SUPPLEMENT ARTICLE

The MAL-ED Study: A Multinational and Multidisciplinary Approach to Understand the Relationship Between Enteric Pathogens, Malnutrition, Gut Physiology, Physical Growth, Cognitive Development, and Immune Responses in Infants and Children Up to 2 Years of Age in Resource-Poor Environments

The MAL-ED Network Investigators[a]

Highly prevalent conditions with multiple and complex underlying etiologies are a challenge to public health. Undernutrition, for example, affects 20% of children in the developing world. The cause and consequence of poor nutrition are multifaceted. Undernutrition has been associated with half of all deaths worldwide in children aged <5 years; in addition, its pernicious long-term effects in early childhood have been associated with cognitive and physical growth deficits across multiple generations and have been thought to suppress immunity to further infections and to reduce the efficacy of childhood vaccines. The Etiology, Risk Factors, and Interactions of Enteric Infections and Malnutrition and the Consequences for Child Health (MAL-ED) Study, led by the Fogarty International Center of the National Institutes of Health and the Foundation for the National Institutes of Health, has been established at sites in 8 countries with historically high incidence of diarrheal disease and undernutrition. Central to the study is the hypothesis that enteropathogen infection contributes to undernutrition by causing intestinal inflammation and/or by altering intestinal barrier and absorptive function. It is further postulated that this leads to growth faltering and deficits in cognitive development. The effects of repeated enteric infection and undernutrition on the immune response to childhood vaccines is also being examined in the study. MAL-ED uses a prospective longitudinal design that offers a unique opportunity to directly address a complex system of exposures and health outcomes in the community—rather than the relatively rarer circumstances that lead to hospitalization—during the critical period of development of the first 2 years of life. Among the factors being evaluated are enteric infections (with or without diarrhea) and other illness indicators, micronutrient levels, diet, socioeconomic status, gut function, and the environment. MAL-ED aims to describe these factors, their interrelationships, and their overall impact on health outcomes in unprecedented detail, and to make individual, site-specific, and generalized recommendations regarding the nature and timing of possible interventions aimed at improving child health and development in these resource-poor settings.

Keywords. MAL-ED; diarrhea; malnutrition.

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National and multinational investments into public health have greatly reduced child mortality from diarrhea and undernutrition over the past 20 years. Between 1990 and 2013, overall child mortality rates decreased from 87 to 51 per 1000 live births, and the associated

prevalence of children underweight fell from 25% to 16% [1]. The progress in decreasing mortality rates has increased attention on childhood morbidity in low-resource areas and the potential, more insidious consequences of undernutrition and infectious diseases on long-term health outcomes.

Early childhood physical growth and cognitive development may be affected by a multitude of economic, biological, environmental, and possible genetic factors. Exposures to putative pathogens at an early stage of physical, immunologic, and cognitive development may adversely disrupt the trajectory of a child's potential development, resulting in long-lasting consequences. As economic achievement in adulthood has been linked to school performance, which is in turn associated with development during the earliest years of life, such long-lasting consequences are likely to have significantly deleterious effects on both individuals as well as their communities [2].

The causes of poor growth and development in early childhood are complex, with a variety of direct and underlying conditions, including a lack of adequate amounts or quality of food [3]; early termination of or insufficient breastfeeding [4], with possible inadvertent introduction of putative pathogens in weaning foods; inadequate diversity of complementary foods, which may lead to specific micronutrient deficiencies [5–8]; diets that contain inhibitors of micronutrient absorption [9, 10]; catabolic states due to infection [11–13]; the inadequate response of the host and the host's gut microbial community to caloric insufficiency; and/or a configuration of the microbiota that is suboptimal for energy/nutrient harvest [14, 15]. The availability of micro- and macro-nutrients for physical and cognitive development and a healthy immune system are a function of both their input and processing, but few studies have attempted to explore longitudinally the sufficiency of food intake alongside disease and infection history with measures of gut function.

Enteric pathogens and their potential role in developing malnutrition have been a focal point for research. Pathogens may be introduced early in life and may damage the absorptive capacity of the intestine, causing protein-energy and micronutrient malnutrition [16]. Enteric infections can compromise the intestinal barrier, increase inflammation, and lead to decreased function. Both micronutrient deficiencies and chronic immune stimulation have also been found to impair growth and to increase susceptibility to infectious diseases [17].

Pioneering studies in Central America documented the impact of childhood infections and diarrhea on malnutrition [18]. Given the relatively high estimates of the prevalence of malnutrition and diarrhea episodes in much of the developing world, there is a great need for detailed data identifying specific enteric pathogens, age-specific incidence, and their association with growth faltering. Malnutrition also has been shown to increase susceptibility to infections and mortality due to diarrhea and other infectious diseases [19–22]. Repeated enteric infections in adults living in unsanitary conditions damage the intestinal tract, which leads to a condition that has been termed "environmental enteropathy or environmental enteric dysfunction" (EE/EED). A description of the clinical characteristics, possible etiology, and diagnosis of EE/EED is included in this supplement [23].

Although EE/EED is thought to contribute to the development of undernutrition in older populations [24, 25], the histology has not been adequately described in infants or young children living in areas of high exposure to enteric pathogens. Plausible mechanisms for how EE/EED may contribute to undernutrition and to growth faltering have been proposed. Repeated exposure to pathogenic bacteria, viruses, and parasites may impact the nutritional status of an individual by competing for available micronutrients, and/or cause villus blunting and thus impair nutrient absorption [16] and compromise the intestinal barrier, leading to increased intestinal permeability to pathogens, endotoxins, and other macromolecules that can result in the chronic stimulation of the immune system [26, 27]. Serum concentrations of micronutrients have been shown to both increase (iron) and decrease (vitamin A and zinc) in response to infection, inflammation, or tissue injury [28]. Both micronutrient deficiencies and chronic immune stimulation have also been found to impair physical growth and to increase the susceptibility to infectious diseases [19]. Additionally, alterations in the gut microbiota, either as a result of enteropathogen infection or the administration of antimicrobials, may influence the structure and functions of the innate and adaptive arms of the immune system, which in turn may reduce the effectiveness of oral and mucosal vaccines [21, 29–32].

To date, there have been few systematic, longitudinal, prospective studies that help define particular windows of vulnerability in infants and young children when infection by a specific pathogen or combination of pathogens could lead to greater deficits in developmental outcomes [33–35]. Definitive histopathological diagnoses of EE/EED in infants and children in developing countries have yet to be made due to ethical challenges associated with obtaining gut biopsies from vulnerable populations in areas with a high incidence of malnutrition. Furthermore, conclusive studies that define associations of physical growth and cognitive development deficits with repetitive specific enteric infections (controlling for dietary intake, levels of macro- and micronutrient deficiencies, and measures of intermediary indicators such as biomarkers of gut function) have not been conducted. There are also limited studies examining these factors on the immune response in children. To address these gaps in knowledge, the study of Etiology, Risk Factors, and Interactions of Enteric Infections and Malnutrition and the Consequences for Child Health (MAL-ED) was established at research sites with a high incidence of diarrheal disease and malnutrition in 8 countries.

MAL-ED Aims

The aim of MAL-ED is to improve scientific understanding of the complex interrelationships between gut microbial ecology, enteropathogen infection, dietary intake, nutritional status, gut physiology, growth, immune function and vaccine response, and cognitive development. Although existing evidence hints at the roles of individual factors, a holistic approach to quantify the interactions between these factors and their potentially synergistic role in multiple and diverse populations has not been explored previously. The primary health outcomes to be evaluated by MAL-ED are physical growth, cognitive development, and immune responses to oral and parenteral vaccines. It is anticipated that knowledge derived from MAL-ED will help the public health community better engineer interventions and their timing to minimize those factors that may contribute to lost lifetime potential.

The central hypotheses of the MAL-ED Network study are as follows:

1. Enteropathogen infection contributes to (*i*) stunting, (*ii*) wasting, and (*iii*) micronutrient deficiencies.
2. Enteropathogen infection causes intestinal inflammation and diminished barrier and adsorptive functions of the gut.
3. In children (≤24 months old), gut dysfunction associated with enteric infections and undernutrition results in (*i*) diminished nutrient absorption from the gut, (*ii*) growth faltering, (*iii*) cognitive impairments, and (*iv*) impaired responses to childhood vaccines.

Each hypothesis is at once superficially simple—with existing evidence—and intractable given the many factors that contribute to the manifestation of the respective health outcomes and the many feedback loops that confound analyses. Underlying these hypotheses is a belief that enteric infections, malnutrition, and gut function interact, rather than act in isolation, to affect physical growth, cognitive development, and immune responses to vaccination (Figure 1).

The MAL-ED Network

Through the MAL-ED study, an international, multidisciplinary collaborative network of researchers was established. Field sites for the study are located in resource-constrained areas (8 countries across 3 continents) and include both urban and rural communities with a history of high incidence of diarrheal disease and malnutrition. The field sites are located in Dhaka, Bangladesh; Fortaleza, Brazil; Vellore, India; Bhaktapur, Nepal; Loreto, Peru; Naushahro Feroze, Pakistan; Venda, South Africa; and Haydom, Tanzania. Collaborating investigators and institutions along with collaborators in the wider MAL-ED Consortium are shown in Table 1.

The Scientific and Administrative Core, based at the Fogarty International Center (National Institutes of Health) and the Foundation for the National Institutes of Health, provides

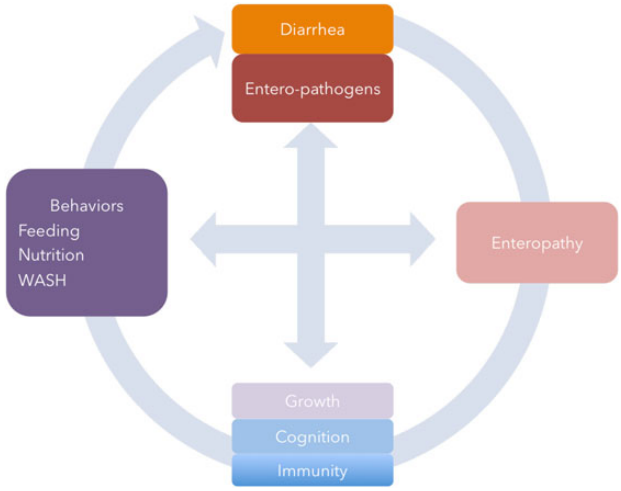

Figure 1. Components of the complex system of interactive relationships of health determinants and outcomes explored by the MAL-ED Study. Abbreviation: WASH, water, sanitary and hygiene interventions.

leadership in coordinating the scientific activities across the network through technical subcommittees (TSCs) for surveillance, microbiology, cognitive development, nutrition, gut function, and vaccine immunogenicity. TSC membership is comprised of subject-matter experts across sites and collaborating institutions and includes epidemiological and statistical support. A common Manual of Procedures containing standardized operating procedures and case report forms used in the study, as well as necessary training and quality assurance/quality control procedures to ensure comparability of results across the sites, was developed following extensive discussion between experts at the field sites and the TSC members. A protocol for real-time data transfer to and from each site to a centralized database was established to collate de-identified data for quality control review and cleaning. The first study subject was enrolled in November 2009 and data and sample collection from study subjects was completed at the end of February 2014.

This supplement describes the MAL-ED study by offering detailed descriptions of each of the 8 field sites [36–43] and specific data collection methodologies for the following categories: surveillance for common infant and childhood illnesses and medication usage, including antibiotics [44], and administered vaccines [45]; growth measurements [46]; breastfeeding and dietary intake assessments [47]; stool collections for microbiological and gut functional assays and antigen detection [48, 49]; blood collections for micronutrients [47] and serological responses to vaccines [45]; urine collections for micronutrients [47] and gut functional assays [50]; and cognitive testing at various ages [51].

Reviews of our current understanding of EE/EED and the scientific tools available to evaluate this condition are also included in the supplement to provide the context in which

Table 1. MAL-ED Consortium, Field Site, Collaborating Institutions, and Companion Project Principal Investigators

Institutions	Principal Investigators
Fogarty International Center	Mark Miller
Foundation for the National Institutes of Health	Michael Gottlieb
Aga Khan University, Karachi, Pakistan[a]	Zulfiqar Bhutta
Christian Medical College Vellore, Vellore, India[a]	Gagandeep Kang and Sushil John
JHSPH Satellite Laboratory, Iquitos, Peru[a]	Margaret Kosek
Federal University of Ceará, Fortaleza, Ceará, Brazil[a,b]	Aldo A. M. Lima and Reinaldo Oria
Walter Reed/AFRIMS Research Unit, Kathmandu, Nepal[a]	Sanjaya Kumar Shrestha
Institute of Medicine, Kathmandu, Nepal[a]	Prakash Sunder Shrestha
University of Venda, Limpopo, South Africa[a]	Pascal Bessong
International Centre for Diarrhoeal Disease Research, Dhaka, Bangladesh[a,b]	Tahmeed Ahmed and Rashidul Haque
Haydom Lutheran Hospital, Haydom, Tanzania[a]	Erling Svensen
JHSPH, Baltimore, MD, USA[c]	Laura Caulfield, Laura Murray-Kolb, and Robert Black
UVA, Charlottesville, VA, USA[d,e]	Richard Guerrant, William Petri[f,g], Eric Houpt[h], Patrick Concannon[f], Stephen Rich[f], Rebecca Dillingham[f]
Walter Reed/AFRIMS, Bangkok, Thailand[i]	Carl J. Mason and Ladaporn Bodhidatta
Center for Genome Sciences and Systems Biology, Washington University School of Medicine, St Louis, MO, USA[e]	Jeffrey I. Gordon[j]
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University of Michigan, Ann Arbor, MI, USA[e]	Felicia Wu[k]

Abbreviations: AFRIMS, Armed Forces Research Institute of Medical Sciences; CO, Colorado; JHSPH, Johns Hopkins Bloomberg School of Public Health; MD, Maryland; MI, Michigan; MO, Missouri; UVA, University of Virginia; VA, Virginia.

[a] Location of birth cohort study site.

[b] Location of case-control study site.

[c] JHSPH is collaborating with the Peru field site.

[d] UVA is collaborating with the Bangladesh, Brazil, South Africa, and Tanzania field sites.

[e] Location of a companion project.

[f] Genome-wide Association Scans for Undernutrition and Growth Impairment and Molecular Markers of Immunity and the Underperformance of Mucosal Vaccines companion project.

[g] PROVIDE.

[h] Next-Generation Molecular Diagnostic Technologies for Developing Countries diagnostic companion project.

[i] Role of the Gut Microbiome in Nutritional Status companion project.

[j] Aflatoxin Exposure and Its Effect on Growth of Children companion project.

[k] AFRIMS is collaborating with the Nepal field site.

protocols for MAL-ED were established [23, 52]. At present, there are limited choices of noninvasive methods to measure gut integrity appropriate for use in the MAL-ED study. Current methods assess the integrity of the intestinal barrier by measuring markers of inflammatory status [53], permeability [54], and absorptive capacity [55]. The relative balance of lactulose to the nonmetabolized sugar mannitol [56] excreted in urine gives an indication of the gut barrier function for both the absorptive capacity (mannitol) and permeability of the gut (lactulose) [57, 58].

METHODS

Overview of Cohort Study Design

The overall design of the project is described with greater detail in the relevant methods articles contained in this supplement [44–51].

Study Population

The MAL-ED study focuses on birth cohorts followed longitudinally (to 24 months of age) in each of the 8 study sites. Each site performed a census of their local community to obtain an assessment of the number of women of reproductive age and the number of children <5 years of age. Using these data, each site defined a catchment area where it was estimated that >200 infants (the target number of children to be enrolled per site) would be born within the enrollment period lasting 2 years.

The inclusion criteria were as follows:

1. Healthy infants enrolled within 17 days of birth.
2. Caregiver report that they had no plans to move out of the catchment area for at least 6 months following enrollment in the study.
3. Willingness of caregiver to be visited in the home twice weekly.

The exclusion criteria were any of the following:

1. The family had plans to move out of the catchment area for >30 consecutive days during the first 6 months of follow-up.
2. The mother was <16 years of age.
3. The mother had another child already enrolled in the MAL-ED cohort study.
4. The child was not a singleton (ie, twins, triplets).
5. The infant had any of the following indications of serious disease:
   a. Hospitalization for something other than a typical healthy birth;
   b. Severe or chronic condition diagnosed by a medical doctor (eg, neonatal disorder; renal, liver, lung, and/or heart disease; congenital conditions); or
   c. Enteropathies diagnosed by a medical doctor.
6. The child's guardian failed to provide signed informed consent.
7. Weight at birth or enrollment was <1500 g.

Human immunodeficiency virus (HIV) infection of mothers or children was not determined in the study cohort. Although HIV infection is recognized as having impact on nutritional status, it was beyond the scope of the study design. The MAL-ED site descriptive articles in this supplement include information regarding rates of HIV in these populations [36–43].

To elucidate the role of seasonal variation related to pathogen exposure, disease etiology, and food availability, subject enrollment occurred at each site over a 2-year period (the earliest enrollment was initiated in November 2009 and the latest enrollment occurred in February 2012). At least 200 children were enrolled per site and followed for 24 months. All sites received ethical approval, as appropriate, from governmental, local institutional, and collaborating institutional ethical review boards. Signed informed consent was obtained from the guardian of each participating child.

Demographic Characteristics, Socioeconomic Status, and Food Access Insecurity

Prior to enrollment, each site conducted a pilot study in an area representative of the study population to determine household characteristics, socioeconomic status (SES), food access insecurity, and general child health status within the MAL-ED catchment communities. At each site, 100 households were administered a standardized questionnaire about the household demographics (head of household, maternal age, marital status, educational attainment, and maternal parity), household environment, asset ownership, and food access insecurity. The questionnaire was based on questions used by the Demographic and Health Surveys and the Food and Nutrition Technical Assistance project [59] and input from the MAL-ED field sites. Additionally, the height and weight of one child 24–60 months of age was measured. This pilot study guided the development of a standardized questionnaire applicable to the MAL-ED cohorts.

Data Collection

At enrollment, each child's date of birth, sex, birth weight (if available) was recorded; information about initiation of breastfeeding was noted; and the child's length, weight, and head circumference were measured. Active surveillance for infectious diseases, general child health information, and basic dietary intake was undertaken by visiting each home twice per week. Additional visits to each household by trained field staff at various intervals were made to collect data about health, vaccinations, and dietary intake, and to measure anthropometry, perform cognitive tests, and collect blood, urine, and monthly surveillance (nondiarrheal) and diarrheal stool samples. Maternal and household characteristics were also recorded (Table 2).

Table 2. Measurement Collection/Questionnaire and Test Administration/Sample Collection Timeline for MAL-ED Cohort Studies

	Child Age (mo) and Sample Type																
Assessment	0	1	2	3	4	5	6	7	8	9	10	11	12	15	18	21	24
Gut integrity				U			U			U				U			U
Gut inflammation	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Incidence and prevalence of enteric pathogens	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Diarrhea illness surveillance	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
Anthropometry	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
Nutrition (breastfeeding and dietary intake)	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
Micronutrients							U	B						B/U			
Cognitive function							T		T					T			T
Household or maternal	I	I	I				I/O/T						I	I	I		I/O
Immunization and vaccine response	I	I	I	I	I	I	I	I/B	I	I	I	I	I	I/B	I	I	I
Other illness surveillance	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I

Abbreviations: B, blood; I, interview; M, measurement; O, observation; S, stool; T, test administration; U, urine.

At enrollment, only the demographics and food access insecurity portions of the SES questionnaire were collected from the households of MAL-ED study children. The complete SES and food access insecurity questionnaire was administered at 6, 12, 18, and 24 months.

Illness Surveillance and Stool Collection

During twice-weekly household visits, caregivers responded to a standardized questionnaire designed to collect a daily record of symptoms of cough, fever, vomiting, diarrhea and medication use. Stool samples were collected during diarrheal episodes (defined as ≥3 loose stools in a 24-hour period and separated by ≥2 diarrhea-free days) and during monthly home visits (nondiarrheal specimens). Subjects experiencing moderate and severe illnesses (including severe diarrhea, dysentery, acute lower respiratory infections, dehydration, and fever) were referred to local health services [44].

Physical Growth

Anthropometric measurements were collected on all children monthly using standardized procedures. The weight-for-age (WAZ), length-for-age (LAZ), and weight-for-length (WLZ) *z* scores are calculated using the World Health Organization (WHO) Multicentre Growth Reference Study Group program [60]. The height and weight of mothers was measured 2 months after delivery.

Microbiology

All stools were analyzed for the presence of bacterial, viral, and parasitic pathogens associated with diarrhea using traditional methods of microscopy, culture, enzyme-linked immunosorbent assay (ELISA), and polymerase chain reaction (PCR) as appropriate to the pathogen [48]. These include *Salmonella*, *Shigella*, *Vibrio*, *Yersinia*, *Aeromonas*, *Campylobacter* and *Plesiomonas*, *Escherichia coli*, rotavirus, norovirus, adenovirus, astrovirus, *Cryptosporidium*, *Giardia*, *Entamoeba histolytica*, *Ascaris*, *Trichuris*, *Strongyloides*, *Cyclospora*, *Isospora*, hookworm, and others. Pathogenic *E. coli* was identified by multiplex PCR amplification of known virulence genes and included enteropathogenic, enterotoxigenic, enteroaggregative, Shiga toxin (1 and 2)–producing, and enteroinvasive strains. Surplus stool samples were archived for use in future studies.

Cognitive Development

The cognitive development of each child was assessed through periodic administration of several validated instruments: the Bayley Scales of Infant Development [61] to assess global capacity (at 6, 15, and 24 months); the Infant Temperament Scale (T. Wachs, personal communication, 2010) to assess infant temperament (at 6 months); and the MacArthur Adapted Communicative Development Inventory: Words and Gestures [62] (at 8, 15, and 24 months) to assess language development [51]. The quality and quantity of stimulation and support available to the child in his or her home environment was assessed with the HOME Inventory [63] (at 6 and 24 months).

Maternal Factors

Maternal factors including mood and reasoning ability, known to be associated with a child's development, were assessed with the Self-Reporting Questionnaire–20 [64] (at 1, 6, 15, and 24 months). Maternal reasoning ability was assessed with the Raven's Combined Progressive Matrices instrument [65] (at 6–8 months) to control for these variables. Information about day and night blindness, and tobacco and alcohol use during pregnancy, was collected at 2 months after delivery.

Nutrition (Breastfeeding Status and Dietary Intake)

Individual nutritional status in the MAL-ED study population was assessed through periodic quantitative and qualitative assessments of the food consumed during the first 2 years of life [47]. Information—organized with a controlled vocabulary—was entered into a searchable database. For the first 8 months, this information was gathered by questioning the caregiver about the extent and duration of exclusive breastfeeding and about the introduction of weaning foods collected during the twice weekly and monthly home visits. When a child was 9 months of age, the caregiver was asked monthly (until 24 months of age is reached) to recall food intake over the past 24 hours to estimate caloric intake of the child and inform assessments of dietary quality and diversity.

Micronutrients

Micronutrient levels were measured in blood samples collected at 7 and 15 months of age. Hemoglobin, ferritin, and plasma transferrin receptor were used to assess levels of iron, lead, zinc, retinol, argentine, and glutamine. Iodine levels were measured in urine collected at 6 and 15 months. Because the acute-phase response to infections are known to affect micronutrient levels, the level of α-1-acid glycoprotein present in blood was measured to serve as a control and enable accurate assessment of micronutrient status in child subjects [28].

Gut Function and Inflammation

The lactulose-mannitol test was administered to study children at 3, 6, 9, 15, and 24 months to evaluate gut permeability and absorptive capacity, respectively. Three additional proteins were also assessed to gauge aspects of gut function: α-1-antitrypsin for gut permeability, neopterin as a marker of T-helper 1 immune activation, and myeloperoxidase, which is indicative of neutrophil activity [66]. Quantitative ELISAs to detect α-1-antitrypsin, myeloperoxidase, and neopterin were performed on all stool samples.

Vaccine Response

During monthly home visits, caregivers provided information about the receipt of childhood vaccinations, including but not limited to those on the schedule of the WHO Expanded Program on Immunization (EPI). Vaccination records were also collected (at 3, 6, 9, 12, 15, 18, 21, and 24 months), and the information source (eg, vaccine card, clinical report) was recorded. The MAL-ED study measured the response to selected parenteral and oral vaccines administered as part of the EPI program in each country. Blood obtained at 7 and 15 months of age was used to evaluate (by ELISA) the level of immune response to pertussis toxin, measles, tetanus toxoid, poliovirus types 1, 2, 3, and rotavirus by ELISA. Poliovirus neutralization titers were also determined.

Case-Control Studies

MAL-ED intensive biweekly household surveillance and efforts to collect clinical specimens and to capture accurate data may create a "Hawthorne effect" that dramatically reduces diarrhea rates and malnutrition [67]. As an adjunct to the cohort study, 2 of the MAL-ED sites (Fortaleza, Brazil [39] and Dhaka, Bangladesh [36]) conducted case-control studies measuring similar variables as described above. In these parallel studies, cases were defined as children from 6 to 18 months of age exhibiting a WAZ score of < -2 compared to community controls. These studies are more fully described in the site-specific articles of this supplement [36, 39].

Data Management and Analysis

The Data Coordinating Center (DCC) of MAL-ED was established at the Fogarty International Center. Members of the DCC contributed to the study design and form development, and developed a double-entry database application, which simultaneously collected and stored MAL-ED data at each site and centrally. In the field and laboratory, data were collected on standardized forms; local data supervisors checked form completeness and accuracy prior to data entry. As an additional quality control measure, forms were double-entered into the local database; discrepancies between first and second entry were resolved by the site data entry supervisor(s).

Data from each site were transferred to a central server at the DCC using data synchronization software. The DCC provided feedback regarding data quality to site investigators through monthly data and quality control reports. In addition, site-specific "issue logs" were maintained in a file sharing Web site that provided real-time, site-specific feedback to identify errors and/or omissions in data entry and to facilitate corrections with minimal time delay. To standardize the definitions of exposure and outcome measures in MAL-ED, the DCC generated datasets that were made available across the MAL-ED Network of investigators. These datasets will serve to uniformly analyze the exposure and outcome relationships of the MAL-ED population.

The MAL-ED Consortium and Companion Projects

The MAL-ED Network provided a scientific and administrative platform from which related projects have leveraged resources including hypothesis-based research and targeted interventional trials. MAL-ED companion projects—together with the cohort and case-control Network studies—constitute the larger MAL-ED Consortium. Companion project institutions and investigators have agreed to abide by the same Research Consortium Agreement (see below), as have all MAL-ED Network investigators. Current MAL-ED companion projects are briefly described below.

Role of the Gut Microbiome in Nutritional Status

Ancillary studies of the human gut microbiome and its role in nutrition are being conducted through a comparative metagenomic study seeking to characterize features of the gut microbiome associated with the development of undernutrition in children identified in the case-control study [36]:

1. Are there identifiable configurations of the gut microbiome associated with undernutrition? If so, the findings may have pathophysiologic and diagnostic implications.
2. How is the microbiome reconfigured with a therapeutic food intervention, and does reconfiguration persist after cessation of the intervention?
3. What is the relationship between diet, the gut microbiome, and environmental enteropathy?
4. Can observations made in one human population be generalized to another?

Answering these questions requires a detailed knowledge of the normal assembly of the microbiome in healthy children in a given cultural setting and an understanding of the variations that exist among these healthy children at given points during their postnatal development.

Genome-wide Association Scans for Undernutrition and Growth Impairment

Genome-wide studies aimed at identifying candidate human genes associated with undernutrition and growth impairment are additionally being conducted. The genetic basis for susceptibility to malnutrition has not been as rigorously studied as the genetic basis for susceptibility to diseases common in the developed world. For obesity, the lipoprotein lipase, β-lactamase, and protein phosphatase 1–like genes have been implicated [68]. In a way, obesity and undernutrition can be considered as extremes of the metabolic spectrum, and it is not unlikely that some of the genetic polymorphisms that predispose to obesity may protect from undernutrition [69]. In addition, genes that influence inflammation and infection may also impact

nutritional state [70, 71]. The adipocytokine leptin not only controls appetite, but also promotes proinflammatory cytokine production, and variation in genes of the immune system can affect susceptibility to infections that contribute to malnutrition [72]. This study aims to narrow the gap in knowledge regarding genetic susceptibility to malnutrition through a genome-wide association scan of malnourished children.

Next-Generation Molecular Diagnostic Technologies for Developing Countries

The Next-Generation Molecular Diagnostic Technologies for Developing Countries project will advance development of enteropathogen target–specific, quantitative, PCR-based assays capable of detecting all of the bacterial, viral, protozoal, and helminthic pathogens being studied in the MAL-ED project. Sample extraction to amplification and detection will be tested on prototype platforms at some of the MAL-ED Network field sites and performance compared with results obtained by more traditional culture, microscopy, ELISA, and biochemical methods. The project deliverables are a series of field-ready protocols for the diagnosis of major enteropathogens that can be deployed efficiently for future epidemiologic projects such as MAL-ED.

Molecular Markers of Immunity and the Underperformance of Mucosal Vaccines

In addition to the vaccine response analyses that will be conducted by MAL-ED for mucosal and parenteral vaccines, the Performance of Rotavirus and Oral Polio Vaccines in Developing Countries (PROVIDE) study is using state-of-the-art immunological methods to assess cellular immunity following vaccination and its association with protection. Additional outcome measures for the study include (*i*) differences in episodes of rotavirus diarrhea between rotavirus vaccinees and nonvaccinees; and (*ii*) change in polio plasma neutralizing antibody titer after immunization. The use of MAL-ED protocols and standardized operating procedures and resources at the MAL-ED Network site in Bangladesh are utilized in this project.

Aflatoxin Exposure and Its Effect on Growth of Children

Aflatoxin exposure has been associated with growth decrements in observational studies. To better assess for this exposure in the context of many other possible growth factors, a subset of blood and urine samples from the MAL-ED study will be assessed for exposures to aflatoxins as an exploratory analysis to investigate the degree of association.

As the data collected from the MAL-ED study populations are explored, associations between factors measured, and health outcomes better defined, it is anticipated that additional opportunities to leverage MAL-ED data and samples and the site's capacities will emerge from diverse research enterprises in the public and private sectors for hypothesis-driven research and intervention studies.

Research Consortium Agreement

A Research Consortium Agreement (RCA) was developed and adopted by all collaborating investigators and their institutions in the MAL-ED Consortium prior to the onset of data collection and/or sharing. The RCA provides the organizational framework for the project including management and authorities, governance structure, methods of dispute resolution, and authority of the Network and its associated advisory committees. The RCA also provides guidance on publication; intellectual property; and data ownership, sharing, and release policies. The intent of these policies is to ensure that the important findings resulting from the study are used to benefit those in low-income countries who are most affected. Clearly delineating these issues, with input from the participating institutions and investigators prior to study initiation, was important to effectively establishing harmonization of the study; having the document in place has helped to facilitate the addition of other studies as companion projects.

DISCUSSION

The multiple interactions of enteric infections, malnutrition, and gut function and their synergistic effect on physical growth, cognitive development, and immune response provide the working hypotheses of MAL-ED. The structural components of this system of relationships and interactions are based on 2 lines of reasoning: (*i*) experiential expert knowledge of biological mechanisms and (*ii*) empirical evidence identifying key components, both risk factors and health outcomes. Together, it is possible to piece together hypothetical ways in which these components interrelate: the components explored by MAL-ED have been described in this introductory article (Figure 1) and are explored in greater detail in the MAL-ED methodological articles of this supplement [44–51].

Empirical evidence to date has usually been derived from studies concentrating on a single outcome. Such a focus ignores the potentially reciprocal and interdependent relationships that we hypothesize exist between the components of this system. The incidence and prevalence of chronic undernutrition manifested as stunting is commonly observed, but the mechanistic causes are poorly understood. Studies to define and quantify the role of particular enteropathogens and their contribution to malnutrition or diminished immune response have been limited by small sample sizes, narrow geographic scope, and/or a lack of robust diagnostic tests [73, 74]. Often they have been predicated on a "one pathogen, one disease" assumption, attributing symptoms to the presence of individual pathogens without consideration for coinfection, timing, or

quantification of pathogens. By definition, pathogens cause damage to their host; however, the ambiguity over the detection, let alone role, of enteric pathogens means that their short- and long-term consequences—especially of chronic and recurrent infections—against the backdrop of this wider system of risks and exposures have not been well characterized to date. Indeed, some enteric pathogens may not cause diarrhea, and therefore are not readily measured or studied in clinical research. Thus the frequency of carriage or infection with these pathogens or their consequences (not associated with diarrhea)—particularly in young children—remains understudied. It is anticipated that the community-based, longitudinal design of MAL-ED will better capture the "average" exposure to enteric pathogens and other factors and more clearly elucidate the consequences of this system of interdependent exposures on child development.

Whether considering single components of this system or the interdependencies, the dilemma with how these components are combined is that the underlying heterogeneity of different populations yields variable rankings of risks and health outcomes [75–89]. Any given case study typically focuses on singular aspects of the system we have described—be that a study of diarrhea, a specific pathogen, socioeconomic status, or growth attainment [90–93]. Between populations, however, the evidence can vary and produce disparate results and expert opinion. Different populations, for example, experience both different pathogens and in different quantities. Add to this a range of diets and behaviors, and the resulting health outcomes of any single population may not be representative of another. In applying a harmonized protocol across 8 geographically, socioeconomically, and culturally diverse populations, MAL-ED expects to identify relationships between health determinants and outcomes that are both uniform across all sites and disparate between sites. Improving the characterization of the similarities and divergence of these relationships would potentially inform decisions about how to most effectively apply as well as adapt interventions to achieve the desired improvements in health outcomes in variable settings. Furthermore, among the important relationships and pathways identified, we expect some to be more or less amenable to intervention. Demographic and socioeconomic characteristics along with general health indicators of each of the 8 MAL-ED research sites are included in the individual papers of this supplement. These papers also contain information about the recruitment and training strategies employed at the sites to implement the MAL-ED protocol [36–43].

As a central objective of MAL-ED, untangling the complicated web of malnutrition and enteric disease is considered a crucial factor in the development of interventions that will improve child health in resource-poor environments. These interventions will ideally account for the multiple and interacting risk factors affecting child development and, given likely limited resources, include consideration of optimal timing and targeting for maximal impact. Improvements in early childhood growth and development can have long-lasting impact, through improved school readiness, educational achievement, and, ultimately, improving the economic potential for individuals and their communities. MAL-ED may provide some insights into targeted interventions at this age.

Notes

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APPENDIX

MAL-ED Network Investigators

Last Name	First Name	Institution	Role in the MAL-ED Network
Acosta	Angel Mendez	A.B. PRISMA, Iquitos, Peru	Data management
Chavez	Cesar Banda	A.B. PRISMA, Iquitos, Peru	Laboratory
Flores	Julian Torres	A.B. PRISMA, Iquitos, Peru	Laboratory
Olotegui	Maribel Paredes	A.B. PRISMA, Iquitos, Peru	Study coordinator
Pinedo	Silvia Rengifo	A.B. PRISMA, Iquitos, Peru	Nutrition
Trigoso	Dixner Rengifo	A.B. PRISMA, Iquitos, Peru	Laboratory
Vasquez	Angel Orbe	A.B. PRISMA, Iquitos, Peru	Psychologist, cognitive development
Ahmed	Imran	Aga Khan University, Naushahro Feroze, Pakistan	Data management
Alam	Didar	Aga Khan University, Naushahro Feroze, Pakistan	Laboratory, nutrition
Ali	Asad	Aga Khan University, Naushahro Feroze, Pakistan	Vaccine response
Bhutta	Zulfiqar A.	Aga Khan University, Naushahro Feroze, Pakistan	Pakistan site PI
Qureshi	Shahida	Aga Khan University, Naushahro Feroze, Pakistan	Laboratory, microbiology
Shakoor'	Sadia	Aga Khan University, Naushahro Feroze, Pakistan	Microbiology
Soofi	Sajid	Aga Khan University, Naushahro Feroze, Pakistan	Operations, surveillance
Turab	Ali	Aga Khan University, Naushahro Feroze, Pakistan	Operations, surveillance, nutrition
Yousafzai	Aisha K.	Aga Khan University, Naushahro Feroze, Pakistan	Cognitive development
Zaidi	Anita K. M.	Aga Khan University, Naushahro Feroze, Pakistan	Pakistan site co-PI, microbiology
Bodhidatta	Ladaporn	AFRIMS, Bangkok, Thailand	Microbiology
Mason	Carl J.	AFRIMS, Bangkok, Thailand	Nepal site PI, vaccine response
Babji	Sudhir	Christian Medical College, Vellore, India	Microbiology supervisor
Bose	Anuradha	Christian Medical College, Vellore, India	Nutrition
John	Sushil	Christian Medical College, Vellore, India	India site co-PI
Kang	Gagandeep	Christian Medical College, Vellore, India	India site PI
Kurien	Beena	Christian Medical College, Vellore, India	Cognitive development supervisor
Muliyil	Jayaprakash	Christian Medical College, Vellore, India	Epidemiology
Raghava	Mohan Venkata	Christian Medical College, Vellore, India	Data management
Ramachandran	Anup	Christian Medical College, Vellore, India	Biochemistry, nutrition
Rose	Anuradha	Christian Medical College, Vellore, India	Epidemiology
Pan	William	Duke University, Durham, NC, USA	DCC, biostatistician
Ambikapathi	Ramya	FIC, NIH, Bethesda, MD, USA	DCC, nutrition
Carreon	Danny	FIC, NIH, Bethesda, MD, USA	DCC, SES, and data management
Charu	Vivek	FIC, NIH, Bethesda, MD, USA	DCC, data management
Dabo	Leyfou	FIC, NIH, Bethesda, MD, USA	DCC, data management
Doan	Viyada	FIC, NIH, Bethesda, MD, USA	DCC, data management
Graham	Jhanelle	FIC, NIH, Bethesda, MD, USA	DCC, data management
Hoest	Christel	FIC, NIH, Bethesda, MD, USA	DCC, vaccine response
Knobler	Stacey	FIC, NIH, Bethesda, MD, USA	Senior scientific program director
Lang	Dennis	FIC, NIH, Bethesda, MD, USA	Senior program coordinator
McCormick	Benjamin	FIC, NIH, Bethesda, MD, USA	DCC, computational biology
McGrath	Monica	FIC, NIH, Bethesda, MD, USA	DCC, QA/QC lead, microbiology, epidemiology
Miller	Mark	FIC, NIH, Bethesda, MD, USA	PI
Mohale	Archana	FIC, NIH, Bethesda, MD, USA	DCC, data management
Nayyar	Gaurvika	FIC, NIH, Bethesda, MD, USA	DCC, data management
Psaki	Stephanie	FIC, NIH, Bethesda, MD, USA	DCC, SES
Rasmussen	Zeba	FIC, NIH, Bethesda, MD, USA	DCC, cognitive development, epidemiology
Richard	Stephanie	FIC, NIH, Bethesda, MD, USA	DCC, surveillance, epidemiology

continued.

Last Name	First Name	Institution	Role in the MAL-ED Network
Seidman	Jessica	FIC, NIH, Bethesda, MD, USA	DCC program manager, gut function, cognitive development, vaccine response
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Blank	Rebecca	FNIH, Bethesda, MD, USA	Scientific program manager
Gottlieb	Michael	FNIH, Bethesda, MD, USA	PI
Tountas	Karen	FNIH, Bethesda, MD, USA	Scientific program manager
Amour	Caroline	Haydom Lutheran Hospital, Haydom, Tanzania	Laboratory
Mduma	Estomih	Haydom Lutheran Hospital, Haydom, Tanzania	Tanzania site co-PI, field site manager
Ahmed	Tahmeed	ICDDR-B, Dhaka, Bangladesh	Bangladesh site PI, nutrition
Ahmed	A. M. Shamsir	ICDDR-B, Dhaka, Bangladesh	Field supervisor
Dinesh	Mondol	ICDDR-B, Dhaka, Bangladesh	Microbiology supervisor
Tofail	Fahmida	ICDDR-B, Dhaka, Bangladesh	Cognitive development
Haque	Rashidul	ICDDR-B, Dhaka, Bangladesh	Bangladesh site co-PI
Hossain	Iqbal	ICDDR-B, Dhaka, Bangladesh	Coordinator, case control
Islam	Munirul	ICDDR-B, Dhaka, Bangladesh	Nutrition
Mahfuz	Mustafa	ICDDR-B, Dhaka, Bangladesh	Field supervisor
Chandyo	Ram Krishna	IOM, Tribuhvan University, Kathmandu, Nepal	Field manager, nutrition
Shrestha	Prakash Sunder	IOM, Tribuhvan University, Kathmandu, Nepal	Nepal site co-PI
Shrestha	Rita	IOM, Tribuhvan University, Kathmandu, Nepal	Psychologist
Ulak	Manjeswori	IOM, Tribuhvan University, Kathmandu, Nepal	Clinical supervisor
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Caulfield	Laura	JHU, Baltimore, MD, USA	JHU site PI, nutrition
Checkley	William	JHU, Baltimore, MD, USA	DCC, epidemiology, statistician
Chen	Ping	JHU, Baltimore, MD, USA	Data management
Kosek	Margaret	JHU, Baltimore, MD, USA	Peru site PI, gut function
Lee	Gwenyth	JHU, Baltimore, MD, USA	Nutrition, gut function
Yori	Pablo Peñataro	JHU, Baltimore, MD, USA	Data management
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Schaefer	Barbara	Pennsylvania State University, University Park, PA, USA	DCC, cognitive development, psychometrics
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Bindá	Alexandre	Universidade Federal do Ceará, Fortaleza, Brazil	Laboratory
Costa	Hilda	Universidade Federal do Ceará, Fortaleza, Brazil	Psychologist
Di Moura	Alessandra	Universidade Federal do Ceará, Fortaleza, Brazil	Nurse study coordinator
Filho	Jose Quirino	Universidade Federal do Ceará, Fortaleza, Brazil	DCC, data management
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Lima	Aldo	Universidade Federal do Ceará, Fortaleza, Brazil	Brazil site PI
Lima	Noelia	Universidade Federal do Ceará, Fortaleza, Brazil	Pediatrician
Lima	Ila	Universidade Federal do Ceará, Fortaleza, Brazil	Laboratory
Maciel	Bruna	Universidade Federal do Ceará, Fortaleza, Brazil	Nutrition
Moraes	Milena	Universidade Federal do Ceará, Fortaleza, Brazil	Nutrition
Mota	Francisco	Universidade Federal do Ceará, Fortaleza, Brazil	Pediatrician
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Quetz	Josiane	Universidade Federal do Ceará, Fortaleza, Brazil	Laboratory
Soares	Alberto	Universidade Federal do Ceará, Fortaleza, Brazil	Data management
Svensen	Erling	University of Bergen, Norway; Haydom Lutheran Hospital, Haydom, Tanzania	Tanzania site PI, cognitive development
Tor	Strand	University of Bergen, Norway	Consultant, nutrition
Patil	Crystal	University of Illinois, Urbana-Champaign, IL, USA	Nutrition

continued.

Last Name	First Name	Institution	Role in the MAL-ED Network
Bessong	Pascal	University of Venda, Thohoyandou, South Africa	South Africa site PI
Mahopo	Cloupas	University of Venda, Thohoyandou, South Africa	Nutrition
Mapula	Angelina	University of Venda, Thohoyandou, South Africa	Psychology supervisor, cognitive development
Nesamvuni	Cebisa	University of Venda, Thohoyandou, South Africa	Nutrition
Nyathi	Emanuel	University of Venda, Thohoyandou, South Africa	Data management
Samie	Amidou	University of Venda, Thohoyandou, South Africa	Laboratory supervisor
Barrett	Leah	UVA, Charlottesville, VA, USA	Study coordinator
Gratz	Jean	UVA, Charlottesville, VA, USA	Laboratory, Tanzania site
Guerrant	Richard	UVA, Charlottesville, VA, USA	UVA PI, surveillance, cognitive development
Houpt	Eric	UVA, Charlottesville, VA, USA	Microbiology, Tanzania site development
Olmsted	Liz	UVA, Charlottesville, VA, USA	Financial manager
Petri	William	UVA, Charlottesville, VA, USA	UVA co-PI, vaccine response
Platts-Mills	James	UVA, Charlottesville, VA, USA	DCC, microbiology
Scharf	Rebecca	UVA, Charlottesville, VA, USA	Cognitive development
Shrestha	Binob	Walter Reed/AFRIMS Research Unit, Kathmandu, Nepal	Data management
Shrestha	Sanjaya Kumar	Walter Reed/AFRIMS Research Unit, Kathmandu, Nepal	Nepal site co-PI, gut function

Abbreviations: AFRIMS, Armed Forces Research Institute of Medical Sciences; DCC, Data Coordinating Center; FIC, Fogarty International Center; FNIH, Foundation for the National Institutes of Health; ICDDR,B, International Centre for Diarrhoeal Disease Research, Bangladesh; IL, Illinois; IOM, Institute of Medicine; JHU, Johns Hopkins University; MD, Maryland; NC, North Carolina; NIH, National Institutes of Health; PA, Pennsylvania; PI, principal investigator; SES, socioeconomic status; USA, United States of America; UVA, University of Virginia; VA, Virginia.