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REVIEW

Microbiome Composition in Pediatric Populations from Birth to Adolescence: Impact of Diet and Prebiotic and Probiotic Interventions

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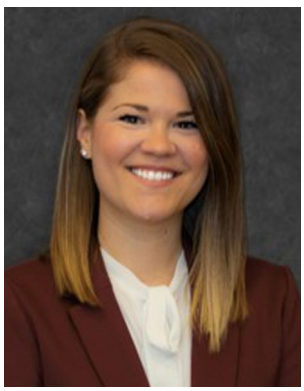
Abstract

Diet is a key regulator of microbiome structure and function across the lifespan. Microbial colonization in the first year of life has been actively researched; however, studies during childhood are sparse. Herein, the impact of dietary intake and pre- and probiotic interventions on microbiome composition of healthy infants and children from birth to adolescence is discussed. The microbiome of breastfed infants has lower microbial diversity and richness, higher Proteobacteria, and lower Bacteroidetes and Firmicutes than those formula-fed. As children consume more complex diets, associations between dietary patterns and the microbiota emerge. Like adults, the microbiota of children consuming a Western-style diet is associated with greater *Bacteroidaceae* and *Ruminococcaceae* and lower *Prevotellaceae*. Dietary fibers and pre- or/and probiotics have been tested to modulate the gut microbiota in early life. Human milk oligosaccharides and prebiotics added to infant formula are bifidogenic and decrease pathogens. In children, prebiotics, such as inulin, increase *Bifidobacterium* abundance and dietary fibers reduce fecal pH and increase alpha diversity and calcium absorption. Probiotics have been administered to the mother during pregnancy and breastfeeding or directly to the infant/child. Findings on maternal probiotic administration on bacterial taxa are inconsistent. When given directly to the infant/child, some changes in individual taxa are observed, but rarely is overall alpha or beta diversity affected. Cesarean-delivered infants appear to benefit to a greater degree than those born vaginally. Infancy and childhood represent an opportunity to beneficially manipulate the microbiome through dietary or prebiotic interventions, which has the potential to affect both short- and long-term health outcomes.

Keywords Infant · Child · Adolescent · Diet · Nutrition · Microbiome



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Introduction

Over the past decade, the essential role that the gut microbiota plays in the developmental programming of the neonate, including growth trajectories, metabolism, and immune and cognitive development, has been demonstrated [1–3]. Thus, fostering the development of the microbiome in the first 1000 days of life is critical to supporting lifelong health. Due to the rapid changes in the gut microbiome in the early postnatal period, most pediatric microbiome research has focused on differences between breast- and formula-fed infants in the first year of life [4]. Few studies have evaluated the microbiota of toddlers and children, and the prevailing thought is that children attain an adult-like microbiota by 3 years of age [5, 6]. However, recent studies suggest that maturation of the gut microbiota is influenced by diet, and differences from an adult-type microbiota persist into later childhood [6, 7]. Therefore, the goal herein was to review the current evidence for the role of dietary intake and pre- and probiotic interventions on the gut microbiota from birth through adolescence.

Early Life (0–2 years)

Breast- and Formula-Feeding

Among pediatric populations, gut microbiota composition of breastfed (BF) and formula-fed (FF) infants is most extensively studied and has been reviewed elsewhere [3, 4]. While heterogeneity exists among demographics, infant age, formula type, and sampling and analytical techniques applied in the published literature, most studies show that both diversity and richness of the microbiome are lower in BF than FF infants [4, 7–10]. Breastfeeding, particularly of longer duration, is associated with a more stable bacterial composition [4, 8] as well as a lower microbiota age [8, 11]. BF infants tend to have higher Actinobacteria [4] and lower Bacteroidetes and Firmicutes than FF infants [2, 6]. Breastfeeding is strongly associated with *Bifidobacterium* [4, 7–9, 11] and *Bifidobacteriaceae* abundance [10]. For example, in the TEDDY (The Environmental Determinants of Diabetes in the Young) cohort, BF infants had higher relative abundance of *B. breve*, *B. bifidum*, and *B. dentium* than FF; while *B. longum* was the most dominant species in this study, it did not differ by feeding group [7]. *Lactobacillus* abundance has also been associated with breastfeeding [9, 11]; however, results vary considerably among published studies [4]. In a recent meta-analysis of seven studies, infants who were not exclusively BF harbored higher relative abundances of *Bacteroides, Eubacterium,* and *Veillonella* [8].

Feeding mode interacts with other perinatal factors to influence the infant gut microbiota. Ho and colleagues reported that non-exclusively BF infants have a lower abundance of Proteobacteria, but only among those delivered via cesarean section (C-section) [8]. However, breastfeeding appears to moderate the detrimental effects of C-section delivery and intrapartum antibiotics on the early microbiota, producing a microbiota profile more similar to that of vaginally delivered infants or those not receiving antibiotics [4]. Geography and ethnicity are also important to take into account. Across five European countries, the effect of country was more pronounced than delivery or feeding method, with dominant bifidobacteria in northern countries and greater early diversification in southern European countries [12]. Within the USA, *Bifidobacterium* abundance differed between white and Hispanic BF and FF infants, but not black infants [9].

Compared to BF infants, the functional capacity of the microbiome of FF infants is more similar to that of adults, consisting of genes related to bile acid synthesis and methanogenesis, but considerable variation exists among recent studies [4]. For example, the BF infant microbiome has an increased abundance of genes associated with

carbohydrate and lipid metabolism and fatty acid biosynthesis than FF [7], although another study reported similar data related to fatty acid biosynthesis genes, but opposite results for carbohydrate and lipid metabolism [8]. Compared to FF infants, the BF infant's microbiome has more genes associated with vitamin and cofactor metabolism [8], free radical detoxification [8], and glutathione metabolism [13]. Discrepancies among the studies could be due to differences in infant age or the inclusion of mixed-feeding infants (MF) in different feeding groups. Thus, more work is needed to understand the functional ontogeny of the infant gut microbiota.

Human milk (HM) contains nutrients, bioactive components, and bacteria that drive the aforementioned differences in the gut microbiota of BF and FF infants. In particular, the human milk oligosaccharides (HMO) are complex glycans that are resistant to digestion and exert a number of functions in the distal gastrointestinal tract of the infant [14]. Over 200 unique HMOs have been identified, and maternal genetics affects the HMO present in milk [4, 14]. HMOs shape the infant gut microbiota by acting as a prebiotic substrate for select beneficial bacteria, such as certain species of *Bifidobacterium*, as well as, acting as a decoy receptor for pathogenic microorganisms [14]. The addition of HMOs and other prebiotics to infant formula over the last decade has likely resulted in some convergence in the microbiota of BF and FF infants [4] and will be discussed later in this review. Along with the HMOs, BF infants receive a continuous source of bacteria from HM [15]. The HM microbiome is dominated by *Staphylococcus* and *Streptococcus*, but also contains *Bifidobacterium, Lactobacillus, Clostridium,* and *Veillonella*, all resident genera found in the early infant microbiome [4, 15–17]. Hundreds of bacterial species are present in HM [15–17], and composition is associated with a variety of maternal factors such as body mass index, delivery mode, geography, and breast pump usage [15]. The microbial compositions of HM and infant feces are strongly associated [16]; thus, the unique microbial composition of each mother's milk may account for some variation in the gut microbiome of BF infants [4, 15]. While HMO and the HM microbiome are most widely studied in relation to the infant microbiota, other HM components, such as IgA, antimicrobials, glycoproteins [18], cytokines [19], phages [20], and fungi [21], likely contribute to development of the early microbiome.

Introduction of Complementary Feeding and Cessation of Breastfeeding

Microbiota composition increases in both diversity and richness during the transition from a milk-based to an adult-like diet [4, 9]. Introduction to complementary foods is accompanied by marked increases in *Lachnospiraceae*, *Ruminococcaceae*, *Blautia, Bacteroides,* and *Akkermansia* [22–25] and decreases in *Bifidobacterium, Veillonellaceae, Lactobacillaceae, Enterobacteriaceae, and Enterococcaceae* [24]. However, early feeding mode continues to remain evident throughout these dietary transitions, influencing infant gut microbiota composition even up to 2 years of age [8, 26]. Whether an infant is BF during solid food introduction influences microbial patterns [10, 12, 24, 25, 27] (Table 1). Continued breastfeeding provides substrates necessary to sustain microbes such as *Bifidobacterium*, *Lactobacillus, Collinsella, Megasphera,* and *Veillonella* [26, 27] (Table 1). Introduction of foods high in protein and fiber increases microbial diversity, but the particular foods most correlated with microbial diversity differ depending on whether the infant is still being breastfed [24]. For example, a greater number of predicted functional changes were identified in FF and MF infants during introduction of solids compared to BF infants, suggesting that breastfeeding may increase the plasticity of the infant microbiome [25].

As energy-yielding substrates change over the first year of life, so does the metabolic capacity of the infant microbiome, with increases in genes associated with starch, central carbon, and pyruvate metabolism [27]. During weaning from HM or formula, milk-associated bacteria decrease and microbes capable of degrading complex polysaccharides, such as *Bacteroidaceae, Lachnospiraceae, and Ruminococcaceae*, increase [24]. Breastfeeding duration influences when these transitions occur; at 12 months, richness and diversity were highest among infants weaned before 6 months and lowest among those still being BF [10]. Similarly, the microbiota of BF infants residing in Italy and Burkina Faso have been shown to cluster fairly close together, despite vast differences in the diets [high fiber vs. high fat/protein] and the environments [urban vs. rural] of the two countries [29]. However, once children were fully weaned, the microbiota of children in Burkina Faso was dominated by Bacteroidetes, while that of Italian children was enriched with Firmicutes [29].

Previously, cessation of breastfeeding, rather than complementary food introduction, was proposed to be the driving force behind the shift toward an adult-like microbiome [27]. However, both contribute to this transition to different degrees among infants [24]. Still, studies investigating changes in the microbiome upon weaning and introduction to solid foods are limited [29]. Additional large, longitudinal cohort studies are needed to explore the compositional and functional changes of the microbiota that accompany dietary shifts in early life.

Beyond the 2 Years of Age

Although studies on gut microbiota composition in children after 2 years of age are more limited, available evidence suggest that the microbiota of young children differs from that

Table 1 Characteristics of studies investigating dietary effects on microbiome composition in infants and children

Country of study, age range, and number of participants	Study design	Method of diet assessment	Method of microbiota assessment	Outcomes	Citations
Sweden y *N* = 98	Cross-sectional	Feeding practices questionnaires assessing BF, FF, MF BF cessation	Metagenomic shotgun sequencing by Illumina HiSeq 2000	BF infants predominate in *Bifidobacterium, Lactobacillus, Collinsella, Megasphaera*, and *Veillonella* BF cessation increased *Bacteroides, Bilophila, Roseburia, Clostridium*, and *Anaerostipes* Newborn and 4-mo microbiota enriched in genes for HMO degradation 12-mo microbiota enriched in genes for complex sugar and starch degradation; increased *B. thetaiotaomicron*	[27]
Denmark 0–3 y *n* = 330	Observational cohort (SKOT)	FFQ at 9-, 18-, and 36-mo visits	Targeted qPCR analysis	Weaning decreased *Bifidobacterium, Lactobacillus,* and increased *Enterobacteriaceae*, *Clostridium* spp., and *Bacteroides* spp.	[26]
USA (North Carolina) 1–4 y and adults *N* = 28	Cross-sectional	Children attended daycares adhering to nutritional requirements defined by local state and federal rules and regulations	Microarray targeting V1–V6 16S rRNA and qPCR	Children had less diverse microbiota than adults Actinobacteria, Bacilli, Clostridium cluster IV (*Ruminococcaceae*), and Bacteroidetes were higher in children than adults	[28]
Italy and Burkina Faso 1–6 y *N* = 29	Cross-sectional	Italian parents completed a detailed medical, diet, and lifestyle survey Burkina Faso parents provided in-depth interview on children's diet and a 3-d dietary questionnaire	V5–V6 16S rRNA by 454-pyrosequencing	Firmicutes-to-Bacteroidetes ratio (F/B ratio) was ~ sixfold higher in Italian than Burkina Faso children 3 genera involved in utilization plant polysaccharides (*Prevotella*, *Xylanibacter* (*Bacteroidetes*), and *Treponema* (*Spirochaetes*) were higher in Burkina Faso children	[29]

Table 1 (continued)

Country of study, age range, and number of participants	Study design	Method of diet assessment	Method of microbiota assessment	Outcomes	Citations
Australia 2–3 y *N* = 37	Cross-sectional	Australian Child and Adolescent Eating Survey (FFQ) 24-h recall	V6–V8 16S rRNA by Illumina MiSeq	Dairy intake negatively associated with *Bacteroidetes*, species richness and diversity and positively with *Erysipelatoclostridium* spp. and the F/B ratio Vegetable protein intake positively associated with *Lachnospira* Soy, pulse, and nut positively associated with *Bacteroides xylanisolvens* Fruit intake negatively associated with *Ruminococcus gnavus*	[30]
USA (Illinois) 4–8 y *N* = 22	Cross-sectional	Nutrient intake assessed by 3-day food diaries Youth and Adolescent (YAQ) FFQ was used for dietary patterns	V3–V4 16S rRNA by Illumina MiSeq	Two dietary patterns were associated with microbial taxa and composition Dietary Pattern 1 (intake of fish, protein foods, refined carbohydrates, vegetables, fruit, juice and sweetened beverages, kid's meals and snacks and sweets) was linked to higher Bacteroidetes, *Bacteroides*, and *Ruminococcus* and lower *Bifidobacterium, Prevotella, Blautia,* and *Roseburia* Dietary Pattern 2 (intake of grains, dairy and legumes, nuts and seeds) was associated with higher *Cyanobacteria* and *Phascolarctobacterium* and lower *Dorea* and *Eubacterium*	[31]
Philippines—Rural (Baybay) and urban (Ormoc City) 7–9 y *N* = 43	Cross-sectional	Parents/guardians interviewed using FFQ modified from Singapore National Dietary Survey and adapted to dietary habits of Filipino children	V6–V8 16S rRNA by 454 pyrosequencing	87.5% of Baybay children fell into P-type cluster (defined by *Prevotellaceae*) and 78.9% of the Ormoc samples were included in the termed BB-type cluster (defined by *Bacteroidaceae*, *Bifidobacteriaceae*, *Ruminococcaceae*, and *Lachnospiraceae*)	[32]
Thailand—Rural (Buriam) and urban (Bangkok) 9–10 y *N* = 45	Comparative cross-sectional	7-day dietary records	V1–V2 16S rRNA by Illumina MiSeq	Bangkok children had higher Actinobacteria, *Bacteroidales* and *Selenomadales* Buriram children had more *Clostridiales*, *Peptostreptococcaceae* and unclassified *Ruminococcaceae* and higher butyrate and propionate	[33]
Netherlands 6–9 y *N* = 281	Cross-sectional	Parent-report FFQ	Metagenomic shotgun sequencing by Illumina sequencing	Higher Bacteroidetes and Actinobacteria (*Bifidobacterium*) in children than adults Negative correlation between high dietary fiber consumption and low plasma insulin levels in children with *Bacteroides* and *Prevotella* enterotypes, but not *Bifidobacterium* enterotype	[34]

Table 1 (continued)

Country of study, age range, and number of participants	Study design	Method of diet assessment	Method of microbiota assessment	Outcomes	Citations
Thailand 8–11 y *N* = 60	Cross-sectional	Self-administered FFQ	qPCR	Vegetables positively correlated with *Lactobacillus* and *Prevotella*; *Bifidobacterium* spp. negatively associated with fish and beef	[35]
China and Malaysia 7–12 y *N* = 210	Cross-sectional	Singapore Health Promotion Board validated FFQ	qPCR	Geographical-related factors (i.e., diet), rather than ethnicity (i.e., Southern Chinese or Malay children) is a major delineator of microbiome changes *Bifidobacterium*, and *Collinsella* positively correlated with refined-sugar-enriched foods; *Collinsella* positively associated with fruits and curry foods	[36]
Bangladesh 8–13 y *N* = 10 USA 12–14 y *N* = 4	Cross-sectional	Not reported	V1–V3 16S rRNA by 454 pyrosequencing	Bangladeshi children had lower *Bacteroides* and higher *Prevotella*, *Butyrivibrio*, and *Oscillospira* Bangladeshi children consumed non-Western diet low in refined-sugar-enriched foods and meat and high in rice, bread, and lentils	[37]
Egypt (Giza) 13.3–14.5 y *N* = 28 USA (Ohio) 10.1–15.7 y *N* = 14	Cross-sectional	Not reported	V4 16S rRNA by Illumina MiSeq	Egyptian consumed Mediterranean-type diet, and American children consumed a Western diet Egyptian children had *Prevotella* enterotype, and American children had *Bacteroides* enterotype Egyptian children had higher fecal SCFAs, microbial polysaccharide degradation-encoding genes, and polysaccharide-degrading genera	[38]

F/B Firmicutes-to-Bacteroidetes ratio, *BF* breastfed, *FF* formula-fed, *FFQ* Food Frequency Questionnaire, *MF* mixed-fed, *SCFA* short-chain fatty acids, *USA* United States of America

of adults [28]. As children consume a more complex diet, associations between dietary patterns and the gut microbiota emerge, and their microbiota composition becomes more similar to adults [28]. How diet affects the gut microbiota can be interrogated at several levels, starting with specific nutrients, such as fiber [31], to categories of foods, or food groups [30, 31], to more complex assessments of dietary intake, such as dietary patterns [30]. A summary of the impact of diet on gut microbiota composition is shown in Table 1 and is discussed below.

Toddlers (2–3 Years of Age)

In Australian 2- to 3-year-olds, both habitual diet, as measured by a Food Frequency Questionnaire (FFQ), and recent dietary intake, as measured by a 24-h recall 3 days prior to fecal sample collection, influenced fecal microbiota composition [30]. Dairy intake was negatively associated with species richness and diversity and Bacteroidetes abundance, but was positively associated with *Erysipelatoclostridium* spp. and the Firmicutes-to-Bacteroidetes ratio [F/B ratio]. Vegetable protein intake was positively associated with abundances of the *Lachnospira*; soy, pulse, and nut intake were positively associated with *Bacteroides xylanisolvens*, and fruit intake was negatively associated with the relative abundance of microbes related to *Ruminococcus gnavus* [30]. Dairy and vegetable-source proteins explained 7–10% of the variation in microbiota composition and fruit intake explained 8%. Among the dairy group, yogurt explained 9% of the variance in microbiota [30].

Young Childhood to Adolescence (4–14 Years of Age)

Moving beyond the first 1000 days of life, Berding and coworkers [31] investigated the temporal stability of the fecal microbiota and whether dietary patterns were associated with microbial taxa and composition in American 4–8-year-olds at 3 time points over a 6-month period. Dietary intakes were assessed over the previous year using the Young Adolescent Questionnaire, and two dietary patterns were identified by principal components analysis (PCA) and factor analysis [31]. Temporal stability of microbiota over the 6-month period was associated with baseline dietary patterns. Dietary pattern 1, defined by intake of fish, protein foods, refined carbohydrates, vegetables, fruit, juice and sweetened beverages, kid's meals and snacks and sweets, was linked to higher relative abundance of *Bacteroidetes*, *Bacteroides*, and *Ruminococcus* and lower *Bifidobacterium*, *Prevotella*, *Blautia,* and *Roseburia* relative abundance. Dietary pattern 2, defined by intake of grains, dairy and legumes, nuts and seeds, was associated with higher *Cyanobacteria* and *Phascolarctobacterium* abundance and lower *Dorea* and *Eubacterium* abundance [31]. Additionally, the intake of snacks and sweets and refined carbohydrates were negatively correlated with both Shannon and the Chao1 Indices, respectively, demonstrating reduced microbial diversity with greater intake of sugars and refined grains.

Residing in rural vs. urban environments can also affect food availability and choices, which has been investigated in a series of studies. A study of Filipino children (7 to 9 years) living in rural (Baybay) and urban (Ormoc) communities showed distinct differences in dietary habits and fecal microbiota composition [32]. Nearly all (94%) of urban children consumed fast food four times per week on average compared to 42% of rural children who consumed fast food less than once per week. Urban-dwelling children also consumed a diet higher in meat, fat, and confectionaries, such as sweetened pastries and biscuits, and lower in complex carbohydrates compared to rural children. Using family-level bacterial composition to execute PCA and clustering analysis in conjunction with a dataset from five other Asian countries, it was observed that 87.5% of rural children fell into the termed P-type cluster [defined by *Prevotellaceae*] and 78.9% of the urban samples were included in the termed BB-type cluster (defined by *Bacteroidaceae*, *Bifidobacteriaceae*, *Ruminococcaceae*, and *Lachnospiraceae*). Additionally, *Prevotellaceae*, including only the genus *Prevotella* and consisting of mostly *Prevotella copri*, were more abundant in the feces of rural children, making up 10% of the total community, whereas it represented < 1% of the fecal microbial sequences in most urban children. These findings may reflect the higher consumption of complex carbohydrates in rural children. [32].

Similarly, Kisuse and colleagues examined differences in dietary habits, fecal microbiome composition, and short-chain fatty acid (SCFA) concentrations of children (9 to 10 years) living in rural (Buriram) and urban (Bangkok) settings in Thailand [33]. Urban children consumed more bread, meat, and beverages and less rice and vegetables than the rural children. Vegetables comprised < 1.0% of total calorie intake in urban children compared to 7.3% in rural children. The fecal microbiome of the rural children displayed significantly greater alpha diversity (Chao1 index). The microbiota of rural children was enriched by bacteria in the order *Clostridiales*, containing families such as *Peptostreptococcaceae* and unclassified *Ruminococcaceae*, compared to higher proportions of *Actinobacteria*, *Bacteroidales,* and *Selenomadales* in urban dwellers. Additionally, rural children had significantly higher fecal butyrate and propionate concentrations, suggesting that the fiber-rich diet in the rural children promotes a microbiota composition with greater fermentative capacity [33].

Greater *Bifidobacterium* abundance in 1- to 4-year-olds compared to adults has been reported [28], and recent studies have shown that the relative abundance of *Bifidobacterium* in older children is related to dietary intake and is

associated with metabolic phenotypes. Studying Dutch children in the KOALA Birth Cohort Study, Zhong and colleagues documented higher levels of *Bifidobacterium* at 6 to 9 years of age compared to adults [34]. They also classified children into three enterotypes and observed that correlations between dietary and metabolic phenotypes were dependent on fecal microbial enterotype. For example, a negative correlation between dietary fiber intake and plasma insulin was only reported in children with *Bacteroides* and *Prevotella* enterotypes, but not the *Bifidobacterium* enterotype [34]. This latter microbiome possesses lower microbial gene richness, alpha diversity, and functional potential for butyrate and succinate production, suggesting that children exhibiting a *Bifidobacterium* enterotype have a less mature gut microbiome [34]. Additionally, a study of 8- to 11-year-olds in Thailand living in two different geographical regions observed that frequency of vegetable intake was positively correlated with *Lactobacillus* and *Prevotella*, while *Bifidobacterium* spp. was negatively correlated with fish and beef intake [35].

A similar study of healthy 7- to 12-year-olds from China and Malaysia, living in three different cities, showed that geographical-related factors (including diet), rather than other potential mediating factors, such as ethnicity (e.g., Southern Chinese or Malay children), was a major delineator of microbiome changes [36]. Four genera (*Bacteroides, Fecalibacterium, Bifidobacterium*, and *Collinsella*) showed significant associations with the 15 food groups under observation. *Bifidobacterium* and *Collinsella* were positively correlated with refined-sugar enriched foods, and *Collinsella* was also positively associated with fruit and curry intake [36].

Parallel to these findings, comparing Bangladeshi and American children (9–14 years), Bangladeshi children exhibited lower levels of *Bacteroides* and higher levels of *Prevotella*, *Butyrivibrio*, and *Oscillospira*, indicative of their consumption of a non-Western diet low in refined-sugar enriched foods and meat and rich in rice, bread, and lentils [37]. Furthermore, the American children consuming Western diets had higher *Bacteroides* abundance than children in Bangladesh [37]. A *Bacteroides* enterotype is more common in adults consuming a Western diet, whereas the *Prevotella* enterotype is more common in those consuming high amounts of fiber [39].

Lastly, a study comparing Egyptian teenagers (mean 13.9 years) consuming a Mediterranean-style diet to American teenagers (mean 12.9 years) consuming a Western diet, found that Egyptian children clustered to the *Prevotella* enterotype and American children clustered to the *Bacteroides* enterotype [38]. Furthermore, the gastrointestinal environment of Egyptian children contained higher levels of SCFAs, microbial polysaccharide degradation-encoding genes, and polysaccharide-degrading genera [38].

Taken together, these findings provide evidence that the microbiome in children and adolescents is shaped to a greater degree by dietary intake [32–38] than by ethnicity [36]. While it is has been postulated that the microbiota after age 3 resembles that of adults [5], emerging evidence suggest that, while the microbiota of children can be assembled into enterotypes [34, 37, 38], differences persist between children and adults. Additionally, children may also be more similar to each other than adults are. For example, in pre-adolescent children (ages 7–12) intragroup similarity in the fecal microbiota was greater in children than adults [40]. Adults also displayed greater abundances of *Bacteroides* spp., while children displayed enhanced *Bifidobacterium* spp., *Faecalibacterium* spp., and members of *Lachnospiraceae* [40]. However, the current literature on the impact of diet in this age group has some noted limitations. Nearly all studies are cross-sectional, they use different types of questionnaires to collect dietary intake data, and many of the studies have compared children living in rural vs. urban settings. While dietary intake differs between rural and urban communities, many other environmental factors are also likely contributing, including socioeconomic status, exposure to agricultural species and routine medical care, which could also be influencing the gut microbiota.

Fiber and Prebiotic Interventions in Children on Gut Microbiota

A consistent finding of the observational studies summarized above is that consumption of a Western-style diet, characterized by low ratio of whole grains-to-refined carbohydrates, detrimentally influences microbiome composition and fecal SCFA concentrations in children [30–37]. Dietary fiber (DF) has documented health benefits for adults, including reducing intestinal transit time, plasma cholesterol and postprandial glycemic response and improving resistance to pathogens and epithelial barrier function [41–43]. The underlying mechanisms of these beneficial effects are not fully known; however, gut microbiome modulation and formation of SCFAs by bacterial fermentation are proposed [43]. DF is also thought to be beneficial for gut health of children [44], although more studies are needed. In the USA, the recommended dietary fiber intake is 14 g/1000 kcal or 25 g for females and 38 g for males. Most Americans only consume about half of the recommended intake (13.5 and 18 g, respectively) [41]. The fiber intake recommendations for children between the ages of 1 and 13 years, range from 5 to 31 g/day, depending on the organization, however, in most cases children are not meeting the recommended fiber intakes [44]. Thus, various strategies have been developed for modulation of gut microbiota, including administration of DFs, pre- or/and probiotics.

In 2009, the Codex Alimentarius Commission defined DF as "carbohydrate polymers with 10 or more monomeric units, which are not hydrolyzed by the endogenous enzymes in the small intestine of humans" [43]. DF includes non-digestible carbohydrates naturally occurring in food, isolated from food or synthesized, the latter two requiring evidence to support their physiological benefit to health [45]. Most countries adopted the 2009 Codex [43] definition by inclusion of carbohydrate polymers with degrees of polymerization between 3 and 9 [46]. DFs have been classified based on their physiochemical properties such as particle size, fermentability, solubility, and viscosity, and these properties influence the functionality of a DF, including its ability to modulate gut microbiota [47]. Soluble and readily fermentable DFs are referred to as prebiotics, which are "a substrate that is selectively utilized by host microorganisms conferring a health benefit." [48]. Most prebiotics are DF, but not all DF are considered to be prebiotics.

Infant Formula and Prebiotics

HMOs are considered prebiotics, which may partly explain the differences in microbiota composition between BF and FF infants [4]. To narrow the gap between HM and infant formula, prebiotics are now routinely added to infant formula. The most studied prebiotics are a 9:1 mixture of short-chain galactooligosaccharides (scGOS) and long-chain fructooligosaccharides (lcFOS). Other prebiotics supplemented to infant formula, either alone or in combination, include GOS, FOS, polydextrose, lactulose, acid oligosaccharides, oligofructose, and inulin [4]. The effect of prebiotics on the composition of infant microbiota has been recently reviewed [4]; most studies show that prebiotics increase the abundance of *Bifidobacterium* and sometimes *Lactobacillus* compared to infants fed control formula [4]. Several studies reported a decrease in opportunistic pathogens, such as *Escherichia coli*, enterococci, and clostridia [4].

Two HMOs, 2′-fucosyllactose (2′-FL) and lacto-*N*-neoteraose (LNnT), are added to infant formula. Both are well tolerated and support age-appropriate growth of infants [49–51]. A multicenter, randomized, double-blind trial compared the fecal microbiota of healthy infants fed formula with 2′-FL and LNnT from < 14 days to 6 months of age to infants consuming with control formula. Findings demonstrated a fecal microbiota closer to that of BF infants in the infants fed formula with HMO, with higher numbers of *Bifidobacterium* and lower potential pathogens than placebo at 3 months of age [51].

DF and Prebiotics in Children

Only a few studies have studied DFs and prebiotics on the gut microbiota in healthy 3–6-year-old children [52] and adolescents (8–15 years) [53–56] (Table 2). As prebiotic fibers, both GOS and inulin-type fructans have been shown to increase abundance of *Bifidobacterium* [52–54]. Several studies have demonstrated that the intake of DFs shape the gut microbes of children; however, their effects on microbiota composition depend on the type of fiber studied. For example, administration of wheat bran extract (5 g/d for 3 weeks) increased fecal *Bifidobacterium* [53], while consumption of soluble corn fiber (SCF; 10 or 20 g/d for 4 weeks) modulated the overall microbiota, increased the alpha diversity and altered the relative abundances of some bacterial genera, including *Parabacteroides* and unclassified *Lachnospiraceae* [54]. This same group also showed that GOS [55] and SCF [56] increased calcium absorption in adolescent girls and boys, demonstrating a health benefit for this age population. The authors proposed that bacterial fermentation of SCF to SCFAs reduced the luminal pH, which increased calcium solubility and transcellular absorption [54]. Calcium absorption was negatively correlated with *Parabacteroides* relative abundance, but positively correlated with *Clostridium* and unclassified *Clostridiaceae* abundance [54]. The authors speculated that the two groups of bacteria were cross-feeding, with the Bacteroidetes (*Parabacteroides*) fermenting SCF to acetate or lactate, and the Firmicutes (*Clostridium*) further fermenting these substrates to butyrate [54]. The limited studies suggest that prebiotic and DF doses of 5–20 g are well tolerated in children, promote the expansion of bifidobacterial populations, and may exert other health benefits. Further large-scale studies are needed with different fiber sources.

Probiotic Interventions in Children on Gut Microbiota

Probiotics are "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" [57]. The most commonly administered probiotic bacteria belong to the genera *Bifidobacterium* and *Lactobacillus*, but can be provided either as single or mixtures of strains. The beneficial effects of probiotics in pediatric populations have been previously reviewed [58–61], although most studies have not been conducted in healthy children. Probiotics shorten the duration of acute gastroenteritis, prevent antibiotic-associated diarrhea, reduce the risk of necrotizing enterocolitis in preterm infants and lower the incidence of eczema in high-risk children [58–61]. The mechanisms of action of probiotics are not fully understood; however, modulation of gut microbiota has been postulated as one of the mechanisms [62].

Two general probiotic approaches have been taken to influence the infant or child microbiota. The first approach is to administer the probiotic to the mother during pregnancy

Table 2 Characteristics of studies investigating effects of dietary fibers and prebiotics on the fecal microbiota of healthy children and adolescents

Country of study, age range, and duration of intervention	Study design and participants/group	Nutrition base and fiber type and amount	Microbiota assessment	Outcomes	Citations
Hungary (Pécs) 3–6 y 24 wk	Randomized, double-blind, placebo-controlled trial Prebiotic ($n=110$) Placebo ($n=109$)	Mixed with food or drink Inulin-type fructans (6 g/d)	Baseline and week 24 qPCR	↑ relative abundances of *Bifidobacterium* and *Lactobacillus* ↔ numbers of total bacteria, relative abundances of *C. perfringens*, *C. difficile,* and *Enterobacteriaceae* ↔ fecal pH and stool consistency	[52]
Belgium (Leuven) y 3 wk	Randomized, double-blind, placebo-controlled crossover trial with 2-wk washout Wheat bran extract and control ($n=29$)	Soft drink Wheat bran extract containing arabinoxylan oligosaccharides (5 g/d)	Baseline and d 19 or 20 FISH	↑ *Bifidobacterium* level ↔ counts of *Lactobacillus/Enterococcus*, *C. histolyticum/C. liteseburense*, *R. rectale/E. rectela* groups, and *F. prausnitzii* ↔ fecal pH, each SCFA levels, percentage of moisture and stool frequency	[53]
USA (Indiana) 10–13 y 3 wk	Randomized, double-blind crossover trial with 2-wk washout GOS 5 g, GOS 10 g and control ($n=20$)	Smoothie drinks GOS (5 or 10 g/d)	Baseline and the end of each treatment DGGE, qPCR	↔ numbers of DGGE bands Change in *Bifidobacterium* counts: GOS 5 g > GOS 10 g, control ↔ bowel movement frequency and stool consistency	[54]
USA (Indiana) 12–15 y 3 wk	Randomized double-blind crossover trial with 7-d washout SCF and control ($n=23$)	Spaghetti, hamburgers, sandwiches and potato chips Soluble maize fiber (12 g/d)	Baseline and the end of each treatment V3–V5 16S rRNA gene by 454 pyrosequencing	↑ proportions of *Parabacteroides*, other Clostridiales and other *Ruminococcaceae* ↓ *Enterococcus*, *Anaerofustis*, *Coprococcus,* and other *Peptostreptococcaseae*	[55]
USA (Indiana) 12–15 y 4 wk	Randomized double-blind crossover trial with 3–4-wk washout SCF (10 g), SCF (20 g) and control ($n=27$)	Muffin and beverage	Before and after each intervention V3–V4 16S rRNA gene by 454 pyrosequencing	↑ Chao 1 and observed OTUs at species level Overall microbiota differed between samples with and without SCF SCF 10 g ↑*Parabacteroides* and unclassified *Lachnospiraceae*, ↓ reclassified *Ruminococcus* SCF 20 g ↑*Parabacteroides* and unclassified *Lachnospiraceae*, ↓ *Bacteroides* and *Lachnospira* Fecal pH: SCF 20 g < control, SCF 10 g ↔ SCFA concentrations	[56]

DGGE denaturing gradient gel electrophoresis, *GOS* galactooligosaccharides, *OTU* operational taxonomic unit, *qPCR* quantitative PCR, *SCF* soluble corn fiber, *SCFA* short-chain fatty acids, ↑indicates significantly increased, ↓ indicates significantly decreased, ↔ indicates no effect

and then to either the mother and/or infant postpartum [63–70] (Table 3), and the second is to administer the probiotic directly to the infant or child [71–84] (Table 4). For the first approach, most studies gave probiotics to the mothers of infants with high-risk of allergy, with the goal of prevention of allergic disease, such as eczema, asthma and allergic rhinitis [63–65, 69, 70]. The impact of maternal probiotic supplementation on the abundances of bacterial taxa were studied [63–70]; however, the results are inconsistent, even when the same probiotic strain was used [63, 64, 69] (Table 3). For example, supplementation of pregnant and lactating women with *L. rhamnosus* GG (LGG), *L. acidophilus* La-5 and *B. animalis* subsp. *lactis* BB-12 from 36-week gestation until 3 months postnatal during breastfeeding did not affect the proportions of bacteria classes and genera of the infants at 3 months and 2 years [67]. In contrast, a Finish study evaluated the effect of administration of *L. rhamnosus* LPR and *B. longum* BL999 to mothers 2 months before and 2 months after delivery. They observed that infants whose mother received probiotics had lower counts of *Bifidobacterium* and a higher percentage of *Lactobacillus/Enterococcus* than placebo at 6 months of age [68]. In addition, several groups investigated the diversity of infant microbiota, reporting that administration of probiotics during pregnancy and lactation, or directly to infants after delivery have no or limited effects on alpha and beta diversity of infant microbiota [65, 67, 70] (Table 3).

Probiotics have been administrated directly to infants and children [71–84] (Table 4). These studies varied in terms of age of the children (newborns to age 18), type of probiotic, dose administered, and duration of the intervention. Despite these differences in study design, no effects of probiotic administration were observed on microbiome alpha or beta diversity between children in probiotic and control groups, with the exception of one study [73]. In that study, formula or *L. reuteri* DSM 17938-supplemented formula was fed for 6 months to newborns born by either vaginal or C-section delivery [73]. The *L. reuteri*-supplemented formula had a limited effect on the microbiota of vaginally born infants; however, the overall microbiota composition of C-section-delivered infants consuming the probiotic-supplemented formula differed from that of placebo and was similar to vaginally delivered infants at 2 weeks of age [73].

Similar to the findings when probiotics were administered to the mother, inconsistent results were observed on the abundances of bacterial taxa when probiotics were supplemented directly to the children; some probiotics affected the proportions of individual bacterial taxa, while others did not (Table 4). These conflicting results may be related to differences in probiotic strain/strains used, the dose use, duration of administration, and the methods used for microbiota analysis. Furthermore, factors that influence the development of gut microbiota, such as delivery mode, children's age, and diet, likely confound the effects of probiotic supplementation in this population [73].

While some encouraging data exist on the efficacy of probiotics on disease prevention, no broad consensus exists to recommend the use of probiotics in these conditions [59]. Although probiotics are safe for use in healthy population; several concerns have been raised related to the administration of probiotics early in life when gut microbiota is not fully established. Long-term consequences of such administration should be carefully evaluated [60].

Future Directions

There is a need for more dietary intervention studies in healthy populations, as the majority of currently published studies describe dietary interventions in the context of disease states, such as obesity, which is represented by microbial dysbiosis [85]. In particular, randomized, controlled clinical trials on the effects of DFs, prebiotics, and probiotics are needed in pediatric populations, particularly in adolescence to young adulthood (15–20 years), where there is a paucity of data available. Additionally, long-term follow-up studies of early-life dietary interventions are needed to determine long-term effects. For example, it is not known whether or not early-life acceleration toward an adult-like microbiome has negative downstream effects on health. None of the reported human studies report effects on host gut gene expression, which is possible to do noninvasively in pediatric populations using exfoliated epithelial cells [86]. Exploring host-microbe molecular cross-talk [87] and incorporating other multi-omic approaches, including the fecal metabolome [88] will further our understanding of the complex relationships between diet, gut microbiota, and human health and disease and can lead to the development of low-cost, safe and efficacious dietary interventions [89, 90]. These "microbiota-directed foods" [90] have the potential to prevent or treat some of the most pressing health nutritional challenges facing the world's population.

Table 3 Characteristics of studies investigating probiotic administration during pregnancy and after delivery on infant fecal microbiota

Country of study, age range, duration of intervention	Study design and participants/group	Nutrition base and probiotic strain and amount	Microbiota assessment	Outcomes	Citation
Australia (Melbourne) Mothers at 36-wk gestation until delivery	Randomized, double-blind placebo-controlled trial Probiotic ($n=59$) Placebo ($n=57$)	Powder in capsules LGG 1.8×10^{10} CFU/d	Infant at 3, 7, 28, and 90 d of age qPCR, T-RFLP	↑ prevalence of species belonging to *B. longum* group at 90 d	[63]
Finland (Turku) Mothers at 36-wk gestation until delivery; infants 0–6 mo	Randomized, double-blind placebo-controlled trial Probiotic ($n=77$) Placebo ($n=82$)	Powder in water LGG 1.0×10^{10} CFU/d	3, 6, and 12 mo of age ($n=96$ infants) FISH	↔ counts of total bacteria *Bifidobacterium* and *Lactobacillus/Enterococcus* at 3, 6, and 12 mo	[64]
Netherlands Mothers at 6 wk before delivery until delivery; infants at 0–1 y	Randomized, double-blind placebo-controlled trial Probiotic ($n=20$–37) Placebo ($n=17$–45)	Powder in water, milk or formula *B. bifidum* W23 + *B. lactis* W52 + *L. lactis* W58 1×10^{9} CFU/strain/d	1 and 2 wk, 1, 3, 12, and 18 mo, 2 and 6 y of age IS-pro	↔ bacterial abundances and diversity, except Shannon diversity for Bacteroidetes and Proteobacteria were lower at 2 wk	[65]
Japan Mothers at 34-wk gestation until delivery; infants 0–6 mo	Open trial Probiotic ($n=122$) Control ($n=26$)	Powder in water, milk or formula *B. breve* M16V + *B. longum* BB536 1×10^{9} CFU/strain/d	4 and 10 mo of age V6–V8 16S rRNA gene by 454 pyrosequencing	Limited change in microbiota composition ↑ proportion of Bacteroidetes at 4 mo	[66]
Norway (Trondheim study) Mother at 36-wk gestation until 3 mo postnatal while breastfeeding	Randomized, double-blind placebo-controlled trial Probiotic (20–37) Placebo (17–45)	Fermented milk LGG (5×10^{10} CFU/d) + *L. acidophilus* La-5 (5×10^{10} CFU/d) + BB-12 (5×10^{9} CFU/d)	3 mo and 2 y of age 16S rRNA gene by 454 Illumina MiSeq	↔ alpha and beta diversity and proportions of bacterial classes and genera at age of 3 mo and 2 y	[67]
Finland (Turku) Mothers at 2 mo before delivery until 2 mo after delivery during breastfeeding	Randomized, double-blind placebo-controlled trial LPR + BL999 ($n=28$) ST11 + BL999 ($n=28$) Placebo ($n=22$)	Powder in water *L. rhamnosus* LPR + *B. longum BL999* or *L. paracasei* ST11 + *B. longum* BL999 (10^{9} CFU/strain/d)	6 mo of age FISH, qPCR	↑ Percentage of Lactobacillus/Enterococcus and ↓count of *Bifidobacterium* in LPR + BL999 at 6 mo of age ↓ Colonization rate of *B. infantis* in LPR + BL999 ↓ Colonization rate of *B. longum* in ST11 + BL999	[68]
Finland (Turku) Mothers at 2–4 wk prior to and until delivery; BF mothers or infants 0–6 mo	Randomized, double-blind placebo-controlled trial Probiotic ($n=46$–53) Placebo ($n=47$–52)	Mother: powder in capsules Infants: powder in water LGG 10^{10} CFU/d	6 and 24 mo of age ($n=96$ infants) FISH	↑ count of *C. perfringens/histolyticum* subgroup at 6 mo ↔ numbers of total bacteria *Bifidobacterium*, *Lactobacillus*, and *Bacteroides* at 6 mo ↑ counts of *Lactobacillus* and *C. perfringens/histolyticum* group at 24 mo ↔ numbers of total bacteria *Bifidobacterium* and *Bacteroides* at 24 mo	[69]
New Zealand (Auckland and Wellington) Mothers at 35-wk gestation until 6 mo postpartum if breastfeeding); infants 5 d–2 y	Randomized, double-blind placebo-controlled trial HN001 ($n=285$) HN019 ($n=50$) Placebo ($n=315$)	Powder in capsules *L. rhamnosus* HN001 *B. animalis subsp. lactis* HN019 9×10^{9} CFU/strain/d	0, 3, 12 and 24 mo of age Metagenomic sequencing by Illumina HiSeq 2500	↑ count of *C. perfringens/histolyticum* subgroup at 6 mo ↔ numbers of total bacteria *Bifidobacterium*, *Lactobacillus*, and *Bacteroides* at 6 mo ↑ counts of *Lactobacillus* and *C. perfringens/histolyticum* group at 24 mo ↔ numbers of total bacteria *Bifidobacterium* and *Bacteroides* at 24 mo	[70]

BB-12 Bifidobacterium animalis subsp. *lactis* BB-12, *CFU* colony-forming unit, *CS* cesarean section, *d* day, *FISH* fluorescent in situ hybridization, *LGG Lactobacillus rhamnosus* GG, *IS-pro* interspace profiling, *mo* month, *qPCR* quantitative PCR, *T-RFLP* terminal restriction fragment length polymorphism, *RT-qPCR* reverse transcription quantitative PCR, *VD* vaginally delivered, *y* year, ↑indicates significantly increased, ↓ indicates significantly decreased, ↔ indicates no effect

Table 4 Characteristics of studies investigating probiotic administration on the fecal microbiota of healthy children under 18 years of age

Country of study, age range, duration of intervention	Study design and participants/group	Nutrition base and probiotic strain and amount	Microbiota assessment	Outcomes	Citation
Germany Infants at birth; 12-mo intervention	Randomized, double-blind placebo-controlled trial Probiotic ($n=11$) Control ($n=11$) EBF ($n=9$)	Formula *B. bifidum* BF3 + *B. breve* BR3 + *B. longum* subsp. *infantis* BT1 + *B. longum* BGT (2.5×10^6 CFU/strain/g)	Monthly during intervention 16S rRNA gene by Illumina MiSeq	↔ alpha and beta diversity ↓ relative abundances of OTUs related to *B. fragilis* and *Blautia* over the first y	[71]
Finland (Tartu) Infants 0–2 mo; 6-mo intervention	Randomized, double-blind placebo-controlled trial Probiotic ($n=12$) Control ($n=13$)	Formula LGG 1.0×10^7 CFU/d	Entry and end of the intervention FISH	↔ colonization frequency and counts of *Lactobacillus/Enterococcus*, *Bifidobacterium*, groups of *C. coccoides*, *C. lituseburense* and *C. butyricum*	[72]
Greece (Athens) Infants ≤3 d; 6-mo intervention	Randomized, double-blind placebo-controlled trial Vaginal (V) or C-section (C) delivery V-Control (VCt) ($n=10$) CCt) ($n=10$) VLr ($n=9$) CLr ($n=11$)	Formula *L. reuteri* DSM 17938 1.2×10^9 CFU/L	2 and 4 mo of age 16S rRNA gene by 454-pyrosequencing	Global microbiota of CSCt differed from others at 2 wk, not at 4 mo *Bifidobacterium* occurrence and abundance: CCt < CLr, VCt, VLr at 2 wk Proportion of unclassified *Enterobacteriaceae*: CCt > CLr, VCt, VLr at 2 wk *Lactobacillus* abundance: CLr > CCt; VLr > VCt at both time points	[73]
China (Shanghai) Infants at 0–7 d; 12-mo intervention	Randomized, double-blind placebo-controlled trial Probiotic ($n=135$) Control ($n=129$)	Formula *B. longum* BB536 10^7 CFU/g	2, 4, and 11 mo of age Selective plating	↑ bifidobacteria level at 2 and 4 mo ↔ count of *Enterobacteriaceae* at 2, 4, and 11 mo	[74]
Spain Infants at 1 mo; 5-mo intervention	Randomized, double-blind placebo-controlled trial Probiotic ($n=46$) Control ($n=46$)	Formula + 0.3 g/100 ml GOS *L. fermentum* CECT5716 1×10^7 CFU/g	3 y of age qPCR	↔ fecal counts of *Lactobacillus*, *Bifidobacterium*, *C. coccoides* group and *B. fragilis* group at 3 y of age	[75]
Chile (Santiago) Infants at 1 mo; 13-wk intervention	Randomized, double-blind placebo-controlled trial Probiotic ($n=48$) FOS ($n=44$) Control ($n=61$) BF ($n=46$)	Formula *L. johnsonii* La1 10^8 CFU/g	7 wk of study and 2 wk postintervention Selective plating FISH	↔ counts of *Bifidobacterium*, *Enterobacteria*, *Bacteroides*, *Enterococcus*, *C. perfringens,* and *C. histolyticum* ↑ number of *Lactobacillus* at 7 wk	[76]
Denmark (ProbiComp Study) Infants at 8–13 mo; 6-mo intervention	Randomized, double-blind placebo-controlled trial Probiotic ($n=103$) Control ($n=98$)	Not reported BB-12 + LGG 10^9 CFU/strain/d	Before and postintervention V3 region of 16S rRNA gene by Ion OneTouch and Ion PGM	↔ overall microbiota ↑ proportion of *Lactobacillus*	[77]

Table 4 (continued)

Country of study, age range, duration of intervention	Study design and participants/group	Nutrition base and probiotic strain and amount	Microbiota assessment	Outcomes	Citation
Italy Infants 12–24 mo; 4-wk intervention	Randomized, double-blind placebo-controlled trial Probiotic ($n=13$) Control ($n=13$)	Fermented milk *L. paracasei* A 1.6×10^{10} CFU/d	Before, during (1, 3, 4 wk) and 1 wk after the intervention Selective plating	↑ counts of *Lactobacillus* after 1 wk ↑ numbers of *Bifidobacterium* ↓ clostridia count after 4 wk ↔ the numbers of enterococci, *Bacteroides* and total anaerobes	[78]
USA. (Washington, DC) Children 1–5 y; 10-d intervention	Randomized, double-blind placebo-controlled trial Probiotic ($n=29$) Control ($n=31$)	Yogurt drink BB-12 10^{10} CFU/d	Prior to and on days 10, 30, 60, and 90 following the initiation of intervention V4 region of 16S rRNA gene by Illumina Genome Analyzer II	↔ overall microbiota and proportion of *Bifidobacterium* ↑ proportions *of Prevotella* and *Sutterella*, ↓ *Allobaculum*, *Collinsella*, *Turicibacter*, *Enterococcus* and *Garnulicatella* after 10 d	[79]
Malaysia Children 2–6 y; 10-mo intervention	Randomized, double-blind placebo-controlled trial Probiotic ($n=55$) Control ($n=61$)	Freeze-dried powder *B. longum* BB536 5×10^{9} CFU/d, 5 d/wk	0 and 10 mo of intervention V3–V4 region of 16S rRNA gene by Illumina MiSeq	Overall microbiota differed between 0 and 10 mo in BB536 group, but not in placebo ↑ Proportion of *Faecalibacterium*	[80]
Finland Children 2–7 y; 7-mo intervention	Randomized, double-blind placebo-controlled trial Probiotic ($n=56$) Control ($n=21$)	Milk LGG 4×10^{8} CFU/d	Beginning and end of intervention Phylogenetic microarray (HITChip)	↑relative abundance of *Lactococcus*, *L. gasseri*, *R. lactaris*, uncultured Mollicutes, *P. melaninogenica,* and *P. oralis* ↓ *E. cylindroides*, *C. ramosum*, and *E. coli*	[81]
Italy Children 5.7 ± 2.6 y; 21-d intervention	Observational trial Probiotic ($n=10$)	Oily suspension LGG 4×10^{8} CFU/d	Beginning and end of intervention Selective plating	↓ total coliform	[82]
Japan (Tokyo) Children 4–12 y; 6-mo intervention	Observational trial Probiotic ($n=23$)	*L. casei* Shirota 4×10^{10} CFU/d	Beginning and end of intervention RT-qPCR	↑counts of *Bifidobacterium* and *Lactobacillus* after 3 and 6 mo of intervention ↓ counts of *Enterobacteriaceae* and *Staphylococcus* after 3 and 6 mo of intervention ↓ detection rate of *C. perfringens* after 6 mo of intervention	[83]
Netherlands (Amsterdam) Children 12–18 y; 6-wk intervention	Observational trial Probiotic ($n=6$) Control ($n=12$)	*L. casei* Shirota 6.5×10^{9} CFU/d	Beginning and end of intervention IS-Pro	↔ Shannon index	[84]

BB-12 Bifidobacterium animalis subsp. *lactis* BB-12, *BF* breastfed, *CFU* colony-forming unit, *C* cesarean section, *Ct* control, *d* day, *EBF* exclusive breastfed, *FF* formula-fed, *FISH* fluorescent in situ hybridization, *FOS* fructooligosaccharides, *LGG Lactobacillus rhamnosus* GG, *IS-Pro* interspace profiling, *mo* month, *OTU* operational taxonomic unit, *qPCR* quantitative PCR, *RT-qPCR* reverse transcription quantitative PCR, *V* vaginal delivery, *wk* week, ↑indicates significantly increased, ↓ indicates significantly decreased, ↔ indicates no effect

Key Findings and Implications for Clinicians

- The gut microbiota in infancy and childhood is more readily shaped by nutrition than during adulthood.
- The microbiome of BF infants is nurtured by human milk components, including HMO, and differs from that of FF infants.
- The addition of HMO and prebiotics to infant formula at concentrations found in human milk promotes the growth of bifidobacteria and narrows the differences between BF and FF infants.
- Prebiotics and dietary fiber at doses of 5–20 g/day modify the gut microbiome of children, increase SCFA production, and may exert other health benefits, including increasing calcium absorption.
- Findings on probiotic administration to pregnant or lactating women or directly to the infant or child are inconsistent, likely due to the variation in the bacterial strains, doses, duration and methods of microbiome analysis.
- Better understanding of diet–microbiome–host interactions is needed, but represents an enormous opportunity to refine dietary interventions with the goal of supporting a healthy microbiome and human well-being.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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