Mechanisms of cross-talk between the diet, the intestinal microbiome, and the undernourished host

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ABSTRACT

Undernutrition remains one of the most pressing global health challenges today, contributing to nearly half of all deaths in children under five years of age. Although insufficient dietary intake and environmental enteric dysfunction are often inciting factors, evidence now suggests that unhealthy gut microbial populations perpetuate the vicious cycle of pathophysiology that results in persistent growth impairment in children. The metagenomics era has facilitated new research identifying an altered microbiome in undernourished hosts and has provided insight into a number of mechanisms by which these alterations may affect growth. This article summarizes a range of observational studies that highlight differences in the composition and function of gut microbiota between undernourished and healthy children; discusses dietary, environmental and host factors that shape this altered microbiome; examines the consequences of these changes on host physiology; and considers opportunities for microbiome-targeting therapies to combat the global challenge of child undernutrition.

KEYWORDS
bile acids; enteropathogens; gastrointestinal motility; glycans; inflammation; intestinal mucus; kwashiorkor; marasmus; metabolomics; severe acute malnutrition; stunting

Undernutrition remains a scourge of global child health

Undernutrition, a pathologic state in which dietary intake fails to meet the body’s energy or nutrient requirements, may arise from insufficient quantity of macronutrients or micronutrients, abnormally high energy expenditures, impaired absorption or assimilation of nutrients, or any combination thereof. Undernutrition afflicts more than 800 million individuals, disproportionately burdening young children. Despite recent progress by the Millennium Development Goals, 159 million of the world’s 667 million children under 5 y of age remain stunted (low height-for-age) and 50 million children exhibit wasting (low weight-for-height), reflecting chronic and acute undernutrition, respectively. Each year, undernutrition claims 3.1 million child lives – 45% of all global child deaths.

Undernutrition is at the heart of a “vicious cycle” in which an altered gut microbiota (“dysbiosis”), often containing enteropathogens from an unsanitary environment, triggers a subclinical constellation of intestinal pathologies that include inflammation, barrier dysfunction, predilection to pathogen invasion, altered transit, and malabsorption. Collectively known as environmental enteric dysfunction (EED), these pathologies promote growth failure and persistent dysbiosis. When this vicious cycle is present during a critical early developmental window, children are at increased risk of life-long co-morbidities including short stature, decreased fitness and earning potential, cognitive impairment, obesity, type 2 diabetes mellitus, and cardiovascular diseases. This article will summarize recent observational and mechanistic studies that have advanced our understanding of precisely how the gut microbiome differs in undernourished children, of the ways in which microbial communities and functions become altered in the nutrient-deprived host, and of mechanisms by which these alterations contribute to the pathophysiologies that perpetuate this vicious cycle (Fig. 1).

Undernourished children have distinct patterns of gut bacterial community configurations

In 1958 P.M. Smythe, working in South Africa, was among the first to study gut bacteria in children with severe acute malnutrition and stunting.
kwashiorkor, a severe edematous form of protein-energy undernutrition. Although his “attempt to show the spread of bacteria up the intestinal tract” was limited by the culture-dependent microbiological techniques available at the time, he detected a surprising number of coliform bacteria in gastric aspirates. Over the next two decades, bacterial overgrowth in the proximal gastrointestinal tract would be confirmed among undernourished children in Guatemala, Aboriginal Australia, Indonesia, Brazil, and the Gambia, and among adults with tropical sprue and acute undernutrition in Bengal. Small bowel biopsies confirmed the co-existence of altered mucosal architecture and inflammation. Although invasive sampling is no longer performed strictly for research purposes, these early studies confirmed both altered bacterial populations and altered intestinal physiology in undernutrition.

Decades later, culture-independent high-throughput microbial DNA sequencing technologies reinvigorated studies that sought to more precisely define the altered fecal bacterial populations in undernourished children, with the hope that such work would shed light on how dysbiosis might contribute to impaired weight gain and how microbiome-targeting therapies might be used to improve a child’s nutritional status. These observational studies revealed two key findings. First, undernutrition is associated with decreased fecal microbial community richness (fewer unique taxa). In one study of preschool-aged Bangladeshi children, stool from undernourished children contained just 57% of the richness found in healthy subjects. Unexpectedly, decreased richness also was detected in stool from children with marasmus, a non-edematous form of severe undernutrition, compared to those with kwashiorkor, a severe edematous form, in a cohort of 87 Ugandan children ages 6–59 months hospitalized for nutritional rehabilitation; healthy subjects were not included in this study. The second key finding is that altered proportional representations of specific bacterial...
groups are found in undernutrition. For example, overabundance of Proteobacteria was present in undernourished children in Bangladesh\textsuperscript{20} and India.\textsuperscript{18} Although functional consequences are difficult to infer from phylum-level changes, fecal microbial communities from both undernourished cohorts included increased proportions of pathogenic taxa within Proteobacteria, including \textit{Enterobacter}, \textit{Escherichia}, \textit{Klebsiella}, and \textit{Shigella}, as confirmed elsewhere.\textsuperscript{19,25} It should be noted that a similar pattern (increased proportions of Proteobacteria with decreased microbial diversity) is found in inflammatory bowel disease.\textsuperscript{26} On the other hand, genera containing potentially beneficial organisms are depleted in the undernourished gut. \textit{Roseburia}, \textit{Faecalibacterium}, and \textit{Butyrivibrio} (important sources of butyrate for colonocytes), as well as \textit{Lactobacillus} and \textit{Bifidobacterium} (which can decrease inflammation, strengthen gut barrier function, inhibit pathogens, and mediate other beneficial effects under certain conditions), are deficient in stool from undernourished children.\textsuperscript{18,20,25} Together, these data suggest that the characteristic fecal microbiota of the undernourished child contains decreased richness, increased relative abundance of genera containing pathogens, and loss of genera containing potentially beneficial organisms.

\textbf{Undernourished children have delayed gut microbiota maturity}

A child’s intestinal microbiota gains complexity over time – most rapidly during the transition from exclusive breast or formula feeding to a diet consisting of a variety of solid foods.\textsuperscript{27,28} As community richness and diversity increase, the functional potential of the microbiota increases as well. For example, acquisition of members of the genus \textit{Bacteroides} broadens the genomic repertoire for carbohydrate hydrolysis, xenobiotic detoxification, and vitamin biosynthesis.\textsuperscript{29} Thus, it was hypothesized that the decreased microbial community richness found in undernourished children might translate to delayed acquisition of important microbial functions. To test this hypothesis, Smith et al. prospectively followed 317 Malawian twin pairs through age 36 months.\textsuperscript{21} Half of these pairs remained well-nourished, but 135 pairs became discordant for acute undernutrition, prompting therapeutic feeding to be administered to both twins. Metagenomic DNA was extracted from multiple (range 4–17) fecal samples over time from both members of nine same-gender healthy twin pairs and 13 same-gender twin pairs discordant for kwashiorkor (308 total metagenomes). Microbial genes isolated from healthy children followed a distinct pattern of maturation with increasing age. Although no specific microbial genes or taxa were consistently discriminatory for kwashiorkor, the rate of microbiome maturation was delayed in children who developed kwashiorkor.\textsuperscript{21} Statistical techniques have not been standardized for complex study designs involving multi-dimensional data sets sampled over time and with more than one subject per household. Nonetheless, this study nicely illustrates the power of large prospective analyses of twin pairs discordant for nutritional phenotypes despite similar genetics and feeding practices.

The concept of microbiota maturity in early postnatal development was further developed by Subramanian et al.,\textsuperscript{22} who collected monthly stool samples from 50 unrelated Bangladeshi children through the first two years of life. In a subset of 12 children with consistently healthy growth, relative abundances of taxa identified by 16S sequencing were regressed against the child’s age at the time of fecal collection, and a set of 24 age-discriminatory taxa were identified using a Random Forests machine-learning algorithm. A “relative microbiota maturity” index and a “microbiota for age Z score” (MAZ) were defined. MAZ positively correlated with weight-for-height \textit{Z} scores, and among 64 severely undernourished children requiring hospital admission for nutritional rehabilitation, microbiota maturity was significantly impaired. Separately, a 25-taxon age-discriminatory model was constructed for the above-mentioned Malawian cohort, in which MAZ also positively correlated with weight-for-height and weight-for-age \textit{Z} scores.\textsuperscript{30} Interestingly, the Bangladeshi and Malawian 25-taxon models had 9 taxa in common, with members of the \textit{Bifidobacterium}, \textit{Faecalibacterium}, and \textit{Lactobacillus} genera among each model’s top 5 most age-discriminatory microbes. These studies were the first to directly correlate gut microbiota maturity to age-based anthropometric indices. The etiology of microbiota immaturity is likely multi-factorial, and will be discussed in the following section.

\textbf{Dietary factors that alter the gut microbiome in undernutrition}

A child’s intestinal microbiota is initially shaped by numerous perinatal factors. These include maternal nutritional status, immunity, and microbiome; delivery and early feeding modalities; antibiotic usage; and
sanitation, hygiene, and pathogen exposure. Post-weaning, food availability and dietary traditions vary worldwide. Individuals in developing regions typically consume cereal- and plant-based diets rich in complex plant polysaccharides, in contrast to the energy-dense animal-derived foods and processed carbohydrates featured in Western diets. The type and amount of dietary carbohydrate are likely partially responsible for the fecal microbial community differences reported between healthy children from sub-Saharan Africa and those from Europe. Compared to healthy Italian children, stool from healthy children in Burkina Faso contained greater proportions of the phylum Bacteroidetes, with specific enrichment of genera (Prevotella and Xylanibacter) that harbor enzymes for metabolizing non-digestible dietary cellulose and xylans, key constituents of the Burkina Faso diet. Thus, a culture’s dietary carbohydrate composition could drive selection for bacteria containing the genomic repertoire to metabolize these nutrients as energy sources.

Mechanistically, the establishment of microbial communities along the intestine’s longitudinal axis is a multifactorial and perhaps even partly random process, and is directed to an extent by dietary carbohydrates. Simple carbohydrates are absorbed in the small intestine, leaving non-digestible complex polysaccharides, most importantly the glycans, as key determinants of microbial populations in the colon. Complex interactions form between dietary glycans, the host, and gut microbes that vary widely in the types of glycans they metabolize. For example, the genus Bacteroides contains an expansive repertoire of glycan-degrading enzymes that metabolize resistant starches, plant cell wall polysaccharides, inulin, and cellulose, into short chain fatty acids and other products that the intestine can absorb. Bacteroides are among the most effective degraders of animal-derived glycoproteins in the colon, which could help explain why the genus is enriched in individuals consuming Western animal-based diets and less abundant in those consuming plant-based diets, and raises the possibility that a microbiota’s inability to convert non-digestible dietary components into energy forms accessible to the host could have deleterious effects on growth. For example, human milk oligosaccharides (HMOs) are glycans that shape the infant gut microbiota. Among their many functions, HMOs serve as metabolic substrates for beneficial microbes including Bifidobacterium longum subspecies infantis. Recent evidence that sialylated (sialyllacto-N-tetraose b) and fucosylated (2-fucosyllactose and lacto-N-fucopentaose I) HMOs may play a role in infant growth via the microbiome was presented by Charbonneau et al., who analyzed breast milk from 88 Malawian mothers. Concentrations of total, fucosylated and sialylated HMOs were higher among mothers of healthy compared to severely stunted 6-month-old infants, and in a second cohort of 215 mothers, total and sialylated HMO concentrations were increased in milk from mothers of healthy versus moderately stunted infants. The authors linked sialylated milk oligosaccharides to lean body mass gain using a mouse model, as described later.

Other glycans are endogenous to the gut and may serve as substrates for microbiota in the context of a glycan-deficient diet. Some bacteria such as Akkermansia muciniphila and Bacteroides thetaiotaomicron can derive carbon and energy from the O-linked glycan structural components of intestinal mucus. When dietary N-linked glycans become limited, these bacteria increase transcription of a variety of enzymes including hexosaminidases, α-fucosidases, and sialidases to metabolize endogenous O-linked glycans. Accordingly, A. muciniphila appears to have a competitive growth advantage in breast milk deprived undernourished neonatal mice, which contain fewer microbial genes that metabolize N-linked glycans. Although it remains to be determined whether enrichment of mucophilic microbes might harm or benefit the undernourished host, the balance and variation of dietary and endogenous polysaccharides appears to influence microbial community composition.

Dey et al. provided further evidence of the impact of regional diets on microbiota function by transplanting six groups of gnotobiotic mice with fecal microbes derived from one of six healthy adults with various ethnic dietary patterns. Each humanized mouse model was challenged successively with diets simulating those of the six ethnicities, and gastrointestinal transit times were approximated as the elapsed time between a bolus gavage of carmine red dye and the initial appearance of dye in the stool. The authors report that turmeric, a staple in the traditional Bangladeshi diet, can alter microbiome composition and function as well as intestinal motility. Turmeric slowed transit by altering bile acid metabolism via mechanisms

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described later. Carmine red has inherent limitations as an indicator of motility, and intestinal transit time is influenced by many factors including dietary polysaccharides, short chain fatty acids, and other microbial-derived metabolites, including those that regulate host production of serotonin and the incretin hormone GLP-1. Nonetheless, given that intestinal motility could influence both appetite and the efficiency of energy extraction from ingested nutrients, these data highlight the intersection of diet, microbiota, and neurogastroenterology as an important area of future research.

Dietary deficiencies also can alter the microbiota. Deletion of the mouse angiotensin I converting enzyme (peptidyl-dipeptidase) 2 (Ace2), which is required for the expression of an enterocyte amino acid transporter, results in tryptophan deficiency with microbial dysbiosis that is due to transcriptional repression of ileal antimicrobial peptides. Compared to tryptophan-sufficient animals, both Ace2-deficient mice and wild type mice maintained on a tryptophan-deficient diet had more profound weight loss and more severe intestinal damage with chemical-induced colitis. These effects were ameliorated by tryptophan supplementation and recapitulated in tryptophan-sufficient gnotobiotic mice colonized by the dysbiotic gut microbes. Thus, deficiency of a specific amino acid may be linked to microbial dysbiosis and enteric dysfunction. Given the variations in dietary intake among children of differing cultures and nutritional states, it will be important to elucidate which of these factors impacts microbiome function and host physiology to a clinically meaningful extent.

**Environmental and host factors that shape the gut microbiome of undernourished children**

For children of diverse genetic backgrounds who live amidst poor sanitation and ubiquitous enteropathogens, diet is just one of many factors that shape the intestinal microbiota. Pathogens can alter microbial populations via multiple mechanisms, including secretion of toxins, competition for nutrients, and promotion of inflammation. *Vibrio cholerae* metabolizes a variety of intestinal mucus components, using altered mucus secretion as a competitive advantage to colonize despite resistance from commensal bacteria. *Salmonella enterica* serovar Typhimurium (S. Typhimurium) expresses virulence genes that induce inflammation, inhibit the growth of commensal communities, and enhance the pathogen’s ability to colonize and invade.

Inflammation alone alters gut microbial communities, as observed in mice with inflammation triggered by dextran sodium sulfate (DSS) or IL-10 deficiency, although possible effects of DSS metabolism by bacteria or compensatory immune changes cannot be excluded. Inflammation may disrupt the microbiota by three key mechanisms. First, inflammation triggers an immune response, releasing antimicrobial peptides into the intestinal lumen; this response innately defends against pathogens but also can target subsets of commensal microbes. Second, inflammation increases luminal oxygen levels. Oxygen normally diffuses from the mucosal capillary network toward the lumen, creating an oxygen gradient that tightly regulates microbes within oxic, microoxic, and anoxic zones. This gradient helps shape microbial ecology, with facultative anaerobes near the mucosal surface and strict anaerobes in the anoxic lumen, and influences bacterial transcriptional programs. The increase in luminal oxygen during inflammation selectively promotes the growth of aerotolerant microbes, especially Enterobacteriaceae and its pathogens. It is not known whether the oxygen gradient is disrupted by the rapid transit rates of non-inflammatory diarrhea. Third, inflammation generates reactive oxygen and nitrogen species which shape microbial populations by facilitating respiration among certain bacteria. Reactive oxygen species combine with luminal sulfur compounds to form the oxidation product tetrathionate. Bacteria such as S. Typhimurium that can utilize tetrathionate as an electron acceptor for respiration have a selective growth advantage over bacteria that cannot. Given that inflammation is a hallmark of EED, these mechanisms likely help shape the microbial dysbiosis observed in undernourished children.

**A disrupted gut microbiota increases enteropathogenic potential**

High-throughput sequencing of the whole bacterial metagenome (not just the 16S rRNA gene) facilitated the initial discovery of fecal microbiome immaturity in undernourished Malawian children, but also proposed potential functional consequences of dysbiosis in the undernourished child. For example, stool from
undernourished Indian and Bangladeshi children contained decreased abundance of bacterial genes involved in nutrient metabolism and an overabundance of genes that mediate virulence and pathogenesis.\textsuperscript{18,19}

Gut microbiota containing low diversity are less resistant to enteropathogens.\textsuperscript{71} In mice, disrupting the microbiota with antibiotics\textsuperscript{72} or inflammation\textsuperscript{73} exacerbates \textit{S. Typhimurium} colitis and colonization potential. Thus, the loss of microbial diversity observed in undernutrition could place children at higher risk of more frequent and more severe enteropathogen infections, perpetuating the vicious cycle of EED, inflammation, and impaired growth. Intriguingly, similar effects can be replicated in mice by gut microbes that are not typically considered to be pathogenic. A low-protein, low-fat chow led to moderate microbes that are not typically considered to be pathogenic. A low-protein, low-fat chow led to moderate growth stunting but not the classic small bowel histopathology seen in EED.\textsuperscript{74} However, repetitive administration of a seven-bacterium combination of members of the Bacteroidales order and commensal \textit{Escherichia coli}, none of which are pathogenic when administered individually, induced robust intestinal histopathology and exacerbated stunting, barrier dysfunction, inflammation, gut microbial dysbiosis, and both colonization and systemic invasion of \textit{S. Typhimurium}. Importantly, the seven-bacterium mixture failed to reproduce any of these effects in normal-weight mice receiving an isocaloric control diet,\textsuperscript{74} illustrating the complex interdependency of microbial, host, and dietary factors in EED.

\textit{Dysbiosis can impact dietary energy harvest, de novo micronutrient synthesis, and bile acid homeostasis}

Energy harvest by gut bacteria contributes substantially to host metabolism. By converting non-digestible dietary components into forms of energy that epithelial cells may absorb, the microbiota contribute to an estimated 10\% of an adult’s caloric requirement.\textsuperscript{75} However, stool from underweight Indian children was deficient in microbial genes that ferment complex plant oligosaccharides and peptidoglycans.\textsuperscript{18} Similarly, decreased abundance of cecal and colonic \textit{Bacteroides} and loss of their genes capable of metabolizing N-linked glycans were observed in a neonatal mouse model of protein-energy undernutrition.\textsuperscript{46} Thus, dysbiosis could result in less efficient energy extraction from the diet.

Gut bacteria are also capable of \textit{de novo} micronutrient biosynthesis. Some microbes can synthesize amino acids by salvaging and recycling nitrogen from a variety of dietary or endogenous sources (e.g., urea, ammonia),\textsuperscript{76} although the extent to which these contribute to an individual’s total protein requirement is poorly defined. Nonetheless, amino acid metabolism is among the most significantly perturbed biological pathways in metabolomic analyses of severely undernourished children\textsuperscript{77} and in undernourished mice,\textsuperscript{74,78} rats,\textsuperscript{79} and pigs.\textsuperscript{80} Similarly, vitamin K and a subset of the water-soluble B vitamins are synthesized by members of the gut microbiota.\textsuperscript{81} Depletion of these strains could theoretically impact the vitamin status of the host. A myriad of vitamin deficiencies are found in undernourished children,\textsuperscript{82} and metabolomic analyses in multiple studies of protein-energy undernutrition have revealed altered concentrations of intermediates in vitamin-metabolizing pathways.\textsuperscript{74,77,78} Whether correcting dysbiosis can ameliorate specific micronutrient deficiencies remains to be explored.

Gut microbes also influence host physiology by regulating the bile acid pool. When glycine- or taurine-conjugated bile acids enter the small intestine, their emulsification properties facilitate uptake of dietary lipids and fat-soluble vitamins and their antimicrobial properties regulate gut microbial communities. Some bacterial genomes encode enzymes that enhance their ability to survive in bile.\textsuperscript{83,84} These enzymes include bile salt hydrolase (BSH),\textsuperscript{85} which enables certain members of \textit{Bacteroides}, \textit{Lactobacillus}, and other genera to remove the glycine and taurine groups from primary bile acids,\textsuperscript{86} and 7-\alpha-dehydroxylase,\textsuperscript{87} which members of genera including \textit{Clostridium} use to convert primary to secondary bile acids. These microbial activities could have profound influences on host physiology including gastrointestinal transit and lipid metabolism. Dey et al. reported that turmeric slows gastrointestinal transit in gnotobiotic mice by increasing concentrations of conjugated bile acids taurohyodeoxycholic acid and tauro-muricholic acid sulfate; transit time was normal if the recipient microbiome was enriched in the BSHs that deconjugate these bile acids.\textsuperscript{47} Furthermore, intestinal expression of cloned BSH alters bile acid concentrations in plasma, liver, and stool, influences transcription of host genes involved in lipid metabolism, and decreases serum
cholesterol, liver triglycerides, and weight gain. Gut microbiota can impact expression of genes related to bile acid transport and metabolism in the ileum and bile acid synthesis in the liver by reducing concentrations of taurine-conjugated β-muricholic acid, a nuclear farnesoid X receptor antagonist. Although it is not yet known the extent to which altered microbial populations contribute to the abnormal bile acid profiles observed in children and mice with protein-energy undernutrition or EED, altered bile acid pools could influence energy metabolism, absorption of dietary fat and fat-soluble vitamins, and ultimately weight gain.

Lessons learned from transplantation of human-derived microbes into gnotobiotic mice

The most convincing evidence to date of a causal link between gut microbial dysbiosis and growth impairment in undernutrition is found in studies of gnotobiotic mice colonized with fecal microbes from healthy vs. underweight children. From the 317-twin Malawian cohort described earlier, the authors selected three twin-pairs ages 16–21 months who were discordant for undernutrition. Frozen stool samples from these six donors were transplanted into separate groups of gnotobiotic mice. In two of the three twin pairs, microbes from the child with kwashiorkor induced greater weight loss vs. microbes from the healthy twin when mice were given a low-protein low-fat chow designed to mimic the donors’ Malawian diet. Discordant weight loss was not observed when mice consumed standard chow. Transmission of donor nutritional phenotype via the microbiota has been confirmed in gnotobiotic mice colonized by microbial communities derived from multiple other undernourished vs. healthy Malawian children, illustrating a direct causality between gut microbes and growth impairment.

Four key concepts have emerged from studies transplanting child-derived fecal microbes into gnotobiotic animals. First, microbiota growth potential is dependent on the age of the donor host. With fecal samples from 19 healthy and undernourished Malawian donors, microbiota from 6-month-old infants induced greater weight gain vs. microbiota from 18-month-old children. Second, many of the taxa observed to be growth-discriminatory in children from Malawi and Bangladesh were also found to be growth-discriminatory when transplanted into mice. In particular, two probiotic species, *B. longum* and *Faecalibacterium prausnitzii*, improved growth trajectories in undernourished mice. Third, growth-discriminatory microbiota have widespread systemic effects that are measurable in multiple ways, including body composition, bone morphology, and metabolite profiling in serum, stool, intestinal lumen, muscle, liver, and brain. These studies reveal perturbations in metabolites associated with carbohydrate and amino acid catabolism, tricarboxylic acid and urea cycle intermediates, acylcarnitines, N-linked glycans, and one-carbon metabolism – pathways that also are among those perturbed in undernourished children and models of undernutrition that employ conventionally reared mice. Fourth, IgA-bound fecal microbes from undernourished hosts can produce pathology resembling EED under certain dietary conditions. Using a fluorescence-activating cell sorting approach, Kau et al. isolated viable IgA-targeted microbes from gnotobiotic recipients of fecal bacteria from a 21-month-old Malawian with kwashiorkor or from the child’s healthy twin. IgA-bound microbes (most prominently, members of the family Enterobacteriaceae) from recipients of the undernourished human donor induced systemic inflammation, weight loss, intestinal epithelial barrier disruption, and mortality when transplanted into another set of gnotobiotic mice maintained on a Malawian diet; this pathology was ameliorated by feeding these mice standard chow or by co-administering IgA-bound microbes from the healthy twin of the child with kwashiorkor. Whether similar effects would be observed following transplantation of the subset of bacteria not targeted by IgA is unknown. Together, these gnotobiotic mouse models provide evidence of a causal link between dysbiosis and growth impairment.

Microbiome-targeting therapies have thus far demonstrated limited efficacy for undernutrition

Given our recent progress in understanding mechanisms by which gut microbes may cause impaired growth in undernourished children, microbiome-targeting therapies hold promise as adjuvant treatments for nutritional rehabilitation. In the outpatient setting, an undernourished child is typically treated with ready-to-use therapeutic food (RUTF) composed of nut paste, sugar, vegetable oil and milk powder...
fortified with vitamins and minerals. However, RUTF can be expensive and often must be imported. Furthermore, long-term outcomes in meta-analyses have demonstrated mixed results. To date, only four large randomized, placebo-controlled trials have sought specifically to improve growth in undernourished children by targeting the gut microbiome during refeeding (Table 1).

Synbiotic 2000 Forte is a fermented milk that contains four probiotic lactic acid bacteria (Pediococcus pentosaceus 16:1, Leuconostoc mesenteroides 23–77:1, Lactobacillus paracasei ss. paracasei F-19, and Lactobacillus plantarum 2362; total of 10^11 colony-forming units) and prebiotic fermentable fibers (oat bran rich in β-glucans, inulin, pectin, and resistant starch; 2.5 g of each). In a randomized, double-blind placebo-controlled efficacy trial enrolling 795 children admitted to a Malawian hospital for nutritional rehabilitation, synbiotic-fortified RUTF, given daily throughout the duration of inpatient and outpatient treatment (median 33 days), improved neither nutritional cure nor other clinical outcomes compared to unfortified RUTF. This probiotic/prebiotic combination had not been identified by preclinical models or observational studies within this patient population. Thus, it would be premature to conclude from this negative result that the microbiome is not amenable to manipulation by probiotics and prebiotics to promote weight gain during refeeding.

As an alternative strategy, elimination of pathogenic elements of the microbiota could be achieved with antibiotics. Promising results were found in a randomized, double-blind, placebo-controlled trial enrolling 2767 Malawian children receiving RUTF as outpatient therapy for severe acute undernutrition. Children receiving placebo had a slightly increased relative risk of both RUTF failure and mortality compared to those receiving either of two oral antibiotic regimens (amoxicillin 80–90 mg/kg/day or cefdinir 14 mg/kg/day, each divided twice daily for seven days) over a 12-week follow-up period. However, two subsequent double-blind placebo-controlled trials did not find antibiotics to be beneficial. First, among 2412 children in Niger with severe acute undernutrition, seven days of amoxicillin (80 mg/kg/day divided twice daily) had no significant effect on nutritional recovery over an eight-week period compared to placebo. Second, among 1778 children who had recovered from severe acute undernutrition in one of four Kenyan hospitals, six months of oral co-trimoxazole (120 mg/day if <6 months of age; 240 mg/day if >6 months) had no effect on mortality during the 12-month study period. It is possible that the beneficial effect on growth observed in the Malawian trial resulted from microbiota restructuring. However, the cohort of 64 undernourished Bangladeshi children studied by Subramanian et al. exhibited only a transient restoration of gut microbiota maturity that was lost four months after inpatient treatment. More importantly, any potential benefit of routine antibiotic use would need to be weighed carefully against the threat of antimicrobial resistance, drug reactions, and other adverse effects, such as increased adiposity that could predispose the undernourished child to metabolic diseases later in life.

What will it take to improve a child’s nutritional status with microbiome-targeting therapies?

Despite the current lack of evidence from clinical trials, preclinical models offer hope that microbiome remodeling could soon help treat childhood EED and undernutrition. In gnotobiotic mice maintained on a representative Malawian diet and colonized by fecal microbes from severely undernourished Malawian children, growth impairment was ameliorated either

| Table 1. Randomized controlled trials that evaluate microbiome-targeting therapies to improve nutritional status in undernourished children. |
|-----------------|-----------------|-----------------|
| Intervention     | Setting         | Number of Study Participants | Result                                                                 |
| Synbiotic 2000 Forte | Malawi         | 795             | No significant effect on nutritional cure                               |
| Amoxicillin or cefdinir | Malawi      | 2767            | Placebo increased risk of treatment failure (RR 1.32 [1.04–1.68] vs amoxicillin; RR 1.64 [1.27–2.11] vs cefdinir) and mortality (RR 1.55 [1.07–2.24] vs amoxicillin; RR 1.80 [1.22–2.64] vs cefdinir) |
| Amoxicillin      | Niger           | 2412            | No significant effect on nutritional recovery                           |
| Co-trimoxazole   | Kenya           | 1778            | No significant effect on mortality                                      |

Note: RR, relative risk followed by 95% confidence interval in brackets.
by co-housing these mice with animals receiving healthy microbiota, facilitating microbial transfer by coprophagy, or by gavaging a consortium of five bacteria cultured from healthy donors (although only *Ruminococcus gravis* and *Clostridium symbiosum* actually colonized recipient mice). Germ-free mice were mildly underweight gain, as evidenced in an elegant study by Schwarzer et al.96 Vitamin-deprived, chronically undernourished by a protein-, fat-, and carbohydrate-deficient diet, monocolonization by one probiotic strain, *Lactobacillus plantarum* NIZO2877, but not *L. plantarum* WJL, restored growth and somatotropic axis signaling to levels observed in conventionally-reared animals with decreased concentrations of insulin-like growth factor-1 (IGF-1) and IGF-1 binding protein 3, which mediate postnatal growth via the somatotropic axis. Recombinant IGF-1 injections increased body weight and longitudinal growth in germ-free but not conventionally-reared mice, whereas treatment with an IGF-1 receptor inhibitor impaired growth in mice with intact microbiota, suggesting that gut microbes promote growth in part through the somatotropic axis. In gnotobiotic mice chronically undernourished by a protein-, fat-, and vitamin-deficient diet, monoclonization by one probiotic strain, *Lactobacillus plantarum* NIZO2877, but not *L. plantarum* WJL, restored growth and somatotropic axis signaling to levels observed in conventionally-reared mice with intact microbiota. Decreased expression of IGF-1 also has been reported in jejunal of undernourished mice with EED, while decreased plasma IGF-1 has been detected in severely undernourished children. Mechanisms by which bacteria affect somatotropic axis signaling remain uncharacterized.

Another preclinical model demonstrated promising results with a prebiotic. Gnotobiotic mice maintained on a representative Malawian diet received a defined 25-strain community (of which 19 strains successfully colonized) isolated from a stunted Malawian infant enrolled in the HMO study by Charbonneau et al.42 Given the range of dietary, environmental, and host factors that may contribute to growth impairment, it is unlikely that a single therapeutic agent will fully restore a healthy balance of microbial functions, systemic metabolites, and micro- and macronutrients in all cases of child undernutrition. Rather, development of low-cost biomarkers from easily accessible body fluids will enable personalized therapies to address an individual’s specific...
functional deficiencies with the appropriate combination of microbiome-targeting agents (Fig. 2). Delivering these therapies will present new challenges, including ensuring the safety of live bacteria ingested by undernourished and immunocompromised children, lowering the cost of large-scale production of prebiotics, and navigating the challenges associated with distribution in resource-constrained settings (e.g., lack of refrigeration, cultural acceptability, sustainable local production). Nonetheless, the remarkable insights gained in the last few years alone provide hope that harnessing the power of gut microbes, their enzymes, and their signaling molecules may soon yield a much-needed breakthrough against the global scourge of childhood undernutrition.

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