdar I, Paul P, Talib VH, Ranga S. The effect of iron therapy on the growth of iron-replete and iron-deplete children. J Trop Pediatr 2003;49:84-8.
14. Dewey KG, Domellöf M, Cohen RJ, 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;132:3249-55.
15. Ramakrishnan U, Nguyen P, Martorell R. Effects of micronutrients on growth of children under 5 years of age: meta-analyses of single and multiple nutrient interventions. Am J Clin Nutr 2009;89:191-203.
16. Hernell O, Lönnerdal B. Iron status of infants fed low-iron formula: no effect of added bovine lactoferrin or nucleotides. Am J Clin Nutr 2002;76:858-64.
17. Bradley CK, Hillman L, Sherman AR, Leedy D, Cordano A. Evaluation of two iron-fortified, milk-based formulas during infancy. Pediatrics 1993;91:908-14.
18. Iannotti LL, Tielsch JM, Black MM, Black RE. Iron supplementation in early childhood: health benefits and risks. Am J Clin Nutr 2006;84:1261-76.
19. Lind T, Lönnerdal B, Stenlund H, Ismail D, Seswandhana R, Ekström EC, et al. A community-based randomized controlled trial of iron and zinc supplementation in Indonesian infants: interactions between iron and zinc. Am J Clin Nutr 2003;77:883-90.
20. Berger J, Ninh NX, Khan NC, Nhien NV, Lien DK, Trung NQ, et al. Efficacy of combined iron and zinc supplementation on micronutrient status and growth in Vietnamese infants. Eur J Clin Nutr 2006;60:443-54.
21. Domellöf M, Hernell O, Abrams SA, Chen Z, Lönnerdal B. Iron supplementation does not affect copper and zinc absorption in breastfed infants. Am J Clin Nutr 2009;89:185-90.
22. Haschke F, Ziegler EE, Edwards BB, Fomon SJ. Effect of iron fortification of infant formula on trace mineral absorption. J Pediatr Gastroenterol Nutr 1986;5:768-73.
23. Esamai F, Liechty E, Ikemeri J, Westcott J, Kemp J, Culbertson D, et al. Zinc absorption from micronutrient powder is low but is not affected by iron in Kenyan infants. Nutrients 2014;6:5636-51.
24. Whittaker P. Iron and zinc interactions in humans. Am J Clin Nutr 1998;68:442S-6S.
25. Friel JK, Andrews WL, Aziz K, Kwa PG, Lepage G, L'Abbe MR. A randomized trial of two levels of iron supplementation and developmental outcome in low birth weight infants. J Pediatr 2001;139:254-60.
26. Craig WJ, Balbach L, Harris S, Vyhmeister N. Plasma zinc and copper levels of infants fed different milk formulas. J Am Coll Nutr 1984;3:183-6.
27. Gera T, Sachdev HP. Effect of iron supplementation on incidence of infectious illness in children: systematic review. BMJ 2002;325:1142.
28. Soofi S, Cousens S, Iqbal SP, Akhund T, Khan J, Ahmed I, et al. 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.
29. Zlotkin S, Newton S, Aimone AM, Azindow I, Amenga-Etego S, Tchum K, et al. Effect of iron fortification on malaria incidence in infants and young children in Ghana: a randomized trial. JAMA 2013;310:938-47.
30. Jaeggi T, Kortman GA, Moretti D, Chassard C, Holding P, Dostal A, et al. Iron fortification adversely affects the gut microbiome, increases

pathogen abundance and induces intestinal inflammation in Kenyan infants. Gut 2015;64:731-42.
31. Harmsen HJ, Wildeboer-Veloo AC, Raangs GC, Wagendorp AA, Klijn N, Bindels JG, et al. Analysis of intestinal flora development in breast-fed and formula-fed infants by using molecular identification and detection methods. J Pediatr Gastroenterol Nutr 2000;30:61-7.
32. Fallani M, Young D, Scott J, Norin E, Amarri S, Adam R, et al. Intestinal microbiota of 6-week-old infants across Europe: geographic influence beyond delivery mode, breast-feeding, and antibiotics. J Pediatr Gastroenterol Nutr 2010;51:77-84.
33. Schwartz S, Friedberg I, Ivanov IV, Davidson LA, Goldsby JS, Dahl DB, et al. A metagenomic study of diet-dependent interaction between gut microbiota and host in infants reveals differences in immune response. Genome Biol 2012;13:r32.
34. Matamoros S, Gras-Leguen C, Le Vacon F, Potel G, de La Cochetiere MF. Development of intestinal microbiota in infants and its impact on health. Trends Microbiol 2013;21:167-73.
35. Kortman GA, Raffatellu M, Swinkels DW, Tjalsma H. Nutritional iron turned inside out: intestinal stress from a gut microbial perspective. FEMS Microbiol Rev 2014;38:1202-34.
36. Mevissen-Verhage EA, Marcelis JH, Harmsen-Van Amerongen WC, de Vos NM, Verhoef J. Effect of iron on neonatal gut flora during the first three months of life. Eur J Clin Microbiol 1985;4:273-8.
37. Mevissen-Verhage EA, Marcelis JH, Harmsen-van Amerongen WC, de Vos NM, Berkel J, Verhoef J. Effect of iron on neonatal gut flora during the first week of life. Eur J Clin Microbiol 1985;4:14-8.
38. Lönnerdal B, Georgieff KM, Hernell O. Developmental physiology of iron absorption, homeostasis, and metabolism in the healthy term infant. J Pediatr 2015;167:S8-14.
39. Sherman MP. Lactoferrin and necrotizing enterocolitis. Clin Perinatol 2013;40:79-91.
40. Krebs NF, Miller LV, Hambidge KM. Zinc deficiency in infants and children: a review of its complex and synergistic interactions. Paediatr Int Child Health 2014;34:279-88.
41. Crane JK, Byrd IW, Boedeker EC. Virulence inhibition by zinc in shigatoxigenic *Escherichia coli*. Infect Immun 2011;79:1696-705.
42. Ashraf AP, Eason NB, Kabagambe EK, Haritha J, Meleth S, McCormick KL. Dietary iron intake in the first 4 months of infancy and the development of type 1 diabetes: a pilot study. Diabetol Metab Syndr 2010;2:58.
43. Lozoff B, Castillo M, Clark KM, Smith JB. Iron-fortified vs low-iron infant formula: developmental outcome at 10 years. Arch Pediatr Adolesc Med 2012;166:208-15.
44. Zlotkin HS, Davidsson L, Lozoff B. Balancing the benefits and risks of iron fortification in resource-constrained settings. J Pediatr 2015;167:S26-30.
45. Domellöf M, Georgieff MK. Postdischarge iron requirements of the preterm infant. J Pediatr 2015;167:S31-5.
46. Buonocore G, Perrone S, Longini M, Vezzosi P, Marzocchi B, Paffetti P, et al. Oxidative stress in preterm neonates at birth and on the seventh day of life. Pediatr Res 2002;52:46-9.
47. Hernell O, Fewtrell MS, Georgieff MK, Krebs NF, Lönnerdal B. Summary of current recommendations on iron provision and monitoring of iron status for breastfed and formula-fed infants in resource-rich and resource-constrained countries. J Pediatr 2015;167:S40-7.
48. Griffin I, Cooke RJ. Iron retention in preterm infants fed low iron intakes: a metabolic balance study. Early Hum Dev 2010;86(Suppl 1):49-53.
49. Gorten MK, Hepner R, Workman JB. Iron metabolism in premature infants. I. Absorption and utilization of iron as measured by isotope studies. J Pediatr 1963;63:1063-71.
50. Ehrenkranz RA, Gettner PA, Nelli CM, Sherwonit EA, Williams JE, Pearson HA, et al. Iron absorption and incorporation into red blood cells by very low birth weight infants: studies with the stable isotope ^{58}Fe. J Pediatr Gastroenterol Nutr 1992;15:270-8.
51. Widness JA, Lombard KA, Ziegler EE, Serfass RE, Carlson SJ, Johnson KJ, et al. Erythrocyte incorporation and absorption of 58Fe in premature infants treated with erythropoietin. Pediatr Res 1997;41:416-23.
52. Dauncey MJ, Davies CG, Shaw JCL, Urman J. The effect of iron supplements and blood transfusion on iron absorption by low birthweight infants fed pasteurized human breast milk. Pediatr Res 1978;12:899-904.
53. Long H, Yi JM, Hu PL, Li ZB, Qiu WY, Wang F, et al. Benefits of iron supplementation for low birth weight infants: a systematic review. BMC Pediatr 2012;12:99.
54. Barclay SM, Aggett PJ, Lloyd DJ, Duffty P. Reduced erythrocyte superoxide dismutase activity in low birth weight infants given iron supplements. Pediatr Res 1991;29:297-301.
55. Braekke K, Bechensteen AG, Halvorsen BL, Blomhoff R, Haaland K, Staff AC. Oxidative stress markers and antioxidant status after oral iron supplementation to very low birth weight infants. J Pediatr 2007;151:23-8.
56. Franz AR, Mihatsch WA, Sander S, Kron M, Pohlandt F. Prospective randomized trial of early versus late enteral iron supplementation in infants with a birth weight of less than 1301 grams. Pediatrics 2000;106:700-6.
57. Steinmacher J, Pohlandt F, Bode H, Sander S, Kron M, Franz AR. Randomized trial of early versus late enteral iron supplementation in infants with a birth weight of less than 1301 grams: neurocognitive development at 5.3 years' corrected age. Pediatrics 2007;120:538-46.
58. Arnon S, Shiff Y, Litmanovitz I, Regev RH, Bauer S, Shainkin-Kestenbaum R, et al. The efficacy and safety of early supplementation of iron polymaltose complex in preterm infants. Am J Perinatol 2007;24:95-100.
59. Joy R, Krishnamurthy S, Bethou A, Rajappa M, Ananthanarayanan PH, Bhat BV. Early versus late enteral prophylactic iron supplementation in preterm very low birth weight infants: a randomized controlled trial. Arch Dis Child Fetal Neonatal Ed 2014;99:F105-9.
60. Taylor TA, Kennedy KA. Randomized trial of iron supplementation versus routine iron intake in VLBW infants. Pediatrics 2013;131:e433-8.
61. Domellöf M. Nutritional care of premature infants: microminerals. In: Koletzko B, Poindexter B, Uauy R, eds. Nutritional care of preterm infants: scientific basis and practical guidelines World Rev Nutr Diet 2014/04/23 ed. Basel: Karger; 2014. p. 121-39.