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Malnutrition as an enteric infectious disease with long-term effects on child development

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Abstract

Malnutrition is a major contributor to mortality and is increasingly recognized as a cause of, potentially lifelong, functional disability. Yet, a rate-limiting step in achieving normal nutrition may be impaired absorptive function due to multiple repeated enteric infections. This is especially problematic in children whose diets are marginal. In malnourished individuals, the infections are even more devastating. This review documents the evidence that intestinal infections lead to malnutrition and that malnutrition worsens intestinal infections. The clinical data presented here derive largely from long-term cohort studies that are supported by controlled animal studies. Also reviewed are the mechanisms by which enteric infections lead to undernutrition and by which malnutrition worsens enteric infections, with implications for potential novel interventions. Further intervention studies are needed to document the relevance of these mechanisms and, most importantly, to interrupt the vicious diarrhea-malnutrition cycle so children may develop their full potential.

Keywords

child development; DALYs (disability adjusted life years); diarrhea; malabsorption; malnutrition

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INTRODUCTION

Malnutrition is well recognized as a widespread health problem with consequences that are both acute and, even more often, long-term. Both acute and underlying effects contribute to mortality, either directly or indirectly (through weakened defenses against other diseases such as malaria, respiratory, or diarrheal diseases). However, the long-term effects, especially from nutritional deficits early in life, on children who don't die, but have their development impaired, may exceed even the troubling mortality.

Not only are the effects of malnutrition complex, its causes are as well. Worrisome food insecurity is obviously critical, but a factor that is potentially even more important (especially for children with marginal intake) is the inability to absorb what they do take in because of repeated or persistent intestinal infections. Hence, the focus of this review is on the importance of understanding the impact and mechanisms of malnutrition and diarrhea, the vicious cycle of enteric infections worsening and being worsened by malnutrition, the costly DALY (disability-adjusted life year) impact, and potential mechanisms and therapeutic implications for breaking this vicious cycle. We argue that viewing malnutrition as an infectious disease with long-term effects on child development will help us better understand and mitigate its still inadequately calculated or appreciated effects on children's development and ability to meet their full human potential.

THE MAGNITUDE OF MALNUTRITION AND DIARRHEA

Global mortality among children under the age of 5 years approximates 9.7–10.6 million deaths each year (or 26,000–29,000 children each day), of whom 18% (i.e., 1.9 million per year or over 5000 per day) die due to diarrhea. Moreover, fully 53% (5.6 million) of these deaths are associated with malnutrition.[1-5] While these numbers reflect improvements in diarrhea mortality over the last 30 years, largely thanks to oral rehydration therapy (ORT), morbidity caused by diarrhea, with its impact on malnutrition, has not decreased and may actually be increasing.1 Furthermore, it is estimated that maternal and childhood undernutrition is the underlying cause of 3.5 million deaths and 35% of the disease burden in children younger than 5 years, accounting for fully 11% of the total global DALYs (disability adjusted life years).6 We suggest that a substantial proportion of global malnutrition is due to impaired intestinal absorptive function resulting from multiple and repeated enteric infections. These include recurrent acute infections as well as persistent infections, even those without overt liquid diarrhea. Furthermore, impaired innate and adaptive host immune responses and disrupted intestinal barrier function due to malnutrition and diarrheal illnesses likely combine to render weaning children susceptible to repeated bouts of enteric infections leading to intestinal injury and, consequently, nutrient malabsorption during the developmentally critical first 2 years of life. Summarized here is the evidence from the existing literature suggesting that the impact of heavy diarrheal burdens and multiple enteric infections in the early formative years of childhood extends long beyond the infection itself and affects both growth and cognitive development in affected children.

IMPORTANCE OF UNDERSTANDING CAUSATION AND IMPACT

The reason the dissection of causal factors as well as the long-term impact of enteric infections and malnutrition is so important is that only a full understanding of both allows us to adequately assign priorities to and assess the full impact of effective interventions. In addition to the full DALY impact of repeated and persistent or even asymptomatic enteric infections on children's development of their human potential,[7-9] we need to factor in a number of important issues that are not typically included in the DALY calculations. These include the quality of life (e.g., of mothers struggling to care for children with diarrhea while

carrying water or wood to survive). Also not counted is the full impact of enteric infections and malnutrition on mortality and morbidity when combined with AIDS, tuberculosis, malaria, or respiratory or other infections, and the societal impact of the contribution of malabsorptive intestinal function to subtherapeutic drug levels and thus drug-resistant pathogens like HIV, *Mycobacterium tuberculosis*, *Plasmodium falciparum*, bacteria, and possibly helminthic and protozoal parasites. Thus, the economic, let alone the human and societal impact of repeated and prolonged enteric infections, is far greater than ever calculated. The 1.1 billion people lacking safe water (1 in 6 people, or 17% of the world's 2005 population, projected to increase to 2.9 billion by 2025) and the 2.6 billion lacking even pit latrines (4 in 10, or 42% of people, projected to be 4.2 billion by 2025) are profoundly affected by the vicious cycle of enteric infections and malnutrition with impaired child development.10 Therefore, any effective interventions, ranging from targeted antimicrobial therapy and key micro- and macronutrient approaches to improved water and sanitation, have far greater cost-benefit effects than ever adequately appreciated.

ENTERIC INFECTIONS AND MALNUTRITION: A VICIOUS CYCLE

Not only does diarrhea impair both weight and height gains, malnourished children have a greater incidence, longer duration, and increased severity of diarrheal illnesses.11 Several reports additionally suggest that even asymptomatic enteric infections can result in growth shortfalls.12–14 The human genetic component and interactions with environmental risk factors that render some children more vulnerable to stunting while their matched neighbors are less affected after similar infection and malnutrition burdens are, as yet, mostly unknown.

Diarrhea and enteric infections impair weight and height gains, physical and cognitive development

Numerous reports have documented the impact of diarrhea and enteric infections on the growth and development of children. Among the most convincing are the studies of Leonardo Mata in Guatemala in the 1960s.15 In his book, *The Children of Santa Maria Cauque*, Mata documented the growth charts of children who thrived in early infancy but, in association with repeated diarrheal and other illnesses, slipped progressively off their predicted growth curves; the cumulative effect of their illnesses led to their being pulled permanently away from any chance of normal growth and development (Figure 1a).15 Clues to the involved mechanisms ranged from malabsorption to acute-phase catabolic and antitrophic responses derived from antibiotic-responsive growth impairment of chicks and other animals.16

Subsequent studies in the rural community of Pacatuba in northeastern Brazil again showed a clear impact of repeated diarrheal illnesses on children's growth (Figure 1b).17,18 Indeed, these and our subsequent studies suggested that diarrheal illnesses in the first 1–2 years of life may account for a persisting 4–5 cm (~8.2 cm) shortfall of growth when the effects of intestinal helminths in just the first 2 years of life are included.19,20 Similar findings of an important impact of diarrhea on childhood growth have been made in Guatemala, West Africa, Mexico, and Bangladesh.21–26 When challenged by the argument that catch-up growth reverses the growth impairment of isolated diarrheal illnesses,27 analyses of weight gains following a diarrheal illness in our studies in Brazil revealed that recurrent diarrhea reduced weight and, albeit less significantly, height gains by 48% and 21%, respectively, when compared with children who did not have recurrent diarrhea17,18,28,29 (Table 1).

While malnourished children (≤3 weight-for-age Z scores) who did not have heavy diarrheal burdens did indeed gain twice the catch-up weight of normally nourished children, increasing incidence of diarrhea progressively ablated that catch-up growth in malnourished

children29,30 (Figure 2). Hence, it is clearly repetitive bouts of diarrhea that have the greatest effects on children's growth. This is likely due to the compounded effects on intestinal absorptive function, which is especially problematic in children who are marginally nourished or experiencing mucosal damage from recent intestinal infections.

Unlike the predominant causes of acute diarrhea, such as enterotoxigenic *Escherichia coli*, rotaviruses, and noroviruses, numerous studies in Asia, Africa, and Latin America show that enteroaggregative *E. coli*, *Cryptosporidium*, and *Giardia* lead among the pathogens causing persistent diarrheal illnesses.13,31–35 Furthermore, intestinal helminthic infections may also impair intestinal function, absorption,36 and growth.37,38 The pathogens associated most frequently with persistent diarrhea in our longitudinal studies in urban shantytowns in northeastern Brazil are shown in Table 2.

Malnutrition increases diarrhea incidence and duration

While diarrheal illnesses, and even enteric infections without overt diarrhea, predispose children to malnutrition and growth shortfalls, malnutrition additionally predisposes to both increased incidence and duration of diarrhea (Table 3).11,19,39 Numerous studies have shown that malnourished children have an increased duration of diarrheal illnesses.40–44,44–46 Although debated by some, others confirm that malnourished children also have an increased incidence of diarrhea.47–49

LONG-TERM EFFECTS OF ENTERIC INFECTIONS: COSTLY DALYS

The impact of heavy diarrheal burdens and enteric infections on physical growth is only part of the picture. Over the lifetime of an impoverished child, the potentially devastating impact of impaired cognitive development that may have lifelong consequences is likely to be far more important than her/his short stature. Thus, the range of negative effects associated with the vicious cycle should be extended to include impaired fitness, cognition, fluency, schooling, and even malabsorption of drugs needed to combat diseases like AIDS, tuberculosis, and malaria, which often coexist with malnutrition and diarrhea.20,50–53 In addition to the average of 8.2 cm growth shortfall from diarrhea and enteric parasites in the first 2 years of life,19 fitness impairment may equate to a 17% decrement in work productivity,20,54,55 and cognitive impairment due to the average diarrhea burden equates to nearly 10 IQ points.20,54,56 We are only beginning to appreciate the greatest and most profound effects on specific areas of cognition, such as on semantic fluency and verbal learning, which require retrieval from adjacent brain regions and thus alter higher level executive brain functions.57 Though the economic consequences have yet to be adequately assessed, clues come from the effects on schooling that persist from early childhood diarrhea in the first 2 years of life into effectively delaying the age at starting school and age for grade several years later.50 In addition, the long-term impact of malnutrition on economic productivity has recently been documented by follow-up studies of 1–2-year-old male children treated with nutrient-dense atole in Guatemala between 1962 and 1977; the individuals now earn 46% more than their peers at ages 25–42 years. Women who had been supplemented as children had gains in schooling and reading comprehension.58 Thus, the DALY impact of diarrheal illnesses is likely 2–6 times greater than previously calculated (when only the mortality and transient limited disability of the brief overt illness were counted).20 The evidence from multiple studies for these long-term consequences of early childhood diarrhea is summarized in Table 4. Cryptosporidial infections predispose to growth shortfalls, even without overt symptomatic diarrhea.13,14,59–62 Enteroaggregative *Escherichia coli* (EAEC) infections lead to increased gut inflammation and growth shortfalls, again, even without overt diarrhea.12 In addition to the cognitive impact studies mentioned above. Berkman et al.63 showed that *Giardia* infections or stunting were associated with measurable reductions in WISC-R cognitive assessments performed at the

age of 9 years. The human cost of impaired development of children's full human potential is beyond quantitative measure. Furthermore, our recent findings suggest that stunting may have an additional effect on cognitive performance, which is independent from that of overt diarrhea; this further suggests that either additional mechanisms are involved or that "asymptomatic" infections may also alter long-term cognitive development through their increasingly recognized effects on growth.12¯14,64 Indeed, the studies mentioned above showing that even asymptomatic enteric infections (such as with *Cryptosporidium*, EAEC, and *Giardia*) are associated with growth shortfalls could explain such an effect of stunting on cognition that is independent of overt diarrhea.

MECHANISMS INVOLVED IN MALNUTRITION-ENTERIC INFECTION INTERACTIONS WITH IMPLICATIONS FOR NOVEL INTERVENTIONS

Several processes are doubtless involved in the mechanisms by which enteric infections cause malnutrition, ranging from well-recognized anorexia and increased catabolic or caloric demands to direct protein and nutrient loss or impaired absorptive function. If malnutrition can be understood as an enteric infection that coexists in a reciprocal relationship with a multitude of other infectious diseases, both gastrointestinal and extraintestinal, another question arises—to what extent do proper nutrition and targeted nutritional interventions serve as prophylaxis against and treatment for infectious diseases? The dramatic role of nutrition in preventing and treating infectious disease morbidity and mortality, along with the benefits of specific interventions with micronutrients such as vitamin A and zinc, have been well described elsewhere. Further examples of promising, targeted nutritional interventions for infectious diseases can be found in work from our laboratories and others, including the benefits and mechanisms of the amino acids glutamine and arginine described below.

Malnutrition and repeated enteric infections reduce nutrient availability due to intestinal malabsorption, increased metabolic needs, increased losses (inflammatory or secretory diarrhea), and disturbed nutrient uptake and transport. These effects are additionally influenced by intestinal host-pathogen-microbiome interactions that are, as yet, poorly understood (e.g., host-pathogen-flora mucosal interactions or nutrient competition). For example, using germ-free and colonized mice and 16S ribosomal RNA gene sequence libraries in obese and lean mice and humans, Gordon et al.65,66 have shown that gut microbial communities affect caloric harvest from dietary sources as well as the expression of host genes that regulate metabolism and storage of these calories.65,66 Gut trophic nutrients (such as zinc, vitamin A, glutamine derivatives, and arginine) and, more directly, oral hydration therapies are now being studied extensively by our group to break the vicious cycle of malnutrition and dehydrating infections.67¯70 These nutrients affect enterocyte turnover, enhance immune responses and rehabilitate the intestinal mucosal barrier following mucosal injury, effects that act in synergy to alter growth and development.71,72

In addition to the reductions in macronutrient/micronutrient and essential amino acid levels, protein-calorie malnutrition aggravated by infection can reduce the availability of conditional amino acids, such as arginine and glutamine, which are considered provisionally essential during catabolic states and also in the post-natal suckling and early post-weaning period, when rapid growth prevails and requirements for key nutrients are even higher (e.g., for glutamine or arginine).71,73 Interestingly, related amino acid transporters (PEPT-1) have been shown to be transiently upregulated during catabolic states such as heavy cryptosporidial infections,74 highlighting their adaptive responses to the increased demand for these amino acids. On the other hand, in cholera-related secretory diarrhea, intestinal aquoporin and zinc transporters are negatively affected.75 The absorptive surface area of the normal mucosa of the small intestinal has been estimated to exceed that of a doubles tennis

court.[30,76,77] Furthermore, the surface epithelium is renewed (i.e., the 'tennis court' is 'repaved') every 3 days. It is this absorptive villous architecture that is often severely disrupted, inflamed, or destroyed by many of several enteric infections, be they protozoa (like *Cryptosporidium*, *Giardia*, *Cyclospora*, or microsporidia), viruses (such as rotaviruses or noroviruses), or certain colonizing bacteria (such as EAEC). Furthermore, malnourished or marginally nourished children have the compounded problem of having inadequate or rate-limiting stores of key nutrients to repair this mucosal damage. As noted above, and in animal models below, not only diarrheal symptoms (incidence and duration), but even the intensity of the infection itself can be worsened in malnourished individuals. Hence, an understanding of these key nutrients is critical to the development of effective, novel interventions to break the vicious cycle described above. Among the most important of these nutrients is glutamine and arginine, which are essential for nucleic acid biosynthesis and for key intermediates of cellular replication.[78-80] Glutamine oxidation by intestinal cells also provides a major energy source for the mucosa.[80-82] Glutamine, like epidermal growth factor (EGF), stimulates crypt cell proliferation and has an additional mitogenic effect on cultured intestinal cells.[83] Moreover, glutamine is required for maximal EGF stimulation of intestinal epithelial cell proliferation.[84] Glutamine may therefore be a unique nutrient for enterocytes, providing fuel for metabolism and integrating signaling to augment the effects of growth factors that govern cellular proliferation and repair.[85] Glutamine's major limitations as an oral therapy are its poor solubility and its tendency to hydrolyze to potentially toxic glutamate. Linking glutamine to alanine solves both drawbacks. Alanyl-glutamine (Ala-Gln) is stable, highly soluble, well tolerated, and at least as effective as glutamine alone in driving sodium cotransport and intestinal injury repair in vitro,[86,87] in animals[88,89] and in patients.[52,90]

Intracellular transport of Ala-Gln presumably occurs via PEPT1, an intestinal H^+-coupled di/tripeptide transporter. Normally, PEPT1 is highly expressed on the apical surface of the small intestine epithelial cells, but not in cells in the healthy colon.[91] However, stimulators of PEPT1 expression and/or transport activity in both small intestine and colon epithelial cells have recently been identified. These include enteric infections such as cryptosporidiosis,[92] malnutrition,[74] EGF,[93] and TNF.[94] Interestingly, PEPT1 is coupled to the sodium-hydrogen exchanger 3 transporter and this may be one mechanism by which Ala-Gln drives sodium absorption and rehydration. One possible intracellular target of Ala-Gln is epidermal growth factor receptor (EGFR)-coupled signal transduction responses. Activation of EGFR profoundly increases epithelial cell growth, motility, and survival and has been shown to counterbalance a number of responses to inflammatory cytokines such as TNF.[95] The importance of understanding how Ala-Gln may influence EGFR and TNF effects on migration, proliferation, and apoptosis in epithelial cells is indicated by the number of gastrointestinal disorders that involve increased mucosal TNF levels, such as infectious diarrhea, inflammatory bowel disease, HIV enteropathy, and *Helicobacter pylori* gastritis.[96-98] Available evidence suggests that glutamine opposes TNF-induced inflammatory effects. Glutamine decreases TNF-mediated bacterial translocation across human intestinal cells[99] and parenteral glutamine treatment decreases levels of the TNF-induced cytokine IL-8 in pancreatitis patients.[100] Similarly, glutamine-deficient human enterocytes produce elevated levels of IL-8 in response to LPS, a highly immunogenic component of gram-negative bacteria cell walls—an effect that is partially reversed by glutamine supplementation.[101] Glutamine may also downregulate TNF responses via induction of heat shock protein-70.[102] In addition to its effects on target gene expression, glutamine prevents apoptosis in enterocytes through mechanisms that are only partially understood. Rhoads et al.[103] showed that glutamine metabolism stimulates anti-apoptotic MAP kinases,[103] a finding that has recently been extended by Larson et al.[104] who showed that extracellular-related kinases (ERK) play a critical role in glutamine-mediated intestinal homeostasis. Evans et al. showed that glutamine prevents apoptosis induced by TNF-related

apoptosis inducing ligand (TRAIL) in human enterocytes through the pyrimidine pathway.[105]

Glutamine, like EGF, inhibits toxin-induced damage to tight junctions and adherens junctions essential to the integrity of intestinal epithelial monolayers. Seth et al.[106] showed that glutamine prevents disruption of epithelial tight junctions by acetaldehyde, a toxic metabolite of ethanol. This protective effect of glutamine was blocked by AG1478, a specific inhibitor of EGFR tyrosine kinase acitivity.[106,107] Furthermore, glutamine rapidly induced tyrosine phosphorylation of EGFR, indicating transactivation of EGFR by glutamine.[106] Recently, EGFR transactivation has also been described with alanyl-glutamine,[108] along with the observation that Ala-Gln also stimulates ERK activation and that this activation is EGFR-dependent. As we continue to extend our knowledge of glutamine physiology and signal transduction in the intestine, new possibilities for targeted therapies for the nutritional consequences of enteric infections and inflammation may be forthcoming.

Nutrients inducing intestinal growth, mucosal repair and barrier function

The lumen of the gastrointestinal tract is lined by a single layer of cells, the epithelium, and the interaction between them is modulated by tight junctions and adherence proteins.[109-111] This fine biological structure separates the environmental lumen from the internal body and it constitutes the intestinal barrier function. It regulates important functions such as intestinal digestion, secretion, and absorption of electrolyte, water and nutrients. The interaction of these cells with the underlying mesenchyme in the lamina propria results in the formation of crypts and villi. Epithelial renewal occurs in the crypts of Lieberkµhn with consequent delivery of these cells to the villus.[110] Several studies have addressed the complexity of this system and understanding the mechanisms involved in the developmental biology of the intestinal barrier function.[110,112-114] Understanding epithelial cell renewal can lead to new strategies to ameliorate the impact of enteric diseases. These include 1) promoting intestinal barrier repair to reduce the impact of malnutrition, diarrheal diseases, and inflammatory bowel diseases; 2) prevent or treat mucositis induced by cancer chemotherapy or radiation therapy; 3) repair intestinal absorptive function in patients with diseases like HIV/AIDS or tuberculosis to improve drug absorption and reduce pressures that foster multidrug resistance; and 4) expanding the surface absorptive area in patients with short-bowel syndromes. Crypt stem cells (located at, approximately, cell position five in the base of the crypt)[115,116] are responsible for the formation of columnar, globet, enteroendrocrine, and Paneth cells. Several host proteins, including the Wnt family, GLP-2, KGF, and EGF, influence stem progenitor activity.[110,111,113,114] However, the specific mechanisms that explain the clonal evolution of these cells are not well understood.

Although the vicious cycle of diarrheal diseases and malnutrition is well documented, its pathophysiology remains poorly understood. Several reports have shown that malnutrition is associated with villus atrophy and disruption of the intestinal barrier function.[117-119] In the last decade the role of nutrients in trophic and cytoprotective effects in intestinal epithelial barrier function have been examined.[71,120] Biological and pharmacological studies now implicate several nutrients and microelements (glutamine and derivatives, arginine, retinol, carotenoids, and zinc) in the regulation of intestinal epithelial proliferation, migration, differentiation, apoptosis, and necrosis, as well as intestinal epithelium transcellular and paracellular transport.

Glutamine, alanylglutamine, and arginine

In vitro and in vivo studies have demonstrated the beneficial effects of glutamine and derivatives in intestinal cell proliferation, migration, differentiation, and reducing apoptosis

and necrosis. In an area of northeastern Brazil endemic for diarrheal diseases and malnutrition, we found low levels of serum glutamine concentrations in children.90 We also found that serum glutamine and arginine concentrations correlate with intestinal barrier disruption, as assessed by lactulose:mannitol excretion ratios in children with intestinal inflammation.121 In these children, arginine and glutamine concentrations correlate with growth (as determined by height-for-age z-scores).121

In addition to improving intestinal barrier function and growth, glutamine and alanyl-glutamine drive electrolyte and water transport, showing potential for a new oral rehydration and nutrition therapy (ORNT).52,90,90,118 Though a Gambian study did not show benefits from 0.25 g/kg body wt/d of glutamine,202 this dose was tenfold lower than that used in the studies in Brazil. For treating diarrhea, Ribeiro et al.122 noted that glutamine-based oral rehydration therapy (ORT) was comparable to standard WHO-recommended glucose-based ORT. Yalcin et al.123 found that glutamine (0.3 g/kg body wt/d) reduced the duration of diarrhea. Although more studies are needed, these recent data from in vitro, in vivo, and human studies suggest a preventive and therapeutic role for glutamine or its derivatives, and possibly for arginine, that may help break the vicious cycle of diarrheal diseases and malnutrition in children in developing areas.

Vitamin A

In vitro studies show a dose-related effect of vitamin A on reducing cell proliferation and increasing cell differentiation and apoptosis. Vitamin A also affects tight junctions and adherence proteins; in vitro studies show increased mRNA and expression of tight junction peptides (claudin-2, occludin, and ZO-1) and adherence proteins (beta-catenin and E-cadherin). A few clinical studies support these in vitro findings.

Quadro et al.70 noted that children with severe vitamin A deficiency and malnutrition had reduced mannitol excretion, which is a measure of intestinal absorptive area. Two other clinical studies showed that children without HIV infection who were treated with vitamin A had reductions in their lactulose:mannitol ratio124 and children with HIV infection treated with vitamin A had reductions in their percentage of lactulose excretion compared to control children.125 Recent data from a double-blind clinical intervention in children without HIV infection from Brazil showed that vitamin A also significantly reduced lactulose excretion compared to placebo-treated controls. Furthermore, the concentration of serum carotenoids, such as lutein, beta-carotene, and beta-cryptoxanthin in children were negatively correlated with lactulose:mannitol ratios, suggesting a functional role of these carotenoids in intestinal barrier function.126

Zinc

A recent meta-analysis on the effects of oral zinc on acute and persistent diarrhea reviewed 22 (16 acute and 6 persistent diarrhea) randomized, controlled trials to compare the efficacy and safety of supplementary oral zinc with placebo in children127. The report noted significant reductions in the duration of acute and persistent diarrhea (by 15% and 15.5%, respectively) in children treated with zinc compared to those treated with placebo. In addition, stool frequency was reduced by 18.8% and 12.5%, respectively, for acute and persistent diarrhea in children treated with oral zinc compared to control children. However, there was a significant increase in the frequency of vomiting in the children taking oral zinc, and this was more frequently associated with the formulation using zinc gluconate than zinc sulfate or acetate. The mechanisms of zinc effects on the morbidity of acute and persistent diarrhea are not fully understood. The data on intestinal barrier function are limited, but a few studies show a decreased percentage of lactulose excretion.128 Zinc supplementation improves tight junction morphology and reduces intestinal paracellular permeability in a

malnutrition model in guinea pigs.129 Zinc supplementation at physiological concentrations of 12.5–20μM significantly enhanced epithelial cell restitution through a transforming growth factor-β (TGF β)-independent mechanism. However, supraphysiological concentrations of zinc are toxic and may cause intestinal cell apoptosis in vitro.130 Data from a cross-sectional study in children from northeastern Brazil did not show that serum zinc concentrations correlate with intestinal barrier function, inflammation, or growth.121 However, the randomized, controlled trial showed significant reductions in the percentage of lactulose excretion in children taking zinc compared to placebo. Children on zinc treatment also had significantly increased z-scores for weight-for-age and weight-for-height.121

Thus, there is strong biological and pharmacological evidence implicating several nutrients and microelements (glutamine and derivatives, retinol and carotenoids, arginine and zinc) in the regulation of intestinal epithelial proliferation, migration, differentiation, apoptosis, and necrosis, as well as intestinal epithelial transcellular and paracellular pathways.71،117،120 Not only have several studies linked micronutrients like vitamin A or zinc deficiencies to intestinal absorptive and barrier disruption,70،124،131 but recent studies also link glutamine and arginine deficiencies, as well as intestinal inflammation, to intestinal malabsorption and barrier disruption.90،123،125،127 Conversely, Anstead et al.132 have shown that reduced leptin with malnutrition is associated with impaired host defenses that can also lead to increased susceptibility to infections that can then disrupt intestinal barrier function. We recently noted that intestinal inflammation and barrier disruption also correlate with growth shortfalls,121 and long-term effects on cognitive function are currently under study. Ultimately, the work on interventions that target these critical functional disruptions and their repair must also include assessments of long-term effects on cognitive development before their true value will be adequately appreciated.133

EVIDENCE FROM ANIMAL MODELS SHOWING THAT INFECTIONS CAUSE GROWTH SHORTFALLS AND MALNUTRITION WORSENS INFECTIONS

The association between growth shortfalls and the breakdown of the intestinal barrier due to diarrheal illnesses with reduced absorptive capability and increased water and electrolyte losses requires in vitro and animal models to dissect the causal relationships and effective interventions. The role of mucosal injury (with or without overt diarrhea) and its causal ties with long-term growth deficits remain poorly understood. Although strong evidence of synergistic interactions between malnutrition and enteric illnesses exists, the mechanisms controlling the intestinal mucosal adaptations to the overlapping two-hit injuries of malnutrition and infection require exploration and dissection in animal models. These accentuated effects of infections in previously undernourished subjects further compound intestinal mucosal damage. Furthermore, malnutrition impairs macrophage phagocytic function.134-136

Some clues come from a model of giardiasis in mice, which has confirmed negative correlations between growth and the load of parasitic infection (*Giardia muris*), effects that were accompanied by villus atrophy and microvillus enzymatic deficits.137 In another study using a model of rotavirus infection and ovalbumin absorption during the suckling period, malnourished infected mice showed a peak uptake of ovalbumen (about 4.5 times higher per g body weight) compared to well-nourished infected mice.138 This increased ovalbumen epithelial permeability was also associated with a greater risk of bacterial translocation. Indeed severe malnutrition associates with increased monocyte IL-6 production, with its concomitant effects of driving hepatic acute-phase protein synthesis.139

Intestinal barrier function, which is dependent on the constant intestinal epithelial cell turnover and the balance between cell proliferation, differentiation, and cell death140،141 is

also profoundly affected by malnutrition and heavy intestinal infections. Epithelial cell migration along the villi and crypts is considered the first step toward mucosal healing142 and plays a critical role in preventing luminal bacterial translocation and septic shock, a devastating consequence of the intestinal barrier breakdown.143,144

Moderate-to-severe malnutrition alone can alter villus and crypt architecture (as seen by villus blunting, crypt derangement, and mitotic arrest, as well as by ultrastructural and functional changes).145 These changes may include reductions in brush borders (reducing the overall intestinal mucosal surface), apical tight junctions (which seals the intestinal epithelial barrier and prevents bacterial invasion of the lamina propria), intestinal motility, gut immune function, and overall mucosal DNA content. Malnutrition per se can also increase lamina propria populations of macrophages and lymphocytes along with increased proinflammatory cytokines, which may further alter intestinal barrier function.118,129 In addition, malnutrition may compromise innate immune barriers, Paneth cell defensins, and goblet cell mucins, the last having been shown to be ameliorated by probiotic interventions.146

Adaptative cellular immune responses have been considered more affected than humoral responses following malnutrition. Although a pro-inflammatory state due to malnutrition has been found, findings are controversial. Anstead et al.132 have shown that leptin deficiency that occurs with malnutrition due to a chronic low-protein diet, leads to reduced plasma TNF-alpha and nitric oxide levels and to reductions in NF-κβ signaling in rats.147

Our findings in a maternal-offspring separation model during the suckling period have also shown increased TNF-alpha mRNA.148 Acute malnutrition has also been associated with a shift of host immune responses away from the protective Th-1 responses (for most bacterial and protozoal infections) toward Th-2 responses, especially when associated with helmintic infections.149 Such inflammatory states due to early maternal separation might have lasting effects on intestinal barrier function.150 Interestingly, an increased inflammatory state in the lamina propria might also impair intestinal barrier function and ultimately lead to increased intestinal permeability and growth deficits.151,152

A model of enteric infections causing malnutrition and of malnutrition worsening infection

Analogous to the vicious cycle described above for children, we recently developed a neonatal mouse model of cryptosporidial infection and of weanling malnutrition that illustrates the bidirectional additive damage to intestinal function by infection and malnutrition. We found that, compared with non-infected controls, cryptosporidial infections in infected mice can cause an approximate 40% decrement in weight gain. In addition, an undernutrition protocol in weanlings without infection can also lead to comparable weight decrements; hence, cryptosporidial infection in suckling mice at an infectious dose of about 10^6 parasites approximated the effects of the weanling malnutrition protocol. When combined with infection, malnutrition approximates the growth impairment of an additional log of oocyst infection in nourished mice. Even with the 10^5 parasite inoculum, the addition of undernutrition increases the effect of deepening mucosal crypts, compared to nourished mice infected with the same oocyst inoculum as shown in Figure 3. Furthermore, the combination of malnutrition and cryptosporidial infection was consistently associated with greater pro-inflammatory responses and more severe mucosal damage with a 10–100-fold increased oocyst burden.148 Hence, cryptosporidial infections not only cause malnutrition, but malnutrition also worsens cryptosporidial infections. Finally, we now have pilot data showing that early interventions with the gut trophic dipeptide, alanyl-glutamine or with L-arginine improves growth and reduces *C. parvum* oocyst shedding (Gomes J. and Castro I., unpublished observations). L-arginine has also been also shown to be beneficial (due to NO generation) against *Cryptosporidium* infections, with accelerated mucosal repair153 (Castro

I, unpublished data). Clearly, further studies addressing potential interactions with nutrients and the immune system to support mucosal repair following enteric infections and malnutrition are needed.

THE HUMAN GENETIC COMPONENT: THRIFTY GENES, EVOLUTION, AND CLUES TO SOLUTIONS

The consequences of the compounding effects of malnutrition and enteric infections (with or without overt diarrhea) at the time of rapid growth and brain plasticity during childhood have not been completely elucidated. Although lasting cognitive decrements have been seen with heavy diarrheal burdens in early childhood54,56 leading to poor schooling,50 it remains challenging to dissect the specific developmental decrements. Relevant to specific cognitive functions affected, we found it was semantic, rather than phonetic, fluency that was most affected, a deficit also seen with early Alzheimer's disease.57 Hence, we examined the APOE4 allele that has been associated with Alzheimer's and cardiovascular disease risk. To our initial surprise, we found that APOE4 was associated with protection against (rather than increased risk for) cognitive deficits, but only among those with heavy diarrheal burdens.154 The interactions of diarrhea and malnutrition with profound developmental disturbances likely have important host-genetic determinants.155 Other studies have demonstrated the benefit of the APOE4 allele during infant development.156,157 These cognitive benefits may be related to higher cholesterol levels often seen with APOE4 as compared with non-APOE4 carriers, especially among the undernourished children158,159 and in young adults.160,161 Additionally, APOE4 carriers were found to have higher educational performance.162 Especially intriguing in light of our work on arginine noted above is the finding of Colton et al.,163,164 which indicated that targeted transgenic insertion of the human ApoE4 gene into ApoEko mice results in an upregulation of the arginine-specific CAT-1 transporter.163,164 Supporting the importance of ApoE in malnutrition is our finding that apoE-deficient mice fail to tolerate weanling malnutrition and have accentuated mucosal damage. Most strikingly, the ApoEko malnourished mice fail to repair mucosal damage or recover from their growth faltering following refeeding, thus showing they have dramatically reduced intestinal adaptive responses. This appeared to be due, at least in part, to blunted IGF-I expression.145

Thus, children bearing the apoE4 allele are relatively protected against cognitive deficits imposed by heavy diarrheal burdens, effects that may relate to impaired mucosal responses to injury or to the ‘thrifty’ benefit of ApoE4 in protecting neuronal and intestinal development at critical rapid growth periods.154,155 These studies, along with others that highlight other thrifty genes165 such as leptin, IL-1ra, ABO, and others,166 may hold key messages for helping protect vulnerable children when they are coping with debilitating diarrhea and malnutrition during their early development. These issues remain widespread and were critical during early human evolution in dangerous environments and during times of food scarcity.

POTENTIAL INTERVENTIONS THAT COMBINE ANTI-INFECTIVE, INNATE AND ACQUIRED IMMUNE ENHANCEMENT, ANTI-INFLAMMATORY, AND INJURY REPAIRING APPROACHES

Given the substantial interactions of enteric infections with each other, the intestinal mucosal structure, immunity, key nutrients, and micronutrients in the constantly renewing epithelial mucosa, one should expect that effective solutions to malnutrition and its consequences must involve multiple approaches. As noted above, the normal adult intestinal mucosal surface area may exceed the size of a doubles tennis court. Yet this huge, critical

absorptive surface is rebuilt every 3–4 days by rapidly renewing epithelial cells. Consequently, the multiple effects shown in vicious and reverberating cycles in Figure 4 illustrate not only interconnected causality, but also multiple targets for intervention.

As noted above, several enteric infections lead to intestinal inflammation and damage. These, in turn, can cause nutrient malabsorption or loss. Furthermore, impaired innate and acquired mucosal defenses may, as so clearly seen in our neonatal murine model of malnutrition and cryptosporidiosis, lead to worsened infection intensity and damage. Severe malnutrition has been associated with compromised T-cell responses, thymic and lymphoid tissue reductions, and a more pro-inflammatory cytokine response.167,168 Leptin deficiency in malnourished individuals may also reduce protective host inflammatory responses.132 Zinc, glutamine, and arginine deficiencies also result in immune suppression.169-171

Outcome measures: biomarkers of intestinal dysfunction and its consequences

Fortunately, there are several biomarkers and outcome measures that can help assess the components of these interactions in order to evaluate causal relationships as well as the effectiveness of potential interventions. Intestinal damage can be assessed by fecal alpha-1 antitrypsin, or lactulose absorption as an indicator of barrier disruption. Mannitol or dxylose absorption, in contrast, reflects the total available absorptive surface area. Hence, lactulose:mannitol excretion ratios following ingestion of test doses of these sugars can suggest the barrier disruption while taking into account the available surface area. To assess inflammation, either qualitative or quantitative fecal lactoferrin or perhaps calpain or calprotectin provide readily measurable markers.

To assess nutritional impact, there are several anthropometric measures including weight-for-age Z scores, arm circumference with skinfold thickness (from which arm muscle or fat areas can be calculated) for acute undernutrition or height-for-age Z scores for chronic undernutrition. In addition, several tests can assess physical fitness and cognitive function. The Harvard Step Test and activity meters have been used to assess effects of intestinal helmintic infections as well as diarrhea.37,54 Well-validated, age-appropriate tests of cognitive function are useful tools for assessing the relationship between early childhood enteric infections and subsequent cognitive shortfalls. Expert observational assessments, like Bailey or Capute testing, are used to assess cognitive development in children under the age of 3 or 4 years, while children older than 4 years can be assessed using Tests of Nonverbal Intelligence (TONI), WISC or other tests.54,56,172-179 To assess innate and acquired immune responses, both specific and nonspecific humoral and cellular immunity can be addressed, using in vitro and in vivo tests of antibody, skin tests, and cytokine responses to vaccines or other immunogens.

Finally, since recall clearly drops after as little as 3–7 days,180 prospective surveillance with twice or thrice weekly household visits is critical to fully assess the frequency of overt symptomatic diarrheal illnesses. Frequent household surveillance of diarrheal diseases for research purposes may have the unintended, but very welcome, consequences of decreasing the community incidence of childhood diarrhea and malnutrition through education, heightened attention to at-risk children, and advocacy.61,196 In addition, the array of diagnostic tools for potential enteric pathogens is expanding rapidly and now includes PCR and even quantitative PCR testing for fecal samples.181-183 Furthermore, it is important to evaluate major potential pathogens, even in infections that were previously considered asymptomatic, since, as noted above, growing evidence suggests that functionally important consequences may occur even in the absence of overt diarrheal symptoms.

Breaking the vicious cycle of enteric infections and malnutrition

Figure 4 shows examples of key interventions needed to break the reverberating vicious cycle of enteric infections and malnutrition. The extent to which these interventions combine or synergize to break this cycle remains to be seen. For example, key repair nutrients may synergize with micronutritients to improve mucosal absorptive and barrier function (as well as children's growth and cognitive development), to improve innate or acquired host defenses, and to reduce the severity and duration of enteric infections or overt diarrheal symptoms. Alternatively, bowel nutrients may enhance the effectiveness of simple antimicrobial approaches, analogous to the impressive effects of single-dose albendazole that reduces but does not eradicate helmintic infections, thereby improving growth and cognitive development.37,184-187 Probiotics may also provide approaches to anti-inflammatory or nutrient delivery effects as well as restore potentially trophic enteric flora and reduce diarrheal rates or duration.65,66,188-193 Finally, the importance of improved water and sanitation may indeed be far greater than ever calculated, in addition to the quality-of-life improvements that accrue with more readily available basic water supplies and sanitary facilities.194

CONCLUSION

It is imperative that malnutrition be understood, at least in part, as an enteric infectious disease that not only exacerbates other enteric infections but has the potential to negatively impact other leading infectious causes of morbidity and mortality and their therapy. Only with this understanding can we adequately address the vicious cycle of infection and malnutrition. Reliable outcome measures serve to document the scope and magnitude of these costly events that profoundly alter the lives of the world's poorest children. Once these costs are fully recognized, the value of effective interventions can begin to be seen as more important than ever before appreciated.

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APPENDIX

Detailed commentary on dietary glutamine as an enteroprotective nutrient

Among the key nutrients for repairing damaged mucosa are glutamine and arginine. These nutrients are necessary for maintaining gut mucosal integrity, because glutamine is essential for nucleic acid biosynthesis and for key intermediates of cellular replication.79 Glutamine oxidation by intestinal cells also provides a major energy source for the mucosa.80-82 The ability of the gut mucosa to metabolize glutamine is perhaps even more important during critical illness, when glutamine depletion may be severe and when oral nutrition is interrupted due to the severity of the illness.79 This may also be important during episodes of diarrhea and malnutrition, when mucosal barrier function is often disrupted.

Glutamine-based oral rehydration therapy (ORT) has been studied in both laboratory and clinical settings. Rhoads et al.197 showed that glutamine promotes electroneutral salt and electrogenic Na^+ absorption in piglet jejunum. In rabbit ileum, Na^+ absorption in the presence of glutamine is even more effective than with glucose, an effect that is maintained with cholera toxin treatment.198–200 Results from clinical trials of glutamine-based ORT versus standard ORT are mixed,68 with one study reporting equivalence201 and two others reporting shorter diarrheal episodes and a 30% reduction in stool volume in patients randomized to glutamine.122,123 Thus, glutamine-based ORT appears at least as effective as glucose-based ORT for rehydration, with the likely benefit of enhanced intestinal barrier repair in patients with diarrhea.

As glutamine serum concentrations were significantly correlated with arginine in these children, the low arginine serum concentrations were also significantly associated with disruption of intestinal barrier function. Furthermore, arginine concentrations were significantly associated with height-for-age z-scores and glutamine was associated with all z-scores measured for height-for-age, weight-for-age, and weight-for-height in these children.121

Glutamine and alanyl-glutamine drive electrolyte and water transport in addition to improving intestinal barrier function and growth, thus showing potential for inclusion in a new oral rehydration and nutrition therapy (ORNT)52,90,90,118 Both clinical studies demonstrated a consistent effect of glutamine and alanyl-glutamine on intestinal barrier function. In the first study a significant decrease was observed in the lactulose:mannitol ratio after 10 days of glutamine (2.75 g/kg/day) compared to a similar dose of glycine.118 In a recent prospective double-blind clinical trial in Brazil, 10-day administration of glutamine or isonitrogenous glycine significantly decreased the percentage of lactulose excretion, meaning intestinal paracellular transport decreased and there were long-term beneficial effects on z-scores for weight-for-age and weight-for-height.121 These last results are consistent with the double-blind intervention trial with a glutamine derivative, alanyl-glutamine, as compared to glycine control for 10 days.90 A third double-blind clinical trial in growth-faltering Gambian infants was performed using glutamine (0.25 g/kg body weight/day) or an isonitrogenous, isoenergetic mix of nonessential amino acids administered daily per mouth for 5 months. This regimen did not improve growth or intestinal barrier function any better than the control regimen.202 However, in this last study the dose of glutamine was tenfold lower than in the other two studies.90,118

An earlier study conducted by Ribeiro et al. (1994)122 in Brazil showed a similar effect of an oral rehydration solution containing 90 mM glutamine compared to the standard formulation recommended by the World Health Organization for children with acute diarrhea. More recently, a double-blind intervention trial performed by Yalçin et al.123 showed a significant decrease in the duration of diarrhea in subjects supplemented with glutamine (0.3 g/kg body weight/day) compared to those receiving a similar dose of placebo cornstarch for 7 days. Although more studies are needed, these recent data from in vitro, in vivo, and human studies suggest glutamine or its derivatives may have a preventive and therapeutic role to play, and they may help break the vicious cycle of diarrheal diseases and malnutrition in children in developing areas.

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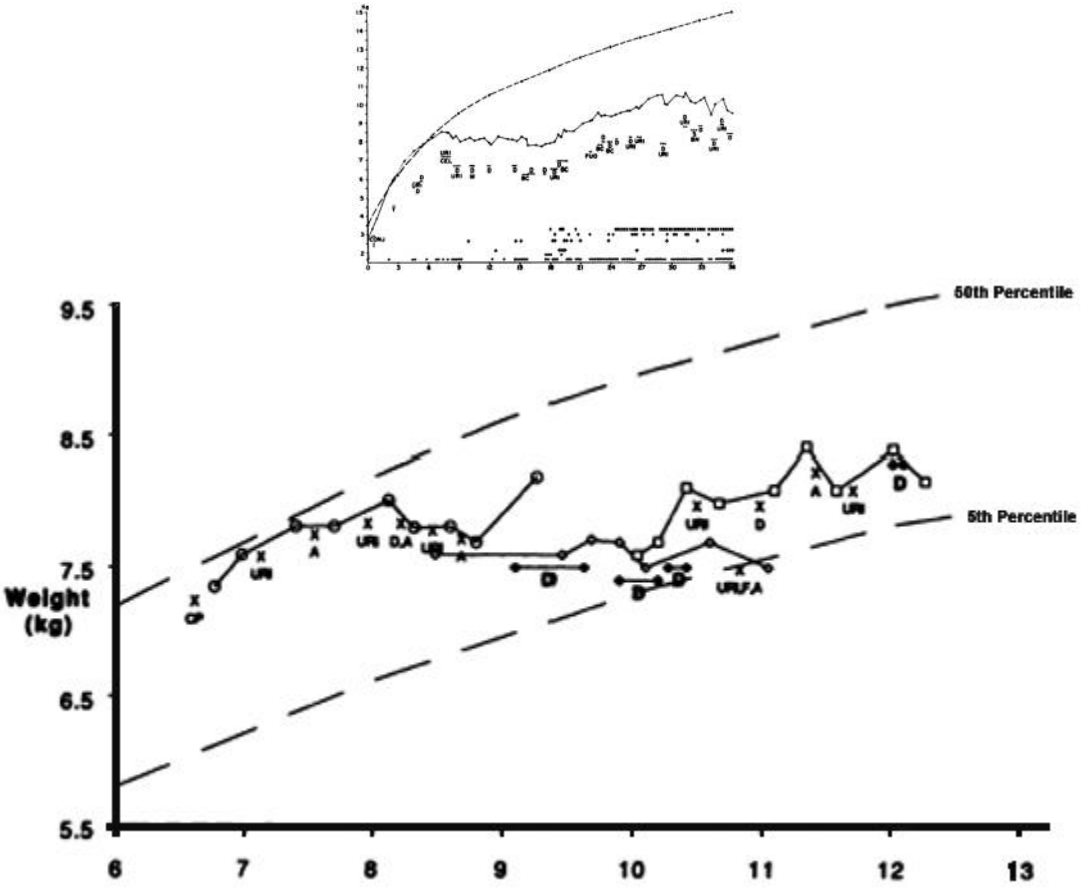

Figure 1. Effects of repeated diarrheal episodes on childhood growth curves

A Illustrative case of a child in Guatemala, from Mata (1978).15 **B** Three illustrative cases from a group of 6–21-month-old girls in Pacatuba, Ceara, Brazil, from Leslie and de Souza (1996).17

Values on the vertical axes are weight in kg; values on the horizontal axes are age in months. D = duration of diarrheal illness

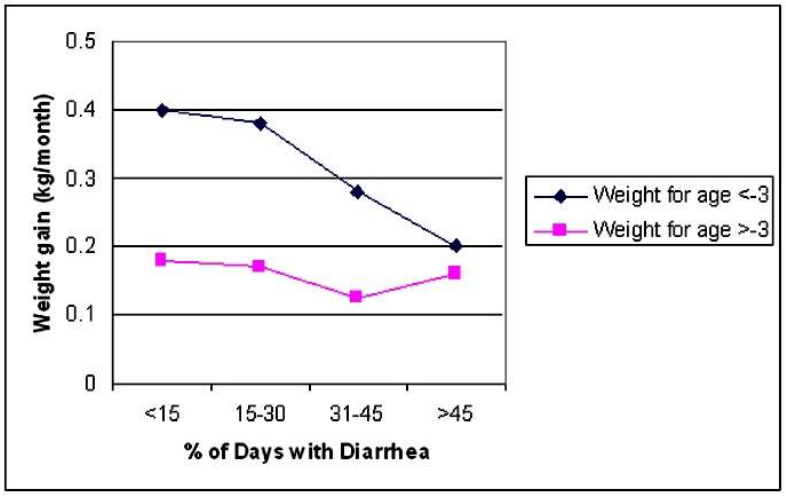

Figure 2. Effect of diarrhea on catch-up growth
Figure from Schorling and Guerrant (1990)29

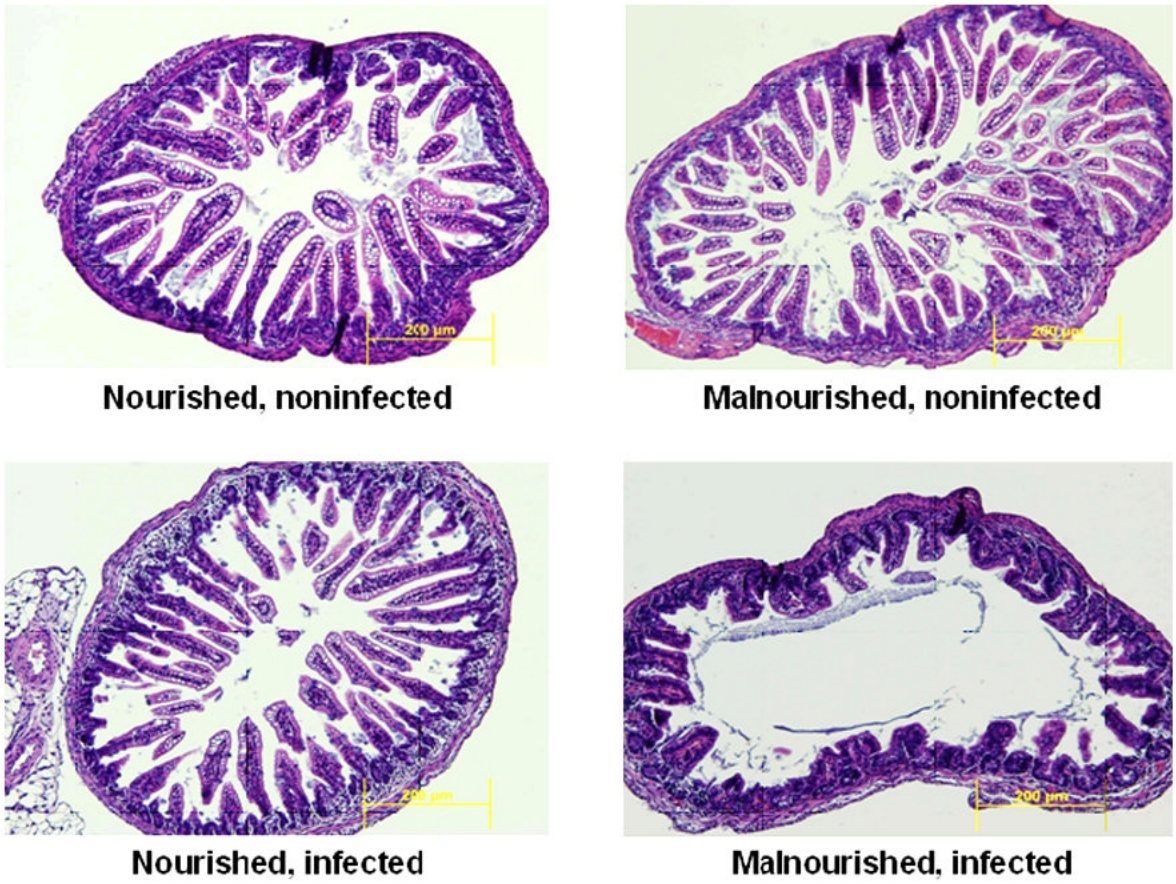

Figure 3. Synergistic effects of malnutrition and *Cryptosporidium* infection on ileal architecture (hemotoxylin and eosin; 10×; at 14 days old; 8 days after infection).
Figure from Coutinho et al. (2008)148

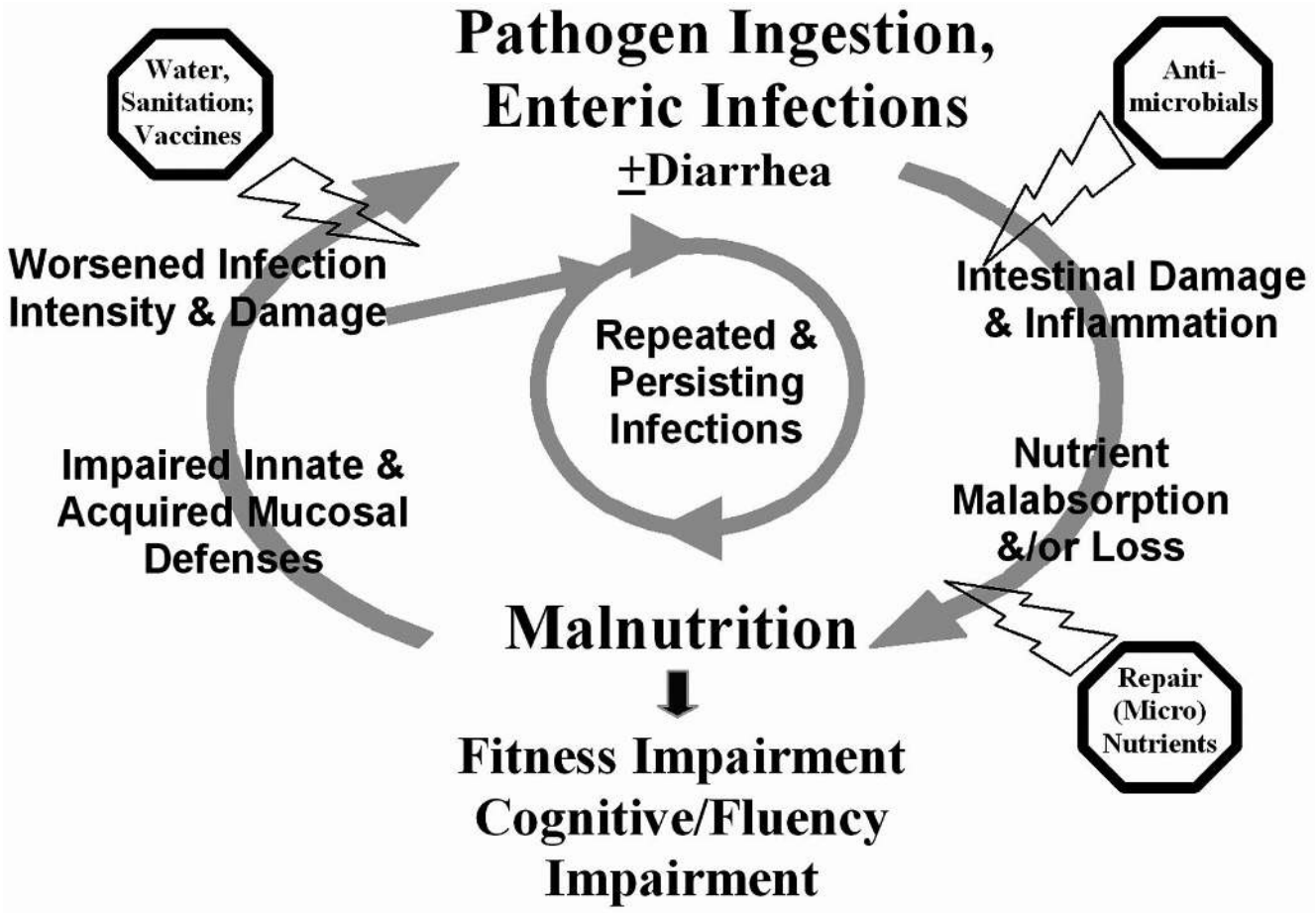

Figure 4. Gut-trophic nutritional approaches to breaking the vicious cycle between malnutrition and diarrhea by repairing the intestinal mucosa

Outcome measures that can help assess these impacts as well as potential interventions include growth and anthropometry (especially HAZ scores), IQ and learning abilities, repeated diarrheal illnesses, intestinal absorptive and barrier function (i.e., using lactulose-mannitol 'permeability', intestinal inflammation (using quantitative fecal lactoferrin testing), and innate and acquired immune function.

Table 1

Recurrent versus non-recurrent diarrhea in relation to height and weight gains.

Diarrheal recurrence	Height gain (cm)	Weight gain (kg)
Non-recurrent	1.74	0.44
Recurrent*	1.38**	0.25***

Data from Schorling and Guerrant (1990)29 and Schorling et al. (1990)39

* >30% prevalence over subsequent 2 cm

** p=0.1 (21% less)

*** p=0.01 (43% less)

Table 2

Emerging causes of persisting diarrhea in shantytowns in Fortaleza northeastern Brazil.

Pathogen	Cases (%) (*n*=127)	Controls (%) (*n*=331)
Enteroaggregative *E. coli*		
AA probe +	32*	14
AA probe −	36*	17
Cryptosporidium	25**	0 5
Giardia lamblia	21**	0 8

Data from Steiner et al. (1998),12 Lima et al. (2000),61 Fang et al. (1995),195 and Moore et al. (2000)196

* $p<0.05$

** $p<0.02$

Table 3

Effect of malnutrition on both the incidence and duration of diarrhea.

	≥3 WAZ	≤3 WAZ	Increase (%)	*P* value*
Episodes/2m	1.9	2.6	37	0.001
Duration (d)	6.7	11.6	73	0.004
Total days of diarrhea	12.1	24.2	100	<0.001

Data from Guerrant et al. (1992)11 and Schorling et al. (1990)39

*Wilcoxon rank sum test. Also valid for moderate MN (<90% HAZ)

Table 4

Effects from early childhood diarrhea/enteric infections on lasting disability.

Disability	Reference
Growth shortfalls (esp. HAZ-2; 8.2 cm by 7 y)	
Cryptosporidial infections increase diarrhea morbidity and nutritional shortfalls to 18 months old	Molbak et al. (1997)59
	Agnew et al. (1998)60
	Lima et al. (2000)61
	Newman et al. (1999)62
Cryptosporidial infections ± diarrhea → decreased weight gain at 1 mo	Checkley et al. (1997)14
Cryptosporidial infections <6 m or stunting → 0.95–1.05 cm deficits at 1 y	Checkley et al. (1998)13
EAEC infections + inflammation → growth shortfall	Steiner et al. (1998)12
Diarrrhea <2 y → 3.6 cm stunted at 7 y (8.2 cm with helminths)	Moore et al. (1998)19
Fitness impairment (=17% decreased work productivity)	
Albendazole → 7% increased HST at 4 mo	Stephenson et al. (1993)37
Diarrhea <2 y → 4–8% decreased HST at 4–7 y	Guerrant (1999)54
4.3% increase HST → 16.6% increased work productivity	Ndamba (1993)55
Cognitive impairment (circa 10 IQ points)	
Diarrhea <2 y decreased WISC coding/digit at 5–9 y	Guerrant et al. (1999)54
Diarrhea <2 y decreased TONI at 6–10 y	Niehaus et al. (2002)56
Giardia or stunting decreased WISC-R at 9 yby 4–10 points	Berkman et al. (2002)63
School performance (circa 1 y)	
Diarrhea <2 y → increased AASS; AFG	Lorntz et al. (2006)50

Abbreviations: AASS, age at starting school; AFG, age for grade; EAEC, enteroaggregative *E. coli*; HAZ-2, height for age Z score at 2 years; HST, Harvard Step Test scores; TONI, Test of Nonverbal Intelligence; WISC, Wechsler Intelligence Scale for Children; →, predisposes to.