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Nutritional Interventions and the Gut Microbiome in Children

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Abstract

The gut microbiome plays an integral role in health and disease, and diet is a major driver of its composition, diversity, and functional capacity. Given the dynamic development of the gut microbiome in infants and children, it is critical to address two major questions: (*a*) Can diet modify the composition, diversity, or function of the gut microbiome, and (*b*) will such modification affect functional/clinical outcomes including immune function, cognitive development, and overall health? We synthesize the evidence on the effect of nutritional interventions on the gut microbiome in infants and children across 26 studies. Findings indicate the need to study older children, assess the whole intestinal tract, and harmonize methods and interpretation of findings, which are critical for informing meaningful clinical and public health practice. These findings are relevant for precision health, may help identify windows of opportunity for intervention, and may inform the design and delivery of such interventions.

Contents

1. INTRODUCTION

Diet and nutritional status modify the composition of the bacteria, archaea, fungi, and viruses residing in the human gut. The diet also provides substrates for these microbes, as well as inhibitors of growth and function, thereby influencing their function. Early in life, critical functions such as immune function and cognitive development are changing the most and are highly sensitive to nutrition. There also is evidence that early gut bacterial patterns may persist and have long-lasting impacts on health, including allergy and obesity in adulthood, which could be prevented by changes in early-life diet (49, 126).

Human gut microbiota: the microbial community in the human gut

Human gut microbiome: the human gut microbiota and its associated genes

Functional profile: functional capacity of microbiota to allow exploration of metabolic, virulence, and other biochemical pathways, predicted from 16S DNA taxonomy such as PICRUSt or inferred from whole-genome sequencing data

In this review, we synthesize the current evidence of the links among nutrition, nutritional interventions, and the gut microbiome in children 0 to 19 years of age, a period of growth and varying susceptibility to disease. We also discuss mechanisms that explain how nutrients, alone or in combinations, may impact the gut microbiome. We then identify research gaps and suggest priority areas for future work.

2. GUT MICROBIOME

In this review, we define the human gut microbiota as the microbial composition of the gastrointestinal tract and the human gut microbiome as the human gut microbiota and its associated genes plus its functional profile (174). The human gut comprises a 1:1 ratio of bacterial cells to human cells (139), a closer approximation than the previously referenced 10:1 ratio at maximum (101). These bacterial cells make up 500 to 1,000 bacterial species at any one time in the gut and contain as many as 2 million bacterial genes, roughly 100 times the number of genes contained in the human genome (49, 158), With its high concentrations of antigens, gut-associated lymphoid tissue, and immune cells, the gut has a role as an immune organ in addition to its role in food digestion and nutrient metabolism (164). Of considerable interest is the impact of diet on the

gut microbiota, which has been shown to change bacterial composition and microbial gene expression in adults (83, 184). Conversely, some studies show that microbiota are resistant to dietary intervention (42). The interplay between the gut microbiome and human health states—including allergy, atopic diseases, autoimmune disorders, and noncommunicable diseases, as well as infectious diseases, vaccine response, and poor growth, particularly in children living in lower-income settings (49, 184)—has been of much interest in recent years. However, there are no studies in healthy children after the first year of life, to our knowledge, to contrast with the few studies done in older children with illness or nutrient deficiency, despite purported links with both immediate and longer-term health outcomes.

2.1. Putative Normal Development of Gut Microbiota in Infants

In **Figure 1**, we summarize the current understanding of gut microbiota colonization and development in early life. Recent reviews and studies dispel the idea of in utero colonization, showing that current evidence is more likely to be an artifact of contamination acquired during sample collection and processing of the low-biomass samples (10, 88). From birth onward, the developing gut microbiome has been shown to be dynamic in bacterial and viral composition (94, 171), and the microbiome stabilizes and resembles that of an adult around 2 to 3 years of age (49); however, some studies have shown that the microbiota continue to change in proportion and diversity across the life span (31, 83, 134).

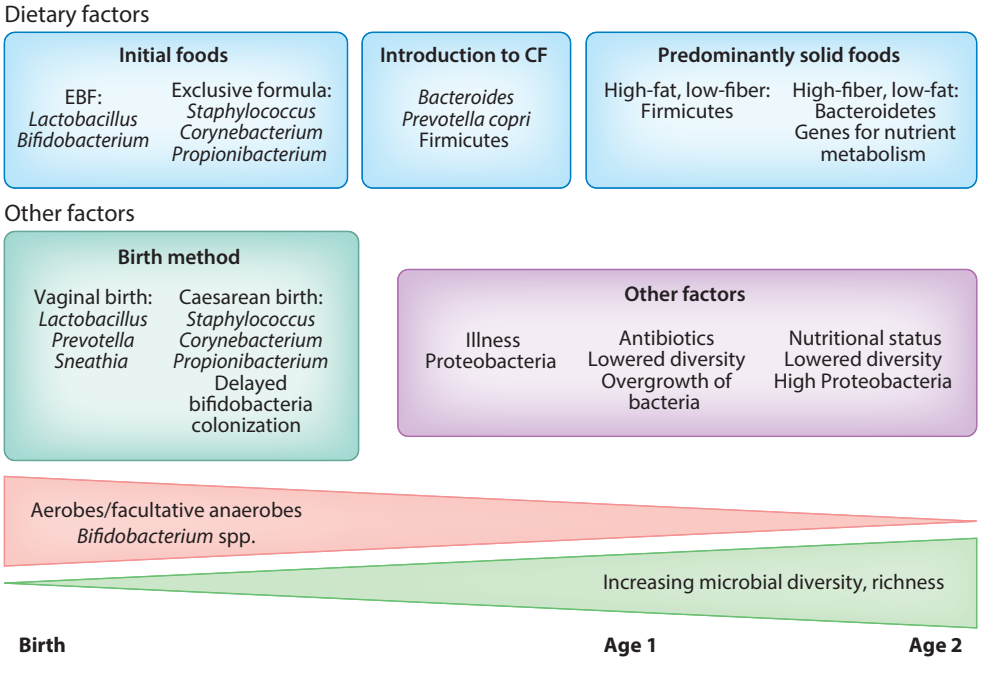

Figure 1

Development of gut microbiota over the first 2 years of life in a healthy child, and factors that enrich certain bacteria, increase or decrease diversity, or have been associated with another effect. Undernourished children tend to have lower microbial maturity (i.e., low microbiota-for-age z score), resulting in prolonged overrepresentation of facultative anaerobes such as Proteobacteria and lower diversity. Figure adapted from images created with BioRender.com. Abbreviations: CF, complementary foods; EBF, exclusive breastfeeding.

The mode of birth—vaginal or Caesarean delivery—informs the first bacterial colonizers of the infant gut (36). Vaginally delivered infants tend to initially acquire bacteria resembling vaginal microbiota, including genera *Lactobacillus*, *Prevotella*, and *Sneathia*, while infants born by Caesarean section are first exposed to skin and harbor related microbes such as *Staphylococcus*, *Corynebacterium*, and *Propionibacterium* (36). Members of the Proteobacteria phylum proliferate in the infant gut for approximately 2 weeks (35, 106); as facultative anaerobes, *Escherichia coli* and *Enterococcus* spp. are able to process the oxygen present in the neonatal gut to allow anaerobic commensal microbiota to flourish, such as *Bacteroides*, *Clostridium*, and in particular, *Bifidobacterium*, spanning the Bacteroidetes, Firmicutes, and Actinobacteria phyla, respectively (35, 93). These four phyla represent the majority of bacteria found in the human gut microbiome after 1 year of age, though up to 10 phyla have been observed (153). This succession of bacteria depends not only on the delivery mode but also on exposure to antibiotics, infant diet, and maternal health (169).

As the breastfed infant matures, *Bifidobacterium* spp. have been observed to compete with Proteobacteria in the guts of infants in higher-income populations (33, 71, 106), particularly in association with breastfeeding, due to substances in breast milk, such as human milk oligosaccharides (HMOs) serving as substrates for *Bifidobacterium* spp. (see Section 3.2) (84) (**Figure 1**). Much of our understanding of the gut microbiome in early childhood stems from studies conducted in high-income settings such as the United States (16, 25, 75, 82, 98, 147, 152, 154, 180), Canada (7, 76), Ireland (113), South Korea (87), Finland (81), the United Kingdom (165), The Netherlands (150), and Singapore (35). From these studies, the taxonomic composition of the gut microbiome transitions from a high abundance of milk-metabolizing microbiota to a greater diversity of taxa when complementary foods are introduced in later infancy and early childhood (6 months to 2–3 years) (6).

Some recent studies suggest that the cessation of breastfeeding may be the major driver in microbial shifts at this stage of development, as bacteria in the infant gut are metabolically equipped to break down simple plant-derived constituents such as starches before solid foods are first consumed (31). The introduction of solid foods encourages the growth of other bacteria, such as *Bacteroides* spp., *Prevotella copri*, and species in Firmicutes, which help to metabolize complex carbohydrates (3, 40). The timing of complementary food introduction may also impact microbial diversity, composition, and short-chain fatty acid (SCFA) concentrations (32).

2.2. Putative Normal Development of Gut Microbiota in Children

Few studies have examined the gut microbiota beyond the first few years of life up to age 19 (31). From cross-sectional and cohort studies, it appears that the rate of gut microbiota development across early life may vary among children and is impacted by dietary habits. Healthy preschool and school-age Dutch children ($n = 61$) aged 2–18 years old were sampled for gut microbial analysis weekly for 18 months and were found to have compositional stability (29), similar to the findings in adults (156). Another study found that certain food groups, such as micronutrient-fortified non-whole-grain foods, were associated with microbial community structure but not diversity among 2–9-year-old American children (63). Another US study (11) among 4–8-year-old children found that correlations between dietary patterns were associated with increases or decreases in certain bacterial species. For example, Dietary Pattern 1 (fish, proteins, refined carbohydrates, vegetables, fruit, juice and sweetened beverages, sweets) was associated with a lower abundance of *Bifidobacterium* spp., of which many species produce helpful SCFAs. Serial fecal sampling in large cohorts of non-Western populations and studies assessing nutrient intake and the microbiota in older children and during puberty, particularly in the context of a randomized trial, to understand microbial development in early life remains a research gap.

3. IMPACT OF NUTRITIONAL INPUTS ON THE GUT MICROBIOME

This review focuses on the impact of foods or food constituents on the gut microbiome throughout childhood. The included trials employed interventions involving whole foods; micronutrient supplementation or fortification; food constituents such as bovine milk fat globule membrane (MFGM); and specially formulated foods, such as early limited formula (ELF), ready-to-use therapeutic foods, ready-to-use supplementary foods (RUSFs), and lipid-based nutrient supplements (LNSs). Criteria for trials eligible for this review are described in **Supplemental Table 1**, **Supplemental Table 2**, and the section titled Methods in the **Supplemental Material**. Other research including cohort, cross-sectional, and laboratory-based experimental studies is also discussed to provide additional evidence and support for mechanistic aspects for each dietary intervention.

Supplemental Material >

Nutritional inputs including diet have the potential to help select for microbiota considered beneficial and to ultimately improve human health, including functional outcomes. The healthy digestive system is a complex, homeostatic microenvironment. It involves commensal gut microbiota as well as intestinal conditions such as oxygen levels, pH, and gastric juices, and it functions in absorption and metabolism of nutrients to promote epithelial integrity (96). Below, we explore how diet may impact the gut microbiota, with a discussion of the foods and nutrients that were part of the interventions being examined in the studies included in this review. It is important to keep in mind that increasing age is concomitant with intestinal development, changes in diet, and environmental exposures, and thus it is challenging to ascribe direct effects of interventions on the microbiome, particularly during early development. This review is structured broadly by the population group targeted by the included studies—mothers or children—and then by the major component of the intervention: breast milk, formula, macronutrients, or micronutrients. We also have arranged each section by life stage as much as possible.

3.1. Maternal Diet and Supplementation

A recent review found that maternal high-fat diets and the intake of fat-soluble vitamins and fiber during pregnancy were associated with differences in gut microbial composition in neonates; however, the limited number of studies included were observational and mostly conducted in overweight or obese mothers, limiting causal inference and generalizability (99). In an observational study among 323 infants, maternal diet during pregnancy, including intakes of high versus low amounts of vegetables, processed meats, and deep-fried foods, was not associated with the infant gut microbiome (136).

Two randomized trials also did not find any differences in infant microbiota from maternal supplementation of ω-3 fatty acids or LNSs compared with the placebo group (73). One study examined the impact of maternal fish oil consumption during the third trimester on the infant gut microbiome, compared with the placebo group (66). Fish oil is rich in both ω-3 and ω-6 long-chain polyunsaturated fatty acids (LCPUFAs), including eicosapentaenoic and docosahexaenoic acids, respectively. ω-3 LCPUFAs may exert anti-inflammatory effects on intestinal immune cells and lead to shifts in the microbiome by reducing oxidative stress and eliminating pathogenic microorganisms that are more resistant to oxidative stress (105) (see **Figure 2**). During pregnancy, LCPUFAs undergo placental transfer to the fetus during the third trimester of pregnancy, whereby they can be used to synthesize brain tissue (64). However, in this study, fish oil consumed by mothers from 24 weeks gestation through 1 week postpartum had no significant effects on infant gut microbial diversity or maturation at 1 week, 1 month, or 1 year of age. Mechanistically, given that nutrients and oxygen are transferred from mother to fetus via placental blood circulation, how microbiota in the neonatal gut may be impacted by maternal diet remains unknown.

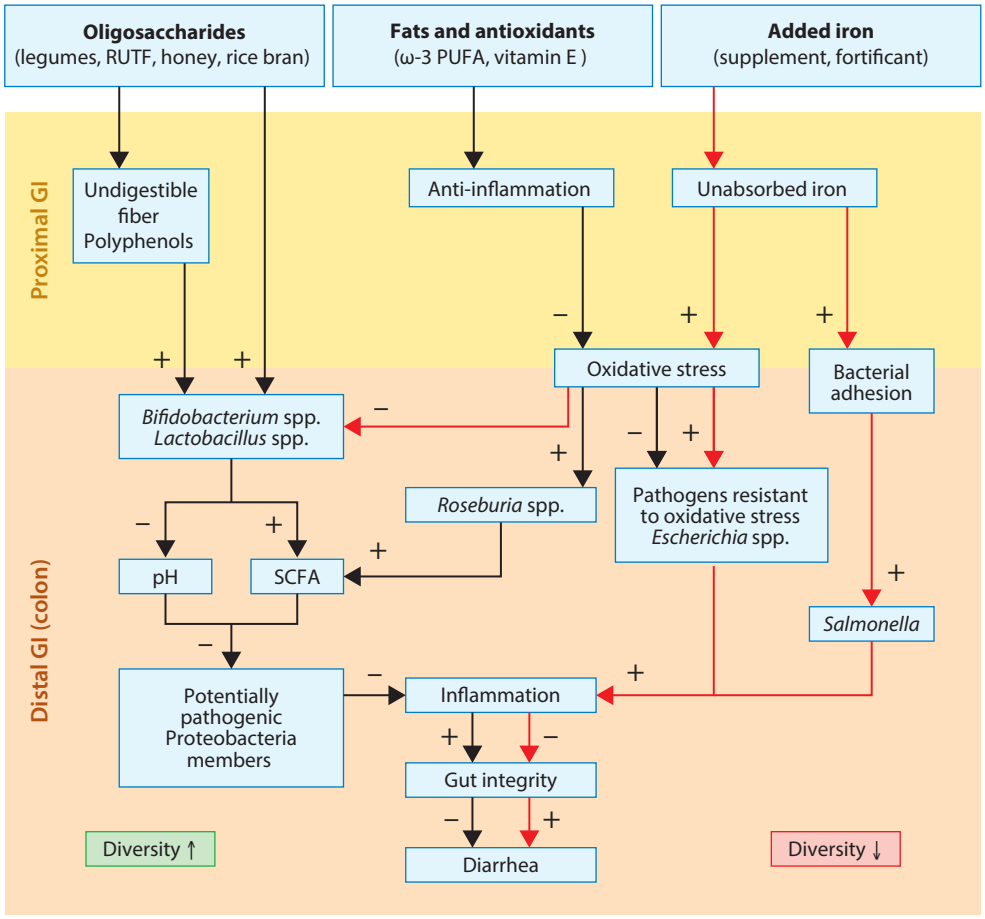

Figure 2

Potential mechanisms for the impact of selected nutritional interventions on gut microbiota, leading to either beneficial effects (e.g., increased microbial diversity, greater *Bifidobacterium* spp.) or detrimental effects (e.g., decreased microbial diversity, greater abundance of potentially pathogenic microorganisms). Figure adapted from images created with BioRender.com. Abbreviations: GI, gastrointestinal; PUFA, polyunsaturated fatty acid; RUTF, ready-to-use therapeutic food; SCFA, short-chain fatty acid.

3.2. Breast Milk and Formula

Infants who are exclusively breastfed tend to have higher proportions of *Bifidobacterium* spp. and *Lactobacillus* spp. in their gut microbiomes, in contrast to infants who are formula fed and who tend to harbor higher abundances of potentially pathogenic bacteria such as members of Proteobacteria (8, 60, 170). Several studies have found that a greater abundance of *Bifidobacterium* spp. is considered beneficial for infant health, whereas a higher proportion of *Enterococcus* spp. is associated with poor health outcomes (8, 30, 100, 104, 115). The mode of consuming breast milk was also recently shown to cause differences in the gut microbiota: Consuming expressed (pumped) breast milk from a bottle was consistently associated with multiple microbiota parameters including enrichment of potential pathogens and depletion of bifidobacteria, in contrast to feeding directly at the breast, in a study of nearly 400 mother–infant dyads (110).

Breast milk is a complex matrix that contains many constituents including lactose, more than 400 different fatty acids, proteins (whey), nucleotides, vitamins, minerals, milk fat globules

(MFGs), and HMOs (44, 86, 92). HMOs can bind to other compounds in milk such as glycoconjugates, forming human milk glycans (HMGs), which reach the infant colon intact and drive bacterial colonization, in particular, colonization of *Bifidobacterium* (44, 92, 182). HMGs escape intestinal digestion and transit to the colon, feeding into the fermentation cycle of the resident *Bifidobacterium* there, allowing these species to proliferate and dominate in a process known as the bifidogenic effect of HMOs (44). In particular, *Bifidobacterium infantis* (ATCC 15697), *Bifidobacterium breve*, *Bifidobacterium longum*, and *Bifidobacterium bifidum* (PRL2010) dominance during infancy is considered a marker of a healthier gut microbiome (44). Furthermore, breast milk itself contains bacteria: Across several cohorts of lactating women, breast milk samples were found to most commonly have *Staphylococcus*, *Streptococcus*, and *Pseudomonas*, with additional taxa varying between women and by setting; these bacteria may help seed the infant gut (101, 123).

Relative abundance: proportions or counts of different classes of microbiota observed

α-diversity: within-sample or within-sample-group taxonomic diversity

Formula consumption, on the other hand, has been associated with potentially negative impacts on the gut microbiota. These include (*a*) changes in pH that allow the overgrowth of bacteria such as *E. coli* and *Salmonella* with resulting disruption to intestinal integrity (96), possibly due to differences in protein type (see Section 3.3.3) and content, and (*b*) lack of free amino acids and certain bioactive compounds in bovine milk that are found in breast milk (20, 44, 132). Infants consuming formula based on cow's milk tend to have higher gut microbial diversity and abundances of Firmicutes, Clostridia, *Enterococcus* spp., Enterobacteriaceae, and *Bacteroides* (93) and a lower abundance of *Bifidobacterium* spp. In one study, of the species in the *Bifidobacterium* genus that were present in formula-fed infants, there were more *Bifidobacterium adolescentis* and *Bifidobacterium pseudocatenulatum*, both of which are associated with the adult intestinal microbiota (44).

The MFGM has received attention for its potential role in infant intestinal maturation, immunity, and brain structure and function (86). The MFGM is derived from the mammary gland epithelium and comprises 60% proteins and 40% lipids, stabilizing the MFG as an emulsion (86). In an effort to replicate human milk, bovine MFGM has been added to infant formula; this was recently examined in the context of the infant gut microbiota and intestinal health (61). In this study, healthy infants ($n = 60$) at 2 months of age were randomized to either bovine MFGM isolate-supplemented infant formula or standard formula for 10 months and their gut microbiota composition was determined using 16S V4 sequencing. Between 2 and 6 months of age, the MFGM formula group had lower amounts of several amino acids and their breakdown products, as well as lower lactate and succinate. At 12 months, infants in the MFGM group had lower *Haemophilus* spp., a genus containing several pathogens, compared with infants receiving a standard formula. However, in contrast to a breastfed (nonrandomized) reference group, the microbiota in the MFGM formula group were more similar to those in the standard formula group, and therefore, findings from the MFGM and standard groups were pooled for analysis and compared with findings from the breastfed group, limiting inference of the effect of MFGM itself from this study. Previous studies in animals such as piglets (85) and mice pups (12) found a greater impact from larger doses of MFGM supplementation on taxonomic relative abundance and diversity of the gut microbiome, compared with control arms without MFGM.

Breast milk and infant formula vary in amounts of whey and casein proteins (20). Infant formula has a higher proportion of cow's milk and is harder to digest. Hydrolyzing cow's milk can improve its digestibility, and hydrolyzed milk is used in ELF, which is given to exclusively breastfed newborns as an intervention to increase enteral intake and avoid complications, such as bilirubinemia or dehydration (41). The effect of ELF on the gut microbiome was examined in a trial ($n = 164$) in newborns with substantial weight loss (≥75% percentile), compared with exclusive breastfeeding as a control (41). ELF did not result in significant differences in α-diversity or community structure compared with exclusive breastfeeding and did not result in lower abundances of *Lactobacillus* spp. or higher abundances of Clostridia, which have been observed in formula-fed infants

in other studies. These findings suggest that ELF may help to improve the health of neonates who have lost substantial weight while not adversely affecting the gut microbiota. Although this study had low enrollment rates of eligible mothers and limited generalizability to certain ethnic and age groups, the findings support the examination of the impact of ELF on the neonatal gut microbiome in future research.

3.3. Macronutrients

In this section, we discuss how macronutrients, that is, carbohydrates, fats, and proteins, may impact the gut microbiota.

3.3.1. Carbohydrates. Carbohydrates include simple mono- and disaccharide sugars and complex oligo- and polysaccharides, such as starch and cellulose (39). Simple sugars are digested and absorbed in the small intestine (SI), while complex or nonglycemic carbohydrates remain undigested until reaching the colon (39). The latter have been shown to modulate the gut microbiota through degradation of glycans, particularly in the form of fiber and oligosaccharides (184). There is limited research on the impact of simple sugars [monosaccharides, such as glucose, galactose, and fructose; disaccharides, such as lactose, sucrose, maltose, and trehalose; and sugar alcohols (polyols)] (39) on gut microbiota, as they do not usually reach the large intestine; more common are studies focused on the impact of artificial sweeteners (184) or the combination of a high-sugar, high-fat Western diet (157) on the gut microbiome. Recently, in a study in mice, researchers found that fructose, which can escape intestinal absorption and reach the colon, decreased the abundance of *Bacteroides thetaiotaomicron* (155) compared with abundance in subjects fed a complex polysaccharide-rich diet. However, the sugar and nutritional content of standard mouse chows and the applicability of the findings to human sugar consumption, metabolism, and gut microbiota were unclear.

In a cohort study among 12–19-year-old adolescents, high fructose intake was associated with lower *Eubacterium* and *Streptococcus*, which are considered beneficial for carbohydrate metabolism (72). Findings from these studies provide some evidence for the benefit of examining the impact of simple sugars on the human gut microbiome in randomized trials, which have not been yet conducted in children.

Complex carbohydrates, including oligo- and polysaccharides and dietary fibers, may function as prebiotics, defined as "a substrate that is selectively utilized by host microorganisms conferring a health benefit" (48, p. 491). These molecules are not digested by humans and are transported to the colon intact, where microorganisms may metabolize and ferment them (108, 114) (see **Figure 2**). Carbohydrate prebiotics include HMOs, fructooligosaccharides, galactooligosaccharides, mannanoligosaccharides, xylooligosaccharides, and dietary fibers, such as insoluble fiber including cellulose and hemicelluloses, for example, β-glucans (from oats). Carbohydrate prebiotics also include soluble fiber such as pectin and inulin, both of which serve as substrates for microorganism fermenters, including *B. longum* subsp. *infantis*, *Bacteroides*, Bacteroidetes, *Lactobacillus*, and *Faecalibacterium prausnitzii* (38).

Prebiotics exert action through both direct and indirect mechanisms, as recently reviewed by Enam & Mansell (38). In brief, carbohydrate molecules similar in structure to host glycans can directly block adhesion to host cells; β-glucans can bind to receptors on phagocytes and natural killer cells to trigger neutrophil phagocytosis; and inulin-type fructans can serve as receptors for gut dendritic cells via Toll-like receptors, C-type lectin receptors, and galectins, which induce anti-inflammatory cytokines (38). Indirectly, prebiotics can select for proliferation of beneficial commensal bacteria whose metabolite by-products can directly affect the gut environment or

host gene expression. For example, in contrast to potentially pathogenic *E. coli* and Clostridia, *Bifidobacterium* spp., *Bacteroides*, and species in the Firmicutes phylum ferment undigested carbohydrates that arrive in the colon into microbial by-products that may be used by the host or other commensal bacteria as substrates (38). Bran (the outer covering of cereals such as wheat, oats, and rice) is a major source of prebiotic soluble fiber as well as other nutrients such as phytochemicals, fatty acids, and phenolics (114). In one trial, 6-month-old infants living in Nicaragua ($n = 47$) and Mali ($n = 48$) received daily supplementation of rice bran (1–5 g per day), added to weaning foods, or no intervention for 6 months (177). Baseline gut microbiota varied in community structure [measured by the Bray–Curtis dissimilarity plotted via nonmetric multidimensional scaling (NMDS)] by country, and this variation led to increasingly distinct microbial responses to rice bran supplementation by 8 and 12 months of age; for example, differences in community structure (separation on the NMDS plot) were more pronounced in the Malian samples at both time points. Genera responsive to rice bran intake also varied by country: In Nicaragua, *Bifidobacterium* spp. and *Veillonella* increased in relative abundance at 8 and 12 months of age, while in Mali, *Lactobacillus* increased in abundance in response to rice bran at 8 and 12 months of age. These responsive taxa are well recognized as carbohydrate digesters. One limitation of this study in comparing these populations is that the entire Mali cohort was exclusively breastfed and born vaginally, while the Nicaraguan sample included formula-fed and a majority of Caesarean-born infants; these baseline dietary differences may have contributed to the variable microbial responses to rice bran. The major strengths of this study are the monthly fecal sampling, high compliance to intervention, analysis of populations in Latin America and West Africa that are underreported in the microbiome literature, and adherence to Earth Microbiome Project protocols. Notably, this study shows how baseline microbial composition can lead to disparate responses to the same dietary intervention, supporting the need for further research in tailored nutrition interventions.

Bray–Curtis dissimilarity: nonphylogenetic metrics measurement of the distance between pairs of communities based on the species they contain

One well-studied by-product of microbial saccharolytic fermentation is the family of SCFAs, including acetate, butyrate, and propionate, which can lower gut pH to make it inhospitable to certain microbes and signal to host immune cells to downregulate inflammatory cytokines (38, 132) (see **Figure 2**). Other microbial by-products include hydrogen gas, secondary bile acids, lactate, folate, indoles, and trimethylamine-*N*-oxide, which are less studied and require further exploration (38, 184).

The introduction of complementary foods, including plant-based food sources of oligosaccharides, such as soybeans and other legumes, fruits, and vegetables (39), help to diversify and increase the richness of the infant gut microbiota as it reaches an adult-like composition (169). Honey is a source of simple sugars, namely glucose, fructose, sucrose, and maltose, as well as prebiotic fructooligosaccharides, and was examined in the context of the gut microbiota in preterm infants (2) (see **Figure 3**). In this study, preterm Egyptian infants ($n = 40$) consumed 5, 10, 15, or 0 g of medical-grade clover honey added to a formula based on cow's milk for 14 days. From quantitative real-time polymerase chain reaction (qRT-PCR) results, *B. bifidum* appeared to respond to honey in a dose-dependent manner while *Lactobacillus* spp. counts were greatest in the 10-g group, with little change in the 5-, 15-, and 0-g groups; this may speak to differences in substrate metabolism in these bacteria. Some limitations include a small sample size (only 10 infants per group), specific bacteria quantified by qRT-PCR (versus next-generation sequencing of the whole bacterial community), short intervention duration, and applicability of findings, given that honey is not recommended for children under 12 months of age due to concerns it may contain bacteria that cause infant botulism (34). However, as a pilot study, these findings offer support for investigating the potential impact of honey and simple sugars on the gut microbiome further, in older age groups. To our knowledge, no other trials have examined the role of carbohydrates in

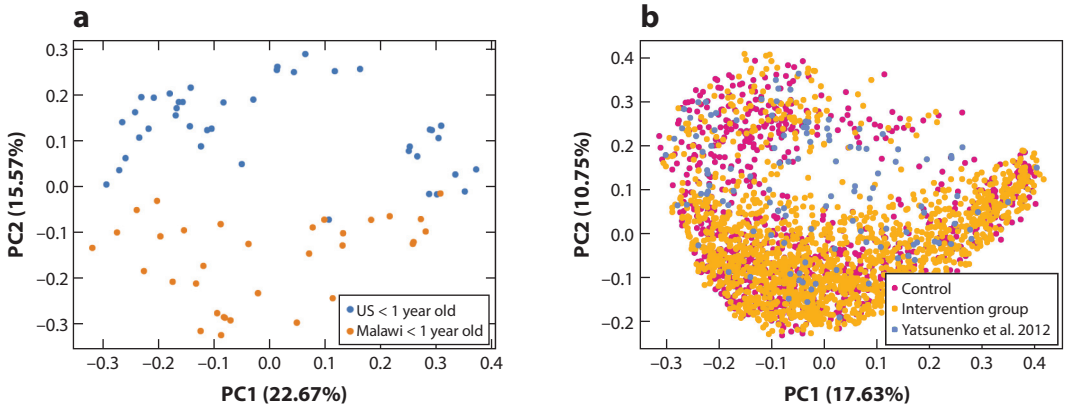

Figure 3

Summary of studies available in Qiita for analyses in the global context. (*a*) Principal coordinates analysis of unweighted UniFrac distances showing community separation of 16S rRNA V4 sequences between Malawian infants, compared with infants in the United States or Venezuela (171), even though samples were processed with identical methods and sequenced on the same runs. Microbial communities showed less separation between Malawian and Venezuelan infants, whose data were similar to Malawian infants and are not shown. (*b*) Combined community separation by intervention for two trial populations in comparison with children under 2 years of age from data reported by Yatsunenko et al. 2012 (*blue*) (171). One trial intervention used legumes among 6-month-old infants, compared with corn–soy blend as a control (117). A second trial intervention included milk fat globule membrane and formula, compared with a control standard formula (61). These two trial interventions were combined into a broad intervention group (*orange*), with their respective controls likewise combined (*magenta*). The principal coordinates on the x and y axes show eigenvalues representing the amount of variation captured. Abbreviation: PC, principal coordinate.

whole foods aside from legumes (see Section 3.3.3), including naturally occurring prebiotics, in term infants or older children or adolescent populations.

3.3.2. Fats. Fatty acids, including monounsaturated fatty acids (MUFAs), polyunsaturated fatty acids (PUFAs) (such as linolenic ω-3 and linoleic ω-6 fatty acids), and saturated fatty acids (SFAs) (39), make up parts of lipid molecules, such as triglycerides, phospholipids, cholesterol, and phytosterol (39). Dietary fats are broken down throughout the gastrointestinal tract. Most triglyceride digestion and absorption occur in the SI (particularly, the duodenum) and are dependent on pancreatic lipase, bile salts from the liver, and bile from the gallbladder. Primary bile salts, including cholate and chenodeoxycholate, are synthesized from cholesterol; commensal microbiota synthesize secondary bile salts, deoxycholate, and lithocholate from primary bile salts, which are further used as a substrate by bacteria or modified by liver cells (39, 132). Approximately 7% of free fatty acids reach the colon intact, where they may impact resident microbiota (127). Foods generally contain mixtures of short-, medium-, and long-chain fatty acids (39).

The mechanisms by which fatty acids affect the gut microbiota remain an area of active research (127), and there is conflicting evidence on the effects of SFAs, MUFAs, and PUFAs on the human gut microbiome. Some studies suggest that SFAs and ω-6 PUFAs promote inflammation and oxidative stress, while ω-3 PUFAs and MUFAs are associated with positive effects such as increases in *Lactobacillus* and *Bifidobacterium* spp. (127, 184). Since metabolization of dietary fat requires oxygen, fat is an unlikely energy source for anaerobic gut bacteria (17). However, higher fat intake can displace dietary fiber and carbohydrates in the diet; therefore, effects on the gut microbiota may be due to dietary substitution or lower carbohydrate substrate availability rather than to higher fat intake. Dietary fat may have a bactericidal effect on cell membranes, resulting in lower

abundance of bacteria (173). Furthermore, dietary fats can induce increased bile acid metabolism by bacteria to respond to increased fat (39, 129).

One study was conducted to analyze the impact of a blend of fish and safflower oil on the gut microbiota for preterm infants ($n = 16$) who had a small-bowel enterostomy [i.e., an opening in the abdominal wall to allow for enteral feeding after abdominal surgery necessitated by inflammatory diseases, such as necrotizing enterocolitis (NEC), a common condition in premature infants] (see Section 4.1), compared with the standard of care (175). The intervention was administered for 9 weeks and resulted in increased microbial α-diversity, lower abundances of potentially pathogenic bacteria (Enterobacteriaceae, *Clostridium*), and enrichment of predicted gene function for carbohydrate metabolism, compared with standard nutritional therapy. These findings require more research due to study limitations, including small sample size, lack of blinding, inability to determine the individual effects of either oil, and the indirect analysis of function predicted from 16S RNA sequencing.

Together, the findings from these studies suggest that ω-3 LCPUFA interventions may improve the diversity, composition, and predicted function of infant gut microbiota, particularly in premature infants with NEC, but that maternal consumption of LCPUFAs during pregnancy does not impact the infant gut microbiome.

3.3.3. Proteins. Dietary proteins are composed of amino acids (AAs) that form peptides, a fundamental component of tissues (39). Essential for skeletal muscle function, growth, health, development, and survival, AAs can provide energy for both host and gut microbiota, and the gut microbiota can alter the bioavailability of AAs to the host (39, 96, 167). In human metabolism, AAs function as substrates of protein synthesis, regulators of protein turnover and enzyme activity, signal transducers, neurotransmitters, transporters, and transcriptional regulators (39).

Digestion of dietary protein begins in the stomach, where proteases cleave proteins into large peptides (96, 167). Peptidases in the SI break down these peptides for subsequent transport and catabolism by enterocytes and luminal bacteria (167, 179). Increasing protein intake usually results in reduced digestibility and greater fermentable substrate in the colon (96, 167). Bacterial conversion of free AAs into polypeptides contributes to AA metabolism and bioavailability in the gut, and availability of AAs such as L-glutamine can regulate small intestinal bacterial metabolism of both essential and nonessential AAs (132). Colonic bacteria, particularly *Bacteroides*, Clostridia, and coliforms, convert both ingested protein and endogenous protein from host enzymes, mucin, and sloughed enterocytes, resulting in by-products such as shorter peptides, AAs, fatty acids (e.g., SCFAs), and gases such as ammonia and hydrogen sulfide (96, 97, 132, 179). Proteolytic activity varies across the intestinal tract: For example, in vitro experiments found that colonic microbiota can degrade bovine serum albumin in the colon more efficiently, compared with microbiota in the ileum, depending on pH levels, carbohydrate availability, and gut model retention time (143). Higher protein intake, such as in the Western diet, is thought to increase pH, decreasing the growth of strict anaerobes such as *Bifidobacterium* spp. and promoting protein-fermenting, potentially pathogenic facultative anaerobic bacteria (96). These bacteria, such as *E. coli* and *Salmonella*, can disrupt the gut barrier and immune system by forming degradation products such as trimethylamine oxide and aromatic and branched-chain AAs; such by-products have been linked to insulin resistance and type 2 diabetes (96). In addition to intake level, protein source, concentration, and AA composition can impact gut microbiota composition and function (179).

Protein sources have been shown to lead to changes in microbial composition and function (96, 179). Plant-based proteins such as legumes are incompletely digested by host enzymes due to the plant cell wall, resulting in their transport to the colon as a prebiotic for microbial fermentation (96, 179). It is difficult to parse out the individual effect of protein, fiber, or other chemicals from

plant-based protein sources, but as a whole, such protein sources tend to be associated with beneficial shifts of bacterial composition including growth of *Lactobacillus* and greater microbial diversity (124, 179). Two trials were conducted to examine the effects of plant-based sources of protein, including cowpea, yellow pea, and common bean, on the gut microbiota using 16S sequencing of the V4 hypervariable region (117, 163). One trial compared roasted cowpea flour (25% protein, 21% fiber), roasted common bean flour (25% protein, 28% fiber), and extrusion-cooked corn–soy blend (CSB) flour (13% protein, 8% fiber) daily for 6 months in 6-month-old children (117). Cowpeas resulted in a higher proportion of *Bifidobacterium* spp. between 9 and 12 months, lower abundance of *Prevotella* between 6 and 12 months, and lower abundance of *Escherichia/Shigella* between 6 and 9 months, compared with common bean and CSB; this was unexpected, given the lower fiber content of cowpeas compared with the common bean.

Another study in children (6–10 years old) examined the effects of four different doses of micronutrient-supplemented, legume-based protein (yellow pea) (i.e., 6, 8, 10, or 12 g) on the 16S V4 region (163). Consumption twice daily for 1 month identified nine discriminatory taxa in the Firmicutes and Bacteroidetes phyla—many associated with plant polysaccharide fermentation and SCFA production (116)—corresponding to changes in the gut microbiome. Three taxa (*Prevotella*, *Oscillospira*, *F. prausnitzii*) were significantly associated with an increase in child linear growth, and there were changes in the relative abundance of all taxa, particularly between the lowest protein dose group (6 g) and the highest (12 g). These results highlight a potential dose–response effect of legumes on the gut microbiota and identify taxa to further investigate in relation to functional outcomes such as linear growth in children.

In contrast to plant proteins, animal-based sources of protein are more digestible in the proximal intestine, and, subsequently, less protein is transported to the colon, resulting in decreased modulation of the colonic microbiota and possible suppression of pathogens (96).

3.4. Micronutrients

In this section, we discuss individual vitamins and minerals (i.e., vitamin A, vitamin E, iron, and zinc), as well as how combinations of micronutrients (i.e., multiple micronutrient fortification/supplementation and lipid-based nutrient supplements) may impact the gut microbiota.

3.4.1. Vitamin A. Vitamin A is a family of retinoid compounds with all-*trans*-retinol biological activity, including retinaldehyde (retinal), retinoic acid, retinyl esters (mainly retinyl palmitate), and carotenoid compounds, such as β-carotene, α-carotene, and β-cryptoxanthin (39). Vitamin A is critical for maintenance of intestinal barrier integrity and regeneration of damaged mucosal epithelium—specifically, the normal proliferation of enterocytes and mucus-producing goblet cell differentiation, which influence the colonization and adherence of gut microbiota (39, 67). Dietary vitamin A can be consumed as provitamin A (carotenoids) from plant sources or as preformed vitamin A (retinyl esters) from animal sources (39). Digestion begins with mastication and includes gastric action via bile salts and pancreatic lipases, which form mixed micelles from dietary lipids in the intestinal lumen for absorption (39). While preformed vitamin A sources are more bioavailable for absorption by host enterocytes for transportation to storage or circulation, provitamin A carotenoids must be processed further to be converted into a retinoid and used by the host (39, 55). Depending on the food matrix, approximately 5–50% of β-carotene is absorbed (13). For example, the presence of dietary lipids increases carotenoid absorption in the SI; if β-carotene is adsorbed to fiber, it can bypass absorption in the upper intestine and be transported to the colon (13). Colonic microbiota may digest the fiber, liberating β-carotene for colonocyte absorption. Carotenoids have been found in the colonic mucosa, mainly in the colon ascendens (112). One

study found that *E. coli* expresses a salt-tolerance enzyme with homology to β-carotene monooxygenase 1, allowing *E. coli* to accumulate β-carotene (26); however, there is no evidence that this β-carotene is cleaved to form retinal for further use.

In a study in mice, *Bacteroides vulgatus*, previously shown to be a growth-discriminatory taxon (144), decreased in abundance with vitamin A repletion and increased in abundance in response to vitamin A deficiency, possibly in conjunction with altered bile acid metabolism (65). The authors reported that these changes were due to the disruption of the *B. vulgatus* retinol efflux system and subsequent influence on retinol and bile aid sensitivity (65). Another study in mice found that mucosal dendritic cells (DCs) sample the gut commensal *B. infantis*, resulting in their increased conversion from vitamin A to retinoic acid and higher numbers of mucosal DCs with tolerogenic properties, such as suppression of T-helper type 1 (Th1) and Th17 cells (79). This effect also varied with the host retinoic acid status (13, 43, 79). However, further research in humans is needed, as rodents are much more efficient at carotenoid metabolism than are humans, and no other animal model represents human carotenoid metabolism completely (59).

A recent study examined the effect of vitamin A supplementation on the gut microbiota in neonates, which found positive as well as sex-specific effects on bacterial composition including greater *Bifidobacterium* spp. (67). A single 50,000 international unit dose at enrollment, compared with placebo, did not alter α-diversity but had a bifidogenic effect in males by 6 to 15 weeks of age, though not in females; the authors hypothesized that this may help explain lower mortality rates observed in males compared with females in this study (9, 145).

3.4.2. Vitamin E. Vitamin E mainly functions as an antioxidant via α-tocopherol activity (39). α-Tocopherol can be sourced from nuts, vegetable oils, dairy, cheese, and eggs and can also be consumed in supplemental form as esterified α-tocopherol. As a free radical scavenger, α-tocopherol breaks the chains of free radicals in cell membranes and plasma lipoproteins to maintain the integrity of LCPUFAs (39). Vitamin E is absorbed along with dietary lipids into intestinal cells, incorporated into chylomicrons, secreted into the lymphatic system, and transported to the liver before being taken up with very-low-density lipoprotein and secreted into the bloodstream bound to α-tocopherol transfer protein; however, many details of its uptake and trafficking are largely unknown (39, 107). After additional metabolism steps, the unbound vitamin E isomer is excreted via feces and urine (107). Vitamin E, along with iron, has been associated with a reduced oxidative potential (118); as such, vitamin E is hypothesized to ameliorate the impact of iron-related inflammation, improving gut microbiota outcomes (37, 152) (**Figure 2**).

One study among iron-deficient 6-month-old infants in the United States found that 18 mg of vitamin E with 15 mg of elemental iron twice daily for 2 months did not result in changes in bacterial diversity but did cause differences in changes in relative abundance over time: Vitamin E with iron resulted in lower Bacteroidetes, especially Bacteroidaceae, and greater Firmicutes, particularly Lachnospiraceae and *Roseburia* spp. *Roseburia* produces butyrate, which can stimulate colonic blood flow (**Figure 2**; see also Section 3.3.2). Iron alone did not create a more pathogenic microbial profile among participants, such as decreased *Lactobacillus* spp. and increased *Escherichia*, as seen in previous studies (see Section 3.4.3) (152). Several factors may contribute to these contrasting findings, including the shorter intervention duration and higher-income setting. To date, no studies have examined how antioxidants may ameliorate the effects of iron on the gut microbiota in lower-income settings.

3.4.3. Iron. Iron is the most abundant micronutrient in the human body and is essential for oxygen transport, redox reactions, metabolism, and electron transport chain mechanisms in humans, as well as metabolism and virulence functions in many bacteria (39, 130). Separate mechanisms

exist for the uptake and absorption of dietary heme iron (10%) and nonheme iron (90%) (39). Beef, a source of heme iron, has been studied in relation to the gut microbiome, which was measured by the 16S V3–V4 region in 6-month-old infants in Canada (125); a study in the United States that pyrosequenced the microbiota also used beef as an intervention (82). From the 16S V3–V4 sequencing data, groups consuming beef as a complementary food had no declines in *Bifidobacterium* spp. and greater bacterial species richness after 4 weeks compared with baseline (125); after 4 months, there were greater abundances of Actinobacteria and Clostridia group XIVa, a butyrate producer (160), and lower Bacteroidetes, a phylum with many polysaccharide fermenters, compared with iron-fortified infant cereals (125). Differences may have been due to the higher bioavailability of iron in the meat groups as well as the unabsorbed iron passing into the colon and the presence of phytates in the iron-fortified cereal groups. Iron bioavailability decreases with the presence of phytates and polyphenols, while ascorbic acid (vitamin C) improves iron bioavailability (39). Gastric acidity and proteolysis may release nonheme iron from its food matrix, with more digestion required to release heme and ferritin (39). Some bacteria can access food-bound iron sources through specialized mechanisms, such as degradation of the polyphenol tannate by *Streptococcus gallolyticus* or *Staphylococcus lugdunensis* (142).

Unabsorbed iron is thought to stimulate growth and virulence of bacterial pathogens, such as *E. coli*, in the gut, while host iron status influences bacterial immune defenses and host inflammatory response (80) (see **Figure 2**). Some bacteria including Lactobacillaceae and *Borrelia burgdorferi* do not depend on iron for growth and instead utilize manganese (172); *Streptococcus* spp. can use either iron or manganese, depending on availability (80). *E. coli* has been shown to use the Feo-uptake system for ferrous iron uptake, while ferric iron is first reduced to ferrous iron by extracellular reductase prior to uptake or is taken up as ferric citrate or bound to bacterial siderophores (4, 80). The change in the amount of available iron during an infection sends signals to virulence genes: Low iron causes siderophores to be depressed and toxins to be upregulated, while higher iron availability can induce bacterial adhesion to intestinal epithelial cells, as in the case of *Salmonella enterica* serovar Typhimurium (80). Iron deficiency can also decrease the production of SCFAs by gut bacteria, including *Roseburia* spp., *Eubacterium rectale*, and *Clostridium* cluster IV members (172).

Of populations vulnerable to iron deficiency, infants and young children most often require oral iron therapy, such as supplementation (82, 125), though their gut microbiomes are still maturing (80). Several studies have demonstrated a negative impact of iron interventions on gastrointestinal symptoms, such as diarrhea (146, 183), as well as changes in the gut microbiota, such as higher Enterobacteriaceae and lower Lactobacillaceae and *Bifidobacterium* spp. from iron supplements, depending on the baseline iron or anemia status (70, 181). To help counteract these effects of iron, randomized trials in children have examined the effects of iron in combination with antioxidants, such as vitamin E (see Section 3.4.2) (152) or freeze-dried raspberries as an antioxidant source (125). For example, in a study in Canada, infants receiving electrolytic, iron-fortified rice cereal with freeze-dried raspberries had increased richness and diversity in their gut microbiomes compared with infants receiving iron-fortified cereal alone after 2 to 4 weeks. However, the group receiving freeze-dried raspberries did not have a significant reduction in reactive oxygen species (125), an initiator of the inflammatory process that is associated with excess iron in the gastrointestinal tract (138); this may be due to the small amounts of freeze-dried raspberries consumed (1.8%) or the potential lowered antioxidant content compared with what is contained in fresh raspberries, which were not consumed by any group (125). In future studies, consideration of other antioxidant sources (e.g., fresh berries or extracts) and varying doses of these antioxidant sources, along with studies among a larger number of infants who are anemic (119) or iron replete, would help to establish potential interactions between iron and antioxidants (see Section 3.4.2) (152).

Other randomized trials using cointerventions with iron included folic acid (73), zinc (82), and multiple micronutrients (MMNs), either as tablets or as part of micronutrient powders (MNPs) (70, 73, 120–122, 151). Forms of iron include ferrous sulfate, ferrous fumarate, NaFeEDTA, and electrolytic iron, which may have varying impacts on the gut microbiota in children; additional variables include dose amount administered, baseline iron and anemia status, cointerventions such as antioxidants and other micronutrients, and study setting (37, 70, 82, 121, 122, 125, 151, 152). Most studies examining MNPs have been conducted in Kenya among 6-month-old infants who were initiating complementary foods; MNPs were administered for 3 to 4 months along with maize porridge (70, 120–122, 151). MNPs varied in micronutrient composition, but all contained vitamin A, folic acid, and vitamin C, and all except one (151) also contained B vitamins, vitamin D, copper, iodine, selenium, and zinc (see **Supplemental Table 2**). From targeted qRT-PCR results, an MNP (*MixMe*), plus 2.5 mg of NaFeEDTA for 4 months, resulted in an increased abundance of *E. coli/Shigella*, sum of *E. coli*, and Enterobacteriaceae:*Bifidobacterium* spp. ratio at 10 months of age, compared with the abundances arising from the same MNP alone (70). Similar results were observed in a trial comparing an MNP (*Sprinkles*) plus 12.5 mg of ferrous fumarate with the same MNP alone and when iron-containing groups were combined and compared with both MNP-only groups (70). In another trial, the iron content (2.5 mg of NaFeEDTA and 2.5 mg of ferrous fumarate) in an MNP had a significant impact on microbiome composition compared with the same MNP alone, including lower abundances of beneficial microbiota such as *Lactobacillus* spp. and *Bifidobacterium* spp., and higher proportions of Clostridiales, while Bacteroidetes remained similar across groups, as ascertained by 16S V3–4 sequencing (121). In an ancillary study, antibiotic treatment did not counteract the effects of iron, which still led to increases in Enterobacteriaceae (122).

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Finally, in another trial, an MNP with iron (12.5 mg of ferrous fumarate) resulted in no between-group differences in diversity or relative abundance, compared with the MNP alone (151). However, the group that received an MNP with iron had increases in Clostridia and decreases in *Bifidobacterium* spp., while the MNP-only group had decreased *E. coli*. Despite differences in MNP formulations and 16S sequencing regions, these studies consistently found that iron led to decreases in beneficial bacteria and increases in possibly harmful bacteria, offering several lines of evidence for the impact of iron on the gut microbiome. Further research is needed to examine the impact of iron on the gut microbiome in other settings.

Iron fortification and supplements have also been examined in older children in studies in other settings in Africa, with both studies using qPCR for analysis. In 6–14-year-old children in Côte d'Ivoire ($n = 60$), biscuits fortified with iron (20 mg of electrolytic iron) resulted in no changes in microbial diversity, an increase in Enterobacteriaceae, a decrease in *Lactobacillus* spp., and no differences in *Bifidobacterium* spp. after 6 months (181); these findings are consistent with previous studies conducted in infants. In another study in South Africa among 6–11-year-old children, consumption of beverages with 50 mg of ferrous sulfate compared with consumption by a placebo group (with both groups receiving vitamin C) resulted in within-group differences in relative abundance; however, no between-group differences were noted despite the higher iron dose (37). It is possible that these findings may be due to differences in enteropathogen burden and sanitation, including quality water and sanitation, diet quality, and infection burden.

Compared with fortificants and supplements, iron present in a whole-food matrix, such as in LNSs, may be beneficial for iron administration without iron-related negative effects such as increased potentially pathogenic bacteria, inflammation, and gastrointestinal disease symptoms such as diarrhea. LNSs are composed of vegetable oil, peanuts, milk powder, sugar, vitamins, and minerals and are consumed in quantities of approximately 54-g sachets or, in the case of small-quantity LNSs (SQ-LNSs), in small quantities of 20 g (~6 g of fat) to help address both macronutrient

β-diversity: between-sample or between-sample-group diversity for assessing differences in community structure

and energy deficiencies and to meet micronutrient requirements (5). Three randomized trials have been conducted to examine the impact of LNSs given directly to infants (1, 22) or given to mothers during pregnancy and then to their infants (73). In two studies on the same cohort, 6-month-old children received either milk-protein-based LNSs, soy-protein-based LNSs, CSB, or no intervention for 12 months (1, 22). Gut microbiota were assessed by sequencing of the 16S rRNA V4 region as well as by qRT-PCR targeting *Bifidobacterium* spp. and *Staphylococcus aureus*. No differences in colonization rates or counts of *Bifidobacterium* spp., *S. aureus*, or other gut bacteria were found in groups receiving the LNSs and CSB supplements compared with a control group receiving no nutritional supplements.

In another trial, SQ-LNSs (20 g of iron) were administered to pregnant women (from <29 weeks gestation), and then to infants aged from 6 to 18 months (73); control arms included administration of MMNs containing 20 mg of iron for mothers during pregnancy and the first 6 months of lactation, or the standard of care, iron and folic acid (containing 60 mg of iron), given to mothers during pregnancy plus a placebo for the first 6 months of lactation. At 12 months, infant gut microbial α-diversity and evenness were higher in the SQ-LNS group compared with either control arm, while no differences in β-diversity or microbiota-for-age z score (MAZ) were observed. However, due to the differences in type, amount, recipient, and dosing schedule of intervention between the intervention arms (with only infants in the SQ-LNS group receiving direct supplementation), it is difficult to identify any one particular nutrient or intervention type that may be causing these effects. Together, these findings suggest that iron supplementation during pregnancy and lactation may not impact the neonatal and infant microbiome.

3.4.4. Zinc. The trace mineral zinc plays catalytic, structural, and regulatory roles in the body, including intestinal health and immune health, and has been widely used as a treatment for severe diarrhea (13, 39). Absorption of zinc, which mainly occurs in the SI in humans, is impacted by either the presence or the absence of substances in foods containing zinc and by food-processing methods. For example, phytates in plant-based sources of zinc, such as seeds, roots, and tubers, inhibit zinc absorption, and other micronutrients such as iron competitively inhibit zinc uptake, while fermentation of grains can improve zinc absorption; additionally, zinc found in animal-source products is more available due to the presence of AAs, such as histidine (137), that enhance zinc solubility (39). Excess zinc, both unabsorbed dietary zinc and endogenous zinc, is excreted through feces in a controlled mechanism to maintain homeostasis. As there are no body stores of zinc, zinc deficiency can occur rapidly after a low-zinc diet (39).

Commensal and pathogenic bacterial colonization and function in the gut may be modulated by zinc, which is essential for many bacteria, such as in virulence factors (27), and is used for animal production due to its antibacterial effect (148). In *E. coli*, zinc is tightly regulated and plays structural and catalytic roles in more than 300 proteins (27), and many bacteria possess heavy-metal efflux systems or perform gene transfer of specific heavy-metal plasmids (148). Bacteria such as *Lactobacillus* spp. also differ in resistance to dietary zinc (95). Studies on zinc and the gut microbiota have been mainly conducted in animal models; the authors of these studies have found differences in bacterial composition, particularly *Lactobacillus* spp., Clostridia, and Enterobacteriaceae, and in diversity depending on species (i.e., murine, piglet, or chick) (128, 148, 159, 176). One study in 2–36-month-old children with antibiotic-associated diarrhea secondary to pneumonia examined the effects of zinc supplements with probiotics for 14 days compared with probiotics alone (168). There were no significant differences in counts of *Bifidobacterium* spp., *E. coli*, or their ratio; these results were mirrored by increases in *Bifidobacterium* spp., decreases in *E. coli*, and increases in their ratio in each intervention group, compared with baseline. Further research using longer supplementation or follow-up duration, with larger sample sizes, may shed more light on these findings.

Another study in 6-month-old children consuming iron-and-zinc-fortified or iron-only-fortified cereals found that zinc may counteract the potentially adverse effects of iron fortification on the gut microbiota (see Section 3.4.3) observed in the iron group (82). These results, found via 16S V1–V3 pyrosequencing, remain to be reexamined in the 16S V4 region (82).

4. SPECIAL CONSIDERATIONS

4.1. Premature and Very Low Birth Weight Infants

Although full-term infants with a normal birth weight follow the succession of microbiological colonizers described above, premature (<37 weeks) and/or very low birth weight (VLBW) (birth weight <1,500 g) infants have a different colonization pattern of microbiota (23). These infants have lower microbial diversity as well as an overgrowth of pathogenic bacteria, particularly Gammaproteobacteria, along with lesser colonization of beneficial or commensal intestinal bacteria, such as *Bifidobacterium* spp. and *Lactobacillus* spp. These differences are likely due to the immature intestinal tract, in addition to other prenatal and postnatal factors, as previously reviewed (23, 53, 54). This microbial composition is considered immature and may not prevent the growth of pathogens, predisposing infants to developing infections, such as NEC (53, 54). Special considerations for dietary intake are required for infants who are born preterm or with VLBW, or for infants who lose substantial weight (weight loss greater than the 75th percentile for age) during the first few days of life (53, 54, 133). Trials investigating the gut microbiota in infants who have these health issues have examined interventions to correct weight loss, such as ELF (see Section 3.3.3) (41) and other interventions used for anti-inflammatory and bactericidal properties, such as supplemental bovine lactoferrin (see Section 3.4.3) (62), in addition to a combination of enteral and parenteral feeding and adjustments for intolerance or illness (57, 161, 162).

4.2. Energy Restriction and Undernutrition

Studies suggest there are differences between the infant gut microbiota in low- and middle-income settings compared with that in high-income settings (56). Children in low-resource areas are often vulnerable to undernutrition due to infections, environmental insults, and inadequate dietary intake, which can be compounded by impairments in digestion, absorption, or utilization, which may be modulated by resident microbiota (104). Undernutrition and impaired growth in children [i.e., lower World Health Organization (WHO) length- or height-for-age, weight-for-age, weight-for-length/height (166)] z scores have been associated with differences in gut microbiota composition and diversity, compared with children who had higher WHO z scores. Evidence from in vivo mouse studies in which the mice received fecal transplants from human gut bacterial populations shows that gut microbiota may cause weight loss and lead to severe acute malnutrition (14, 47, 74, 144).

Microbial diversity, as a function of diet, has been examined in a comparative study of 15 European children who consumed a higher-energy Western diet and 14 children in rural Africa who consumed a high-fiber, low-fat diet. The European children had a higher abundance of Firmicutes and Bacteroidetes phyla and the exclusive presence of other specific bacterial species compared with the African children (28).

Several human studies have shown that undernourished children have an immature gut microbiome for their chronological age, as reflected by MAZ (14, 144, 149), in addition to decreases in bacterial diversity and increases in potentially pathogenic microbes, such as those in the Proteobacteria phylum (58, 109). In a recent randomized trial of microbiota-directed complementary foods (MDCFs) in Bangladesh, children ($n = 63$) aged 12 to 18 months with moderate acute malnutrition were randomized to MDCF prototypes containing varying amounts

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of chickpea flour, peanut flour, soy flour, raw banana, powdered skimmed milk, sugar, soybean oil, and micronutrients (see **Supplemental Table 2**) (MDCF-1: all included; MDCF-2: no milk; MDCF-3: no peanut flour, raw banana, or milk). All prototypes were compared with an RUSF composed of rice, lentils, powdered skimmed milk, sugar, soybean oil, and the same micronutrients (45). These foods were designed and optimized on the basis of the results of human and animal experiments that identified the ingredients best suited for promoting microbiota into an age-appropriate and healthy state.

In previous studies conducted in gnotobiotic mice and piglets, MDCF prototypes targeted underrepresented, weaning-phase bacteria and not only changed the abundance of these bacteria but also improved plasma biomarkers and mediators for growth, bone health, neurodevelopment, and immune function. In the aforementioned trial in children in Bangladesh, the number of enteropathogens remained similar across groups, and there were no changes in relative abundance in the MDCF-1 group between baseline and end point (45). However, MDCF-2 resulted in higher *F. prausnitzii* and Clostridiales and decreases in *B. longum* compared with baseline. Children in the MDCF-3 arm had greater *Streptococcus* spp., while the RUSF control exhibited less *Enterococcus faecalis* compared with baseline. These results suggested that the arm consuming chickpea flour, peanut flour, soy flour, raw banana, sugar, and soybean oil but not milk (MDCF-2) achieved the most gut microbial maturity and an increased abundance of proteins associated with length- or height-for-age, compared with the arm consuming MDCFs containing similar ingredients but including milk (MDCF-1) or not including peanut flour (MDCF-3). These results were used to inform a larger study among 12- to 18-month-old Bangladeshi children ($n = 118$), which found that twice-daily consumption of MDCF-2 for 3 months was linked to 21 associated gut bacterial taxa that were also positively correlated with weight-for-length z scores compared to an RUSF (21). However, the variation in ingredient(s) in each study arm limits inference of causal effect due to the presence, absence, or particular amount of each ingredient.

4.3. Pediatric Inflammatory Bowel Disease Management

The majority of studies investigating the impact of nutrition and the gut microbiome in the context of inflammatory bowel diseases (IBDs), including Crohn's disease (CD) and ulcerative colitis (UC), have examined pre-, pro-, and synbiotics interventions and/or have been conducted in adult cohorts (24, 91, 103, 135, 140). Briefly, it is postulated that the gut microbiota may be implicated in the development of IBD, as animal studies have suggested that signs of colitis are lessened or nonexistent in germ-free or antibiotic-treated mice (24). Specific gut taxa were enriched in mice models of IBD (colitis induced by dextran sodium sulfate) and were associated with increased inflammation (24). Human microbiota-associated mice (germ-free mice colonized with gut microbiota transplanted from a human) have also been used to determine the role of microbiota in IBD (131); such transplants have resulted in an altered balance of regulatory T cells leading to exacerbated colitis (15).

Studies in humans have shown that gut microbial presentation in IBD includes reduced microbial richness and diversity, increased abundances of *Bacteroides* and Enterobacteriaceae, and decreased abundances of Firmicutes (24, 131). This presentation results in a form of dysbiosis that may distinguish between IBD types and severity (140) as well as mediate host responses to IBD treatments (77). In children, a case-control study among patients with IBD and healthy controls found that the gut microbiota correlated with specific levels of inflammation and that six groups of gut bacteria related to *E. rectale* and *Bifidobacterium* spp. predicted the response to anti–tumor-necrosis factor-α medication (77). One review found that fecal transplants increased the

likelihood of clinical remission in UC, but the small amount of evidence found, all among adults, was rated as low quality by the review authors (69).

Few studies, however, have examined dietary or food-based interventions in pediatric IBD. One dietary intervention analyzed is the CD exclusion diet (CDED), which has been compared with the current standard of care, exclusive enteral nutrition (EEN) (90). Though EEN has been shown to be superior at inducing remission of CD compared with steroids, has few side effects, and is the recommended first-line treatment, the 6–8-week treatment course involving this completely liquid diet via nasogastric tube has remained underused (89, 90). Therefore, the alternative CDED was developed: a high-protein, low-fat, whole-food (including foods with specific fibers and starches as substrates for SCFA production as well as lean protein), multistage diet that excludes foods thought to generate a high-Proteobacteria/low-Firmicutes presentation of dysbiosis (e.g., red meat, bread, additives, and animal fat) (89). In a 12-week trial among 4–18-year-old children with mild/moderate CD, consuming CDED-appropriate foods along with partial enteral nutrition (PEN) ($n = 25$) was associated with sustained reductions in inflammation, improvement in intestinal impermeability, and lower fecal Proteobacteria (assessed by 16S V4–V5 ribosomal sequencing) among responders ($n = 25$ at week 6 or $n = 21$ at week 12) compared with EEN ($n = 21$). Though both diets induced CD remission, this study found that CDED was better tolerated and maintained the changes in inflammation and Proteobacteria abundance compared with EEN, wherein the microbiota reverted back to the dysbiotic baseline composition over time. Although the CDED pattern is more complicated than EEN, involving multiple stages with steadily decreasing amounts of PEN and the reintroduction of table foods, these promising results have the potential to alter the clinical management of IBD.

5. SYNTHESIS AND DISCUSSION

In this review, we synthesized the available evidence from nutritional intervention trials that tested a diversity of dietary interventions on microbiome-related outcomes among infants and children and discussed supporting evidence from laboratory and animal studies, as well as observational data from human studies, with the aim to guide future research and to enhance interpretability to inform diet-related policies. Comparability is poor as the included studies differed in the type and duration of intervention, were conducted in different pediatric populations with or without preexisting conditions, and in various settings that may have influenced baseline microbial composition. While studies investigating the infant microbiota found few effects of maternal dietary changes during pregnancy, direct dietary interventions impacted diversity and composition of the gut microbiota in children.

An emphasis should also be placed on harmonizing protocols and assessment methodology, such as utilizing the Earth Microbiome Project (50) and Human Microbiome Project protocols (153). It is difficult to separate study biases from real biological phenomena by examining studies individually versus including the whole global context. Such analysis can be attempted using a tool such as Qiita (51), provided that study data are publicly available with standards-compliant sample metadata to facilitate across-study comparability in future analyses. Of the 19 studies that performed 16S rRNA sequencing, only two trials that sequenced the 16S V4 region were available for further analyses in Qiita (61, 117). We demonstrate this comparison using these two studies; results are shown in **Figure 3**.

We compared the gut microbiota from these two trial populations with subgroups from a 2012 landmark microbiome cohort study (171) (**Figure 3**). We found striking microbial separation between Malawian- and US-born infants in the 2012 study (171) (**Figure 3*a***), but data were lacking to deeply examine the effects of nutrition interventions when including the two recent trials (61, 117) in comparison with the 2012 study (**Figure 3*b***).

Despite much progress in microbiome bioinformatics and an ever-increasing breadth in the types of populations and health states studied, many research gaps need to be addressed and questions need to be answered in the field of nutrition and the gut microbiome, particularly in children. These gaps and questions are summarized in the subsections below. Published study protocols (52, 111) and trial registrations at ClinicalTrials.gov show several future studies (e.g., NCT03755583, NCT03440346, NCT03139773, NCT02658500, NCT03085277, and NCT02634749) that aim to address some of these gaps, the results from which will further our knowledge of the influence of nutritional interventions on the gut microbiome along with functional capacity in the near future.

5.1. Discovery-Based Gaps

To address discovery-based gaps, future researchers will need to (*a*) identify diet-responsive microbiota and determine if they effect physiological outcomes in host (46); (*b*) understand the role of whole food interventions, compared with specific dietary components, that are most amenable to real-world application; (*c*) elucidate sex differences in microbiota, from early development through old age; (*d*) include metabolite and gene expression profiling, along with microbial sequencing; (*e*) establish the microbiome's resilience to inputs other than dietary intakes, including antibiotics and environment (49); (*f*) improve replication and reproducibility, such as using the same dietary intervention across differing populations, as well as test different dietary interventions in the same population; (*g*) determine the role of food processing and preparation, to understand how cooked or raw foods impact the gut microbiota (the effect of cooking has been recently studied but remains an underresearched area) (18); (*h*) expand research to the full microbial community, including fungi, viruses, protozoa, and archaea; and (*i*) include specific disease states and populations, to understand the role of the microbiota in certain disease states (78), including inflammatory bowel diseases, infectious diseases, noncommunicable diseases, and double-burden of malnutrition, and in the context of non-Western cultures and associated diets (31, 78). Such studies also need to include more diverse populations (78).

5.2. Methodological Challenges

Methodological challenges faced by researchers include (*a*) standardizing and harmonizing microbiome analysis protocols (184), including sampling method, targeted or untargeted sequencing approaches, varying quality control guidelines for data analysis, and different bacterial genome databases and bioinformatics tools (78); (*b*) moving from animal models to human studies, considering that the correlative nature of studies on food/microbiome/host physiology interactions has been concluded mainly from mice models (78) [few human studies have included a mechanistic component with gut microbial transplant into germ-free mice (49), and human feeding studies are needed to confirm relevance before nutritional recommendations can be made (46)]; (*c*) profiling the functional capacity of microbes and integrating proteomic and metabolic data with existing DNA methods, which is often studied in vitro and not necessarily representative of the human gut (46, 78); (*d*) designing and using dietary interventions, given the variance in compliance, which requires large cohorts with long time periods for follow-up (78); (*e*) reconsidering the term dysbiosis by defining it as an absolute compared with a comparative, and as a deviation from the norm, not a specific pattern (46); (*f*) developing statistical solutions for realistic human studies involving random serial stool sampling, which is crucial for participant retention and successful completion of studies; (*g*) including appropriate sample size and power calculations for microbiome studies, which have been developed for cross-sectional studies (19) but require further consideration for longitudinal and complex study designs; and (*h*) compiling study results and sequence data into

one database, for pooled analyses of similarly processed sequences and to understand global phenomena while minimizing study biases.

5.3. Interventional/Therapeutic Gaps

Future research should address the following five interventional and therapeutic gaps. First, stability and resilience need to be addressed. While short-term dietary changes can alter the gut microbiota, long-term dietary patterns are associated with more stable microbiota conformations and are difficult to alter (46); what remains to be determined is which dietary patterns have the most or the least plasticity and whether this plasticity also depends on baseline microbial populations and past dietary patterns (49). A second gap is underresearched nutrients and foods in intervention studies, in diverse populations. Although this review identified several trials of micronutrient powders (MNPs) and comparatively few on supplementation with individual vitamins, minerals, and whole foods, a need remains to investigate MNPs both in replicative studies in Kenya and across other countries and continents. Third, longitudinal, long-term human studies should be conducted. Longer-term feeding studies in populations such as human infants beyond the age of 2–3 years, during puberty and adolescence, in early adulthood, and in later life are needed to understand the dynamics of an aging microbiome. Fourth, precision nutrition and health should be addressed. The gut microbiome likely plays a role in the intra- and interindividual variability in an individual's response to a given dietary intervention, and including the gut microbiota as a variable in developing models for personalized nutrition to optimize health and nutrition status must be tested (141, 178). Fifth, the big picture in future health recommendations should be considered. Recommending food and diet (lifestyle) changes requires a comprehensive approach and support from a multifaceted team of dietitians and nutrition scientists, psychologists, physicians, and biostatisticians (78).

6. CONCLUSIONS AND FUTURE DIRECTIONS

Most studies to date in children have focused on the first year of life; few randomized trials have been conducted among older children and in adolescents (summarized in **Figure 4**). Of interest would be longer-term follow-up of older children who received dietary interventions in early life, to examine lasting changes on the gut microbiota and links to functional outcomes. Furthermore, the most common interventions included different infant formulas, MNPs, PUFAs, and iron-fortified foods, while few studies directly assessed the individual effect of vitamins A, D, and E and zinc (**Figure 4**; see also **Supplemental Table 1**). Similarly, only a limited number of studies to date have examined the impact of whole foods (e.g., honey, legumes, beef) on the pediatric gut microbiome.

Supplemental Material >

Findings from past randomized trials require replication across other populations to understand their impacts on the gut microbiota. Future studies should involve additional intervention types, such as vitamin D and B vitamins, and address how cooking methods may influence the impact of nutritional interventions (individual food or diet) on the gut microbiota, a factor that has only recently begun to be investigated (18). Considering the evidence around the potentially adverse effects of iron fortification and the gut microbiome, further research is needed to examine how iron-biofortified foods may impact the gut microbiome; the effect of iron is currently under investigation in randomized trials in India among infants and young children (68, 102). Finally, all trials were conducted using fecal samples, which represent the colonic microbiota; our current understanding of the impact of nutritional interventions on microbiota residing throughout the gastrointestinal tract including the SI and oral cavity is limited.

a

Typical microbiome development in a healthy, breastfed infant across childhood

No dietary interventions after 12 months of age in healthy children

Birth | **12 months** | **19 years**

VitA
(Huda 2019)
versus placebo
1 bolus at birth
@ 6, 11, 15 weeks; 2 years

LCPUFA or **VitD**
(Hjelmsø 2020)
versus placebo
maternal supplementation
(from 24 weeks GA): daily
@ 1, 4 weeks; 1 year

SQ-LNS
(Kamng'ona 2020)
versus MMNs or
IFA + placebo
daily
@ 1, 6, 12, 18, 30 months

Formula + MFGM
(He 2019)
versus standard formula
daily
@ 2, 4, 6, 12 months

LNS, CSB
(Cheung 2016, Aakko 2017)
versus no intervention
daily
@ 6, 9, 12, 15, 18 months

Fe+ cereal ± fruit
(Qasem 2017)
versus meat
daily
@ 4–6, 4.5–7 months

MNP + ↑Fe++
(Tang 2017, Paganini 2017,
Paganini 2019a, Paganini 2019b)
versus MNP ± ↓Fe++
daily
@ 6–6.5, 6.5, 7, 9, 10 months

Cowpea, common bean
(Ordiz 2020)
versus CSB
daily
@ 6.5, 7.5, 9, 10.5, 12 months

Rice bran
(Zambrana 2019)
versus no intervention
daily
@ 6, 7, 8, 9, 10, 11, 12 months

% Abundance	≈	≈	↓↑*	↓↑*	nd*	≈	≈↓↑*	≈↓↑*	≈↓↑*	≈↓↑*
α-diversity	≈	≈	≈	≈	≈*	nd	≈↑*	≈↑*	≈	≈
β-diversity	≈	≈	≈	nd	≈	nd	≈	nd	≈	↑
MAZ	≈	≈	nd	nd	≈	nd	nd	nd	nd	nd
Function	nd	nd	nd	≈↓↑*	nd	nd	nd	nd	nd	nd

b

ELF
(Flaherman 2018)
versus EBF
daily, after BF
@ birth; 1, 4 weeks
Newborn weight loss

Lactoferrin formula (+probiotics)
(Grzywacz 2020)
versus probiotics only
daily
@ birth; 1, 2, 3, 4 weeks
<31 GA

PUFA
(Younge 2017)
versus no intervention
daily
@ 2 months; 9-week tp
<37 GA, jejunostomy or ileostomy

Honey
(Aly 2017)
versus no intervention
daily
@ 3 days; 2, 7 weeks
<34 GA

Zn + probiotics
(Xiang 2019)
versus probiotics only
daily
@ 2–36 months; 14-day tp
AAD 2° to pneumonia

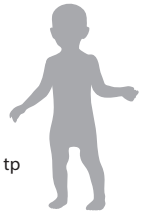
MNP+Fe++
(Jaeggi 2015)
versus MNP only
daily
@ 5.5, 6, 10 months
Anemia (67%)

Fe + VitE
(Tang 2016)
versus Fe + placebo
daily
@ 9–24 months, 8-week tp
ID, ± anemia

MDCF 1/2/3
(Gehrig 2019)
versus RUSF
daily
@ 12–18 months; 14-day tp
MAM

MDCF 2
(Chen 2021)
versus RUSF
daily
@ 12–18 months; 14-day tp
MAM

CDED+PEN
(Levine 2019)
versus EEN
daily
@ 4–18 years; 6-, 12-week tp
CD



$FeSO_4$ + VitC
(Dostal 2014)
versus placebo + VitC
daily
@ 6–11 years; 2-, 12-, 38-week tp
ID (~18%); anemia (~20%)

↑Yellow pea
(Varkey 2020)
versus ↓yellow pea
daily
@ 6–10 years, 30-day tp
Stunting

Fe+ biscuits
(Zimmermann 2010)
versus no-Fe biscuits
daily
@ 6–14 years; 6-month tp
Anemia (75%)

% Abundance	≈	≈↓↑*	↓*	↓↑*	↓↑*	↓↑*	↓↑*	≈↓↑*	≈↓↑*	≈↓↑*	≈↓↑*	≈↓↑*	≈↓↑*
α-diversity	≈	≈↑*	≈↑	nd	nd	nd	≈	nd	nd	≈	nd	≈	nd
β-diversity	≈	≈	nd	nd	nd	nd	nd	nd	nd	↑*	nd	nd	nd
MAZ	nd	nd	nd	nd	nd	nd	nd	↑*	nd	nd	nd	nd	nd
Function	nd	↓↑*	↓↑*	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

Healthy enterocyte | Bacteroidetes | Firmicutes | Proteobacteria | *Bifidobacterium* spp. | *Lactobacillus* spp.

(*Caption appears on following page*)

Figure 4 (*Figure appears on preceding page*)

Summary of effects of nutritional interventions on gut microbiota in (*a*) apparently healthy children and in (*b*) children with poor nutrition status, illness, or inflammation. No studies have analyzed apparently healthy children after 12 months of age; in panel *b*, the health state is labeled below each study in bold font (percentages in parentheses indicate the proportion of the population that presented with that health state, while no percentage indicates that the entire population presented with the health state). The comparison is shown in the third line, beginning with "versus." The frequency of dietary intervention is shown on the fourth line (e.g., daily). The microbiome sampling time point is presented at weeks, months, or years of age. Some studies in panel *b* included a range of ages at baseline, with a subsequent sample collection after a specific time period; these are indicated by tp. Key: ≈, no change; ↓, decrease; ↑, increase. An asterisk denotes that details on specific changes by taxon or by group are available in **Supplemental Table 2**. Figure adapted from images created with BioRender.com. Abbreviations: 2°, secondary; AAD, antibiotic-associated diarrhea, BF, breastfeeding; CD, Crohn's disease; CDED, Crohn's disease exclusion diet; CSB, corn–soy blend; EBF, exclusive breastfeeding; EEN, exclusive enteral nutrition; ELF, early limited formula; Fe+, iron-fortified; Fe++, iron fortificant; $FeSO_4$, ferrous sulfate; GA, gestational age; ID, iron deficiency; IFA, iron and folic acid; LC, long-chain; LNS, lipid-based nutrient supplement; MAM, moderate acute malnutrition; MAZ, microbiota-for-age z score; MDCF, microbiota-directed complementary food; MFGM, milk fat globule membrane; MMN, multiple micronutrient; MNP, micronutrient powder; nd, not determined; PEN, partial enteral nutrition; PUFA, polyunsaturated fatty acid; RUSF, ready-to-use supplementary food; SQ-LNS, small-quantity lipid-based nutrient supplement; tp, time point; VitA/C/D/E, vitamin A, C, D, or E; Zn, zinc.

Supplemental Material >

This review has discussed the current literature base on diet, nutrition, and the gut microbiome in children and has highlighted several areas for future research. Advancing technologies in the field of microbial analyses coupled with decreasing costs of sequencing allow for a higher resolution of outcomes and more targeted points for nutritional interventions. Harmonizing methods and interpreting findings across different studies remain major challenges in microbiome research and are critical for this body of research to inform meaningful clinical or public health practice. Designing such dietary interventions to target the pediatric gut microbiota across a range of health states will have implications for child health and functional, clinical outcomes, particularly in vulnerable populations, as well as for future dietary programs and policies.

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S.M. is an unpaid board member for and has an equity stake in a diagnostic start-up that is commercializing point-of-care technology for nutritional status, partially developed in his research laboratory as a faculty member at Cornell University. The other authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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