# 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 (BF) infants has lower microbial diversity and richness, higher Proteobacteria, lower Bacteroidetes and Firmicutes than those formula-fed (FF). 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, 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.

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## Keywords

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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, 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 life-long 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, 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 intra-partum 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 U.S., *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 composition 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, anti-microbials, 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 increase microbial diversity, but the particular foods most correlated to 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 [28]. 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 [28].

Previously, cessation of breastfeeding, rather than complementary food introduction, was proposed to be the driving force behind the shift towards 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 [28]. 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 of adults [30]. 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 [30]. 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 [31, 32], to more complex assessments of dietary intake, such as dietary patterns [32]. 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-hour recall three days prior to fecal sample collection, influenced fecal microbiota composition [32]. 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* [32]. 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 [32].

**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 [33]. 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. [33].

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 [34]. 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 [34].

Greater *Bifidobacterium* abundance in 1- to 4-year-olds compared to adults has been reported [30], 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 [35]. 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 [35]. 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 [35]. Additionally, a study of 8- to 11-year-olds in Thailand living in two different geographic 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 [36].

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 [37]. 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 [37].

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 [38]. Furthermore, the American children consuming Western diets had higher *Bacteroides* abundance than children in Bangladesh [38]. 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 [40]. Furthermore, the gastrointestinal environment of Egyptian children contained higher levels of SCFAs, microbial polysaccharide degradation-encoding genes, and polysaccharide-degrading genera [40].

Taken together, these findings provide evidence that the microbiome in children and adolescents is shaped to a greater degree by dietary intake [33-38, 40] than by ethnicity [37]. 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 [35, 38, 40], 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) intra-group similarity in the fecal microbiota was greater in children than adults [41]. Adults also displayed greater abundances of *Bacteroides* spp., while children displayed enhanced *Bifidobacterium* spp., *Faecalibacterium* spp., and members of *Lachnospiraceae* [41]. 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 [31-38]. 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 [42-44]. The underlying mechanisms of these beneficial effects are not fully known; however, gut microbiome modulation and formation of SCFAs by bacterial fermentation are proposed [44]. DF is also thought to be beneficial for gut health of children [45], although more studies are needed. In the U.S., the recommended dietary fiber intake is 14g/1,000 kcal or 25g for females and 38g for males. Most Americans only consume about half of the recommended intake (13.5 and 18g,

respectively) [42]. 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 [45]. 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" [44]. DF includes non-digestible carbohydrates naturally occurring in food, isolated from food or synthetized, the latter two requiring evidence to support their physiological benefit to health [46]. Most countries adopted the 2009 Codex [44] definition by inclusion of carbohydrate polymers with degrees of polymerization between 3 and 9 [47]. 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 [48]. 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." [49]. 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 fructooligossacharides (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 [50-52]. A multicenter, randomized, double-blind trial compared the fecal microbiota of healthy infants fed formula with 2′-FL and LNnT from < 14 d- to 6 month-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 month-of-age [52].

### 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 [53] and adolescents (8-15 years) [54-57] (Table 2). As prebiotic fibers, both GOS and inulin-type fructans have been shown to increase abundance of *Bifidobacterium* [53-55]. 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* [54], 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* [55]. This same group also showed that GOS [56] and SCF [57] 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 [55]. Calcium absorption was negatively correlated with *Parabacteroides* relative abundance, but positively correlated with *Clostridium* and unclassified *Clostridiaceae* abundance [55]. 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 [55]. 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" [58]. 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 [59-62], 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 [59-62]. The mechanisms of action of probiotics are not fully understood; however, modulation of gut microbiota has been postulated as one of the mechanisms [63].

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 and then to either the mother and/or infant postpartum [64-71] (Table 3), and the second is to administer the probiotic directly to the infant or child [72-85] (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 [64-66, 70, 71]. The impact of maternal probiotic supplementation on the abundances of bacterial taxa were studied [64-71]; however, the results are inconsistent, even when the same probiotic strain was used [64, 65, 70] (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 weeks 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 [68]. 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 [69]. 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 [66, 68, 71] (Table 3).

Probiotics have been administrated directly to infants and children [72-85] (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 [74]. 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 [74]. 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 [74].

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 [74].

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 [60]. 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 [61].

## 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 [86]. 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 towards 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 non-invasively in pediatric populations using

exfoliated epithelial cells [87]. Exploring host-microbe molecular cross-talk [88] and incorporating other multi-omic approaches, including the fecal metabolome [89] 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 [90, 91]. These "microbiota-directed foods" [91] have the potential to prevent or treat some of the most pressing health nutritional challenges facing the world's population.

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## Biography

## References

1. Li M, Wang M, Donovan SM. Early development of the gut microbiome and immune-mediated childhood disorders. Semin Reprod Med. 2014;32:74–86. [PubMed: 24390924]

2. Wang M, Monaco MH, Donovan SM. Impact of early gut microbiota on immune and metabolic development and function. Semin Fetal Neonatal Med. 2016;21:380–387. [PubMed: 27132110]

3. Milani C, Duranti S, Bottacini F, Casey E, et al. The first microbial colonizers of the human gut: Composition, activities, and health implications of the infant gut microbiota. Microbiol Mol Biol Rev. 2017;81(4). ph: e00036–17. doi: 10.1128/MMBR.00036-17.

4. Davis EC, Wang M, Donovan SM. The role of early life nutrition in the establishment of gastrointestinal microbial composition and function. Gut Microbes. 2017;8:143–171. [PubMed: 28068209]

5. Yatsunenko T, Rey FE, Manary MJ, et al. Human gut microbiome viewed across age and geography. Nature. 2012;486(7402):222–227. [PubMed: 22699611]

6. Derrien M, Alvarez A-S, deVos WM. The gut microbiota in the first decade of life. Trends Microbiol. 2019;27:997–1010. [PubMed: 31474424]

7. Stewart CJ, Ajami NJ, O'Brien JL, Hutchinson DS, Smith DP, Wong MC. Temporal development of the gut microbiome in early childhood from the TEDDY study. Nature. 2018;562:583–588. [PubMed: 30356187]

8. Ho NT, Li F, Lee-Sarwar KA, et al. Meta-analysis of effects of exclusive breastfeeding on infant gut microbiota across populations. Nat Commun. 2018;9:4169. doi: 10.1038/s41467-018-06473-x [PubMed: 30301893]

9. Savage JH, Lee-Sarwar KA, Sordillo JE, et al. Diet during pregnancy and infancy and the infant intestinal microbiome. J Pediatr.2018;203:47–54.e4.doi: 10.1016/j.jpeds.2018.07.066. [PubMed: 30173873]

10. Forbes JD, Azad MB, Vehling L, et al. Association of exposure to formula in the hospital and subsequent infant feeding practices with gut microbiota and risk of overweight in the first year of life. JAMA Pediatr. 2018;172(7):el81161.

11. Stearns JC, Zulyniak MA, de Souza RJ, et al. Ethnic and diet-related differences in the healthy infant microbiome. Genome Med. 2017; 9:32. doi: 10.1186/s13073-017-0421-5. [PubMed: 28356137]

12. Fallani M, Young D, Scott J. Intestinal microbiota of 6-week-old infants across Europe: Geographic influence beyond delivery mode, breast-feeding, and antibiotics. J Pediatr Gastroenterol Nutr. 2010;51:77–84. [PubMed: 20479681]

13. Yang R, Gao R, Cui S, et al. Dynamic signatures of gut microbiota and influences of delivery and feeding modes during the first 6 months of life. Physiol Genomics. 2019;51:368–378. [PubMed: 31226006]

14. Donovan SM, Comstock SS. Human milk oligosaccharides influence neonatal mucosal and systemic immunity. Ann Nutr Metab. 2016;69(Suppl 2): 42–51. [PubMed: 28103609]

15. Moossavi S, Sepehri S, Robertson B. Composition and variation of the human milk microbiota are influenced by maternal and early life factors. Cell Host Microbe. 2019;25:324–335. [PubMed: 30763539]

16. Williams JE, Carrothers JM, Lackey KA, et al. Strong multivariate relations exist among milk, oral, and fecal microbiomes in mother-infant dyads during the first six months postpartum. J Nutr. 2019;149:902–914. [PubMed: 31063198]

17. Hunt KM, Foster JA, Forney LJ. Characterization of the diversity and temporal stability of bacterial communities in human milk. PLoS One. 2011;6(6):e21313. doi: 10.1371/journal.pone.0021313 [PubMed: 21695057]

18. Pacheco AR, Barile D, Underwood MA, Mills DA. The impact of the milk glycobiome on the neonate gut microbiota. Annn Rev Anim Biosci. 2015;3:419–445. [PubMed: 25387230]

19. Sitarik AR, Bobbitt KR, Havstad SL, Fujimura KE, Levin AM, Zoratti EM. Breast milk TGFB is associated with neonatal gut microbial composition. J Pediatr Gastroenterol Nutr. 2017;65(3):e60–e67. doi: 10.1097/MPG.0000000000001585 [PubMed: 28827481]

20. Duranti S, Lugli GA, Mancabelli L. Maternal inheritance of bifidobacterial communities and bifidophages in infants through vertical transmission. Microbiome. 2017;5:66. doi: 10.1111/1462-2920.14705. [PubMed: 28651630]

21. Boix-Amoros A, Puente-Sanchez F, du Toit E. Mycobiome profiles in breast milk from healthy women depend on mode of delivery, geographic location, and interaction with bacteria. Appl Environ Microbiol 2019;85(9). pii: e02994–18. doi: 10.1128/AEM.02994-18. [PubMed: 30824446]

22. Koenig JE, Spor A, Scalfone N. Succession of microbial consortia in the developing infant gut microbiome. Proc Natl Acad Sci U S A. 2011;108(Suppl 1):4578–4585 [PubMed: 20668239]

23. Vallès Y, Artacho A, Pascual-García A. Microbial succession in the gut: directional trends of taxonomic and functional change in a birth cohort of Spanish infants. PLoS Genet. 2014;10(6):el004406. doi: 10.1371/journal.pgen.1004406

24. Laursen MF, Bahl Mf, Michaelsen KF, Licht TR. First foods and gut microbes. Front Microbiol. 2017;8:356. doi: 10.3389/fmicb.2017.00356. [PubMed: 28321211]

25. Thompson AL, Monteagudo-Mera A, Cadenas MB, Lampl ML, Azcarate-Peril MA. Milk- and solid-feeding practices and daycare attendance are associated with differences in bacterial diversity, predominant communities, and metabolic and immune function of the infant gut microbiome. Front Cell Infect Microbiol. 2015;5:3. doi: 10.3389/fcimb.2015.00003. [PubMed: 25705611]

26. Bergstrom A, Skov TH, Bahl MI. Establishment of intestinal microbiota during early life: a longitudinal, explorative study of a large cohort of Danish infants. Appl Environ Microbiol. 2014;80(9):2889–2900. [PubMed: 24584251]

27. Bäckhed F, Roswall J, Peng Y, et al. Dynamics and stabilization of the human gut microbiome during the first year of life. Cell Host Microbe. 2015;17(6): 852. doi: 10.1016/j.chom.2015.05.012 [PubMed: 26308884]

28. De Filippo C, Cavalieri D, Di Paola M, et al. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. Proc Natl Acad Sci USA. 2010;107(33): 14691–14696. [PubMed: 20679230]

29. Fallani M, Amarri S, Uusijarvi A. Determinants of the human infant intestinal microbiota after the introduction of first complementary foods in infant samples from five European centres. Microbiology. 2011;157(Pt 5):1385–1392. [PubMed: 21330436]

30. Ringel-Kulka T, Cheng J, Ringel Y, et al. Intestinal microbiota in healthy U.S. young children and adults – a high throughput microarray analysis. PLoS One 2013;8, e64315. doi: 10.1371/journal.pone.0064315. [PubMed: 23717595]

31. Berding K, Holscher HD, Arthur AE, Donovan SM. Fecal microbiome composition and stability in 4- to 8-year old children is associated with dietary patterns and nutrient intake. J Nutr Biochem. 2018;56:165–174. [PubMed: 29571010]

32. Smith-Brown P, Morrison M, Krause L, Davies PSW. Dairy and plant based food intakes are associated with altered faecal microbiota in 2 to 3 year old Australian children. Sci Rep.2016; 6(1):32385. doi: 10.1038/srep32385 [PubMed: 27694811]

33. Nakayama J, Yamamoto A, Palermo-Conde LA. Impact of Westernized diet on gut microbiota in children on Leyte Island. Front Microbiol. 2017;8.doi: 10.3389/fmicb.2017.00197

34. Kisuse J, La-Ongkham O, Nakphaichit M, et al. Urban diets linked to gut microbiome and metabolome alterations in children: A comparative cross-sectional study in Thailand. Front Microbiol. 2018;9: doi: 10.3389/fmicb.2018.01345

35. Zhong H, Penders J, Shi Z. Impact of early events and lifestyle on the gut microbiota and metabolic phenotypes in young school-age children. Microbiome 2019;7:2. doi: 10.1186/s40168-018-0608-z. [PubMed: 30609941]

36. La-Ongkham O, Nakphaichit M, Leelavatcharamas V, Keawsompong S, Nitisinprasert S. Distinct gut microbiota of healthy children from two different geographic regions of Thailand. Arch Microbiol. 2015;197:561–573. [PubMed: 25644242]

37. Khine WWT, Zhang Y, Goie GJY et al. Gut microbiome of pre-adolescent children of two ethnicities residing in three distant cities. Scientific Report, 2019;9(1):7831. doi: 10.1038/s41598-019-44369-y

38. Lin A, Bik EM, Costello EK, et al. Distinct distal gut microbiome diversity and composition in healthy children from Bangladesh and the United States. PLoS One. 2013;8:e53838, doi: 10.1371/journal.pone.0053838. [PubMed: 23349750]

39. Arumugam M, Raes J, Pelletier E, et al. Enterotypes of the human gut microbiome. Nature 2011;473: 174–180. [PubMed: 21508958]

40. Shankar V, Gouda M, Moncivaiz J, et al. Differences in gut metabolites and microbial composition and functions between Egyptian and U.S. children are consistent with their diets. MSystems. 2017;2(1) pii: e00169–16. doi: 10.1128/msystems.00169-16

41. Hollister EB, Riehle K, Luna RA, et al. Structure and function of the healthy pre-adolescent pediatric gut microbiome. Microbiome 2015;3:36. doi: 10.1186/s40168-015-0101-x. [PubMed: 26306392]

42. Slavin JL. Position of the American Dietetic Association: health implications of dietary fiber. J Am Diet Assoc. 2008;108:1716–1731. [PubMed: 18953766]

43. Mobley AR, Jones JM, Rodriguez J, Slavin J, Zelman KM. Identifying practical solutions to meet America's fiber needs: proceedings from the Food & Fiber Summit. Nutrients. 2014;6:2540–2551. [PubMed: 25006857]

44. Verspreet J, Damen B, Broekaert WF, Verbeke K, Delcour JA, Courtin CM. A critical look at prebiotics within the dietary fiber concept. Annu Rev Food Sci Technol. 2016;7:167–190. [PubMed: 26735801]

45. Korczak R, Kamil A, Fleige L, Donovan SM, Slavin JL. Dietary fiber and digestive health in children. Nutr Rev. 2017;75:241–259. [PubMed: 28586481]

46. Codex Alimentarius Committee. Guidelines on nutrition labelling CAC/GL 2-1985 as last amended 2010 Joint FAO/WHO Food Standards Programme, Secretariat of the Codex Alimentarius Commission. Rome, Italy: FAO 2010.

47. Jones JM. CODEX-aligned dietary fiber definitions help to bridge the 'fiber gap'. Nutr J. 2014;13:34. [PubMed: 24725724]

48. Holscher HD. Dietary fiber and prebiotics and the gastrointestinal microbiota. Gut Microbes 2017;8172–184.

49. Gibson GR, Hutkins R, Sanders ME, et al. Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. Nat Rev Gastroenterol Hepatol. 2017;14(8):491–502. [PubMed: 28611480]

50. Puccio G, Alliet P, Cajozzo C, et al. Effects of infant formula with human milk oligosaccharides on growth and morbidity: a randomized multicenter trial. J Pediatr Gastroenterol Nutr. 2017;64(4):624–631. [PubMed: 28107288]

51. Reverri EJ, Devitt AA, Kajzer JA, Baggs GE, Borschel MW. Review of the clinical experiences of feeding infants formula containing the human milk oligosaccharide 2'-fucosyllactose. Nutrients. 2018;10(10). pii: E1346. doi: 10.3390/nu10101346. [PubMed: 30241407]

52. Vandenplas Y, Berger B, Carnielli VP, et al. Human milk oligosaccharides: 2'-fucosyllactose (2'-FL) and lacto-N-neotetraose (LNnT) in infant formula. Nutrients. 2018;10(9). pii: E1161. doi: 10.3390/nu10091161. [PubMed: 30149573]

53. Lohner S, Jakobik V, Mihályi K, et al. Inulin-type fructan supplementation of 3- to 6-year-old children is associated with higher fecal *Bifidobacterium* concentrations and fewer febrile episodes requiring medical attention. J Nutr. 2018;148:1300–1308. [PubMed: 29982534]

54. François IE, Lescroart O, Veraverbeke WS, et al. Effects of wheat bran extract containing arabinoxylan oligosaccharides on gastrointestinal parameters in healthy preadolescent children. J Pediatr Gastroenterol Nutr. 2014;58:647–653. [PubMed: 24368315]

55. Whisner CM, Martin BR, Nakatsu CH, et al. Soluble corn fiber increases calcium absorption associated with shifts in the gut microbiome: A randomized dose-response trial in free-living pubertal females. J Nutr. 2016;1461298–1306.

56. Whisner CM, Martin BR, Schoterman MH, et al. Galacto-oligosaccharides increase calcium absorption and gut bifidobacteria in young girls: a double-blind cross-over trial. Br J Nutr. 2013;110:1292–303. [PubMed: 23507173]

57. Whisner CM, Martin BR, Nakatsu CH, et al. Soluble maize fibre affects short-term calcium absorption in adolescent boys and girls: a randomised controlled trial using dual stable isotopic tracers. Br J Nutr. 2014;112:446–456. [PubMed: 24848974]

58. Hill C, Guarner F, Reid G, et al. Expert consensus document. The international scientific association for probiotics and prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol. 2014;11:506–514. [PubMed: 24912386]

59. Cuello-Garcia C, Brozek JL, Fiocchi A, et al. Probiotics for the prevention of allergies: A systematic review and meta-analysis of randomized controlled trials. J Allergy Clin Immunol. 2015; 36:952–961.

60. Szajewska H What are the indications for using probiotics in children? Arch Dis Child. 2016;101:398–403. [PubMed: 26347386]

61. Szajewska H Short- and long-term effects of probiotics administered early in life. Nestle Nutr Workshop Ser Pediatr Program. 2011;68:65–78.

62. Sansotta N, Peroni DG, Romano S, et al. The good bugs: the use of probiotics in pediatrics. Curr Opin Pediatr. 2019;31:661–669. [PubMed: 31356353]

63. Suez J, Zmora N, Segal E, Elinav E. The pros, cons, and many unknowns of probiotics. Nat Med. 2019;25:716–729. [PubMed: 31061539]

64. Lahtinen SJ, Boyle RJ, Kivivuori S, et al. Prenatal probiotic administration can influence Bifidobacterium microbiota development in infants at high risk of allergy. J Allergy Clin Immunol. 2009;123:499–501. [PubMed: 19135234]

65. Rinne M, Kalliomaki M, Arvilommi H, Salminen S, Isolauri E. Effect of probiotics and breastfeeding on the bifidobacterium and lactobacillus/enterococcus microbiota and humoral immune responses. J Pediatr. 2005;147:186–191. [PubMed: 16126047]

66. Rutten NB, Gorissen DM, Eck A, et al. Long term development of gut microbiota composition in atopic children: impact of probiotics. PLoS One. 2015;10(9):e0137681. doi: 10.1371/journal.pone.0137681 [PubMed: 26378926]

67. Enomoto T, Sowa M, Nishimori K, et al. Effects of bifidobacterial supplementation to pregnant women and infants in the prevention of allergy development in infants and on fecal microbiota. Allergol Int. 2014;63:575–85. [PubMed: 25056226]

68. Dotterud CK, Avershina E, Sekelja M, et al. Does maternal perinatal probiotic supplementation alter the intestinal microbiota of mother and child? J Pediatr Gastroenterol Nutr. 2015;61:200–207. [PubMed: 25782657]

69. Grze kowiak Ł, Grönlund MM, Beckmann C, Salminen S, von Berg A, Isolauri E. The impact of perinatal probiotic intervention on gut microbiota: double-blind placebo-controlled trials in Finland and Germany. Anaerobe. 2012;18:7–13. [PubMed: 21979491]

70. Rinne M, Kalliomäki M, Salminen S, Isolauri E. Probiotic intervention in the first months of life: short-term effects on gastrointestinal symptoms and long-term effects on gut microbiota. J Pediatr Gastroenterol Nutr. 2006;43:200–5. [PubMed: 16877985]

71. Murphy R, Morgan XC, Wang XY, et al. Eczema-protective probiotic alters infant gut microbiome functional capacity but not composition: sub-sample analysis from a RCT. Benef Microbes. 2019;10:5–17. [PubMed: 30574802]

72. Bazanella M, Maier TV, Clavel T, et al. Randomized controlled trial on the impact of early-life intervention with bifidobacteria on the healthy infant fecal microbiota and metabolome. Am J Clin Nutr. 2017;106:1274–1286. [PubMed: 28877893]

73. Vendt N, Grünberg H, Tuure T, et al. Growth during the first 6 months of life in infants using formula enriched with *Lactobacillus rhamnosus* GG: double-blind, randomized trial. J Hum Nutr Diet. 2006; 19(1):51–58. [PubMed: 16448475]

74. Garcia Rodenas CL, Lepage M, Ngom-Bru C, Fotiou A, Papagaroufalis K, Berger B. Effect of formula containing *Lactobacillus reuteri* DSM 17938 on fecal microbiota of infants born by cesarean-section. J Pediatr Gastroenterol Nutr. 2016;63:681–687. [PubMed: 27035371]

75. Wu BB, Yang Y, Xu X, Wang WP. Effects of *Bifidobacterium* supplementation on intestinal microbiota composition and the immune response in healthy infants. World J Pediatr. 2016;12:177–182. [PubMed: 25846071]

76. Maldonado-Lobón JA, Gil-Campos M, Maldonado J, et al. Long-term safety of early consumption of *Lactobacillus fermentum* CECT5716: A 3-year follow-up of a randomized controlled trial. Pharmacol Res. 2015;95-96:12–9. [PubMed: 25697549]

77. Brunser O, Figueroa G, Gotteland M, et al. Effects of probiotic or prebiotic supplemented milk formulas on fecal microbiota composition of infants. Asia Pac J Clin Nutr. 2006;15:368–376. [PubMed: 16837430]

78. Laursen MF, Laursen RP, Larnkjær A, Michaelsen KF, Bahl MI, Licht TR. Administration of two probiotic strains during early childhood does not affect the endogenous gut microbiota composition despite probiotic proliferation. BMC Microbiol. 2017;17(1):175. doi: 10.1186/s12866-017-1090-7 [PubMed: 28818050]

79. Marzotto Ml, Maffeis C, Paternoster T, et al. *Lactobacillus paracasei* A survives gastrointestinal passage and affects the fecal microbiota of healthy infants. Res Microbiol. 2006;157:857–866. [PubMed: 16934438]

80. Tan TP, Ba Z, Sanders ME, et al. Safety of *Bifidobacterium animalis* subsp. lactis (B. lactis) strain BB-12 supplemented yogurt in healthy children. J Pediatr Gastroenterol Nutr. 2017;64:302–309. [PubMed: 28114246]

81. Lau AS, Yanagisawa N, Hor YY, et al. *Bifidobacterium longum* BB536 alleviated upper respiratory illnesses and modulated gut microbiota profiles in Malaysian pre-school children. Benef Microbes. 2018;9:61–70. [PubMed: 29065707]

82. Korpela K, Salonen A, Virta LJ, Kumpu M, Kekkonen RA, de Vos WM. *Lactobacillus rhamnosus* GG intake modifies preschool children's intestinal microbiota, alleviates penicillin-associated changes, and reduces antibiotic use. PLoS One. 2016;ll(4):e0154012. doi: 10.1371/journal.pone.0154012

83. Mogna L, Del Piano M, Mogna G. Capability of the two microorganisms *Bifidobacterium breve* B632 and *Bifidobacterium breve* BR03 to colonize the intestinal microbiota of children. J Clin Gastroenterol. 2014;48(Suppl 1): S37–39. [PubMed: 25291125]

84. Wang C, Nagata S, Asahara T, et al. Intestinal microbiota profiles of healthy pre-school and school-age children and effects of probiotic supplementation. Ann Nutr Metab. 2015;67:257–266. [PubMed: 26496372]

85. El Manouni El Hassani S, de Boer NKH, et al. Effect of daily intake of *Lactobacillus casei* on microbial diversity and dynamics in a healthy pediatric population. Curr Microbiol. 2019;76:1020–1027. [PubMed: 31187207]

86. Power SE, O'Toole PW, Stanton C, Ross RP, Fitzgerald FG. Intestinal microbiota, diet and health. Br J Nutr. 2014;111:3870403

87. Chapkin RS, Zhao C, Ivanov I, et al. Non-invasive stool-based detection of infant gastrointestinal development using gene expression profiles from exfoliated epithelial cells. Am J Physiol Gastrointest Liver Physiol. 2010;298:G582–589. [PubMed: 20203060]

88. Schwartz S, Friedberg I, Ivanov I, et al. A metagenomic study of diet-dependent interaction between gut microbiota and host in infants reveals differences in developmental and immune responses. Genome Biol. 2012;13:R32. doi: 10.1186/gb-2012-13-4-r32. [PubMed: 22546241]

89. Chilloux J, Neves AL, Boulangé CL, Dumas M-E. The microbial-mammalian metabolic axis: A critical symbiotic relationship. Curr Opin Clin Nutr Metab Care. 2016;19:250–256 [PubMed: 27137897]

90. Zhang N, Ju Z, Zuo T. Time for food: The impact of diet on gut microbiota and human health. Nutrition 2018;52–52:800–885.

91. Barratt MJ, Lebrilla C, Shapiro H-Y, Gordon JI. The gut microbiota, food science and human nutrition; a timely marriage. Cell Host Microbe. 2017; 22:134–141. [PubMed: 28799899]

**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/d 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 are needed, but represent an enormous opportunity to refine dietary interventions with the goal of supporting a healthy microbiome and human wellbeing.

**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 | Citation |
|---|---|---|---|---|---|
| Sweden 0-1 y N=98 | Cross-sectional | • Feeding practices questionnaires assessing<br>• BF, FF, MF<br>• BF cessation | Metagenomic shotgun sequencing by Illumina HiSeq 2000 | • BF infants predominate in *Bifidobacterium, Lactobacillus, Collinsella, Megasphaera,* and *Veillonella*<br>• BF cessation increased *Bacteroides, Bilophila, Roseburia, Clostridium,* and *Anaerostipes*<br>• Newborn and 4 mo microbiota enriched in genes for HMO degradation<br>• 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. 36 mos. visits | Targeted qPCR analysis | • Weaning decreased *Bifidobacterium, Lactobacillus,* and increased *Enterobacteriaceae, Clostridium* spp. and *Bacteroides* spp. | 26 |
| U.S. (North Carolina) 1-4y 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 & qPCR | • Children had less diverse microbiota than adults<br>• Actinobacteria, Bacilli, Clostridium cluster IV (*Ruminococcaceae*), and Bacteroidetes were higher in children than adults | 30 |
| Italy and Burkina Faso 1-6 y N=29 | Cross-sectional | • Italian parents completed a detailed medical, diet, and lifestyle survey<br>• 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 ~6-fold higher in Italian than Burkina Faso children<br>• 3 genera involved in utilization plant polysaccharides *(Prevotella, Xylanibacter* (*Bacteroidetes*), and *Treponema* (*Spirochaetes*) were higher in Burkina Faso children. | 28 |
| Australia 2-3 y N=37 | Cross-sectional | • Australian Child and Adolescent Eating Survey (FFQ)<br>• 24 hr 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 | 32 |

| Country of Study, Age Range and Number of Participants | Study Design | Method of Diet Assessment | | Method of Microbiota Assessment | Outcomes | | Citation |
|---|---|---|---|---|---|---|---|
| | | | | | • | Vegetable protein intake positively associated with *Lachnospira* | |
| | | | | | • | Soy, pulse, and nut positively associated with *Bacteroides xylanisolvens* | |
| | | | | | • | Fruit intake negatively associated with *Ruminococcus gnavus* | |
| U.S. (Illinois) 4-8 y N=22 | Cross-sectional | • | Nutrient intake assessed by 3-day food diaries. | V3-V4 16S rRNA by Illumina MiSeq | • | 2 dietary patterns were associated with microbial taxa and composition | 31 |
| | | • | Youth and Adolescent (YAQ) FFQ was used for dietary patterns | | • | 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* | |
| 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 pyro-sequencing | • | 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*) | 33 |
| 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* | 34 |
| | | | | | • | Buriram children had more *Clostridiales*, *Peptostreptococcaceae* and unclassified *Ruminococcaceae* and higher butyrate and propionate | |
| 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 | 35 |

| Country of Study, Age Range and Number of Participants | Study Design | Method of Diet Assessment | Method of Microbiota Assessment | Outcomes | Citation |
|---|---|---|---|---|---|
| | | | | • Negative correlation between high dietary fiber consumption and low plasma insulin levels in children with *Bacteroides* and *Prevotella* enterotypes, but not *Bifidobacterium* enterotype | |
| 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 | 36 |
| 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<br><br>• *Bifidobacterium*, and *Collinsella* positively correlated with refined-sugar enriched foods; *Collinsella* positively associated with fruits and curry foods | 37 |
| Bangladesh 8-13 y N=10 U.S. 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*<br><br>• Bangladeshi children consumed non-Western diet low in refined-sugar enriched foods and meat and high in rice, bread, and lentils | 38 |
| Egypt (Giza) 13.3-14.5 y N=28 U.S. (Ohio) 10.1-15.7 y N=14 | Cross-sectional | Not reported | V4 16S rRNA by Iliumina MiSeq | • Egyptian consumed Mediterranean-type diet & American children consumed a Western diet<br><br>• Egyptian children had *Prevotella* enterotype and American children had *Bacteroides* enterotype<br><br>• Egyptian children had higher fecal SCFAs, microbial polysaccharide degradation-encoding genes, and polysaccharide-degrading genera | 40 |

Abbreviations: F:B, Firmicutes to Bacteroidetes ratio; BF, breastfed; FF, formula-fed; FFQ, food frequency questionnaire; MF, mixed-fed; SCFA, short chain fatty acids; U.S., United States

**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, Duration of Intervention | Study Design and Participants/Group | Nutrition Base and Fiber Type and Amount | Microbiota Assessment | Outcomes | Citation |
|---|---|---|---|---|---|
| Hungary (Pécs) 3-6 y 24 wk | • Randomized, double-blind, placebo-controlled trial<br>• Prebiotic (n=110)<br>• Placebo (n=109) | • Mixed with food or drink<br>• Inulin type fructans (6 g/d) | • Baseline and week 24<br>• qPCR | • ↑ relative abundances of *Bifidobacterium* and *Lactobacillus*<br>• ↔ numbers of total bacteria, relative abundances of *C. perfringens*, *C. difficile* and *Enterobacteriaceae*<br>• ↔ fecal pH and stool consistency | 53 |
| Belgium (Leuven) 8-12 y 3 wk | • Randomized, double-blind, placebo-controlled crossover trial with 2 wk washout<br>• Wheat bran extract and control (n=29) | • Soft drink<br>• Wheat bran extract containing arabinoxylan-oligosaccharides (5 g/d) | • Baseline and d19 or 20<br>• FISH | • ↑ *Bifidobacterium* level<br>• ↔ counts of *Lactobacillus/ Enterococcus*, *C. histolyticum/C. liteseburense*, *R. rectale/E. rectela* groups, and *F. prausnitzii*<br>• ↔ fecal pH, each SCFA levels, percentage of moisture and stool frequency | 54 |
| U.S. (Indiana) 10-13 y 3 wk | • Randomized, double-blind cross-over trial with 2 wk washout<br>• GOS 5g, GOS 10g and control (n=20) | • Smoothie drinks<br>• GOS (5 or 10 g/d) | • Baseline and the end of each treatment<br>• DGGE, qPCR | • ↔ numbers of DGGE bands<br>• Change in *Bifidobacterium* counts: GOS 5g > GOS 10g, control<br>• ↔ bowel movement frequency and stool consistency | 55 |
| U.S. (Indiana) 12-15 y 3 wk | • Randomized double–blind cross-over trial with 7 d washout<br>• SCF and control (n=23) | • Spaghetti, hamburgers, sandwiches and potato chips<br>• Soluble maize fiber (12 g/d) | • Baseline and the end of each treatment<br>• V3-V5 16S rRNA gene by 454 pyrosequencing | • ↑ proportions of *Parabacteroides*, other Clostridiales and other *Ruminococcaceae*<br>• ↓ *Enterococcus*, *Anaerofustis*, *Coprococcus* and other *Peptostreptococcaseae* | 56 |
| U.S. (Indiana) 12-15 y 4 wk | • Randomized double–blind cross-over trial with 3-4 wk washout | • Muffin and beverage | • Before and after each intervention<br>• V3-V4 16S rRNA gene by | • ↑ Chao 1 and observed OTUs at species level<br>• Overall microbiota differed between | 57 |

| Country of Study, Age Range, Duration of Intervention | Study Design and Participants/Group | Nutrition Base and Fiber Type and Amount | Microbiota Assessment | Outcomes | Citation |
|---|---|---|---|---|---|
| | • SCF (10 g), SCF (20 g) and control (n=27) | | 454 pyrosequencing | samples with and without SCF<br><br>• SCF 10g ↑*P arabacteroides* and unclassified *Lachnospiraceae,* ↓ reclassified *Ruminococcus*<br><br>• SCF 20g ↑*Parabacteroides* and unclassified *Lachnospiraceae,* ↓ *Bacteroides* and *Lachnospira*<br><br>• Fecal pH: SCF 20g < control. SCF 10g<br><br>• ↔ SCFA concentrations | |

Abbreviations: 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; ↓ indicated significantly decreased; ↔ indicates no effect

**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<br>• Probiotic (n=59)<br>• Placebo (n=57) | • Powder in capsules<br>• LGG<br>• $1.8\times10^{10}$ CFU/d | • Infant at 3, 7, 28 and 90 d of age<br>• qPCR, T-RFLP | • ↑ prevalence of species belonging to *B. longum* group at 90 d | 64 |
| Finland (Turku) Mothers at 36 wk gestation until delivery; infants 0-6 mo | • Randomized, double-blind placebo controlled trial<br>• Probiotic (n=77)<br>• Placebo (n=82) | • Powder in water<br>• LGG<br>• $1.0\times10^{10}$ CFU/d | • 3, 6 and 12 mo of age (n=96 infants)<br>• Fish | • ↔ counts of total bacteria *Bifidobacterium* and *Lactobacillus/ Enterococcus* at 3, 6 and 12 mo | 65 |
| Netherlands Mothers at 6 wk before delivery until delivery; infants at 0-1 y | • Randomized, double-blind placebo controlled trial<br>• Probiotic (n=20-37)<br>• Placebo (n=17-45) | • Powder in water, milk or formula<br>• *B. bifidum* W23 + *B. lactis* W52 + *L. lactis* W58<br>• $1\times10^{9}$ CFU/ strain/d | • 1 and 2 wk, 1, 3, 12, and 18 mo, 2 and 6 y of age<br>• IS-pro | • ↔ bacterial abundances and diversity, except Shannon diversity for Bacteriodetes and Proteobacteria were lower at 2wk | 66 |
| Japan Mothers at 34 wk gestation until delivery; infants 0-6 mo | • Open trial<br>• Probiotic (n=122)<br>• Control (n=26) | • Powder in water, milk or formula<br>• *B. breve* M16V + *B. longum* BB536<br>• $1\times10^{9}$ CFU/ strain/d | • 4 and 10 mo of age<br>• V6-V8 16S rRNA gene by 454 pyrosequencing | • Limited change in microbiota composition<br>• ↑ proportion of Bacteroidetes at 4 mo | 67 |
| Norway (Trondheim study) Mother at 36 wk gestation until 3 mo postnatal while breastfeeding | • Randomized, double-blind placebo controlled trial<br>• Probiotic (20-37)<br>• Placebo (17-45) | • Fermented milk<br>• LGG ($5\times10^{10}$ CFU/d) + *L. acidophilus* La-5 ($5\times10^{10}$ CFU/d) + BB-12 | • 3 mo and 2 y of age<br>• 16S rRNA gene by 454 Illumina MiSeq | • ↔ alpha- and beta-diversity and proportions of bacterial classes and genera at age of 3 mo and 2y | 68 |

| Country of Study, Age Range, Duration of Intervention | Study Design and Participants/Group | Nutrition Base and Probiotic Strain and Amount | Microbiota Assessment | Outcomes | Citation |
|---|---|---|---|---|---|
| | | $(5\times10^9$ CFU/d) | | | |
| Finland (Turku) Mothers at 2 mo before delivery until 2 mo after delivery during breastfeeding | • Randomized, double-blind placebo controlled trial<br><br>• LPR+BL999 (n=28)<br><br>• ST11+BL999 (n=28)<br><br>• Placebo (n=22) | • Powder in water<br><br>• *L. rhamnosus* LPR + *B. longum BL999* or<br><br>• *L. paracasei* ST11 + *B. longum* BL999 ($10^9$CFU/ strain/d) | • 6 mo of age<br><br>• FISH, qPCR | • ↑ Percentage of Lactobacillus/ Enterococcus and ↓count of *Bifidobacterium* in LPR+BL999 at 6 mo of age<br><br>• ↓ Colonization rate of *B. infantis* in LPR +BL999<br><br>• ↓ Colonization rate of *B. longum* in ST11 + BL999 | 69 |
| 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<br><br>• Probiotic (n=46-53)<br><br>• Placebo (n=47-52) | • Mother: powder in capsules<br><br>• Infants: powder in water<br><br>• LGG<br><br>• $10^{10}$CFU/d | • 6 and 24 mo of age (n=96 infants)<br><br>• FISH | • ↑ count of *C. perfringens/ histolyticum* subgroup at 6 mo<br><br>• ↔ numbers of total bacteria *Bifidobacterium*, *Lactobacillus*, and *Bacteroides* at 6 mo<br><br>• ↑ counts of *Lactobacillus* and *C. perfringens/ histolyticum* group at 24 mo<br><br>• ↔ numbers of total bacteria *Bifidobacterium* and *Bacteroides* at 24 mo | 70 |
| New Zealand (Auckland and Wellington) Mothers at 35 wk gestation until 6 mo postpartum if breastfeeding); infants 5d – 2 y | • Randomized, double-blind placebo controlled trial<br><br>• HN001 (n=285)<br><br>• HN019 (n=50)<br><br>• Placebo (n=315) | • Powder in capsules<br><br>• *L. rhamnosus* HN001<br><br>• *B. animalis subsp. lactis* HN019<br><br>• $9\times10^9$ CFU/ strain/d | • 0, 3, 12 and 24 mo of age<br><br>• Metagenomic sequencing by Illumina HiSeq2500 | • ↑ count of *C. perfringens/ histolyticum* subgroup at 6 mo<br><br>• ↔ numbers of total bacteria *Bifidobacterium*, *Lactobacillus*, and *Bacteroides* at 6 mo<br><br>• ↑ counts of *Lactobacillus* and *C. perfringens/ histolyticum* group at 24 mo<br><br>• ↔ numbers of total bacteria *Bifidobacterium* | 71 |

| Country of Study, Age Range, Duration of Intervention | Study Design and Participants/Group | Nutrition Base and Probiotic Strain and Amount | Microbiota Assessment | Outcomes | Citation |
|---|---|---|---|---|---|
| | | | | and *Bacteroides* at 24 mo | |

Abbreviations: 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, vaginal delivered; y, year; ↑indicates significantly increased; ↓ indicated 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<br>• Probiotic (n=11)<br>• Control (n=11)<br>• EBF (n=9) | • Formula<br>• *B. bifidum* BF3 + *B. breve* BR3 + *B. longum* subsp. *infantis* BT1 + *B. longum* BGT<br>• ($2.5 \times 10^6$ CFU/strain/g) | • Monthly during intervention<br>• 16S rRNA gene by Illumina MiSeq | • ↔ alpha- and beta-diversity<br>• ↓ relative abundances of OTUs related to *B. fragilis* and *Blautia* over the first y. | 72 |
| Finland (Tartu) Infants 0-2 mo; 6 mo intervention | • Randomized, double-blind placebo controlled trial<br>• Probiotic (n= 12)<br>• Control (n=13) | • Formula<br>• LGG<br>• $1.0 \times 10^7$ CFU/d | • Entry and end of the intervention<br>• FISH | • ↔ colonization frequency and counts of *Lactobacillus/ Enterococcus, Bifidobacterium*, groups of *C. coccoides, C. lituseburense* and *C. butyricum* | 73 |
| Greece (Athens) Infants 3d; 6 mo intervention | • Randomized, double-blind placebo controlled trial<br>• Vaginal (V) or C-section (C) delivery<br>• V-Control (VCt) (n=10)<br>• CCt) (n=10)<br>• VLr (n-9)<br>• CLr (n=11) | • Formula<br>• *L. reuteri* DSM 17938<br>• $1.2 \times 10^9$ CFU/L | • 2 and 4 mo of age<br>• 16S rRNA gene by 454-pyrosequencing | • Global microbiota of CSCt differed from others at 2 wk, not at 4 mo<br>• *Bifidobacterium* occurrence and abundance: CCt < CLr, VCt, VLr at 2 wk<br>• Proportion of unclassified *Enterobacteriaceae*: CCt > CLr, VCt, VLr at 2 wk<br>• *Lactobacillus* abundance: CLr > CCt; VLr > VCt at both time points | 74 |
| China (Shanghai) Infants at 0-7 d; 12 mo intervention | • Randomized, double-blind placebo controlled trial<br>• Probiotic (n=135)<br>• Control (n=129) | • Formula<br>• *B. longum* BB536<br>• $10^7$ CFU/g | • 2, 4 and 11 mo of age<br>• Selective plating | • ↑ bifidobacteria level at 2 and 4 mo.<br>• ↔ count of *Enterobacteriaceae* at 2, 4 and 11 mo | 75 |

| Country of Study, Age Range, Duration of Intervention | Study Design and Participants/Group | Nutrition Base and Probiotic Strain and Amount | Microbiota Assessment | Outcomes | Citation |
|---|---|---|---|---|---|
| Spain Infants at 1 mo; 5 mo intervention | • Randomized, double-blind placebo controlled trial<br><br>• Probiotic (n=46)<br><br>• Control (n=46) | • Formula + 0.3 g/100ml GOS<br><br>• *L. fermentum* CECT5716<br><br>• 1x10$^7$ CFU/g | • 3 y of age<br><br>• qPCR | • ↔ fecal counts of *Lactobacillus, Bifidobacterium, C. coccoides* group and *B. fragilis* group at 3 y of age | 76 |
| Chile (Santiago) Infants at 1 mo; 13 wk intervention | • Randomized, double-blind placebo controlled trial<br><br>• Probiotic (n=48)<br><br>• FOS (n=44)<br><br>• Control (n=61)<br><br>• BF (n=46) | • Formula<br><br>• *L. johnsonii* La1<br><br>• 10$^8$ CFU/g | • 7 wk of study and 2 wk post-intervention<br><br>• Selective plating<br><br>• FISH | • ↔ counts of *Bifidobacterium, Enterobacteria, Bacteroides, Enterococcus, C. perfringens* and *C. histolyticum*<br><br>• ↑ number of *Lactobacillus* at 7 wk | 77 |
| Denmark (ProbiComp Study) Infants at 8–13 mo; 6 mo intervention | • Randomized, double-blind placebo controlled trial<br><br>• Probiotic (n= 103)<br><br>• Control (n=98) | • Not reported<br><br>• BB-12 + LGG<br><br>• 10$^9$ CFU/ strain/d | • Before and post-intervention<br><br>• V3 region of 16S rRNA gene by Ion OneTouch and Ion PGM | • ↔ overall microbiota<br><br>↑ proportion of *Lactobacillus* | 78 |
| Italy Infants 12-24 mo; 4 wk intervention | • Randomized, double-blind placebo controlled trial<br><br>• Probiotic (n=13)<br><br>• Control (n=13) | • Fermented milk<br><br>• *L. paracasei* A<br><br>• $1.6 \times 10^{10}$ CFU/d | • Before, during (1,3, 4 wk) and 1 wk after the intervention<br><br>• Selective plating | • ↑ counts of *Lactobacillus* after 1 wk<br><br>• ↑ numbers of *Bifidobacterium*<br><br>• ↓ clostridia count after 4 wk<br><br>• ↔ the numbers of enterococci, *Bacteroides* and total anaerobes | 79 |
| U.S.A. (Washington, DC) Children 1-5 y; 10 d intervention | • Randomized, double-blind placebo controlled trial<br><br>• Probiotic (n=29)<br><br>• Control (n=31) | • Yogurt drink<br><br>• BB-12<br><br>• 10$^{10}$ CFU/d | • Prior to and on days 10, 30, 60 and 90 following the initiation of intervention<br><br>• V4 region of 16S rRNA gene by Illumina Genome Analyzer II | • ↔ overall microbiota and proportion of *Bifidobacterium*<br><br>• ↑ proportions *of Prevotella* and *Sutterella,* ↓ *Allobaculum, Collinsella, Turicibacter, Enterococcus* and | 80 |

| Country of Study, Age Range, Duration of Intervention | Study Design and Participants/Group | Nutrition Base and Probiotic Strain and Amount | Microbiota Assessment | Outcomes | Citation |
|---|---|---|---|---|---|
| | | | | *Garnulicatella* after 10 d | |
| Malaysia Children 2-6 y; 10 mos intervention | • Randomized, double-blind placebo controlled trial<br>• Probiotic (n=55)<br>• Control (n=61) | • Freeze-dried powder<br>• *B. longum* BB536<br>• $5 \times 10^9$ CFU/d, 5 d/wk | • 0 and 10 mo of intervention<br>• 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<br>• ↑ Proportion of *Faecalibacterium* | 81 |
| Finland Children 2-7 y; 7 mos intervention | • Randomized, double-blind placebo controlled trial<br>• Probiotic (n=56)<br>• Control (n=21) | • Milk<br>• LGG<br>• $4 \times 10^8$ CFU/d | • Beginning and end of intervention<br>• Phylogenetic microarray (HITChip) | • ↑relative abundance of *Lactococcus, L. gasseri, R. lactaris,* uncultured Mollicutes, *P. melaninogenica* and *P. oralis*<br>• ↓ *E. cylindroides, C. ramosum,* and *E. coli* | 82 |
| Italy Children 5.7 ± 2.6 y; 21 d intervention | • Observational trial<br>• Probiotic (n=10) | • Oily suspension<br>• LGG<br>• $4 \times 10^8$ CFU/d | • Beginning and end of intervention<br>• Selective plating | • ↓ total coliform | 83 |
| Japan (Tokyo) Children 4-12 y; 6 mo intervention | • Observational trial<br>• Probiotic (n=23) | • *L. casei* Shirota<br>• $4 \times 10^{10}$ CFU/d | • Beginning and end of intervention<br>• RT-qPCR | • ↑counts of *Bifidobacterium* and *Lactobacillus* after 3 and 6 mo of intervention<br>• ↓ counts of *Enterobacteriaceae* and *Staphylococcus* after 3 and 6 mo of intervention<br>• ↓ detection rate of *C. perfringens* after 6 mo of intervention | 84 |
| Netherlands (Amsterdam) Children 12-18 y; 6 wk intervention | • Observational trial<br>• Probiotic (n=6)<br>• Control (n=12) | • *L. casei* Shirota<br>• $6.5 \times 10^9$ CFU/d | • Beginning and end of intervention<br>• IS-Pro | • ↔ Shannon index | 85 |

Abbreviations: BB-12, *Bifidobacterium animalis* subsp. *lactis* BB-12; BF, breast-fed; CFU, colony-forming unit; C, cesarean section; Ct, control; d, day; EBF, exclusive breast-fed; FF, formula-fed; FISH, fluorescent in situ hybridization; FOS, fructooligossacharides; 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; ↓ indicated significantly decreased; ↔ indicates no effect