Stunting Persists despite Optimal Feeding: Are Toilets Part of the Solution?

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Abstract
Children in developing countries have an average length-for-age that is already below the World Health Organization standard at birth and show a further decline in linear growth over the first 24 months of life; however, complementary feeding interventions have only a modest impact on growth. Children living in conditions of poor sanitation and hygiene are frequently exposed to pathogenic microbes through feco-oral transmission. Acute diarrhea represents only the tip of the ‘enteric disease iceberg’, with a substantial underlying burden of chronic, subclinical enteropathy. Environmental enteric dysfunction (EED) is characterized by disturbance in small intestinal structure and impaired gut barrier function, enabling microbial translocation and chronic systemic inflammation, which may impair growth. Gut damage appears to arise early in infancy and markers of intestinal inflammation, intestinal permeability and systemic immune activation are inversely associated with linear growth. Reducing feco-oral microbial transmission by improving water, sanitation and hygiene (WASH) may theoretically prevent or ameliorate EED and improve linear growth; ongoing trials are exploring this hypothesis. Given the complex interplay of factors leading to stunting, multisectoral interventions are likely required. Improving WASH in addition to infant feeding may be one approach to improve the growth and developmental potential of infants in developing countries.

Globally, 165 million children under 5 years of age are stunted [1]. Stunting, defined as a height-for-age more than 2 standard deviations below the World Health Organization (WHO) reference median, is associated with both short- and long-term risks. Stunted children have increased morbidity and mortality, particularly from pneumonia and diarrhea, with an estimated 14–17% of global
child deaths below 5 years attributable to stunting [2]. Long-term, children who are stunted have reduced motor and cognitive development, poorer performance at school, lower adult economic productivity and an increased risk of nutrition-related chronic diseases [2, 3].

Intrauterine growth restriction is a risk factor for stunting. An estimated 20% of child stunting has in utero origins, based on a recent birth cohort meta-analysis of postnatal growth among infants born small for gestational age [4]. Children in developing countries therefore have an average length-for-age that is already below the WHO standard at birth, then show a further decline in linear growth over the first 24 months of life, with little or no catch-up thereafter [5]. An ambitious global target of 40% reduction between 2010 and 2025 in the proportion of children under 5 years who are stunted is unlikely to be reached; in Africa, where stunting prevalence has remained relatively stable over the past 20 years, the absolute number of stunted children is actually likely to increase by 2025 [6].

**Does Inadequate Diet Explain Stunting?**

Is the solution to poor linear growth not obvious: children need to consume adequate quantities of a nutrient-rich, varied diet? Whilst this appears logical, the quality and quantity of infant diets does not account for the high prevalence of stunting seen in developing countries. A meta-analysis of 42 effectiveness studies and efficacy trials undertaken in developing countries among children in the 6–24 months age range, when growth faltering peaks, showed only a modest impact of complementary feeding interventions on growth [7]. Generally, studies that provided complementary foods in food-insecure regions such as Africa and South Asia, with or without educational messages about child feeding, showed some benefit; micronutrient fortification alone showed little or no impact on growth. Overall, effect sizes for growth were modest; even the most successful interventions improved height-for-age by only around +0.7 z scores, which is approximately one third of the average growth deficit. Taken together, the sobering fact is that no nutrition trial or program has ever normalized the linear growth of children in developing countries.

**The Impact of Enteric Infections on Stunting**

Despite its extraordinarily high prevalence, the pathophysiology of stunting remains poorly understood. It is clear that multiple factors contribute to stunting [8], but the relative importance of each is not known, and it remains unclear
which would be most tractable to interventions. Given the high infectious disease burden in developing countries, attention has focused on the impact of recurrent infections on growth. These associations were elegantly demonstrated several decades ago by Leonardo Mata [9] in his rural Guatemalan studies, in which children who thrived in early infancy subsequently showed progressive declines in growth with each episode of intercurrent illness. Diarrhea remains one of the most frequent recurrent infections among children in developing countries, but the impact of diarrhea on both short- and long-term linear growth has been difficult to ascertain. Some studies report a significant impact of diarrhea on height, whilst others indicate that catch-up linear growth occurs between episodes, leading to little or no long-term deficit in height attributable to diarrhea; the discrepancies between findings likely reflect differences in study design and population characteristics. In a recent study of 7 longitudinal cohorts of infants under 24 months of age, cumulative diarrheal burden had a small but measurable effect on linear growth; a child experiencing the average diarrheal burden (equivalent to 23 days per year) was 0.38 cm shorter at 2 years of age compared to a child without diarrhea [10]. Taken together, diarrhea does have an impact on growth, but provided children have a disease-free period for recovery and access to adequate diet, catch-up growth can occur; diarrheal disease is therefore not sufficient to account for the degree of stunting that is typical among infants in developing countries.

Environmental Enteric Dysfunction – A Role for the Gut in Stunting?

Children living in impoverished conditions are undoubtedly frequently exposed to pathogenic microbes through feco-oral transmission; it is therefore likely that acute diarrhea represents only the tip of the ‘enteric disease iceberg’ (fig. 1), with a substantial underlying burden of chronic, subclinical enteropathy [11]. Indeed, it has been recognized for many decades that people living in conditions of poverty almost universally have a small intestinal pathology, characterized by villous atrophy and a mucosal inflammatory infiltrate, which has been attributed to living in conditions of poor sanitation and hygiene [12, 13]. Originally named tropical enteropathy, the current preferred term is environmental enteric dysfunction (EED), reflecting the broad disturbance in intestinal structure and function that likely arises from an apparent environmental insult [14].

Studies conducted in the Gambia some 30 years after the original published biopsy findings of tropical enteropathy, refocused attention on the gut and suggested that, rather than being a purely incidental finding, small intestinal enter-
Enteropathy may actually be an important underlying cause of stunting [15–18]. The challenge in investigating EED is that endoscopy and small intestinal biopsy are not feasible or ethical in otherwise healthy infants, so studies have relied almost entirely on surrogate markers. Most have assessed intestinal absorptive capacity and permeability by measuring the ratio of lactulose to mannitol in urine collected over several hours following an oral challenge dose. Mannitol, a monosaccharide, should be passively absorbed by a healthy small intestinal mucosa and reduced mannitol recovery in urine therefore indicates impaired absorptive capacity; lactulose, a disaccharide, should be precluded from absorption by tight junctions between epithelial cells, and increased recovery in urine therefore indicates abnormal gut permeability. Infants in the Gambia were found to have normal lactulose:mannitol (L:M) ratios for the first 3 months of life, then a progressive rise to 12–15 months of age, when the L:M ratio was around 5-fold greater than in age-matched UK counterparts [18]. The elevated L:M ratio was driven partly by impaired absorptive capacity and, to a greater extent, by increased intestinal permeability. Infant length growth was inversely related to the L:M ratio, with the timing of onset of linear growth failure coinciding with the age at which intestinal permeability started to increase. Further studies from the Gambia [15–17] and elsewhere [19, 20] confirmed that abnormal intestinal permeability is common among infants in underprivileged settings; although EED appears to improve from around 15 months of age, L:M ratios remain elevated even in adulthood compared to people living in developed countries.

Fig. 1. The enteric disease iceberg. Diarrhea may be conceptualized as being the tip of the ‘enteric disease iceberg’, with a much greater burden of chronic, subclinical enteropathy (termed EED) underlying clinically overt episodes of acute diarrhea. This figure is schematic and is not meant to depict relative proportions of diarrhea and enteropathy.
What would plausibly link impaired intestinal permeability with growth failure in infancy? It is increasingly recognized that intestinal barrier function is critical to health. A single layer of intestinal epithelium separates the ±100 trillion commensal microbes that populate the gut from the systemic circulation. Increased intestinal permeability enables microbes and bioactive macromolecules to cross the gut epithelium (a process termed microbial translocation) and activate macrophages and dendritic cells in local mesenteric lymph nodes and Kupffer cells in the liver. Activation of the innate immune system leads to elaboration of cytokines such as interleukin-6 (IL-6), IL-1β and tumor necrosis factor-α (TNF-α). In response to these proinflammatory cytokines, particularly IL-6, the liver synthesizes acute-phase proteins such as C-reactive protein (CRP).

**Intestinal Barrier Function and Chronic Inflammation**

Fig. 2. The pathway of intestinal microbial translocation. With loss of intestinal barrier function, microbes and microbial-associated products such as flagellin and lipopolysaccharide are able to translocate across the impaired gut epithelium to local mesenteric lymph nodes and to the liver, where they activate pattern recognition receptors on innate immune cells to cause elaboration of cytokines such as interleukin-6 (IL-6), IL-1β and tumor necrosis factor-α (TNF-α). In response to these proinflammatory cytokines, particularly IL-6, the liver synthesizes acute-phase proteins such as C-reactive protein (CRP).
an acute-phase protein produced by the liver, were elevated [15]. Infants also had high concentrations of lipopolysaccharide (also called endotoxin), a major component of the Gram-negative bacterial outer membrane, and antibodies against endotoxin (EndoCAb) in the systemic circulation. It was hypothesized therefore that chronic exposure to bacteria and bacterial constituents due to microbial translocation leads to a state of chronic inflammation that is metabolically expensive, repartitioning nutrients away from growth and towards immune activation.

A series of recent studies has further explored the hypothesis that EED and chronic inflammation underlie stunting [21–24]. The Malnutrition and Enteric Diseases (Mal-ED) study enrolled 8 birth cohorts from countries in South America, Africa and Asia, undertook monthly anthropometry and evaluated three biomarkers of intestinal inflammation (neopterin, myeloperoxidase and α1-antitrypsin) in stool samples from 661 infants collected at 3, 6 and 9 months of age [21]. At all sites, infants had elevated markers of intestinal inflammation, and each of the biomarkers predicted subsequent decline in length-for-age over the following 6 months, highlighting again the association between gut pathology and linear growth deficits. A study of 39 urban and 105 rural Chinese infants (age 5–7 months) evaluated levels of fecal calprotectin, a zinc- and calcium-binding protein abundant in neutrophils, which is a well-validated marker of intestinal inflammation [23]. Fecal calprotectin concentrations were higher in rural compared to urban infants and, in the rural group only, regression analysis using Monte Carlo simulation to account for missing data showed a significant inverse relationship between calprotectin and length-for-age z scores. A study of 202 Zimbabwean infants undertaken by our group evaluated levels of intestinal fatty acid-binding protein, a cytosolic protein in enterocytes that is released into the blood following villous damage [24]. Intestinal damage was evident from 3 months of age, peaked at 12 months, and exceeded levels seen in European children with untreated celiac disease, which has similar biopsy findings to EED. Infants who became stunted by 18 months of age had evidence of chronic inflammation from as early as 6 weeks of age, and the concentration of CRP over the first year of life predicted stunting in multivariate analysis. One mechanism through which inflammation may drive stunting is suppression of the growth hormone (GH) axis. Levels of insulin-like growth factor 1 (IGF-1), which is produced by the liver in response to GH, were inversely associated with each inflammatory marker evaluated [24]. It is well known in children with chronic inflammatory diseases such as Crohn’s disease and juvenile idiopathic arthritis that proinflammatory cytokines suppress IGF-1 levels and impair linear growth, but it appears that in otherwise healthy infants, a similar relationship may exist in the context of low-grade inflammation. Taken together, these recent studies confirm that gut damage occurs early in infancy, that stunting is characterized
by chronic inflammation and is at least partly mediated by suppression of the GH-IGF-1 axis.

What causes such extensive intestinal damage in early life? Studies fairly consistently show that, whichever surrogate marker is used, infants develop enteropathy from around 3 to 6 months of age. Although WHO guidelines recommend exclusive breastfeeding (EBF) for the first 6 months of life, it is well recognized that EBF rates are poor in many settings, with introduction of potentially contaminated non-breast milk liquids and foods early in life. We recently showed that infants, observed in a rural Zimbabwean setting to identify the potential vectors of feco-oral microbial transmission, regularly mouth their own hands (which were frequently visibly dirty and were rarely washed by mothers) and often ingest soil and chicken feces as part of exploratory behavior [25]. There are therefore multiple potential routes of pathogenic microbial exposure for infants living in contaminated conditions, at a time when the normal commensal microbiota is being established.

There is emerging interest in the role of the microbiota in malnutrition. The microbiota emerges from a founding population transmitted from the mother at the time of birth and during breastfeeding, and diversifies over the first 1–2 years of life to form a stable community of organisms [26]. Intestinal commensal organisms may have important functions in energy harvesting, nutrient synthesis and utilization, mucosal immune development and regulation of intestinal inflammation. A recent Malawian study convincingly demonstrated a role for the microbiota in the pathogenesis of severe acute malnutrition (SAM) [27]. Gnotobiotic mice transplanted with fecal microbiota samples from infants with kwashiorkor and fed a typical Malawian diet developed weight loss and metabolic disturbances characteristic of kwashiorkor; introduction of ready-to-use therapeutic food (RUTF) led to a change in the microbiota and weight gain. Another recent study from Malawi recruited clinically stable children treated for SAM with RUTF in the community [28]. Children randomized to a 7-day course of antibiotics in addition to RUTF had lower mortality and better nutritional recovery than children receiving placebo, despite no overt evidence of infection. It is conceivable in this population that antibiotics operate, at least in part, by modulating the intestinal microbiota, thereby improving weight gain. A recent meta-analysis of data from 10 randomized controlled trials enrolling children with a range of underlying conditions in low- and middle-income countries showed a significant impact of antibiotics on both weight and height gain; the authors speculated that the growth-promoting effects of antibiotics may be due to alterations in the intestinal microbiota, although none of the trials specifically investigated this hypothesis [29].

Taken together, there is emerging evidence that both intestinal damage and composition and function of the microbiota may be important factors underly-
ing malnutrition in early life, although further studies are required to investigate the specific role of the microbiota in stunting. There are multiple potential overlapping causes of enteropathy in developing countries, including persistent diarrhea, HIV, mycotoxin exposure and micronutrient deficiencies, all of which may interact to compound intestinal damage [30]. EED appears to be an almost universal finding among infants living in impoverished conditions and is relatively slow to resolve once established. Could improvements in environmental conditions potentially prevent onset of EED in early life?

**A Role for Toilets in Reducing Stunting?**

Reducing feco-oral microbial transmission by improving water, sanitation and hygiene (WASH) may theoretically prevent or ameliorate EED and improve linear growth. A study from rural Bangladesh, which categorized children as living in ‘clean’ or ‘dirty’ homes based on water quality and sanitary and hand washing infrastructure, found that the height-for-age z score of children in clean homes was 0.54 (95% CI 0.06–1.01) greater than those in dirty homes after adjusting for confounding factors [22]. There was also a trend towards lower L:M ratios and lower EndoCAb concentrations in those from clean homes, suggesting that the height differences between groups may have been mediated by EED.

Several recent studies have explored the association between open defecation and stunting. The starting point for these analyses was the observation that wealth does not adequately explain the prevalence of stunting in different countries. India, in particular, has higher rates of stunting than many African countries, despite individuals on average being wealthier in India. In a regression analysis of data on sanitation coverage and child height from 65 developing countries, the author concluded that differences in height between countries, even after accounting for economic indicators, are explained by rates of open defecation [31]. An ecological analysis from India, where more than 50% of the population still practices open defecation, reported a 0.7% increase in stunting and severe stunting for each 10% increase in open defecation across 112 districts for which data were available [32]. However, despite adjusting for potential confounders, these studies are severely limited by the potential for residual confounding inherent in observational and ecological studies.

A recent systematic review of the impact of WASH interventions on child nutritional status identified 5 cluster-randomized controlled trials which evaluated solar disinfection of water, provision of soap or improvement in water quality; there were no trials of improved sanitation [33]. Although no individual
trial showed a significant effect of any intervention on height, a meta-analysis of data from 4,627 children under 5 years of age showed a small but significant impact on height-for-age z score (mean difference 0.08; 95% CI 0.00–0.16). The quality of evidence from these trials was judged to be generally low, and all reported fairly short-term (9–12 months) outcomes, leading the authors to conclude that further high-quality data are required. It is recognized that WASH interventions can be challenging to evaluate in randomized trials because of difficulties in blinding, frequent reliance on self-reported outcomes and consequent potential for observer and reporter bias [34].

Two large community-based trials are currently underway to evaluate the impact of WASH interventions on stunting in developing countries. The Sanitation Hygiene Infant Nutrition Efficacy (SHINE) study in Zimbabwe is a community-based, cluster-randomized factorial trial evaluating the impact of improved WASH and/or infant and young child feeding (IYCF) on stunting and anemia (clinicaltrials.gov identifier NCT01824940). In total, 4,800 pregnant women will be recruited from two contiguous districts of rural Zimbabwe and their infants followed to 18 months of age. WASH interventions include provision of a Blair ventilated improved pit latrine and two Tippy Tap hand washing devices in the homestead (fig. 3), together with chlorination of water and a clean play space to protect crawling infants from geophagia and inges-
tion of chicken feces. Behavior change interventions promoting use of these hardware items are provided by Village Health Workers. IYCF interventions include education about optimal complementary feeding practices and provision of Nutributter, a lipid-based nutrition supplement that provides additional calories and effectively closes the micronutrient gap, from 6 months of age. The WASH Benefits study (clinicaltrials.gov identifiers NCT01704105 and NCT01590095) comprises 2 parallel cluster-randomized controlled trials, one in Kenya and one in Bangladesh, which are evaluating the impact of individual and combined water quality, sanitation, hand washing and nutritional interventions on child growth and diarrhea. Both SHINE and WASH Benefits have intensive substudies evaluating potential pathways through which these interventions may operate, and the extent to which WASH interventions impact environmental microbial contamination. WASH Benefits and SHINE will be among the few randomized controlled trials of toilets that have evaluated child health outcomes, and aim to provide high-quality evidence to inform public health programming, given that 40% of the global population still lacks access to safe sanitation.

There is increasing interest in multisectoral interventions to tackle stunting, through both nutrition-specific and nutrition-sensitive programs. However, there is currently a paucity of evidence to guide which strategies are likely to be successful. It is hypothesized that interventions need to be targeted to the first 1,000 days to have maximal impact on growth and development (www.thousanddays.org), particularly if the aim is to prevent onset of EED. Improvements in WASH and nutrition are, however, likely only two interventions within a complex interplay of factors that must be targeted to optimally promote healthy growth and improve long-term developmental potential of children in developing countries [8].

Disclosure Statement

AJP and JHH are both investigators on the SHINE trial. The authors have no declarations of interest to declare.

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References


15 Campbell DI, Elia M, Lunn PG: Growth faltering in rural Gambian infants is associated with impaired small intestinal barrier function, leading to endotoxemia and systemic inflammation. J Nutr 2003;133:1332–1338.


