CHAPTER 1.

ENVIRONMENTAL ENTERIC DYSFUNCTION (EED) BACKGROUND

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1.1 EED History and Overview

For many years it has been known that the digestive system, and in particular the small bowel, of people who reside in poor regions do not function well. The first English language description of this disorder was written by William Hillary in the mid-1700s. His case series of malabsorption in European expatriates in Barbados [2] (reviewed by Booth [3]) also reported glossitis (most likely caused by concomitant folate deficiency) and diarrhea. Over a century later, Patrick Manson re-described this disorder, again focusing on expatriates in the Dutch West Indies, and used the term tropical "Sprouw," from a Dutch word describing what was almost certainly celiac disease (gluten-sensitive enteropathy) in Europe [4]. The onset of this malady in previously healthy Europeans residing in the tropics, and reports of epidemic malabsorption in Allied troops in Southern Asia during the Second World War [5, 6], gave rise to the concept of gut lesions caused by "environmental" factors. Therefore, this enteropathy was modeled as an acquired, likely infectious, problem [7]. This acquired/environmental concept was reinforced by secular variances in the incidence of tropical malabsorption syndromes, alternately referred to as tropical enteropathy (TE), in areas previously endemic for this disorder [8, 9].

The early descriptions of tropical enteropathy did not define the true extent of the problem. Most notably, these initial reports focused on adults. The realization that children were affected by what was probably the same disorder was delayed. Second, while various causes of this intestinal dysfunction have been postulated, the definitive cause or causes remain(s)

elusive. Indeed, interest and investigation into the problem itself waxes and wanes with discoveries of new enteric pathogens. This has implications for our systematic review of the problem. For example, giardiasis would be a lead candidate as a cause of tropical enteropathy in view of the chronicity of the illnesses it can cause, and the ability of *Giardia* to injure the mucosa of the small bowel. However, *Giardia lamblia* was not widely accepted as a human pathogen until the 1970s. Hence, prior investigations would have discounted this agent as being a cause of enteropathy. Third, there have been regional differences in the reported incidence of tropical enteropathy. Early communications suggested that individuals in Africa were seemingly less affected than were individuals in other areas, and that many cases of adult malabsorption were attributed to biliary and pancreatic disorders [10]. However, more recent reports suggest that Africans are, indeed, susceptible to tropical enteropathy [11-15]. Also, diagnosis in early studies was made by varying methodologies, including those based on response to empiric treatments (antibiotics, antiparasitics, micronutrients) and repatriation to countries in which tropical enteropathy is not endemic; it is often unclear from which intervention(s) responders benefitted. Finally, enteric dysfunction in resource-poor settings has often eluded, and continues to elude, useful definition. Instead, we are forced to rely on histopathologic findings from the small bowel, and these findings are variably specific. These abnormalities include villous blunting, increased crypt:villous ratios, and presence of intraepithelial lymphocytes (IEL). However, the reliance on biopsies skews disease detection and data acquisition towards adults who more frequently undergo invasive testing [16]. These definitional issues continue to hinder advancement in the field, and to impose challenges in identifying useful biomarkers.

The nomenclature used to describe the entity of interest has shifted in response to ongoing efforts to better understand and describe the disease. As early as 1984, the term "environmental enteropathy" was used synonymously with "tropical enteropathy" [17], although not commonly until 2004 [18]. Most recently, "environmental enteric dysfunction" (EED) has

been suggested as nomenclature for the entity of interest [16]. We adopt this term in this book as it provides specificity with regards to the origin of intestinal dysfunction (i.e., environmental opposed to genetic or other factors) and focuses on functional alterations of consequence [19]. The term EED refers to functional shortcomings of the gut with and/or without histological correlates, and obviates the need to rely on the histology inferred by the term "enteropathy"[1]. We acknowledge that EED might differ in disease burden, etiology, and effects in children and adults in different regions. However, independent of case definition or underlying criteria, suboptimal intestinal function in children in resource-poor environments is common, and it is important to determine its true incidence, spectrum of host injury, mechanisms of pathophysiology, diagnostic ascertainment, clinical consequences, and mitigation via interventions whether prevention or treatment. Therefore, it is now time to measure the extent of this dysfunction and its consequences. To do so, we need to consider the technology that will be required to investigate the problem.

1.2 An Old Problem Requiring New Knowledge

There is a compelling need for research on enteric dysfunction in children in resource-poor environments, but scientific advancement in the field has been, in fact, quite modest. The Web of Science (formerly ISI Web of Knowledge) demonstrates a paucity of publications related to EED (Figure 1).

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[1] Strictly speaking, the "-pathy" suffix is from the Greek word for disease or suffering, but in many connotations refers to histopathologically defined disorders.

Figure 1. List of plausibly relevant literature from 1980 to 2010.

A search of *Thomson Reuters Web of Science™* ALL Databases Collection[1] on 27 August 2014, spanning 1980-2010 and using any of the following search terms: "tropical enteropathy", "environmental enteropathy", "tropical sprue", "environmental enteric dysfunction", "tropical malabsorption syndrome", or "tropical malabsorption." Topic was used for the query, with the phrase enclosed in quotation marks. Search filters for language or document type were not applied.
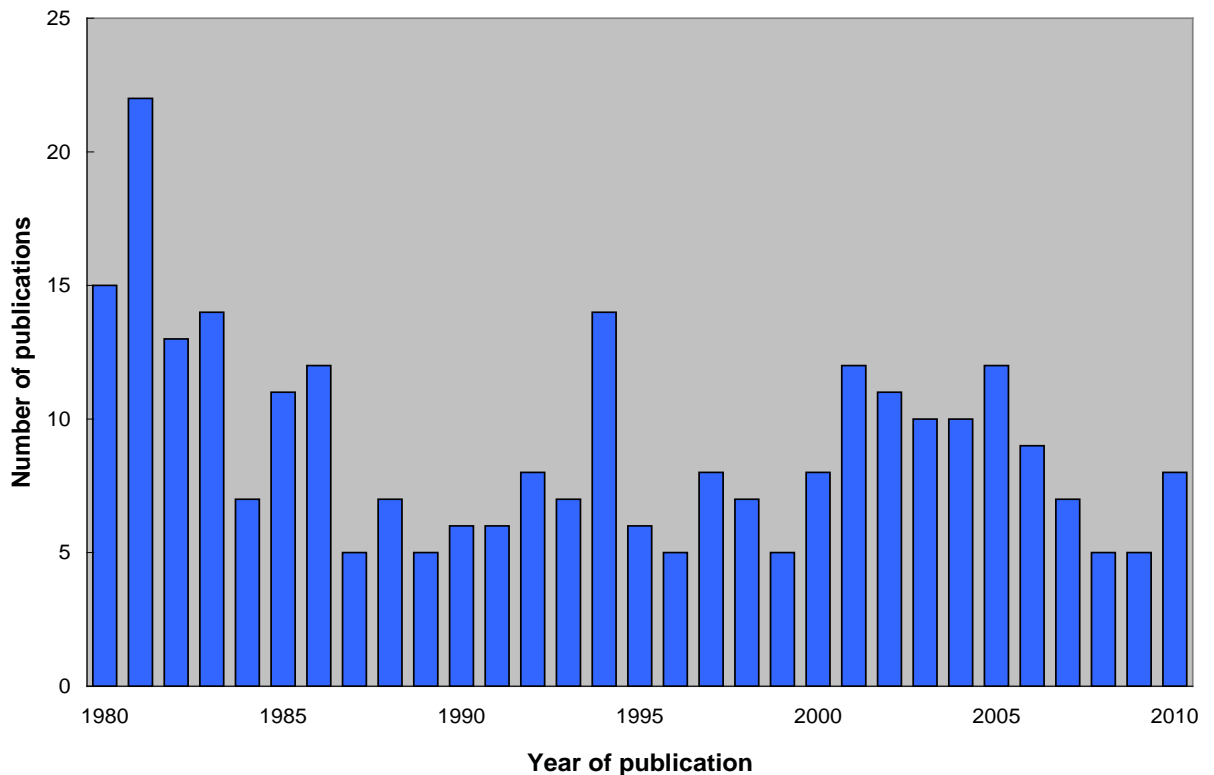
The lack of progress on EED contrasts with recent dramatic advances in mortality reduction caused by acute enteric infections. The annual number of deaths attributed to diarrhea among children under five years of age fell from an estimated 4.6 million in 1980 [20] to about 700,000 in 2011 [21]. Most of the mortality reduction in the latter two decades of the past century was likely a result of refinements in, and/or increased use of, oral rehydration

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[1] Searches were conducted using the *Thomson Reuters Web of Science*™ ALL Databases Collection and reflect only the data as indexed by the database. Publication data may be incomplete. The *Thomson Reuters Web of Science*™ ALL Databases Collection consisted of Inspec® (1898-present), MEDLINE® (1950-present), SciELO Citation Index (1997-present), Science Citation Index Expanded (1970-present), Social Sciences Citation Index (1970-present) and Art & Humanities Citation Index (1975-present).

therapy(ORT) [22, 23]. Globally, current ORT coverage rates are moderate and have stabilized over the past decade [24]; more recent reductions in diarrhea-related mortality likely stem from general improvements in socioeconomic development, nutrition, and water/sanitation infrastructure [25]. Coverage rates for access to improved water sources, particularly in urban settings, are on track to meet international development goals [26]. However, access to adequate quantities of water, water of sufficient quality for drinking, and improved sanitation facilities are limited and only slowly improving [26-28]; the same lack of progress applies to hand hygiene prevalences [29]. Improved water sources, sanitation, and hygiene can avert 58% of childhood diarrhea-related deaths in low- and middle-income countries [27]. A 34% reduction in risk of diarrheal disease has been demonstrated with interventions to improve water quality, and a 28% reduction in diarrheal disease has been observed with improvements in sanitation, particularly sewer interventions [30]. Promotion of hand hygiene with soap is estimated to contribute to a 23% reduction in diarrheal disease risk [29].

Treatment with zinc can reduce the duration and severity of acute episodes of diarrhea and reduce the risk of future episodes [31-33]. Since 2004, zinc has been a recommended therapeutic for children suffering from diarrheal episodes in resource-limited settings. Safe and effective rotavirus vaccines are now available and recommended for widespread use [34-36]. Coverage rates for these relatively "new" interventions are low, but their implementation is accelerating. Major advances might also be on the horizon in the form of effective cholera, typhoid, *Shigella,* and enterotoxigenic *Escherichia coli* (ETEC) vaccines [37-39].

Despite reductions in mortality risk associated with acute diarrhea, enteric infections remain prevalent. Over 1.7 billion episodes of diarrhea occur among children under five years of age each year [21] and incidence rates have not changed markedly in the past several decades [40]. Repeated episodes of infection in the developing gut are probably deleterious [41]. Expanded implementation of prevention and treatment measures could further diminish diarrhea

mortality and could additionally reduce morbidity and disease burden. However, these advances against acute enteric infections now raise the relative importance of chronic intestinal dysfunction as a hindrance to optimal childhood health [42].

However, this problem is much more complex: emphasis on acute enteric infection fails to address the consequences of recurrent, persistent, and chronic diarrhea, as well as the long-term consequences of diarrhea on stunting and on disability-adjusted life years. Also, a new variety of agents, in particular enteroaggregative *Escherichia coli*, and *Cryptosporidia* spp., which each require special technologies to identify, have recently and repeatedly been associated with persistent diarrhea and stunting. Additionally, several epidemiologic studies suggested that the frequency of episodes of acute diarrhea impairs long-term health in populations outside Brazil [18, 43, 44]. In view of the successes of oral rehydration and the rotavirus vaccine, it was reasonable to assume that the problem of enteric infections in children would be solved. Moreover, simple infrastructural management strategies, particularly the provision of appropriate village waste containment facilities [45] were determined to be important protective measures against chronic diarrhea. We now recognize that enteric inflammation is a determinant of stunting and long-term gastrointestinal dysfunction, even independent of the presence of the more easily enumerable episodes of diarrhea [45].

The renewed interest in EED is aptly timed for many different reasons. As noted above, we are in an era in which we know how to treat and prevent acute diarrhea. Therefore, chronic intestinal injury and inflammation now pose larger proportional threats to population and childhood well-being. Also, translational research has created new proteomic, metabolomic, and genomic technology with which to study human pathophysiology. The value of re-examining and expanding diagnostic capabilities is two-fold. First, such efforts will shed light on incidence rates within communities and assess factors associated with differences in disease rates in various

populations. Second, such tools might accurately identify children at risk of developing EED, who have EED (at its various clinical stages), and as a way to monitor treatment for EED.

We recognize that other priorities compete with EED for resources in the field of child health. First, there is the imperative to continue to reduce mortality from acute diarrhea. Indeed, the Child Health and Nutrition Research Initiative of the Global Forum for Health Research methodology de-emphasized research on chronic enteric conditions in favor of implementing available strategies to continue to reduce acute childhood diarrhea mortality [46, 47]. That analysis was weighted towards interventions that could be implemented by 2015 to meet Millennium Development Goals. EED intervention studies require longer investigation periods than do disorders with known etiologies and outcomes that are apparent in the short term. For example, the pathophysiology of EED is not well understood, technologies sufficient to assess the process in individual hosts or within populations are not broadly available, and interventions to mitigate consequences of clinical importance are not yet established. Work to understand and control EED should be viewed as complementing, rather than competing with, continued efforts to reduce the burden of acute enteric illnesses. It was with this concept in mind that we embarked on our review of the EED literature, with a goal of providing a platform on which future EED efforts, especially those related to biomarkers and diagnostics, could be built.

1.3 Pathophysiologic Processes and Consequences of EED

The gut is an organ that is central to health and development. Healthy intestinal functioning optimizes childhood physical and intellectual well-being and development, and enables children to achieve adult stature. Several lines of evidence indicate that suboptimal functioning of the human gut, manifesting as EED, leads to poor health for children in resource-poor settings. First, investigators in Fortaleza, Brazil have demonstrated that episodes of

recurrent or persistent diarrhea are major determinants of childhood growth impairment [48], and subsequent reduced growth and intellectual capacity at school entrance [49, 50]. These investigations were seminal because they provided a new metric for calculating the burden of enteric-related disease other than counting deaths or numbers of episodes of loose stools. This is also important because stunting can be associated with subclinical intestinal inflammation [15, 51].

Multiple aspects of intestinal pathophysiology contribute to poor health, growth and development (Figure 2) and physiologic derangements in these processes are deleterious (Figure 3). First, injured intestinal mucosa absorbs nutrients poorly [52] and represents one potential EED-mediated growth-failure pathway [15, 53]. Chronic intestinal inflammation is very common in the tropics [54] and can damage intestinal epithelial integrity and disrupt tight junctions, resulting in intestinal permeability defects. Multiple studies demonstrate increased intestinal porosity in children in the tropics [15, 44, 55-58]. Increased intestinal permeability can, in turn, lead to translocation or the nonphysiologic uptake of intestinal luminal contents, including microbes and microbial products, into the bloodstream [59]. Microbial translocation can, in turn, mediate systemic inflammation as can chronic intestinal inflammation. Stunting is often associated with subclinical intestinal inflammation [15, 51]. Systemic inflammation can penalize growth by interrupting bone growth potential, release of growth hormone binding proteins at the level of the liver [60], suppressing appetite, and increasing metabolic requirements [61]. Such mechanistic attribution remains hypothetical at this juncture.

Figure 2. The vicious cycle of intestinal dysfunction, infectious disease susceptibility, poor growth, and development.

NCDs= non-communicable diseases. WaSH=water, sanitation and hygiene. EED= environmental enteric dysfunction.
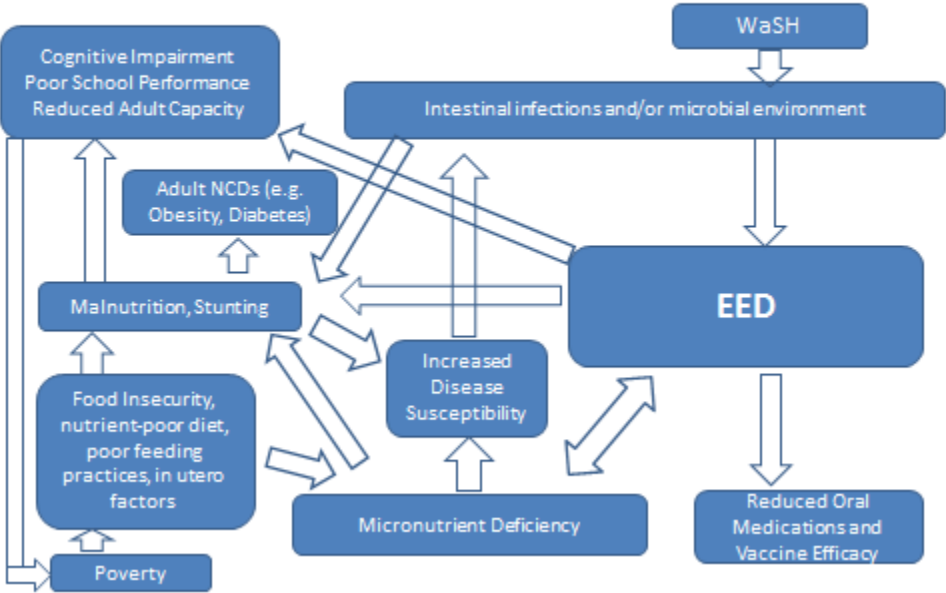
Adapted from Denno DM [62], Guerrant RL, et al. [63] and Mata L [64].

Figure 3. EED-related pathophysiological processes result in stunting and/or growth shortfall.

Growth can be affected by disturbances in a variety of intestinal and other physiologic processes resulting from EED. GH=growth hormone.
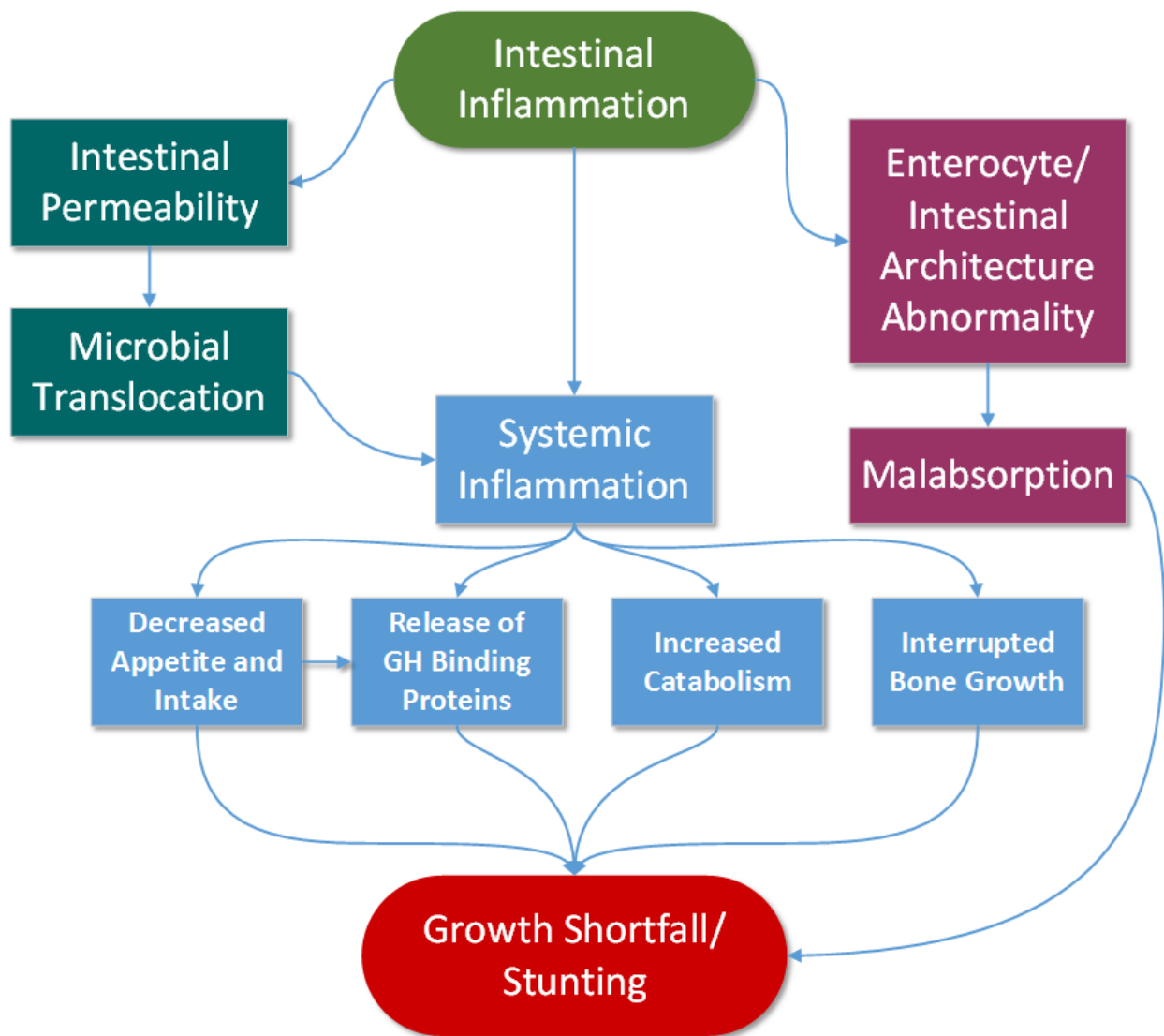
The first several years of childhood represent a critical period that influences life-long nutritional and health status and human potential [65]. Stunting is an especially important cause of adverse consequences in resource-poor regions, and stunting that occurs in the first two

years of life is particularly consequential [48, 66-72]. Childhood stunting is associated with cognitive impairment, poor school performance, and reduced adult capacity [73-76]. Clinical complications of childhood stunting also include predisposition to obesity and diabetes and other chronic diseases in later adulthood—a double burden increasingly afflicting populations in resource-limited settings [65]. Moreover, undernutrition contributes to over one-third of childhood deaths [77].

Poor nutrition among girls can have a particularly profound effect on future reproductive and fetal/neonatal health. For example, maternal stunting can be a risk factor for obstructed labor, stillbirth, and neonatal mortality [78]. Furthermore, maternal anthropometry also influences future generational growth, morbidity and mortality [79].

Dysfunctional guts can hinder the efficacy of oral vaccinations and absorption of medications. This may pose a threat to oral enteric vaccine strategies and absorption of medications for chronic infections such as tuberculosis and HIV/AIDS [80]. Indeed, failure of oral polio vaccine to induce protective immunity in children at risk is a major challenge to disease eradication [81].

Increasing dietary nutrient provision does not necessarily or completely resolve growth failure in resource-poor settings [82]. Gut function is an important antecedent to healthy human growth and development. Strategies are needed to ascertain gut inflammation, increased permeability, and decreased absorption. Strategies are also needed to measure and address the persistence, progression, and resolution of these types of dysfunction. Chronic intestinal inflammation and inability to absorb nutrients may be the most actionable manifestations of enteric dysfunction in children. Intervention studies to mitigate EED will require appropriate, robust and reproducible methods to identify children with intestinal dysfunction, ideally before penalties to growth and development accrue.

A coordinated effort to control EED in children in resource-poor regions could markedly reduce childhood undernutrition, poor development, and mortality. In the past decade, we have seen an explosion in our understanding and abilities to diagnose an analogous disorder, i.e., celiac disease, which has histopathologic similarities to EED. We have learned the genetic and environmental, i.e., dietary, risk factors for celiac disease, and also determined that this disorder is not confined to individuals of northern European ancestry, as previously thought. Furthermore, the diagnosis and management of celiac disease, including partial mitigation of growth consequences, has advanced, at least in high-income countries. Similar data are emerging regarding the more challenging disorders of inflammatory bowel disease, i.e., Crohn's disease and ulcerative colitis [83]. We can apply lessons learned from these chronic intestinal inflammatory states to the study of EED.

There is reason to be optimistic about renewed research and development relevant to EED. Emerging technologies can interrogate human processes and microbial populations to an extent not predicted even a few years ago. These technologies can shed new light on EED, an entity that has traditionally required tissue for diagnosis.

1.4 The Role of Biomarkers and Diagnostics in EED

Much as pioneering studies on mechanisms of intestinal secretion in cholera [84] formed the basis for giving oral rehydration solution to children with diarrhea worldwide, it is critical to identify the mechanisms underlying intestinal dysfunction in children in resource-poor areas. Beyond searches for specific etiologic pathogens or nutritional deficiencies, biomarkers and diagnostics will need to be broadly considered in favor of pursuing a better understanding of EED as a disorder. "Discovery" efforts will be needed to generate sufficient information to move forward.

Broad-based research is urgently needed to invigorate the field. For example, multi-dimensional assessments of individuals and populations will be necessary to measure intestinal dysfunction, and identify factors that precipitate and perpetuate EED. We believe that the utility of biomarkers in EED needs to extend beyond the concept of diagnostics. Diagnostics, as most commonly used to assess the human gut, usually seek specific pathogens, or belong to a limited panel of tests of gut inflammation and/or absorptive function. It is likely that rigorous and systematic analyses of EED require a better understanding of the microbial population in the gut and detailed assessments of intestinal inflammation, absorption, permeability, translocation, and subsequent systemic inflammatory cascade as well as the precipitants and consequences of EED, such as nutrient deficiencies. As such, we sought to systematically search, review and portray the existing diagnostics/biomarkers literature related to EED as a basis of knowledge to leverage further investigation.

1.5 Scientific Basis for this Review

In view of increasing understanding of the role of gut health and function in promoting overall health and development in childhood and beyond, it is logical to catalyze efforts to accurately identify and predict children with EED and to refine the sensitivity and specificity of promising biomarkers. While a single marker with optimal operating characteristics would be a welcome tool, it is unlikely that a single test can be used worldwide to detect and predict EED with the necessary precision. A panel of tests, perhaps in conjunction with clinical characteristics, may be necessary, akin to the Jones criteria used to diagnose acute rheumatic fever.

Currently, there is no consensus on the best way to measure intestinal function either invasively or noninvasively. Additionally, there is no evidence that the entities of interest, i.e., intestinal inflammation and/or poor function associated with stunting, are caused by a single

etiology or that a common pathogenesis underlies all cases. Also, we do not know if intestinal inflammation precedes growth failure, if the disorder might actually begin *in utero*, or if any therapies can reliably and uniformly restore linear growth. Nonetheless, there is now consensus that stunted children are not only at risk for unhealthy consequences during childhood, they are more likely than their non-stunted peers to develop chronic disorders that extend into adulthood, including obesity, type II diabetes, and hypertension [85, 86]. Clearly there is a need to reassess whether existing markers of intestinal dysfunction can better define the disorder of interest, and anticipate its development in children. We therefore attempted to first identify the current state of the knowledge, then delineate gaps in that knowledge, and, finally, sought to determine which tests might have the most utility.

Table 1. Spectrum of etiologies, outcomes, and biomarkers/diagnostics in EED.

These lists of causes, results, and techniques to identify EED were used to define manuscripts of potential interest in the list generated by the search terms.

Potential drivers (causes) of EED	Pathogens, food insecurity, immune activation, specific nutrient deficiencies, environmental hygiene
Potential consequences of EED	Recurrent diarrhea, persistent diarrhea, intestinal inflammation with and without diarrhea, stunting
Possible biomarkers/diagnostics of EED	Biopsies, sugar clearance tests (measuring absorption and permeability of the gut), breath hydrogen tests, nutrient challenge tests, fecal analyses for leakage or non-absorption, serologic analyses for inflammation or evidence of gut permeability