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Gut Microbiota Development: Influence of Diet from Infancy to Toddlerhood

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Key Messages

- The gut microbiota develops drastically during the first year of life and is influenced by a range of external factors, with diet being a major player.
- Breastfeeding versus formula feeding strongly affects gut microbiota composition and metabolism during early infancy. Breastfeeding promotes the dominance of specific human milk oligosaccharide-degrading *Bifidobacterium* species in the gut, which may contribute to protection against infectious and immune-related diseases. Formula feeding results in a more diverse gut microbiota with a higher prevalence of opportunistic pathogenic bacterial taxa and proteolytic metabolism in the gut.
- Complementary feeding and the increasing dietary fiber and protein intake induces a shift from the milk-adapted gut microbiota and metabolism toward one with vastly increased diversity and proportions of the fiber-degrading bacterial families *Lachnospiraceae*, *Ruminococcaceae,* and *Bacteroidaceae,* and their major metabolic end products short chain fatty acids. Inadequate maturation of the gut microbiota during complementary feeding is associated with poor growth and development in early life.

Keywords

Gut microbiota · Diet · Breastfeeding · Complementary feeding

Abstract

Early life is a critical period as our gut microbiota establishes here and may impact both current and future health. Thus, it is of importance to understand how different factors govern the complex microbial colonization patterns in this period. The gut microbiota changes substantially during infancy and toddlerhood in terms of both taxonomic composition and diversity. This developmental trajectory differs by a variety of factors, including term of birth, mode of birth, intake of antibiotics, presence of furred pets, siblings and family members, host genetics, local environment, geographical location, and maternal and infant/toddler diet. The type of milk feeding and complementary feeding is particularly important in early and late infancy/toddlerhood, respectively. Breastfeeding, due to the supply of human milk oligosaccharide into the gut, promotes the growth of specific human milk oligosaccharide (HMO)-utilizing *Bifidobacterium* species that dominate the ecosystem as long as the infant is primarily breastfed. These species perform saccharolytic fermentation in the gut and produce metabolites with physiological effects that may contribute to protection against infectious and immune-related diseases. Formula feeding, due to its lack of HMOs and higher protein content, give rise to a more diverse gut microbiota that contains more opportunistic pathogens and results in a

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more proteolytic metabolism in the gut. Complementary feeding, due to the introduction of dietary fibers and new protein sources, induces a shift in the gut microbiota and metabolism away from the milk-adapted and toward a more mature and diverse adult-like community with increased abundances of short chain fatty acid-producing bacterial taxa. While the physiological implication of these complementary diet-induced changes remains to be established, a few recent studies indicate that an inadequately matured gut microbiota may be causally related to poor growth and development. Further studies are required to expand our knowledge on interactions between diet, gut microbiota, and health in the early life setting.

Key Insight

The healthy fetus is devoid of microbial organisms, but upon birth our gastrointestinal (GI) tract becomes colonized with a multitude of microbes, gradually developing into a complex microbial community during the first year of life. This community, termed the gut microbiota, interacts with our gut epithelium, immune, and nervous systems and influences our metabolism. During the last decades, the gut microbiota has been linked with a range of gut inflammatory, infectious, allergic, autoimmune, neurological, and metabolic diseases. Thus, understanding the processes that govern initial colonization and establishment of microbes in our GI tract is of great importance. A key factor influencing the gut microbiota development in early life is diet. This article review our current knowledge of how the type of milk feeding and complementary feeding affects the microbial ecosystem in the gut and how this may impact current and future health.

Current Knowledge

Type of milk-feeding and complementary feeding dictates the microbial succession throughout infancy and into early toddlerhood. Breastfeeding keeps the gut microbiota in a state of low diversity dominated by human milk oligosaccharide-utilizing *Bifidobacterium* species that produce various metabolites characteristic of saccharolytic fermentation. Formula feeding allows for a somewhat more diverse gut microbiota to colonize and is characterized by higher prevalence of potential pathogenic taxa such as *Clostridium* and *Enterobacteriaceae* and a higher degree of proteolytic fermentation. The gradual cessation of milk-based feeding and progression in complementary feeding, characterized by increased consumption of dietary fibers and proteins, results in a further diversification and maturation of the microbial community in the gut with increased abundance of the bacterial families *Lachnospiraceae*, *Ruminococcaceae,* and *Bacteroidaceae,* as well as expansion of bacterial products from saccharolytic and proteolytic metabolism.

Practical Implications

The breastfeeding promoted *Bifidobacterium* species produce metabolites that lower intestinal pH and thus creates an environment hostile to pathogenic bacteria, they exert anti-inflammatory properties, protect the gut epithelium and regulate the immune system. These properties may result in reduced the risk of infectious and immune-related diseases for breastfed infants harboring these species. Formula fed infant are more frequently colonized with opportunistic pathogenic and toxin-carrying bacterial species and exhibit a mostly proteolytic metabolism in the gut, which may influence the risk of infectious, but also metabolic and immune-related diseases. Lack of diet-promoted maturation of the gut microbiota during complementary feeding is associated with poor growth and neuro, bone and immune development, although the evidence base for this is extremely limited at present. Further studies disentangling the complex interactions between infant diet, gut microbiota and health are warranted in order to approach clinical implication.

Recommended Reading

Laursen MF, Bahl MI, Licht TR. Settlers of our inner surface: factors shaping the gut microbiota from birth to toddlerhood. *FEMS Microbiol Rev*. 2021;1:1–14 [1].

Gut Microbiota Development in Early Life

Despite recent controversies, the general consensus is that the healthy fetus is devoid of microbial organisms [2]. During and following birth the neonatal external body surfaces and gastrointestinal (GI) tract rapidly becomes colonized with a multitude of microorganisms, reaching >10^8 microorganisms per gram feces in a matter of hours to days [3] and over weeks to months increase up to 10^{11}–10^{12} microorganisms per gram feces [4, 5], comparable to the microbial densities found in the adult gut [6, 7]. The infant gut microbiota undergoes drastic changes during the first years of life in terms of taxonomic

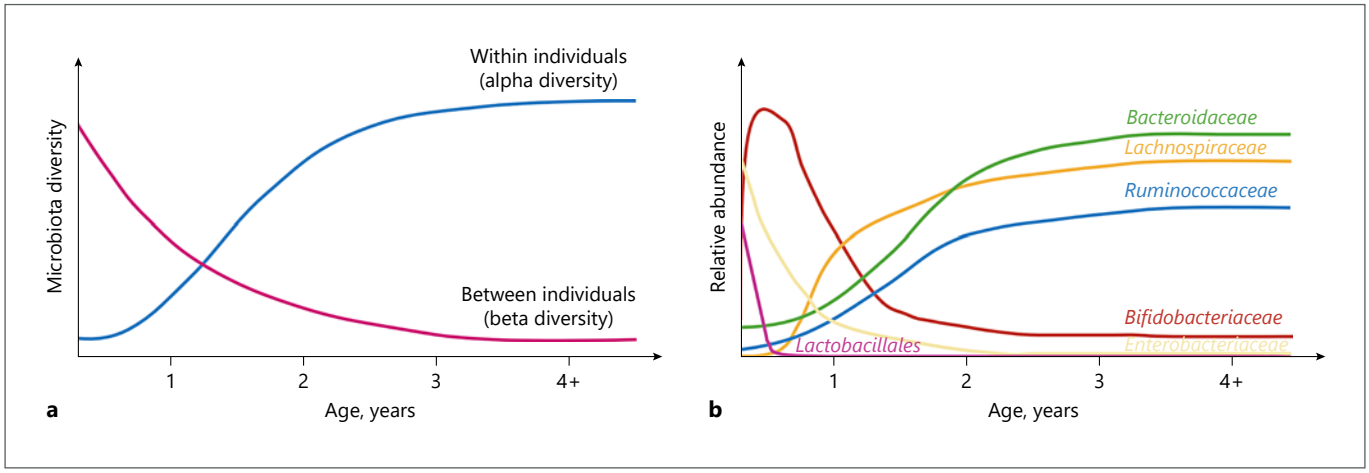

Fig. 1. Development of the gut microbiota in early life. **a** Development of gut microbiota diversity with age [97]. **b** Development of the average gut microbiota composition with age, showing the relative abundance of the major microbial families/orders [11, 13].

composition and diversity (Fig. 1). As a function of age, the infant becomes exposed to and colonized by a range of new microbes, increasing diversity within the individual (termed alpha diversity). However, differences in gut microbiota composition between individuals (termed beta diversity), is initially high, resulting from individual exposures to various different environmental sources of microbes, but these differences gradually decrease as selective forces, such as diet, converge microbiota compositions (Fig. 1a). Thus, the individual infant continuously acquires new microbial taxa with age, but when comparing across infants the gut microbiota gradually becomes taxonomically more similar. Aerotolerant and facultative anaerobes, such as lactic acid bacteria (*Lactobacillales* genera, such as *Streptococcus*, *Enterococcus* and *Lactobacillus*) and *Enterobacteriaceae* are among the major initial colonizers of the neonatal gut, but their proportions rapidly decrease within days to weeks and the gut microbiota in breastfed (BF) infants becomes dominated by anaerobic breast milk-promoted *Bifidobacteriaceae*. As the gut microbiota develops during infancy and early childhood, abundance of other anaerobic bacterial families, such as *Bacteroidaceae*, *Lachnospiraceae,* and *Ruminococcaceae* increase (Fig. 1b). In early life, the gut microbiota is unstable and less resilient to perturbation than the adult gut microbiota [8], but the ecosystem develops in alpha diversity until around 3–5 years of age, where a stable adult-like microbiota has established [9]. However, before this state is reached, the gut microbiota is more susceptible to modulation by external factors. Factors influencing the infant gut microbiota development include (as reviewed in [10]); term of birth, mode of birth, intake of antibiotics, presence of furred pets, host genetics, siblings and family members, geographical location/population, growing up environment (i.e., urban vs. rural), maternal diet (through breast milk), and infant diet (Fig. 2). While all of these individually have shown impact, infant diet has been identified as a major contributor to gut microbiota development in early life [11, 12]. In this regard, early infancy diet, such as formula versus breast milk has been relatively well studied, whereas much less is known about the effects complementary feeding on infant gut microbiota composition [13]. Given the dominating influence of breastfeeding on gut microbial composition as well as its protective effects against infectious and some metabolic diseases [14], and the involvement of gut bacteria in regulation of host immune system and metabolism [15], it is of great importance to obtain a deep understanding of the potential microbe-mediated host effects of feeding mode in early infancy. Also, the complementary feeding period (6–24 months of life) coincides with a critical period in gut microbiota development, transitioning away from the influence of milk-based diet. As early childhood development of a mature and diverse adult-like gut microbiota, which is stable and resilient toward perturbation, appears to be an important asset of health [16], assessing how dietary changes in early life affect gut microbiota trajectory is of great value.

Early Infancy Feeding and Gut Microbiota

Breast milk is the recommended first nutrition for the infant, providing all necessary nutrients to support growth and development, as well as passive immunity to protect against infectious diseases during infancy [17]. After lactose and lipids,

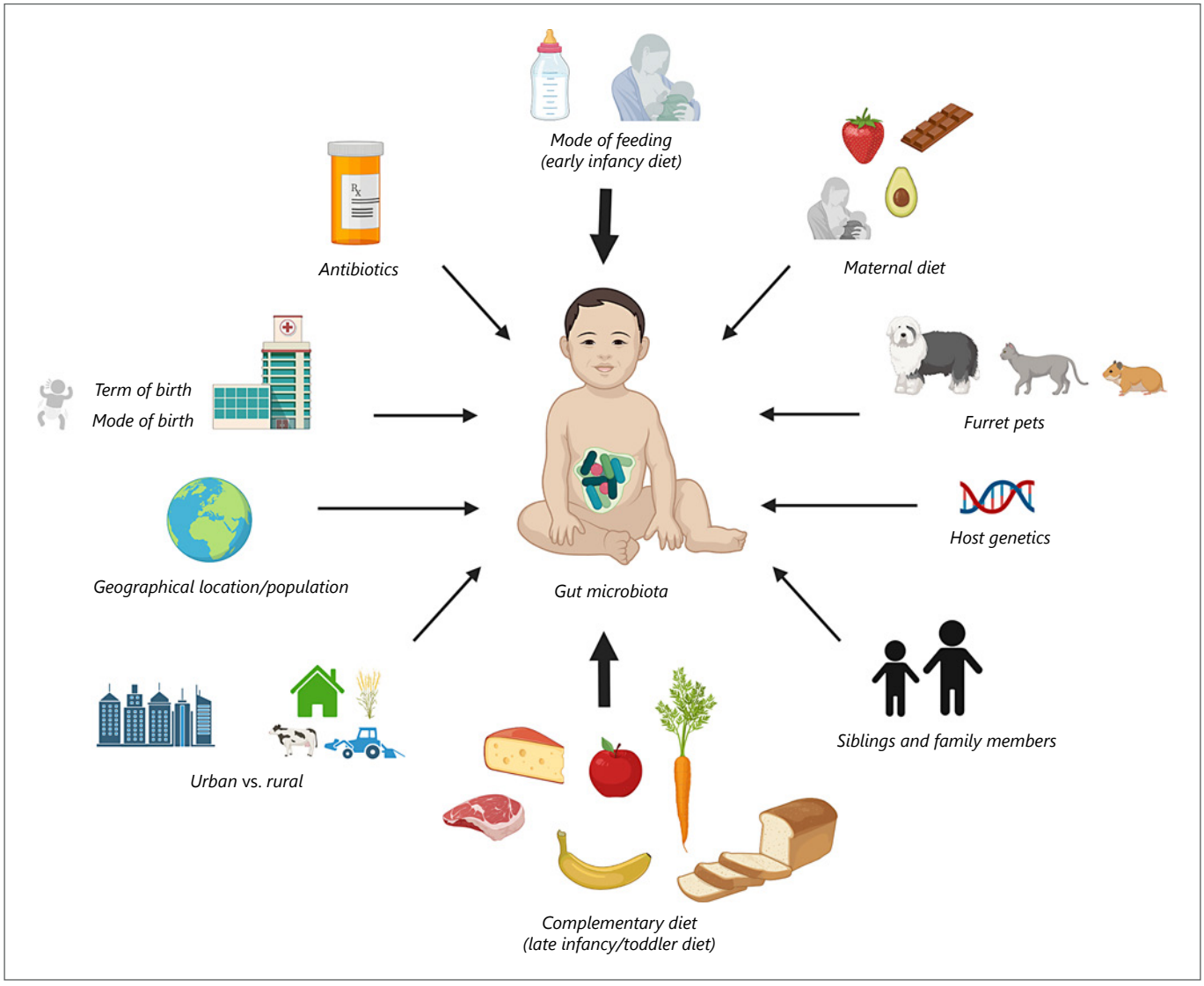

Fig. 2. Factors influencing the gut microbiota in early life. Gut microbiota vary by term of birth, mode of birth, oral antibiotics, mode of feeding, maternal diet, presence of furred pets, host genetics, presence of siblings and family members, complementary diet, and local environment, such as urban versus rural and geographical location/population [10]. Mode of milk feeding and complementary diet has been identified as the strongest determinants of infant gut microbiota composition [11, 12, 89] as indicated by the arrow sizes.

human milk oligosaccharides (HMOs) represent the third most abundant component of breast milk, reaching 20–25 g/L in colostrum and ranging between 5 and 15 g/L in mature milk [18]. HMOs are short saccharides composed of 5 monomeric building blocks (Glucose, Galactose, N-acetylglycosamine, Fucose, and Sialic acid). From these 5 building blocks, >200 different HMOs structures have been identified [19]. Whereas lactose, protein, and lipid serve as important macronutrients for the BF infant, HMOs are virtually ingestible by the infant's own GI saccharolytic enzymes. Therefore, HMOs pass through the upper GI tract and reach the colon largely intact. Throughout the GI tract, they can exert direct biological functions, such as antiadhesion of pathogens and modulation of epithelial cell responses [18], but in the colon they also serve as metabolic substrates for gut bifidobacteria. Some of these bifidobacteria harbor an arsenal of membrane transporters and saccharolytic enzymes which cleave and internalize HMOs [20] to implement the monomers into the central catabolic pathways, leading to the main end products lactate, acetate, formate, and 1,2-propandiol [21–24] (Fig. 3a). Although other bacteria (e.g., *Bacteroides*, *Lactobacillus,* and recently the *Roseburia*/*Eubacterium* group) exhibit some de-

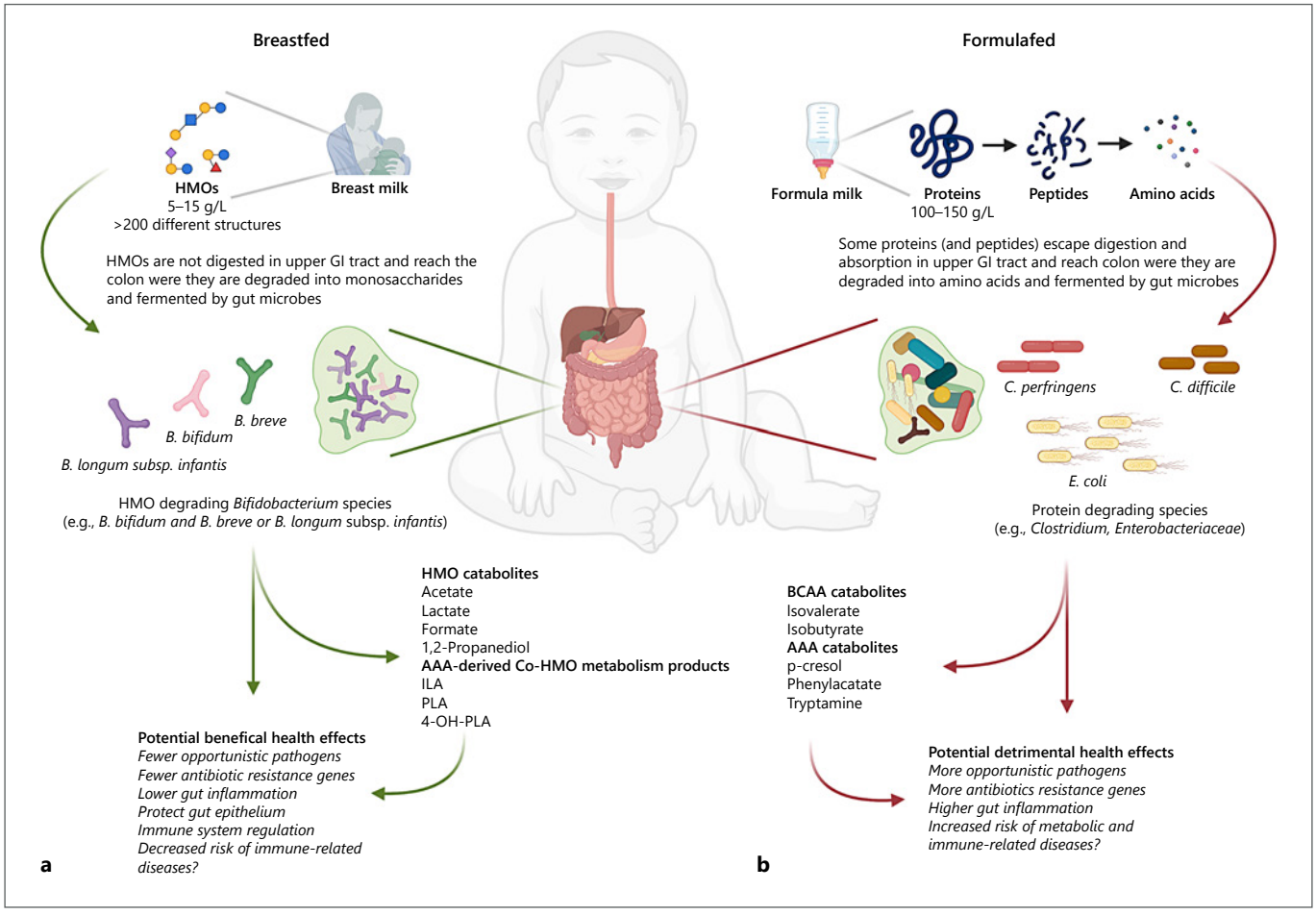

Fig. 3. Mode of feeding influences early infancy microbiota and saccharolytic versus proteolytic fermentation in the gut with potential implications for health. **a** Breast milk contains HMOs, which upon ingestion by the infant pass undigested through the upper GI tract until they reach the colon. Here, they are digested by specific HMO-degrading *Bifidobacterium* species into various metabolites with potential beneficial health effects. **b** Formula milk contain excess proteins, some of which, upon ingestion, is incompletely digested and absorbed in the upper GI tract and reaches the colon. Partly digested protein, peptides, and individual amino acids are metabolized by gut microbes, including opportunistic pathogenic bacteria, such as *Clostridium* and *Enterobacteriaceae* species, yielding various amino acid catabolites, some of which have potential detrimental health effects. HMO, human milk oligosaccharide; AAA, aromatic amino acid; BCAA, branched chain amino acid; GI tract, gastrointestinal tract; ILA, indolelactate; PLA, phenyllactate; 4-OH-PLA, 4-hydroxypheyllactate.

gree of HMO utilization [25–27], the extensive arsenal of enzymes and transporters is restricted to specific species within the *Bifidobacterium* genus. Whereas some species, such as *B. bifidum*, employ extracellular saccharolytic enzymes to degrade HMOs and internalize mono- and disaccharides, other species such and *B. breve* and especially *B. longum* subsp. *infantis* transport the intact HMO structures and degrades them inside the cell [20]. The ability of these species to efficiently utilize HMOs makes them dominant members of the gut of BF infants [11, 28, 29]. Importantly, other *Bifidobacterium* species commonly found in the adult gut (e.g., *B. adolescentis*, *B. catenulatum,* and *B. angulatum*) cannot utilize HMOs and therefore are less abundant and/or infrequent members of the gut microbiota of BF infants [20]. Thus, due to the HMO content, breast milk represents a strong selective factor for shaping the early infancy microbiota, dominated by specific *Bifidobacterium* species (Fig. 3a). Through HMO catabolism, these *Bifidobacterium* species produce metabolites with potential host-health effects. Acetate and lactate are 2 main end products of bifidobacterial HMO metabolism and are responsible for the low pH found in feces from BF infants [24, 30, 31], which is likely to suppress the growth of oppor-

tunistic pathogenic species within *Clostridiaceae*, *Enterobacteriaceae,* and *Staphylococcaceae* [30, 32]. Further, *Bifidobacterium*-produced acetate was found to inhibit enteropathogenic *E. coli* infection in a mouse model, by maintaining the epithelial barrier function upon insult [33]. HMO-utilizing *Bifidobacterium* species are also main producers of a limited set of the aromatic amino acid catabolites, namely indolelactate (ILA), phenyllactate, and 4-hydroxypheyllactate (4-OH-PLA), in the gut of BF infants [29]. These metabolites are produced when the bacteria grow on HMOs (and have access to the aromatic amino acids tryptophan, tyrosine, and phenylalanine, also contained in breast milk), and interact with various receptors expressed in immune cells, exhibit immunomodulatory potential and protect the gut epithelium [29, 34–36]. This may contribute to the anti-inflammatory status observed in the GI tract of BF infants with *Bifidobacterium*-rich communities [37]. However, despite being BF, some individuals (e.g., infants born preterm, by C-section or in population with high consumption of antibiotics which disrupts vertical transmission from the maternal gut) lack *Bifidobacterium* species, such as *B. longum*, *B. breve,* and *B. bifidum* [38, 39]. Lack of *Bifidobacterium* is associated with immune dysregulation [40] and development of asthma [41] and autoimmune diseases [42], but early life intervention with a HMO-degrading *B. longum* subsp. *infantis* strain in BF infants modulates immune responses away from the allergy and autoimmunity associated immune-phenotypes [40]. In both full term [31] and preterm [43, 44] BF infants lacking bifidobacteria, oral administration of HMO-utilizing *Bifidobacterium* spp. were found to decrease the abundance of opportunistic pathogens, antibiotic resistance genes, and reduce intestinal inflammation, demonstrating the importance of this group of bacteria for infant health (Fig. 3a).

On the contrary, exclusively formula-fed (FF) infants harbor a more diverse microbiota with lower abundances of HMO-utilizing *Bifidobacterium* species, often with increased abundances of *Clostridium* species (*C. difficile* and *C. perfringens*) and *Enterobacteriaceae* species (e.g., *E. coli*) [45–47] (Fig. 3b). The lack of HMOs and higher protein content in formula is likely to explain these observations. Although many infant formula products are supplemented with fructo-oligosaccharides and/or galacto-oligosaccharides, these are not as selective since they can be utilized by most *Bifidobacterium* species (including the adult-associated *B. adolescentis* and *B. catenulatum*) [48, 49] and additional can stimulate growth of various *Lactobacillus*, *Streptococcus,* and *Bacteroides* species [50, 51]. Studies of the fecal metabolome in exclusively FF (even when formula is containing galacto-oligosaccharides) versus exclusively BF infants, suggest that proteolytic rather than saccharolytic metabolism dominates in the FF gut [52, 53]. Whereas remnants of HMO metabolism (e.g., fucose), their direct catabolic end products (e.g., lactate and 1,2 propanediol) and aromatic amino acid derived co-HMO metabolism products [29, 35] (e.g., indolelactate and 4-hydroxypheyllactate) dominate in BF infants, various different protein fermentation products such as isovalerate, isobutyrate, phenylacetate, p-cresol and tryptamine dominate in FF infants [52, 53] (Fig. 3). Some of these metabolites (e.g., p-cresol and phenylacetate) are converted in the liver to metabolites with detrimental effects, such as p-cresol-sulfate, which is a uremic toxin [54] and phenylacetateglutamine which may contribute to development cardiovascular diseases [55]. Phenylacetate has also been shown to promote accumulation of fat in the liver [56]. Further, bacterially produced tryptamine in FF infant gut may shift tryptophan metabolism away from serotonin and influence immune system development [57]. Biogenic amines produced by bacterial amino acid metabolism (such as tryptamine and histamine) can cause GI symptoms and allergic reactions [58, 59]. In addition *C. perfringens* and *C. difficile* that often colonize the gut of FF infants are potential toxin-carrying opportunistic pathogens [60, 61] and the gut microbiota of FF infants has a higher abundance of antimicrobial resistance genes [62, 63]. Last, FF infant have higher levels of inflammation markers than BF infants [64].

In summary, breastfeeding, due to the presence of HMOs, promotes growth of beneficial bifidobacteria in the infant gut, which produces metabolites that may contribute to prevention of GI infections and support immune system development. By contrast, formula feeding leads to a gut microbiota with higher abundances of opportunistic pathogens and a mostly proteolytic gut metabolism, leading to potentially detrimental health effects (Fig. 3). However, it has recently become technically and legally possible to add synthetically produced HMOs (e.g., 2′FL) to infant formula in order to stimulate a BF-like gut microbiota and metabolism [65]. A recent double-blinded randomized controlled trial investigated how addition of 2 HMOs, namely 2′FL and LN*n*T, to infant formula influence the gut microbiota, and compared HMO-supplemented and unsupplemented formula groups with a reference group of BF infants [66]. The addition of HMOs to the formula resulted in a microbiota configuration that was more similar to that of the BF infants. Infants receiving HMO-supplemented formula, similar to the BF infants, had higher relative abundances of *Bifidobacterium* and lower relative abundances of *Enterobacteriaceae* and *Peptostreptococcaceae* (the bacterial family that includes *C. difficile*) compared to infants receiving unsupplemented formula. Although promising, this study is the first of its kind in investigating the influence on the gut microbiota and confirmatory studies are re-

quired. Nonetheless, this strategy may constitute an avenue to improve the gut microbiota in infants that for some reason cannot be BF.

Complementary Feeding and Gut Microbiota

At some point during infancy milk-based feeding is no longer adequate to cover the nutritional requirements of the infant. Therefore, supplementation with additional foods, alongside milk-feeding, is needed. The period of transition from exclusive milk-feeding toward eating family foods is termed complementary feeding and usually covers age 6–24 months [67, 68]. It is recommended by WHO to start complementary feeding around 6 months of age [67]; however, in some countries/populations is it not uncommon to introduce other foods as early as 2–3 months of age [69]. Early complementary feeding (before 3 months of age) has been linked to increased risk of GI and respiratory infections, obesity, and allergies, but this may rather be attributed to shorter duration of breastfeeding [70]. However, late introduction to complementary feeding can also be problematic as it may result in feeding problems, inadequate nutrition and growth [70], and failure to induce oral tolerance [71]. Recent evidence suggests that the infant gut microbiota development may be causally linked to healthy growth [72, 73] and protection against food allergies [74, 75].

Despite extensive progression, during the last decade, in our understanding of gut microbial succession in early life, and our continuously expanding knowledge about microbial capacity to consume various dietary compounds, we know surprisingly little about causal effects of diet on gut microbiota during complementary feeding. As outlined above, the complementary feeding period coincides with a phase of drastic changes in the gut microbiota (Fig. 1), including a rapid decline in HMO-degrading *Bifidobacterium* species. The prominent increase in alpha diversity and appearance/bloom in *Bacteroidaceae*, *Lachnospiraceae,* and *Ruminococcaceae* species, mirror the increased complexity of the diet with the introduction of fibers from various fruits, vegetables, cereals/porridge, and breads as well as new protein sources that is, in the form of meats, dairy products, and legumes/lentils. As dietary fibers and secondly (incompletely digested) proteins/peptides are the main sources of energy for gut microbes [76] these macronutrients would be expected to have most impact on the microbial composition. Carbohydrates (dietary fibers) are preferred energy-sources for microbes, but in shortage of these a higher degree of proteolytic fermentation occurs in the gut (as illustrated in the example of metabolism in the gut of FF infants mentioned above). This balance not only depends on diet but will also vary with passage of luminal content through the colon, as dietary fibers will gradually become depleted [76]. The main catabolic end products of dietary fiber metabolism are the short chain fatty acids (SCFAs) acetate, butyrate, and propionate [77]. Whereas the former (together with lactate, formate, and succinate) is produced in high quantities in early infancy (e.g., by *Bifidobacterium, Lactobacillus, and Enterobacteriaceae* spp.), butyrate and propionate concentrations are initially very low but increase with infant age [24]. In addition, products of protein fermentation, such as branched chain fatty acids (BCFAs) are almost undetectable during breastfeeding, but follow a similar pattern of increase with age [24]. These changes in SCFAs and BCFAs are coinciding with the introduction of solid foods and cessation of breastfeeding [24]. Consistent with the typical gut microbiota developmental pattern, some key *Lachnospiraceae* (*Anaerostipes*, *Roseburia*, and *Eubacterium*) and *Ruminococcaceae* (*Faecalibacterium*, *Gemminger*, and *Subdoligranulum*) species are butyrate producers [24, 78], whereas *Bacteroides* are common propionate producers [79]. These species harbor an extensive catalog of enzymes (glycosyl hydrolases) for degradation of dietary fibers [80] into these SCFAs [79]. In addition, some *Bacteroides* and *Clostridium* species may utilize various amino acids from dietary proteins to form BCFAs [81]. Thus, complementary feeding is very likely to causally affect microbiota composition and metabolism. As SCFAs have a range of physiological effects, including influence on intestinal barrier function, host metabolism, immune system, and nervous system [82], these are plausible mediators of microbiota-host interactions during complementary feeding affecting host health (Fig. 4). Much less is known about the physiological effects of BCFAs [83].

Longitudinal studies, designed with multiple samplings around the period of first introduction to solid foods, have demonstrated increased alpha diversity and abundance of *Lachnospiraceae* genera (such as *Blautia*) after introduction of solid foods [84, 85]. Supporting this, earlier introduction to complementary foods is associated with higher microbial alpha diversity throughout infancy, including higher abundance of the butyrate producing *Lachnospiraceae* genera *Roseburia* [86]. In a randomized controlled trial [87] the authors compared the gut microbiota in infants that were weaned traditionally (with spoon feeding) to infants weaned with a baby-led approach (no spoon feeding, but only complementary "finger foods" consumed), with complementary diet assessed by 3-day dietary records. The baby-led weaned infants were introduced to solid foods roughly 3 weeks later (6 months of age) and consumed significantly less fruits and vegetables and total dietary fiber at 7 months of age, which was associated with a reduced alpha diversity and lower abundance of specific *Lachnospiraceae* (*Roseburia facies* and *Eubacterium rec-*

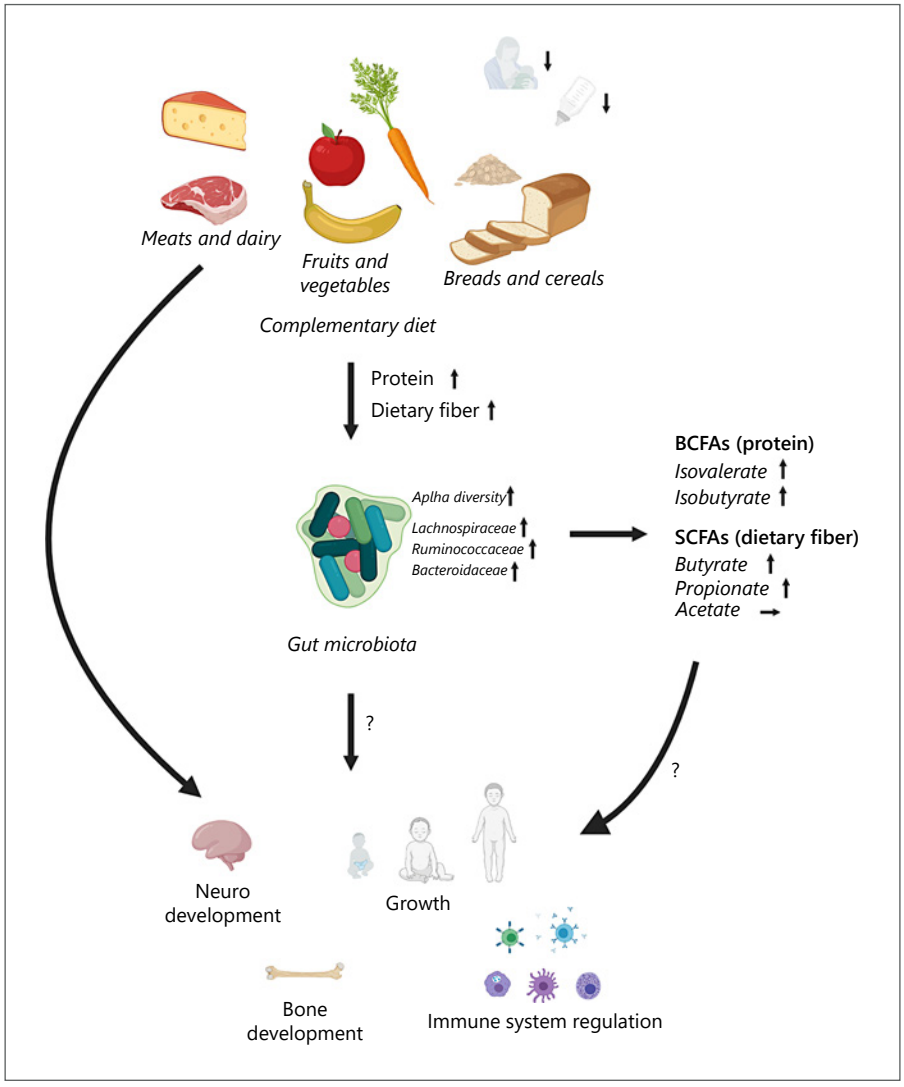

Fig. 4. Complementary feeding increases gut microbial diversity and production of BCFAs and SCFAs with potential implication for growth, neuro, bone, and immune development. As complementary feeding progresses, the milk-based component is gradually replaced by other foods such as meats and dairy, fruits and vegetables, and bread and cereals, which are directly and possibly indirectly (through the gut microbiota) impacting growth and development. These foods contain protein and dietary fibers that modulate the infant gut microbiota, increasing alpha diversity and the abundance of key bacterial families that produce SCFAs (note that acetate is also produced in high amount during early infancy) and BCFAs during complementary feeding. This "natural" development of the gut microbiota and its metabolites is associated with healthy growth, neuro, bone development, and appropriate immune system regulation. BCFAs, branched chain fatty acids; SCFAs, short chain fatty acids.

tale) and *Ruminococcaceae* (*Faecalibacterium prausnitzii*) species during complementary feeding [87]. In addition, independent of the feeding groups, consumption of breads and cereals, as well as meat products at 7 months of age were positively associated with alpha diversity at 12 months of age [87].

A study including 9 months old Danish infants assessed the complementary feeding diet by 7-days dietary records and a used multivariate statistical analysis to generate the dietary pattern "family foods" [88, 89], describing the infant's progression in complementary feeding (from milk-based toward family foods). Progression in complementary feeding, characterized by higher dietary fiber and protein intake, correlated significantly with gut microbial alpha diversity [89]. Specifically, consumption of rye bread and cheese and meat products were main food groups driving these associations [89], suggesting that these complementary foods are contributing to the diversification of the infant gut microbiota, at least in this cohort. Importantly, these associations were not solely mediated by cessation of breastfeeding, as they were found in both partially BF and partially FF infants [13]. Progression in complementary feeding also correlated positively to the abundance of several *Lachnospiraceae* and *Ruminococcaceae* spp., but negatively to *Bifidobacterium* [13], thus marking the transition of the breast milk promoted *Bifidobacterium*-rich gut community toward the fiber and protein promoted (more diverse) gut microbial community, characterized by butyrate, propionate, and BCFA-producing bacteria (Fig. 4).

Indeed, interventions with meat as a main complementary food compared to dairy [90] or cereals [91] has revealed

significantly increased abundance of butyrate-producing *Lachnospiraceae* genera. A recent 7-week intervention study comparing a refined grain cereal product to a whole grain cereal product as first complementary food, demonstrated a significant increase in *Bacteroides* and *Lachnoclostridium* (*Lachnospiraceae* family) and a decrease in *Escherichia* (*Enterobacteriaceae* family) over time only in the whole grain cereal group. However, other intervention with specific complementary foods such as legumes versus corn-soy flour [92] or meat versus cereals [93] show limited differential impact on the gut microbiota, possibly due to these infants still receiving a high proportion of breast milk in their diet or other study participant characteristics. Ultimately, more randomized controlled trials are needed to establish direct causal proof of links between specific complementary foods and specific microbial taxa. Nonetheless, as outlined below, a few recent studies indicate that specific *Lachnospiraceae* species affected by complementary foods may influence growth and development and protect against food allergies (Fig. 4).

Stunted undernourished Bangladeshi infants/toddlers have an immature gut microbiota compared to same age healthy well-nourished counterparts [94], likely as a result of inadequate and/or delayed complementary feeding. Transplantation of the gut microbiota, using fecal samples from stunted, malnourished versus healthy infants, into germ-free mice (devoid of microbes) has revealed differential growth patterns, recapitulating the growth phenotypes observed in these infants [73]. Oral introduction of some of the *Lachnospiraceae* species (such as *Ruminococcus gnavus* and *Clostridium symbiosum*) that appear during complementary feeding in healthy infants was shown to promote growth and bone development in mice previously transplanted with the malnourished gut microbiota [73]. Furthermore, complementary food intervention in stunted malnourished Bangladeshi toddlers rationally designed to repair the immature gut microbiota suggest that a combination of banana, chickpea, soy, and peanut matures the gut microbiota (increase abundance of some *Lachnospiraceae* and *Ruminococcaceae* species) and influence blood markers of healthy growth, bone, immune, and neurodevelopment [72, 95]. However, despite having a similar energy, macronutrient and fiber content, other rationally designed microbiota-directed complementary food combinations were not effective, illustrating the need for a deeper understanding of the effects of specific food combinations.

Recent studies have demonstrated that blood concentrations of food specific immunoglobulin E (IgE), an important mediator in food allergy, is elevated in germ-free mice compared to conventional (colonized) counterparts after weaning onto an antigen-containing diet [75]. In addition, colonization of previously germ-free mice with gut commensal microbes inhibit the increase in food-specific IgE and disruption of the gut microbiota by oral administration of antibiotics to conventional mice increases food-specific IgE concentrations in blood [75]. Thus, gut microbes play a causal role in regulation of IgE levels during the food antigen exposure and may affect the risk of developing food allergy. Another study performed gut microbiota transplantation from healthy or cow's milk allergic 6-month old infants into germ-free mice and challenged them with the cow's milk allergen β-lactoglobulin [74]. In contrast to the mice colonized with the cow's milk allergic infant gut microbiota, mice colonized with the healthy infant gut microbiota were protected against allergic responses. In the healthy infant gut microbiota, *Anaerostipes coccae*, a *Lachnospiraceae* species that increases during complementary feeding and associates with consumption of vegetables and fruits [96], was identified as a bacterial taxon that mediated this protection. By comparing mice colonized with cow's milk allergic microbiota to mice mono-colonized with *A. caccae* it was demonstrated that this species protected the mice against allergic food response [74]. While results from these studies are encouraging, it must be emphasized that our current knowledge on the effects of different complementary foods on gut microbiota and thereof potential health impact is still extremely limited.

Future Directions

Undoubtedly, diet plays a major role for the succession of the infant gut microbiota, yet we still have a very incomplete understanding of the details. There is a need for well-designed and well-powered interventions in various different populations in order to assess the impact of formula supplemented with different HMOs on gut microbiota and metabolome in early infancy, including relevant measures of host impact, such as prevalence of infectious and immune-related diseases, during and after intervention. There is a lack of studies with detailed dietary recordings coupled to measurements of the gut microbiota at a finer resolution scale. In example, to improve our knowledge on dietary factors shaping the infant gut microbiota, we need prospective cohort studies with detailed information about infant diet, obtained from multiple consecutive days of dietary recordings throughout periods of complementary feeding, combined with dense fecal sampling and state of the art microbiome (obtaining species and strain level resolution) and metabolome profiling, as well as deep genomic and phenotypic characterization of fecal microbial isolates to decipher molecular mechanisms. These studies

should be accompanied by intervention studies, in relevant study populations, designed to introduce specific complementary foods using frequent longitudinal sampling and the state-of-the-art methods of microbiome and metabolome profiling. Hopefully, this will lead to identification of key diet-microbe-metabolite interactions that beneficially impact aspects of host physiology, such as metabolism and immunology. Ultimately, if evidence is adequate, specific dietary intervention should be tested in disposed/susceptible study populations (e.g., disposed to allergic or autoimmune diseases or individuals at risk of malnourishment and stunting) to provide proof of applicability.

Summary and Conclusions

The infant gut microbiota undergoes significant temporal development during the first years of life. A multitude of factors such term of birth, mode of birth, antibiotics, contact with furred animals and siblings/family members, local environment, geographical location, and maternal and infant diet affect the trajectory of this development. Of these, infant/toddler diet has been identified as one of the most influential factors. The neonatal gut microbiota is initially characterized by random colonization of environmental microbes, but in a matter of days to weeks the gut ecosystem and the type milk-feeding selects for microbes adapted to the GI environment and equipped to consume the indigestible (or incompletely digested) constituents of breast or formula milk. Breast milk feeding selects for specific HMO-degrading *Bifidobacterium* species that vastly dominate the gut ecosystem and produce metabolites with beneficial effects likely contributing to the protection against GI infections and supporting immune system development. On the contrary, formula-milk feeding is less selective and the gut microbiota of FF infants is more diverse with a higher frequency and proportion of protein degrading opportunistic pathogenic species. This may increase risk of GI infections and the apparent mostly proteolytic metabolism is possibly detrimental to health as some of the metabolites are associated with kidney, liver, and cardiovascular diseases in adults.

As the infant transitions into complementary feeding the gut microbiota diversifies along with the increasing amounts of dietary fiber and proteins in the diet. Specifically, members of the bacterial families *Lachnospiraceae*, *Ruminococcaceae,* and *Bacteroidaceae* capable of consuming various dietary fibers from fruits, vegetables, breads and cereals, and possibly proteins from meat and dairy sources, increase in abundance during complementary feeding. This leads to a shift in gut metabolism with the appearance or increase of the SCFAs butyrate and propionate as well as the BCFAs. While the physiological relevance of these bacterial taxa and metabolites remains to be established in this early life context, recent research suggests that they may be important for healthy growth and development and protection against food allergies.

Clearly, the question of when to start introducing various complementary foods in order to achieve optimal health and gut microbiota progression is central. Early introduction, combined with reduced or lack of breastfeeding is presumably not beneficial, as it will disrupt the *Bifidobacterium* dominated gut community, associated with protection against infectious and immune-related diseases. On the other hand, very late introduction as observed in populations where food is scare and breastfeeding of the infant is prolonged without adequate complementary foods would also be expected to be adverse, given the inability of the gut microbiota to mature when deprived of key nutrients and the obvious negative implications for growth and development. Undoubtedly, further studies are required to improve our understanding of the intriguing links between complementary feeding, gut microbiota, and health.

Conflict of Interest Statement

The author declares no conflict of interest.

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