Environmental Enteric Dysfunction: Pathogenesis, Diagnosis, and Clinical Consequences

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Stunting is common in young children in developing countries, and is associated with increased morbidity, developmental delays, and mortality. Its complex pathogenesis likely involves poor intrauterine and postnatal nutrition, exposure to microbes, and the metabolic consequences of repeated infections. Acquired enteropathy affecting both gut structure and function likely plays a significant role in this outcome, especially in the first few months of life, and serve as a precursor to later interactions of infection and malnutrition. However, the lack of validated clinical diagnostic criteria has limited the ability to study its role, identify causative factors, and determine cost-effective interventions. This review addresses these issues through a historical approach, and provides recommendations to define and validate a working clinical diagnosis and to guide critical research in this area to effectively proceed. Prevention of early gut functional changes and inflammation may preclude or mitigate the later adverse vicious cycle of malnutrition and infection.

Keywords. biomarkers; developing countries; enteropathy; malabsorption; stunting.

The development of intestinal biopsy devices more than half a century ago, such as the Crosby-Kugler biopsy capsule [1], allowed per os sampling of intestinal mucosa in living individuals. For the first time, small bowel tissue could be safely and repeatedly obtained from living individuals, averting reliance on postmortem specimens [2]. “Tropical enteropathy” (i.e., jejunitis) was identified when jejunal biopsies from asymptomatic adults in South Asia demonstrated leaflike villus morphology, villus blunting, and mucosal inflammation [3], in stark contrast to the delicate fingerlike villi in healthy adults in industrialized countries [2]. Expatriates studied in developing countries had similar changes in their small bowels, sometimes with altered stool consistency or increased frequency, and weight loss, but no easily professed illness [4, 5]. These findings and symptoms resolved after repatriation [6]. Conversely, the small-bowel morphology of asymptomatic South Asians who moved to New York City resembled the Western pattern [7]. Taken together, these data suggested that enteropathy was a reversible response to environmental conditions unique to the “tropical” locale, and formed the basis for another descriptor, “subclinical enteropathy.”

Reduced villus:crypt ratios, with diminished surface area of mature absorptive intestinal epithelial cells, could plausibly hinder nutrient uptake. Indeed, when expatriates with enteropathy were studied, moderately reduced intestinal function was observed, such as...
absorption of various carbohydrates, fat, and vitamin B₃ [4, 5, 7]. This constellation was therefore accorded yet another name, “subclinical malabsorption.” It was arbitrarily distinguished from tropical sprue by the magnitude of change, with much greater reductions in villus:height ratios in tropical sprue, accompanied by overt clinical signs and symptoms that often responded to folic acid and/or broad-spectrum antibiotics [8]. Remarkably, the relationship of subclinical malabsorption to tropical sprue remains uncertain to this day. In addition, before 1970 in south India, tropical sprue occurred in epidemic clusters, suggesting an infectious etiology [8]. However, although such outbreaks have disappeared, tropical enteropathy and sporadic cases of tropical sprue are still observed [9].

We know astonishingly little about intestinal structure and function in children in developing countries. The small bowel from stillborn fetuses or infants who died in the perinatal period has long, delicate (i.e., normal) villi, but in somewhat older infants they are flatter and more leaflike [10, 11], indicating the acquired nature of villus alterations and the likely role of environmental postnatal exposures to as yet unidentified factors. Reduced mucosal surface area and damage to the epithelial cell brush border could impair nutrient absorption and lead to malnutrition, a process that is itself associated with mucosal abnormalities [12]. Early functional changes could serve as a prelude to, and be exacerbated by, multiple drivers of malnutrition, including poor diet due to poverty and food insecurity, and infections with specific enteric pathogens. One major consequence of this process is linear growth faltering leading to stunting, which is a common occurrence in children in developing countries. Stunting is associated with increased morbidity, developmental delays, and excess risk of mortality associated with infections [13]. Acquired enteropathy and its associated functional defects might play significant roles in the evolution of this condition [14, 15].

These various observations compel us to focus on the function of the childhood gut as a determinant of individual and population health. A usable case definition for the underlying gut lesion is critical to confirm or refute this potential linkage, and, hopefully, interdict its consequences when the association is established.

Assessment of Intestinal Function in Young Children
Tropical enteropathy was originally defined by gross and histological alterations, but it is unlikely that single jejunal biopsies, let alone serial sampling to study the evolution of mucosal changes, will be feasible and acceptable in young children who are not yet clinically ill. However, if the initial physiological perturbation is malabsorption and/or mucosal inflammation, before there are clear-cut clinical consequences, then the assessment of intestinal function or surrogate biomarkers of changes in the histological appearance of the mucosa might more sensitively define the condition and provide a way to assess its progression. This assessment is feasible as many studies have documented changes in carbohydrate absorption tests such as D-xylose in many asymptomatic young children in these settings [16, 17]. For example, in Bangladeshi children aged 2–64 months, mean D-xylose absorption was two-thirds of the value in children in the United States [16]. In another study of clinically healthy rural Thai children, aged 2.5–9 years, half had test results below the threshold for normal D-xylose absorption [17]. Because of these functional deficits, we have termed this entity “environmental enteric dysfunction” (EED) as a more accurate descriptor than environmental enteropathy, the term now being commonly used in the literature [14, 15].

Increasingly, dual sugar absorption tests [18] have been used to assess intestinal permeability (gut barrier integrity) as a marker of abnormal mucosal function [19]. Typically, lactulose and mannitol are given together by mouth, and the ratio of their uptake or excretion is reported [20]. Lactulose is too large to cross normal mucosa unless intestinal permeability across the tight junctions between epithelial cells is excessive. Mannitol, on the other hand, is handled like other monosaccharides that are taken up in proportion to small-bowel absorptive capacity. Absorbed lactulose and mannitol are then filtered at the glomerulus and not reabsorbed by the kidney. Therefore, their relative concentrations in urine and the absolute amount of mannitol, respectively, measure intestinal permeability and small-bowel absorptive capacity. Increased lactulose to mannitol (L:M) ratios (relative to healthy reference values) among asymptomatic children as well as children with diarrhea are common in Africa [21], Asia [22], and South America [23]. Using a variant of the L:M test in which rhamnose is substituted for mannitol, gut permeability was abnormal in 36% of a group of Australian Aboriginal children without diarrhea, but exposed to environmental hazards similar to developing countries—as compared to none of a group of non-Aboriginal control children [24]. The results, although compromised by the lack of appropriate local controls, are consistent with many other reports. For example, investigators have found an inverse relationship between L:M and height-for-age in asymptomatic Gambian children 2–5 years of age [25], and in interval length gains in 8- to 64-week-old Gambian infants [26]. However, as is often the case, other studies have failed to find similar associations [27]. Potential reasons for these discrepancies include lack of methodological standardization, differences in selection criteria (inclusion or exclusion of breastfed babies), whether subjects are fasting or not, the dose of sugars administered, what sample is collected, which assay methods are used, and even how results are reported (arithmetic vs geometric means). There has been considerable speculation about the cause of increased permeability, ranging from microbial contamination of water and food, microbial overgrowth in proximal small bowel, constant
exposure to bacterial components such as endotoxin, or ingestion of other immunologically active or toxic organic or inorganic contaminants in water or food. Unfortunately, these all remain speculative, and a rational strategy to prevent the functional abnormality is not yet possible.

Another potential surrogate measure for overall mucosal function is plasma citrulline concentration, which appears to reflect enterocyte mass and function [28]. As a nonprotein amino acid minimally present in the diet, citrulline levels in blood depend on de novo synthesis by proximal gut epithelial cells. It is reduced in short bowel, acquired immunodeficiency, and villus atrophy syndromes, all of which have decreased epithelial cell surface area. Measuring increases in citrulline after an oral dose of a precursor, alanine-glutamine dipeptide, is another proposed evaluation method. Neither citrulline levels nor generation of citrulline from precursors have yet been critically examined as markers of EED [14], but an exploration of their utility to reflect reduced epithelial surface area and function is warranted.

Morphometric and immunohistochemical analyses of small intestinal biopsies from Gambian children, most of whom were malnourished and had diarrhea, suggest that EED is a T-cell–mediated lesion [29]. Specifically, increased CD8+ γδ− intraepithelial T cells and lamina propria CD4+ and CD8+ T cells and decreased CD25+ T regulatory cells, together with villus atrophy and crypt hyperplasia, were observed. These alterations were accompanied by increased expression of Th1 proinflammatory cytokines such as tumor necrosis factor alpha (TNF-α) and interferon gamma (IFN-γ) and decreased levels of regulatory cytokines such as transforming growth factor beta (TGF-β). As malnutrition worsened, TGF-β+ cell density diminished, with a shift from a regulatory to a proinflammatory environment, assessed as the ratio of interleukin 10/TGF-β to TNF-α/IFN-γ, which decreased from 3:1 to <1:1. The most malnourished children had the most proinflammatory profile. Although T cells and crypt epithelium displayed an “activated” CD69+ and/or CD45-DR+ phenotype, the cause has remained unknown. Mucosal CD19+ B cells and syndecan-1+ mature plasma cells also increased, but as malnutrition worsened their density trended downward. Remarkably, almost all our knowledge of inflammatory mucosal changes in children with EED comes from these studies of 2- to 5-year-old Gambian children, published 10–20 years ago, before many new investigative tools were available. It is not known whether or not inflammation directly drives mucosal dysfunction and contributes to nutritional deficits (eg, as in inflammatory bowel disease), or is a response to microbial and antigen translocation across the mucosal barrier secondary to increased permeability. Nor is it clear whether immune activation is initially an appropriate adaptive response to potentially harmful gut organisms or inflammatory signals derived from microbes that ultimately becomes excessive, with adverse consequences manifested by reduced host defenses and poor responses to oral vaccines and, of course, stunting. Moreover, the existence of specific unrecognized pathogens within the intestinal microbiomes of affected children cannot be ruled out at the present time.

The Challenge of Diagnosing of EED

No single biomarker currently known reliably indicates the presence of early functional bowel deficits contributing to early growth faltering. Similarly, there is no clear evidence pointing to the cause(s) of EED that precede significant malnutrition, when the incidence of enteric infection with known effects on gut structure and function increases. To establish diagnostic criteria, it will be necessary as soon after birth as possible, and serially thereafter, to assess carefully chosen parameters of function and injury. These include assessment of absorptive function, mucosal permeability, inflammation, and immune activation (Table 1). The first 2 have been discussed above; how might inflammation and immune activation be assessed? Calprotectin, a stable calcium and zinc binding protein produced by neutrophils and monocytes, may be a good marker of inflammation in EED, as it is known to be present in stool in high concentration in inflammatory bowel disease [30]. As a recent example of this approach for EED, intestinal inflammation in young children has been evaluated by combining the measurement of 3 biomarkers (α1-antitrypsin, myeloperoxidase, and neopterin) into a composite disease activity score [31]. Additional signals initiated by microbial [32] or antigen [33] translocation may be indirectly measured as the level of circulating cytokines [29], or antibodies that recognize and neutralize the core antigen of lipopolysaccharide (LPS) [33] or serum levels of LPS binding protein (LBP), a hepatocyte-derived acute-phase protein synthesized in high concentration in response to microbial or LPS stimulation. Immune activation has been assessed by assay of stool neopterin [34], a protein produced

Table 1. Biomarkers to Assess Environmental Enteric Dysfunction

<table>
<thead>
<tr>
<th>Category</th>
<th>Biomarkers</th>
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<tbody>
<tr>
<td>1. Intestinal absorption and mucosal permeability</td>
<td>D-xylene, mannitol, or rhamnose absorption; lactulose paracellular uptake; α1-antitrypsin leakage into gut lumen</td>
</tr>
<tr>
<td>2. Enterocyte mass and function</td>
<td>Plasma citrulline and/or conversion of alanyl-glutamine to citrulline, lactose tolerance test (as a marker of brush border damage)</td>
</tr>
<tr>
<td>3. Inflammation</td>
<td>Plasma cytokines, stool calprotectin, myeloperoxidase, or lactoferrin</td>
</tr>
<tr>
<td>4. Microbial translocation and immune activation</td>
<td>Stool neopterin, plasma LPS core antibody and/or LPS binding protein, circulating soluble CD14</td>
</tr>
</tbody>
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Abbreviation: LPS, lipopolysaccharide.
by IFN-γ-stimulated macrophages serving as a marker of activation of cellular immunity, or circulating soluble CD14, a secreted protein derived from monocytes/macrophages when the cell surface ligand CD14 binds LPS [35]. Although such tests appear feasible, and in concert are likely to provide relevant information, other parameters, not yet identified, may later turn out to be more useful. This area of research remains an exciting topic likely to rapidly evolve as increasing attention is devoted to it. However, it should be emphasized that early postnatal evaluation is critical, given the growing recognition that almost a third of early stunting may be attributable to intrauterine growth retardation and early postnatal factors [36]. In addition to establishing baseline status, serial evaluations will be necessary to determine which parameters (most likely multiple assessments examined together) can best predict subsequent stunting. For this reason, assays to be employed will need to be feasible in the very young and acceptable to parents or guardians, in addition to being sensitive and specific for the reason they are selected. Each test has constraints of sensitivity, specificity, and reproducibility, the nature of sample required, and the simplicity of assay; however, standardization of methodology for performance, sample collection, and detection will diminish inherent test variability and individual differences in results. Miniaturization of assays to reduce the volume of blood required and creative use of breath tests may help avoid the use of timed urine samples, which, while simple in theory, are difficult in practice to ensure complete collections.

Recent studies suggest that the gut microbiome modulates mucosal immune and inflammatory responses, maintains intestinal epithelial integrity, and determines nutritional status [37]. Because altered microbiome composition in stool is implicated in the pathogenesis of enteric diseases characterized by dysregulated immune and inflammatory responses (eg, inflammatory bowel disease and celiac disease), colonization with proinflammatory microbial consortia has been proposed as a cause of EED. Where-as characteristics of the microbiome might someday be diagnostic of EED, it is entirely possible that observed changes are secondary to host factors such as age or genetics, or represent the consequence of an altered mucosal environment due to poor diet, malnutrition, repeated enteric infections, or antibiotic use [38]. If this leads to increased intestinal permeability, the resulting microbial or antigen translocation may initiate mucosal inflammation and immune activation, resulting in mucosal injury, villus atrophy, an exacerbation of underlying EED, and clinically relevant malabsorption, followed by changes in the microbiome. These different mechanisms would suggest different therapeutic approaches supporting the need for a better understanding of the sequence of events underlying EED, and, by extension, stunting, and what are primary drivers vs secondary effects.

Although there are already many possible parameters to employ, some of which are shown in Table 1, a defined, robust set of tests will need to be identified and validated. Direct studies to determine feasibility in very young subjects will be essential to choose, optimize, and validate the best panel. As a starter, refining the methodology to include lactulose and mannitol or rhamnose, possibly D-xylene, plasma citrulline, selected circulating cytokines and soluble CD14 levels, and possibly LPS core antibody and LBP, as well as judiciously sampling blood and breath and stool calprotectin and neopterin, would simultaneously assess absorption, permeability, enterocyte mass, inflammation, microbial translocation, and immune activation. Technical developments to miniaturize the required sample size without significantly reducing accuracy (eg, development of transcutaneous assays) would greatly advance the potential for research and subsequent clinical use.

It has long been known that once the spiral of infection–malnutrition–infection is initiated, the frequency of diarrhea and other bacterial, viral, or protozoal infections increases [39]. These episodes have direct and indirect effects, (ie, through cytokine-mediated metabolic alterations) [40] that add to the injury of baseline EED and previous infections. In many cases, this proceeds to severe malnutrition and death, either primarily from the infection or malnutrition, or from processes to which malnutrition contributes. It is not clear at this time whether exposure of the small bowel to “commensal” microbes or biologically active molecules such as LPS or other antigens derived from them, or toxic chemicals in the environment can initiate the process. But given the highly contaminated environment in which these children live, a reasonable initial focus should be on microbial drivers, even though a single-candidate monomicrobial etiology is unlikely.

Significance of EED

There is ample evidence of a spectrum of functional changes of the small bowel of children in resource-limited settings, where environmental hygiene is generally poor, that is acquired after birth and is variably prevalent in different geographic locales. If early EED and its functional consequences are risk factors for more severe outcomes, and its causes were identified, then this entity would be an attractive target for interventions, before significant malnutrition or severe and recurrent diarrhea develops, to mitigate morbidity and reduce mortality. By preventing impairment of intestinal absorptive function, better nutritional status may be sustained and permeability defects and consequential inflammation from microbial translocation diminished, allowing the immune system to respond more effectively to infectious challenges and better respond to orally administered vaccines.

To accomplish this goal, a feasible, standard, and ethical way to define EED is necessary to identify likely causes and consequences of early gut functional or mucosal changes. Such a definition would allow us to propose and test interventions to
prevent EED in these settings, and thereby reduce stunting, macro- and micronutrient deficiencies, poor development including cognitive function, and mortality, and improve long-term human potential among survivors. The outcome variable of most relevance will be healthy growth, and for reasons of prevalence of poor early childhood growth in these populations, simplicity, and clinical importance, linear growth is most appealing. Whether expression of growth data as a deviation from the normal curve (z scores), or as interval growth velocity, will be the most sensitive indicator of EED and its impact on intestinal absorption, permeability, inflammation, and immune activation can be determined in the course of future studies. Prevention is ethically and economically preferable to treatment, particularly for potentially lethal disorders; therefore, a concerted effort to unravel the causes and identify strategies to avoid EED and avert its consequences is the most rational way to proceed.

Notes

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