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Malnourished Microbes: Host–Microbiome Interactions in Child Undernutrition

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Abstract

Childhood undernutrition is a major global health burden that is only partially resolved by nutritional interventions. Both chronic and acute forms of child undernutrition are characterized by derangements in multiple biological systems including metabolism, immunity, and endocrine systems. A growing body of evidence supports a role of the gut microbiome in mediating these pathways influencing early life growth. Observational studies report alterations in the gut microbiome of undernourished children, while preclinical studies suggest that this can trigger intestinal enteropathy, alter host metabolism, and disrupt immune-mediated resistance against enteropathogens, each of which contribute to poor early life growth. Here, we compile evidence from preclinical and clinical studies and describe the emerging pathophysiological pathways by which the early life gut microbiome influences host metabolism, immunity, intestinal function, endocrine regulation, and other pathways contributing to child undernutrition. We discuss emerging microbiome-directed therapies and consider future research directions to identify and target microbiome-sensitive pathways in child undernutrition.

Contents

1. THE BURDEN AND PATHOPHYSIOLOGY OF CHILD UNDERNUTRITION

Stunting: a form of chronic undernutrition classified as a length-for-age Z-score <−2

Environmental enteric dysfunction (EED): a disorder of the small intestine commonly presenting in low-resource settings and characterized by villous blunting, inflammation, and barrier defects

Insulin-like growth factor (IGF-1): a hormone central to the somatotropic axis that has important functions in stimulating skeletal muscle growth, particularly in early life

Childhood undernutrition underlies 45% of all child deaths globally and has a lifelong impact on health (11). Despite decreasing prevalence in recent years, more than 1 in 5 children under the age of 5 around the world exhibit at least one form of chronic or acute undernutrition (141). Stunting [defined as a length-for-age Z-score (LAZ) < −2], which affects 22% children globally, is associated with poorer cognitive development, reduced lifelong economic productivity, and greater chronic disease risk in adulthood (103). Wasting (defined as a weight-for-height Z-score < −2) affects nearly 7% of children around the world and is associated with high mortality in addition to poor long-term cognitive and health deficits (17, 81, 82, 88). Nutritional therapies are insufficient to fully restore growth deficits and associated pathophysiological consequences of undernutrition. Stunting is reduced by only 12–14% by standard early life nutritional supplements (36) while high mortality, hospital readmission, and long-term growth deficits occur following severe wasting (17, 73, 81). Hence, there is a hidden pathophysiological burden associated with childhood undernutrition that is incompletely addressed by current therapies.

Child undernutrition is associated with disturbances to multiple early-life biological systems. Intestinal function is disturbed in both stunted and wasted children in a condition termed environmental enteric dysfunction (EED) (102). This intestinal pathology impairs nutrient absorption and may contribute to systemic inflammation, thereby impairing early life growth. Broad deficits in host immunity are also observed in child undernutrition, leading to impaired ability to fight infection, especially in pathogen-dense environments (13). Hormonal differences exist between well-nourished and undernourished children, especially those associated with growth [growth hormone (GH) and insulin-like growth factor 1 (IGF-1)] and appetite regulation (leptin and ghrelin) (9, 59). Host metabolism is altered in undernutrition, whereby amino acid, lipid, and energy metabolism are perturbed (86). These disturbed physiological systems suggest a complex pathophysiology that underlies child undernutrition involving interlinked biological systems. In addition to suboptimal nutrition, microbial exposures in early life may contribute to these

perturbed pathways observed in child undernutrition. High enteropathogen carriage is associated with reduced linear and ponderal growth and EED, while diarrhea and respiratory infections are also strongly associated with child undernutrition (1, 24, 27, 111). A growing body of evidence suggests that, in addition to pathogens, the commensal gut microbiome influences early life growth (109). The assembly of a complex microbiome in early life is essential for immune training, colonization resistance against pathogens, metabolism of human milk oligosaccharides (HMOs) and other nutrients, intestinal structuring, and endocrine signaling. Hence, disruptions to the gut microbiome in childhood can impair these pathways that contribute to healthy child growth.

Human milk oligosaccharides (HMOs): complex sugars found in human milk that are primarily digested by the gut microbiome

Here, we review the existing research examining how the early life gut microbiome influences pathways driving child growth, in the context of undernutrition. We explore data from both human and animal studies that demonstrate the mechanistic pathways by which gut microbial colonization in early life influences infection susceptibility, metabolism, hormone signaling, immunity, intestinal function, and other pathways that influence child growth, and we touch on lesser-studied areas including epigenetic interactions with the gut microbiome in undernutrition.

2. HEALTHY AND MALNOURISHED MICROBIOME SUCCESSION IN EARLY LIFE

2.1. Healthy Gut Microbiome Development During Childhood

Children acquire a complex microbiome at birth, which predominantly originates from the maternal gut, vaginal, oral, and skin microbiomes, in addition to environmentally acquired organisms (47). The assembly of the intestinal microbiome is critical for intestinal development, immunity, and the metabolism of nutrients that cannot otherwise be metabolized by the host. In exclusively breastfed children, the diversity of this initial infant gut microbiome remains quite low and is composed predominantly of *Escherichia coli*, *Bifidobacteria*, and *Bacteroides* species. In the following 2–3 years of life, the gut microbiome undergoes a patterned assembly that is primarily shaped by exclusivity of breastfeeding followed by the introduction of complementary foods, which leads to the rapid expansion and diversification of the gut microbiome. However, a number of environmental factors can influence this microbial assembly, including geography, delivery mode, antibiotic exposure, gestational age, and other maternal and household factors (127). The postnatal accumulation of these microbial communities within the gastrointestinal tract drives the maturation of immune, metabolic, and endocrine pathways, in addition to intestinal structuring and colonization resistance against pathogens, each of which can contribute to normal child growth (109). These growth-defining pathways may therefore be impaired if this microbial succession is perturbed through inadequate nutrition, hygiene, or antibiotic exposure. Few studies have longitudinally examined the succession and assembly of the gut microbiome in children who remain well nourished in low-resource settings versus those who become wasted or stunted (110, 129). This dependence on microbiome databases derived from high-income settings therefore limits the interpretability of the malnourished microbiomes generated from individuals in low- and middle-income countries (LMIC) where gut microbiomes, and the species/strains within, are less well characterized (96). However, growing evidence from cross-sectional and short-term longitudinal studies in LMIC reveals microbiome differences that differentiate undernutrition from healthy growth.

2.2. Gut Microbiome Signatures in Child Stunting

Stunting is a chronic form of undernutrition driven by several genetic and environmental factors. Up to 30% of stunting occurs in utero, as demonstrated by the strong effect of birth weight

MALNOURISHED MICROBIOMES BEYOND THE GUT

Microbiomes at body sites beyond the gut may also contribute to child growth and undernutrition. A study from Madagascar and the Central African Republic provided evidence that the oral microbiome was associated with child stunting (135, 136). Stunted children displayed a greater abundance of oropharyngeal microorganisms, including *Lactobacillus salivarius*, in the duodenum and stool compared with nonstunted controls, suggesting that decompartmentalization of the gastrointestinal tract may contribute to the intestinal changes observed in child undernutrition and to poorer growth itself via reduced nutrient absorption (136) or the inflammatory action of oral microorganisms outside of their ecological niche. Maternal microbiomes during pregnancy have also been associated with poor child growth. Periodontitis is an oral dysbiotic disease that drives a local dysfunctional immune response and is negatively associated with low birth weight (31). In the maternal oral cavity during pregnancy, relative abundance of *Actinomyces naeslundii* was negatively associated and *Lactobacillus casei* was positively associated with birth weight and pregnancy duration (32), both of which are strongly predictive of child undernutrition. An expanding body of literature demonstrates a consistent vaginal microbiome signature of preterm birth that is strongly linked with low birth weight and subsequent stunting (48). A highly diverse, *Lactobacillus*-deficient vaginal microbiome is strongly associated with preterm birth in Western settings and with reduced newborn LAZ in sub-Saharan Africa (40, 43). The mechanism by which the vaginal microbiome affects preterm birth and birth weight is not known; however, it is likely that an altered vaginal microbiome co-occurs with classical infectious triggers of preterm birth including chorioamnionitis, bacterial vaginosis, and urogenital infections, thereby contributing to an inflammatory urogenital environment, which collectively may restrict fetal growth or trigger early birth.

on stunting risk (28), after which an array of environmental factors contributes to postnatal linear growth (133). Few studies have longitudinally examined the acquisition and assembly of the gut microbiome in healthy versus stunted infants; however, several cross-sectional studies provide some evidence of disrupted gut microbial signatures in stunting. Secondary analysis of cohorts from Malawi and Bangladesh found that reduced microbiome diversity and increased abundance of *Acidaminococcus* were both associated with stunting severity and future linear growth deficits, respectively (51). A handful of other small cross-sectional studies have identified varying differences in fecal microbiome composition in stunted versus healthy children across a range of LMIC cohorts (41, 124, 130, 144). A larger multisite cross-sectional analysis from the Central African Republic and Madagascar found that the fecal microbiomes of stunted children were enriched in *Escherichia coli/Shigella* and *Campylobacter* species and depleted in butyrate-producing species (135, 136). Furthermore, higher rates of small intestinal bacterial overgrowth were observed in stunted children, characterized by enrichment of oropharyngeal microbes in the duodenum (see the sidebar titled Malnourished Microbiomes Beyond the Gut). Due to the histopathological changes that characterize EED being observed in the small intestine of stunted children, there is growing focus on the microbiome of the small intestine measured in duodenal aspirates over the fecal microbiome, which more closely resembles communities in the colon. Chen et al. (25) found a negative correlation between bacterial load in the small intestine and LAZ in a cohort of stunted children from Bangladesh. The duodenal abundance of a *Veillonella* species, a *Streptococcus* species, and *Rothia mucilaginosa* were also negatively correlated with LAZ.

Despite the chronic pathophysiological nature of linear growth faltering over the first 2 years of life, large longitudinal studies concurrently mapping microbiome succession and linear growth have largely been lacking. We recently reported compositional and functional development of the gut microbiome in a cohort of 335 children from rural Zimbabwe followed from 1 to 18 months of age (110). We found that functional metagenomic composition, but not taxonomic composition,

of the gut microbiome could predict both attained linear growth and future growth velocity, whereby B vitamin and nucleotide biosynthesis pathways were among the most predictive features. These data suggest that the functional potential of the gut microbiome is a more powerful indicator, or indeed influencer, of child growth and that standard compositional assessment of the infant gut microbiome alone may be insufficient to identify associations with linear growth. A longitudinal cohort from Malawi found similar outcomes, whereby 16S sequencing was unable to identify associations between compositional diversity, maturity, or species abundance and LAZ (68). A longitudinal study from Peru of children from 6–24 months of age, as part of the Malnutrition and Enteric Disease (MAL-ED) cohort, demonstrated some associations between compositional diversity and linear growth, albeit only in children who were born stunted and were severely stunted at sampling (113). Another study from the MAL-ED Peru cohort suggests that the gut microbiome may mediate the effect of diarrhea and *Campylobacter* infection on linear growth (113, 114). In addition to the effect of the bacterial component of the gut microbiome, the abundance of bacteriophages—viruses that can regulate microbiome composition—differ in stunted versus nonstunted infants (35, 74). Collectively, the gut microbiome of stunted children appears to be distinct from that of well-nourished children; however, this varies across geographical cohorts. Hence, no consistent compositional or functional microbiome signature of stunting currently exists.

Functional potential of the gut microbiome: the genes encoded by the gut microbiome that determine its activity and function

Severe acute undernutrition (SAM): a form of acute malnutrition classified as a weight-for-height Z-score <3, mid-upper arm circumference <115 mm, or the presence of nutritional edema

Microbiota-for-age Z-score (MAZ): a metric of microbiota maturity during early life that is strongly associated with child growth

2.3. Gut Microbiome Signatures in Child Wasting

Wasting is a form of undernutrition that can reflect both chronic nutritional deficits and acute illness and frequently coincides with stunting. Wasting can be classified as moderate [moderate acute malnutrition (MAM)] or severe [severe acute malnutrition (SAM)], depending on thresholds of weight-for-height Z-score (WHZ), mid-upper arm circumference (MUAC), and/or the presence of edema. SAM additionally presents in two primary forms: edematous SAM (previously known as kwashiorkor) and nonedematous SAM (previously known as marasmus), which are treated either through community-level feeding programs or through in-patient care if related complications such as acute infections, lack of appetite, shock, or edema are present (also known as complicated SAM). Children with edematous and nonedematous SAM have differing clinical outcomes, whereby those with nonedematous SAM display higher mortality and hospital readmission following in-patient treatment for SAM in certain settings (17).

Despite improvements in nutritional therapies to treat SAM, mortality in complicated SAM requiring hospital admission ranges from 10% to 40% (17, 73) while growth deficits can persist for up to 7 years (81) in addition to elevated risk of cognitive deficits and chronic disease risk later in life (81, 82, 88). The gross morphological changes that have been observed in the intestines of children with SAM suggest that the gut microbiome may be influential (4, 76, 105, 109). Although microbiome studies of complicated SAM are confounded by broad-spectrum antibiotic treatment that forms part of World Health Organization treatment guidelines, evidence from community SAM cases suggests disturbances to the gut microbiome compared with healthy growing children. Recent studies show that children with SAM have reduced gut microbial diversity and an enrichment in *Enterobacteriaceae* (89), while twins discordant for SAM share disturbed gut viromes compared with concordant healthy growing twin pairs (108). A landmark study from a cohort in Bangladesh in 2014 created a metric of microbiome maturation, termed the microbiota-for-age Z-score (MAZ), which was markedly reduced in children with SAM and was highly correlated with anthropometric measures of nutritional recovery following SAM (129). This and more recent follow-up studies have allowed for the refinement of a so-called microbiome ecogroup, made up of 15 bacterial taxa that exhibit consistent covariation in the first 2 years of life across various geographical settings and can be used as an indicator of immature microbiome development and to

measure the success of microbiome recovery following MAM or SAM (107). Standard therapeutic feeds tend to only temporarily restore microbiome maturity, as assessed by MAZ (129), suggesting that MAZ may have potential as an indicator of future MAM/SAM relapse or that nutritional therapies targeting the gut microbiome may aid nutritional recovery. Indeed, these indicators of microbiome maturity have informed new microbiota-directed nutritional interventions in MAM that have, in pilot studies, improved nutritional recovery to a greater extent than standard therapeutic foods (26); however, large-scale randomized trials are still lacking. A number of intestinal differences have been also observed between children with edematous and nonedematous SAM (3, 38, 79) and these may partly explain differences in their nutritional recovery. Recent evidence shows that alpha diversity of children with nonedematous SAM is significantly reduced and is associated with reductions in relative abundances of *Prevotellaceae*, *Lachnospiraceae*, and *Ruminococcaceae* versus those with edematous SAM (21). Collectively, these data appear to suggest consistent microbiome immaturity and reduced diversity in children with SAM versus healthy growing children. This dysbiotic microbiome is likely a result of the acute nutritional state in addition to comorbidities and antibiotic treatment associated with treatment.

Lipopolysaccharide (LPS): an endotoxin produced by gram-negative bacteria that stimulates inflammation if translocated across the intestinal epithelial barrier

3. MICROBIOME-MEDIATED PATHWAYS DEFINING GROWTH

A growing body of evidence from observational human studies shows differences in microbiome composition between well-nourished and undernourished children, as outlined above. In addition, preclinical and clinical studies have begun to uncover the pathophysiological mechanisms by which a disturbed gut microbiome in early life may contribute to poor child growth. These include gut microbiome influence on immunity, metabolism, intestinal function, and endocrine signaling, among other pathways (**Figure 1**).

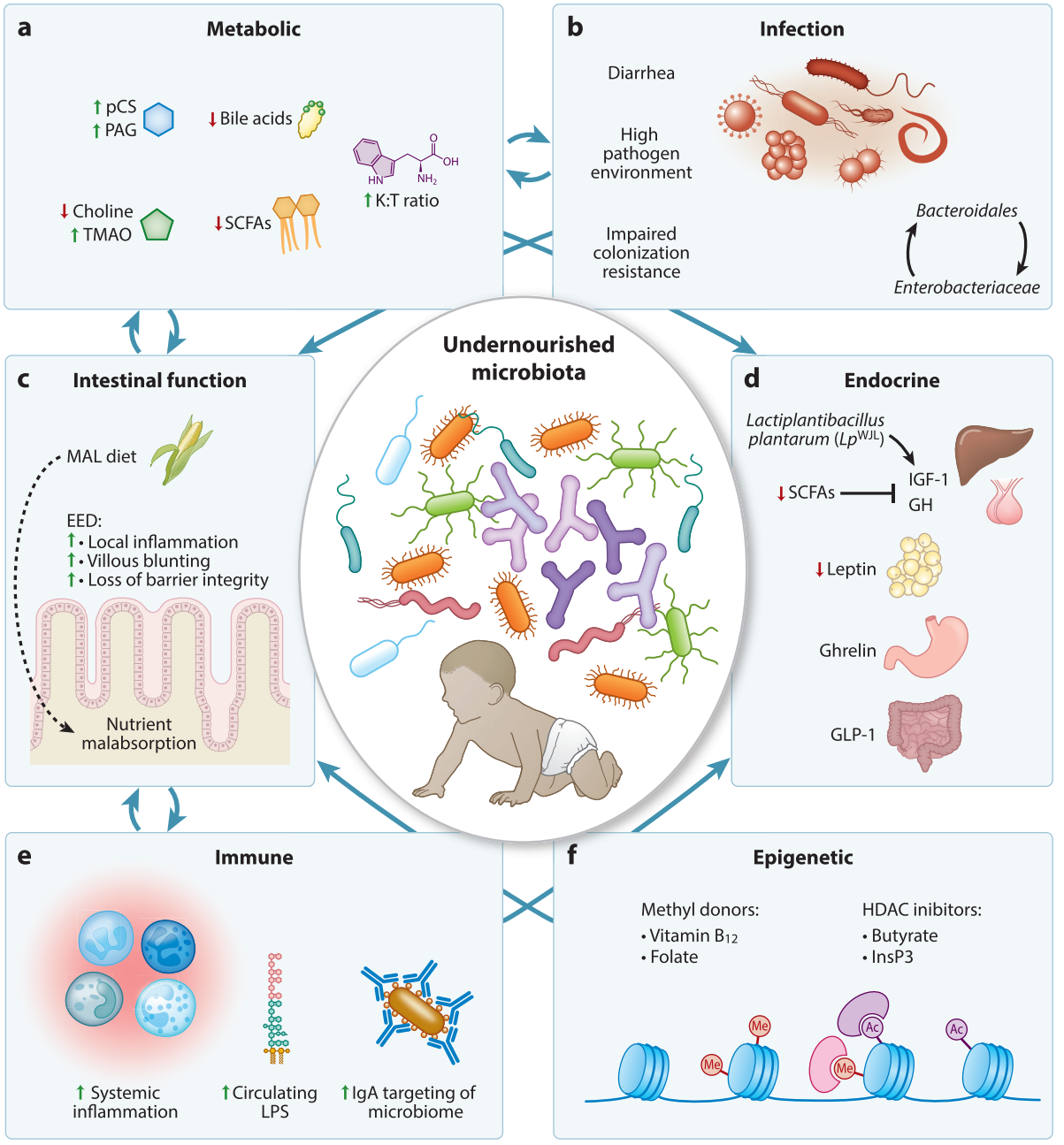
3.1. Intestinal Structure and Function

The presence of a commensal gut microbiota is essential for the normal structural, functional, and transcriptional development of the intestinal barrier in early life. Extensive evidence from preclinical studies demonstrates that germ-free mice pups exhibit delayed maturation of the intestinal barrier, which is associated with spontaneous colitis, greater susceptibility to enteric infection, and nutrient malabsorption (54, 71). However, clinical studies suggest that chronic exposure to enteropathogens during early life can disrupt intestinal structure and function (4, 105). EED is a gastrointestinal phenomenon that is common in low-resource settings and is frequently observed in children with undernutrition. EED is usually subclinical, localized to the small intestine, and characterized by increased intestinal permeability, villous atrophy and blunting, changes in the mucosal barrier, malabsorption, local inflammation, and crypt elongation (102). This loss of barrier function also allows the translocation of microbes and their by-products, with the most frequently studied being lipopolysaccharide (LPS), from the gut into systemic circulation, thereby stimulating systemic inflammation. This combination of nutrient malabsorption, intestinal inflammation, and chronic systemic inflammation is hypothesized to prevent healthy child growth.

EED is commonly attributed to chronic exposure to enteropathogens in settings with poor water, sanitation, and hygiene (WASH) (78). However, large, randomized trials improving WASH during pregnancy and early life in such settings fail to reduce common biomarkers of EED in young children, plausibly due to a concurrent failure to reduce their enteropathogen carriage (52, 112). In addition to pathogen carriage, disruption of the commensal microbiome may contribute to EED. A large analysis of 611 children at 6, 18, and 30 months of age found that there was an inverse association between microbiota diversity/maturity and three biomarkers of EED [calprotectin, alpha-1-antitrypsin, and regenerating family member 1 beta (REG-1B)], suggesting that

immaturity of the gut microbiota may drive intestinal inflammation and barrier dysfunction (76). Elevated abundances of particular taxa within the microbiome, including *Megasphaera*, *Mitsuokella*, and *Sutterella*, have also been associated with EED (92).

Animal models of EED lend insight into the mechanisms that may drive phenotypes of the disorder. Some models are able to replicate certain aspects of EED by applying low-protein diets



(*Caption appears on following page*)

Figure 1 (*Figure appears on preceding page*)

Microbiome-driven pathways contributing to child undernutrition. The composition and function of the gut microbiome is altered in undernourished versus well-nourished children. Evidence, primarily from animal studies, shows that this altered intestinal microbial environment influences multiple overlapping biological systems and pathways that define both linear and ponderal growth. Six of these systems/pathways are illustrated here, many of which overlap with each other but which share a proposed common causal basis: an undernourished microbiota. (*a*) An undernourished gut microbiome alters the production of microbial metabolites, including elevated pCS and PAG and reduced choline and SCFAs, some of which can contribute to intestinal function and IGF-1 production. (*b*) Reduced diversity and altered composition in the gut microbiome of undernourished children allow the colonization of enteropathogens, which contribute to diarrhea and both intestinal and systemic inflammation. (*c*) The disturbed microbial environment within the gut leads to EED, characterized by blunted villi, permeability, and intestinal inflammation. (*d*) Both systemic inflammation induced by the gut microbiota and reduced production of SCFAs dampen production of IGF-1 and GH, hormones critical for bone and skeletal muscle growth. Leptin, which is predictive of mortality in SAM, and ghrelin, a hormone involved in satiety, both have associations with gut microbiome composition. (*e*) The damaged gut barrier in EED, which may be driven by an undernourished microbiome, allows the translocation of LPS and microbes into circulation, thereby inducing chronic systemic inflammation. The altered gut microbiome also impairs vaccine responses and other immune phenotypes associated with undernutrition. Targeting of the microbiota by IgA, a secretory antibody, is increased in chronic undernutrition. (*f*) The microbiota also produces methyl donors, HDAC inhibitors, and other compounds that modify epigenetic signaling both in the intestine and globally and which may contribute to epigenetic modifications observed in child undernutrition. Abbreviations: AMP, antimicrobial peptide; EED, environmental enteric dysfunction; GH, growth hormone; GLP-1, glucagon-like peptide 1; HDAC, histone deacetylase; IgA, immunoglobulin A; IGF-1, insulin-like growth factor 1; InsP3, inositol-1,4,5-trisphosphate; K:T, kynurenine-to-tryptophan; LPS, lipopolysaccharide; MAL, malnutrition; PAG, phenylacetylglutamine; pCS, *p*-cresyl sulfate; SAM, severe acute malnutrition; SCFA, short-chain fatty acid; TMAO, trimethylamine-*N*-oxide.

and intestinal insults such as LPS or indomethacin (116). However, with growing evidence of a potential gut microbial contribution to EED, novel models leveraging interactions between aberrant microbial states and altered nutrient environments have successfully recapitulated several EED phenotypes. For example, gut physiology and the microbiome are linked via the expression of angiotensin I converting 2 enzyme (ACE2), a protein expressed on the luminal surface of differentiated epithelial cells. Mice lacking ACE2 display EED-like pathology, disrupted amino acid metabolism, and disturbances to the gut microbiome compared with wild-type mice when challenged with dextran sodium sulphate (DSS), an irritant that disrupts the intestinal epithelial barrier and results in colitis (58). The EED phenotypes during DSS challenge can be transferred to wild-type animals following fecal transplant from ACE2-deficient mice, implicating the disrupted microbiome. Dietary tryptophan, and its metabolite nicotinamide, can rescue the phenotypes in ACE2-deficient mice by stimulating antimicrobial peptide production via mammalian target of rapamycin (mTOR) activity, thereby maintaining microbiome homeostasis. Brown et al. (15) previously reported that a low-protein, low-fat diet induced microbiome disruption in the small intestine of mice, characterized by elevated species richness in the small intestine in addition to expansion of Bacteroidetes and Proteobacteria. In combining the undernourished diet with a consortium of *Bacteroidales* and *E. coli* species, the authors produced phenotypes of EED including intestinal permeability, increased villus and crypt atrophy, increased jejunal cytokine [interleukin (IL)-6 and MCP-1] release into the lumen, and increased bacterial adherence to small intestine epithelial cells. This phenotype relied on both the microbiome alteration and malnourished diet, suggesting that EED phenotypes result from complex microbial–nutrient interactions. More recently, a simplified EED model combining a low-protein, low-fat diet with an adherent invasive *E. coli* was reported to induce similar EED and growth phenotypes in addition to impaired vaccine responses (10). These phenotypes were dependent upon the changes induced in the wider gut microbiome, whereby antibiotic administration negated the combined effect of the diet and *E. coli*.

The major limitation of such models, however, is that EED pathology is largely restricted to the small intestine, where the gut microbiome is less dense, and indeed less studied, than in the

colon, which is the source and predominant target of fecal transplant–based models. Small intestinal bacterial overgrowth (SIBO) is associated with undernutrition in human cohort studies (42, 136, 138); however, compositional analysis of the upper intestinal microbiome in children is largely lacking due to sampling difficulties and ethical concerns with sampling from healthy control children. Chen et al. (25) sequenced duodenal aspirates from 80 children with biopsy-confirmed EED and identified bacterial strains that could recapitulate several EED phenotypes when transferred to germ-free mice, thereby providing causal evidence for the role of the small intestine microbiome in EED. However, the lack of duodenal sampling from well-nourished children, due to ethical constraints, limits the interpretation of these results. Despite these limitations, these data collectively suggest a potential causal role of disrupted intestinal microbial communities in the pathology of malnutrition-associated intestinal dysfunction.

3.2. Infection

Robust evidence from large multicenter studies of diarrhea and child undernutrition suggest that a high burden of enteropathogens is associated with poor growth in early life (1). Indeed, diarrhea and symptomatic respiratory infections are associated with both stunting and wasting (24, 27, 111). The healthy and diverse gut microbiome provides colonization resistance against intestinal pathogens through niche exclusion (44). An impaired gut microbiome may therefore provide niches for pathogen colonization. However, the gut microbiome may also mediate infection susceptibility at distant sites, including the lungs (140). Assembly of a healthy gut microbiome in early life may therefore contribute to colonization resistance against pathogens in poor hygiene environments, thereby preventing infection burden and associated poor growth.

Reduced gastrointestinal microbial diversity in the context of stunting can be predictive of increased diarrhea incidence. Stunted children from Peru exhibit compromised trajectories of gut microbiome diversification throughout the first 2 years of life. During this time, stunted children display greater reductions in microbial diversity and slower recovery of diversity following diarrheal episodes (113). Therefore, the impaired assembly of the gut microbiome may exacerbate the infection–stunting cycle. A study from the same cohort found that *Campylobacter* carriage was associated with an altered gut microbiota (114). Furthermore, the species within the gut microbiome associated with *Campylobacter* carriage were independently associated with reductions in LAZ, suggesting that impaired gut microbiome composition facilitates pathogen colonization and concurrent growth deficits.

Animal models provide further evidence of a microbiome-induced effect on pathogen colonization in the context of undernutrition. Murine models of EED induced by malnourished diets and disturbed gut microbiomes have greater susceptibility to infection versus well-nourished mice, suggesting that malnutrition-associated microbiota disruptions may permit pathogen colonization and infection burden (15). Susceptibility to growth deficits following *Giardia lamblia* infection is also dependent on the gut microbiome in animal models. Human studies report that *Giardia* and associated enteroinvasive pathogens, in addition to those involved in mucosal disruption, have the most profound influence on systemic inflammation, gut inflammation, and impaired growth (1). In mice, *Giardia* interacts with *Enterobacteriaceae* in the small intestine to restrict growth during protein malnutrition, an effect that is lost following treatment with antibacterials exhibiting no antigiardial activity (8), suggesting a critical role of the dysbiotic microbiome in this process.

This interaction suggests that particular pathogens or pathobionts depend on members of the commensal gut microbiota to colonize and proliferate. This is evident in human studies of stunting, whereby cross-feeding between *Enterobacteriaceae* and *Bacteroidales* spp. is associated with child stunting (62). In vitro, synergistic growth occurs between *Enterobacteriaceae* and *Bacteroidales* spp. uniquely in malnourished conditions low in protein and iron. In such circumstances, *Bacteroidales*

spp. utilize diet- and mucin-derived sugars, and *Enterobacteriaceae* spp. enhance bioavailability of iron, a micronutrient essential for proliferation of particular pathogens. *Bacteroidaceae* and *Enterobacteriaceae* were strongly correlated in undernourished children, but not in well-nourished children. Another *Bacteroidales* species, *Bacteroides fragilis*, also relies on the surrounding microbiome to induce growth effects in mice. An enterotoxigenic strain of *B. fragilis* containing the bft toxin induced weight loss and impaired energy metabolism in germ-free mice who were colonized with the microbiota from stunted children but not in those colonized with microbiota from well-nourished children (137). These data suggest that the effects of pathogens and infections on child undernutrition may be reliant on the composition and function of the commensal gut microbiome and that strategies to reduce the pathogen burden in settings with high prevalence of child undernutrition must consider the commensal microbiome in addition to pathogen burden.

Metabolic phenotypes: the profile of small molecules, including amino acids, lipids, and other metabolites, that are present within a biological sample and reflect host and microbial metabolism

Short chain fatty acids (SCFAs): metabolites produced by fermentation of dietary polysaccharides by the gut microbiota that have a range of beneficial functions for intestinal, metabolic, and immune health

3.3. Host and Microbial Metabolism

Disturbances in host protein, fat, and energy metabolism have long been reported in undernourished children. However, the metabolic capacity of the gut microbiome substantially extends the biotransformational capacity of the mammalian host, expanding the diversity of substrates that can be processed and increasing the range of molecules to which the host is exposed. Many of these microbial-derived metabolites can exert local effects in the gut and at the gut wall and, following absorption, can also impact on host processes in peripheral tissues. Hence, changes to the gut microbiome in early childhood may influence metabolic phenotypes by altering both the metabolic by-products provided to the child and the host metabolic pathways themselves, therefore affecting growth.

Similar to the MAZ score used to map childhood microbiome maturity, a model of metabolic maturity, termed the phenome-for-age Z-score (PAZ), was constructed from urine samples of children from Peru, Tanzania, and Bangladesh and was used to track biochemical age and its association with growth (50). Three of the eight metabolites used to calculate PAZ—*p*-cresyl sulfate (pCS), phenylacetylglutamine (PAG), and hippurate—were related to gut microbial metabolism. Interestingly, growth-constrained infants were found to be biochemically immature (i.e., their biochemical age was lower than their chronological age) from as early as 3 months of life compared with the infants who were not constrained. This highlights the importance of the microbiota in the development of the overall metabolic capacity of the host and its subsequent impact on growth.

A major function of the healthy gut microbiota is to ferment dietary substrates that cannot be digested by the host. During breastfeeding, this primarily involves the microbial digestion of HMOs. HMOs are significantly lower in the breast milk of mothers with stunted infants, and structurally similar bovine milk oligosaccharides can restore growth in mice in models of undernutrition (23). However, this effect is dependent upon the microbiota, whereby growth in germ-free mice is not recovered following milk oligosaccharide supplementation. Following weaning, the gut microbiota switches to the metabolism of nondigestible carbohydrates, resistant starch, mucins, and proteins. The main end products of saccharolytic activity (i.e., the breakdown of carbohydrates) are the short-chain fatty acids (SCFAs), acetate, propionate, and butyrate. These all contribute to the daily energy requirements of the host. Butyrate is the principal energy source for colonocytes, providing 80% of their required energy, while acetate is metabolized in systemic areas such as muscle, and propionate is used in the liver for ATP production (30). This microbial activity provides a mechanism to unlock energy from the diet that can be used to support growth. Moreover, SCFAs can have other beneficial effects such as reducing colonic pH to increase colonization resistance against potential pathogens (19) and improve mineral absorption. Furthermore, butyrate has been shown to promote the development of the intestinal barrier

by increasing AMP-activated protein kinase (AMPK) activity and the assembly of tight junction proteins (99). Several studies have observed a functional shift in the malnourished microbiota away from carbohydrate fermentation and SCFA production toward proteolytic metabolism. For example, moderately malnourished children from Indonesia were observed to have lower fecal propionate and butyrate and a higher fecal pH compared with those of well-nourished children (67). In pig models of undernutrition, the gut microbiome produces less butyrate and is causally linked to reduced hepatic fatty acid metabolism (β-oxidation) (22). Similarly, in a metabolomic study of children from Northeastern Brazil, stunted infants excreted higher amounts of microbial–host cometabolites arising from the microbial degradation of amino acids (85). This included pCS and PAG, which are derived from the microbial breakdown of tyrosine and phenylalanine, respectively. Gut malabsorption due to malnutrition-pathogen-related EED (55) is likely to drive this observation. This increases the availability of amino acids in the small intestine, which contributes to the small intestinal bacterial overgrowth seen in stunted children (135, 136) and increases the amino acids reaching the colon for bacterial metabolism. In this regard, the malnourished microbiota competes with the developing host for amino acids that are essential for healthy development. Interestingly, while SCFAs promote epithelial barrier integrity, pCS has been found to impair it, potentially contributing to the leaky gut phenotype observed in malnourished individuals. In addition, pCS is produced by *Clostridioides difficile* and other pathogens to restrict the biodiversity of the intestinal microbiota (97), potentially contributing to the dysbiotic microbiota observed in undernourished infants (129). Two other amino acid metabolites, indolelactate and *N*-acetylglutamate, derived from tryptophan and glutamate metabolism, respectively, were positively associated with linear and ponderal growth metrics between 2–24 months of age in a cohort of children from Bangladesh (134). *Bifidobacterium longum*, which was also positively associated with growth, encoded a large proportion of microbial metabolic pathways involved in the production of these compounds that may help to support early life growth.

As well as competing with the host for amino acids, an increase in the microbial breakdown of dietary choline has been seen with protein malnutrition and stunting. In a mouse model of protein deficiency, urinary choline was reduced while the microbial products of choline metabolism, trimethylamine and dimethylamine, were increased (84). Similarly, in children from Malawi, the trimethylamine-*N*-oxide (TMAO)-to-choline ratio in serum was positively associated with linear growth failure (121), and lower choline and betaine excretion were associated with stunting in infants from Brazil (85). As well as being important for muscle acquisition, choline availability is also necessary for the generation of *S*-adenosylmethionine (SAMe), which is key for DNA methylation and development. Importantly, choline is an essential nutrient for skeletal muscle (87) and neurological development and brain function (34). Bile acids are an example of transgenomic metabolites arising from the combinatorial metabolism of the host liver and gut microbiota with an important role in digestion. Primary bile acids, such as cholic acid and chenodeoxycholic acid, are synthesized in the liver before being conjugated, usually with glycine or taurine, and secreted into the bile. Following release into the gut, a small proportion of bile acids can reach the colon where the microbiota deconjugate them and convert them into secondary bile acids, such as deoxycholic acid. These modified bile acids can be excreted in the feces or recycled back to the liver. Interestingly, children with EED have a lower amount of total serum bile acids compared with those without EED, with specific differences in taurochenodeoxycholic acid, tauromuricholic acid, and glycoursodeoxycholic acid (122). In addition to digestion, bile acids also have an important role in metabolic regulation. By acting as ligands for receptors expressed throughout the body (e.g., FXR, PXR, VDR, and TGR5), bile acids can modulate the expression of several host pathways and functions such as energy homeostasis, glucose, and lipid metabolism. Moreover, these bacterial-associated metabolites also possess antimicrobial characteristics and both inhibit and promote the

growth of specific pathogens. For example, variation in bile acid metabolism, particularly bacterial bile salt hydrolase activity, has been implicated as an important trigger for excystation of *Cryptosporidium* oocysts (75). Furthermore, children protected from amebiasis were observed to have lower fecal amounts of the secondary bile acid, deoxycholic acid. It was shown that administration of this bile acid to mice was sufficient to increase granulocyte-monocyte progenitors and provide protection against amebiasis (16). This suggests that bile acids may have a potential role in shaping the protection against and susceptibility to different enteric infections commonly seen in malnourished settings.

In addition to alterations in microbial metabolism, host metabolic alterations are observed in child undernutrition that may be partly driven by a dysbiotic microbiome. For example, oral microbes isolated from the small intestines of stunted children impair lipid absorption in in vitro and in vivo models, thereby providing a potential causal role of the small intestine microbiome and SIBO in undernutrition (136). Loss of intestinal barrier function can lead to increased translocation of the microbiota and their products, including LPS, from the gut into the systemic circulation. LPS translocation can result in chronic systemic inflammation, which activates the tryptophan-kynurenine pathway, via indoleamine 2,3-dioxygenase induction. Indeed, the plasma kynurenine-to-tryptophan (K:T) ratio has been correlated with plasma LPS and LPS-binding protein (LBP) in children from Peru and Tanzania (55, 77) and additionally associated with linear growth deficits. Activation of the kynurenine pathway can dampen inflammation and promote tolerance but can dysregulate tryptophan pathways that are important for serotonin production and NAD^+ and nicotinamide generation, which are key for growth. Increased immunological tolerance can also have implications for mounting effective responses to pathogens, which can also be impacted by tryptophan deprivation (80), and may contribute to the persistence of such infections in the undernourished gut.

3.4. Endocrine Responses

Child undernutrition is associated with disruptions to endocrine signaling (59), including to leptin and ghrelin, which mediate appetite and energy metabolism, and to GH and IGF-1, which collectively constitute the somatotropic axis. Plasma IGF-1 is lower in stunted versus nonstunted children both in later childhood (12) and throughout the first 18 months of life (104), a phenomenon that is associated with chronic inflammation. Low levels of leptin are predictive of mortality in children with SAM (9). Emerging data suggest that the gut microbiome directly and indirectly influences both local hormone production in the gut, such as glucagon-like peptide-1 (GLP-1) (139), and systemically by mediating the production of leptin, ghrelin, and IGF-1 (57, 101), each of which mediate metabolism and nutritional status.

The microbiome regulates production of GLP-1 by producing SCFAs, which suppress the expression of the proglucagon (139). GLP-1 is a gut-derived hormone responsible for stimulating insulin secretion, reducing gastric emptying, and increasing satiety, thereby influencing metabolism and nutritional status. In the absence of the microbiome, in germ-free mice, GLP-1 production is elevated in a hypothesized adaptive mechanism to increase nutrient adsorption when the microbiome is disrupted or absent, an effect that is reversed by SCFA administration (139). Microbially derived SCFAs also impact IGF-1 production. IGF-1 is produced in liver and adipose tissue and impacts bone formation, bone mass, and skeletal growth, especially in early life. When germ-free mice are colonized with a microbiome, the concentration of serum IGF-1 in plasma increases (143). Similarly, the use of antibiotics reduces IGF-1 concentrations, and SCFA supplementation restores the concentration of IGF-1 during antibiotic treatment. Non-SCFA-producing bacterial species can also stimulate growth in experimental animals via stimulation

of the somatotropic axis. Specific strains of *Lactobacillus plantarum* restore linear growth, femur length, IGF-1 production and activity, and sensitivity of peripheral tissue to GH in both *Drosophila* and mice (120). The activity of the *Lactiplantibacillus plantarum* (Lp^{WJL}) strain on IGF-1 production and growth is dependent upon stimulation of nucleotide-binding oligomerization domain-containing protein 2 (NOD2) in intestinal epithelial cells (119). Commensal intestinal bacteria can also prevent muscle wasting in the context of infection. Commensal *E. coli* sustain IGF-1 signaling in skeletal muscle independently of host metabolism, caloric uptake, or inflammation, thereby preventing muscle wasting following intestinal or lung infections (117).

Hormones involved in satiety and metabolism have been correlated with the abundance of particular commensal taxa within the gut microbiome in both animal and human studies. *Bifidobacterium* and *Lactobacillus* abundance are positively correlated with leptin concentrations in animal models, while ghrelin is positively correlated with *Bacteroides* and *Prevotella* abundance (106). Ghrelin levels are also negatively correlated with *Bifidobacterium*, *Lactobacillus*, and *Blautia coccoides–Eubacterium rectale* in malnutrition models (106). A cross-sectional observational cohort study of Gambian children between 6 and 24 months ($n = 60$) identified a number of strong associations between gut microbiome taxa and metabolic hormone concentrations in acutely malnourished children (89), using network analyses that modeled gut microbiome and hormone interactions. *Escherichia/Shigella* was highly predictive in discriminating between healthy controls and malnourished patients through its interaction with ghrelin and ghrelin receptor, while interactions between *Lactobacillus mucosae* and the leptin/leptin receptor were also highly discriminatory between malnourished and well-nourished children. *Enterobacteriaceae* and IGF-1 interactions also discriminated MAM from SAM. These interactions are likely bidirectional, whereby leptin can stimulate mucin production and alter gut microbiome composition, while the inflammatory activity of particular LPS-producing *Enterobacteriaceae* may impair IGF-1 and GH signaling.

3.5. Immunity and Inflammation

The microbiota is in reciprocal, dynamic communication with the host immune system throughout life, and this communication is disrupted by undernutrition (5, 13). There is cumulative evidence that undernourished children are immunologically distinct from their adequately nourished peers, with smaller thymuses, qualitatively and quantitatively different immune cell composition and distribution, chronically elevated proinflammatory mediators in their systemic and intestinal milieu, and epigenetic marks on immune genes driven by adverse pre- and postnatal exposures (13, 14, 115). To date, a majority of these immune phenotypes in child undernutrition have been studied in the context of infection and pathogen carriage. However, emerging data suggest that the diversity of commensal and conditionally pathogenic organisms residing within the gut microbiome may drive distinct immune phenotypes in child undernutrition, independently of pathogens.

Intestinal inflammation in undernutrition is characterized by biomarkers of EED such as calprotectin and myeloperoxidase, which are produced by microbially activated innate immune cells in gut tissue that may be activated by a disrupted gut microbiome (109). Animal models of EED demonstrate that gut dysbiosis of nutrient-deficient versus sufficient mice leads to higher intraepithelial lymphocyte numbers and proinflammatory cytokine secretion in the small intestine (15). Despite increased jejunal immune activation, nutrient-deficient animals are less able to contain *Salmonella typhimurium* infection in the intestine, resulting in dissemination of bacteria to the spleen and liver and proinflammatory mediator secretion in the liver. Child undernutrition is also associated with chronic systemic inflammation (4, 91, 104, 131). Although recurrent symptomatic infections can trigger a systemic inflammatory phenotype, murine models demonstrate that this can also occur in the absence of overt infection, through systemic spread of the gut microbiota

and/or their components into circulation across the dysfunction gut barrier, as observed in EED. Healthy mice weaned onto a protein-, iron-, and zinc-deficient diet developed microbiota dysbiosis and weight loss compared with nutrient-sufficient pups, corresponding to a failure to contain LPS when administered to the colon; elevated systemic, cecal, and colonic (but not ileal) proinflammatory cytokines; and greater secretion of proinflammatory cytokines upon cutaneous LPS challenge, in the absence of infection (98). Differences in weight gain and proinflammatory immune responses were normalized in this model by antibiotic treatment targeting gram-negative (LPS-positive) but not gram-positive (LPS-negative) commensals (98). The selectivity of the antibiotic treatment effect in this model supports a role for disseminated proinflammatory immune responses to gram-negative microbiota constituents translocated from the gut into circulation in the weight and height deficits that characterize undernutrition.

One way in which chronic systemic inflammation driven by microbial translocation may impact anthropometry is via suppression of growth factor signaling and bone/skeletal muscle growth. Evidence from animal studies demonstrates that sialylated HMOs, which rely on microbiota metabolism, increase osteoblast-driven bone formation and that this process is mediated by Th2-polarized immune responses, namely, increased eosinophil recruitment and eotaxin concentrations in the large intestine (29). There is consistent evidence from pediatric cohort studies in LMIC that inflammatory mediators are also inversely associated with IGF-1 (45, 65, 83, 104, 131). Inflammatory mediators including sCD14, complement protein 2, macrophage inflammatory protein 1B, and LBP were negatively associated with weight and/or MUAC gain among children recovering after hospital admission for SAM (90). Animal and in vitro models provide mechanistic evidence for a direct effect of inflammatory mediators on growth. Transgenic mice that constitutively overexpressed the proinflammatory cytokine IL-6 had reduced circulating IGF-1, had slower growth rates, and were 30–50% smaller than their nontransgenic littermates, a phenotype that could be partially rescued by administration of an IL-6-blocking antibody (33). Together with IL-6, IL-1β and tumor necrosis factor alpha (TNFα) were found to downregulate growth hormone receptor (GHR) expression, GH signaling via suppressor of cytokine signaling 3 (SOCS3), and IGF-1 production in human hepatic cell lines in vitro and in murine liver in vivo (145). Whether a disturbed gut microbiota or the presence of overt intestinal pathogens are driving these inflammation-induced IGF-1 and GH deficits are unknown. However, the potential for targeting the microbiota as a means of normalizing inflammation-driven growth defects has been explored in a clinical trial of a therapeutic legume-based feed designed to enhance the diversity and maturity of the gut microbiome among undernourished Bangladeshi children (26, 49). Consistent with previous observations, 30 children with SAM could be discriminated at baseline from 21 healthy children by a plasma proteome associated with lower bone ossification and osteoblast differentiation, and higher acute-phase inflammatory response, including C-reactive protein (CRP), IL-6, and intermediary proteins in the proinflammatory nuclear factor kappa B (NF-κB) signaling pathway. Following the intervention, WHZ and microbiota maturity increased despite limited evidence for a difference in overall enteropathogen carriage or absolute fecal microbiota diversity; these changes were associated with a shift in the plasma proteome toward signatures of healthy growth and reduced inflammation, suggesting that gut microbiome–targeted interventions can reduce malnutrition-associated inflammation (26).

The ability of a dysbiotic microbiome to induce intestinal and systemic inflammation in child undernutrition may also depend on antibody-mediated control of gut microbiome composition. Secretory immunoglobulins in the intestine, particularly immunoglobulin A (IgA), selectively bind microbiome constituents, thereby regulating microbiome composition (63). Stunted children were found to have a greater proportion of bacteria targeted by IgA in their stool than did nonstunted control children from Madagascar and the Central African Republic (61). In addition

to quantitative differences in IgA targeting of the gut microbiota, IgA also targets a different subset of intestinal microbes in healthy children versus children with SAM (70), whereby IgA tended to target *Enterobacteriaceae* in children with SAM while targeting a broader range of commensal organisms in healthy children. The IgA^+-extracted microbiota from children with SAM induced EED-like pathology and systemic inflammation when transplanted into mice, an effect that was dependent upon an undernourished diet. In a separate model, mice fed a low-protein, low-fat diet with or without bacterial gavage to mimic EED had impaired IgA targeting of *Lactobacillus* spp. despite similar relative abundance of *Lactobacilli* in the jejunum, total fecal IgA, and percentage of total IgA-targeted bacteria relative to well-nourished controls (60). These differences were due to adaptation of *Lactobacilli* carbohydrate metabolism to changing diet rather than differences in IgA titres or avidity. Collectively, these observations highlight the plasticity of immune–microbiota interactions at the gut barrier, with evidence that adaptations of the microbiota and tissue-resident immune cells may compromise immune-mediated containment of microbes and their antigens in EED, thereby contributing to faltered growth.

Oral vaccine responses are impaired among undernourished children (95) and reflect some of the immune deficits present in undernutrition. Murine models provide some clues for how microbiome dysbiosis in undernutrition may impair oral vaccine immunogenicity (10, 39); however, these paradigms require further exploration in human cohort studies. Upon prime-boost oral vaccination against enterotoxigenic *E. coli* (ETEC), mice with an EED-like phenotype induced by dietary deficiency and adherent *E. coli* colonization had lower vaccine-specific $CD4^+$ T cells in the small intestine and lower levels of vaccine-specific IgA relative to mice fed a nutrient-sufficient diet and to uncolonized mice fed either the deficient or sufficient diet (10). These differences were microbiota dependent since numbers of small intestine vaccine-specific T cell numbers in ETEC-colonized mice normalized after 3 weeks of broad-spectrum antibiotic treatment (10). Impaired vaccine responses in the EED phenotype were due to expansion of microbiota-dependent $ROR\gamma T^+FOXP3^+$ (retinoic acid receptor–related orphan receptor γT, forkhead box P3) T regulatory cells (Tregs) capable of suppressing vaccine responses in the small intestine; vaccine-specific $CD4^+$ T cell proliferation and IgA titres were restored in the small intestine, and percentage weight gain increased upon conditional depletion of the Tregs (10). In a MAM model in which mice were colonized with the stool microbiota of Bangladeshi children and fed a diet representative of that consumed by the donor children, prebiotic and micronutrient supplementation led to higher ratios of fecal cholera toxin (CT)-specific IgA to total IgA and more germinal center B cells in mesenteric lymph nodes upon cholera vaccination than those in nonsupplemented mice (39). However, the effect of the nutrient supplement was not universal across the four stool microbiota samples used: Prebiotic- and micronutrient-supplemented animals colonized with some fecal communities had CT-IgA responses similar to those of nonsupplemented control animals, and CT-IgA responses were elevated only when these hyporesponsive animals were cohoused with animals colonized with responsive stool microbiota (39). Collectively, these studies provide proof of concept for how microbiota-targeted therapies could restore impaired immune phenotypes, including impaired vaccine response, in undernourished children but also highlight inherent immune and microbial heterogeneity, which should be taken into consideration when designing translational studies and population-level interventions.

3.6. Epigenetics

Epigenetic modifications, which refer to environmentally induced changes to gene expression independent of changes to DNA sequence, may contribute to the metabolic and intestinal pathophysiology observed in child undernutrition and the associated heightened risk of chronic diseases in later life. Children with chronic and acute malnutrition display altered global DNA

methylation profiles on metabolic and immune-related genes versus healthy controls (64, 100, 118, 123, 132). Disturbances in DNA methylation have also been observed locally, in the intestines, whereby children with EED show hypermethylation of epithelial metabolism and barrier function genes and hypomethylation of immune response and cell proliferation genes in DNA methylation arrays from small intestinal biopsies (56).

DNA methylation, and indeed other epigenetic modifications including histone modifications and noncoding RNA, are induced by nutritional compounds. Methionine, choline, betaine, folate, and vitamin B_{12} are all dietary sources of methyl donors, which feed into the one-carbon pathway, critical to epigenetic regulation. Indeed, maternal folic acid supplementation increases infant birth weight, while infant vitamin B_{12} status is predictive of both linear and ponderal growth (66, 128). The gut microbiome, however, also synthesizes a number of these methyl donors, including folate and vitamin B_{12}, at quantities similar to dietary intake, suggesting that the composition and function of the gut microbiome in infancy may impact epigenetic modifications involved in child undernutrition. This effect may act via epigenetic modifications in the intestines. A murine study of dietary methyl donor deficiency produced reduced growth and intestinal pathophysiology similar to child stunting (2). Intriguingly, however, succinylsulfathiazole, an antibiotic that targets bacterial folic acid production, also induced EED-like pathology, as observed by increased depth of intestinal crypts. Hence, microbial production of folic acid, a methyl donor involved in intestinal epigenetic modifications, may contribute to the intestinal pathophysiology observed in child undernutrition. Indeed, the gut microbiota drives distinct methylation profiles of the intestinal epithelium in infancy (94), whereby conventional mice display enrichment of methylated genes involved in immune responses and cellular proliferation and regeneration versus germ-free mice. This suggests that the acquisition of a healthy gut microbiome in the neonatal period may be critical for the normal methylation patterns that define healthy intestinal development, a physiological process required for healthy growth, and that disruption of this process may contribute to EED.

Methyl donors are not the only epigenetic-modifying compounds produced by the gut microbiota. Commensal gut bacteria, including *E. coli*, can stimulate the activity of histone deacetylase 3 (HDAC3) in the intestine through production of an inositol metabolite, inositol-1,4,5-trisphosphate (InsP3) (142). Activation of HDAC3 by this microbial compound promotes epithelial repair in mice. Butyrate, a major SCFA produced by the gut microbiota, is an inhibitor of HDACs, thereby influencing epigenetic modifications (20). Significantly lower levels of butyrate have been observed in children who died from SAM versus those who survived, in two separate studies (6, 18). Butyrate, however, has several other functions beyond HDAC inhibition. Other murine studies point to other potential indirect effects of an altered gut microbiota on epigenetic modifications in child undernutrition. Transplantation of stool samples from children with edematous SAM produces wasting phenotypes in mice but also leads to reduced circulating levels of methionine and cysteine, both of which are critical to one-carbon metabolism and downstream epigenetic modifications (125).

Despite the limited evidence in human studies of child undernutrition, the growing evidence for the effect of the gut microbiome on epigenetic mechanisms driving intestinal function and metabolism warrants further research in this area. Leveraging the gut microbiome as a source of methyl donors and HDAC inhibitors and as a potent site of host epigenetic regulation via probiotic or microbiota-targeted foods has potential as a novel target for combatting child undernutrition.

4. MICROBIOTA-TARGETED TREATMENTS OF UNDERNUTRITION

The growing evidence of the role of the gut microbiome in early life growth provides a novel target for nutritional and pharmacological treatments for child undernutrition. Current nutritional

strategies for the prevention of stunting and wasting do not fully resolve the short- and long-term growth deficits or associated clinical outcomes. Small-quantity lipid-based nutrient supplements from 6–24 months of age reduce prevalence of stunting by 12% and severe stunting by 17% (36, 37). Community management of SAM using ready-to-use therapeutic food (RUTF) significantly improves nutritional recovery; however, high mortality and long-term growth deficits persist following complicated SAM (17, 81). Collectively, current treatments address infection burden, via antibiotics, and host nutritional recovery via simple sugars, lipids, and micronutrients; however, these treatments do not specifically target the gut microbiome, which may contribute to improved growth. Indeed, current recommendations for complicated SAM, which include antibiotic treatment in all cases, may impair gut microbiome recovery. A growing number of pilot trials, however, report clinically beneficial effects of microbiota-targeted interventions in child undernutrition (**Table 1**).

A small number of probiotic trials report varied effects on child growth in settings with a high burden of undernutrition. A large randomized clinical trial in 795 children with SAM found no effect of a combined multispecies probiotic and prebiotic treatment on nutritional recovery, mortality, or associated clinical symptoms, although a trend toward reduced outpatient mortality was observed (72). Smaller trials suggest potential benefits of other probiotic species in SAM and other forms of undernutrition (46). *Bifidobacterium longum* subsp. *infantis* has great potential as a potential probiotic to enhance growth due to its positive association with growth in low-resource settings (134). A trial of children with SAM in Bangladesh randomized 62 participants to a *B. infantis* probiotic (*Bifidobacterium infantis* EVC001), a probiotic and a purified HMO (lacto-*N*-neotetraose), or a placebo for 4 weeks following clinical stabilization and acute phase management in hospital (7). Weight-for-age Z-score (WAZ) and MUAC were significantly greater in the probiotic arms at the study end point, 8 weeks following treatment initiation. Trials of *Lactobacillus rhamnosus* GG (LGG) and a combination of *Bifidobacterium animalis* subsp. *lactis* and LGG during SAM treatment also demonstrate evidence for reduced infection incidence and outpatient diarrhea (21, 53, 69). The potential for particular probiotics to be employed as standard treatments for SAM will rely heavily on cost, sustainability, and safety, due to the clinical instability and high mortality of children with complicated SAM. Furthermore, rational selection of probiotics is required to identify those that can suitably colonize the impaired intestine of undernourished children and target specific pathways that can improve growth recovery. Further data from animal studies may help to inform future clinical trials of suitable probiotics, such as those targeting endocrine growth pathways (120).

Modified nutritional therapies targeting the gut microbiome have potential as sustainable and cost-effective therapies for child undernutrition. Evidence from SAM suggests that recovery of microbiome maturity is short-lived following RUTF feeding and tends to revert back to an immature state 3–4 months after treatment (129). Hence, modified supplementary foods that target the gut microbiome in SAM recovery and for the prevention of stunting may support enhanced growth. A pilot trial assessing the addition of the microbiota-fermentable carbohydrates inulin or cowpea flour to F75 and F100 therapeutic milks during treatment for SAM found no improved effect on nutritional recovery versus controls; however, the supplemented feeds may prevent the temporary loss of species diversity during in-patient treatment, which is partially driven by antibiotic treatment (18). Complementary feeding with cowpea can significantly reduce some of the LAZ deficits in children at risk of stunting (126); however, it induces only modest changes in gut microbiome composition, including increased *Bifidobacteria* and reduced *Escherichia/Shigella* (93), suggesting that the beneficial effect on growth may be independent of the gut microbiome. More recently, promising data from a cohort in Bangladesh reported significantly enhanced effects of a microbiota-directed complementary food (MDCF) on growth in children recovering

Table 1 Randomized trials of microbiota-targeted interventions in child undernutrition

Authors	Year	Site	Subjects	Number of participants	Intervention	Intervention duration	Growth outcomes	Other outcomes
Gehrig et al. (49)	2019	Community setting, Bangladesh	MAM; 12–18 months	63	Microbiota-directed therapeutic food (MDCF-2)	4 weeks	No difference in anthropometric indicators versus RUSF	Increased circulating proteins involved in bone growth and neurodevelopment
Chen et al. (26)	2021	Community setting, Bangladesh	MAM; 12–18 months	118	Microbiota-directed therapeutic food (MDCF-2)	3 months	Improved rate of growth (WHZ and WAZ)	Increased circulating proteins involved in bone growth and neurodevelopment
Barratt et al. (7)	2022	Hospital setting, Bangladesh	SAM; 2–6 months	62	Synbiotic [*Bifidobacterium infantis* (EVC001) + lacto-*N*-neotetraose]	During hospitalization and nutritional recovery (28 days total)	Improved WHZ and WAZ 3 weeks after intervention	None
Kerac et al. (72)	2009	Hospital setting, Bangladesh	SAM; 5–168 months	795	Synbiotic (Synbiotic 2000 Forte)	During hospitalization and nutritional recovery (median 33 days)	No difference in nutritional cure or growth rate	None
Grenov et al. (53)	2017	Hospital setting, Uganda	SAM; 6–59 months	400	Probiotic (*Bifidobacterium animalis* subsp. *lactis* and *Lactobacillus rhamnosus*)	During hospitalization and 8–12 weeks after discharge	No difference in nutritional recovery	Reduced diarrhea during outpatient treatment
Kara et al. (69)	2019	Hospital setting, Turkey	Weight and height below −2 SD WHO standards; 6–59 months	100	Probiotic (*L. rhamnosus* GG)	3 months	Significant increase in BMI and BMI Z-score	Reduced infection episodes
Stephenson et al. (126)	2017	Community setting, Malawi	All children; 6 months	355	Cowpea flour or common bean flour	6 months	Less reduction in LAZ between 6–9 months with cowpea flour	None
Calder et al. (18)	2021	Hospital setting, Uganda	SAM; 6–60 months	58	Cowpea flour or inulin	During hospitalization (median 14–18 days across groups)	None	Loss in species diversity temporarily prevented during inpatient treatment with cowpea
Famouri et al. (46)	2014	Community setting, Iran	WAZ <5th percentile or WHZ <10th percentile; 12–60 months	84	Synbiotic (FOS and *Bacillus coagulans*)	6 months	Increase in weight	None

Abbreviations: BMI, body mass index; FOS, fructooligosaccharide; LAZ, length-for-age Z-score; MAM, moderate acute malnutrition; SAM, severe acute malnutrition; RUSF, ready-to-use supplementary food; WAZ, weight-for-age Z-score; WHO, World Health Organization; WHZ, weight-for-height Z-score.

from MAM compared with standard RUTF therapy (26). By rationally designing a complementary feed combination (MDCF-2) consisting of locally available, culturally acceptable foods that enhanced the maturation of the early life gut microbiome in humanized animal models, Chen and colleagues (49) reported a nutritional intervention that significantly improved WHZ and WAZ throughout 3 months of treatment. Furthermore, MDCF-2 had a lower caloric density compared with RUTF (204 kcal versus 247 kcal per 50-g daily dose) and simultaneously led to an enrichment of plasma proteins involved in bone growth and neurodevelopment. These promising data suggest that microbiota-directed complementary foods may enhance growth recovery following child undernutrition to a greater extent than current therapies. Whether these small, but significantly greater, growth improvements correspond to sustained growth improvements, cognitive benefits, or reduced infection and chronic disease risk in the long term are unknown. However, these data provide a framework through which region-specific complementary foods targeting the gut microbiome could be designed to improve child growth.

5. FUTURE PERSPECTIVES AND CONCLUSIONS

Growing evidence from clinical trials in high-income settings support the efficacy of microbiota-targeted treatments in a variety of infectious, gastrointestinal, and metabolic conditions. As more than 1 in 5 children around the world are either stunted or wasted, research that aims to further characterize the microbiome-mediated pathophysiological pathways that drive child undernutrition is essential to inform better treatments. Large-scale randomized trials of microbiota-targeted interventions to combat both stunting and wasting are warranted to build upon data from existing pilot trials. Child undernutrition involves disturbances to a number of physiological systems, including metabolic, immune, and endocrine systems, and many of these are intricately connected with the gut microbiome and therefore may be amenable to microbiome-targeted interventions. Future potential microbiome-targeted therapies will be complementary to existing therapies that target host nutritional requirements and infection burden. Furthermore, it will be essential to tailor these interventions to the specific requirements of the country or region to create sustainable interventions that are cost-effective and culturally acceptable.

A number of outstanding questions remain in this field, as indicated below. Future research efforts addressing these and other outstanding questions in this field will help to inform new microbiome-focused interventions to tackle the global burden of child undernutrition.

FUTURE ISSUES

1. What is the influence of the nonbacterial component of the gut microbiome (fungi, viruses, parasites, archaea) on pathways mediating child growth?
2. What factors determine probiotic colonization within the malnourished gut and how can these be optimized to aid colonization?
3. Birth weight is strongly predictive of future child stunting. Can maternal microbiomes be modified during pregnancy to increase birth weight and prevent preterm birth?
4. Compositional and functional differences have been observed between the gut microbiomes of stunted and nonstunted children. How does the microbiome of stunted children metabolize micronutrients and macronutrients essential for growth?
5. Can fecal transplants persistently restore the gut microbiome and improve clinical recovery following complicated severe acute malnutrition?

6. What are the long-term effects of microbiota-directed therapeutic foods on growth and associated clinical outcomes?
7. How does the impaired gut microbiome impact neurodevelopment in malnourished children?

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