

Nutritional and Health-Related Environmental Studies (NAHRES)

The Efficacy of Amino Acid Supplementation in Treating Environmental Enteric Dysfunction Among Children at Risk of Malnutrition

Brief summary

Environmental Enteric Dysfunction (EED) is caused by subclinical infection due to enteric pathogens that thrive in conditions of poor sanitation and hygiene. It is characterized by villous atrophy, crypt hyperplasia, increased intestinal permeability, inflammatory cell infiltrate, and possibly nutrient malabsorption. The increased gut permeability, often measured by oral lactulose and mannitol test, is related to gut barrier dysfunction, with translocation of pathogenic organisms and endotoxins. EED is known to be an important component of the causal origin of undernutrition and stunting in infants in low-and-middle income countries (LMICs) as it reduces the efficacy of nutritional interventions with food supplements to restore normal growth and allow catch-up growth. In order to improve these conditions that contribute to poor child growth it is required to minimise EED and improve nutrition by combining improvements in water quality, sanitation and nutritional intervention. Protein supply is a major contributor to support normal growth and catch-up growth, but very few data are available on the impact of EED on protein and amino acid requirement, protein digestion and amino acid absorption and metabolic availability to support growth. Targeted nutrient therapies (such as supplementation with specific amino acids) may be one such approach. The objective of this CRP is to use a combination of nuclear techniques to assess the response of EED to a short course of targeted amino acid supplementation. Specifically, the CRP will aim to 1) develop a combined minimally invasive protocol for the application of the dual stable isotope tracer method for protein digestion (DSIT) and the 13-C Sucrose Breath Test (13-C SBT) to assess nutrient absorption in the context of EED; 2) test the applicability of combined DSIT and 13-C SBT to understand the interaction between EED and protein metabolism and; 3) assess the efficacy of amino acid supplementation on the treatment of EED in children. The CRP will provide the evidence base to enable Member States to formulate policies to improve optimal child growth and development. Further, the CRP results would contribute to the revision of the global clinical management and feeding regimens for stunted children complicated with or without EED, for the promotion of linear growth and the reduction in morbidity and mortality of vulnerable children in LMICs.

Background

More than 150 million children < 5 years of age, or approximately a fifth of the global population, are estimated to be stunted (defined as a length/height-for-age Z-score (LAZ/HAZ) < -2 using WHO Child Growth Standards). Stunting has been linked to an increased risk of morbidity, diminished cognitive development, poorer educational outcomes, lower economic productivity and earnings in adulthood, and is an important risk factor for childhood death. Despite the large global burden of stunting, its etiology, including the role of environmental enteric dysfunction (EED), remains largely unknown. EED (previously termed tropical or environmental enteropathy) is an acquired subclinical condition of the small intestine first recognized in the 1960s in apparently healthy adults living in LMICs. The condition is characterized by abnormal morphology and physiology of the small intestine, with mucosal inflammation, villous blunting, altered barrier integrity, and reduced intestinal absorptive capacity. Both early and recent investigations into EED, including the Malnutrition and Enteric Disease (MAL-ED) consortium, have demonstrated that the condition is practically ubiquitous among populations living in LMICs (4, 5). Currently, EED is thought to result from chronic exposure to enteropathogens due to living in poor water, sanitation, and hygiene (WASH) conditions (e.g., contaminated water, lack of basic sanitation and handwashing infrastructure).

While highly prevalent, much remains unknown regarding the diagnosis and therapy of EED. It is postulated that EED may limit responses to dietary interventions, explaining the limited effect of nutritional supplementation alone to improve growth outcomes. Proposed mechanisms linking EED and subsequent poor growth and development include the following: decreased nutrient intake,

altered nutrient absorption, increased intestinal permeability with subsequent bacterial translocation and immune stimulation, increased energy expenditure due to chronic inflammation, and the growth-inhibiting effects of chronic inflammation (e.g., inhibition of IGF-1). Furthermore, nutrient malabsorption and chronic inflammation from EED are hypothesized to affect brain development, inducing lasting negative effects on cognition and educational achievement (although evidence for this is much more limited compared to growth).

Protein supply is a major contributor to support normal growth and catch-up growth, but very few data are available on the impact of EED on protein and amino acid requirements, protein digestion and amino acid absorption and metabolic availability to support growth. Under these conditions, there is a need of non-invasive methods to characterize EED, to measure intestinal permeability, and to record the efficacy of protein supply to support infant recovery and growth. Targeted nutrient therapies (such as supplementation with specific amino acids) may be one such approach.

Protein quality refers to the ability of a dietary protein, when eaten in the required daily quantity, to meet the daily indispensable amino acid (IAA) requirements. The latter have been shown to be 2-3 times higher than previously thought, by the 2007 WHO/FAO/UNU Expert Consultation. The amino acid composition of the protein (also called its score) is therefore important. Another important dimension of protein quality is its digestibility and absorption. This varies by food proteins. Since IAA digestion and absorption occurs in the small intestine, faecal samples that are collected to represent what is not digested/absorbed give a false value of IAA digestibility, as bacteria in the colon can modify the amount of nitrogen or IAA that are present. Ideally, digestibility and absorption should be measured at the level of the small intestine. This is called ileal digestibility and is measured for each IAA. When the IAA ileal digestibility is multiplied by the IAA score of the protein, this yields the DIAAS; the IAA with the lowest DIAAS value represents the protein DIAAS value.

Disaccharidases, including sucrose-isomaltose (SI) are crucial for the digestion and the subsequent absorption of carbohydrates. Lower SI activity measured by SBT in children with intestinal mucositis compared to healthy controls. Lower SI activity detected in Australian Aboriginal children with and without diarrhoeal disease compared to non-Aboriginal children (Ritchie et al 2009). Villus atrophy in coeliac disease is associated with reduced expression and activity levels of intestinal disaccharidases including SI. Standard measure of intestinal disorders associated with carbohydrate malabsorption due to primary or secondary defects in SI is based on endoscopic small bowel biopsies and assessment of disaccharidase activities ex-vivo.

Two IAEA CRPs are providing data that inform these frameworks. The first CRP is evaluating the digestibility of legume proteins in adults and children using a dual tracer (deuterium and ¹³C-carbon) stable isotope technique. It will generate information on the quality of protein derived from legumes commonly consumed in LMICs and how this contributes to meeting protein requirements. It is worth pointing out that, in this CRP, it was found that the specific digestibility of sulphur AA and threonine is low and in the range of 50 %. Whether EED is contributing to this low digestibility and absorption is unknown. The second CRP is evaluating stable isotopes techniques for accurately quantifying gut dysfunction in the context of EED. The ¹³C sucrose breath test optimised in this CRP provides a tool to quantify the degree of gut dysfunction noninvasively in children with EED.

This advance potentially allows case selection and rapid evaluation of sensitive functional outcome of nutritional interventions. The aim of this CRP is to use a combination of nuclear techniques to assess the response of EED to a short course of targeted amino acid supplementation.

The CRP will be implemented in TWO key steps:

STEP I: Develop and test in both animal model and in children a combined minimally invasive protocol for the application of the dual stable isotope tracer method for protein digestion (DSIT) and the ¹³-C Sucrose Breath Test (¹³-C SBT) to assess nutrient absorption in the context of EED.

STEP II: Intervention studies to test the efficacy of supplementation with a suite of amino acids in the treatment of EED in children in LMICs.

Overall Objective

To use a combination of existing nuclear techniques to assess the response of EED to a short course of targeted amino acid supplementation among children.

Specific Research Objectives (purpose)

1. To develop and test the applicability of a combined minimally invasive protocol for the dual stable isotope tracer method for protein digestion (DSIT) and the 13-C Sucrose Breath Test (13-C SBT) to assess nutrient absorption in the context of EED.
2. To assess the interaction between EED and protein metabolism
3. To assess the efficacy of amino acid supplementation on the treatment of EED in children.

Expected outcome

New information on the efficacy of interventions to treat EED generated and used as the basis to provide guidance on the design and implementation of programmes to reduce stunting in LMICs.

Expected outputs

1. A combined minimally invasive protocol for the application of the dual stable isotope tracer method for protein digestion (DSIT) and the 13-C Sucrose Breath Test (13-C SBT) to assess nutrient absorption in the context of EED.
2. New data on the interaction between EED and protein metabolism.
3. New data on the efficacy of amino acid supplementation on the treatment of EED in children.
4. Publications in the form of scientific reports, peer-reviewed papers and conference abstracts.

Analytical techniques to be used

This CRP will primarily apply, in combination, two non-invasive stable isotope techniques, namely a dual tracer (deuterium and 13-carbon) method for assessment of protein digestion and a stable isotope based (13-carbon sucrose) breath test to assess nutrient absorption as an indicator of intestinal function. The dual tracer stable isotope technique enables a quick, non-invasive and accurate assessment of the digestion and absorption of protein from the diet. A test food is labelled with an isotope (typically deuterium or 13C) at the point of food preparation or during production in the field. For example, to determine amino acid absorption from a plant, deuterium is added to irrigation water during growth. Subsequently, a test meal is prepared from the labelled edible portion of the test food, which is then consumed by the study participant along with another isotope (carbon-13)-labelled highly digestible protein source. Blood samples are collected before the test meal is consumed and again five, six, seven and eight hours after the test meal and the blood amino acid concentration is analysed. The appearance of labelled amino acids in the blood relative to the test meal are used to calculate the digestibility of the legume protein. The 13C Sucrose Breath Test (13C-SBT) is based on the simple principle that, in the intestine, sucrose is broken down by a brush border enzyme called sucrase into glucose and fructose. When these are oxidized for use by the body, carbon-13 dioxide (13CO₂) and water are produced. In abnormal circumstances, as in EED, sucrase enzyme activity and therefore 13CO₂ production may be reduced or delayed.

Proposal submission forms

Research institutions in Member States interested in participating in this CRP are invited to submit proposals directly to the Research Contracts Administration Section (NACA) of the International Atomic Energy Agency or to Mr Victor Owino: V.Owino@iaea.org. The forms can be downloaded from the CRA website: <https://www.iaea.org/services/coordinated-research-activities/how-to-participate>

For more information about research contracts and research agreements, please visit our web-site.

Selection criteria

Member States will be selected to participate in the CRP based on:

1. Demonstration of the existence of suitably qualified staff and adequate laboratory capacity to implement the activities.
2. Previous work in the area of EED and protein metabolism will be added advantage.
3. Demonstration of strategic partnerships both locally and internationally for technical and analytical support will also be a strength.

Deadline for submission of proposal

Proposals must be received no later than 31 December 2020. Transmission via Email is acceptable if all required signatures are scanned.

For additional information, please contact:

Mr Victor Owino
Nutrition Specialist
Nutritional and Health-related Environmental Studies Section
Division of Human Health
International Atomic Energy Agency (IAEA) A-1400 Vienna, Austria
Phone: + 43 1 2600 21657
V.Owino@iaea.org

References

1. Owino V, Ahmed T, Freemark M, Kelly P, Loy A, Manary M, Loechl C. Environmental Enteric Dysfunction and Growth Failure/Stunting in Global Child Health. *Pediatrics*. 2016;138: e20160641.
2. Butler RN, Kosek M, Krebs NF, Loechl CU, Loy A, Owino VO, Zimmermann MB, Morrison DJ. Stable Isotope Techniques for the Assessment of Host and Microbiota Response During Gastrointestinal Dysfunction. *J Pediatr Gastroenterol Nutr*. 2017;64:8-14
3. Black RE, Allen LH, Bhutta ZA, Caulfield LE, de Onis M, Ezzati M, et al. Maternal and child undernutrition: global and regional exposures and health consequences. *Lancet*. 2008;371(9608):243-60.
4. Petri WA, Naylor C, Haque R. Environmental enteropathy and malnutrition: do we know enough to intervene? *BMC Med*. 2014;12(1):187.
5. Syed S, Ali A, Duggan C. Environmental Enteric Dysfunction in Children: A Review. *J Pediatr Gastroenterol Nutr*. 2016;63(1):6-14.
6. Lunn PG, Northrop-Clewes CA, Downes RM. Intestinal permeability, mucosal injury, and growth faltering in Gambian infants. *Lancet*. 1991;338(8772):907-10.
7. Kosek M, Haque R, Lima A, Babji S, Shrestha S, Qureshi S, 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*. 2013;88(2):390-6.
- Humphrey JH. Child undernutrition, tropical enteropathy, toilets, and handwashing. *Lancet*. 2009;374(9694):1032-5.
8. Lin A, Arnold BF, Afreen S, Goto R, Huda TM, Haque R, et al. Household environmental conditions are associated with enteropathy and impaired growth in rural Bangladesh. *Am J Trop Med Hyg*. 2013;89(1):130-7.
9. Bhutta ZA, Ahmed T, Black RE, Cousens S, Dewey K, Giugliani E, et al. What works? Interventions for maternal and child undernutrition and survival. *The Lancet*. 2008;371(9610):417-40.
10. Kupka R, Manji KP, Bosch RJ, Aboud S, Kisenge R, Okuma J, et al. Multivitamin supplements have no effect on growth of Tanzanian children born to HIV-infected mothers. *J Nutr*. 2013;143(5):722-7.
11. Blakstad MM, Smith ER, Etheredge A, Locks LM, McDonald CM, Kupka R, et al. Nutritional, Socioeconomic, and Delivery Characteristics Are Associated with Neurodevelopment in Tanzanian Children. *J Pediatr*. 2019;207:71-79.e8.(doi):10.1016/j.jpeds.2018.10.066. Epub Dec 14.
12. Louis-Auguste J, Besa E, Zyambo K, Munkombwe D, Banda R, Banda T, et al. Tryptophan, glutamine, leucine, and micronutrient supplementation improves environmental enteropathy in Zambian adults: a randomized controlled trial. *The American Journal of Clinical Nutrition*. 2019.