


Review

The Human Microbiome and Child Growth – First 1000 Days and Beyond

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The assembly of microbial communities within the gastrointestinal tract during early life plays a critical role in immune, endocrine, metabolic, and other host developmental pathways. Environmental insults during this period, such as food insecurity and infections, can disrupt this optimal microbial succession, which may contribute to lifelong and intergenerational deficits in growth and development. Here, we review the human microbiome in the first 1000 days – referring to the period from conception to 2 years of age – and using a developmental model, we examine the role of early microbial succession in growth and development. We propose that an ‘undernourished’ microbiome is intergenerational, thereby perpetuating growth impairments into successive generations. We also identify and discuss the intertwining host–microbe–environment interactions occurring prenatally and during early infancy, which may impair the trajectories of healthy growth and development, and explore their potential as novel microbial targets for intervention.

Growth and Development: A Microbial Perspective

The first 1000 days – the period from conception to 2 years of age – represents a critical window of early childhood growth and development. This prenatal and early postnatal period is defined by rapid maturation of metabolic, endocrine, neural, and immune pathways, which strongly influence and support child growth and development. These pathways develop in tandem and are highly interdependent, with a complex program of assembly reliant on internal and external cues. When these developmental pathways are challenged by adverse environmental insults, such as infection or suboptimal feeding, the trajectory of child growth can be perturbed, leading to malnutrition, which can manifest as overnutrition (overweight or obesity) or as undernutrition – **stunting** (see [Glossary](#)) or **wasting**. An emerging perspective of human developmental biology includes the trillions of microbes (microbiota) and their genes (**microbiome**) that reside within the human body, and which assemble and stabilize during the first 2 years of life [1]. Emerging evidence suggests that the colonization of microbes in the human body during early life plays a critical role in the establishment and maturation of developmental pathways [2] and that disruption of this optimal microbial **succession** may contribute to lifelong and intergenerational deficits in growth and development (Figure 1).

Here, we review the role of the human microbiota in healthy growth and development during the first 1000 days. We focus on undernutrition, which remains an enormous public health challenge: 155 million children are stunted (low height-for-age), and 52 million children are wasted (low weight-for-height); global targets to end malnutrition in all its forms by 2030 are unlikely to be achieved (<https://sustainabledevelopment.un.org/sdg2>). We identify and discuss the intertwining host–microbe–environment interactions that occur prenatally and during early infancy that may impair the trajectories of healthy growth and development, and we explore their potential as novel microbial targets for intervention.

Highlights

Undernutrition is influenced by infections, subclinical pathogen carriage and metabolic impact of ‘dysbiotic’ commensal gut microbial communities (gut microbiota) in infants during the first 1000 days.

Delayed or immature assembly of the gut microbiota underlies severe acute malnutrition in children.

The gut microbiota affects the somatotropic axis through regulation of IGF-1 and growth hormone production, thereby affecting growth.

There is emerging knowledge of the influence of prenatal microbial communities on fetal and postnatal growth.

The gut microbiota plays an influential role in inflammation and enteropathy, which may be linked to growth faltering.

The first 1000 days provides a window of opportunity for modulating the microbiota through interventions such as diet, antibiotics, probiotics, prebiotics, or fecal microbiota transplantation to promote healthy growth and development.

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Days 0–270: Pregnancy

During pregnancy, fetal growth and development (Box 1) are profoundly influenced by the *in utero* environment and interactions at the fetal–maternal interface. Approximately 20% of stunting has *in utero* origins, due to preterm birth, **small-for-gestational age (SGA)**, or both [3]. Growth deficits *in utero* have been associated with maternal and placental inflammation and infection, suggesting a prenatal role of microbes in fetal growth [4]. Biogeographical analysis of the maternal microbiota at varying body sites suggests that the microbiota composition of pregnant women is distinct from that of nonpregnant women, and it changes throughout pregnancy [5,6]. Hence the prenatal microbiota may play an important role in the *in utero* environment that influences both the duration of pregnancy and the trajectory of fetal growth.

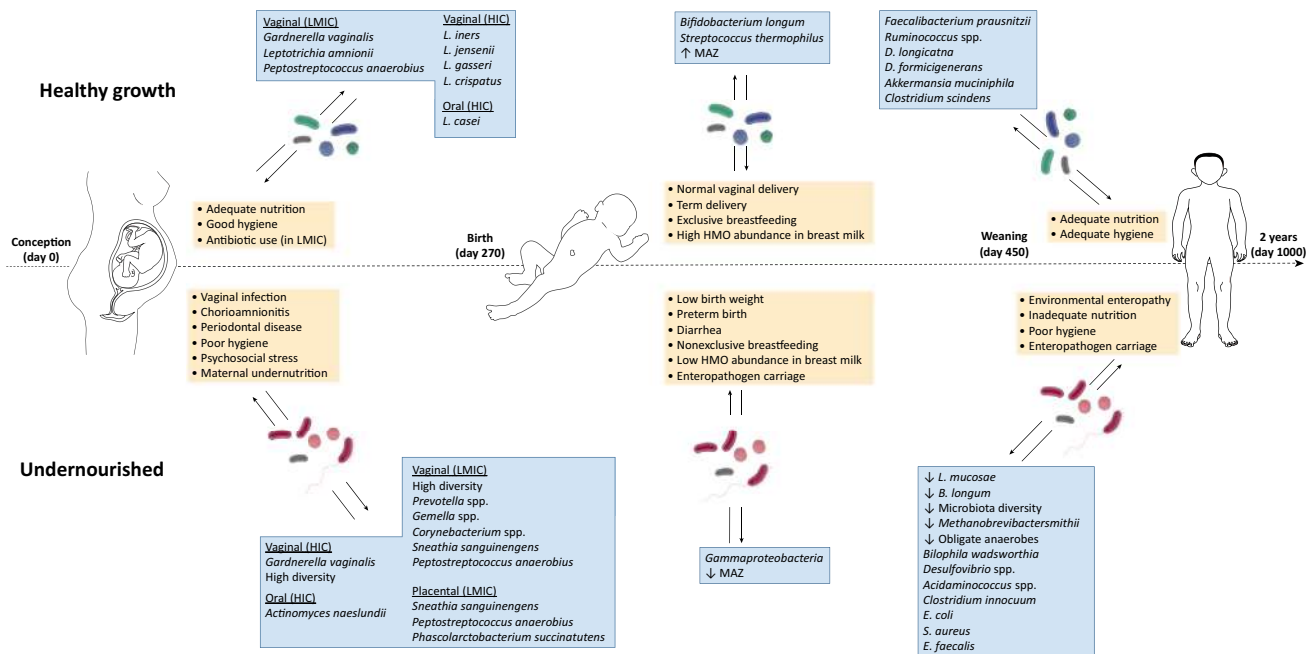
The Maternal Microbiota

The vaginal microbiota plays a key role in colonizing the infant at birth during normal vaginal delivery. However, emerging evidence also suggests that microbes in the vaginal tract may interact with the developing fetus, thereby affecting prenatal growth and duration of pregnancy. Vaginal infections, most commonly bacterial vaginosis, represent an important route of transmission for pathogens to invade the *in utero* environment and stimulate the inflammatory cascade associated with SGA and preterm birth [7]. However, characteristic patterns of the overall vaginal microbiota, rather than individual pathogens, have also recently been associated with reduced fetal growth. In urbanized cohorts from high-income countries, the vaginal microbiota during pregnancy is typically dominated by one of four species of *Lactobacillus* [7–9]. However, geographical differences appear to exist in the vaginal microbiota of pregnant women. A recent study of 1107 women in rural Malawi reported that a diverse *Lactobacillus*-deficient vaginal microbiota was most common in this setting [10]. Furthermore, this *Lactobacillus*-deficient vaginal microbiota could be further divided into four distinct subtypes, one of which was characterized by high abundances of *Prevotella* spp., *Gemella* spp., and *Corynebacterium* spp. This specific subtype was associated with significantly reduced newborn length-for-age Z-score (LAZ), which may have been partially driven by shorter duration of pregnancy. Hence the maternal vaginal microbiota may play an important role in the prenatal programming that influences growth.

Other ecological niches during pregnancy harbor communities of microbes that may influence fetal growth and birth outcomes. Gut microbial translocation increases during pregnancy [11]. Indeed, maternal inflammatory bowel disease, which may be partially driven by gut dysbiosis, is associated with preterm birth and low birth weight (LBW) [12]. Preterm birth in high-income settings is also associated with reduced maternal **gut microbiota** diversity and lower abundance of *Bifidobacterium*, *Streptococcus*, and *Clostridium* [13,14]. Several studies have also observed close similarity between the maternal oral microbiota and the placental and infant oral microbiota, suggesting that maternal–fetal microbial transmission may occur [15]. Intriguingly, maternal periodontal infection is associated with a disturbed oral microbiome and has been associated with preterm birth and SGA in some studies, suggesting that the oral cavity may act as a storehouse of microbes, which may ultimately interact with the developing fetus [16–18]. The abundance of *Actinomyces naeslundii* in maternal saliva is negatively associated, and *Lactobacillus casei* positively associated, with birth weight [19]. The incidence of preterm birth and LBW is four to five times greater in certain low- and middle-income countries (LMICs) compared with high-income settings, and periodontal disease is common due to poor oral health [20–22]. Maternal periodontal infection therefore may induce a chronic inflammatory environment *in utero*, leading to preterm birth and SGA or impaired infant growth.

Glossary

- Enteropathogens:** microorganisms found in the intestinal tract that cause disease.
- Enteropathy:** disease associated with the intestines.
- Environmental enteric dysfunction (EED):** a chronic condition of intestinal inflammation and blunting of intestinal villi, observed in children living in impoverished settings.
- Gut microbiota:** the community of microbes residing within the intestinal tract.
- Human milk oligosaccharides (HMOs):** complex sugars found in human breast milk that are digested by gut microbes.
- Microbiome:** the genes contained within the microbiota.
- Prebiotic:** a substrate that is selectively utilized by host microorganisms conferring a health benefit.
- Probiotic:** live microorganisms that, when administered in adequate amounts, confer a health benefit on the host.
- Ready-to-use therapeutic food (RUTF):** an energy-dense therapeutic food comprised primarily of peanuts, sugar, skimmed milk, oil, vitamins, and minerals given to children with severe acute malnutrition.
- Severe acute malnutrition (SAM):** refers to a child with a weight-for-height Z-score <−3, low mid-upper arm circumference (MUAC <11.5 cm), or the presence of bilateral pitting oedema.
- Small-for-gestational age (SGA):** refers to newborns weighing below the tenth percentile for gestational age.
- Stunting:** refers to a child with a length-for-age Z-score <−2.
- Succession:** the process by which a biological community or ecosystem evolves over time.
- Wasting:** refers to a child with a weight-for-height Z-score <−2.



Trends in Microbiology

Figure 1. Key Bacterial Taxa at Different Stages of 1000 Days That Contribute to Healthy versus Undernourished Growth. Current evidence suggests that a number of bacterial signatures are associated with either undernutrition or healthy growth during the first 1000 days. During pregnancy, a vaginal microbiota with low diversity and rich in *Lactobacillus* is associated with term birth and normal birth weight in high-income settings. Conversely, a more diverse vaginal microbiota, rich in *Prevotella* spp., *Gemella* spp., and *Corynebacterium*, is associated with reduced newborn LAZ. Healthy growth is associated with greater *Bifidobacterium longum* and *Streptococcus thermophilus* in the first 6 months of life, which are less prevalent in early-life undernutrition. Breastfeeding during this period is associated with greater *Bacteroides* and *Bifidobacterium*. In later childhood, higher *Akkermansia muciniphila*, *Methanobrevibacter smithii*, *Faecalibacterium prausnitzii*, *Lactobacillus*, and obligate anaerobes are associated with healthy growth, whilst *Escherichia coli*, *Staphylococcus aureus* and other species are associated with severe acute malnutrition. A two-way interaction exists between an immature microbiome and the risk factors contributing to undernutrition, whereby diarrhea, nutrition, birth weight, and other factors both influence and are influenced by the 'undernourished' microbiome. Image adapted from Servier Medical Art under a CC-BY license. HICs, high-income countries; LMICs, low- and middle-income countries; HMO, human milk oligosaccharide; MAZ, microbiota-for-age Z-score; *E. coli*, *Escherichia coli*; *S. aureus*, *Staphylococcus aureus*; *D. longicatna*, *Dorea longicatna*.

The Fetal Microbiota

Microbial invasion of the amniotic cavity is associated with preterm birth and other adverse birth outcomes; however, the hypothesis of fetal exposure to a more complex placental microbiota in healthy pregnancies remains under debate [15,23,24]. Similar to the vaginal microbiota, taxa within the placental microbiota have been associated with both preterm birth and LBW, suggesting that disruption of the normal microbial ecosystem *in utero* may affect growth and duration of pregnancy [4,25]. A recent study of 1391 pregnant women in Malawi, the largest of its kind, found that the presence of *Sneathia sanguinengens* and *Peptostreptococcus anaerobius* in both vaginal and placental samples was associated with a lower newborn LAZ, using culture-independent techniques [26]. *S. sanguinengens*, in addition to *Phascolarctobacterium succinatutens*, and an unidentified *Lachnospiraceae* sp. were also inversely associated with newborn head circumference for age Z-score.

The first stool of the infant (meconium) was previously thought to be sterile, yet numerous studies have since reported a complex meconium microbiota signature [27,28]. Similar to the placental and amniotic microbiota, 16S rDNA is more prevalent in the meconium of preterm infants, suggesting a potential role of prenatal microbial exposure in growth and length of gestation [29]. However, it is yet unclear whether this microbial presence is a cause or effect of

Box 1. Microbial Influence on Neurodevelopment in Undernutrition

In addition to increased mortality, immune defects and later-life chronic disease risk, childhood undernutrition is also associated with impaired neurodevelopment and subsequent cognition in later life. Some 75% of brain growth occurs during the first 1000 days, after which synaptic proliferation and pruning occurs throughout adolescence and into the third decade of life [104]. Child head circumference is a strong predictor of IQ and hence is employed as an additional anthropometric measure alongside WHZ, LAZ, and MUAC to assess children's nutritional status. The brain during childhood has enormous metabolic capacity comprising 5–10% of total body mass and accounting for up to 50% of the body's basal metabolic energy rate and hence is particularly susceptible to the reduced energy intake associated with undernutrition [104]. Due to the capacity of intestinal microbial communities to regulate nutritional energy harvest, the gut microbiota may play a regulatory role in neurodevelopment during the first 1000 days. Germ-free mice display impaired blood–brain barrier formation and myelination, suggesting an essential role for the microbiota in structural and functional neurodevelopment [38,105]. Child cohort studies from LMICs have reported that diarrhea and enteric infection are predictive of cognitive delay in later childhood [106,107]. Furthermore, EED biomarkers have also been associated with neurodevelopment in LMICs. Plasma citrulline, a marker of gastrointestinal mucosal surface area, is positively associated with higher gross motor development scores; however, novel inflammatory markers of EED also demonstrated positive associations [108]. Hence the concurrent maturation of both the gut microbiota and central nervous systems within the first 1000 days highlights the potential for gut microbiota interventions to optimise child neurodevelopment in LMICs.

preterm birth and impaired growth. Prenatal microbial exposure may also have long-term influence on offspring immune development, which may impact growth trajectories postnatally. Transient colonization of germ-free mice during pregnancy primes innate immune maturity in their germ-free offspring, resulting in better protection against infection [30]. As infection plays an important role in impaired growth, these results suggest that translocation of intestinal microbial products from mother to fetus plays an essential role in the immune maturity and plausibly the growth phenotypes of offspring after birth.

Days 270–450: Infancy (First 6 Months of Life)

During and immediately after birth, the newborn is exposed to complex microbial communities in the external environment. Both the composition and function of the early infant microbiota is primarily defined by birth mode, the maternal microbiota, antibiotic exposure, and early-life feeding practices. As breastfeeding status profoundly influences infant growth and microbial succession in early life, the milk–microbiota interaction may act as a primary target for intervention through which faltering growth could be addressed.

The Preterm Microbiota

Microbial succession in the infant is profoundly influenced by gestational age. It is unclear, however, whether the 'immature' preterm microbiota plays a causal role in reduced growth and elevated risk of infectious and inflammatory disorders, such as sepsis and necrotizing enterocolitis, or whether the preterm microbiota assembles as an adaptive response to such stressors. Recent findings suggest that the preterm gut microbiota follows a three-phased pattern of assembly, beginning with a facultative anaerobe-dominated composition (primarily Bacilli; Phase 1) followed by expansion of obligate anaerobes and fermentation-based metabolism dominated by Gammaproteobacteria (Phase 2) and Clostridia (Phase 3) [31]. Transition between phases is strongly influenced by postmenstrual age (PMA), nutrition, and medication usage, and delayed transition to P3 is strongly associated with reduced weight-for-age Z-score [32]. The observation that PMA strongly drives gut microbial transition in a nonrandom manner suggests that developmental factors within the mucosal immune system or intestinal metabolic environment may drive host-microbial homeostasis and mutualism. The accumulation of Paneth cells in the small intestine in later PMA stages is essential for antimicrobial peptide production, which may initiate host tolerance to a complex microbiota [33]. In order to define microbiota 'immaturity' in these contexts, further examination of

neonatal microbiota assembly in both preterm and term infants is required in relation to growth. However, these data have important implications for undernourished infants, whose 'immature' gut microbiota signatures and immunophenotypes are similar to those of preterm infants, suggesting that optimization of early-life infant–microbe mutualism may help to stimulate healthy growth and development.

Maturation of the Healthy Gut Microbiota

The microbiota of the healthy newborn closely matches the maternal stool, vaginal, or skin microbiota, depending on delivery mode. The first colonizers of the infant gut microbiota are typically facultative anaerobes, followed by the accumulation of obligate anaerobes, including *Bifidobacterium*, *Bacteroides*, and *Clostridium* during the following 6 months [34–36]. The diversity of the microbiota remains narrow in early infancy and is dominated by species involved in **human milk oligosaccharide (HMO)** metabolism in breastfed infants. It has been estimated that 25–30% of the infant bacterial microbiota originates from breast milk [37]. Succession of the early-life microbiota plays important roles in growth and maturation of the endocrine, mucosal immune and central nervous systems [38–40]. Germ-free mice exhibit significantly reduced weight and length prior to weaning compared with conventionally raised animals [41]. This may be due to a number of factors, including reduced capacity for energy harvest from the diet; however, it has also been theorized that microbiota-induced interactions with insulin-like growth factor 1 (IGF-1), that remain uncharacterized, may also play a role in early-life growth.

One of the challenges of the microbiome field, however, is in defining a 'normal' or 'healthy' microbiome, which can vary distinctly between gender and across different ages, geographical regions, and medical contexts. In order to identify an 'abnormal' microbiome and its associations with adverse health outcomes, it is essential to contextualize the 'healthy' microbiome to the appropriate setting. In the context of malnutrition in LMIC, a healthy microbiome is considered in those of healthy WHZ (weight-for-height Z-score) or LAZ, yet may differ distinctly to that of healthy growing infants in high-income settings. Hence, future developments in the field will require careful consideration of environmental setting.

A landmark study developed an index to assess maturation of the gut microbiota in the first 2 years of life in the context of malnutrition, known as the microbiota-for-age Z-score (MAZ). Using a reference cohort of children from Bangladesh with normal growth, the authors used a machine-learning model to identify 25 bacterial taxa that were discriminant for age and healthy growth [42]. The most age-discriminatory taxa in the first 6 months of infancy included several *Bifidobacterium* and *Streptococcus* species, namely *Bifidobacterium longum* and *Streptococcus thermophilus*. An identical model was applied to a Malawian cohort in which *B. longum* was also the most discriminatory taxon for age [43].

The role of the early-life microbiome in growth may be dependent on both the composition and function of the acquired microbiome. Metagenomic analysis of the infant microbiome suggests that the functional potential of the microbiome may play essential roles in nutritional status in addition to composition alone. For example, the inheritance of starch utilization genes and bile acid metabolism genes, which may differ between individuals, likely plays an essential role in nutrient absorption and hence growth in early life [9,44].

Breast Milk and Early Gut Microbiota Maturation

Microbial succession in preterm infants appears to be rescued by breastfeeding [45], suggesting an essential role for breast milk in normal infant microbiota assembly. Furthermore, growth is

also significantly greater in breast-fed compared to formula-fed preterm infants following discharge from hospital [46]. Hence the impact of breastfeeding on infant microbiota assembly may enhance growth and developmental pathways. Breast milk harbors a diverse microbiota, which varies by maternal weight and delivery mode, and between populations, but is most commonly characterized by Proteobacteria (primarily *Pseudomonas*), *Staphylococcus*, and *Streptococcus*, and is compositionally distinct to the skin, oral, and gut microbiome [47,48].

The relationship between the breast-milk microbiota and infant growth has not been investigated; however, recent studies support the essential role of HMOs in defining early-life growth phenotypes. Genetic factors influence HMO production whereby carriers of an active fucosyltransferase 2 (*FUT2*) gene, known as secretors, produce more HMOs, both fucosylated and sialylated structures [49]. Maternal secretor status hence influences infant microbiota composition, whereby *Bifidobacterium* is more abundant in infants of maternal secretors [50,51]. Maternal secretor status *per se* has not been associated with infant growth [52]; however, individual HMOs have been associated with infant growth and anthropometry in both high-income and low-income settings [53,54]. In a cohort of Gambian mothers and infants 3'-sialyllactose was positively associated whilst sialyllacto-*N*-neotetraose was negatively associated with weight-for-age Z-score (WAZ). Furthermore, difucosyllacto-*N*-hexaose a, lacto-*N*-fucopentaose I and III were positively associated with LAZ.

Charbonneau *et al.* dissected the mechanisms by which HMOs interact with the infant microbiota to regulate growth [49]. Mothers of stunted infants in Malawi exhibited a significantly lower abundance of HMOs in breast milk at 6 months, particularly sialylated HMOs, including sialyllacto-*N*-tetraose b, which were the most growth-discriminatory. The undernutrition phenotypes were recapitulated in animals by colonizing germ-free mice and piglets with a consortium of organisms cultured from the stool of a child with severe stunting, and feeding the animals a suboptimal 'Malawian diet'. However, supplementing the animals with bovine milk oligosaccharides that were structurally similar to HMOs promoted weight gain, lean mass, and bone volume in the animals. The growth effects were not observed in germ-free animals, suggesting a microbiota-dependent effect. Hence, HMOs play a unique role in shaping the infant microbiota in early life and mediating growth.

Days 450–1000: Childhood (6 Months to 2 Years)

Following breastfeeding, the introduction of solid foods initiates a rapid increase in the structural and functional diversity of the infant microbiota, creating a mature, adult-like state. This mature microbiome is dominated by species capable of degrading glycans, mucin, and complex carbohydrates as well as the production of short-chain fatty acids. Concurrently, the period from introduction of complementary foods (6 months of age) to 2 years of age represents a crucial period for child growth, particularly linear growth [55]. In low-income settings, however, food insecurity and environmental exposures in conditions of poor water, sanitation, and hygiene pose a risk of exposure to pathogens (Box 2) and undernutrition in this period, which may perturb the intertwining gut microbial and growth pathways (Figure 2).

Microbiota Maturity

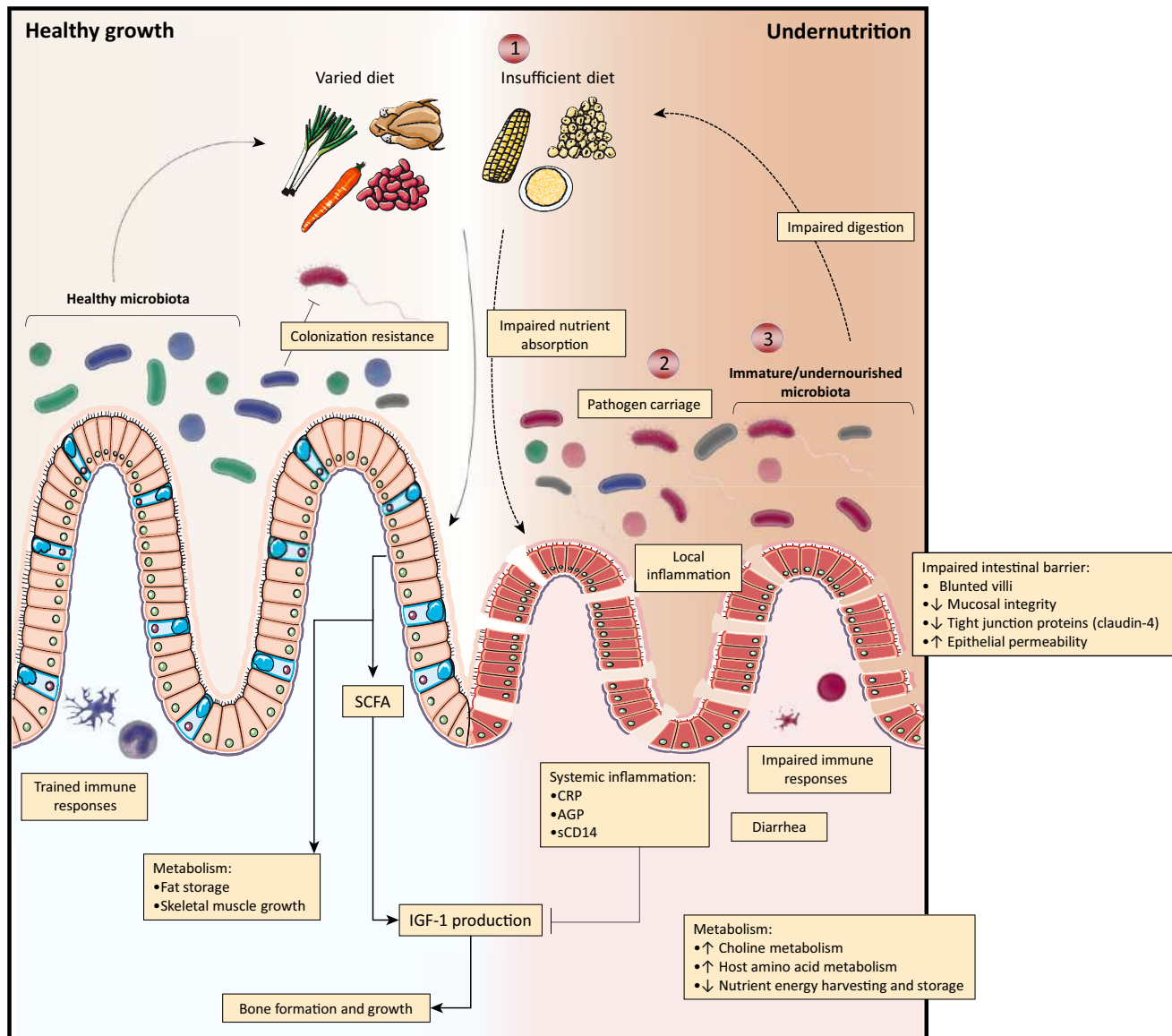
Subramanian *et al.* carefully dissected this maturation process and its role in growth through development of the MAZ in a cohort of infants from Bangladesh. *Faecalibacterium prausnitzii*, *Ruminococcus* species and *Dorea* species (*Dorea longicatna* and *Dorea formicigenerans*) were among the most age-discriminatory species from 6 to 24 months of age in healthy infants [42]. Children with **severe acute malnutrition (SAM)** (WHZ <−3) exhibited significantly lower MAZ, indicating microbiota immaturity compared with healthy children.

Box 2. The Role of Infection, Enteropathogens, and Diarrhea in Undernutrition

Beyond 6 months, children in LMICs become exposed to a greater number of pathogens through water, solid foods, soil, and the surrounding environment. This elevated exposure to pathogenic organisms may disturb normal gut microbiota assembly and hence impair growth. Large multicountry studies have observed that carriage of **enteropathogens** begins very early in infancy and is almost ubiquitous among children in impoverished LMIC settings in the first 2 years of life, yet pathogen carriage is often subclinical [109]. Recent data from the MAL-ED study in seven different LMICs observed that infants carried on average at least one enteropathogen in nondiarrheal stools [110]. A higher number of pathogens was inversely associated with both ponderal and linear growth in the first 2 years of life. *Giardia* and associated enteroinvasive pathogens, and those involved in mucosal disruption, appeared to have the most profound influence on systemic inflammation, gut inflammation, and impaired growth [111]. In animal models the pathogenic inflammatory effect of *Giardia* during undernutrition is dependent upon its interaction with the host microbiota and is amplified through coinfection with enteroaggregative *Escherichia coli* [112]. This interaction between common enteropathogens and the microbiota remains underexplored, and little is known about whether enteropathogens disturb the commensal microbial composition and function and whether this interferes with the host-microbiota pathways that mediate growth. Wagner *et al.* conducted an elegant study that described the role of *Bacteroides fragilis* as a pathobiont, whereby the presence or absence of the *B. fragilis* toxin (*bft*) discriminated between enterotoxigenic and nontoxigenic strains [113]. Transfer of a culture collection from stool containing a strain of toxigenic *B. fragilis* from a stunted child to germ-free mice induced significant weight loss and impaired host energy metabolism compared to animals colonized with stool from a healthy donor carrying a nontoxigenic strain. Removal of the toxigenic strain prevented weight loss in the animals; however, addition of this strain to the healthy donor's culture collection did not induce weight loss when transferred to the animals. These results suggest that the presence of certain pathobionts is not sufficient to induce malnutrition phenotypes alone but rather are dependent upon expression of virulence factors and interaction with the larger intestinal microbial community.

Germ-free animal studies have provided further evidence of the mechanisms by which microbiota immaturity contributes to undernutrition through interaction with the diet. A study of twin pairs discordant for oedematous SAM in Malawi failed to identify a distinct microbiota signature of edematous-SAM [56]. However, following transfer into germ-free mice, the 'undernourished' microbiota induced significant weight loss in the animals when administered in combination with a nutrient-insufficient 'Malawian diet'. Weight loss was associated with perturbed amino acid, carbohydrate, and TCA cycle metabolism in both urinary and fecal metabolic profiles, which were only partly restored by ready-to-use therapeutic food. These findings suggest that the intestinal microbiota in undernutrition may trigger a catabolic state, including disturbed amino acid metabolism, which may contribute to weight loss and associated metabolic disturbances. Interestingly, however, weight and lean mass can be recovered in mice via colonization with a consortium of weight-discriminatory taxa [43].

The programmed maturation of the microbiota in early childhood appears to influence linear as well as ponderal growth. Stunting – defined as LAZ < -2 – is the most common form of undernutrition worldwide [55]. A small study in India examined the longitudinal succession of the infant microbiota from birth to 2 years, reporting that reduced relative abundance of *B. longum* and *Lactobacillus mucosae* in addition to elevated relative abundance of *Desulfovibrio* spp. was associated with stunting [57]. In a secondary analysis of data from children aged 0–2 years in Malawi and Bangladesh, Gough and colleagues reported that reduced microbiota diversity, elevated *Acidaminococcus* spp. abundance, and elevated glutamate fermentation pathways were all predictive of future linear growth deficits [58]. Furthermore, in-depth characterization of the fecal and upper gastrointestinal (duodenal and gastric) microbiome from infants in the Central African Republic and Madagascar found that taxa of oropharyngeal origin were overrepresented in these lower gastrointestinal regions of stunted infants. These findings suggest that decompartmentalization of the gastrointestinal tract occurs in stunting whereby oral taxa translocate to lower regions and may plausibly play a role in linear growth deficits and its associated inflammation [117].



Trends in Microbiology

Figure 2. The Pathways by Which Microbes in the Intestinal Lumen Interact with Host Growth in Healthy versus Malnourished Children. The commensal gut microbiota regulates a number of processes that affect child growth in the first 1000 days. The structural and functional integrity of the intestinal barrier (mucus layer, antimicrobial peptides, epithelium and tight junctions) is tightly regulated by gut microbial composition in healthy infants but becomes perturbed in undernutrition. A healthy microbiome also provides colonization resistance against invading pathogens. The interaction between the commensal microbiome and the innate immune system maintains immune homeostasis and recognition of antigens. The host microbiome also regulates somatotrophic axis (GH/IGF-1) activity to stimulate growth in early life, through mechanisms that remain unknown. The microbiome plays a critical role in nutrient and host metabolism, thereby affecting digestion, absorption, and energy storage. A dysbiotic microbiome in early life may impair each of these pathways related to growth whereby an immature microbiota fails to protect the intestinal barrier leading to villous blunting, mucus degradation, intestinal permeability, and impaired immune responses. These intestinal impairments may contribute to environmental enteric dysfunction (EED), chronic systemic inflammation, infectious morbidity and diarrhea, each of which may impair the trajectories of growth. A combination of 'hits' may be required to induce EED and undernutrition phenotypes, including both an insufficient or inadequate diet (1), pathogen carriage (2), and/or a dysbiotic microbiome (3). Microbiome dysbiosis also may impair metabolism of key nutrients, including essential amino acids, thereby preventing normal growth. A disturbed gut microbiota composition may impair the normal production of growth hormones. Image adapted from Servier Medical Art under a CC-BY license. SCD14, soluble cluster of differentiation 14; AGP, alpha-1-acid glycoprotein; CRP, C-reactive protein; IGF-1, insulin-like growth factor 1; SCFA, short-chain fatty acids.

Microbial and Host Metabolism

Both SAM and stunting are associated with disturbed host metabolic phenotypes, particularly energy metabolism, nutrient metabolism, and amino acid turnover [59]. Little mechanistic evidence however has demonstrated if and how the dysbiotic microbiota observed in undernutrition contributes to these altered metabolic processes. In experimental mice, zinc- and protein-deficient diets induced major changes in the intestinal microbiota following weaning, accompanied by disturbed energy metabolism and upregulated dietary choline processing [60]. Furthermore, the microbiota and microbial-derived metabolites fail to recover during catch-up growth following a period of undernutrition, suggesting that undernutrition may persistently disrupt gut microbial metabolism [61]. Stunting in Brazilian children was associated with greater abundance of urinary phenylacetylglutamine (PAG), 4-cresyl sulfate (4-CS), and 3-indoxyl sulfate (3-IS), which are microbial metabolites of the amino acids phenylalanine, tyrosine, and tryptophan, respectively [62]. Reductions in essential amino acids are also associated with the microbiome in wasting [63]. Hence undernutrition appears to be associated with, and plausibly mediated by, greater proteolytic activity of the host microbiota. Further research is warranted to delineate the host-induced versus microbial-induced changes to host metabolism observed in undernutrition. Million *et al.* also observed that SAM was associated with depletion of obligate anaerobes and the methanogenic archaeal species *Methanobrevibacter smithii*, which appeared consistent across five cohorts from Africa and Asia [42,56,64,65]. The authors hypothesized that this dysbiosis decreases fecal antioxidant capacity and hence impairs microbial nutrient energy harvesting, thereby exacerbating malnutrition.

Disturbances in microbial and host metabolism may be driven by differences in microbiome function as well as composition, through the acquisition of particular microbial genes. Using genome-scale metabolic models (GEMs), it has been shown that particular metagenomic pathways involved in fatty acid and amino acid metabolism are less abundant in cases of malnutrition, suggesting that metagenomes may influence nutritional status [63].

Microbiota Impact on Endocrine Pathways

There is some evidence that the effect of the microbiota on growth phenotypes is mediated through indirect influence on the somatotrophic axis. Inflammatory proteins such as C-reactive protein (CRP) and alpha-1 acid glycoprotein (AGP), which are stimulated by infection, are inversely associated with IGF-1 and linear growth [66]. However, germ-free mice gain significantly less weight and body length during lactation compared with conventionally raised animals [41]. Interestingly, these effects become more pronounced following weaning and in the presence of a depleted diet and appear to be attributed to significantly reduced skeletal growth. IGF-1, a mediator of the effects of growth hormone (GH), was also significantly depleted in germ-free animals, an effect that was strongest following weaning, supporting the essential role of the microbiota in endocrine-mediated growth pathways. In both *Drosophila* and mice, particular strains of *Lactobacillus plantarum* restore normal growth, IGF-1 production and activity, and sensitivity of peripheral tissue to GH [41,67], the mechanism of which is unclear. Recent evidence suggests that short-chain fatty acids restore bone mass, growth, and IGF-1 in animals following growth deficits induced by antibiotics [39]. Hence, normal microbial products of fermentation may play a regulatory role in somatotrophic axis stability and growth phenotypes in early life, which may have implications for stunting and wasting observed in infants.

Environmental Enteric Dysfunction

An attractive hypothesis surrounding the mechanisms of growth faltering suggests that a condition termed **environmental enteric dysfunction (EED)**, also called environmental **enteropathy**, which is characterized by subclinical structural and functional small intestinal changes, mediates the suppression of early-life growth pathways through impaired nutrient absorption (via villous blunting) and chronic inflammation [68]. However, data on the impact of EED on stunting remain heterogeneous, and the role of the microbiota in EED has not yet been established. However, substantial evidence suggests an essential role for the gut microbiota in priming the structural integrity of the intestinal barrier in early life (Box 3), and recent reports indicate that microbiota dysbiosis in LMIC settings may trigger EED and hence undernutrition [69]. One of the challenges of associating EED with the microbiota is the lack of easy microbiota sampling of the upper gastrointestinal tract where EED occurs. A characteristic fecal microbiota signature of EED does not exist, but some evidence supports the overabundance of *Megasphaera* and *Sutterella* in EED, which have also been associated with coeliac and Crohn's disease, respectively [70]. Animal studies have provided deeper insight into the microbial composition of the upper gastrointestinal tract in experimental EED and undernutrition [71,72]. Hashimoto *et al.* reported that angiotensin I converting enzyme (peptidyl-dipeptidase A) 2 (Ace2) regulates amino acid metabolism, gut microbial homeostasis, and antimicrobial peptide production [73]. Ace2 deficiency induced enteropathy in mice under conditions of protein malnutrition, which could be transferred to other animals through fecal transplantation and restored following dietary tryptophan treatment. Brown *et al.* developed a novel model of EED and undernutrition through comprehensive 16S rDNA sequencing and metabolic phenotyping of the murine small intestine [74]. A cocktail of nonpathogenic Bacteroidales species and *Escherichia coli* in combination with an undernourished diet produced growth deficits, impaired tolerance to pathogen challenge, and characteristic features of enteropathy (reduced villous height and tight-junction protein expression, increased intestinal permeability, and intestinal inflammation). Mucosal immune responses may play an essential role in mediating the effects of a 'dysbiotic' microbiota in EED and undernutrition [75]. In SAM, immunoglobulin A

Box 3. Microbiota Priming of Infant Gut Structure

The intestinal barrier functions as a selective gateway to prevent the translocation of microbes and their toxins, whilst allowing nutrients from the intestinal lumen into the systemic circulation. This barrier comprises several protective layers beginning with a mucus layer produced by goblet cells, which contains a concentration gradient of antimicrobial peptides. An epithelial monolayer, sealed by tight junctions, lies beneath the mucus layer, acting as a physical barrier. Finally the underlying lamina propria contains a plethora of immune cells that contribute to the innate immune system. If these layers are compromised, bacteria and their toxins such as lipopolysaccharide (LPS) can translocate across the impaired barrier eliciting a local and systemic inflammatory response in the host. Impaired intestinal barrier function, intestinal and systemic inflammation may underlie both stunting and SAM [68]. These intestinal pathologies, referred to as environmental enteric dysfunction (EED), in addition to chronic pathogen exposure and inflammatory stimuli may impair innate and acquired immune responses, thereby increasing infectious morbidity and undernutrition [75]. However, recent evidence suggests that the commensal intestinal microbiota acts as an additional primary layer within this intestinal barrier to assist with the selective absorption of nutrients and protection against invading pathogens. Indeed, the acquisition of a commensal microbiome is essential for priming this complex gut structure. Studies using human intestinal organoids (HIOs) demonstrate that exposure of nonpathogenic *E. coli* to HIOs induces transcriptional responses associated with antimicrobial defence and epithelial barrier production in addition to the production of antimicrobial peptides, the maturation of enterocytes, and the formation of a mucus layer [114]. Genome-wide analysis of murine infant intestinal epithelial cells (IECs) has also identified 'functional' DNA methylation signatures which are microbiota-dependent and involved in the maturation of the transcriptional programming of IECs postnatally [115]. The dependence of infant intestinal structural programming on the introduction and interaction of a commensal microbiota suggests that 'dysbiotic' microbial communities may impair this programmed intestinal structuring in the first 1000 days, thereby contributing to EED, as has been observed in similar conditions including necrotizing enterocolitis [116]. The impaired intestinal structure observed in EED has been hypothesized to play a causal role in child undernutrition via inflammatory mechanisms [68], and animal studies have reported mechanistic evidence by which specific microbial communities interact with diet to produce EED phenotypes and impaired growth [74].

(IgA) appears to target a consortium of species within the intestinal microbiota dominated by Enterobacteriaceae, which, if isolated and transferred to germ-free mice, induces weight loss and enteropathy [76]. Microbes with less affinity to IgA, or the IgA-targeted fraction from healthy donors, do not induce such an effect. Hence, immune-microbiota communication appears to be dependent upon nutritional status, which may mediate responses to infection and future growth.

Intergenerationality of a Malnourished Microbiome

Undernutrition and its associated sequelae are perpetuated across generations. Mothers with short stature have higher risk of stunted children [55]. Much of this intergenerationality may be attributed to epigenetic modifications that impair offspring growth. Here, we propose that intergenerationality of an 'undernourished' microbiome also contributes to growth impairments across generations. Intergenerational transmission of a dysbiotic microbiota has been proposed in metabolic disorders, including obesity [77] and in enteropathic conditions such as colitis [78]. Furthermore, the deleterious effects of nutrient deficiencies on the gut microbiota, namely dietary fiber, are reversible within a single generation, but become largely irreversible if reintroduced into the diet in subsequent generations [79]. Hence diet-induced microbial extinctions may occur in states of undernutrition, which are compounded across generations and which contribute to the cycle of intergenerational undernutrition.

Intervening in the Malnourished Microbiota during the First 1000 Days and Beyond

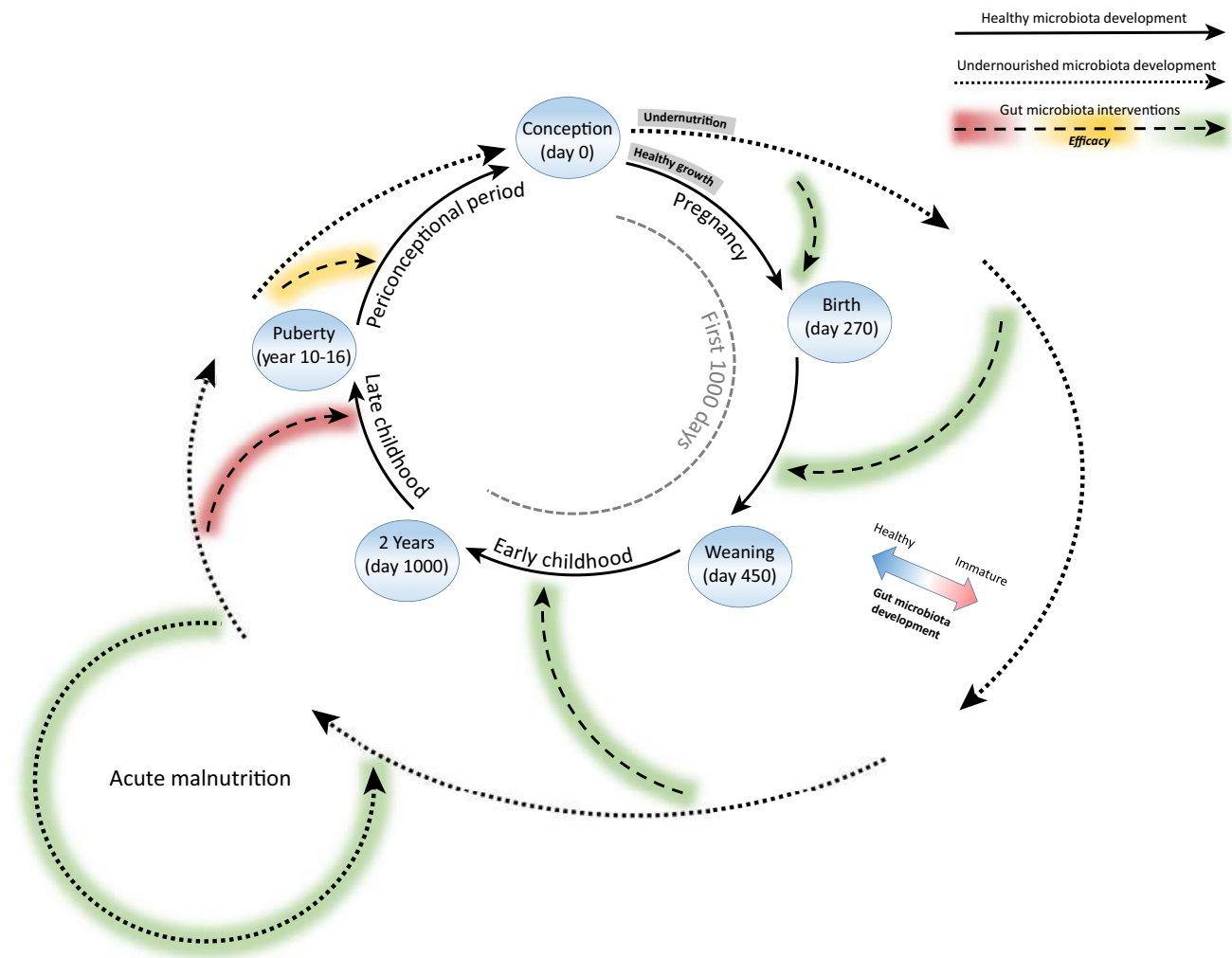
The growing evidence for a causal role of disturbed gut microbiota composition and function in child malnutrition warrants intervention studies using microbiota-targeted therapies to prevent or treat undernutrition. However, the cyclical process of undernutrition raises questions about what is the most effective period in which to target interventions. The first 1000 days contain windows of opportunity within which a disturbed microbiota may be amenable to intervention (Figure 3).

Prenatal Preventative Interventions

The evidence that host-microbe interactions *in utero* may influence fetal and infant growth trajectories raises the possibility that manipulation of the maternal microbiota during pregnancy could impact infant growth. Treatment for maternal periodontal disease, for example, may reduce the risk of low birth weight [80]. Conversely, poor water, sanitation, and hygiene (WASH), and hence exposure to a more pathogenic environment, is associated with preterm birth and low birthweight, suggesting that intensive WASH interventions may improve fetal growth [81]. Antibiotic use during pregnancy has been associated with LBW in high-income countries [82], and with increased birth weight, length, and reduced preterm birth in LMIC, plausibly due to the reduction of pathogens in the female reproductive tract that stimulate preterm birth or SGA [83]. Moreover, antibiotic use during pregnancy continued to exert beneficial effects on postnatal growth in a recent Malawian study, leading to reductions in stunting up to 5 years of age [84]. The effect of **probiotics** and **prebiotics** on birth outcomes remains unclear. Maternal probiotic intake during pregnancy has been associated with reduced risk of pre-term delivery [85]. However, a recent meta-analysis found no effect of either maternal prebiotics or probiotics on birth weight or other birth outcomes [86].

Postnatal Preventative Interventions

A meta-analysis of antibiotic trials in LMICs has shown benefits for ponderal and linear growth, which may be mediated by effects on the gut microbiota [87]. In high-income settings,



Trends in Microbiology

Figure 3. Microbiota Interventions during the Life Cycle. The life cycle contains windows of opportunity in which microbiota-targeted therapies may be more amenable to intervention in order to enhance growth. Undernutrition is a cyclical process that is perpetuated across generations. Interventions that target the microbiota during certain windows in the life cycle may help to improve child growth. From the point of conception, the mother's microbiota may influence fetal growth and duration of pregnancy. Optimizing maternal oral health, hygiene, and nutrition may help to improve the microbiome during pregnancy in the mouth, vagina, and gut in order to prevent inflammatory stimuli or translocation of bacteria and toxins to the fetus thereby inhibiting fetal growth. Microbiota assembly in early infancy may be a critical period in which interventions could be successful. Probiotic interventions soon after birth can significantly increase weight and prevent infection. The following 2 years represent a period of microbial assembly in which the microbiome could be targeted to optimize healthy composition. From the period of 2 years to adolescence, microbiota-targeted interventions may be less effective for growth; however, in cases of severe acute malnutrition, certain therapies could be effective. The preconception period may represent a further window of opportunity in which to target the maternal microbiome to optimize fetal growth. Green arrows represent windows of opportunity in which the microbiota may be most amenable to interventions, amber represents periods in which the effects of such interventions are less clear, and red arrows represent periods in which microbiota-targeted interventions are unlikely to have a substantial impact on child growth.

observational evidence suggests that probiotics can improve growth in preterm or LBW infants [88] but many interventions have failed to report a beneficial effect [89]. Conversely, several trials report positive effects of probiotics on weight gain in children at risk of undernutrition in LMIC settings [90–92]. A recent intervention trial among 4500 newborn infants in India reported significantly reduced rates of sepsis and death following 7-day treatment with an oral symbiotic (*Lactobacillus plantarum* + fructo-oligosaccharides) beginning on day 2–4 of life; the

intervention also significantly increased weight in infants [91]. These results were obtained among a cohort of healthy weight, term infants and hence raise the possibility that such interventions may have an even greater effect in preterm or LBW infants at higher risk of sepsis and undernutrition. Complex carbohydrates are readily fermented by the intestinal microbiota, producing short-chain fatty acids (SCFAs) and other metabolites beneficial for intestinal epithelial integrity. Locally sourced legumes containing such fibers have demonstrated some ability to reduce deficits in LAZ, potentially through amelioration of EED [93].

Treating Undernutrition

Gut microbiota-targeted interventions may not only prevent but also help to treat both acute and chronic undernutrition. Current **ready-to-use therapeutic foods (RUTFs)**, however, are insufficient to persistently restore microbiota maturity, which reverts to an immature state 3–4 months post-treatment [42]. These findings have crucial implications for approaches to SAM treatment. Mortality remains as high as 42% in children hospitalized with complicated SAM, and relapse occurs in a substantial proportion after discharge [94]; furthermore, children treated for SAM exhibit long-term growth deficits [94,95]. Hence, future interventions should examine the use of combined nutrition plus microbiota-targeted treatments to persistently restore microbiota maturity and healthy growth.

The use of antibiotics in all cases of complicated SAM was adopted into WHO guidelines in 1999; however, their benefit for nutritional recovery remains uncertain. One large study of 2767 children with uncomplicated SAM in Malawi reported that the addition of antibiotics to standard RUTF significantly improved nutritional recovery and reduced mortality [96]. However, similar large trials among children with uncomplicated SAM in Niger, and complicated SAM in Kenya, reported no benefits of antibiotic treatment on mortality or nutritional recovery [97,98]. Data from probiotic intervention studies provide promising evidence for their potential in undernutrition; however, their potential as therapeutic interventions following SAM remains unclear [99]. Future studies require careful consideration of dosage, timing of intervention, and selection of probiotic strains capable of colonizing the undernourished intestine and suitable to the specific setting [100]. Finally, as has been observed through the restoration of metabolic disruption in overnutrition, complete recolonization of the intestinal microbiota through fecal microbiota transplantation (FMT) from healthy donors poses the potential to restore intestinal function, metabolic homeostasis, and growth where children with SAM fail to respond to standard treatment (<https://clinicaltrials.gov/ct2/show/NCT03087097>). Indeed, future undernutrition therapies should consider dual complementary approaches that combine nutritional and microbiota interventions to optimize treatment.

Beyond 1000 Days

Windows of opportunity to enhance growth may also exist beyond the first 1000 days (Figure 3). The assembly and maturation of the gut microbiota has largely occurred by the age of 2 or 3 years, hence strategies to target the gut microbiota after this period may have less impact. However, the periconceptional period in adolescent females, beginning 14 weeks prior to conception [101], may represent an additional window of opportunity in which to optimize nutrition and the gut microbiota [102], which may have significant benefits for health and nutrition behaviors [103], suggesting potential for microbiota-targeted therapies as well. Hence, an expanded view of early life that includes this preconception period, may help to optimize nutritional and microbiota-targeted therapies to prevent the intergenerational cycle of malnutrition.

Concluding Remarks

As DNA-sequencing technologies continue to undergo rapid advances, the knowledge that has been gained about coevolution of humans with our microbial symbionts has grown enormously. Arguably the most pressing focus for this emerging knowledge is global child health. Despite under-5 mortality rates falling by half since 1990, undernutrition continues to underlie 45% of all child deaths, and there are few current effective preventive interventions. One-quarter of under-5 children globally are stunted and, due to population growth, the absolute number of stunted children in sub-Saharan Africa is increasing. Thus, the renewed focus on global public health is for children not just to survive but to thrive. Substantial evidence suggests that the trajectories of child growth and development are primed during the first 1000 days, or even earlier. Hence, this early-life period represents a critical window in which to focus mechanistic research and interventions. Here we have reviewed the rapidly growing knowledge of how the human microbiota regulates growth and developmental trajectories during the first 1000 days. The concurrent assembly of the microbiota alongside endocrine, immune, and metabolic pathways indicates tight regulatory interdependence between microbiota and host underlying growth and development. Despite promising findings regarding microbiota-induced effects on child growth, a number of outstanding questions surrounding the mechanisms of these interactions remain (see Outstanding Questions). Deeper insight is required into how the microbiota of the infant responds to prenatal influences, high pathogen burden, and different dietary patterns. Better understanding these host–microbiota interactions in early-life growth will inform targeted interventions to reduce global morbidity and mortality associated with child undernutrition.

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Outstanding Questions

What is the composition and function of the small intestinal microbiota in the context of undernutrition? Characterizing the microbiota in the upper gastrointestinal tract, where EED and most nutrient absorption occurs, would provide promising insights that may not be observed by studying the fecal microbiota alone.

What is the composition of the breast milk microbiota in mothers of children with and without undernutrition? How does it relate to the child's gut microbiota composition?

What are the proportional contributions of the microbiome in prenatal versus postnatal growth?

What is the role of the infant gut microbiome in stunting? Most studies examining the microbiome in undernutrition have focussed on SAM, yet few large longitudinal studies have examined the influence on linear growth faltering, which is more common.

How does the recovery of the microbiome affect immune function after nutritional rehabilitation for SAM?

Does an altered microbiome play a role in the clinical outcomes associated with oedematous malnutrition (kwashiorkor)? Oedematous-SAM remains an enigmatic condition, which may have microbial influence.

Can a microbiota maturity index be used to predict clinical outcomes in stunting or in SAM in order to identify high-risk patients?

What is the role of the maternal vaginal, gut, and oral microbiomes in seeding the infant microbiome in the context of undernutrition, and does this intergenerational transfer influence postnatal growth?

What probiotics are effective in colonizing the 'undernourished' gut in both stunted or SAM children?

What microbiota-targeted intervention (antibiotics, diet, probiotics, FMT) is most effective and sustainable for resolving undernutrition and associated outcomes? Are combined therapies more effective?

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