Infant Nutritional Status and Markers of Environmental Enteric Dysfunction are Associated with Midchildhood Anthropometry and Blood Pressure in Tanzania

Lindsey M. Locks, ScD, MPH[1], Ramadhani S. Mwiru, ScD, MSc[2], Expeditho Mtisi, PhD, MSc, MS[3], Karim P. Manji, MBBS, MMED, MPH[4], Christine M. McDonald, MS, ScD[5], Enju Liu, PhD[6], Roland Kupka, ScD[1,7], Rodrick Kisenge, MD, MMed, PhD[4], Said Aboud, MD, PhD[8], Kerri Gosselin, MD[5], Matthew Gillman, MD, SM[1,9], Andrew T. Gewirtz, PhD[10], Wafaie W. Fawzi, DrPH, MBBS, MPH, MS[1,11,12], and Christopher P. Duggan, MD, MPH[1,4,12]

Objective To assess whether growth and biomarkers of environmental enteric dysfunction in infancy are related to health outcomes in midchildhood in Tanzania.

Study design Children who participated in 2 randomized trials of micronutrient supplements in infancy were followed up in midchildhood (4.6-9.8 years of age). Anthropometry was measured at age 6 and 52 weeks in both trials, and blood samples were available from children at 6 weeks and 6 months from 1 trial. Linear regression was used for height-for-age z-score, body mass index-for-age z-score, and weight for age z-score, and blood pressure analyses; log-binomial models were used to estimate risk of overweight, obesity, and stunting in midchildhood.

Results One hundred thirteen children were followed-up. Length-for-age z-score at 6 weeks and delta length-for-age z-score from 6 to 52 weeks were associated independently and positively with height-for-age z-score and inversely associated with stunting in midchildhood. Delta weight-for-length and weight-for-age z-score were also positively associated with midchildhood height-for-age z-score. The 6-week and delta weight-for-length z-scores were associated independently and positively with midchildhood body mass index-for-age z-score and overweight, as was the 6-week and delta weight-for-age z-score. Delta length-for-age z-score was also associated with an increased risk of overweight in midchildhood. Body mass index-for-age z-score in midchildhood was associated positively with systolic blood pressure. Serum anti-flagellin IgA concentration at 6 weeks was also associated with increased blood pressure in midchildhood.

Conclusions Anthropometry at 6 weeks and growth in infancy independently predict size in midchildhood, while anti-flagellin IgA, a biomarker of environmental enteric dysfunction, in early infancy is associated with increased blood pressure in midchildhood. Interventions in early life should focus on optimizing linear growth while minimizing excess weight gain and environmental enteric dysfunction. *(J Pediatr 2017;187:225-33).*

Trial registration ClinicalTrials.gov: NCT00197730 and NCT00421668.

The prevalence of childhood overweight and obesity is increasing in almost every region of the world.[1] At the same time, several low- and middle-income countries continue to confront high rates of undernutrition and impaired child growth, and are thus simultaneously facing a dual burden of malnutrition among children. In low-resource settings in particular, where 156 million children under 5 years of age experience chronic undernutrition,[2] the widespread prevalence of undernutrition and impaired growth in early life may have profound impacts on long-term health outcomes. Children who suffer from undernutrition in early life are more likely to have reduced adult stature, impaired cognitive development, lower earning potential, and if they are women, also a higher risk of birth complications.[3] Furthermore, the developmental origins of health and disease paradigm proposes that perturbations in the homeostasis of a

BMIZ	Body mass index-for-age z-score
EED	Environmental enteric dysfunction
HAZ	Height-for-age z-score
LAZ	Length-for-age z-score
LPS	Lipopolysaccharide
WAZ	Weight-for-age z-score
WHO	World Health Organization
WLZ	Weight-for-length z-score

From the [1]Department of Nutrition, Harvard T.H. Chan School of Public Health, Boston, MA; [2]Management and Development for Health; [3]African Academy for Public Health; [4]Departments of Pediatrics and Child Health, Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania; [5]Division of Gastroenterology, Hepatology and Nutrition; [6]Clinical Research Center, Boston Children's Hospital, Boston, MA; [7]UNICEF Headquarters, New York, NY; [8]Department of Microbiology and Immunology, Muhimbili University of Health Sciences, Dar es Salaam, Tanzania; [9]Department of Population Medicine, Harvard Medical School, Boston, MA; [10]Institute of Biomedical Sciences, Georgia State University, Atlanta, GA; [11]Department of Epidemiology; and [12]Department of Global Health and Population, Harvard T.H. Chan School of Public Health, Boston, MA

Supported by the National Institutes of Health (R01 HD043688-01, R01 HD048969-01, K24HD058795, K24 DK104676, and 2P30 DK040561), Bill and Melinda Gates Foundation (OPP1066203), and the Feed the Future Food Innovation Lab for Nutrition which is funded by the United States Agency for International Development (USAID; AID-OAA-L-10-00006). C.D. serves as an Editorial Board member for *The Journal of Pediatrics*. The other authors declare no conflicts of interest.

http://dx.doi.org10.1016/j.jpeds.2017.04.005

developing fetus or infant result in long-term changes affecting that individual's risk of future diseases.[4] Several studies have documented an increased risk of cardiometabolic risk factors, such as high blood pressure, dyslipidemia, and insulin resistance, among individuals who were born at a low birth weight[5-8] and among those with a low body mass index-for-age z-score (BMIZ) in early childhood.[6,9,10] The relative importance of perinatal versus infant growth in long-term health outcomes, however, is unknown. Furthermore, much of the evidence on the relationship between early life growth and adult health outcomes comes from wealthy nations,[5,9,11] despite the growing evidence that the relative influence on growth in early life on later-life health outcomes may be population specific.[12] Specifically, there is limited research from sub-Saharan Africa, where the risk factors for poor growth as well as chronic disease outcomes are likely quite different from those in developed nations.

Recent data suggest that environmental enteric dysfunction (EED), a subclinical condition of the small intestine characterized by villous atrophy, crypt hyperplasia, increased intestinal permeability, inflammatory cell infiltrate, and malabsorption, is associated with growth failure and stunting.[13-15] Some have also hypothesized that EED may increase the risk for cardiometabolic diseases in later life, including insulin resistance and hypertension.[16] Although the gold standard for assessing EED is small bowel biopsy, the invasive and expensive nature of these procedures has led researchers to pursue biomarkers that are more suitable for widespread use in community-based settings. Our group has assessed previously the use of anti-flagellin and anti-lipopolysaccharide (LPS) IgA and IgG as biomarkers of EED. We found that anti-LPS and anti-flagellin IgA and IgG concentrations increased over the first year of life in Tanzanian infants, that the concentrations in Tanzanian infants were significantly higher than in healthy controls in Boston, and that elevated anti-LPS and anti-flagellin IgA and IgG concentrations were associated with an increased risk of underweight in infancy.[17] In our current study, we assess whether these biomarkers continue to be associated with growth and health outcomes in midchildhood.

Given the limited research on how nutritional status and EED in early infancy relate to long-term growth and cardiometabolic risk factors in children in low-resource settings, we collected data from a cohort of children in Dar es Salaam, Tanzania, to assess the relative importance of infant growth and nutritional status at age 6 weeks of age and change from 6 to 52 weeks of age, as well as biomarkers of EED in infancy, on health and growth outcomes in midchildhood.

Methods

The study sample included children born in Dar es Salaam, Tanzania, who participated in 1 of 2 randomized controlled trials of multiple micronutrient supplementation to infants. The first trial (ClinicalTrials.gov: NCT00197730) randomized 2387 infants born to HIV-infected mothers to either daily administration of multiple micronutrients (vitamins B complex, C, and E) or placebo at 6 weeks of age.[18] Randomization of infants occurred between August 2004 and November 2007; follow-up ended in May 2008. The micronutrient supplements did not show an effect on mortality, morbidity, or child growth.[18,19] The second trial (ClinicalTrials.gov: NCT00421668) was implemented with a 2 × 2 factorial design assessing the effect of zinc, zinc plus multivitamins (the same combination of vitamins B complex, C, and E as described), multivitamins alone, or placebo among 2400 infants born to HIV-negative women.[20] The second trial found that zinc supplementation reduced the risk of acute respiratory and diarrheal infections,[20] but that neither supplement alone nor in combination had an effect on rates of stunting, wasting, or underweight.[21] The 2 studies were designed to allow for pooled analyses—they were conducted in overlapping clinics with similar staff, they used identical inclusion/exclusion criteria (other than maternal HIV status), and they collected the same sociodemographic and clinical data on all mothers and children. In both trials, infants were randomized at 6 weeks of age, and mothers were asked to bring the children to the clinic for follow-up visits every 4 weeks after randomization. At each monthly follow-up visit, a trained study nurse measured child anthropometry using standard techniques.[22] Weight was measured on a digital infant balance scale with 10-g precision (Tanita, Arlington Heights, Illinois) and length with 1-mm precision using a rigid length board with an adjustable foot piece.

For the current study, we identified children who participated in the 2 original trials who met the following criteria: children with complete physical descriptions of their home addresses on file, who had anthropometric data at 6 weeks, who had participated in their original trial through 15 months of age, and who were available for contact during the follow-up study recruitment period of June to August 2014. From the 2387 children in the first trial, a list of all children who fulfilled these criteria was generated and simple random sampling was used to select children for follow-up. From the 2400 children in the second trial, we selected from 269 children who had participated in an enteric disease substudy because these children had provided blood specimens at both 6 weeks and 6 months of age.[17] Further inclusion criteria in the substudy was that children had length-for-age z-score (LAZ) > −2 at 6 weeks.

Laboratory Assessments

Microtiter plates were coated with purified *Escherichia coli* flagellin (100 ng/well) or purified *Escherichia coli* LPS (2 mg/well). Serum samples from study participants were diluted 1:200 and applied to wells coated with flagellin or LPS. After incubation and washing, the wells were incubated with anti-human IgA (KPL, Milford, Massachusetts) or IgG (GE Healthcare, Little Chalfont, United Kingdom) coupled to a horseradish peroxidase. The quantification of total immunoglobulins was performed with the use of the colorimetric peroxidase substrate tetramethylbenzidine, and absorbance (optical density) was read at 450 nm with the use of an enzyme-linked immunosorbent assay plate reader. Data are reported as optical density-corrected data by subtracting background concentrations, which were determined from the readings in samples that lacked serum.