**Technical meeting on the 'Application of Stable Isotope Techniques in Environmental Enteric Dysfunction (EED) Assessment', IAEA Headquarters Vienna, 31 May to 3 June 2016 (TM-52335)**

**Setting:**

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[1]. 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[2]. 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[3,4], 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.

**Why:**

- EED adversely affects intestinal structure and function and is characterised by villi blunting, chronic gut and systemic inflammation, altered gut permeability, bacterial translocation and nutrient malabsortption[5].
- EED is linked to defects in carbohydrate[6], amino acid[7,8], fatty acid[9] and vitamin B12[10] absorption. Gut mucosal damage can affect lactase activity, thereby limiting the utilisation of lactose, the major carbohydrate in breast milk[11]. Some amino acids, especially glutamine, which are involved in maintenance of gut integrity show an overall lower concentrations in malnourished pigs compared with reference models [8]. Increased arginine catabolism without an increase in arginine flux has been observed in Indian women of childbearing age compared to their peers Jamaican and American women.[12]. Pancreatic lipase activity is diminished in EED[9]. These effects are more pronounced in severe acute malnutrition (SAM) with kwashiorkor[6,9,10]. How much of altered nutrient metabolism is attributable to undernutrition on one hand and EED on the other remains to be investigated.
- Insulin growth factor (IGF-I) is diminished in EED, likely due to increased levels of systemic cytokines, cortisol and Insulin growth factor binding-protein that act in combination to inhibit protein synthesis and growth failure[13].
- Altered gut microbiota affects gut function, however, the causal pathways require further elucidation.
- EED affects host intestinal immunity, which could be enhanced by for example oral poliovirus vaccine[14].
- EED affects vaccine efficacy via reduced gut absorptive function, micronutrient deficiencies and pathogen-vaccine competition[15].
- EED is exacerbated by poor infant feeding practices such as early introduction of complementary foods[11]. Emerging evidence suggests that exclusive breastfeeding is protective against gut inflammation associated with EED[16].
- Despite the significance of EED to infant and child nutrition and health, biomarkers and simple diagnostic techniques for the definition and classification of EED are lacking. The gold standard for diagnosing EED, intestinal biopsy, is too invasive[5].

**Hypothesis:**

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. Localizing and assessing the extent of the gastro-intestinal damage may be possible (reduced digestive/absorptive intestinal capacity, raised small and large intestinal permeability, bacterial translocation 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.

**What is the need?**

- Better understanding of the underlying causes of EED
- Point-of-care test in EED diagnosis that can be applied in a resource limited environment
- Combination of labelled substrates to assess gastrointestinal transit time and small-intestinal bacterial overgrowth
- Bacterial translocation through single cell labelling
- Validation of the potential diagnostic techniques.

**Purpose of meeting:**

In light of the foregoing, the IAEA will host a meeting of about 15 experts from universities and research organizations from 31 May -3 June 2016 in Vienna, Austria to design a Coordinated Research Project (CRP) on the application of stable isotope techniques in EED assessment. The CRP will be based on the recommendations of the Technical Meeting on EED, the Microbiome and Undernutrition that was held at the IAEA Headquarters, Vienna, 28 to 30 October 2015. The meeting concluded that several gaps in knowledge such as the classification of EED and better understanding of the underlying causes of EED require urgent attention. The IAEA was encouraged to foster the use of stable isotopes for assessments in three main areas: digestive and absorptive capacity of the gut, bacterial translocation, and body composition as a proxy indicator of dietary quality since changes in food nutrient composition (especially protein, lipids and sugars) easily modify lean mass accrual and adiposity. This may have implications on risk of morbidity (mostly related to overweight and obesity in the context of nutrition transition). The specific aims of the meeting will be:-

- To define the specific research questions, and then decide on what the CRP will look like: which studies to be conducted and which stable isotope techniques will be deployed.
- To review and critically analyse the viability of recommendations of the October 2015 EED TM with specific focus on application of stable isotope techniques
- To review and update emerging work in EED diagnosis since the October 2015 EED TM
- To identify the most suitable stable isotope method and substrate combination to be used to answer identified research questions.
- To develop a coordinated research project proposal, based on identified research questions, for the use of stable isotope techniques in assessment of EED

The following thematic areas will inform discussions at the meeting:

*Potential Biomarkers of EED:*

While nutrient 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 factors related EED, namely:

- intestinal permeability and nutrient absorption (e.g., lactulose/mannitol or lactulose/rhamnose tests, $^{13}C$ sucrose breath test)

- bacterial translocation (e.g., lipopolysaccharides)
- intestinal inflammation (e.g., myeloperoxidase, calprotectin)
- systemic inflammation (e.g., C-reactive protein, alpha 1-acid glycoprotein, cytokines)
- functional enterocyte mass (conversion of arginine to citrulline to assess damaged gut)
- intestinal repair (e.g., promoter glucagon-like peptide-2 [GLP-2])
- mucosal immune underachievement (e.g., kynurenine/tryptophan ratio)
- alterations in microbiota diversity (e.g. single cell labelling).

*Potential stable isotope techniques to assess one or more of EED underlying causes:*

- $^{13}$C-sucrose breath test is a promising future technique to assess gut digestive and absorptive function
- Combined $^{13}$C and $H_2$ breath tests to assess colonic fermentation and small intestinal bacterial overgrowth
- $^{13}$C and $^{2}$H as an intrinsic label (including potential intrinsic labelling of foodstuffs) to assess carbohydrate and protein/amino acid (e.g. 13C-algae) digestion and absorption
- Bacterial translocation through single cell labelling

**Further research questions:**

- Study by Prentice in the Gambia indicates that there is some spontaneous recovery from EED in later childhood/adolescence without specific intervention. How much recovery is possible is not yet known.
- How much of altered nutrient metabolism is due to undernutrition vs EED.
- What is the causal direction: (i.e. altered microbiota affecting gut function and bacterial translocation, or vice versa?).

**Organization hosting the meeting:**

The IAEA is a technical agency that complements the work of other UN agencies, Non-Governmental Organizations and other major players in nutrition and health by encouraging the use of nuclear techniques to develop and evaluate interventions used to combat malnutrition in all its forms throughout the life course. The IAEA has supported the use of stable isotope techniques and related techniques to assess breastfeeding patterns and to measure body composition, physical activity and metabolic risk factors for non-communicable diseases in school children and adolescents. Additionally, the IAEA has supported the application of $^{13}$C-urea breath test used to diagnose and monitor *Helicobacter pylori* infection in the stomach.

**References**


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