

Markers of Environmental Enteric Dysfunction Are Associated with Poor Growth and Iron Status in Rural Ugandan Infants

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ABSTRACT

Background: Environmental enteric dysfunction (EED), characterized by altered intestinal permeability/inflammation, microbial translocation, and systemic inflammation (SI), may be a significant contributor to micronutrient deficiencies and poor growth in infants from low-resource settings.

Objective: We examined associations among EED, SI, growth, and iron status at 6 mo of age.

Methods: We performed a cross-sectional analysis of 6-mo-old infants ($n = 548$) enrolled in a Ugandan birth-cohort study (NCT04233944). EED was assessed via serum concentrations of anti-flagellin and anti-LPS immunoglobulins (Igs); SI was assessed via serum concentrations of α 1-acid glycoprotein (AGP) and C-reactive protein (CRP); iron status was assessed via serum concentrations of hemoglobin (Hb), soluble transferrin receptor (sTfR), and ferritin. Associations were assessed using adjusted linear regression analysis.

Results: At 6 mo, ~35% of infants were stunted [length-for-age z score (LAZ) < -2] and ~53% were anemic [hemoglobin (Hb) < 11.0 g/dL]. Nearly half (~46%) had elevated AGP (> 1 g/L) and ~30% had elevated CRP (> 5 mg/L). EED and SI biomarkers were significantly correlated ($r = 0.142$ – 0.193 , $P < 0.001$ for all). In adjusted linear regression models, which included adjustments for SI, higher anti-flagellin IgA, anti-LPS IgA, and anti-LPS IgG concentrations were each significantly associated with lower LAZ [β (95% CI): -0.21 ($-0.41, 0.00$), -0.23 ($-0.44, -0.03$), and -0.33 ($-0.58, -0.09$)]. Furthermore, higher anti-flagellin IgA, anti-flagellin IgG, and anti-LPS IgA concentrations were significantly associated with lower Hb [β (95% CI): -0.24 ($-0.45, -0.02$), -0.58 ($-1.13, 0.00$), and -0.26 ($-0.51, 0.00$)] and higher anti-flagellin IgG and anti-LPS IgG concentrations were significantly associated with higher sTfR [β (95% CI): 2.31 ($0.34, 4.28$) and 3.13 ($0.75, 5.51$)].

Conclusions: EED is associated with both low LAZ and iron status in 6-mo-old infants. Further research on the mechanisms by which EED affects growth and micronutrient status is warranted. *J Nutr* 2020;150:2175–2182.

Keywords: environmental enteric dysfunction, systemic inflammation, growth, iron, anemia, Uganda

Introduction

Stunting, or a length-/height-for-age z score (LAZ/HAZ) > 2 SDs below the WHO Child Growth Standards median (1), affects ~22% of children < 5 y of age globally (2). Stunted children experience a myriad of threats to their health and well-being, including increased risk of morbidity and mortality, diminished cognitive development, poorer educational outcomes, and lower economic productivity and earnings in adulthood (3). However, despite the large global burden of stunting, questions

remain regarding its pathogenesis. Specifically, the role of pre- and postnatal subclinical intestinal inflammation/permeability and systemic inflammation (SI) [i.e., environmental enteric dysfunction (EED)] remains poorly understood.

EED is thought to arise from chronic exposure to environmental pathogens and toxins (4) and is widespread among children living in conditions of poor water, hygiene, and sanitation (WASH) (5). It is postulated that EED may underlie persistently high rates of stunting across low- and

middle-income countries (6–9) and may limit responses to dietary interventions, explaining the limited effect of nutritional supplementation alone to improve growth outcomes (10). EED may also contribute to the burden of micronutrient deficiencies (11–13) and reduced immunogenicity of oral vaccines (14–16) in these settings.

SI (i.e., the release of proinflammatory cytokines and activation of the innate immune system) is considered an important pathway by which EED inhibits growth (17, 18). In EED, microbial translocation due to altered barrier integrity drives intestinal inflammation, which further exacerbates gut dysfunction and promotes SI. In turn, SI can further perturb immune function, cause anorexia, and suppress the production of insulin-like growth factor I (IGF-I), creating a cycle of malnutrition, infection, and immune dysfunction (19–21).

Currently, there is no consensus regarding which biomarkers should be used to assess EED. Markers characterizing different domains of the condition have been proposed, including those assessing intestinal permeability (22), intestinal inflammation (23), and microbial translocation (24). Anti-flagellin and anti-LPS immunoglobulins (Igs), which are used to indicate a host's response to microbial translocation, have been supported by their elevated presence in a range of diseases associated with gastrointestinal inflammation such as short bowel syndrome and Crohn's disease (25–27). Furthermore, we have previously linked these biomarkers to poor growth in Tanzanian infants (24), and in Uganda, we observed a relation between these biomarkers in pregnant women and lower infant birth length/LAZ, as well as shorter gestation (28).

The hypothesized link between EED and stunting has been supported by several studies (5, 19, 29–31); however, others have failed to demonstrate such an association (32, 33). Furthermore, few studies have accounted for SI during analysis, and there remains a dearth of studies assessing the effect of EED on micronutrient status. The primary goal of this study is to examine the relations between EED (serum concentrations of anti-flagellin and anti-LPS IgA and IgG), SI [serum concentrations of α 1-acid glycoprotein (AGP) and C-reactive protein (CRP)], growth [LAZ, weight-for-length z score (WLZ), and weight-for-age z score (WAZ)], and iron status [serum concentrations of hemoglobin (Hb), inflammation-adjusted soluble transferrin receptor (sTfR), and inflammation-adjusted ferritin], at 6 mo of age in a sample ($n = 548$) of infants from 16 subcounties in rural northern and southwestern Uganda.

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Abbreviations used: AAT, α 1-anti-trypsin; AGP, α 1-acid glycoprotein; ANC, antenatal care; aOR, adjusted OR; BRINDA, Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia; CRP, C-reactive protein; EED, environmental enteric dysfunction; HAZ, height-for-age z score; Hb, hemoglobin; HFIAS, Household Food Insecurity Access Scale; Ig, immunoglobulin; IGF-I, insulin-like growth factor I; LAZ, length-for-age z score; MPO, myeloperoxidase; NEO, neopterin; OD, optical density; SI, systemic inflammation; sTfR, soluble transferrin receptor; UBCS, Uganda Birth Cohort Study; WASH, water, sanitation, and hygiene; WAZ; weight-for-age z score; WLZ, weight-for-length z score.

Methods

Approvals

Study approval was obtained from the Makerere University Research Ethics Committee at the School of Public Health in Kampala, Uganda; the Uganda National Council for Science and Technology in Kampala, Uganda; the Tufts Health Sciences Institutional Review Board in Boston, Massachusetts; and the Harvard TH Chan School of Public Health Institutional Review Board in Boston, Massachusetts. Written consent was obtained from all mothers prior to enrollment.

Description of cohort

Participants in this observational, cross-sectional study were a subset of infants enrolled in a birth-cohort study, the Uganda Birth Cohort Study (UBCS; NCT04233944). The UBCS, which was conducted from 2014 to 2016 by the US Agency for International Development (USAID) Feed the Future Innovation Lab for Nutrition at Tufts University, recruited and followed ~5000 pregnant women in 16 subcounties in rural southwestern (Bugangari, Buyanja, Bwizi, Kebisoni, Kibiito, Nyamweru, Rugyeyo, and Ruhija) and northern (Aduku, Agoro, Agweng, Apac, Atanga, Atyak, Ayer, and Parombo) Uganda. Home visits were conducted every 3 mo from pregnancy until the infant turned 6 mo of age.

Extensive household, maternal, and infant data were collected within the UBCS, including in-depth information on demographics, household characteristics, agricultural production, WASH practices, food security [using the Household Food Insecurity Access Scale (HFIAS)] (34), nutritional and health status of women through pregnancy, birth outcomes, and anthropometry for women and their infants. Maternal height and infant length were measured to the nearest 0.1 cm using a portable height board (ShorrBoard® infant/child/adult portable height-length measuring board; Weigh and Measure, LLC); weight was measured to the nearest 0.1 kg using an electronic scale (Seca model 874; Seca Corporation). All anthropometry measurements were taken in triplicate and averaged.

Hb concentrations were measured at 6 mo of age using a finger-prick blood sample and a portable hemoglobinometer (HemoCue 301; HemoCue America). Blood samples for biomarker analysis were collected via venipuncture by a trained phlebotomist (BD Vacutainer; Becton Dickinson). Samples were then transported on ice to facility laboratories, where serum was separated, placed into aliquots, and frozen at -20°C .

The flow diagram for the study is presented in Figure 1. Of the 5044 households enrolled in the UBCS, 1700 had a maternal serum sample collected at birth and an infant sample collected at 6 mo of age. From these, infant serum samples from the 6-mo visit with adequate sample volume ($n = 781$) were selected and analyzed for anti-flagellin and anti-LPS Igs; 688 were further analyzed for biomarkers of SI and iron status. Infants missing 6-mo covariate/anthropometry data ($n = 140$) were excluded from analysis. A total of 548 infants were therefore included in this study. Of these, 488 infants had Hb assessed at 6 mo of age.

Laboratory analysis

Serum samples were analyzed for concentrations of anti-flagellin and anti-LPS IgA and IgG at Georgia State University (Atlanta, GA) via previously described ELISA methods (25). Briefly, serum samples were diluted 1:200 and applied to wells coated with either flagellin (100 ng/well) or LPS (2 μg /well). Wells were then incubated with anti-human IgA (KPL) or IgG (GE Healthcare) coupled to HRP. The quantification of total Igs was conducted using the colorimetric peroxidase substrate tetramethylbenzidine, and absorbance [optical density (OD)] was read at 450 nm using an ELISA plate reader. Concentrations of serum biomarkers are reported as OD-corrected data; higher values suggest increased intestinal permeability and microbial translocation indicative of EED.

Samples were further analyzed for AGP, CRP, sTfR, and ferritin at the VitMin Lab in Willstaett, Germany, via previously described sandwich ELISA methods (35). Ferritin and sTfR were adjusted

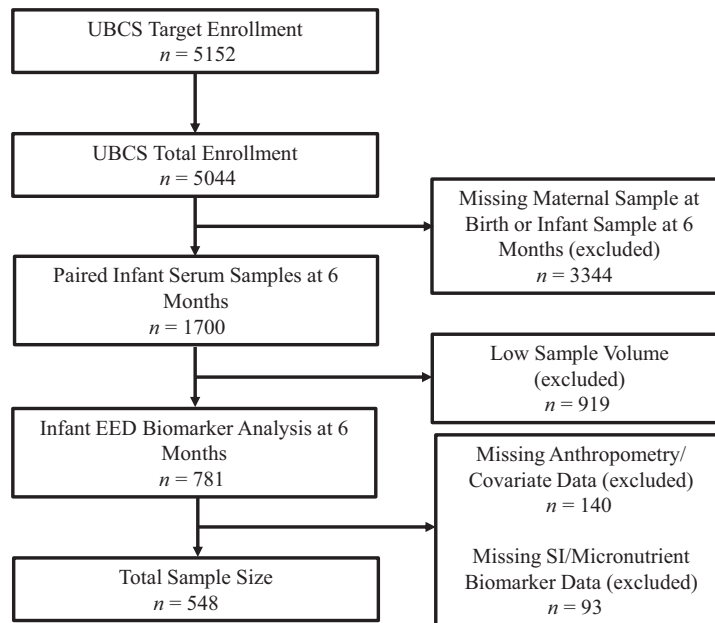


FIGURE 1 Flow diagram for cross-sectional study of infants from northern and southwestern Uganda. EED, environmental enteric dysfunction; SI, systemic inflammation; UBCS, Uganda Birth Cohort Study.

for inflammation using the Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) method (36).

Statistical analysis

Background characteristics, including household, maternal, and infant characteristics, were calculated and presented as means \pm SDs or n (%) for continuous and categorical outcomes, respectively. Growth outcomes at 6 mo of age, including LAZ, WAZ, and WLZ, were calculated using the WHO Multicenter Growth Reference Study growth standards (1). Outliers, defined as LAZ <6 or ≥ 6 , WAZ <6 or ≥ 5 , and WLZ <5 or ≥ 5 , were set to missing.

EED, SI, and iron status biomarker concentrations are presented as medians (IQRs). Mann-Whitney U tests were used to assess differences in biomarker concentrations between moderately (< -2 to ≥ -3 SDs) and severely (< -3 SDs) stunted infants and infants ≥ -1 SD. Because of their skewed distribution, EED and SI biomarkers were ln-transformed prior to correlation and regression analyses. Pearson correlation coefficients were calculated to assess relations between EED biomarkers (anti-flagellin and anti-LPS IgA and IgG) and SI biomarkers (AGP and CRP).

Adjusted linear regression models were used to assess the association between EED and SI biomarkers and infant growth. Adjusted models controlled for significant predictors of LAZ at 6 mo of age ($P < 0.10$ in unadjusted analyses), including maternal age, maternal height, household head educational level, infant sex, infant birth weight, household food security status (HFIAS), improved water source (yes/no), AGP (EED models only), and subcounty clustering.

Finally, adjusted linear regression models, controlling for infant sex, age, and subcounty clustering, were developed to assess the association between EED biomarkers and iron status biomarkers, including Hb (grams/deciliter), inflammation-adjusted sTfR (milligrams/liter), and inflammation-adjusted ferritin (micrograms/liter). All statistical analyses were carried out using STATA 15 software (StataCorp). For all analyses, a P value <0.05 was considered statistically significant.

Results

Background characteristics

Background characteristics for 548 infants are presented in Table 1. Over half of households ($\sim 59\%$) were located in the

North. The majority had an improved water source, a mud/dirt floor, and no electricity. Mothers were ~ 27 y old, ~ 1.6 m tall, and had an average of ~ 5 y of education.

Half ($\sim 49\%$) of infants were male, and nearly all ($\sim 94\%$) were breastfeeding at 6 mo of age. Mean LAZ, WAZ, and WLZ at 6 mo of age were -1.37 ± 1.70 , -0.56 ± 1.34 , and 0.52 ± 1.48 , respectively. Over one-third ($\sim 35\%$) of infants were stunted, and over half ($\sim 53\%$) were anemic (Hb <11 g/dL) at this age. Based on established cutoffs (37), $\sim 46\%$ had elevated AGP (>1 g/L) and $\sim 30\%$ had elevated CRP (>5 mg/L).

EED, SI, and micronutrient biomarkers

Median (IQR) EED, SI, and iron status biomarker concentrations are presented in Table 2. At 6 mo of age, median concentrations of CRP were significantly higher in moderately stunted infants versus nonstunted infants. Furthermore, anti-flagellin IgG, anti-LPS IgA, anti-LPS IgG, and inflammation-adjusted ferritin were significantly higher in severely stunted infants versus nonstunted infants.

Table 3 shows the coefficient matrix of EED biomarkers (anti-flagellin and anti-LPS Igs) and measures of SI (AGP and CRP). Correlations among EED biomarker concentrations were high, ranging from 0.887 between anti-flagellin IgA and anti-LPS IgA ($P < 0.0001$) to 0.436 between anti-flagellin IgA and anti-LPS IgG ($P < 0.0001$). AGP and CRP were highly correlated with each other ($r = 0.671$, $P < 0.0001$). Furthermore, significant, modest correlations were observed between EED biomarkers and SI biomarkers ($P < 0.001$ for all), ranging from 0.193 between anti-LPS IgA and AGP ($P < 0.0001$) to 0.142 between anti-LPS IgG and AGP ($P < 0.001$).

Associations between EED and SI biomarkers and growth

Associations between EED and SI biomarkers and growth measures are presented in Table 4. In adjusted linear

TABLE 1 Background characteristics for 548 infants from northern and southwestern Uganda participating in a birth cohort study¹

Characteristics	Values
Household characteristics	
Location, North	325 (59.3)
Household head education, y	6.5 ± 3.1
Food secure ²	228 (41.6)
Improved water source ³	411 (75.0)
Mud or dirt floor	500 (91.2)
Electricity	5 (0.9)
Maternal characteristics	
Age, y	27.3 ± 6.4
Height, cm	159.4 ± 6.5
MUAC, cm	26.4 ± 2.4
Education, y	4.9 ± 2.9
Nulliparous	96 (17.5)
ANC visits during pregnancy	3.4 ± 1.1
Infant characteristics at 6 mo	
Sex, male	267 (48.7)
Birth weight, kg	3.3 ± 0.5
Breastfeeding	516 (94.2)
LAZ	-1.37 ± 1.70
WAZ	-0.56 ± 1.34
WLZ	0.52 ± 1.48
Stunted (LAZ < -2)	191 (34.9)
Moderately stunted (< -2 to ≥ -3 SDs)	100 (18.2)
Severely stunted (< -3 SDs)	91 (16.6)
Wasted (WLZ < -2)	24 (4.4)
AGP >1 g/L	253 (46.2)
CRP >5 mg/L	166 (30.3)
Hb <11 g/dL ⁴	261 (53.5)

¹Values are *n* (%) or means ± SDs. AGP, α1-acid glycoprotein; ANC, antenatal care; CRP, C-reactive protein; Hb, hemoglobin; HFIAS, Household Food Insecurity Access Scale; LAZ, length-for-age z score; MUAC, midupper arm circumference; WAZ, weight-for-age z score; WLZ, weight-for-length z score.

²Defined using HFIAS methodology (34).

³Piped water, public tap, tube well/borehole, or protected well/spring.

⁴*n* = 488.

regression models [controlling for maternal age, maternal height, household head educational level, infant sex, infant birth weight, HFIAS, improved water source, AGP, and sub-county clustering], higher anti-flagellin IgA, anti-LPS IgA, and

anti-LPS IgG concentrations were each significantly associated with lower LAZ at 6 mo of age [β (95% CI) *z* score: -0.21 (-0.41, 0.00), -0.23 (-0.44, -0.03), and -0.33 (-0.58, -0.09) respectively].

TABLE 2 EED, SI, and iron status biomarker concentrations by stunting status for 548 Ugandan infants aged 6 mo old¹

	All (<i>n</i> = 548)	Nonstunted (≥ -1 SD) (<i>n</i> = 225)	Moderately stunted (< -2 to ≥ -3 SDs) (<i>n</i> = 100)	<i>P</i>	Severely stunted (< -3 SDs) (<i>n</i> = 91)	<i>P</i>
EED, OD						
Anti-flagellin IgA	0.92 (0.65, 1.24)	0.92 (0.62, 1.17)	0.83 (0.63, 1.19)	0.75	0.94 (0.72, 1.36)	0.09
Anti-flagellin IgG	1.80 (1.59, 2.06)	1.76 (1.54, 2.01)	1.77 (1.61, 1.98)	0.75	1.90 (1.62, 2.17)	0.020*
Anti-LPS IgA	0.98 (0.70, 1.34)	0.95 (0.66, 1.27)	0.90 (0.66, 1.19)	0.49	1.06 (0.72, 1.56)	0.046*
Anti-LPS IgG	1.82 (1.54, 2.09)	1.78 (1.49, 2.02)	1.81 (1.55, 2.09)	0.36	1.90 (1.61, 2.16)	0.035*
SI						
AGP, g/L	0.95 (0.66, 1.39)	0.90 (0.65, 1.34)	1.00 (0.67, 1.45)	0.33	1.01 (0.65, 1.41)	0.27
CRP, mg/L	1.78 (0.57, 6.77)	1.49 (0.56, 5.18)	2.59 (0.75, 8.65)	0.016*	2.33 (0.74, 8.40)	0.06
Iron ²						
Ferritin, μg/L (adj)	14.90 (8.59, 26.92)	13.41 (7.81, 23.21)	14.77 (9.40, 28.73)	0.18	19.86 (9.89, 36.26)	0.003*
sTfR, mg/L (adj)	9.96 (7.22, 15.50)	9.79 (7.34, 15.77)	9.99 (7.24, 14.39)	0.36	10.12 (6.54, 15.66)	0.26
Hb, ³ g/dL	10.9 (10.0, 11.7)	10.9 (10.2, 11.7)	10.8 (9.9, 11.5)	0.38	10.9 (9.9, 11.8)	0.93

¹Values are medians (IQRs). *P* values were obtained from Mann-Whitney test compared with nonstunted (≥ -1 SD) infants. **P* < 0.05. adj, adjusted; AGP, α1-acid glycoprotein; BRINDA, Biomarkers Reflecting Inflammation and Nutrition Determinants of Anemia; CRP, C-reactive protein; EED, environmental enteric dysfunction; Hb, hemoglobin; OD, optical density; SI, systemic inflammation; sTfR, soluble transferrin receptor.

²Iron biomarkers adjusted for inflammation using the BRINDA method (36).

³*n* = 488.

TABLE 3 Correlation coefficient matrix of EED biomarkers (anti-flagellin and anti-LPS Igs) and measures of SI (AGP and CRP) for 548 Ugandan infants aged 6 mo old¹

	Anti-flagellin IgA, OD	Anti-flagellin IgG, OD	Anti-LPS IgA, OD	Anti-LPS IgG, OD	AGP, g/L
Anti-flagellin IgA, OD					
Anti-flagellin IgG, OD	0.500***				
Anti-LPS IgA, OD	0.887***	0.439***			
Anti-LPS IgG, OD	0.436***	0.731***	0.501***		
AGP, g/L	0.191***	0.157**	0.193***	0.142**	
CRP, mg/L	0.173***	0.168**	0.163**	0.181***	0.671***

¹Values are Pearson correlation coefficients. All biomarkers were log-transformed before analysis. ** $P < 0.001$, *** $P < 0.0001$. AGP, α 1-acid glycoprotein; CRP, C-reactive protein; EED, environmental enteric dysfunction; Ig, immunoglobulin; OD, optical density; SI, systemic inflammation.

In adjusted models controlling for the same covariates (excluding AGP), higher AGP and CRP concentrations were significantly associated with lower LAZ at 6 mo of age [β (95% CI) z score: -0.29 ($-0.47, -0.11$) and -0.11 ($-0.16, -0.06$), respectively]. No significant associations were observed between EED or SI biomarkers and WAZ or WLZ at 6 mo of age.

Associations between EED biomarkers and iron status

As shown in Figure 2, EED biomarkers were significantly negatively correlated with Hb concentrations ($P < 0.05$ for all). Table 5 shows the associations between EED biomarkers and Hb, inflammation-adjusted sTfR, and inflammation-adjusted ferritin using adjusted linear regression models. Controlling for infant sex, age, and subcounty clustering, higher anti-flagellin IgA, anti-flagellin IgG, and anti-LPS IgA concentrations were each significantly associated with lower Hb levels [β (95% CI): -0.24 ($-0.45, -0.02$), -0.58 ($-1.13, 0.00$), and -0.26 ($-0.51, 0.00$), respectively]. Furthermore, higher anti-flagellin IgG and anti-LPS IgG concentrations were significantly associated with higher sTfR concentrations [β (95% CI): 2.31 ($0.34, 4.28$) and 3.13 ($0.75, 5.51$), respectively]. Finally, higher anti-LPS IgA was significantly associated with higher ferritin (β : 2.74 ; 95% CI: $0.36, 5.12$).

Discussion

In this cross-sectional analysis of rural Ugandan infants where stunting was common ($\sim 35\%$), we examined relations among putative biomarkers of EED, SI, growth, and iron status at 6 mo of age. In adjusted linear regression models, EED was associated with lower LAZ, even after controlling for SI, suggesting that intestinal permeability and translocation may relate to linear growth apart from SI. Furthermore, we found evidence of a significant association between EED biomarkers and iron status, particularly lower Hb concentrations as well as higher inflammation-adjusted sTfR concentrations.

EED in this study was assessed via anti-flagellin and anti-LPS Igs biomarkers, which are used to indicate a host's response to microbial translocation (i.e., the passage of immunogenic microbes and/or microbial products through the epithelial barrier into the lamina propria) (38). The bacterial protein flagellin, which mediates bacteria motility, and LPS, a major structural component of bacteria, are normally present in the gut lumen. However, they are typically excluded from absorption by the gut epithelium except for cases of intestinal-barrier dysfunction. In such cases, the translocation of flagellin and LPS across the intestinal mucosa can activate an immune response, resulting in the generation of anti-flagellin and anti-LPS antibodies.

Previous studies assessing anti-flagellin and anti-LPS Igs and growth have shown mixed results. Results from this study are supported by a case-control study conducted in

TABLE 4 Associations between EED (anti-flagellin and anti-LPS Igs) and SI (AGP and CRP) biomarkers and anthropometric measures for 548 Ugandan infants aged 6 mo old¹

	LAZ		WAZ		WLZ	
	β (95% CI)	P	β (95% CI)	P	β (95% CI)	P
EED, OD						
Anti-flagellin IgA	-0.21 ($-0.41, 0.00$)	0.048*	0.00 ($-0.21, 0.22$)	0.98	0.18 ($-0.07, 0.43$)	0.14
Anti-flagellin IgG	-0.29 ($-0.87, 0.28$)	0.30	0.04 ($-0.44, 0.53$)	0.85	0.40 ($-0.05, 0.85$)	0.08
Anti-LPS IgA	-0.23 ($-0.44, -0.03$)	0.029*	-0.02 ($-0.23, 0.18$)	0.84	0.18 ($-0.10, 0.45$)	0.19
Anti-LPS IgG	-0.33 ($-0.58, -0.09$)	0.011*	0.02 ($-0.43, 0.47$)	0.92	0.38 ($-0.15, 0.92$)	0.15
SI						
AGP, g/L	-0.29 ($-0.47, -0.11$)	0.004*	-0.03 ($-0.26, 0.19$)	0.52	0.14 ($-0.17, 0.45$)	0.35
CRP, mg/L	-0.11 ($-0.16, -0.06$)	0.000*	-0.06 ($-0.14, 0.02$)	0.13	0.01 ($-0.08, 0.10$)	0.83

¹Values are β -coefficients (95% CIs) and P values from linear regression models with a continuous biomarker term. All biomarkers were ln-transformed before analysis. Adjusted models controlled for maternal age, maternal height, household head educational level, infant sex, infant birth weight, household food security status (HFIAS), improved water source (yes/no), and subcounty clustering. EED models also controlled for inflammation (i.e., AGP). * $P < 0.05$. AGP, α 1-acid glycoprotein; CRP, C-reactive protein; EED, environmental enteric dysfunction; HFIAS, Household Food Insecurity Access Scale; Ig, immunoglobulin; LAZ, length-for-age z score; OD, optical density; SI, systemic inflammation; WAZ, weight-for-age z score; WLZ, weight-for-length z score.

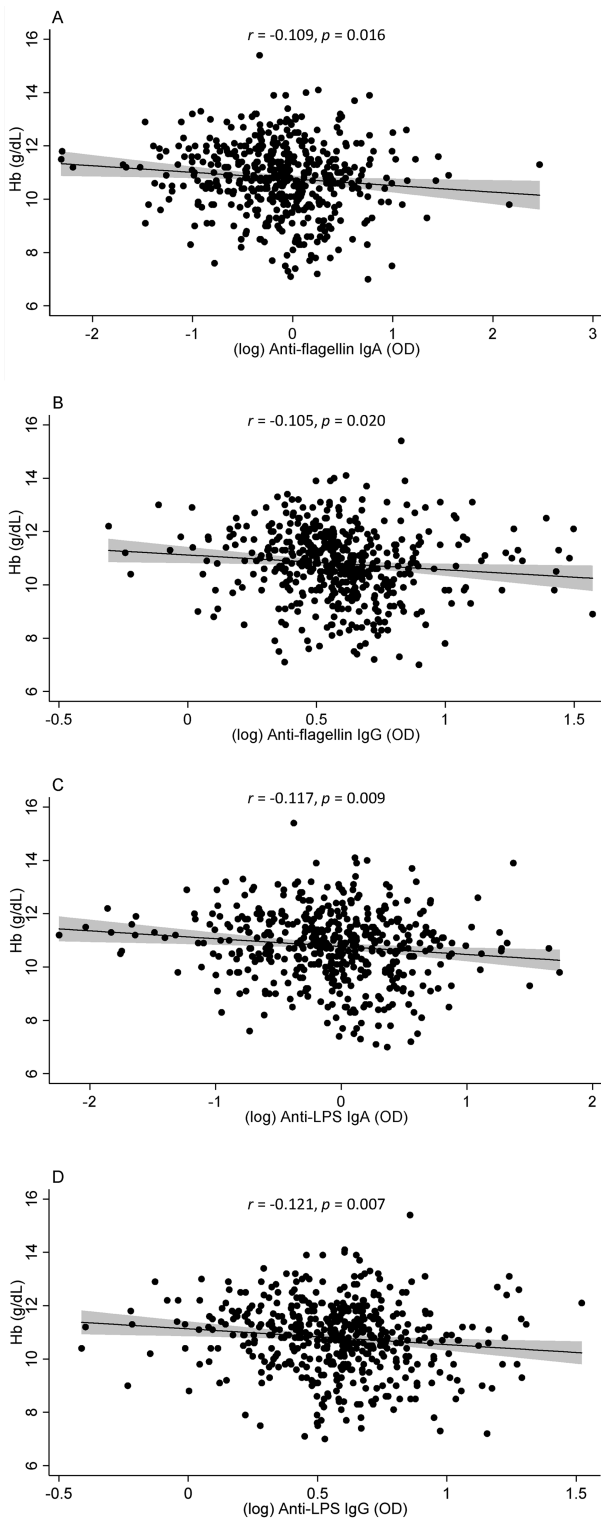


FIGURE 2 (A–D) EED biomarkers (log-transformed anti-flagellin and anti-LPS Igs) and their correlation with hemoglobin concentrations for 488 Ugandan infants aged 6 mo old. Graphs show the best-fit trend line with 95% CIs (gray area). EED, environmental enteric dysfunction; Hb, hemoglobin; Ig, immunoglobulin; OD, optical density.

Fortaleza, Brazil, by Guerrant et al. (31) which reported that anti-flagellin IgA and anti-LPS IgA were significantly correlated with lower HAZ at the time of enrollment ($r = 0.15$, $P = 0.011$, and $r = 0.14$, $P = 0.017$, respectively). Notably, however,

these biomarkers were not predictive of subsequent growth. Furthermore, in Pakistani children ($n = 380$), Syed et al. (39) found that increased anti-flagellin IgA and anti-LPS IgA at 6 and 9 mo predicted declines in linear growth during the subsequent 18 mo of life. However, our results differ from those of McDonald et al. (24), which showed no association between these biomarkers and subsequent stunting in a sample ($n = 590$) of Tanzanian infants, although a relation was observed between these biomarkers and subsequent underweight. Likewise, several studies have assessed microbial translocation via endotoxin and plasma IgG-endotoxin core antibody and showed an association with linear growth (19, 40); however, others have not observed such an association (20, 41, 42).

SI, which impairs immune function and leads to the suppression of IGF-I, was also associated with lower LAZ at 6 mo of age, which is consistent with previous studies. In a case-control study of 202 HIV-unexposed Zimbabwean infants, Prendergast et al. (20) found higher concentrations of CRP [adjusted OR (aOR): 3.06; 95% CI: 1.34, 6.99; $P = 0.008$] and AGP (aOR: 7.87; 95% CI: 0.74, 83.74; $P = 0.087$) during infancy were associated with stunting. However, in the same study, the authors found no evidence that EED, assessed via serum concentrations of intestinal fatty acid binding protein (I-FABP), was associated with stunting. Furthermore, in a prospective cohort study of 380 Pakistani infants by Iqbal et al. (43), ferritin assessed at 6 and 9 mo was associated with decreasing LAZ (6 mo: $\beta = -0.882$, $P < 0.0001$; 9 mo: $\beta = -0.714$, $P < 0.0001$) as were CRP ($\beta = -0.451$, $P = 0.039$) and AGP ($\beta = -0.443$, $P = 0.012$) at 9 mo.

We further explored the hypothesis that EED may be associated with poor iron status by examining the association between EED biomarkers and Hb, inflammation-adjusted sTfR, and inflammation-adjusted ferritin biomarkers. We found that higher EED biomarker concentrations were significantly associated with lower Hb concentrations and higher inflammation-adjusted sTfR concentrations, which may be suggestive of increased iron deficiency due to gastrointestinal malabsorption. Notably, these results are consistent with findings from The Etiology, Risk Factors and Interactions of Enteric Infections and Malnutrition and the Consequences for Child Health and Development (MAL-ED) study, which found that anemia risk was associated with both the lactulose to mannitol ratio z score (1.15; 95% CI: 1.01, 1.31) and myeloperoxidase (MPO) (1.16; 95% CI: 1.01, 1.34) (44).

However, we also found higher EED biomarker concentrations to be associated with higher ferritin concentrations, and severely stunted infants had significantly higher ferritin concentrations compared with nonstunted infants. Ferritin is considered a positive acute-phase protein whereby serum concentrations increase in subclinically infected/inflamed individuals irrespective of iron status. After adjusting for inflammation using the BRINDA method (36), anti-LPS IgA was positively associated with ferritin in our study, perhaps suggestive of incomplete adjustment for systemic inflammation. A study by Fahim et al. examined the association between fecal markers of EED, including MPO, neopterin (NEO), and $\alpha 1$ -anti-trypsin (AAT), and zinc and iron status in 222 Bangladeshi children and found that elevated AAT concentrations were associated with lower ferritin concentrations. However, no associations between MPO or NEO and zinc or iron status were observed (45). Given our findings and the relative dearth of studies on EED and micronutrients, further research is needed to better understand these relations.

TABLE 5 Associations between EED biomarkers (anti-flagellin and anti-LPS Igs) and iron status (Hb, sTfR, and ferritin) for 548 Ugandan infants aged 6 mo old¹

	Hb, ² g/dL		sTfR, ³ mg/L (adj)		Ferritin, ³ µg/L (adj)	
	β (95% CI)	P	β (95% CI)	P	β (95% CI)	P
Anti-flagellin IgA, OD	−0.24 (−0.45, −0.02)	0.034*	0.31 (−0.72, 1.34)	0.53	1.94 (−0.87, 4.47)	0.16
Anti-flagellin IgG, OD	−0.58 (−1.13, 0.00)	0.049*	2.31 (0.34, 4.28)	0.025*	7.65 (0.28, 15.58)	0.06
Anti-LPS IgA, OD	−0.26 (−0.51, 0.00)	0.047*	0.53 (−0.39, 1.45)	0.24	2.74 (0.36, 5.12)	0.027*
Anti-LPS IgG, OD	−0.60 (−1.28, 0.09)	0.08	3.13 (0.75, 5.51)	0.013*	4.87 (−1.07, 10.80)	0.10

¹Values are β-coefficients (95% CIs) and P values from linear regression models with a continuous biomarker term. EED biomarkers were ln-transformed before analysis. Models controlled for infant sex, age, and subcounty clustering. *P < 0.05. adj, adjusted; BRINDA, Biomarkers Reflecting Inflammation and Nutrition Determinants of Anemia; EED, environmental enteric dysfunction; Hb, hemoglobin; Ig, immunoglobulin; OD, optical density; sTfR, soluble transferrin receptor.

²n = 488.

³sTfR and ferritin adjusted for inflammation using the BRINDA method (36).

Several strengths and limitations of this study are worthy of mention. Because this was a substudy to a larger study, we were able to control for multiple potentially confounding factors in our multivariable models, particularly with regard to linear growth. We were further able to control for SI. However, with regard to iron status, we were unable to control for other potential contributors to anemia, such as lack of delayed chord clamping and dietary intake. Another key limitation is that EED in this study is inferred based on anti-flagellin and anti-LPS Igs alone, failing to capture other domains of EED, such as intestinal inflammation and absorption. Furthermore, EED and SI biomarkers were measured in a small subset of infants enrolled in the UBCS and at only 1 point in time (i.e., at 6 mo of age). Therefore, as this was a cross-sectional study, we cannot examine prospective relations or establish cause and effect. Finally, although results were statistically significant, it is worth noting that the EED biomarkers explain only a relatively small percentage of the variability in LAZ at 6 mo of age.

In conclusion, this observational, cross-sectional study from rural Uganda found that EED biomarkers were associated with both low LAZ and iron status in children 6 mo of age. These results contribute to the growing body of literature on the potential adverse consequences of EED during infancy and underscore the need to reduce exposure to enteropathogens and toxins during this critical period of growth. Furthermore, as the associations were observed even after controlling for SI, more research on the mechanisms by which intestinal permeability and translocation affect growth and micronutrient status is warranted.

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