



INTERNATIONAL ATOMIC ENERGY AGENCY

Report on Technical Meeting (TM 52335)

on

**Application of Stable Isotope Techniques in
Environmental Enteric Dysfunction (EED) Assessment**

31 May – 3 June 2016

IAEA HQ, Vienna, Austria

TABLE OF CONTENTS

RATIONALE AND BACKGROUND	3
DAY 1: TUESDAY, 31 MAY 2016.....	5
OPENING SESSION:	5
IAEA’s Activities in Nutrition	5
Objectives and expected outcomes of the meeting.....	6
Overview of recent activities on EED.....	7
Looking forward in EED at the BMGF.....	9
DAY 2: WEDNESDAY 1 JUNE 2016:.....	17
DEFINING THE SCOPE AND PRIORITY AREAS FOR THE PROPOSED IAEA COORDINATED RESEARCH PROJECT	17
Stunting is life-long - But does stunting matter? Want to know not need to know. Long- term health consequences are more important. What are they?	18
Does EED cause adverse functional outcomes? Is it adaptive or maladaptive?	18
DAY 3: THURSDAY 2 JUNE 2016	20
DAY 4: FRIDAY 3 JUNE 2016:.....	25
DESIGN OF COORDINATED RESEARCH PROJECT ON THE USE OF STABLE ISOTOPE TECHNIQUES TO ASSESS EED.	25
Funding and participating institutions.....	26
Funding opportunities.....	26
Participating institutions	26
Potential institutions:.....	26
Potential institutions to provide technical support/advice –paired with Contractees:.....	27
Conclusions and way forward	27
REFERENCES.....	29
ANNEX 1: MEETING AGENDA.....	30
ANNEX 2: MEETING PARTICIPANTS.....	34

RATIONALE AND BACKGROUND

Retarded linear growth, widely referred to as stunting, is rampant in low- and middle-income countries, affecting a total of 159 million children under the age of five years. Stunting, defined as height-for-age z score less than -2 standard deviations of the World Health Organization Child Growth standards develops in the first 1000 days of life, and becomes largely irreversible if no appropriate interventions are in place. Evidence from the Gambia suggests that some spontaneous recovery may occur in childhood/adolescence, but the magnitude of this recovery is unknown. The consequences of stunting include increased infant and child mortality and morbidity; increased risk of overweight, obesity and non-communicable diseases later in life; and low psychomotor development and lost economic potential (Hoddinott et al., 2013). Inadequate nutrition and recurrent infection are the primary drivers of stunting. However, evidence now shows that all known nutritional interventions combined may only partially prevent stunting (Bhutta et al., 2008). Poor hygiene and absence of adequate sanitation may play a role but evidence to support a causal relationship is largely lacking. Living in poor sanitary conditions may induce gut dysfunction (Keusch et al., 2013, Mbuya and Humphrey, 2016), referred to as environmental enteric dysfunction (EED). EED affects presumably 50-95% of all children under the age of 5 years in resource poor settings. Retarded growth, altered gut microbiota, and decreased vaccine responsiveness are considered the most important consequences of EED.

The technical meeting on environmental enteric dysfunction (EED) organized and hosted by the International Atomic Energy Agency (IAEA) in Vienna, Austria from October 28–30, 2015 identified gaps including need for a clear classification and understanding of causal pathways underpinning EED. The meeting recommended the development of practical, simple, and affordable tools to diagnose and characterize EED to allow better targeting of interventions in vulnerable populations. Established stable isotope techniques were recommended for the assessment of absorptive capacity/permeability of the gut, bacterial translocation and body composition as a proxy indicator of dietary quality and morbidity.

The IAEA hosted a follow up technical meeting from 31 May-3 June 2016 during which 15 experts from research and academic institutions, the Bill and Melinda Gates Foundation and IAEA Technical Officers from Nutritional and Health-related Environmental Studies Section (NAHRES) discussed the design of a new coordinated research project (CRP) aimed to foster the use of stable isotopes in the assessment of EED. The following experts participated in the meeting: Mr Michael Freemark, Duke University, USA, Mr Douglas Morrison, Glasgow University, UK, Mr Richard Elliot, Bill and Melinda Gates Foundation, USA, Mr Ross Butler, University of South Australia, Ms Margaret Kosek, John Hopkins Bloomberg School of Public Health, Mr Jahoor Farook, Baylor College of Medicine, Mr Ali Asssad, Aga Khan University, Pakistan (via Webex), Mr Robert Bandsma, The Hospital for Sick Children, Canada, Mr Paul Kelly, Queen Mary University of London/University of Zambia, Mr

Kenneth Brown, Bill and Melinda gates Foundation, USA, Mr Daniel Tome, AgroParisTech, France, Mr Tahmeed Ahmed, icddr-b, Bangladesh, Mr Simon Murch, Warwick University, UK, Mr Alexander Loy, University of Vienna, Ms Cornelia Loechl, IAEA, Mr Victor Owino, IAEA and Ms Souheila Abbeddou, IAEA.

This report presents the key deliberation points from the meeting and is organized to chronologically follow the order of proceedings with a few exceptions.

DAY 1: TUESDAY, 31 MAY 2016

OPENING SESSION:

Ms Cornelia Loechl, Section Head, Nutritional and Health-Related Environmental Studies (NAHRES) Section welcomed participants and summarized IAEA's Program in Nutrition.

IAEA's Activities in Nutrition

The IAEA fosters the use of stable isotopes to enhance Member State (n=168) capabilities to combat malnutrition and environment related nutrition issues for better health throughout the life course. Stable isotope techniques (safe and non-radioactive) provide key information to develop and evaluate interventions aimed at combatting malnutrition. Specifically, the techniques can be used to objectively assess:

- Body composition;
- Exclusive breastfeeding;
- Total daily energy expenditure;
- Micronutrient bioavailability;
- Vitamin A status;
- Protein and amino acid bioavailability and metabolism;
- **Helicobacter pylori** infection.

Currently IAEA's activities in nutrition cover the following thematic areas:

- Maternal, infant and young child nutrition;
- Prevention and control of non-communicable diseases;
- Agriculture for improved nutrition;
- Health effects of the environment;
- Quality assurance.

The IAEA works in partnership with major players in nutrition, including the UN family (UN Network for SUN, FAO, UNICEF, UN Task Force on non-communicable diseases and WHO regional offices) and other partners (Bill and Melinda Gates Foundation, International Malnutrition Task Force, Valid International, HarvestPlus, Global Alliance for Improved Nutrition and Micronutrient Initiative).

The IAEA support to member countries is channelled through two funding mechanisms, namely, coordinated research projects (CRPs) and Technical Cooperation projects. CRPs are funded for 4–5 years following calls for proposal to answer a specific research question. Typically 5–8 countries participate and each receives EUR 10 000–15 000 annually and participates in regular coordination meetings. Technical cooperation are member state driven and are linked to country development priorities – funding is channelled through procurement of equipment, training courses, expert missions and fellowships.

She highlighted TWO new projects for 2016–2017 technical cooperation project cycle where EED will be assessed: 1) Inter-Regional project brings together 13 countries (Bangladesh, Benin, Burkina Faso, Senegal, Mauritania, Tanzania, Malawi, Madagascar, Philippines,

Myanmar, Vietnam, Bolivia and Guatemala in collaboration with UNICEF, World Bank, Inter-American Development Bank, Care International) to commonly and with contextual variation contribute to the evidence base to improve programmes targeting the reduction in stunting through the application of stable isotopes to assess nutrient absorption in the context of environmental enteric dysfunction; breastfeeding patterns; body composition as an indicator of nutritional status, reflecting the quality of the diet and morbidity. 2) Regional project is bringing together 8 African countries (Burkina Faso, Kenya, Ethiopia, Malawi, Uganda, Tanzania, Zambia, and Democratic Republic of Congo) in the use of stable isotopes to evaluate the impact of moderate acute malnutrition (MAM) and severe acute malnutrition (SAM) treatment programmes on health outcomes to improve understanding of relative success of current approaches.

Reference was made to the IAEA Human Health Campus website (<http://humanhealth.iaea.org>) for more information on IAEA's work in nutrition

Objectives and expected outcomes of the meeting

Ms Loechl stated that the meeting was a follow up of the Technical Meeting on EED, Microbiome and Undernutrition held 28-30 October 2015. The objective of the current meeting was to prepare a proposal for a new CRP on the 'Application of Stable Isotope Techniques in EED Assessment'

The main outcome of the meeting would be a proposal clearly laying out a test or series of tests for EED that include stable isotope techniques to diagnose and characterize EED. Criteria for identification of such a test include:

- Desirable characteristics, e.g. specificity, sensitivity, safety;
- Application context (research, clinical, community);
- Domains to be addressed.

An outline of the CRP proposal should include:

1. Background Situation Analysis;
2. Nuclear Component;
3. CRP Overall Objective;
4. Specific Research Objectives;
5. Outcomes;
6. Expected Outputs;
7. Methodological Considerations;
8. Analytical Techniques;
9. Potential Participating Institutions;
10. Other Resources Required.

The following steps were laid out as necessary during the meeting for realizing the new CRP:

- Update on recent activities on EED;
- Update on technical developments in EED – promising biomarkers;
- Defining scope and priority areas for the proposed CRP based on broader research agenda:
 - Research questions;

- Measurements/study design;
- Biomarkers and techniques;
- Potential research institutions;
- Funding opportunities and potential institutions;
- Drafting of the CRP proposal.

The meeting progressed with a series of presentations to update on activities in EED since 2015.

Overview of recent activities on EED – Chair – Mr. Michael Freemark, Duke University, USA

Mr. Victor Owino summarized the findings of the October 2015 IAEA meeting, which characterized EED and its clinical domains, pathogenesis, and histologic, biochemical, and microbiological features. EED presents as a continuum of features falling into six domains (Gut leakiness/permeability, Microbial translocation, Gut inflammation, Systemic inflammation, Dysbiosis, and Nutrient malabsorption). The domains are important because they represent anatomic and pathophysiologic basis of EED; can be the basis for understanding link between EED and stunting; can act as entry points for measurement/diagnosis and treatment of EED.

He discussed the use of stable isotopes for characterization of gut structure and function, microbial translocation, microbiota diversity and function, nutrient bioavailability, body composition and energy expenditure. Other techniques highlighted during the meeting include omic approaches, transcriptomics, metabolomics of stool as measure of function. He then discussed a variety of interventions for prevention and treatment of EED, including WASH, pro- and pre-biotics, micronutrient supplementation, anti-inflammatory agents and antibiotics.

Research questions arising from the meeting and other related events include:

- What are the other effects of EED?
- Neurocognitive development;
- Metabolic dysfunction and link to risk for non-communicable diseases (NCDs);
- Which of the SIX EED domains ought to be prioritised;
- What role do specific breastmilk components (circulating glutamic acid, glutamine) play;
- What is the role of maternal EED during pregnancy?
- What is the role of leptin in EED (related to appetite, inflammation and growth)?

The 2015 technical meeting concluded that:

- A test for EED is urgently needed to facilitate evaluation of interventions;
- Identification of candidate markers for EED (e.g., markers of small and large intestinal permeability, epithelial function and inflammation, bacterial translocation) was needed urgently;
- Development and validation of a set of tests using stable isotopes to assess EED is important;
- Identifying potential interventions that can reverse or prevent EED (e.g. nutrients, water, sanitation and hygiene, WASH, drugs and/or probiotics) should be explored.

The meeting has been disseminated through various pathways including:

1. Owino et al. Environmental enteric dysfunction and growth failure/stunting in global child health. Accepted as Perspective: *Pediatrics*;
2. Butler et al. Stable isotope techniques for the assessment of host and microbiota response during gastrointestinal function. Provisionally accepted: *Journal of Pediatric Gastroenterology & Nutrition*;
3. Abbeddou et al. Technical meeting on environmental enteric dysfunction (EED), microbiome and undernutrition. Accepted: *Sight & Life Magazine*;
4. Websites:
 - i) IAEA Technical Cooperation; Nuclear Sciences and Applications;
 - ii) Scaling Up Nutrition Movement; CMAM Forum; IAEA News;
 - iii) Centre and UN Radio.

Mr. Doug Morrison in summarising post technical meeting debates among researchers from IAEA, University of Vienna and University of Glasgow, discussed the role of microbial dysbiosis as a potential consequence, and/or a possible trigger, of gut inflammation; this led to a discussion of a possible vicious cycle in EED pathogenesis. He raised the possibility that critical components of the diet might modify microbiome maturity and function. However, the association between microbiota diversity and functionality is more complicated, and changes in diversity might have or not consequences on functionality. This is partly explained by the redundancy in function of microbiota. Opportunities for application of stable isotopes include:

- Inflammation in gut: inflammation in the gut conditions the gut microbiota composition and function. Potential methods to assess inflammation include terminal electron acceptors, add nitrates/nitrites, pro-inflammatory;
- iNOS pathway and subsequent production of nitrates and nitrites. ¹⁵N Arginine can be used to track nitrite production. For gut inflammations, urine ¹⁵N, gut urinary nitrates, may be useful, NO in breath likely, early marker gut inflammation;
- Understanding of how do particular microbomes survive modified redox conditions (e.g. facultatively anaerobic pathogens such as Salmonella). Potential substrates include labeled tetrathionate that measures hydrogen sulfide production;
- ¹³C, ¹⁵N or Deuterium labeled resistant starch can be used to follow carbon into microbiota, microbiota RNA/DNA;
- Understanding of function of microbiota, in large vs. small intestine;
- Microbiota/tumors;
- Permeability: isotopic techniques, location of gut lesion and the magnitude of permeability? Other methods have been developed to assess permeability of the gut. Unless refined information is needed, stable isotope techniques may not be the way forward;
- Role of mucus in gut protection/additional protection. Until recently, biopsy was the only method to assess thickness and probably role of mucus. Mucous functionality could also be assessed using stable isotope techniques. One limitation in assessing the barrier properties of gastrointestinal mucus to particle transport is that particle diffusion depends on surface charge (mucus is negatively charged).

Mr. Richard Elliott summarized the findings of two recent meetings organized by the Bill and Melinda Gates Foundation and what the future looks like in the EED arena. He described a number of new and innovative approaches to understanding EED pathogenesis using animal models, in vitro experimental systems, and human investigations. Studies will test new technologies to assess bone growth, muscle protein metabolism, small bowel

histology, and the roles of anti-inflammatory agents and breast milk constituents in EED treatment.

Looking forward in EED at the BMGF

- BMGF has interest in protein/ amino acids, including intake, digestibility, uptake, utilization, and downstream effects (e.g. Tryptophan)
- General/Biomarkers:
 - Define set of biomarkers to validate against biopsies. Priority biomarkers are those which showed association with enteric dysfunction including linear growth faltering. especially to diagnose, prevent or treat a child with EED. Biomarkers enable us to diagnose and define EED for measurement in clinical trials;
 - Ability to use stable isotope approaches to get good quantitative and dynamic measures;
 - How to measure mucus layer integrity- is there an opportunity using stable isotope technology (SIT)?
 - Application of stable isotopes in measuring microbiome flux: a great opportunity, be able to follow flux of carbon through microbial community, to deconvolute relationships between members of the community (primary, secondary feeders), etc., understand dynamics of the system. For example, labeled human milk oligosaccharides (HMOs) via fermentation technology, use as a probe to evaluate how HMOs are utilized by bacterial (inside/outside feeders, etc.);
 - Need tools to help us understand functional domains of gut microbiome; what role is each bacterial member playing in the community? Can we build a functional framework to understand the gut microbiome based on functionality?
 - Stable isotope techniques should fill basic knowledge gaps in addition to useful clinical or preclinical tools (e.g. how to easily measure, define primary, secondary, tertiary, etc. interactions of microbiome).
- Further gaps to consider:
 - Accurately measuring gestational age;
 - Beyond passive permeability, how can we accurately and specifically measure active transport in the gut?
 - Epigenetic markers needed?
- Animal Models:
 - Developing/using stable isotope based tools in preclinical models to help explore, validate approach that are unethical in humans;
 - BMGF partners in the Gut Health Model consortium eager to help develop/use such tools, building of synergies where appropriate will be useful.

The afternoon session began with a rundown by **Margaret Kosek** on the current status of biomarkers of EED and the inherent difficulties in defining this entity using presently available measurement techniques. She delivered a comprehensive history and evaluation of a series of biomarkers directed at several domains in the putative pathogenic pathways that lead to this disorder. The discussions that ensued resulted in robust debate which highlighted the

lack of a universal definition of EED. This further emphasized that the spectrum of nutritional compromise, extent of mucosal damage and regional inflammation combined to produce variable systemic mucosal inflammation and probably personalized changes in microbiome composition and activity. Biomarkers are linked to EED domains to measure:

- Intestinal Inflammation (myeloperoxidase [MPO], calprotectin, Neopterin [NEO], alpha-anti-trypsin [AAT]);
- Intestinal repair and regeneration (Reg1B, GLP-2);
- Intestinal Permeability (Lactulose:Mannitol [LM], Lactulose:Rhamnose [LR], Polyethylen glycols [PEGs]);
- Malabsorption/efficiency of uptake and utilization of key nutrients (macronutrients, amino-acids [AAs] as well as micronutrients [MN]);
- Bacterial translocation;
- Small bowel bacterial-overgrowth;
- Microbiome (lots of data, poorly standardized reporting);
- Mucosal immune response (oral vaccine hypo-responsiveness);
- Systemic immune activation (Alpha 1-acid glycoprotein [AGP], C-reactive protein [CRP], IL-6, EndoCAb, sCD14, ferritin, numerous).

Biomarkers highlighted since the TM in October 2015 by EED domain were:

- Permeability:
 - LM test, differences in permeability by age and sex, LMZ score;
 - LR, either the standard 6h test or the validated short (1 h) test.
- Microbiome- microbiome-by-age z score [MAZ];
- AAs, plasma AA's low in children at risk of EED (Semba et al., 2016).

Further work has shown that:

- Glucagon-like peptide-2 (GLP2) is important for intestinal repair;
- Pathogen pressure vs pathogen density are important for assessing risk of EED;
- Age affects predictability of biomarkers and sometimes, sex;
- Microbiome MAZ;
- Tryptophan is low in children Tanzania and Peru;
- Stunted children lower levels of all 9 amino acid than non-stunted children;
- AA metabolism markers predict growth;
- LM not associated to linear growth in many countries, and when it was associated, the effect sizes were too small.

Conclusion:

- There is heterogeneity in normal ranges of biomarkers by age, and at times sex;
- There is heterogeneity in ranges of biomarkers across different sites with probable high prevalence of EE;
- Collaborations to permit cross validation important to determine promise of biomarker, understanding of common vs regional features of EE.

The next component of the afternoon session was directed at four presentations proposing newer biomarkers that may incorporate stable isotope biomarkers in conjunction with measurements and interventions not necessarily incorporating stable isotopes.

The first of these was delivered by **Margaret Kosek** on the use of the kynurenine/tryptophan ratio in EED. This presentation emphasized the utility of this ratio as an index of host response in EED:

- Tryptophan metabolites regulated by Indoleamine 2,3-dioxygenase 1 (IDO1);
- IDO1 activity favors immune suppression and tolerance;
- K/T ratio has a low correlation with systemic inflammation indicators (AGP and CRP), thus it is not yet another inflammation biomarker;
- Kynurenine: tryptophan (KT ratio) is associated with oral polio virus 1 (OPV1) vaccine failure. Increase of 1 SD in kynurenine associated with 1.90 times higher risk of OPV1 failure (95%CI 1.18-3.10) in a model that controlled for length-for-age z score (LAZ) at time of biomarker measure, breastfeeding, number of days of diarrhea up to date of primary vaccine administration, and diarrhea at the time of primary immunization;
- A cutoff of 50% of K/T ratio predicts OPV1 vaccine failure with a specificity greater than 50% and a sensitivity of 80%;
- Enterocytes major producer of IDO1 (and hence Kynurenine) (Dai and Zhu, 2010);
- Tryptophan levels notably low in a significant subset of population with EED (e.g. Tanzania and Peru);
- Usual amino acid intakes for weaned children meet minimal requirements. For example, In Peru, at 9-15 months, the % < Estimated Average Requirement (EAR) was 2% for Valine, Threonine and Leucine, and was 9% for Lysine. At 16-24 months, the % < EAR was 0% for all AA. For Tanzania, at 9-15 month, the % < EAR was 1% for Lysine, and at 16-24 months, the % < EAR was 0% for all AA;
- Recent enteropathogen exposure and diarrhea are determinants of plasma tryptophan level while dietary intake is not.

The following priorities remain to be addressed:

- Measure additional analytes on metabolic pathways of tryptophan degradation;
- Integrate microbiome analysis- can we determine host v microbiome origin of increased IDO1 activity;
- Confirm growth findings:
 - Nutritional intervention trial.
- Confirm vaccine failure findings:
 - Pakistan rotavirus trial;
 - Southern Sudan cholera vaccine.
- Measure other AA's, particularly neutral AA's;
- Incorporate tryptophan and downstream neuroactive metabolites of tryptophan in analysis of cognitive testing and neurodevelopment.

The second presenter, **Farook Jahoor**, gave a succinct delivery of the use of arginine-citrulline as a novel biomarker of a measure of the host mucosal surface area and aspects of its immune effector response. The meeting was briefed as follows:

- Decreased plasma citrulline correlates well with reduced enterocyte mass, independently of nutritional and inflammatory status;
- Citrulline Flux correlates well with urinary mannitol excretion in American, Indian and Jamaican women;

- Citrulline Flux correlates well with citrulline concentration in American and Indian but not Jamaican Women (Kao et al., 2016);
- Citrulline flux has been shown to positively correlate with citrulline concentration in septic patients with good renal function;
- The above suggest that plasma citrulline concentration is a good marker of gut function, but citrulline flux was able to distinguish among healthy women from India, Jamaica and the States;
- Salivary citrulline concentration is a good proxy for plasma concentration and hence, a possible marker of gut function;
- Enteral arginine conversion to citrulline correlates with urinary mannitol excretion in American, Indian and Jamaican women;
- Enteral arginine conversion to ornithine correlates negatively with urinary mannitol excretion in American, Indian and Jamaican women;
- Mannitol excretion correlates positively with arginine flux and enteral arginine converted to citrulline, and negatively with enteral arginine hydrolysed to ornithine, suggesting that:
 - i) Certain parameters of arginine metabolism are good indicators of gut function;
 - ii) A possible test of gut function is an oral dose of ^{13}C -Arginine followed by isotopic enrichment of salivary citrulline.

The relationship between gut microbiota and gut function was also discussed and the following conclusions made:

1. The Bacteroides-dominant subjects have a greater gut absorptive capacity than Prevotella-dominant subjects and those subjects whose communities fell within the Bacteroides with Clostridia genera;
2. Citrulline and arginine fluxes increase as the prevalence of Bacteroides increased and Prevotella decrease (Kao et al., 2016);
3. The positive correlations between urinary mannitol excretion with body weight and with gut Bacteroides suggest a relationship between gut Bacteroides and body weight, which is mediated through gut absorptive capacity.

Michael Freemark then gave a presentation of the importance of insulin growth factor-1 (IGF-1) and leptin in the regeneration and subsequent homeostasis of the EED mucosa and the relationship to the whole body outcomes of EED:

- IGF-1 produced by small intestine, mediates GLP-2 action, and controls adaptive re-growth of crypts and villi after fasting. Leptin produced by white adipose tissue; reflects energy reserves;
- IGF-1 and leptin decline with *weight loss*; IGF-1 low in *growth failure* in EED and inflammatory bowel disease (IBD). Ratio of IGF-1/leptin might be used as surrogate marker of ratio of lean body to fat mass;
- Reductions in IGF-1 cause growth failure/stunting, while hypoleptinemia predisposes to immune dysfunction and infection;
- Reductions in IGF-1 and leptin reversed by treatment/recovery, paralleling clinical reversal;
- Both IGF-1 and leptin may be measured in field;
- But neither IGF-1 nor leptin is a unique/pathognomonic marker of EED.

Two potential implications were presented for the use of IGF1 and leptin as EED biomarkers:

1. **IGF-1 and leptin as screening tools for nutritional status:**

- Edema;
- Infants and pregnant women;
- What is the value of IGF-1 / leptin ratio?

2. **Pathogenesis of morbidity/mortality:**

- In combo with stable isotopes and other biomarkers, IGF-1 and leptin integrate markers of small intestine dysfunction with systemic complications:
 - Growth failure/stunting (IGF-1);
 - Immune dysfunction (hypoleptinemia);
 - Long-term risk of excess adiposity (leptin excess);
 - Cognitive dysfunction (IGF-1).

Point of care assays for leptin, IGF-1, IL6:

- Efforts are underway to develop rapid, portable, inexpensive, and stable microassays for leptin and adiponectin in fingerstick blood samples at ambient temperatures;
- However, primary barriers to clinical field testing are:
 - Cost;
 - Response time;
 - Assay stability;
 - Blood collection and sample handling.

Mark Manary discussed the importance of host RNA as a biomarker of EED:

- Associations with L/M in rural Malawian children;
- Stunting is predicted by L/M ratio;
- 18 transcripts of interest (differentially expresses across population), could be biomarkers;
- 7 transcripts of particular interest (CD53, CDX1, HLA-DRA, Muc12, Reg1A, S100A8, TNF etc.);
- Machine learning models [random forest model using **CD53, HLA-DRA, MUC12 and TNF**] to predict EED, 84%, 83% sensitive respectively for severe EED and no EED;
- Six risk factors identified:
 1. WHZ < 0;
 2. Age < 24 mo.;
 3. No clean water source;
 4. No pit latrine at home;
 5. Child sleeps with domesticated animals;
 6. Had diarrhea in last 7 days;
- When a child has more than 5–6 of these, he/she is very unlikely to have a normal L/M;
- When a panel of six mRNAs - **CDX1, HLADRA, MUC12, REG1A, S100A8, TNF**, are added to the clinical risk factors and when modeled in random forest, this will identify those with severe EED with 84% sensitivity and 73% specificity;
- In the same population, L/M ratio was significantly correlated with kynurenine/tryptophan, taurine and serotonin.

The adoption of stable isotopes into some of these proposed biomarkers was robustly discussed. These combined presentations provided a basis for establishing the need for using objective indices in different domains of the EED pathogenesis to help define this syndrome in the future.

The final presentation in this component was made by **Asad Ali** on the morning of Thursday 2 June 2016 via Webex. He discussed alanine-glutamine interventions as a management strategy for EED. The meeting heard that:

- Glutamine (Gln) is the most abundant amino-acid in the body (Pochini et al., 2014);
- Gln is pivotal in several metabolic pathways;
- Gln helps recovery of intestinal integrity from many pathological conditions;
- Gln may become “conditionally essential” during severe illness;
- Gln deprivation induces a reversible atrophy of crypt domains in mouse enteroids;
- Five randomized control trials from low and middle income countries of glutamine-based oral rehydration therapy (ORT) for acute diarrhea showed that glutamine was at least as good as glucose-based ORT, and 3 of the 5 studies suggested an additional benefit from glutamine on stool volume or diarrhea duration.

Alanyl-Glutamine (Ala-Gln)

- More soluble and stable in solution than glutamine;
- Well-tolerated in patients;
- Drives intestinal sodium cotransport as effectively as glucose and glutamine;
- Ala-Gln improved intestinal barrier function and weight gain in undernourished Brazilian children (Lima et al., 2007);
- The first study enrolled 107 undernourished children from a poor community and randomized them to receive 10 days of oral Ala-Gln (24g/day) or isonitrogenous glycine. Children’s weights, heights, and intestinal barrier function were measured up to 4 months later. 4 months after therapy, children randomized to glutamine exhibited superior weight gain and intestinal barrier function (as measured by lactulose excretion) than those children randomized to glycine.

IMAGINE: Intervention and Mechanisms of Alanyl-Glutamine for Inflammation, Nutrition, and Enteropathy is a study seeking to answer the following questions:

- What is the **minimum dose** (12g, 6g, or 3g) of Ala-Gln that produces a clinically significant improvement in gut integrity (L:M) and weight gain?
 - 140 Brazilian children, ages 2 months to 5 years;
 - Risk of underweight ($WAZ \leq -1$), wasting ($WHZ \leq -1$), or stunting ($HAZ \leq -1$);
 - 4 month follow-up.
- What are **mechanisms** by which Ala-Gln provides these benefits?
 - Reduced intestinal inflammation (fecal cytokines, *REG1B);
 - Altered metabolism (urine metabonomics);
 - Enhanced absorption (fecal energy).
- Conclusion:
 - Role of glutamine supplementation in recovery of intestinal mucosa in disorders like IBD, short gut, post chemotherapy etc. is well established;

- Ala-Gln dipeptide is a stable and more feasible compound to deliver glutamine to infants;
- While preliminary data on the role of Ala-Gln supplementation in EED is promising, more definitive studies are needed and some are ongoing.
- The following questions arose from discussion:
 - What is intake of Gln in countries, is intake deficient in low resource settings?
 - How does dose compare to intakes, why would small dose have an effect that would last so long?
 - What is the effect of Ala-Gln intervention on gut microbiome, maybe next study?
 - Is there a shortage of Gln during acute diarrhea?
 - Is Gln a modulator of immuno-tolerance?

Douglas Morrison, discussed the use of a variety of stable isotope tests to interrogate the function of the gut and its local immune response. Some tests were directed at interrogating the degree of damage and/or maturity of the small intestinal mucosa. Others were designed to determine the regional mucosal immune response. These tests might also be used to ascertain gut motility and absorptive dysfunction and in concert with other stable isotope studies, both micronutrient and macronutrient, be built into a suite of biomarkers to aid in the classification of EED and a more comprehensive future functional definition of the spectrum of this disorder in different global regions. Tentative links between various stable isotope tests and some of the already investigated biomarkers such as gut permeability and the newer proposed probes were discussed. Overall the entire afternoon session gave some insights into ways the mechanistic aberrations leading to and maintaining EED might be probed and subsequently used to better define the condition.

Stable isotope application in gut function assessment is based on what we want to know:

1. Infection, inflammation, immune status / function (L-[guanidine-¹⁵N₂]arginine and L-[ureido-¹⁵N] citrulline to track *INOS* pathway: L-Arginine, Ornithine, Citrulline pathway);
2. Motility;
3. Digestion – villus maturity (¹³C sucrose breath test [SBT]);
4. Absorption – nutrient requirements (¹³C starch, ¹³C/²H triglycerides (MTG), ¹³C/²H Fatty acids, ¹³C/²H protein);
5. Malabsorption (¹³C xylose breath test [BT], ¹³C sorbitol BT, ¹³C lactose BT (H2));
6. Permeability [region of permeability and lesion size as proxy for LPS and inflammation]:
 - i. Sugar permeability tests (urinary L:M, L:R, 3 or 4 sugar test);
 - ii. ⁵¹Cr EDTA;
 - iii. PEG 3500/400;
 - iv. Oligosaccharide (?).
7. Gut microbiome: Stable isotope to label up bacteria, delivery of label across bacterial, enrichment indicative of turnover rates, can even use labelled water, a long point away for POC diagnostic, but it is a research tool;
8. Impact on host.

The following questions are relevant in the application of stable isotopes to assess impact on host:

- Mechanistic and functional outcomes;
- Do stable isotope tests + additional biomarkers allow additional / improved stratification;
- Does stratification of patients lead to targeted / improved outcomes;
- Stable isotopes are invaluable research tools, allow non-invasive tests (potentially) but work needed before new generation of breath tests come online;
- Importance of assessing several domains of EED;
- Linking biomarkers is important because of the diversity of phenotypes/symptoms in a population;
- Clinical outcomes.

DAY 2: WEDNESDAY 1 JUNE 2016:

DEFINING THE SCOPE AND PRIORITY AREAS FOR THE PROPOSED IAEA COORDINATED RESEARCH PROJECT - Chair: Mr. Robert Bandsma – The Hospital for Sick Children, Canada

The day began with a presentation on alanine and glutamine intervention by Asad Ali (this is already described above. Richard Elliot presented on the way forward based on BMGF previous meetings on EED and mentioned the foundation's interest in amino acid metabolism (this is presented above under day 1). **Margaret Kosek** gave a brief overview of discussions from the first day before. The meeting then progressed to a brainstorming session on research questions.

Paul Kelly laid out research questions relevant to EED from a clinical perspective and opportunities for intervention.

Finagle's Laws of Information:

- The information you have is not what you want;
- The information you want is not what you need;
- The information you need is not what you can obtain;
- The information you can obtain costs more than you want to pay.

Research Questions:

- Clinical outcomes of EED;
- Health consequences on NCDs?
- What is effect of helminth gut infections?
- Is stunting the right outcome to target, measure? Is stunting the right marker?
- Prevention criteria: cheap, safe, efficacious, etc.;
- Malnutrition enteropathy: strong case for treatment;
- High levels LPS in SAM;
- Biomarkers of damage in SAM, consistent signal;
- Goblet cells: are they reduced in EED? Counts are the same, but not sure about quality/health of cells.

Opportunities to intervene:

- Pathogen burden: WASH trials;
- Repair microbiota: repair dysbiosis;
- Mucosal healing: fix leaky gut;
- Extra nutrients: provide more nutrients;
- Antimicrobial interventions;
- Anti-inflammatory;
- Tryptophan and other amino acids?
- Suggested clinical research priorities:
 - Await WASH/nutrition studies;
 - Long term cohort studies or exposure and real outcome (correlate NCDs with height;
 - Trials on novel interventions, but need better biomarkers:
 - Adult height correlates with gut permeability;
 - Increase surface area can increase LPS in some circumstances;

- Exploratory clinical trials.
 - Gln/Try/Leu, (AMAZE, IMAGINE trials);
 - Steroids for gut inflammation;
 - Translocation: rifaximin;
 - Systemic inflammation: anti-cytokines;
 - Malabsorption: targeted nutrients;
 - Microbiome: microbiome transplants, prebiotics.

Stunting is life-long - But does stunting matter? Want to know not need to know. Long-term health consequences are more important. What are they?

Mr. Kenneth Brown outlined research questions relevant to EED from a public health perspective:

- What is the causality between EED and malnutrition?
- What is the case definition of EED?
- Do we want to diagnose/prevent/treat EED or stunting; currently most biomarkers available are associated with stunting;
- Dynamic proteomics? Labelled amino acids;
- Can we think about EED as a risk factor?
- What is the role of sucrose breath test?

Does EED cause adverse functional outcomes? Is it adaptive or maladaptive?

The afternoon session focused on prioritizing of research questions. A number of important domains relevant to EED were identified, namely:

- Nutrient intake (including breast milk intake);
- Nutrient digestion and absorption (macronutrient and micronutrient);
- Translocation;
- Epithelial barrier function (gut leakiness);
- Gut and systemic inflammation;
- Nutrient metabolism, energy needs (Host metabolic response);
- Microbiome.

A total of 18 research questions were generated and matched with the relevant EED domain addressed as in Table 1 below.

It was agreed that the following THREE domains will be given first priority in the proposed CRP. The domains will be investigated in a stepwise manner as outlined here:

- Dietary intake (breast milk using the standardized deuterium dose to the mother technique; and non-breastmilk by direct measure of food intake)
Potential secondary outcome: anorexia;
- Gut function and integrity
 - i. Absorption (macronutrient and micronutrient digestion and malabsorption)
Potential secondary outcome: Bacterial metabolism of nutrients
Microbiome and host metabolism.

- ii. Translocation (mucosal integrity/permeability; role of intestinally derived LPS or labelled bacteria);
- iii. Inflammation (gut and systemic inflammation);
- Host metabolic response:
 - i. Growth (IGF, Collagen, Leptin);
 - ii. Metabolism (tryptophan and protein synthesis).

The day ended with a presentation on funding opportunities which is presented later in this report.

DAY 3: THURSDAY 2 JUNE 2016

The day started with a continuation of prioritization of research questions and progressed to consider design issues related to the CRP on the use of stable isotopes in EED assessment.

Further deliberations focused on prioritizing the research questions into which ones are of utmost urgency in terms of contribution to knowledge gap. Table 2 presents the list of research question chosen in order of priority and the most relevant stable isotope technique that may be deployed to answer the questions.

TABLE 1: EIGHTEEN POTENTIAL RESEARCH QUESTIONS MATCHED BY RELEVANT EED DOMAIN ADDRESSED AND FEASIBILITY FOR ASSESSMENT USING STABLE ISOTOPE TECHNIQUES

EED Domain	Question	Feasibility for using stable isotopes	Remarks
1 Dietary intake including breastmilk intake	What is the role of breastmilk lipid content and composition in EED and how could stable isotopes play a role in evaluating this?	Yes	Fatty acid transfer from mother to baby only as additional benefit when using stable isotopes
	Is EED associated with unmet nutrient requirements in the high risk population?	Yes	
2 Absorption and digestion	Absorption of nutrients: is there malabsorption of micronutrients in general (global), or specific micronutrients?	Yes	Zinc, vitamin A, Se, riboflavin, B12/folate
	Can stable isotopes be used to trace micronutrients fate in EED?	Yes	
	Is there significant macronutrient malabsorption and what is the role of digestion versus trans epithelial transport?	Yes	Priority highest for peptides, especially for essential AA, then lipids
	Do serial changes in disaccharidase function correlate with biochemical markers of growth and fat deposition?	Yes	
	How does EED change active transport in the intestine?	Yes	Breath test is not specific in case the molecule is glucose
	Can evidence of malabsorption of breast milk components be identified?	Yes	
3 Leakiness/ Permeability	Is there a biomarker specific to the mucosal integrity and permeability domain that can be assessed using stable isotopes?	Yes	Labeled LPS
	Can mucin production be assessed using stable isotopes?	?	No additional advantage of using stable isotopes
4 Gut inflammation	What is the role of the inflammatory component of EED on growth?	Yes	labelled arginine and tryptophan
	Could stable isotopes be used to assess intestinal proliferation and inflammation simultaneously?	?	Heavy water labelling or BT for epithelial cell proliferation, replacing biopsy
	Could stable isotopes be used to determine CCR9 and alpha 4 beta 7 integrin synthesis?	Yes	Protein enrichment
5 Translocation	Can we better understand the role of intestinally derived LPS or labelled bacteria in EED by giving labelled endotoxins?	Yes	Labeled LPS
6 Systemic inflammation	No specific question		

7 Host metabolic response	What is the host metabolic response (energy and protein metabolism, enterocyte production) in children with EED?	Yes	Heavy water labeling for protein synthesis, C labeled proteins for catabolism, deuterated AA
	Can tryptophan metabolism be assessed using intravenous versus oral isotopes and how is it related to protein synthesis?	Yes	Classical amino acid flux
8 Microbiome	Can the dynamics of gut microbiome community feeding and cross-feeding be assessed using stable isotopes?	Yes	Ideal for basic research
	What is the relation between the microbiome and host metabolism in EED?	Similar to the above	Ideal for basic research question
	How does a newborn acquire a gut microbiome and is there a role for stable isotopes to assess this?	No	Difficult

TABLE 2: LIST OF HIGH PRIORITY RESEARCH QUESTIONS

EED Domain	Question	Priority	Stable isotope options
Dietary intake	<p>What is the dietary (breast milk and complementary foods) intake in children with EED using stable isotopes?</p> <p>Is EED (based on L:M ratio) associated with energy intake/expenditure?</p>	High (is a risk factor for EED)	<p>Breast milk intake as an important component of infant and young children using deuterium.</p> <p>Food other than breastmilk (using standard dietary assessment tools [Direct observation, Quantitative 24 hour recall])</p> <p>Biomarkers of food intake such as choline for flesh intake, urinary 1- and 3-methylhistidine.</p>
Absorption and digestion	<p>Is there significant macronutrient malabsorption and what is the role of digestion versus trans epithelial transport?</p> <p>Do subjects with EED have lower protein digestion and absorption than subjects without EED?</p>	Highest for peptides, essential AAs, then lipids	<p>15N labeled protein such as albumin, or at least atriptide)</p> <p>13 C Spirulina</p>
	<p>Is there significant malabsorption of micronutrients in EED and is the malabsorption in EED global or specific?</p>	High	<p>Stable isotopes of zinc and vitamin A (<i>already one study underway in Bangladesh</i>)</p> <p>Stable isotopes of Selenium, Riboflavin, Vitamin B12 and Folate</p>
Leakiness	<p>Is there a biomarker specific to the mucosal integrity/permeability domain that can be assessed using stable isotopes?</p> <p>A question on the dose and host-adaptive-response to be considered in the design</p>	High	<p>Labeled LPS in combination with marker of systemic inflammation and oxidation</p> <p>Micro RNA in blood (host, exogenous)</p>
Gut inflammation	<p>What is the role of the gut inflammatory component of EED on growth?</p>	High	<p>15N labeled arginine and tryptophan</p>

Translocation	Can we better understand the role of intestinally derived LPS or labelled bacteria in EED by giving labelled endotoxins?	High	Labeled LPS (<i>safety and ethics approval may be issues to consider</i>)
Host metabolic response	What is the host metabolic response (growth [IGF, collagen X, leptin], energy and protein metabolism, enterocyte production)? Do subjects with EED have high resting energy expenditure?	Highest	Deuterium labeling for protein synthesis C labeled proteins for catabolism Deuterated amino acids
	Can tryptophan metabolism be assessed using iv versus oral isotopes and how is it related to protein synthesis?		High Amino acid flux study
Microbiome	Does EED modify bacterial metabolism of nutrients either in vitro or in vivo?	High	In vivo/vitro: repeated sampling after feeding ¹³ C/ ¹⁵ N labeled substrate (AA, carbohydrates, lipids) to see microbiota community are labeled/metabolism of the bacteria Difficult to implement in a field setting (research)
	What is the relation between the microbiome and host metabolism in EED?		Highest related to the one above C labeled proteins for catabolism Deuterated amino acids

DAY 4: FRIDAY 3 JUNE 2016:

DESIGN OF COORDINATED RESEARCH PROJECT ON THE USE OF STABLE ISOTOPE TECHNIQUES TO ASSESS EED. - The session was chaired by Mr Mark Manary, Washington University, St Louis.

The main discussion was on the inclusion criteria for individuals participating in a study linking EED to growth (stunting as the main outcome). After lengthy deliberations, it was agreed as follows (a detailed protocol is annexed separately).

A validated questionnaire such as the water, assets, maternal education and income index (WAMI) is preferred. When measured across eight sites, a 25% difference in WAMI was associated with a change in HAZ of 0.3 (95% CI 0.22, 0.55) cross-sectionally. Advantages of this strategy include ease of application, validation in a multisite study from different regions, and quantified relationships between two biomarkers of EED, LMZ and the number of pathogens in a non-diarrheal stool collected at the time of the LM test. The WAMI score was inversely associated with LMZ, with a 1 unit increase in WAMI (from lowest to highest) associated with a 0.43 lower LMZ. There was a statistically significant interaction between WAMI and age, with the greatest effect of WAMI on LMZ occurring among the older (9 and 15 month old) children. The number of pathogens in stool is directly correlated with the LMZ.

The selection of participants in real time faces the technical challenge of performing the principal biomarkers in real time and the lack of a single biomarker to adequately address the multiple components of the pattern of functional deficits (permeability, bacterial translocation, intestinal inflammation, systemic inflammation, metabolic alterations) that are believed to result in the acquisition of linear growth deficits. The traditional approach of the selection of a case and control population is challenging, given known changes in disease activity over relatively short periods of time (as measured by biomarkers) whereas the outcome of statural growth over the interval subsequent to the biomarker measure when controlling for baseline anthropometrics requires a minimal period of 3 months, with a 6 month period likely providing more meaningful measures of enduring linear growth deficits.

High risk nine month old children may be identified by demographic and behavioural risk factors listed above or on the basis of local data from the location from which the study is proposed. For example, a risk child might have a WAMI index in the bottom quartile (0.25 or below). To avoid the enrolment of children undergoing acute food deprivation or wasting, and therefore acute or chronic malnutrition children with a WHZ of <-2 will be excluded from enrolment. At baseline a physician exam will be done to exclude active symptomatic illness (diarrhoea, acute lower respiratory infection [ALRI], symptomatic malaria, tuberculosis [TB], etc.). Control children might be in the upper quartile of the WAMI index and be screened for acute, chronic and congenital illnesses. They will be enrolled at the same site as the at risk population.

Children exhibiting stuntedness, HAZ <-2 , is not a sufficient criterion for defining risk of EED, and children with severe stunting, HAZ <-3 , should be excluded for risk of attainment of the nadir in linear growth and non-responsiveness to further environmental insults/EED.

As EED is thought to exist as a process of dynamic injury and response with gradations of severity, a post hoc severity assessment employing the currently employed biomarkers of

EED will be attempted. The principal severity of the outcome assessment will be the change in linear growth for 3 months following the performance of the tests of EED. In addition in order to understand components of a multifactorial process that include intestinal inflammation, permeability defects, compromised epithelial cell metabolic capacity, and systemic inflammation. This will allow the reference population to serve as disease activity 0 and the activity of EED in the effect group to be comprised of mild/moderate and severe enteropathy.

Funding and participating institutions

Funding opportunities

Douglas Morrison presented on a call for proposal from the Biotechnology and Biological Sciences Research Council (BBSRC). Global Infections (GCRF Foundation Awards) theme will be relevant to EED. The Foundation Awards 600K UK pounds.

Cornelia Loechl presented on how IAEA funding for CRP works. Typically there are 5–8 research contract holders from Member States who receive each between 30 000–40 000 Euros over a total of 3–4 years. Including convening of research coordination meetings and technical contract services, total CRP budget is typically about 500 000–600 000 Euros over the CRP period. The timeline for the proposed CRP on EED is foreseen as follows: September/October 2016 IAEA internal approval; then publish call for proposals; identify research groups by end of 2016; hold 1st coordination meeting early 2017.

Ken Brown and Richard Elliot outlined the following entry points for exploration of funding opportunities that align with BMGF strategy:

- EED work package;
- Nutrition work package;
- Discovery package.

Participating institutions

The following countries/institutions were considered based on available capacity to deliver the CRP outcomes, at least in the first steps.

Potential institutions:

- Sierra Leone – Injala University;
- Zambia - University of Zambia;
- Bangladesh, ICDDR-B;
- Peru, ONG;
- India - Saint John’s Medical College of Bangalore;
- Malawi - University of Malawi;
- Bolivia – Instituto de Investigacion en Salud y Desarrollo;

- Uganda – Mwanamugimu Nutrition Unit, Mulago Hospital.

Potential institutions to provide technical support/advice –paired with Contractees:

- Agro-Paris Tech, France [can support sample analysis, especially for amino acids and protein];
- Sick Kids, Canada – **Malawi** [Sick Kids can also perform proteomics and stable isotope assays];
- University of South Australia [can support with stable isotope assays];
- University of Glasgow, UK [can support stable isotope assays];
- John Hopkins, USA – **Peru and Bolivia;**
- Washington University, St Louis, USA – **Sierra Leone;**
- Queen Mary University of London, UK - **Zambia;**
- University of Vienna, Austria [can support stable isotope assays to understand microbiota physiology];
- Duke University [can support systematic metabolomic analysis of amino acids, fatty acids/fatty acid metabolites, acylcarnitines and a variety of metabolic and GI hormones, growth factors];

Conclusions and way forward

1. Three domains will be given first priority in the proposed CRP. The domains will be investigated in a stepwise manner as outlined here:
 - Dietary intake (breast milk using the standardized deuterium dose to the mother technique; and non-breastmilk by direct measure of food intake)
Potential secondary outcome: anorexia;
 - Gut function and integrity:
 - i. Absorption (macronutrient and micronutrient digestion and malabsorption)
Potential secondary outcome: Bacterial metabolism of nutrients
Microbiome and host metabolism;
 - ii. Translocation (mucosal integrity/permeability; role of intestinally derived LPS or labelled bacteria);
 - iii. Inflammation (gut and systemic inflammation).
 - Host metabolic response:
 - i. Growth (IGF, Collagen, Leptin)
 - ii. Metabolism (tryptophan and protein synthesis)
2. A validated questionnaire such as the water, assets, maternal education and income index (WAMI) will be used as an entry criterion to classify children according to the level of risk for EED.
3. Other screening criteria can be based on risk factors such as L:M, pathogen pressure (TaqMan test), food insecurity, and animal source food intake.
4. The draft CRP proposal will be finalised by IAEA Secretariat for eventual approval and publishing to identify potential participants.

5. Douglas Morrison will draft a one page concept to be submitted as an expression of interest for the GCRF Foundation Awards.
6. Further suggestions were made for future consideration outside the scope of the CRP:
 - i. A descriptive review of similarities and differences between EED and IBD and other related diseases;
 - ii. Assessing the efficacy and acceptability of microbiome transplant to treat EED;
 - iii. Assessment of the effect of maternal protein restriction during pregnancy on offspring outcomes;
 - iv. Assessing the role of beneficial bacteria in the context of EED;
 - v. Linkage of the CRP to an animal model.

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ANNEX 1: MEETING AGENDA



Technical Meeting (TM-52335)
on
‘Application of Stable Isotope Techniques in Environmental
Enteric Dysfunction (EED) Assessment’
31 May – 3 June 2016
IAEA HQ, Vienna, Austria
Meeting room: Room M0E100, M Building

Background

Retarded linear growth, widely referred to as stunting, is rampant in low- and middle-income countries, affecting a total of 159 million children under the age of five years. The consequences of stunting include increased infant and child mortality and morbidity; increased risk of overweight, obesity and non-communicable diseases later in life; and low psychomotor development and lost economic potential. Inadequate nutrition and recurrent infection are the primary drivers of stunting. However, evidence now shows that all known nutritional interventions combined may only partially prevent stunting. Poor hygiene and absence of adequate sanitation may play a role but evidence to support a causal relationship is largely lacking. Living in poor sanitary conditions may induce gut dysfunction, referred to as environmental enteric dysfunction (EED). Retarded growth, altered gut microbiota, and decreased vaccine responsiveness are considered the most important consequences of EED. Developing non-invasive, practical, simple, and affordable tools to diagnose and characterize EED (with initial focus on clinical diagnosis, but ultimately aiming to have this at the community level) will allow better targeting of interventions to combat undernutrition in vulnerable populations. Using stable isotopes has significant potential in improving our understanding of EED and potentially could provide a non-invasive diagnostic test.

Purpose of the Meeting

To prepare a proposal for a Coordinated Research Project (CRP) on the ‘Application of Stable Isotope Techniques in Environmental Enteric Dysfunction (EED) Assessment’

The CRP should include the following sections:

1. Background Situation Analysis
2. Nuclear Component
3. CRP Overall Objective
4. Specific Research Objectives
5. Outcomes
6. Expected Outputs

7. Methodological Considerations
8. Analytical Techniques
9. Potential Participating Institutions
10. Other Resources Required

Tuesday, 31 May 2016

09:00 – 09:30 Opening session: Welcome, Nutrition at the IAEA, meeting objectives and expected outcomes, *Ms Cornelia Loechl, Section Head, Nutritional and Health-Related Environmental Studies (NAHRES) Section*

09:30 – 10:00 Discussions

10:00 – 10:30 Administration matters

10:30 – 11:00 Coffee Break

Overview of recent activities on EED - Chair: Mr Michael Freemark, Duke University, USA

11:00 – 11:20 IAEA Technical Meeting on EED (October 2015) *Mr Victor Owino, Nutrition Scientist, NAHRES*

11:20 – 11:30 Discussions

11:30 – 11:50 EED TM follow up discussions among researchers from IAEA, University of Vienna and University of Glasgow on opportunities for applying stable isotopes in EED assessment – *Mr Douglas Morrison, University of Glasgow, UK*

11:50 – 12:00 Discussions

12:00 – 12:30 Bill and Melinda Gates Foundation Gut Function Biomarker Consortium (March 2016) and Animal Models for the study of Gut Health (May 2016) Meetings – *Mr Richard Elliot, Bill and Melinda Gates Foundation, USA*

12:30 – 12:45 Discussions

12:45 – 14:00 Lunch Break

Update on technical developments in EED - Chair: Mr Ross Butler, University of South Australia, Australia

14:00 – 14:20 Review on EED biomarkers – *Ms Margaret Kosek, John Hopkins Bloomberg School of Public Health, USA*

14:20 – 15:00 Discussions

15:00 – 15:40 Discussions on promising EED Biomarkers

1. Kyrunenine/Tryptophan Ratio (10 minutes) - *Ms Margaret Kosek, John Hopkins Bloomberg School of Public Health, USA*

2. Plasma arginine/citrulline flux (10 Minutes) – *Mr Jahoor Farook, Baylor College of Medicine, USA*

3. Alanine-glutamine supplementation: N-methylnicotinamide (MNND) and β -aminobutyric acid (BAIBA) as markers of catch up growth in the context of systemic inflammation (10 minutes) – *Mr Asad Ali, Aga Khan University, Pakistan*

4. Insulin-like growth factor (10 minutes) – *Mr Michael Freemark, Duke University, USA*

5. Host RNA as a biomarker of EED (10 minutes) – *Mr Mark Manary, Washington University in St. Louis, USA*

15:50 – 16:10 Coffee Break

16:10 – 16:40 Application of breath tests in gut function assessments in the context of EED – *Mr Douglas Morrison, Scottish Universities Environmental Research Centre, UK*

16:40 – 17:10 Discussions

17:10 – 17:40 Wrap-up of Day 1

Wednesday, 1 June 2016

Defining the scope and priority areas for the proposed IAEA coordinated research project - Chair: *Mr Robert Bandsma, The Hospital for Sick Children, Canada*

09:00 – 09:30 Summarizing discussions from day 1 on recent evidence, gaps and biomarkers on EED and nutrition – *Ms Margaret Kosek, John Hopkins Bloomberg School of Public Health, USA*

09:30 – 10:00 Discussions

10:00 – 10:30 Coffee Break

10:30 – 10:45 What are the research questions relevant to EED from a clinical perspective? *Mr Paul Kelly, Queen Mary University of London/University of Zambia*

10:45 – 11:00 What are the research questions relevant to EED from a public health perspective? *Mr Ken Brown, Bill and Melinda Gates Foundation, USA*

11:00 – 12:30 Plenary Discussion on research questions and prioritizing research questions

12:30 – 14:00 Lunch Break

14:00 – 15:30 Funding opportunities and needs:
i) Biotech and Biol Sciences Res Council (BBSRC) – Global Infections (GCRF Foundation Awards) – *Mr Douglas Morrison, Scottish Universities Environmental Research Centre, UK*
ii) Other funding opportunities – *ALL*
iii) Thoughts on fundraising – *ALL*

15:30 – 16:00 Coffee Break

16:00 – 17:30 Plenary discussion on the research agenda for the IAEA Coordinated Research Project based on the following guiding themes:

Research question

Assumption addressed/priority

Measurement options

Matching study design

Candidate EED Biomarkers (in order of priority)

Techniques to employ (in order of priority)

Thursday, 2 June 2016

Defining the scope and priority areas for the proposed IAEA coordinated research project - Chair: Mr Thameed Ahmed, ICDDR-B, Bangladesh

09:00 – 10:30 Continued plenary discussion on the research agenda for the IAEA Coordinated Research Project based on the above guiding themes.

10:30 – 11:00 Coffee Break

11:00 – 12:30 Continued plenary discussion on the research agenda for the IAEA Coordinated Research Project based on the above guiding themes.

12:30 – 14:00 Lunch Break

Drafting of the Coordinated Research Project (CRP) Proposal – GROUP WORK - Chair: Mr Victor Owino, NAHRES

Split into working groups

14:00 – 15:30 Group work: Drafting the CRP proposal

15:30 – 16:00 Coffee Break

16:00 – 17:30 Group work: Drafting the CRP proposal

Friday, 3 June 2016

Plenary Discussions to refine Draft CRP Proposal – Chair: Mr Asad Ali, Aga Khan University, Pakistan

09:00 – 10:30 Plenary Group presentations of CRP Proposal drafts

10:30 – 11:00 Coffee Break

11:00 – 12:30 Plenary discussion to refine draft CRP Proposal/refining the CRP proposal

12:30 – 14:00 Lunch Break

14:00 – 15:30 Discussions on potential partners

15:30 – 16:00 Recommendations, next steps and closing - Ms Cornelia Loechl, Section Head, NAHRES

ANNEX 2: MEETING PARTICIPANTS



Technical Meeting
on
**Application of Stable Isotope Techniques in Environmental Enteric
Dysfunction (EED) Assessment
(TM-52335)**

IAEA Headquarters Vienna, Austria
31 May – 3 June 2016
Meeting room: MOE100 (M-Building, ground floor)

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