Report

Technical Meeting on Environmental Enteric Dysfunction, the Microbiome and Undernutrition

Nutritional and Health-Related Environmental Studies Section

Division of Human Health

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A. ACRONYMS

AA	Amino acid
AAT	Alpha-1 anti-trypsin
ACF	Action Contre la Faim
AGP	Alpha 1-acid glycoprotein
BMFG	Bill & Melinda Gates Foundation
CMAM	Community management of Acute Malnutrition
COG	Clusters of Orthologous Groups
CRP	C-reactive protein
CRPs	Coordinated Research Projects
D_2O	Deuterium water
DNA	Deoxyribonucleic acid
DP4	Dipeptidyl peptidase-4
EE	Environmental enteropathy
EED	Environmental enteric dysfunction
EndoCAb	Endogenous endotoxin-core antibody
ETH	Swiss Federal Institute of Technology
GH	Growth hormone
GI	Gastrointestinal
GLP-2	Glucagon-like peptide-2
HAZ	Height-for-age z-score
HIV	Human immunodeficiency virus
IAEA	International Atomic Energy Agency
IBD	Inflammatory bowel disease
IgA	Immunoglobulin A
IGF-I	Insulin growth factor
IGFBP	Insulin growth factor binding-protein
IL-1 β	Interleukin-1β
IUGR	Intrauterine growth retardation
IYCF	Infant and young child feeding
LAZ	Length-for-age z-score
LCPUFA	Long chain polyunsaturated fatty acids
L:M	Lactulose/Mannitol
L:R	Lactulose/Rhamnose
LIC	Low income countries
LMIC	Low and middle income countries
LPS	Lipopolysaccharides
MAL-ED	The Interactions of Malnutrition & Enteric Infections: Consequences for Child Health and Development
MAM	Moderate acute malnutrition
MNP	Micronutrient powder
MPO	Myeloperoxidase
mRNA	Messenger ribonucleic acid
OPV	Oral polio and rotavirus vaccines

PCR	Polymerase chain reaction
PGE3	Prostaglandins E3
PROVIDE	Performance of Rotavirus and Oral Polio Vaccines in Developing Countries
PUFA	Polyunsaturated fatty acid
RDA	Recommended daily allowance
rRNA	Ribosomal ribonucleic acid
SAM	Severe acute malnutrition
SIBO	Small intestine bacterial overgrowth
SBT	Sucrose breath test
SCFA	Short-chain fatty acids
SD	Standard deviation
SHINE	Sanitation, Hygiene, Infant Nutrition Efficacy Project
SIBO	Small intestinal bacterial overgrowth
T3	Triiodothyronine
TM	Technical meeting
TNF	Tumor necrosis factor alpha (TNF-α)
WASH	Water, Sanitation and Hygiene
WAZ	Weight-for-age z-score
WHZ	Weight-for-height z-score

B. EXECUTIVE SUMMARY

Stunting, a multifactorial condition, develops as a result of sustained inadequate nutrition and infection. Socioeconomic and environmental factors are considered to be important components of stunting. The observation that growth retardation can actually occur in apparently well fed children has prompted a search for other factors causally related to stunting. Poor hygiene and absence of adequate sanitation may play a role but evidence to support a causal relationship is largely lacking. It is thought that living in poor sanitary conditions may induce gut dysfunction, referred to as environmental enteric dysfunction (EED). The mechanisms by which EED impacts upon linear growth are unknown but evidence supports a role for microbial translocation, gut and systemic inflammation, alteration in gut microbiota diversity and nutrient malabsorption. It is thought that the inflammation of EED is deleterious and may result in severe acute malnutrition in the extreme end of the EED spectrum. A technical meeting on EED took place from 28-30 October 2015, at the International Atomic Energy Agency (IAEA) in Vienna. Nearly fifty experts from a diverse set of professions participated in the three-day meeting to discuss current research, developments and experiences in diagnosing and evaluating EED. Topics covered included defining EED, identifying biological pathways and consequences of EED on the microbiome and undernutrition, prevention and treatment of EED, with a particular focus on the role of stable isotope techniques in EED diagnosis.

This report presents a summary of key discussion points arising from the meeting.

B.1. Current knowledge on EED, the microbiome and effects on growth and functional outcomes

Environmental enteropathy is a combination of infection-undernutrition induced failure of the mucosal barrier of the gut and was first recognised in 1960 after Peace Corps workers manifested gut discomfort that was reversible upon their return home. It has been recently referred to as environmental enteric dysfunction (EED) to reflect the numerous gut function deficits associated with it. EED is thought to affect 50-95% of all children under the age of 5 years in resource poor settings. Although most cases are asymptomatic and hardly diagnosed, there is compelling evidence to support the association of EED with: 1) nutrient malabsorption; 2) linear growth faltering; 3) microbial translocation; 4) alterations in gut microbiota diversity; 5) gut and systemic inflammation; 6) reduced effect of vaccines and 7) severe acute malnutrition. Further, EED is reversible, seasonal and highly associated with low socio-economic status (as an example, there is an association between EED and Gross Domestic Product). There is no universal agreement on a case definition of EED. This is due to several reasons including multiple pathways leading to EED and the spectrum of symptoms among subjects with EED.

B.1.1. EED and undernutrition

EED is strongly associated with stunting and other forms of undernutrition. Stunting is known to emanate from long-term unfavourable conditions of nutrition and health but today poor sanitary conditions are also considered to be an important part of the problem. It is thought that living in poor sanitary conditions may lead to gut function disorders and chronic inflammation, both of which are evident in EED. The mechanisms by which EED impacts growth are unknown but nutrient malabsorption, microbial translocation, gut and systemic inflammation, increased gut permeability and altered gut microbiota and metabolome are

sometimes observed in EED. These are key entry points for the development of potential biomarkers to diagnose EED.

B.1.2. Gut microbiome and EED

The gut microbiota community is largely acquired at birth, develops quickly during breastfeeding period and continues to develop at the end of the complementary feeding period toward the adult-like pattern. It plays an important role in host development (e.g. immunity, metabolism, gut motility) and is influenced by several factors, such as: mode of delivery (vaginal versus caesarean), breast milk composition, dietary diversity, host lifestyle, genotype, pathobiology, physiology, age, the environment and immune system.

Unfavourable nutritional conditions result in gut microbiota immaturity which cannot be recovered by current nutritional interventions such as therapeutic food in the case of severe acute malnutrition (SAM). There is compelling evidence that gut immaturity is in turn related to the pathogenesis of undernutrition and plays an important role in treatment outcome of SAM. The mechanisms by which the microbiome affects gut health and nutritional status is not entirely clear, however there is evidence that some gut intestinal pathogens have specific mechanisms of action that make a link with EED biologically plausible – e.g., mucin degradation. Additionally, certain taxa of gut microbiota can be beneficial and harmful under different environmental conditions (e.g., complexity of the microbiota community, pathogen susceptibility, and dietary shifts). Data on the role of the gut microbiota on inflammation are scarce. More studies are needed to elucidate the causal/consequential relationship between EED and the microbiota.

B.1.3. Pathogenesis of stunting in EED

Stunting reflects combined effects of low birth weight and postnatal growth failure. The response of the body to malnutrition and EED is similar. Insulin growth factor (IGF-I) regulates growth and other functions in the body during pregnancy. IGF-1 is deficient in malnourished pregnant women leading to intrauterine growth retardation (IUGR). Malnutrition is furthermore associated with an increase in cortisol and insulin growth factor binding protein (IGFBP) levels, which results in a decrease in IGF-I and reduced growth. Similarly, inflammation of the small intestine in EED is associated with high C-reactive protein and may be accompanied by release of cytokines such as IL-6 that reduce appetite and food intake and impair production and action of chondrocyte growth factors. Stress-induced activation of the hypothalamic-pituitary-adrenal axis stimulates a rise in cortisol and IGFBP-1, which inhibit IGF-1 action and induce chondrocyte apoptosis. A reduction in hepatic growth hormone (GH) receptor expression and inhibition of GH signalling by fibroblast growth factor 21 and possibly zinc deficiency, further limit IGF-1 production and thereby contribute to growth failure. However, in the case of IUGR, undernutrition and in EED, re-feeding results in an abnormal increase in body fat (abdominal and visceral fat) and may pre-dispose to type 2 diabetes in later life. Therefore risks of metabolic dysfunction from nutrition repletion must be balanced against potential benefits for cognitive function.

B.1.4. Energy and nutrient requirements in EED

Malnutrition and deficiencies in certain macro- and micronutrients inhibit intestinal mucosal growth and alter gut barrier functions. A number of nutrients may have beneficial effects on the management of EED. Vitamin A, zinc and some amino-acids (AA) such as glutamine, threonine, leucine and cysteine are potentially involved in improving gut barrier function and absorption. Furthermore, AA have been shown

to be involved in gastrointestinal repair. Zinc, probiotics, plant flavonoids and n-3 polyunsaturated fatty acids (PUFA) have been associated with reduced inflammation. In contrast, available evidence suggests that iron potentially shifts the microbiome towards a more pathogenic profile and increases gut inflammation.

The relationship between EED and nutrients is bidirectional. Inflammation and reduced absorption, both of which are evident in EED, may result in an increased energy and nutrient requirements in children with EED. Current international recommendations for protein, fat and energy intake for children with moderate acute malnutrition are higher than average for broad population (10% high-quality protein, 25-35% fat for energy density). Reaching the physiological requirements in zinc (estimated to be 0.74 mg/d in 9 m-old children) has only been achieved in malnourished children with oral intakes well above those needed by infants and young children in high income settings. Studies showed that children with EED might have up to 50% higher requirements for dietary zinc.

B.2. Use of stable isotopes in evaluating gut dysfunction

Stable isotopes have been used to assess gut dysfunction (small intestine bacteria overgrowth, celiac disease and chemotherapy induced small intestinal damage in rats) with different substrates (starch and other carbohydrates, mixed triglycerides, fatty acids, proteins, etc.). An example of a diagnostic ^{13}C breath test is the ^{13}C-urea breath test used to diagnose and monitor *Helicobacter pylori* infection in the stomach. The high specificity and high sensitivity associated with the test makes it the ideal non-invasive diagnostic test. The ^{13}C-sucrose breath test is a promising future technique to assess gut function and has been used in Australia to measure the absorptive capacity of the small intestine. It was validated against the small intestinal permeability test (lactulose: rhamnose ratio). The glucose hydrogen breath test is currently the most accurate non-invasive test to diagnose small intestine bacterial overgrowth (SIBO). Combined ^{13}C and H_2 breath tests could also be used to assess fermentation and SIBO with higher specificity by correcting for the gastric emptying rate. Combined stable isotopes better distinguished between lactose digesters and non-digesters. Labelling of macronutrients with ^{13}C and ^{2}H is furthermore being tested as an intrinsic label to assess carbohydrate and protein bioavailability. Other uses of dual stable isotope techniques include studying the kinetics of the glucose metabolism, starch digestion and fermentation. Combination of $^{13}C/^{2}H$ has the potential to be used to assess AA bioavailability from different food sources.

B.3. Biomarkers of EED

While malabsorption, gut barrier dysfunction, and gut inflammation are overlapping components of EED, it is difficult to identify specific markers of each that could be used solely for EED diagnosis. An ideal EED biomarker should be highly associated with stunting and could be classified according to the underlying causes of EED, namely: 1) intestinal permeability and nutrient absorption (e.g., lactulose/mannitol or lactulose/rhamnose tests, ^{13}C sucrose breath test), 2) bacterial translocation (e.g., lipopolysaccharides), 3) intestinal inflammation (e.g., myeloperoxidase, calprotectin and neopterin), 4) systemic inflammation (e.g., C-reactive protein, alpha 1-acid glycoprotein, cytokines), 5) functional enterocyte mass (citrulline to assess damaged gut), 6) intestinal repair (e.g., promoter glucagon-like peptide-2 [GLP-2]), 7) mucosal immune underachievement (e.g., kynurenine/tryptophan ratio) and 8) alterations in microbiota diversity.

B.4. On-going interventions to address EED

The following areas for interventions were considered to be important: a) water, sanitation and hygiene (WASH), including reduction of exposure to faeces and contact with animals; b) probiotics and prebiotics; c) dietary diversity and recommended breastfeeding practices; d) nutrients such as zinc, PUFA and AA; e) anti-inflammatory agents; and f) antibiotics in the context of severe acute malnutrition and infection. Some of these aspects (especially WASH, infant feeding practices and nutrients) are already being tested individually or combined in large randomized controlled studies in a number of countries, which should provide more information about their effectiveness and guide further orientation of interventions.

B.5. Conclusion and recommendations

The meeting concluded that several gaps in knowledge exist that need attention, such as the classification of EED and better understanding of the underlying causes of EED. There was a consensus on the importance of developing practical, simple, and affordable tools to diagnose and characterize EED to allow better targeting of interventions in vulnerable populations. It was recommended for the IAEA to foster the use of stable isotopes for assessments in three main areas: bacterial translocation, absorptive capacity of the gut/permeability and body composition as a proxy indicator of dietary quality and morbidity.

C. INTRODUCTION

A technical meeting (TM) on environmental enteric dysfunction (EED) took place from 28-30 October 2015, at the International Atomic Energy Agency (IAEA) in Vienna. About fifty experts, drawn from diverse professional backgrounds including nutrition; gastroenterology; immunology; water, sanitation and hygiene (WASH); stable isotope applications; policy and development; and other key players in the community nutrition field met to explore the links between EED, undernutrition and the microbiome. Experts considered current knowledge and gaps on EED definition and classification, biological pathways and consequences of EED, as well as the next steps to take in addressing the problem, including diagnosis of EED, and how to draw leverage from the IAEA's expertise in stable isotope techniques in addressing the problem of EED.

EED seems to be highly prevalent in low and middle income countries (LMIC), it is acquired early in life but it is reversible, seasonal and is associated with socio-economic status. The lack of evidence on the effect of EED on growth and cognitive development, and strategies to prevent and/or treat EED is overwhelming. Furthermore, a clear gold standard for diagnosis of EED has not yet been defined.

EED is strongly associated with stunting and other forms of undernutrition. Stunting, or a child's height-for-age below two standard deviations from the median of the World Health Organization (WHO) reference population, is a multifactorial condition. While inadequate nutrition and infections are among factors thought to play major roles in a child's stunting, socioeconomic and environmental factors are also considered to be very important. Recent predictive models (Bhutta et al. 2008)[1] showed that all evidence-base interventions (assuming 90% coverage), aimed at preventing stunting, can lead up to only one-third reduction in stunting. The observation that growth retardation can actually occur in apparently well fed children has prompted a search for other causally related factors. Poor hygiene and absence of adequate sanitation are part of the puzzle; evidence to support this is however largely lacking. It is thought that living in poor sanitary conditions may induce gut function disorder, originally named environmental enteropathy (EE), but more recently referred to as EED in order to reflect its related functional deficits. The mechanisms by which EED impacts growth are unknown, but microbial translocation, gut and systemic inflammation and nutrient malabsorption seem to be plausible pathways.

The goal of the IAEA is to enhance the capabilities of Member States to manage all forms of malnutrition. The IAEA contributes towards the global health nutrition agenda by promoting the use of stable isotope techniques in developing and evaluating nutrition interventions, which are more accurate and precise than conventional techniques, safe to use across all ages, and can be used in a community setting. In line with its core mandate to promote peaceful uses of nuclear technology for improved human health and development, the IAEA organized a TM to facilitate a better understanding of the relationships between EED, the microbiome and undernutrition.

[1] BHUTTA, Z.A., AHMED, T., BLACK, R.E., et al., What Works? Interventions for Maternal and Child Undernutrition and Survival, Lancet, 371 (2008) 417-440.

C.1. Meeting objectives

The objectives of the meeting were:

1- To discuss current knowledge and gaps on the causes and consequences of EED
2- To share experiences related to the implementation and evaluation of programs to prevent and treat EED in infants, children, and adults
3- To discuss technical issues and strategies related to the management of EED and undernutrition, including tests for diagnosis
4- To identify knowledge gaps in the field of EED where the IAEA can add value by supporting the use of stable isotope techniques

C.2. Meeting format

The meeting was structured into 7 plenary sessions, 5 breakout sessions linked to the plenary presentations and a 6-member panel which deliberated on on-going field interventions to draw lessons and gaps related to EED. All the three sessions (plenary, breakout sessions and panel discussion) revolved around the following themes:-

1- Latest knowledge on EED and undernutrition
2- Latest knowledge on the gut microbiome
3- Effects of EED on growth, body composition and functional outcomes
4- Use of stable isotopes evaluating gut function
5- Biomarkers for EED
6- Overview and progress of on-going intervention trials on EED

Further details may be found under:

https://nucleus.iaea.org/HHW/Nutrition/EED_Technical_Meeting/index.html

Breakout sessions were intended to allow participants to discuss and explore the content of presentations in detail, make suggestions and query the next steps in EED research, diagnosis, and treatment. Breakout sessions are reported under each regular session that discussed the same theme.

The meeting was also provided with an overview of the IAEA activities and the human health campus, as well as the current TM objectives, outputs and concluding remarks.

D. REPORT ON THE REGULAR AND BREAKOUT SESSIONS

D.1. Opening session – Opening remarks by the IAEA secretariat

The IAEA's broad mandate is to promote the use of nuclear technology for peace, health, prosperity and sustainable development. The IAEA supports its 167 Member States in combating malnutrition in all its forms in addition to addressing environmental issues related to health. Through the promotion of the use of

stable isotopes, the IAEA supports the design and evaluation of nutrition interventions covering: infant and young child nutrition, childhood obesity, adolescent and maternal nutrition, healthy ageing, diet quality and health effects of the environment. IAEA's support is channelled through coordinated research projects (CRPs) whose topics are identified by the IAEA secretariat in collaboration with field experts. The second support mechanism is the Member State driven Technical Cooperation (TC) programme; projects are funded in 2 year cycles. Funding is channelled in the form of fellowships, expert missions, scientific visits and purchase of equipment and consumables.

The TM on EED fulfilled the mandate of IAEA through facilitating knowledge sharing and supporting research into issues relating to malnutrition and its consequences. The meeting was organized to gain a better understanding of the relationships between EED, the microbiome and undernutrition, identify gaps in research, and explore potential applications and next steps in diagnosing and treating EED.

D.2. First session - Latest knowledge on EED and undernutrition

The objective of the session was to provide an update on what is known about the biological pathways underpinning the association between EED and undernutrition. The session further aimed to update on an earlier meeting convened by the Bill and Melinda Gates Foundation (BMGF) in March 2015 that discussed key questions and factors related to developing interventions to prevent and treat EED in children.

Current knowledge on EED with a focus on intestinal failure in EED was discussed. EED, previously referred to as environmental enteropathy (EE), is a combination of infection-undernutrition induced failure of the mucosal barrier of the gut. It was first defined in 1960 after Peace Corps workers manifested gut discomfort that was reversible upon their return home. Biopsy of the small bowel mucosa of living individuals using Crosby–Kugler capsule identified a difference in the mucosal architecture between apparently healthy adults living in Western industrialized countries and adults living in developing countries. The causes of EED have not been identified, but there is evidence that EED is reversible, seasonal and highly associated with low socio-economic status (as an example, there is an association between EED and Gross Domestic Product). EED affects approximately 90% of the normal population of children in low- and middle-income countries (LMICs). EED is not an infection but is associated with barrier disruption and macrophage activation with more of a virus response. It is also apparent that EED is associated with reduction in specific biomarkers such as mucin transcripts, growth factor and protein kinase. There is a gap in identifying a specific biomarker for EED.

Until today, there is no consensus on the definition of EED. Definitions can be based on:

1- Morphology (short, fused, expanded villi compared to long slender villi in healthy subjects)
2- Gut function based on biochemical tests such as the dual sugar intestinal permeability test
3- Epithelial architecture (defects in cell adhesion)

Although most cases of EED are asymptomatic and hardly diagnosed, there is strong evidence supporting the association of EED with; 1) nutrient malabsorption; 2) growth faltering[2]; 3) microbial translocation; 4) alterations in microbiota diversity; 5) gut and systemic inflammation; and 6) reduced efficacy of vaccines[3].

In addition to the very accessible dual sugar test which assesses gut intestinal absorption/permeability and is the closest to the Gold Standard to assess EED, other tests have been used at a clinical level. These can be summarized as:

1- Morphological, such as biopsy or imaging Fluorescein leakage *in vivo* by laser endomicroscopy
2- Markers of gut leaking (e.g. alpha1-anti trypsin [AAT])
3- Microbial translocation (e.g. lipopolysaccharides [LPS] ; Tumor Necrosis Factor [TNF-α] as a biomarker of systemic inflammation)
4- Faecal and tissue mRNA to characterise the upregulated and/or downregulated genes associated with EED.
5- Markers of gut inflammation (e.g. myeloperoxidase, calprotectin and neopterin[4])
6- Loss of Heparan sulfate as a biomarker in Kwashiorkor. Heparan sulfate and syndecan-1 are essential in maintaining murine and human intestinal epithelial barrier function[5].

Nutrients play a role in protecting against development and progression of EED. Some have been characterised such as protective peptides, mucin and micronutrients (zinc). Infection also seems to impact on how EED presents. For example, in human immunodeficiency virus (HIV), inflammation is disproportionate to the degree of bacterial translocation.

The link between intestinal infection and inflammation in severely malnourished (SAM) children was discussed. Specific pathogens have been associated with EED (various parasites [e.g., *Cryptosporidium*, *Amoeba*], *Giardia*, bacteria). SAM is associated with diminished immunity due to reduced thymus size. Furthermore, SAM is associated with reduced acute phase protein response to bacterial infection and increased faecal markers of intestinal inflammation and permeability such as neopterin and myeloperoxidase levels[4]. Elevation of TNF-α with *Shigella* infection has been noted, especially in the tropics. TNF-α is also elevated in colitis and Crohn's disease, and is associated with linear growth suppression in these children. Amadi et al (2005)[6] demonstrated better recovery when children with SAM were given an elemental amino acid formula. Golden et al (1990)[7] demonstrated effacement of glomerular filtration process in the kidneys of children with Kwashiorkor, in addition to loss of heparan sulfate which is essential in maintaining murine and human intestinal epithelial barrier function. Kwashiorkor seems to be characterized with more permeability and to a lesser extent, systemic and mucosal inflammation.

[2] CAMPBELL, D.I., ELIA, M., LUNN, P.G., Growth Faltering in Rural Gambian Infants Is Associated with Impaired Small Intestinal Barrier Function, Leading to Endotoxemia and Systemic Inflammation, J. Nutr., 133 (2003) 1332-1338.
[3] NAYLOR, C., LU, M., HAQUE, R., et al., Environmental Enteropathy, Oral Vaccine Failure and Growth Faltering in Infants in Bangladesh, EBioMedicine, 2 (2015) 1759-1766.
[4] KOSEK, M., HAQUE, R., LIMA, A., et al., Fecal Markers of Intestinal Inflammation and Permeability Associated with the Subsequent Acquisition of Linear Growth Deficits in Infants, Am. J. Trop. Med. Hyg., 88 (2013) 390-396.
[5] AMADI, B., FAGBEMI, A.O., KELLY, P., et al., Reduced Production of Sulfated Glycosaminoglycans Occurs in Zambian Children with Kwashiorkor but Not Marasmus, Am. J. Clin. Nutr., 89 (2009) 592-600.
[6] AMADI, B., MWIYA, M., CHOMBA, E., et al., Improved Nutritional Recovery on an Elemental Diet in Zambian Children with Persistent Diarrhoea and Malnutrition, J. Trop. Pediatr., 51 (2005) 5-10.
[7] GOLDEN, M.H., BROOKS, S.E., RAMDATH, D.D., et al., Effacement of Glomerular Foot Processes in Kwashiorkor, Lancet, 336 (1990) 1472-1474.

A meeting convened by the Bill and Melinda Gates Foundation in March 2015 considered key factors to inform the design of intervention trials to address EED, food product profile and clinical trial design. Biomarkers of inflammation, permeability, microbiota and tissue damage are not adequately understood. The meeting suggested that interventions to address EED could consist of different components, alone or in combination: nutrition, WASH, drugs and probiotics. Study designs could be based on prevention or treatment or a combination of both. The potential of using imaging techniques was also considered.

What are the gaps in knowledge of EED?

1) The need for a clear case definition of EED
2) EED consists of a continuum of features (histology, inflammation, microbial biomarkers, host genomics, etc.) that could be quantified using a score in order to allow diagnosis across populations, age groups, geographical regions, etc.
3) Interaction between EED and nutrients and causal/consequential nature of the relationship
4) Could EED treatment be achieved through mechanisms that enhance mucin production?

D.2.1. Future work from research and clinical groups

1- Identify candidate markers for EED. Faecal gene transcripts have so far shown promising results; recent advances in research show that 13 genes are associated with EED and its associated pathways. These genes might be age and population specific.
2- Identify preventive and treatment measures
3- Identify key nutrients that can reverse (or prevent) EED
4- Identify potential interventions that can reverse or prevent EED (nutrition, WASH, drugs and/or probiotics). Future work from developmental agencies such as the BMGF is to upscale promising interventions.

D.2.2. Breakout session – What are the implications of EED for later outcomes?

Two groups discussed EED and its main measurable consequences in an intervention trial. From a public health point of view, the main consequences are 1) an increased risk of stunting, and associated impaired cognitive development and metabolic deficiency; and 2) vaccine failure and later infection susceptibility. Drug absorption efficiency could be affected by EED; unless more data are available, drug absorption cannot be used as an outcome. Another limiting factor to use drug absorption as a marker is its variation depending on the chemical nature of the drug.

There is evidence associating EED and linear growth. However, many confounding factors which are not related to EED may lead to growth faltering. To answer questions on the direct link between EED and later nutritional and health outcomes, efforts should be made to define a clear set of criteria to classify EED, as has been done in other conditions e.g., inflammatory bowel disease (IBD). Such a set of criteria should be limited to the definition of actual features across the continuum of EED that can be quantified using a score that is universally accepted in order to allow diagnosis across populations, age groups, geographical regions, etc. This would include developing a standardised approach for EED researchers to score various features (histology, inflammation, host biomarkers, microbial biomarkers, microbial translocation, host genomics, etc.); such a criterion could be based on index scoring or domain clustering.

D.3. Second session - Latest knowledge on the gut microbiome

The objective of this session was to have an in-depth understanding of the gut microbiome in general and, specifically, how the gut microbiota influences host health. The following aspects were further contemplated: how individual microbiota members interact with the host and how single cell interactions can be understood using stable isotope tracers. The session aimed to address the link between EED and microbiota on the pathobiology of SAM. Although there is no clear evidence on causal pathways leading to EED, several possible causes have been suggested and studied. Much has indeed been learned on the role of the gut microbiome in the evolution of EED.

Microorganisms play an important role in host health. They differentially colonize locations along the digestive tract with equally differential physiological activities and interactions with the host. The gut microbiota is acquired at the time of birth; is influenced by birth mode (vaginal versus caesarean), breast milk composition, dietary diversity, host lifestyle, genotype, pathobiology, physiology, age, environment and immune system; and plays an important role in host development[8] (e.g., immunity, metabolism, gut motility, etc.). Gut microbiota has key roles in the host: 1) breakdown of dietary fibre, 2) conversion of endogenous metabolites such as bile acids, 3) helping the host with colonisation resistance and immunity and, 4) metabolism of non-nutrients such as polyphenols.

The gut microbiota has a high taxonomic diversity as well as metabolic potential. Microbial taxa abundance is variable across individuals, but functional categories are static (Clusters of Orthologous Groups, COGs) with higher conservation of functional potential than microbial diversity across humans. Certain taxa of gut microbiota can be beneficial and harmful under different environmental conditions (e.g., complexity of the microbiota community, pathogen susceptibility, dietary shifts). For example in work by Derrien et al (2004)[9], *Akkermensia muciniphila* was shown to be a mucin-degrading commensal.

Research exploring the relationship between nutrient deprivation and gut microbial community has indicated that malnutrition can influence the microbial community composition, which is in turn correlated with host weight loss. The role of plant metabolites such as isoflavones (daidzein and genistein) may be important in influencing the microbiota but more work needs to be done.

Nutrient deprivation/malnutrition is associated with several immunological responses by the host that are indicative of a general/untargeted response. Much work has shown that nutrient deprivation is associated with a decrease in epithelial barrier function and elevated detection of indicators for bacterial translocation (LPS, bacterial 16S rRNA gene in the blood). The intestinal microbiota plays an important role in SAM treatment outcome. Kwashiorkor is associated with lower microbiota diversity; further, the diversity gained with treatment is not sustained.

The objective of recent studies has been to reveal the function of individual microbiota members in health and disease by single cell stable isotope probing. Microbiota may use host-derived substrates or dietary nutrients depending on the microbiota complexity. Mucosa degrading microorganisms may feed on host-

[8] BACKHED, F., Programming of Host Metabolism by the Gut Microbiota, Ann. Nutr. Metab., 58 Suppl 2 (2011) 44-52.
[9] DERRIEN, M., VAUGHAN, E.E., PLUGGE, C.M., et al., Akkermansia Muciniphila Gen. Nov., Sp. Nov., a Human Intestinal Mucin-Degrading Bacterium, Int. J. Syst. Evol. Microbiol., 54 (2004) 1469-1476.

derived metabolites and may be traced through isotope labelling of the mucus. For example, *Akkermansia* and *Bacteroides* are thought to depend on host-derived threonine. Berry et al. (2015)[10] developed a technique that allows activity measurements of microbial communities on a single-cell level. As such it is important to study the functions of the microbiota at the single cell scale; this information can otherwise be lost or overlooked at a population level due to the structure and dynamism of microbial communities.

Biomarker characterization (e.g. 16S rRNA or functional genes) of gut microbial composition can be used to differentiate healthy hosts and those with gut dysfunction. Through the application of stable isotopes the interactions between microbial populations, diet and host can be explored. With the use of isotopically labelled nutrients (e.g., ^{15}N, ^{13}C in amino acids and carbohydrates, or deuterium [D_2O]) the labelled atoms can be traced to assimilating microbial cells, metabolites and host tissues. These isotope pools can be explored using single-cell microscopic techniques (nanoscale resolution secondary ion mass spectrometry, NanoSIMS; RAMAN microspectroscopy) and mass-spectrometry based characterisation of metabolites (e.g., in the intestinal lumen, host blood). The use of stable isotopes has been successfully implemented in a few studies in efforts to characterize host-microbe interactions and shows great promise to improving our understanding of host-microbe interactions.

Drawing from a case study of Zambian children admitted with SAM and diarrhoea, it is apparent that lipopolysaccharides (LPS), a biomarker of bacterial translocation, is significantly elevated in SAM (unpublished data). Growth factor (IGF1) is inversely correlated with LPS concentration. Tumour necrosis factor (TNF-α), a pro-inflammatory cytokine, is reduced in SAM. Furthermore, SAM is associated with high levels of gut permeability and translocation. False-positive coeliac antibodies are associated with SAM severity. Exploration of the role of stable isotopes in labelling of bacterial translocation was proposed. Based on what is known, it was thought that reference to EED as asymptomatic is a misnomer; EED should be defined within a spectrum.

D.3.1. Where are the gaps in the evidence?

1) Research on EED is largely focused on the small intestine, however, much remains unknown about the relationship between the small and large intestinal microbiome in EED. Evidence was presented for bacterial translocation but only at the 16S rRNA level and LPS (detected in blood indicative of bacterial translocation).
2) What is the role of mucus in EED and how do diet and the gut microbiota influence mucus production and integrity?
3) What is the role of parasites in the EED phenotype?
4) What are the research priorities?

D.3.2. Breakout session – Synthesise learning, gaps and other research on EED – gut microbiome interactions

Two groups discussed the current knowledge and gaps on interactions between EED and microbiome.

The relationship between EED and gut microbiome is bidirectional. The microbiome is affected by environmental, health and nutritional conditions. The mechanism by which the microbiome affects gut

[10] BERRY, D., MADER, E., LEE, T.K., et al., Tracking Heavy Water (D2O) Incorporation for Identifying and Sorting Active Microbial Cells, Proc. Natl. Acad. Sci. U. S. A., 112 (2015) E194-E203.

health and nutritional status is not entirely clear, however there is evidence that some GI pathogens have specific mechanisms of action that make a link with EED biologically plausible – e.g., mucin degradation. There are emerging examples of specific synergies/interactions between specific gut pathogens resulting in gut damage.

A few studies were conducted on the role of the gut microbiota on inflammation. It can be confounded by variations in environmental exposures and the microbial ecology, amongst others. Gnotobiotic mouse model is a successful animal model that was used to: 1) determine the effect of diet and different nutrients on microbiota composition and activity; 2) to define interrelationships of the gut microbiome with the immune system in IBD.

Most of the details concerning our gut microbiota and its interaction with EED remain obscure. More studies are needed to elucidate the causal/consequential nature of the relationship between EED and the microbiome. Pathogens may work alone or their effects may be dependent on symbiotic dependence on others, or be augmented/diminished by others. It is unclear, what the most important pathogenesis mechanisms between the microbiome and EED are. Additionally, factors that define the location (small vs. large intestine) of the different microbiota are largely unknown. Advances in technology can benefit studies to locate the microbiota: small intestinal microbiota may be identified through fluid sampling with capsule endoscopy or nasojejunal tube and colonoscopy may benefit the understanding of large intestinal histology and function.

D.4. Third session - Effects of EED on growth, body composition and functional outcomes

This session considered EED and linear growth failure (stunting), the impact of infection and inflammation on intrauterine growth restriction and the energy and nutrient requirements in EED.

Worldwide 165 million children under 5 years of age are stunted, mainly in Sub-Saharan Africa and South-East Asia. Child stunting and other forms of malnutrition are estimated to underlie nearly 3.1 million child deaths annually. Stunting increases the risk of mortality by four times in children under 5 years of age, however its importance from a public health point of view remains, because of the high risk that a stunted child would not reach its cognitive development potential, which could result in lower working capacity at an individual level and less growth capacity at nation level.

The attempt to prevent stunting showed limited impact, far below the ambitious Millennium Development Goals, which aimed at halving undernutrition in all its forms by 2015. In a simulated model, Bhutta et al. (2008)[1] suggested that only up to a third of stunting in children younger than 5 years could be prevented if populations can access ten of the evidence-based nutrition interventions at 90% coverage. There is an increasing body of evidence that EED plays an important role in the puzzle of undernutrition. EED has been defined epidemiologically as "a phenomenon of community stunting and impaired intestinal function". This definition is based on the high association between growth Z-scores and gut permeability measured as the lactulose/mannitol ratio (increase in L:M ratio is correlated with decrease in z-score from the Gambian study[11]). In some studies, half of the stunting could be explained by EED. Stunting is also associated with

[11] LUNN, P.G., NORTHROP-CLEWES, C.A., DOWNES, R.M., Intestinal Permeability, Mucosal Injury, and Growth Faltering in Gambian Infants, Lancet, 338 (1991) 907-910.

a number of EED markers such as systemic inflammation markers (e.g., C-reactive protein [CRP]) and causes (animal exposure, geophagy, poor WASH but not diarrhoea, lower socio-economic status, previous therapeutic feeding, male sex, low birthweight, maternal lack of education, early marriage and pregnancy)[12,13,14]. In a simple model, it seems as though growth failure from adverse environment is mediated via microbial translocation and nutrient malabsorption that occur in a bidirectional leakage pattern.

Additionally, observations from Sweden showed that children with low birth-weight and length have significant, albeit incomplete catch-up growth compared to children born with the same condition in LIC[15]. This is probably due to chronic inflammation in the latter. Thus, stunting reflects combined effects of low birth weight, inadequate catch-up growth and postnatal growth failure[16]. The response of the body to malnutrition and to EED is similar. During pregnancy, insulin growth factor (IGF-I) and possibly other growth factors regulate growth and many other functions in the body. In LIC, IGF-I expression may be deficient (women with small placenta due to early marriage and pregnancy; low body mass index, BMI), the IGF-1 gene may express polymorphism/mutation or its production may be blocked by the inflammatory products such as monokines IL-1 or IL-6 (due to infections such as HIV positive mothers), which can result in intrauterine growth retardation (IUGR). Mouse and rabbit models of IUGR show that in experimental conditions, IUGR can be prevented by overexpressing IGF-1 protein in the placenta, with the help of a targeted gene transfer[17]. Malnutrition results in increased growth hormone (GH), decreased insulin and leptin with consequently reduced triiodothyronine (T3) and growth. The alteration of GH and IGF-I secretion could represent an adaptive response to malnutrition, with the result of a diversion of scarce substrate from growth to acute metabolic needs. During malnutrition, cortisol and insulin growth factor binding protein (IGFBP) increase, which also result in a decrease in IGF-I[18]. Similarly, Inflammation of the small intestine in EED is associated with high CRP and may be accompanied by release of cytokines such as IL-6 that reduce appetite and food intake and impair production and action of chondrocyte growth factors[19,18,20,21,22,23]. Stress-induced activation of the hypothalamic-pituitary-adrenal axis stimulates a rise in

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cortisol and IGFBP 1, which inhibit IGF-1 action and induce chondrocyte apoptosis[24,25]. A reduction in hepatic GH receptor expression[26] and inhibition of GH signalling[27,28] by fibroblast growth factor 21 and possibly zinc deficiency, further limit IGF-1 production and thereby contribute to growth failure. IUGR in a mouse model was caused by inflammation, which was induced by localised cystitis[29]. This is not to say that EED and stunting are the same. Children with IUGR may suffer from permanent and direct epigenetic effects that can be long lasting and not fully overcome, while EED is in many cases reversible. However, in the cases of IUGR, in undernutrition and EED, refeeding results in abnormal increase in body fat (abdominal and visceral fat)[30], which may predispose to metabolic dysfunction and the development of type 2 diabetes. The risks of metabolic dysfunction from nutrition repletion must be balanced against potential benefits for cognitive function.

In the context of a randomized clinical trial in Malawi[31], a concept model looking at newborn length for age z-score (LAZ) shows that child length at birth is proportional to the length of pregnancy. Infections of mothers were strongly associated with the length of children; for example, HIV infection of the mother increased inflammation in the child and caused smaller and lighter placentas. Increase in length drives weight gain, however the head growth is least affected (in line with the fact that children are born with 0 weight for age z-score [WAZ] and -1 LAZ).

Prevalence of EED in children under 5 years of age is estimated to be as high as 50-95% in low income settings[32,33,34]. Therefore, nutritional interventions should: 1) improve gut-barrier; 2) support immune function; 3) feed the gut (the microbiome); 4) support digestion and absorption and 5) target inflammation[32]. A number of nutrients have shown potential in achieving these targets[35]. Vitamin A, zinc and some amino-acids (AA) such as glutamine, threonine, leucine and cysteine are potentially involved in improving gut barrier function and absorption, and in the AA case, they are involved in gastrointestinal repair. Zinc, probiotics, plant flavonoids and n-3 PUFA have been able to lower inflammation. Zinc and vitamin A deficiency is associated with abnormal L:M ratio[35], related to impaired gut barrier function. Supplementation with alanyl-glutamine in Brazil showed a beneficial effect on GI barrier, improved WAZ

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and WHZ, but decreased absorptive capacity[36,37] (an opposite effect when the subject is HIV positive[38]). Interventions to increase long-chain poly-unsaturated fatty acid (LCPUFA) intake in LIC could have immune benefits in non-breastfed infants[39]. n-3 fatty acid levels are low in LMIC, but could be improved by increased consumption of fish, soybean and rapeseed oil. LCPUFA interventions can have immune benefits in non-breastfed infants[40].

Children with EED might have higher energy and nutrient requirements than the recommended daily allowance. Current international recommendations for protein, fat and energy intake for children with moderate acute malnutrition (MAM) are higher than average for broad population (10% high-quality protein, 25-35% fat for energy density). Amino acid (AA) metabolism is very important for growth and repair in the GI tract. During stress and inflammation other pathways are opened and some AA are reduced more than others. Pig models of malnutrition allow detailed analysis of protein and AA metabolism[41,42]. Pig Studies show that the levels of glutamate and threonine are low under stress, while glutamine is used up during stress. Glutamine, an important AA which can repair the damaged gut, comprises a third of the respiratory metabolism in the gut. The gut accounts for a quarter of the glutamine in the whole body. The levels of free AA in breast milk are low but interestingly glutamine and glutamate increase substantially during the first three months of infant life[43]. The consequences thereof on the gut and brain receptors are unknown but could be linked to appetite regulating effects.

D.4.1. Gaps in knowledge and way forward:

1) How important is EED in determining the stunting risk compared to other factors?
2) Research on EED and potential interventions that can improve EED and ameliorate growth can be done on animal models. Piglets may be ideal based on lessons from the University of Copenhagen. Further studies testing the effect of nutritional interventions (e.g., n-3 PUFA, specific AA, zinc, vitamin A, multi-micronutrient supplementation, probiotics) to improve EED on growth are needed.
3) Research on the effect of maternal infection on the microbiome of the infant is needed to determine the type of post-conception intervention that can improve IUGR.
4) Research on the link between pre-natal growth restriction and later growth and cognitive development is needed.

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D.4.2. Breakout session – Is the maternal microbiome in EED associated with the development of EED in the child?

Two groups discussed existing evidence on the effect of maternal nutritional status on infants. The groups considered this effect as potentially mediated by maternal EED, as well as some of the gaps in the evidence.

Stunting begins early in life, as evidenced by differences in length at birth between children in high-income and LMIC. Additionally, it is known that mothers with short stature have stunted babies. However, the contribution of maternal EED and their microbiome on the development of EED in the child is unknown. It is plausible that the maternal microbiome has an effect on the microbiome of the child, as it could be transmitted during labour, in the course of co-habitation (people living in the same home have similarities in microbiota, for example), or via breastmilk. However, pathogens that contribute to EED may differ between the mother and child. The International Lipid-based Nutrient Supplements (iLiNS-Dyad Malawi)[31] study found abnormal maternal vaginal microbiota in Malawi, but how this is associated with EED in children is unknown. It is hypothesised that EED in the mother, with accompanying systemic inflammation, could contribute to IUGR or prematurity in the child.

Currently, there are significant gaps in research about the maternal contribution to EED in the child and the effect of the maternal microbiome on the growth and development of the microbiome in the child. Data regarding the prevalence of EED in pregnant women is also lacking.

D.4.3. Breakout session – Synthesise learning, gaps and other research on EED – nutrient interactions

Two groups considered existing knowledge on interactions between EED and nutrients as well as some of the environmental factors leading to EED.

Malnutrition and deficiencies in certain macro- and micronutrients inhibit intestinal mucosal growth and alter gut barrier functions. Other food components such as contaminants can directly cause gut inflammation. Furthermore, poor and undiversified dietary practices have been shown to lead to the onset and intensification of EED. Some nutrients, such as zinc are associated with gut integrity. Currently, zinc is used to treat diarrhoea, and may also have a positive effect in reversing EED. Zinc deficiency can cause intestinal inflammation.

Once established, EED affects the absorption of key nutrients – a critical issue in people who are already suffering from lack thereof. Nutrient malabsorption caused by interacting metabolites can also lead to EED development or progression. Additionally, the EED effect on the microbiome can alter the recovery of some nutrients and also might affect some nutrient neo-synthesis.

More research is needed to understand how the different elements associated with EED interact, and more specifically whether EED is a cause or a consequence of nutrient malabsorption. Given the limited evidence on the positive role of some nutrients in reversing EED, more research to identify which nutrients are beneficial and in what dosages would be helpful in developing pathways for preventing and treating EED. On the preventative side, further research is needed to identify the extent to which dietary versus other environmental factors affect EED.

D.5. Fourth session – Use of stable isotopes evaluating gut function

An overview on the use of stable isotopes in functional measurement and nutritional assessment was given by five speakers from different fields of development and application of stable isotope techniques.

Stable isotopes have been widely used for the assessment of different aspects of gut dysfunctions (enteric infections, gut transit times, small intestine bacteria overgrowth [SIBO], coeliac disease, indirectly, and chemotherapy induced small intestinal damage in rats) with different substrates (starch and other carbohydrates, mixed triglycerides, fatty acids, proteins, etc.). The ^{13}C urea breath test is used to diagnose *Helicobacter pylori* and represents the paradigm of a diagnostic stable isotope breath test. This test is based on the principle that *H. pylori* produces high amounts of the enzyme urease that can cleave a labelled ^{13}C-urea to $^{13}CO_2$ and NH_3. The labelled CO_2 can be measured in the breath. The test has high specificity and sensitivity. The ^{13}C-sucrose breath test (SBT) is a promising test for the assessment of brush-border enzyme sufficiency. Intrinsic labelling using ^{13}C and ^{2}H is also being tested to investigate carbohydrate and protein bioavailability. For example, starch digestion and fermentation can be quantified by measuring ^{13}C-glucose and ^{13}C- short chain fatty acids (SCFA) appearance in blood and urine.

^{14}C-D-xylose breath test has been used in detecting SIBO. However, variations in gastrointestinal transit reduce both sensitivity (false negatives) and specificity (false positives) of the test because the microbiome can also yield CO_2 from xylose fermentation. This can be more pronounced in subjects with severe gastrointestinal motor dysfunction. Chang et al (1995)[44] succeeded in increasing the accuracy of the ^{14}C D-xylose breath test by correction with the gastric emptying rate, a retention ratio of a radioactive substance over a period of 30 min. This technique involves radiation exposure; Uchida et al (1995)[45] established a non-invasive method for the evaluation of rat gastric emptying and oro-caecal transit time at the same time by using ^{13}C acetic acid and lactose ^{13}C-ureide. In addition combination of isotopes could be used to determine different transit time such as: ^{13}C acetate/$^{13}C(^{2}H)$ octanoate to assess liquid and solid gastric emptying; and H_2/ lactose ^{13}C-ureide to determine oro-caecal transit time. The combination of ^{2}H or ^{13}C labelled substrates to assess colonic fermentation and gastric emptying can help determine oro-caecal transit time and gastrointestinal (GI) transit time of a particular subject to correct for these confounders in breath test results.

Additionally, using combination of stable isotopes (e.g., ^{13}C lactose and ^{2}H glucose) has shown to better distinguish between lactose digesters and non-digesters. Other uses of dual stable isotopes are to study the kinetics of the metabolism of glucose and starch digestion and fermentation. Combination of $^{13}C/^{2}H$ can be used to assess amino acid bioavailability from different food sources.

A promising use of the breath test is being developed in the context of EED. The ^{13}C-sucrose breath test has been used in Australia to assess the digestive and absorptive capacity of the small intestine[46]. It was validated against the small intestinal permeability test (lactulose: rhamnose ratio). The test could

[44] CHANG, C.S., CHEN, G.H., KAO, C.H., et al., Increased Accuracy of the Carbon-14 D-Xylose Breath Test in Detecting Small-Intestinal Bacterial Overgrowth by Correction with the Gastric Emptying Rate, Eur. J. Nucl. Med., 22 (1995) 1118-1122.
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furthermore be used to assess SIBO and to measure fermentation profiles (colon). The principle of the ^{13}C sucrose breath test is based on the difference in the functionality between damaged and undamaged small intestine. In the undamaged GI tract, the enzyme responsible for sucrose digestion, sucrase, is highly expressed resulting in greater digestion and absorption leading to higher cumulative $^{13}CO_2$ (during 90 min) compared to the damaged GI tract. The test has been validated against the lactulose:rhamnose permeability test, and can distinguish between diarrhoea and healthy children with high specificity and sensitivity. After setting a cut off for "normal" at 90 min-cumulative $^{13}CO_2$ excretion, the test could also classify children with EED from healthy children. SBT could also be used to assess the efficacy of interventions targeting EED such as probiotic administration on the GI tract.

Using stable isotopes has significant potential in improving our understanding of EED and potentially could provide a non-invasive diagnostic test. Localizing and assessing the extent of the GI damage may be possible (, reduced digestive/absorptive intestinal capacity, raised small and large intestinal permeability and altered fermentation patterns of the colon). Some practical challenges are still limiting the wide application of stable isotope techniques, such as costs and availability of stable isotopes tracers and cost, running and maintenance of instrumentation. However, newer spectroscopic technologies offer the potential point of care instrumentation solutions.

A study among Aboriginal groups in Australia showed that children with EED have a higher risk of being iron deficient. However, many trials supplementing iron in Africa (Pemba study, MNP study in Ghana, studies by the ETH group in Ivory Coast and Kenya) and Asia (Pakistan) showed an increased risk of diarrhoea and malaria in endemic areas, a negative shift in gut microbiome associated with an increase in enterobacteria and faecal calprotectin and higher rates of hospitalization and mortality. Iron is a growth limiting nutrient for many gut bacteria. For most enteric pathogens iron acquisition plays an essential role in colonization and virulence. A particularly virulent strain of *E. coli*, which was recently identified, had a plasmid with 3 different iron uptake systems. Thus, there is heavy genomic investment in iron acquisition by many pathogenic bacteria. Available evidence suggests that iron potentially shifts the microbiome towards a more pathogenic profile and increases gut inflammation. Beneficial bacteria require little or no iron. Iron fortification reduces iron deficiency anaemia, but there is a need to find safer ways to deliver iron. Reduction of iron dose with increased absorption through the addition of phytase; and addition of prebiotics seem to be potential solutions. Studies on iron fortification can also benefit from the emerging field of application of stable isotope labelling for understanding the role of microbiome and/or metabolic pathways.

Zinc is a key micronutrient in EED. Zinc deficiency can lead to anorexia, impaired growth, reduced immune function, increased GI permeability and decreased barrier function, increased secretory processes and diarrhoea. These symptoms are also common in EED, and may be associated with reduced growth and immunostimulation/inflammation. Zinc absorption is crudely controlled by dietary zinc intake, dietary phytate, age and presence of EED. Physiological requirements (estimated to be 0.74 mg/d in 9 m-old children) have only been achieved in malnourished children with oral intakes well above those needed by infants and young children in westernized settings. Studies showed that children with EED might have up to 50% higher requirements for dietary zinc. The inflammation and dysfunction of the gastrointestinal tract in EED may lead to excessive zinc secretion, impaired zinc reabsorption, or both. Data on the relationship of endogenous zinc losses and markers of gut function are limited. Stable isotopes should be used to address research gaps in zinc metabolism in EED and to assess zinc requirements in children with EED.

D.5.1. Identified key gaps in the use of stable isotopes in EED and nutrient absorption herein

1. Understanding of iron and zinc absorption (maybe focusing on extracellular zinc pool).
2. Ability to assess intestinal transit time/gastric emptying rate.
3. Development of a combination of substrates and/or isotopes to assess GI inflammation and dysfunction.
4. Requirement for a point-of-care test that can be applied in a resource limited environment.

D.5.2. Breakout session – Role of stable isotopes in EED assessment

Promising stable isotope techniques to improve our understanding of EED include: labelling bacteria and labelled nutrients (fats, proteins, carbohydrates, lipids versus amino acids, nucleic acids) to understand the host-microbiota interactions in the gut. Doubly labelled water would be of value to assess body composition and energy expenditure in EED. In terms of EED diagnosis, ^{13}C labelled substrates may be used to assess gut permeability; combined tracers may be used to assess nutrient absorption correcting for variations in gastric emptying. Labelled microbiota may also potentially be used to directly assess bacterial translocation. A ^{13}C breath test was considered an attractive diagnostic target because of its non-invasive nature and potential for field deployability, especially with commercially available spectroscopic-based instruments. To date, no single test yet has the sensitivity or specificity to be deployed in EED. The ^{13}C-SBT has potential for assessing mucosal sufficiency but requires further validation in the field. The potential for combined ^{13}C and H_2 breath tests holds some promise to resolve small and large intestinal events, although complications of SIBO on substrate utilisation need to be fully understood. In the post-genomic era, improved characterisation of the small intestinal microbiome may yield novel and highly selective molecular targets for infectious bacteria, thus potentially yielding a highly sensitive and specific test.

D.6. Fifth session – Biomarkers for EED

The objective of the session was to review methods on assessing EED in the field and review knowledge on application of EED biomarkers including stable isotope techniques.

The EED spectrum is wide in terms of its causes, symptoms and consequences. Therefore, a lot of potential markers were tested in different clinical trials. In this session, speakers went through some of these biomarkers:

- **Vaccine response.** The link between EED, oral vaccine failure and growth faltering in children was discussed based on lessons from the PROVIDE study[47]. Oral polio (OPV) and rotavirus vaccines failure was observed in 20% of children. There was GI inflammation in 80% of the cases as indicated by the elevated levels of calprotectin, myeloperoxidase and alpha-1 anti-trypsin. Biomarkers of Rotarix response (IgA) explained 24% of change in HAZ in 6-24 weeks of age infants.
- **Intestinal inflammation** (calprotectin, myeloperoxidase, neopterin)
- **Systemic inflammation** (alpha 1-acid glycoprotein [AGP], cytokines)
- **Intestinal barrier**: permeability as assessed by lactulose/mannitol test. Limitations of L/M test: reflects only one condition, there is a lack of standardized methods and fasting/lengthy urine collection problematic.
- **Bacterial translocation** (LPS)
- **Nutrient absorption** (L:M test)
- **Functional enterocyte mass** (citrulline to assess the damaged gut)
- **Enterocyte regeneration** (repair)
- **Mucosal immune underachievement** (reproducible, cheap, point of care test, safe, and non-invasive)

The kynurenine/tryptophan (KT) ratio is based on the activity of indoleamine 2, 3, deoxygenase (IDO-1) enzyme[48]. In a cohort study in Peru (unpublished data), change of 1SD in tryptophan concentration was associated with a 0.2 SD change in HAZ. This association is stronger than systemic inflammation markers such as AGP. KT captures the IFN-y and IL-10, both of which are associated with oral vaccine failure (OPV, tetanus and rotavirus).

- **Cognitive outcomes**?
- **Metabolic syndrome**?
- **Faecal biomarkers** (Calprotectin and some candidate mRNA).

Faecal host transcripts can be used as biomarkers of EED. Stool biomarkers may reflect multiple aspects of gut function. However, stool mRNA is bacterial and is limited by PCR inhibitor in the stool. This may be overcome by use of Droplet Digital PCR[49].

- **Microbiome biomarkers**

[47] NAYLOR, C., LU, M., HAQUE, R., et al., Environmental Enteropathy, Oral Vaccine Failure and Growth Faltering in Infants in Bangladesh, EBioMedicine, 2 (2015) 1759-1766.
[48] MUNN, D.H., MELLOR, A.L., Indoleamine 2,3 Dioxygenase and Metabolic Control of Immune Responses, Trends Immunol., 34 (2013) 137-143.
[49] DINGLE, T.C., SEDLAK, R.H., COOK, L., et al., Tolerance of Droplet-Digital Pcr Vs Real-Time Quantitative Pcr to Inhibitory Substances, Clin. Chem., 59 (2013) 1670-1672.

It was suggested that biomarkers of EED should predict growth failure in addition to oral vaccine response/mucosal immune underachievement, cognitive outcomes and metabolic syndrome.

D.6.1. Key points

- ⇨ Biomarkers are age dependent, which makes the choice of the biomarker very critical.
- ⇨ Biomarkers such as faecal markers (mRNA) might additionally be population specific.
- ⇨ The role of the microbiome in biomarker's response is unknown.

D.6.2. Breakout session – methods on assessing EED in the field and knowledge on application of EED biomarkers

Four parallel group discussions had an in depth discussion based on the following questions:

How do we achieve a better definition and classification of EED?

The intended purpose and descriptive domains need to be taken into account in an effort to develop a definition for EED; diagnosis of EED; outcome of an intervention; identification of risk factors for public health and policy; and investigating the aetiology or outcomes of EED. Several mechanisms/pathways forming the basis for the diagnosis of EED may be part of a specific definition of the condition. While malabsorption, gut barrier dysfunction and gut inflammation are all important components of EED, it is difficult to identify specific markers of each that could be used solely for EED diagnosis.

Additionally, it was agreed that EED definition should exclude the following since they are outcomes: stunting, and infections. Changes in the microbiome may play a causal role in EED and should be considered, at least initially. Also, EED definition in the context of interventions should exclude coeliac diseases, diarrhoea episodes and low-birth weight infants due to the different pathogenesis.

What biomarkers could be/are universally applicable across the EED spectrum?

An ideal biomarker of EED should be highly associated with stunting and could be classified according to underlying causes of EED, namely:

1- Intestinal permeability
   Lactulose/mannitol or lactulose/rhamnose tests are widely used. Both rhamnose and mannitol are small molecules. Polyethylene glycol with different molecular weight, or alpha-1-antitrypsin could be alternatives in this test. Labelled substrates (carbohydrates, proteins and lipids) can be developed to assess intestinal permeability.
2- Bacterial translocation, caused by the permeability changes leading to a leaky gut (virus, food allergens and food components can also pass).
   Lipopolysaccharides (LPS), blood DNA and food antigens.
3- Intestinal inflammation
   Myeloperoxidase (MPO), calprotectin, neopterin, stool IL-1B, IL-8
4- Systemic inflammation
   CRP, AGP, IL1, IL6, TNF, IGF-1, endogenous endotoxin-core antibody (EndoCAB)
   Vaccine response marker could be used as a predictor of other inflammations.
5- Intestinal repair

Markers of intestinal growth such as the growth promoter glucagon-like peptide-2 (GLP-2)

What are the low cost methods in use for the assessment of EED?

The goal should be to identify the methods/tools which have the potential for wide applicability and perhaps portability. Sensitivity and specificity of the test should be balanced against costs. There will be a requirement to validate diagnostic tests.

D.7. Sixth and seventh sessions – Overview and progress of on-going intervention trials on EED

The cause of EED is postulated to be inflammation from chronic exposure to insanitary environmental conditions leading to gut and systemic inflammation, altered absorptive gut cells and shift in the gut microbiota. These pathways form the basis for interventions targeting EED, which include pro- and prebiotics, anti-inflammatory treatment, supplementation with zinc and LCPUFA as well as anthelmintic treatment among others. Other therapeutic options for prevention and management of EED, for example, antibiotics in the context of SAM were recognized but were not discussed at length.

- *Prebiotics and Probiotics*

Probiotics are living micro-organisms that can confer a health benefit onto the host organism, if administered in adequate amounts. Most of the research done on probiotics has been conducted in-vitro or on laboratory animals. Health claims of probiotics include improved GI barrier function and modulated immune system, plasma lipid profiles and erythrocyte morphology. Efficacy data are available on a few species. The leading probiotic organisms in the food market belong to two genus; *Lactobacillus* and *Bifidobacterium*. Prebiotics are non-digestible food ingredients that beneficially affect host health via colonic microflora changes. Examples of prebiotics include galactooligosaccharides and *L. casei* Shirota. Claimed benefits of prebiotics range from stimulation of immune system and prevention of diarrhoea to reduced inflammation. Efficacy trials to test the effect of pre- and probiotics on EED and general health are needed.

- *Anti-inflammatory agents*

EED is associated with gut and systemic inflammation, which is similar to inflammatory bowel disease (IBD), an innate immune response that can result from a lack of balance between pathogens and host response. In the case of EED, children are continuously living in environments with high pathogen exposure and dysbiosis; enteric inflammation might be a protective/adaptive mechanism that remains imbalanced leading to growth failure. In the case of IBD, patients respond well to immunomodulation. To test the role of anti-inflammatory treatment on EED and growth, 44 stunted Kenyan children with SAM were treated daily using mesalazine for 28 days (21 days of full treatment)[50]. There was no difference in adverse events between groups. Mesalazine seemed to protect against adverse liver function. There were no differences in nutritional (recovery from SAM) and most of the inflammatory outcomes. There was a trend to lower erythrocyte sedimentation rate (ESR), lower faecal calprotectin, CRP and Endocab antibodies. These

[50] JONES, K.D., HÜNTEN-KIRSCH, B., LAVING, A.M., et al., Mesalazine in the Initial Management of Severely Acutely Malnourished Children with Environmental Enteric Dysfunction: A Pilot Randomized Controlled Trial, BMC Med., 12 (2014) 1-14.

findings are important regarding the safety of the treatment (if the inflammation was protective one would expect a worsening in the situation of the children, although this is premature as a conclusion) and the intervention was too time-limited to show an effect on linear growth[51].

- *Nutrients and anti-helminth*

Zinc, albendazole, multiple micronutrients and fish oil have been tested for their potential effects on EED. In the first trial[52] testing zinc and albendazole treatment in comparison to a control group, all groups showed an increase in L:M ratio, demonstrating that they all got worse during the intervention period. However, L:M ratio increased significantly more in the placebo group compared to both treatment groups. Supplementation with multiple micronutrients or fish oil did not affect the L:M test differently compared to the control group[53]. Additional trials using Rifamixin[54] + Lactobacillus GG[55] did also not affect the L:M test. Interventions targeting children at the beginning of the complementary feeding period, e.g. when children are more mobile, might show better results.

- *WASH, nutrition and behaviour change communication*

There are many environmental factors that may contribute to the prevalence of EED. It is hypothesized that environmental contamination (especially faecal) and nutrition are directly linked to the prevalence of EED; managing contamination and improving nutrition could help reducing EED. Two major areas that are considered to potentially improve EED status include modifications in hygiene and nutrition management. Three on-going sets of interventions were presented (MAL-ED[56], SHINE[57], WASH Benefits[58]). They aim to identify the contributions of hygiene and nutrition to EED including the analyses of outcomes (physical and cognitive) and of the sustainability of implemented practices. The success of intervention efforts and cohort follow-up has been variable and has many challenges including political unrest, and infrastructure support.

The WASH Benefits trial is a two country cluster randomized trial of improved water quality, sanitation, hygiene, and nutrition interventions with sites in rural Kenya and Bangladesh. In a subsample of approximately 1100 children per country, urine, blood and stool samples are being collected and biomarkers of EED including the urinary lactulose:mannitol test and faecal markers of inflammation and permeability (neopterin, alpha-1 antitrypsin, and myeloperoxidase) will be measured. The MAL-ED study is a multi-site birth cohort study designed to improve the understanding of interactions between undernutrition and enteric

[51] PETRI, W.A., JR., NAYLOR, C., HAQUE, R., Environmental Enteropathy and Malnutrition: Do We Know Enough to Intervene?, BMC Med., 12 (2014) 187.
[52] RYAN, K.N., STEPHENSON, K.B., TREHAN, I., et al., Zinc or Albendazole Attenuates the Progression of Environmental Enteropathy: A Randomized Controlled Trial, Clin. Gastroenterol. Hepatol., 12 (2014) 1507-1513.e1501.
[53] SMITH, H.E., RYAN, K.N., STEPHENSON, K.B., et al., Multiple Micronutrient Supplementation Transiently Ameliorates Environmental Enteropathy in Malawian Children Aged 12-35 Months in a Randomized Controlled Clinical Trial, J. Nutr., 144 (2014) 2059-2065
[54] TREHAN, I., SHULMAN, R.J., OU, C.-N., et al., A Randomized, Double-Blind, Placebo-Controlled Trial of Rifaximin, a Nonabsorbable Antibiotic, in the Treatment of Tropical Enteropathy, The American journal of gastroenterology, 104 (2009) 2326-2333.
[55] GALPIN, L., MANARY, M.J., FLEMING, K., et al., Effect of Lactobacillus Gg on Intestinal Integrity in Malawian Children at Risk of Tropical Enteropathy, Am. J. Clin. Nutr., 82 (2005) 1040-1045.
[56] http://mal-ed.fnih.org/?p=886
[57] The Sanitation Hygiene Infant Nutrition Efficacy (SHINE) Trial Team, The Sanitation Hygiene Infant Nutrition Efficacy (Shine) Trial: Rationale, Design, and Methods, Clin. Infect. Dis., 61 (2015) S685-S702.
[58] http://www.washbenefits.net/

infections and measures the consequences of these adverse events in early life on child growth and development. The cohort enrolled 2145 children in 8 countries: Brazil, Peru, Tanzania, South Africa, India, Bangladesh, Nepal and Pakistan. The Sanitation Hygiene Infant Nutrition Efficacy (SHINE) trial is a proof-of-concept, 2x2 factorial, cluster-randomized community-based trial in two rural districts of Zimbabwe that will test the independent and combined effects of protecting babies from faecal ingestion.

Environmental contamination and human nutrition are factors that are actively being explored in intervention studies as contributors to the prevalence of EED. Ongoing research is exploring the identification of phenotypic markers for field-based assessment of EED and the roles of environmental contamination and nutrition. The results from these trials will allow NGOs and governments to better understand the benefits and costs of investments in WASH and/or nutritional interventions aimed at reducing both stunting and EED.

D.7.1. Gaps in the evidence

1) What is the long-term impact of intervention efforts on EED prevalence?
2) What are the best assessment methods for EED to use in field-based studies?
3) How are EED assessment measurements linked?
4) How do different sources of faecal contamination contribute to EED and can this be monitored using microbial source tracking?
5) What is the impact of enteropathogen pressure on EED?
6) Are the impacts of improved hygiene and nutrition linked and to what extent do either have an impact on EED outcomes?
7) Is it possible to model the impacts of environmental improvements using biomarkers?

D.8. Eight session – Panel discussion

A panel consisting of representatives from research institutions, funding agencies and non-governmental organizations discussed interventions and programs to address EED.

Exposure to poor sanitation and hygiene is postulated to be at the origin of chronic inflammation and EED. Interventions to improve WASH and reduce exposure of children to faeces could potentially reduce EED. CARE International is implementing two interventions in this context. The first intervention consists of installing playpens in the community to limit children's contact with animals and limit their exposure to faeces. The second is behaviour change communication to raise awareness with caregivers on the risk of geophagy (soil usually contaminated by animal faeces) for child health and development.

In the same context of improving hygiene through behaviour change communication, Action Contre la Faim (ACF) launched a strategy to integrate health centres in an effort to improve hygiene in the household and community. The initiative is included in the community management of Acute Malnutrition (CMAM) and is expected to reduce length of stay and relapse rate of SAM children in the program. The same organization (ACF) is aiming to improve MAM management through interventions that target intestinal infections in addition to the nutrition one. A controlled study will compare the effect of administration of antibiotics/prebiotics on recovery rate after 3 months of administration vs. the standard management of MAM.

Other interventions have also been tested such as antibiotics and multiple micronutrients. There is some evidence from laboratory animals that legume proteins might be associated with reduced gut inflammation. In Malawi, a community trial in collaboration with the US-government aims at distributing complementary foods combining legumes with cereals and assesses immunological function. L:M test is used to assess EED in addition to other markers of inflammation. The Malawian group emphasizes the importance of nutrition as a core intervention in reducing gut inflammation and improved growth, although it is not sufficient as a sole intervention. Combating EED and stunting needs an integrated approach to improve maternal health, infant and young child feeding practices (IYCF), WASH, etc.

WASH might not be the only pathway or promising intervention to control EED. Aerosols are another common way of infections. In other terms, crowded households might be at high risk of infections and exchange of viruses and other infectious agents. WASH might be less effective if the crowding cycle has not been broken. Control of household air pollution from solid fuel, or clean cooking, as a way to control the burden of infectious diseases, has been tested in Laos (the World Bank, WB). Maternal education is a link that can improve the outcomes of interventions targeting EED and stunting in an indirect way. Maternal education has long term effects and results can only be seen over generations, more focused work in identifying proximal causes of EED and stunting needs to be done.

One of the agencies investing heavily in nutrition is the WB. The WB invests in upscaling evidence-based interventions. The challenge with interventions targeting EED such as WASH is that numerous impact evaluations have been done, but outcomes have not been achieved. The on-going trials in Africa and Asia could potentially answer several questions on the effectiveness of this initiative and on the right combinations of interventions to improve child nutritional status and health. Stunting is a multifactorial problem, hence the importance of synergies between different pillars (nutrition, health, education, environment). But in a world with limited resources, we should help governments make the right prioritization.

Evaluation of interventions is a key point in the decision to continue or upscale the interventions. Evaluation includes: 1) adherence to the intervention and its coverage; 2) effectiveness (reaching the targeted outcomes); 3) cost-effectiveness and 4) situation context analysis. Organisations recognised the importance of measuring EED, and the direct effect of the intervention targeting the reduction of children's exposure to animal faeces on their growth and development. An innovation to develop a tool to measure EED (or other outcomes) is of utmost importance if we want to upscale evidence-based interventions. The evidence should furthermore include specificities of the target population such as age, dose of treatment, duration, etc. Interventions also need to be adjusted to the context and tested in the target community before any upscaling can occur. Interventions to limit population exposure to livestock might, for example, work in Africa but not in Pakistan, where livestock is a key part of the local economy. Livestock is a source of livelihood in communities. Separating it from the home environment, unprotected, would put the animals at risk and consequently put the family livelihood at risk. In order to succeed, researchers must find the most relevant system to target livestock-human contamination. Additionally, some of the interventions might turn out to be very expensive (e.g., investment in housing if crowding, which so far has been under-studied or not studied at all, turns out to be a potential pathway to inflammatory state). Research needs to give answers on the pathways leading to EED in different contexts, before a huge economic investment can be mobilised.

Although some of the implementation groups think that actual diagnosis of EED can probably not be done in the field, using a tool for diagnosis and research seems to be of interest. Using markers such as faecal biomarkers is cheap and they can be collected at a large population level. A tool to monitor community sanitation should be used. The use of proxies can respond to important questions.

D.8.1. Key outcomes

- Poor sanitation and hygiene is postulated to be at the origin of chronic inflammation and EED;
- Improved WASH and reduced exposure of children to faeces could potentially reduce EED;
- WASH and Nutrition integrated approach might improve outcomes of SAM children, including shorter treatment period and fewer relapse
- WASH practices may be improved through behaviour change communication;
- Efforts are underway to test non-WASH interventions including antibiotics, probiotics, legume metabolites (protein and isoflavones) to reduce gut inflammation;
- Combating EED and stunting needs an integrated approach to improve the following: maternal education and health, IYCF practices, WASH; as well as address crowding in households. The question is: What is the right mix of interventions to maximise effectiveness?
- A quick and simple tool to measure EED is urgently needed to facilitate evaluation of interventions;
- Interventions targeting separation of humans from livestock should be context sensitive and identify a relevant system to address livestock-human contamination in order to minimize the risk to family livelihoods.

E. MEETING OUTPUTS, CONCLUSIONS AND RECOMMENDATIONS

E.1. Co-definition of EED

There is no universal agreement on the case definition of EED. This is due to several reasons including the multiple pathways leading to EED and the spectrum of symptoms among subjects with EED. A framework of an EED case definition was proposed to facilitate the identification and diagnosis of EED in field and clinical settings.

1- EED is a combination of infection-undernutrition induced failure of mucosal barrier of the gut; it is reversible, seasonal and is associated with socio-economic status.
2- EED is a spectrum triggered by undernutrition and pathogen exposure, which, in the presence of additional environmental conditions would lead to SAM or other forms of malnutrition.

E.2. Essential descriptive domains for assessing EED

1. Altered gut permeability
2. Nutrient malabsorption
3. Gut inflammation
4. Systemic inflammation
5. Intestinal leakiness
6. Bacterial translocation
7. Alterations in microbiota/microbiome composition

E.3. Essential outcomes of interventions on EED

1. Linear growth and other associated outcomes such as cognitive development
2. Vaccine efficiency
3. Body composition
4. Energy expenditure

E.4. What are the priority aspects of EED biomarkers?

The meeting resolved that the following issues need urgent attention:

E.4.1. Development of a suite of tests to characterize and classify EED

These include tests using stable isotopes to assess a) nutrient absorption, namely protein bioavailability; b) permeability defect, ideally with an indication of size and location of lesions; and c) the microbiota/microbiome in the small intestine versus colon to localize the site of damage. Other promising tests were proposed, such as Dipeptidyl peptidase-4 (DP4) enzyme activity in gut lumen as a proxy of GI functionality.

1) Assessment of intestinal permeability (ideally informing about the size and location of intestinal lesions)

Urinary L:M and L:R are widely used but are insensitive to lesion size. A substrate containing a range of molecule sizes may have greater utility for determining lesion size. Many prebiotics, for example fructooligosaccharides, contain a wide molecular weight range (180 – 10000 Da) and the profile of plasma or urinary excretion may reflect lesion size. This remains to be tested and may be complicated by enterohepatic recycling and renal clearance. Small molecule may still be required to assess absorptive cell mass and efficiency of urinary clearance (M or R).

2) Assessment of epithelial function

The ^{13}C SBT appears to reflect epithelial sucrase sufficiency, which may be an indirect marker of epithelium maturity and integrity. There is evidence to suggest reduced sucrase activity in villus atrophy. Gastric emptying and oro-caecal transit time are potential confounders that may need to be corrected for in the breath test results. Breath H_2 appearance from concomitant fermentable carbohydrates (or sucrose itself) may allow separation of small intestinal $^{13}CO_2$ release to be differentiated from bacterial $^{13}CO_2$ release (SIBO). Coincidence would suggest SIBO whilst $^{13}CO_2$ prior to H_2 would suggest regional separation of sucrose digestion and carbohydrate fermentation.

3) Assessment of epithelial inflammation

Myeloperoxidase (MPO), calprotectin, neopterin and stool IL-1B, IL-8 all show promise for the assessment of gut inflammation. C-reactive protein is an established marker of systemic inflammation. Increased urinary nitrate/nitrite has been observed in enteric infections screening-detected coeliac disease as a marker of increased NO production in an inflamed mucosa. Urinary nitrate/nitrite is an inexpensive, non-invasive assay which may have utility in assessing inflammation in EED.

4) Assessment of large intestinal permeability

Multi-sugar urine excretion tests show some potential for localising permeability changes. In one study, the combination of raffinose/mannitol ratio and sucrose/raffinose ratio appears to be an indication of the distribution of intestinal damage. The use of larger molecules (raffinose is a trisaccharide) appears to improve the site-specific information available. Whether a substrate with a range of molecular weights can yield information on site-specific permeability remains to be tested but the potential to combine urinary information with breath H_2 excretion, may yield improved diagnostic accuracy because it potentially allows correction for transit time variations.

E.4.2. Investigate the colonic microbiome in the context of EED

Epithelial cells in the small intestine are primarily mucus-secreting cells, whereas differentiated cells of the columnar epithelium are absorptive cells, removing water and electrolytes from the mucus[59]. In the small intestine, microbiota might promote mucosal barrier function. Due to high levels of substrate availability, the cecum and colon are sites of the anaerobic organic matter breakdown (fermentation). Fluorescein could be a potential assessment tool for the small intestine barrier function, but another test for colon absorption function should be developed.

[59] KUNZELMANN, K., MALL, M., Electrolyte Transport in the Mammalian Colon: Mechanisms and Implications for Disease, Physiol. Rev., 82 (2002) 245-289.

E.4.3. Most effective next steps

A strategic group to follow up on the meeting outputs regarding development of a set of tests using stable isotopes to assess EED.

E.5. What lessons can inform future interventions?

Large community intervention trials to improve hygiene and/or dietary practices have been implemented in different settings in LMIC. A few of these interventions are already completed and some are on-going (WASH, SHINE). Other interventions of interest are: pre- and probiotics, liver cytosol antibodies, microbiome, trophic factors, anti-inflammatories, antibiotics, and LCPUFA. However, the intervention results are quite meagre so far, showing that we do not have a good handle on the biology of EED. There is a need for better understanding of the underlying causes of EED, and the association between EED, the microbiome and nutrition so that interventions can be targeted.

Interventions can be categorized into the following: 1) preventive (vs. treatment) depending on the age of the intervention participants (pre-conception, pregnancy, infants less than 6 months of age, and children and toddlers); 2) household vs. community level; 3) different environments (e.g., how environmental conditions in one continent affect inflammation differently than in another continent); and 4) different exposure through the food chain (breast milk, toxins, heavy metals, household air pollution from solid fuel, etc.).

In some settings, 99% of children live with EED. EED is a continuum and should be measured as such to identify the appropriate treatment /intervention for the child. Using markers of systemic inflammation could be a good start. In other terms, interventions should target the shift in the curve so that everyone is at a lower risk of EED. In this context, special distribution or mapping of risks and/or biomarkers can be a good decision-making basis for interventions. For example, ACF targets communities with high wasting and stunting; if wasting is more prevalent, it is targeted through improved SAM treatment; if stunting is more prevalent, it is targeted through preventive actions. While it is easy to standardize cut-offs for defining growth indicators (stunting or wasting), it may not be so straight forward with EED.

E.6. What suggestions or recommendations for the IAEA to support future work relating to stable isotopes and EED?

There was a consensus on the importance of developing practical, simple, and affordable tools to diagnose and characterize EED for better targeting of interventions in vulnerable populations. It was recommended that the IAEA foster the use of stable isotopes for assessments in three main areas: firstly, bacterial translocation; secondly, absorptive capacity of the gut/permeability; and thirdly, body composition as a proxy indicator of dietary quality and morbidity.

E.7. What are the gaps in knowledge and actions to be taken to address EED?

The following areas should inform research in the EED field:

1) Identify candidate markers for EED (e.g., markers of small and large intestinal permeability, epithelial function and inflammation, bacterial translocation);

2) Development and validation of a set of tests using stable isotopes to assess EED;
3) Identify potential interventions that can reverse or prevent EED (nutrition –role of specific nutrients, WASH, drugs and/or probiotics);
4) How can iron be delivered more safely to women of reproductive age and children to improve iron status without negative shift in the microbiota?
5) How can stable isotopes be used to address research gaps for zinc and EED? What are the "pathophysiological" requirements for children with EED and the daily intakes needed to meet this? Of what importance are endogenous zinc stores in conditions of high inflammation?
6) How to bring different expertise and backgrounds to work in synergy on EED?

F. ANNEXES

Annex 1: Meeting agenda

DAY 1 - Wednesday, 28 October 2015

09:00 – 09:30 **Welcome and opening remarks: the IAEA, meeting objectives and logistics** *Cornelia Loechl (IAEA Scientific Secretariat)*
Participant introductions and meeting expectations *Jo Zaremba (Facilitator)*

09:30 – 10:45 **SESSION 1: Latest knowledge on EED and undernutrition**
Chair: Paul Kelly (University of Zambia, Zambia)

1. **Intestinal barrier failure in EED: What do we know?** *Paul Kelly (University of Zambia, Zambia)*
2. **Pathobiology of EED** *Mark Manary (Washington University School of Medicine, USA)*
3. **Intestinal infection and inflammation in severely malnourished children** *Simon Murch (The University of Warwick, UK)*
4. **Overview of BMFG convening on EED** *Alexis Katsis (Bill and Melinda Gates Foundation, USA)*

Plenary: Questions & Answers

10:45 – 11:15 **Coffee break**

11:15 – 12:45 **SESSION 2: Latest knowledge on the gut microbiome**
Chair: Douglas Morrison (Scottish Universities Environmental Research Centre, UK)

1. **Influence of gastrointestinal microbiota on health** *Michael Blaut (German Institute of Human Nutrition, Germany)*
2. **Understanding the role of gut microbiota in severe acute malnutrition** *Paul Kelly (University of Zambia, Zambia)*
3. **The intestinal microbiome in action - revealing the functions of individual microbiota members in health and disease by single cell stable isotope probing** *Alexander Loy (University of Vienna, Austria)*
4. **Enteropathy in severe acute malnutrition** *Paul Kelly (University of Zambia, Zambia)*

Plenary: Questions & answers

12:45 – 13:45 **Lunch Break**

13:45 – 14:30 **BREAKOUT SESSION A: Learning, gaps & other research on EED & the gut microbiome**

14:30 – 15:15 **SESSION 3: Effects of EED on growth, body composition and functional outcomes**
Chair: Tahmeed Ahmed (iccdr,b, Bangladesh)

1. **Energy and macronutrient requirements in children 0-5 years with EED** *Kim Michaelsen (University of Copenhagen, Denmark)*
2. **Link between EED and stunting: the new frontier** *Sophia Agapova (Columbia University, USA)*

Plenary: Question & Answers

15:15 – 15:45 **Coffee Break**

15:45 – 16:30 **SESSION 3 continued**

3. **Pathogenesis of linear growth failure (stunting) in EED** *Michael Freemark (Duke University, USA)*
4. **The impact of infection and inflammation on intrauterine growth restriction** *Per Ashorn (University of Tampere, Finland)*

Plenary: Questions & Answers

16:30 – 17:30 **BREAKOUT SESSION B: Learning & gaps on the effect of EED on growth and nutrient requirements & feedback session**

17:30 – 17:45 **Closing**

DAY 2 – Thursday, 29 October 2015

09:00 – 09:15 **Summary of previous day and reflection**

09:15 – 10:15 **SESSION 4: Use of stable isotopes evaluating gut function**
Chair: Alexander Loy (University of Vienna, Austria)

1. **Stable isotopes and gut function**
   Douglas Morrison (Scottish Universities Environmental Research Centre, UK)
2. **New combination non-invasive tests for mucosal dysfunction, inflammation and gastrointestinal microbiome composition and activity** *Ross Butler (University of South Australia, Australia*
3. **Stable isotope techniques to investigate oral-fecal transit time, lactose malabsorption and starch digestion/fermentation** *Marion Priebe (University of Groningen, The Netherlands)*

Plenary: Questions & Answers

10:15 – 10:45 **Coffee break**

10:45 – 11:30 **SESSION 4 continued: PLENARY – Questions & Answers and Discussion**

4. **Assessment of zinc metabolism in EE using stable isotopes,** *Nancy Krebs (University of Colorado, USA)*
5. **Lessons from iron fortification trials in Ivory Coast, Kenya and Pakistan with a focus on the gut microbiome** *Michael Zimmerman (ETH Zurich, Switzerland)*

Plenary: Questions & Answers

11:30 – 12:30 **SESSION 5: Biomarkers for EED**
Chair: Margaret Kosek (John Hopkins Bloomberg School of Public Health, USA)

1. **Environmental enteropathy, oral vaccine and growth faltering in infants in Bangladesh**
   William Petri (University of Virginia, USA)
2. **Biomarkers of EED including kynurenine tryptophan ratio**
   Margaret Kosek (John Hopkins Bloomberg School of Public Health, USA)
3. **Use of fecal host transcripts as biomarkers of EED**
   Sophia Agapova (Columbia University, USA)

Plenary: Questions & Answers

12:30 – 13:30 **Lunch Break**

13:30 – 15:00 **BREAKOUT SESSIONS C & D:**
C. Review of low cost non-invasive methods for assessment of EED in field settings with a focus on stable isotope techniques
D. Review of latest knowledge on EED biomarkers

15:00 – 15:30 **CHAIR Summary of Sessions 1 – 5**
Chair: Mark Manary (Washington University School of Medicine, USA) and Jo Zaremba (Facilitator)

15:30 – 16:00 **Coffee Break**

16:00 – 17:00 **SESSION 6: Overview and progress of on-going interventions trials on EED**
Chair: Christine Stewart (University of California, Davis, USA)

1. **Biomarkers of EED in the MAL-ED Study** *Margaret Kosek (John Hopkins Bloomberg School of Public Health USA)*

2. **The Sanitation Hygiene Infant Nutrition Efficacy (SHINE) trial in rural Zimbabwe: an overview** *Mduduzi Mbuya (Zvitambo Institute for Maternal & Child Health Research, Zimbabwe)*
3. **The WASH Benefits Trial: an evaluation of the impact of water, sanitation, hygiene and nutritional interventions on child growth, development and environmental enteric dysfunction in Kenya and Bangladesh** *Christine Stewart (University of California, Davis, USA)*

Plenary: Questions & Answers

17:00 - 17:15 **Closing**

DAY 3 – Friday, 30 October 2015

09:00 – 09:15 **Summary of previous day and reflection**

09:15 – 10:15 **SESSION 7: Overview of interventions on EED**
Chair: Paul Kelly (University of Zambia, Zambia)

1. **Influence of pro/pre-biotics and other interventions on gut health** *Nagendra Shah (The University of Hong Kong, Hong Kong)*
2. **Results of three interventions to ameliorate EED** *Kevin Stephenson (Columbia University, USA)*
3. **Mesalazine in the initial management of SAM children with EED** *Kelsey Jones (Imperial College, UK)*

Plenary: Questions & Answers

10:15 – 10:45 **Coffee break**

10:45 – 12:15 **BREAKOUT SESSION E: Review of interventions on EED**

12:15 – 13:30 **Lunch Break**

13:30 – 14:30 **SESSION 8: Overview and progress of on-going operational research on EED**
Chair: Christine Stewart (University of California, Davis, USA)

1. **Benefits of a household WASH package to Community Management of Acute Malnutrition (CMAM) program – The OUADINUT Study** *Mathias Altmann and Antonio Vargas (ACF-IN)*
2. **Malnutrition and childhood infections in Africa: developing strategies against child malnutrition** *Mathias Altmann and Antonio Vargas (ACF-IN)*
3. **Ways to operationalize WASH-Nutrition synergies in implementation** *Susanna Smets (World Bank – Vienna)*
4. **The legumes and growth project** *Ken Maleta (University of Malawi, Malawi)*

14:30 – 16:00 **BREAKOUT SESSION F: Synthesis, Next Steps, Recommendations (working coffee)**

16:00 – 16:30 **SESSION 9: Summarizing actions, the way forward, and recommendations for the IAEA**
Chair: Mark Manary (Washington University School of Medicine, USA)

16:30 – 17:00 **Closing**
Cornelia Loechl

Annex 2: Meeting participants

Name	Affiliation	Country
Ross BUTLER	University of South Australia School of Pharmacy and Medical Sciences Division of Health Sciences, GPO Box 2471 Adelaide	Australia
Johanna BECKMANN	University of Vienna	Austria
Buck HANSON	University of Vienna Division of Microbial Ecology Department of Microbiology and Ecosystem Science	Austria
Alexander HASLBERGER	University of Vienna Department of Nutritional Research	Austria
Alexander LOY	University of Vienna Division of Microbial Ecology Department of Microbiology and Ecosystem Science	Austria
Susanna SMETS	World Bank Vienna	Austria
Robert BANDSMA	The Hospital for Sick Children, Division of Gastroenterology, Hepatology and Nutrition. University of Toronto	Canada
Nagendra Prasad SHAH	Food and Nutritional Science School of Biological Sciences, The University of Hong Kong	China
Kim FLEISCHER MICHAELSEN	Department of Nutrition, Exercise and Sports, University of Copenhagen.	Denmark
Henrik FRIIS	Department of Nutrition, Exercise and Sports, University of Copenhagen	Denmark
Dennis Sandris NIELSEN	University of Copenhagen Department of Food Science	Denmark
Per ASHORN	University of Tampere, School of Medicine	Finland
Mathias ALTMANN	Action contre la Faim, France	France
Claude SAINT JORE-DUPAS	Nutriset S.A.S	France
Martin SCHWARZER	Functional genomics of host/intestinal bacteria interactions group IGFL – Ecole Normale Supérieure de Lyon	France
Mamane ZEILANI	Nutriset S.A.S	France
Michael BLAUT	Department of Gastrointestinal Microbiology, German Institute of Human Nutrition	Germany
Marion PRIEBE	University of Groningen	Germany

Gagandeep KANG	Christian Medical College, Division of Gastrointestinal Sciences	India
James Alexander BERKLEY	KEMRI-Wellcome Trust Research Programme CGMRC	Kenya
Rosie CRANE	KEMRI-Wellcome Trust Research Programme CGMRC	Kenya
Musa MULONGO	KEMRI-Wellcome Trust Research Programme CGMRC	Kenya
Kenneth M. MALETA	University of Malawi, College of Medicine	Malawi
Asad ALI	Aga Khan University, Department of Pediatrics and Child Health	Pakistan
Margaret KOSEK	Asociacion Benefica Prisma, Unidad de Investigaciones Biomedicas Peru	Peru
Antonio VARGAS	Accion contra la hambre	Spain
Daniela PAGANINI	Human Nutrition, ETH Zürich, Swiss Federal Institute of Technology	Switzerland
Michael ZIMMERMANN	Human Nutrition, ETH Zürich, Swiss Federal Institute of Technology	Switzerland
Kelsey JONES	Imperial College, Section of Paediatrics and Centre for Global Health Research	United Kingdom
Douglas MORRISON	Stable Isotope Biochemistry Laboratory, Scottish Universities. Environmental Research Centre	United Kingdom
Simon MURCH	Dept of Paediatrics, University Hospital Coventry and Warwickshire	United Kingdom
Sophia AGAPOVA	Columbia University, College of Physicians and Surgeons	United States of America
Fayrouz ASHOUR	Department of Nutrition and Food Science, University of Maryland	United States of America
Michael FREEMARK	Duke University, Division of Pediatric Endocrinology and Diabetes	United States of America
Alexis KATSIS	Bill & Melinda Gates Foundation, Seattle	United States of America
Nancy KREBS	University of Colorado School of Medicine, Department of Pediatrics	United States of America
Mark MANARY	Washington University School of Medicine in St. Louis St. Louis Children's Hospital	United States of America
Jennifer ORGLE	CARE USA Nutrition at the Center Program	United States of America
William PETRI	Division of Infectious Diseases & International Health University of Virginia	United States of America
Kevin STEPHENSON	Washington University in St Louis	United States of America

Christine STEWART	Department of Nutrition, University of California, Davis	United States of America
Indi TREHAN	Washington University in St. Louis	United States of America
Paul KELLY	Department of Internal Medicine, University of Zambia	Zambia
Chiza KUMWENDA	University of Zambia School of Agricultural Sciences Department of Food Science and Nutrition	Zambia
Mduduzi NN MBUYA	Implementation Science Zvitambo Institute for Maternal and Child Health Research	Zimbabwe
Cornelia LOECHL	IAEA Scientific Secretary Nutritional and Health-related Environmental Studies Section – Division of Human Health, IAEA	Austria
Victor OWINO	Nutritional and Health-related Environmental Studies Section – Division of Human Health, IAEA	Austria
Christopher WEGNER	Nutritional and Health-related Environmental Studies Section – Division of Human Health, IAEA	Austria
Kirsten GLENN	Nutritional and Health-related Environmental Studies Section – Division of Human Health, IAEA	Austria
Souheila ABBEDDOU	Nutritional and Health-related Environmental Studies Section – Division of Human Health, IAEA	Austria
Joanna K ZAREMBA	Markets, Private Sector Engagement, Livelihoods and Environment	United Kingdom