Appendix 1: Unified Phase 2 Study Protocol

Phase 2 study protocol: The ^{13}C-SBT, LR test, and D_2O dilution to assess body composition will be completed during a single visit with each participant. The study team will screen for recent diarrhea, antibiotic usage, and anti-inflammatory usage in the past month (anti-inflammatories are known to induce transient intestinal permeability[1] as well as enterocyte injury). If any of these are reported by the parents, the appointment will be schedule one month from the date of the diarrheal episode or antibiotic/anti-inflammatory use.

Guardians will be asked to fast their child for one hour prior to the appointment. Time will be given for the child to settle and adjust to their surroundings, including time for the child to play with, and become familiar with, the breath sampling equipment. Two baseline breath collection measurements will be completed, using a cannula apparatus for breath collection, consisting of a piece of PVC tubing taped just underneath the child's nostril and controlled with a 3-way tap, or, if the field team prefers it, a face mask and breath collection bag. Exhaled breath will then be transferred to Labco Exetainers (evacuated tubes Labco Order No 428W or 439W, Burlington, North Carolina, USA) either by positive displacement (syringe) or using evacuated exetainer (bag). A baseline urine sample will be collected, and, for sites using deuterium dilution technique with analysis of saliva samples to assess body composition[2] (Bangladesh, India, Kenya, and Zambia), a baseline saliva sample will be collected.

To avoid $^{13}C_{12}$-sucrose loss, the $^{13}C_{12}$-sucrose solution and the LR solution will be administered separately. First, the 40 mg/mL $^{13}C_{12}$-sucrose solution will be administered at a dosage of 10µL per kg body weight (spiked in 1mL of drinking water). For example, for a child weighing 10kg, the dosage will be 100µL spiked in 1mL of drinking water. This will be followed a 2mL chaser of drinking water to rinse the vial.

Secondly, a 9mL of drinking water spiked with 1g lactulose and 0.2g rhamnose will be administered, and again a 2 mL chaser of drinking water will be used to rinse the vial. The total volume (1mL sucrose solution + 2mL chaser + 9mL LR solution + 2mL chaser) is 14 mL.

If the child spits out, vomits, or fails to swallow all the sugar solution, the test will be canceled and re-scheduled. After the child has consumed the sugars, a standard dose of deuterium oxide (D_2O) based on IAEA protocols will also be administered to the child. As soon as both the sugar solutions and the D_2O have been administered the child will be encouraged to drink water. After 90 minutes, the child will be given a standardized meal. The choice of standardized meal will be site-specific but may include egg, legumes, or rice and will not include any sugary foods or dairy products.

Breath sample collection: Using a stopwatch, breath samples will be collected every 15 minutes for the first 90 minutes following the administration of the sugar solution, and then every 30 minutes until 240 minutes (4 hours) have passed.

Urine sample collection: All urine passed in the 30-120 minutes of the test will be collected and combined for analysis, and any urine passed in the 120-300 minutes of the test will be collected and combined separately. The volume of all voids will be recorded. Both 2- and 5-hour LR tests are common in the literature[3–5], with some evidence that 2-hour urine collections better capture small intestinal, rather than colonic, absorption[5]. Here, both measures will be collected to enhance comparability with prior reports. 5-hour recoveries of lactulose and rhamnose will be calculated as the weighted average of the 30-120 and 120-300-minute samples. Samples will either be

stored with chlorhexidine or will be collected without preservative and stored immediately at -80. We will examine whether chlorhexidine results in interference during LC-MS/MS analysis.

Saliva sample collection: For sites measuring D_2O via saliva, the sample will be collected between 180 and 210 minutes of the test.

Plasma sample collection: After the breath/urine collection is complete, a second visit will be scheduled with study families to collect a plasma sample. Families will be asked to fast the child for 6 hours prior to the blood draw, and 2mL of blood will be collected in K2 EDTA. Following collection, the sample will be centrifuged and stored at -80C pending analysis.

Anthropometry and questionnaire data: The length, weight, and head circumference of each child will be measured, as will the height and weight of their mothers. Standard questionnaires to capture key socio-economic[6] and demographic information, child dietary diversity (including recent consumption of C4 foods (e.g. maize, sugar cane, and sorghum or millet), and household food insecurity[7], will be administered. Three months following the initial test, a third appointment will be made with the family to re-measure anthropometry, and to administer a questionnaire asking about morbidity over the past three months. Key data are summarized in **Appendix 2**.

Additional site activities: In addition to coordinated study activities, some activities will only be undertaken by one, or a sub-group, of study sites. In one site (Peru), the ^{13}C-SBT test will be repeated after 2 days on 40 children, to assess test reproducibility. Several secondary EE biomarker assays of interest were also identified, to be assayed by sites resource permitting. These include, in order of priority, plasma fatty acid binding protein (FABP); plasma LPS binding protein (LPS-BP); and stool alpha-1-antitypsin (AAT) and stool myeloperoxidase (MPO).

Laboratory analyses: Breath samples from four sites (Peru, Jamaica, Kenya, and Zambia) will be sent to Dr. Roger Yazbek at the South Australian Breath Analysis Research Laboratories (SABAR Lab) where they will be analyzed via ABCA Isotope Ratio Mass Spectrometry. To minimize of inter-platform variability in dual-sugar testing[8], urine samples for lactulose and rhamnose will be performed either on a single platform, on two or more standardized platforms (to be determined). Breath sample analysis for Bangladesh and India will be analyzed at Saint John's Research Institute, Bangalore.

Deuterium dilution testing: Based on available instrumentation, one site (India) will assess body composition analysis through D_2O analysis of urine, and four sites (Peru, Jamaica, Bangladesh, Kenya, and Zambia) will use saliva. Deuterium equilibrates faster in saliva compared to urine[9] and deuterium dilution analysis in saliva requires a higher dose and different instrumentation (Fourier Infrared Spectroscopy versus Isotope Ratio Mass Spectrometer) compared to urine sampling. However, the collection of urine or saliva for total body water analysis has been standardized and validated, with comparable performance demonstrated in infants younger than those we propose to measure in this study[10]. Deuterium enrichment of urine will be assessed using a Delta V advantage Isotope Ratio Mass Spectrometer (Thermo Fisher scientific Inc)[11]. Saliva samples will be measured for their deuterium enrichment in duplicate by Fourier Transformed Infrared Spectrophotometry (4500t FTIR, Agilent Technologies, CA, USA).

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