10-1-2022

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ORIGINAL RESEARCH

CURRENT DEVELOPMENTS IN NUTRITION

Nutrition in Health and Disease

Choline, DHA, and Diarrheal Disease Associated with Growth Faltering in a Case-Control Study

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ABSTRACT

Background: Children with recurrent infectious diarrhea are susceptible to growth faltering. DHA and choline may play a role in this relationship due to their involvement in lipid metabolism, gut immunity, and inflammatory pathways.

Objectives: This study aimed to characterize the contributions made by DHA and choline status and enteric damage in young children in the association between diarrheal illness and child growth.

Methods: A longitudinal case-control study was conducted among children aged 6–36 mo ($N = 195$) in Cap-Haitien, Haiti. Mother-child dyads were recruited from community health posts and outpatient clinics. Cases were defined as children experiencing acute diarrhea within the last 3 d and matched to healthy controls. Child anthropometry, dietary intake, and blood and stool samples were collected at baseline and follow-up. Plasma DHA, choline, and betaine were determined by LC-MS/MS methods ($n = 49$) and intestinal fatty acid–binding protein (I-FABP) by ELISA ($n = 183$). Multivariate regression models were applied with mediation analyses to examine associations and adjust for confounding factors.

Results: At baseline, mean plasma DHA concentrations (1.03 μg/mL; 95% CI: 0.91, 1.15) were not significantly different between cases and controls, nor was there a difference in mean plasma choline concentrations (4.5 μg/mL; 95% CI: 3.8, 5.1). Mean plasma I-FABP concentrations were significantly higher at follow-up in cases (3.34; 95% CI: 3.28, 3.40) than controls (3.20; 95% CI: 3.13, 3.27; $P = 0.002$). In adjusted multilinear regression models, higher plasma DHA concentrations at follow-up were associated with a negative change in weight-age *z* score ($P = 0.016$), and follow-up I-FABP was inversely associated with height-age *z* score ($P = 0.035$). No interaction or mediation effects were found.

Conclusions: I-FABP concentrations were significantly higher in cases as compared with controls at follow-up, suggesting ongoing enteric damage and increased risk for malnutrition. Plasma DHA and I-FABP may have a role in childhood growth outcomes. *Curr Dev Nutr* 2022;6:nzac140.

Keywords: Haiti, intestinal fatty acid–binding protein, underweight, stunting, childhood diarrhea

Manuscript received March 28, 2022. Initial review completed June 17, 2022. Revision accepted September 1, 2022. Published online September 12, 2022.
Source of support: Washington University Office of the Vice Chancellor of Research.
Author disclosures: The authors report no conflicts of interest.
Supplemental Table 1 is available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/cdn/.
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Abbreviations used: ASF, animal source food; COVID-19, coronavirus 2019; EED, environmental enteric dysfunction; FC, fecal calprotectin; HAZ, height-age *z* score; HUJ, Hôpital Universitaire Justinien; I-FABP, intestinal fatty acid–binding protein; LMIC, low- or middle-income country; WASH, water, hygiene, and sanitation; WAZ, weight-age *z* score; WHZ, weight-height *z* score.

Introduction

Diarrheal disease and undernutrition are 2 predominant causes of morbidity and mortality in children living in low- and middle-income countries (LMICs) (1–3). A nationally representative study conducted in Haiti found that approximately 38% of infants aged 6–11 mo experienced a diarrheal illness within the past 2 wk (3). Undernutrition in the form of stunting is also widely prevalent among young children in Haiti, affecting 21.9% (3). Repeated diarrheal disease often exacerbates undernutrition and may magnify underlying energy and nutritional deficits (4). This unforgiving cycle of repeated diarrheal disease and compounding undernutrition has the potential to result in a condition known as environmental enteric dysfunction (EED), a subclinical disorder of the small intestine characterized by villous blunting and crypt hyperplasia (5, 6). The presence of EED is a postulated etiology of stunted growth in children living in LMICs (5, 6). Thus, the potential for public health nutrition interventions that alleviate these diarrheal complications warrants investigation to improve health and mitigate disease (7).

Interventions comprising nutrient-dense foods can significantly affect child growth and development (8). One randomized controlled trial

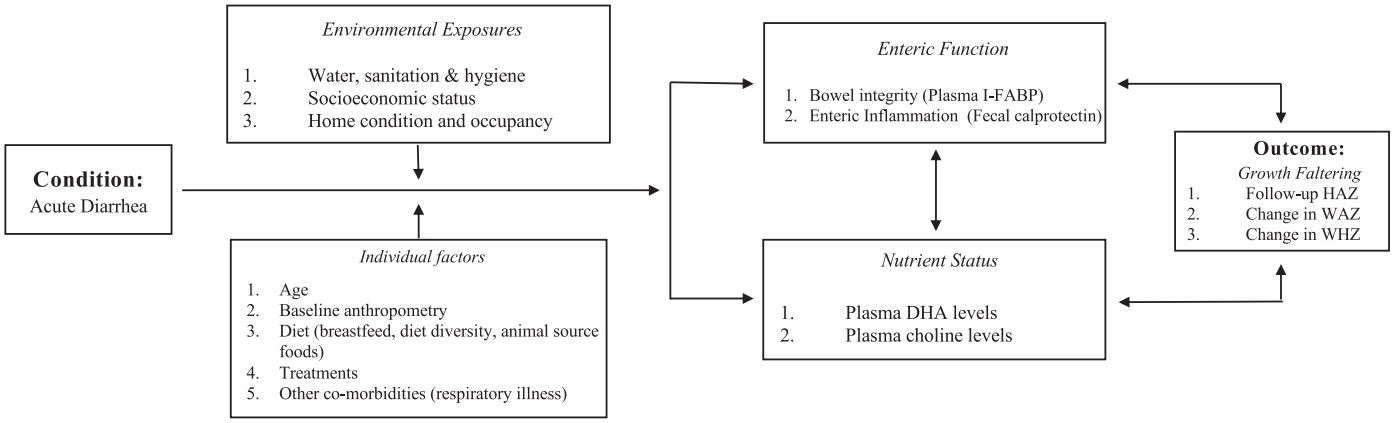

FIGURE 1 Conceptual diagram and mediating mechanisms involved in growth outcomes. HAZ, height-age *z* score; I-FABP, intestinal fatty acid–binding protein; WAZ, weight-age *z* score; WHZ, weight-height *z* score.

conducted in Ecuador targeted eggs as an abundant source of choline to children early in the complementary feeding period (ages 6–9 mo), a phase known to have the highest risks for growth faltering and diarrheal disease (9). Findings showed significant reductions in stunting and increased concentrations of DHA and choline status for children in the egg group (10, 11). Choline is an essential nutrient for neurotransmitter synthesis, lipid transport, and 1-carbon metabolism. Choline deficiencies have been shown to alter specific immune system processes resulting in increased inflammatory responses within the intestine (12). Similarly, evidence supports the role of DHA, an ω-3 long-chain PUFA, as an anti-inflammatory mediator of the immune system (13, 14). Coinciding with this function, DHA is vital for linear growth and brain and eye development (15–18). A deeper understanding of how these nutrients influence diarrhea, enteric health, and child growth may be critical in LMIC contexts.

A longitudinal case-control study was conducted to gather preliminary data on these complex interactions across nutrition, diarrhea, and EED factors (**Figure 1**). We hypothesized that plasma DHA, choline, and enteric health would mediate childhood growth outcomes in those with diarrheal illness.

Methods

Study design and participants

We conducted a case-control study with a 1-mo longitudinal outcome assessment in Cap-Haitien, Haiti. Participants were recruited from Hôpital Universitaire Justinien (HUJ), community clinics (Fort St Michel and La Fossette), and associated community health posts. Inclusion criteria were children 6–36 mo of age and caregivers >18 y old. Those who could not provide consent or required immediate medical care were excluded. Children with symptoms suggestive of coronavirus 2019 (COVID-19) infection were referred to the pediatrician at HUJ. Cases were chosen on the basis of acute diarrhea 3 d before enrollment. Acute diarrhea was defined as ≥3 liquid or semiliquid stools in 24 h by self-report (19). Those who fit the study inclusion criteria but had no history of acute diarrhea were assigned to the control group.

Enrollment occurred between December 2020 and May 2021. Additional measures were taken to improve the safety of the staff and study participants during the COVID-19 pandemic. These included specific COVID-19 symptomatic questionnaires at each visit time point and the provision of face masks for everyone >3 y old. Staff were trained on proper hand-washing techniques. Hand sanitizer was provided for all staff, caregivers, and children. Data and specimen collection stations were thoroughly disinfected between participants. Enumerators, nurses, and the phlebotomist were all supervised by the on-site field coordinator to ensure that all these measures were followed.

Ethical review

Study approvals were obtained from the Washington University Institutional Review Board and the National Bioethics Committee in Haiti (Comité National de Bioéthique). Written informed consent was obtained from all caregivers. Caregivers were also asked for permission to use their data in future research. Refusal did not affect their eligibility for the study. Caregivers were not paid to participate but were compensated for travel costs to the study sites. Participants did not directly benefit from the study; however, we hope that the results will help children with diarrheal disease in the future.

Data were anonymized after the initial visit by using different identification numbers at enrollment. All data collected on paper forms were kept in a locked cabinet and were not accessible to members outside the research team. Electronic data were password protected and deidentified. Our field coordinator, a trained registered nurse, will inform participants in small groups of the results.

Survey management

Trained nurses completed baseline and follow-up assessments with the child and caregiver in their native Creole language (**Supplemental Table 1**). In addition, written surveys were conducted and entered into the REDCap tools (Research Electronic Data Capture) housed at Washington University (20, 21). Double data entry was completed on 10% of participants selected at random to assess for interrater reliability with a goal of >90% agreement. Inconsistencies, if found, were reviewed and corrected.

Measures

Outcomes.

Anthropometric measurements were obtained following the WHO standardized reference manual (22, 23). Weight (Seca model 874 digital scale) and length (ShorrBoard stadiometer) were measured twice. Measurements were repeated a third time when differences were >0.1 kg or >0.7 cm, and the 2 closest were averaged. We subtracted 0.7 cm from the length for children >2 y old when standing height was not measured (24). The WHO Anthro Survey Analyser Software (version 3.2.2) was used to calculate height-age *z* score (HAZ), weight-age *z* score (WAZ), and weight-height *z* score (WHZ) (23, 25).

Exposures and sample collection.

Plasma samples were collected in lithium heparin-containing BD Vacutainers and placed on ice. After centrifugation at 1100–1300 × *g* for 20 min, aliquots were stored at −20 °C (26).

A random subset of cases and controls was selected for nutrient analysis, excluding hemolyzed samples. Unfortunately, product cost changes limited our initial planned analysis. Paired plasma choline, DHA, and betaine were measured using modified LC-MS/MS methods at the Washington University Metabolomics Facility (27). The betaine:choline ratio was used to measure potential flux into 1-carbon metabolism and away from lipid synthesis as choline was metabolized to betaine. Higher betaine:choline ratios suggest an increase in 1-carbon metabolism (28). When this occurs, it limits the amount of choline used for phosphatidylcholine synthesis, and previous studies suggested that this may have a role in growth faltering (29).

Stool samples were collected at the time of the visit. If the child did not or was unable to provide a stool sample during the study visit, caregivers were given a clean collection cup containing a sterile spoon and instructed to return with a sample. Equal amounts of stool and stabilization solution (Monarch DNA/RNA Preservation Reagent, T2011L; New England Biolabs) were mixed and stored at −20 °C.

All samples were transported on ice from HUJ to the Kuhlmann Laboratory at Washington University in St Louis via the Haitian National Laboratory. Upon arrival, samples were placed in the −80 °C freezer.

To evaluate for intestinal damage, we detected plasma intestinal fatty acid–binding protein (I-FABP) in duplicate. I-FABP was detected using an ELISA (Human FABP2 DuoSet; R&D Systems) according to the manufacturer's protocol, substituting BSA for normal goat serum. I-FABP is a marker of enteric damage since it is highly expressed in the small intestinal enterocytes and immediately released into the circulation during acute insults (30). To evaluate intestinal inflammation, we detected fecal calprotectin (FC), a major protein found in the cytosol of monocytes and neutrophils (31). FC was determined in duplicate on preserved stool samples using ELISA (Hycult Biotech) according to the manufacturer's protocol; a separate proprietary buffer (K 0001.C.100; Immunodiagnostic) provided optimized results. Both markers have been associated with EED (6).

Covariates.

Age was determined using the difference between the date of birth and the date of the initial encounter. The following information was collected at baseline: socioeconomics, demographics, drinking water source, sanitation (drinking water, toilet type, number of people using the toilet, and waste disposal), and hygiene (hand-washing techniques). Maternal level of education, occupation, home ownership, house composition, and household income were utilized to evaluate socioeconomic status. Child health and morbidity questionnaires inquired about any history of asthma, congenital anomalies or developmental delays, a 2-wk symptom recall, and vaccination status. Dietary intake was evaluated by breastfeeding history, vitamin and mineral supplementation, and a 24-h FFQ, previously validated in other studies in Cap-Haitien and catchment communities (32). Based on the FFQ, foods were categorized into groups: breastmilk; milk, cheese, yogurt; bread, rice, pasta, potatoes, cereal; roots and tubers; beans and legumes; red meat, chicken; eggs; seafood; dark leafy green vegetables; all other vegetables; fruits; oil and butter; and bonbon sel (thin flour cracker), juice, soda, and other sweets. Animal source foods (ASFs) included red meat, chicken, eggs, seafood, and milk products. Self-reported dietary recalls are inherently subject to social desirability bias (33, 34). To temper possible social desirability bias, caregivers were interviewed by local Haitian women trained to minimize expressing any judgments or opinions. Nevertheless, bias may still be present and should be considered when interpreting results.

Statistical analysis

All continuous variables were assessed for normal distribution and outliers using histograms, scatter plots, and box plots. Those with nonnormal distributions were $\log_{10}$ transformed, which included I-FABP and FC. Baseline continuous variables were assessed for significant differences using independent samples *t* test or Mann-Whitney *U* test when nonparametric tests were indicated. A paired samples *t* test was used to compare continuous baseline variables with those from follow-up. Continuous variables were compared by Pearson correlations or Spearman ρ when nonparametric tests were indicated. Categorical variables were evaluated using χ^2 analysis. For longitudinal analyses, we included the difference in WAZ/underweight (WAZ < −2) and WHZ because of the increased probability that weight could change in 1 mo. For follow-up, only HAZ/stunting (HAZ < −2) was examined as the linear growth outcome due to the low probability of change in 1 mo (22). Difference-in-difference changes were determined by subtracting baseline values from follow-up and comparing changes across groups. This method was employed for WAZ, WHZ, and plasma and stool biomarkers.

Two sets of multivariate models were tested: *1*) logistic regression to examine the association between the nutritional biomarkers and underweight and stunting at 1-mo follow-up and *2*) linear regression models for primary exposures on anthropometric *z* scores. Using the logistic regression model allowed us to assess for trends in the nutritional biomarkers concerning the smaller sample size. In the first analysis, the crude OR was determined and adjusted for by variables where the association was potentially evident ($P < 0.15$). Then, models were assessed by omnibus tests of model coefficients (significant $P < 0.05$), Nagelkerke R^2 for variability, and goodness of fit by Hosmer and Lemeshow. The Benjamini and Hochberg procedure was used to control for a maximum false discovery rate of 0.05 (35).

In the multilinear regression analysis, we examined the primary exposure and *z* score outcomes with the coefficients for all covariates to provide insight into the varying contributions of factors. Regression modeling diagnostics were applied to test the underlying assumptions, examine collinearity, and optimize model fit. Stepwise regression analysis was done for age, sex, socioeconomic and demographic factors,

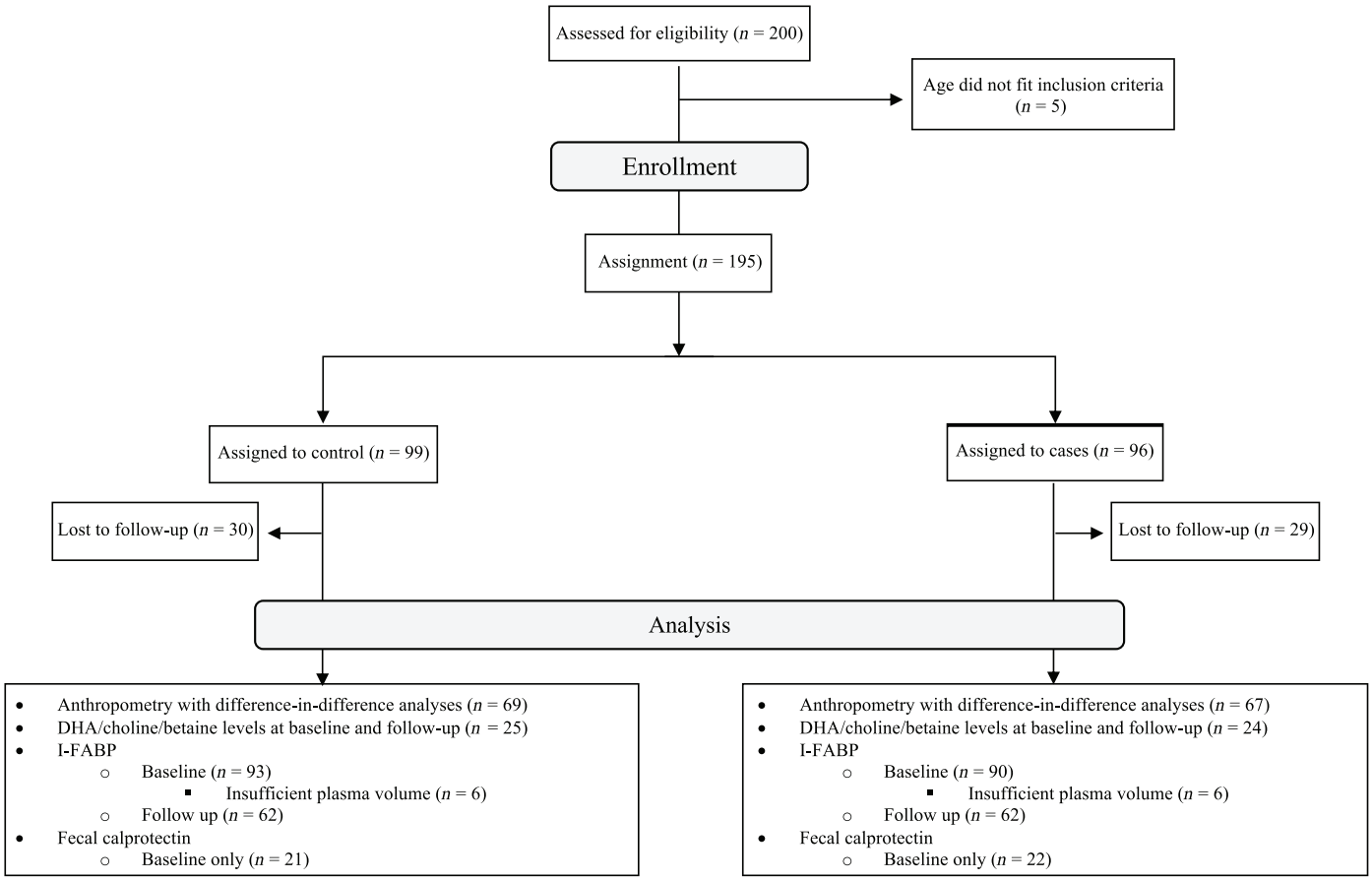

FIGURE 2 Flow diagram illustrating progression and biomarker analyses in cases and controls. Specific sample size per variable is shown. Children who met the inclusion and exclusion criteria were assessed for ≥3 liquid stools over 24 h in the last 3 d. Those who met this definition of diarrhea were assigned to the cases, and those who did not were considered controls. I-FABP, intestinal fatty acid–binding protein.

WASH variables (water, hygiene, and sanitation), and dietary intake variables. Covariates were retained in final models when $P < 0.15$ in association with growth outcomes (follow-up HAZ, change in WAZ, change in WHZ), with few exceptions. The child's last dietary intake before the venous draw was assessed, and the time of blood draw was obtained, which was adjusted for in analyses where significant interactions were found. Analyses were repeated with and without the inclusion of outliers. Outliers tested for differences in analyses included I-FABP concentrations >15,000 pg/mL and <200 pg/mL, and difference in I-FABP >5000 pg/mL or < −5000 pg/mL was noted. Our main exploratory variables (enteric biomarkers, DHA, choline, and betaine) were tested in models as interaction terms and using structural equation modeling. Type I error was set to be 2-sided and at a value of 0.05. All analyses were completed using SPSS software (version 27.0; IBM).

Results

Participant enrollment and baseline characteristics

A total of 195 children were enrolled in the study (case: $n = 96$; control: $n = 99$), and 136 children returned for the follow-up assessment (Figure 2). The difference between the number of participants lost to follow-up among cases and controls was nonsignificant. At baseline, our cases and controls appeared similar (Table 1). However, symptoms associated with acute gastroenteritis, such as vomiting and poor appetite, were significantly higher in the cases compared with controls. WASH practices and dietary intake of breastmilk, fruits, vegetables, and ASF were comparable between groups. Intake of bonbon sel, equivalent to a thin cracker made of white flour, was reported in 43% of all participants and was a significant component of the diet.

Child anthropometry

There were no significant differences in anthropometric *z* scores or undernutrition indicators between groups at baseline, nor were there significant differences in growth when the difference-in-difference analysis was applied between cases and controls (Table 2).

Biomarkers of nutrition and enteric damage

Plasma DHA and choline concentrations were nonsignificant when compared between cases and controls (Table 3). However, plasma concentrations of I-FABP were significantly higher in cases than in controls at follow-up.

TABLE 1 Baseline characteristics by group[1]

	Control ($n = 99$)	Case ($n = 96$)	*P* Value
Child			
Age,[2] mo	18.8 ± 8.3	17.1 ± 7.5	0.114
Sex: female	56.9	43.1	0.075
Dietary intake			
Currently breastfeeding	47.5	52.5	0.243
Times breastfeed, 24 h	14.4 ± 5.8	13.3 ± 6.3	0.182
Bonbon sel	42.7	46.2	0.645
Animal source foods	48.7	51.3	0.478
Eggs	15	12	0.478
Fish	4.1	11.7	0.129
Morbidities, 14-d recall			
Vomiting	14.6	31.3	0.029[3]
Suppressed appetite	34	54.2	0.005[3]
Nasal congestion/rhinorrhea	68.7	63.5	0.448
Cough	51.5	56.3	0.594
Difficulty breathing/wheezing	15.2	15.6	0.507
Rash	23.7	19.8	0.509
Fever, >38.0 °C	2.1	5.3	0.248
Days with diarrhea[2]	4.7 ± 3.7	2.8 ± 1.6	0.013[3]
Vaccinations received			
Polio	100	97.3	0.117
Rotavirus	91.5	91.9	0.437
Typhoid	38.2	47.1	0.095
Maternal			
Maternal age,[2] y	29 ± 6.7	25.7 ± 5.7	0.086
Completion of secondary school and higher	50.6	47.9	0.7
Household			
Household occupancy	5.9 ± 2.1	6.0 ± 2.2	0.784
Drinking bottled water	86.9	89.6	0.557
Electricity	22.4	46.9	0.001[3]
Material floor is rock or dirt	9.2	22.9	0.023[3]
Flush toilet	11.2	3.1	0.15
Number of people using toilet[2,4]	2.8 ± 1.3	2.7 ± 1.1	0.886

[1]Values are presented as mean ± SD and percentages. *T* test and χ^2 test were used to assess for statistical significance unless otherwise specified.
[2]Mann-Whitney *U* test.
[3]$P < 0.05$.
[4]Control, $n = 42$; case, $n = 34$.

Paired analyses of the entire cohort showed mean plasma DHA concentrations at baseline (1.03 μg/mL; 95% CI: 0.91, 1.15) to be significantly higher than those at follow-up [0.73 μg/mL; 95% CI: 0.63, 0.83; $t(48) = 4.9$, $P < 0.001$]. Similar findings were also noted with plasma choline concentrations, with mean baseline values (4.5 μg/mL; 95% CI: 3.8, 5.1) significantly higher than those at follow-up [3.0 μg/mL; 95% CI: 2.6, 3.5; $t(48) = 5.97$, $P < 0.001$]. These findings were consistent between the case and control groups, with the difference-in-difference

TABLE 2 Anthropometric data compared between cases and controls at baseline and 1-mo follow-up[1]

	Baseline			Follow-up			Change from Baseline		
	Control ($n = 97$)	Case ($n = 96$)	*P* Value	Control ($n = 69$)	Case ($n = 67$)	*P* Value	Control ($n = 69$)	Case ($n = 67$)	*P* Value
HAZ	–1.10 ± 1.23	–1.14 ± 1.31	0.78	–1.18 ± 1.28	–1.28 ± 1.15	0.63	–0.05 ± 0.69	–0.03 ± 0.54	0.9
WAZ	–0.87 ± 1.33	–1.00 ± 1.16	0.47	–0.81 ± 1.17	–0.98 ± 1.03	0.38	0.03 ± 0.51	0.08 ± 0.53	0.61
WHZ	–0.37 ± 1.23	–0.53 ± 1.01	0.33	–0.26 ± 0.97	–0.46 ± 0.98	0.22	0.01 ± 0.78	0.05 ± 0.71	0.77
Stunted	20.4	25	0.44	21.7	25.4	0.61	−1.3	−0.4	0.34
Underweight	21.4	17.7	0.51	15.9	14.9	0.87	5.5	2.8	0.84
Wasted	9.2	10.4	0.77	4.3	4.5	0.97	4.9	5.9	0.79

[1]Values are presented as mean ± SD and percentages. HAZ, height-age *z* score; WAZ, weight-age *z* score; WHZ, weight-height *z* score.

TABLE 3 Biomarkers of nutrition, enteric damage, and enteric inflammation compared between cases and controls at baseline and 1-mo follow-up[1]

	Baseline					Follow-up				
	Controls		Cases			Controls		Cases		
	n	Mean (95% CI)	*n*	Mean (95% CI)	*P* Value[2]	*n*	Mean (95% CI)	*n*	Mean (95% CI)	*P* Value[3]
Plasma DHA, μg/mL	25	0.95 (0.79, 1.10)	24	1.11 (0.91, 1.31)	0.19	25	0.64 (0.52, 0.76)	24	0.82 (0.65, 0.99)	0.17
Plasma choline, μg/mL	25	4.02 (3.39, 4.66)	24	4.94 (3.80, 6.07)	0.27	25	2.53 (2.21, 2.85)	24	3.58 (2.70, 4.46)	0.14
Plasma betaine, μg/mL	25	8.02 (6.70, 9.24)	24	7.29 (6.15, 8.44)	0.40	25	7.65 (6.62, 8.67)	24	6.88 (5.74, 8.02)	0.22
Betaine:choline ratio	25	2.35 (1.88, 2.81)	24	1.63 (1.00, 2.27)	0.19	25	3.18 (2.74, 3.62)	24	2.46 (1.86, 3.05)	0.049[4]
Log I-FABP, pg/mL	93	3.15 (3.10, 3.20)	90	3.22 (3.10, 3.38)	0.13	62	3.20 (3.13, 3.27)	62	3.34 (3.28, 3.40)	0.002[4]
Log FC, ng/mL	21	3.43 (2.91, 3.94)	22	3.39 (2.91, 3.87)	0.77					

[1]Statistical significance was determined by Student *t* test and was considered for $P < 0.05$, except where indicated. FC, fecal calprotectin; I-FABP, intestinal fatty acid–binding protein.
[2]Statistical significance was determined by Mann-Whitney *U* test and was considered for $P < 0.05$ for plasma choline and betaine:choline ratio at baseline.
[3]Statistical significance was determined by Mann-Whitney *U* test and was considered for $P < 0.05$ for plasma DHA, plasma choline, and plasma betaine at follow-up.
[4]$P < 0.05$.

changes of plasma choline and DHA nonsignificant when compared between groups. In contrast, the mean betaine:choline ratio at baseline (2.0; 95% CI: 1.7, 2.3) was significantly lower than the ratio at follow-up [2.8; 95% CI: 2.5, 3.2; $t(48) = -5.6$, $P < 0.001$]. The difference-in-difference changes of plasma betaine and the betaine:choline ratio were nonsignificant between groups.

After log transformation, plasma I-FABP concentrations at baseline were significantly lower than those at follow-up [$t(59) = 3.3$, $P = 0.002$] in the cases but not the controls. The difference-in-difference changes for plasma I-FABP were nonsignificant in cases as compared with controls.

Analyses of plasma I-FABP concentrations in association with age showed that children in the control group had I-FABP concentrations that inversely correlated with age at follow-up (Spearman correlation $r = -3.25$, $P = 0.009$). Decreasing I-FABP concentrations with age were not appreciated in the case group, where plasma I-FABP concentrations remained high regardless of age ($r = 0.008$, $P = 0.95$).

Nutritional biomarkers in association with underweight and stunting

In the first set of multivariate models, plasma biomarkers of nutrition were assessed after adjustment for maternal education, maternal age ≤19 y, ASF intake at baseline, and time of blood draw to determine the association with underweight status at follow-up (**Table 4**). Adjusted analyses did confirm that covariates—including breastfeeding, maternal education, adolescent mother, ASF intake at baseline, and time of blood draw—were significantly associated with underweight status. A similar multivariate analysis was done to assess the relationship of nutritional biomarkers with stunting at follow-up after adjusting for age, breastfeeding at baseline, and time of blood draw. Plasma DHA, choline, and betaine concentrations were nonsignificant in association with stunting. In addition, the goodness of fit using Hosmer and Lemeshow was nonsignificant in both models.

Determinates of growth faltering

The second multivariate model found that sociodemographic determinants contributing to growth faltering included age, source of drinking water, days with diarrhea, and intake of ASF (**Table 5**). Case-control group assignment did not significantly contribute to growth faltering ($P > 0.15$). After adjusting for multiple comparisons, plasma DHA concentrations at follow-up were significantly associated with growth outcomes. I-FABP concentrations at follow-up were inversely related to HAZ ($P = 0.035$). Factors and biomarkers identified as significant in

TABLE 4 Association between nutritional biomarkers and underweight or stunted at the 1-mo follow-up[1]

	Underweight			Stunted		
	Crude OR (95% CI)	Adjusted OR (95% CI)[2]	Adjusted *P* Value	Crude OR (95% CI)	Adjusted OR (95% CI)[3]	Adjusted *P* Value
Baseline						
Plasma DHA, μg/mL	0.29 (0.03, 2.61)	0.08 (0.001, 4.16)	0.207	0.64 (0.14, 3.40)	0.69 (0.11, 4.47)	0.840
Plasma choline, μg/mL	0.70 (0.39, 1.26)	0.43 (0.14, 1.31)	0.233	1.10 (0.83, 1.45)	1.16 (0.85, 1.58)	0.513
Betaine:choline ratio	1.96 (0.90, 4.26)	3.81 (0.91, 16.0)	0.136	1.43 (0.76, 2.69)	1.40 (0.72, 2.74)	0.644
Follow-up						
Plasma DHA, μg/mL	0.12 (0.01, 2.65)	0.09 (0.002, 3.27)	0.282	3.32 (0.54, 20.6)	8.68 (0.80, 94.2)	0.456
Plasma choline, μg/mL	0.25 (0.05, 1.37)	0.10 (0.01, 0.90)	0.120	1.24 (0.85, 1.80)	1.48 (0.93, 2.34)	0.288
Betaine:choline ratio	2.19 (1.02, 4.70)	5.65 (1.34, 23.8)	0.108	1.09 (0.65, 1.84)	0.95 (0.51, 1.77)	0.949

[1]OR and 95% CI were determined by logistic regression and adjusted *P* values by the Benjamini-Hochberg procedure.
[2]Adjusted for maternal education, adolescent mother, intake of animal source foods at baseline, and time of blood draw.
[3]Adjusted for age, breastfeeding at baseline, and time of blood draw.

TABLE 5 Multilinear regression model for predicting change in growth parameters[1]

	Height-Age *Z* Score[2]		Weight-Age *Z* Score		Weight-Length *Z* Score	
	β (SE)	*P* Value	*β* (SE)	*P* Value	*β* (SE)	*P* Value
Child age, mo	–0.05 (0.02)	0.008[3]	0.02 (0.02)	0.371		
Baseline nutrition						
Breastfeeding			0.29 (0.30)	0.402		
ASFs	0.85 (0.32)	0.01[3]				
Egg intake					0.35 (0.17)	0.058
Bonbon sel					–0.22 (0.09)	0.026[3]
Morbidities						
Vomiting, baseline					0.37 (0.17)	0.057
Days with diarrhea, baseline	0.10 (0.05)	0.056			–0.01 (0.01)	0.076
Days with diarrhea, follow-up					0.06 (0.06)	0.331
Household						
Drinking bottled water	–1.34 (0.53)	0.016[3]				
Drinking public pump water					–1.42 (0.25)	<0.001[3]
Number using toilet			0.37 (0.09)	0.024[3]		
Biomarkers						
DHA, follow-up			–1.97 (0.40)	0.016[3]		
I-FABP, pg/mL, follow-up	–1.37 (0.63)	0.035[3]				
I-FABP, pg/mL, difference					$7.25e^{-5}$ (0.00)	0.055
Adjusted R^2	0.28	0.001[3]	0.85	0.038[3]	0.73	0.001[3]

[1]Values are coefficient *β* (SE) for the difference in growth parameters from baseline to the 1-mo follow-up unless otherwise specified. ASF, animal source food; I-FABP, intestinal fatty acid–binding protein.
[2]Height-age *z* score at 1-mo follow-up.
[3]$P < 0.05$.

regression models were nonsignificant in mediation analysis with structural equation modeling.

Discussion

In this longitudinal case-control study, we examined the relationship between plasma DHA and choline concentrations on growth faltering in children with and without diarrheal illness. Findings showed that plasma DHA and I-FABP concentrations were associated with growth outcomes in young Haitian children after adjusting for confounding factors. Moreover, plasma I-FABP was significantly higher in those with diarrheal illness and remained elevated at the follow-up time point. This study contributes to the limited evidence base for these nutrients and their role in enteric health, diarrhea, and child growth. In our view, it suggests a crucial need for ongoing investigation into the potential impacts of integrated nutrition and diarrhea prevention interventions.

DHA status of young children may indicate different processes at the nexus of nutrition and infection. Our study found that children in Haiti had lower plasma DHA concentrations (mean: 1.03 μg/mL; 95% CI: 0.91, 1.15) than healthy children of similar ages from other contexts (36). These concentrations matched the plasma lipid profiles of severely malnourished Pakistani children (37). However, the pathophysiology for low plasma DHA concentrations in children with undernutrition and diarrheal illness is still unclear. Plasma DHA has been shown to be influenced by diet (10), though nutrient losses in the stool may occur particularly in those with underlying enteropathy (6). Our study showed that higher concentrations of plasma DHA at follow-up were negatively associated with a change in WAZ but not stunting or HAZ. These findings diverge somewhat from other studies showing an association with stunting (18), though this may be explained by the shorter follow-up period, lower intake of foods containing DHA, and high prevalence of diarrhea in our sample.

Plasma DHA concentrations were nonsignificantly higher in cases than controls. Although this did not align with our original hypothesis, the increase in the systemic inflammatory response in children with acute illness may justify these results. In a cross-sectional study of 1609 children aged 6–23 mo with moderate acute malnutrition, researchers noted that whole blood DHA concentrations were negatively correlated with C-reactive protein, which is generally elevated during systemic inflammation. Conversely, they noted a positive association between DHA concentrations in whole blood and the presence of diarrhea (38). Since plasma DHA concentrations fluctuate more than whole blood DHA concentrations, it is plausible that plasma DHA may increase to attenuate the inflammatory reactions in response to infection (39–41).

Plasma choline concentrations and the association with growth outcomes differed from a study by Semba et al. (29), which reported that HAZ positively correlated with serum choline concentrations and negatively with betaine and betaine:choline ratio. Nevertheless, we found that the betaine:choline ratio was significantly lower in cases as compared with controls at the follow-up visit, which may be explained by choline homeostasis pathways, where the oxidation of choline to betaine is reduced in response to a choline-deficient diet to ensure that choline is available for lipid metabolism (28). To our knowledge, no studies have evaluated changes in plasma choline in the setting of acute diarrheal illness in young children, thus warranting more extensive trials for further investigation.

Malnutrition and recurrent diarrheal illness are associated with morphologic and functional changes within the intestine (42). In previous studies, plasma I-FABP was linked to EED and growth faltering

and, as such, can be a valuable, sensitive, and immediate marker for intestinal damage (30, 43). Regarding age-based differences in I-FABP concentrations in the control group, our findings mirror those of other studies, where I-FABP concentrations decrease after 12 mo of age (44). Persistent elevation of I-FABP in cases suggests ongoing intestinal damage in these malnourished children after an intestinal insult. Additionally, I-FABP concentrations were, on average, higher in children 3–12 mo of age living in LMICs when compared with healthy US children and adults (44, 45). This suggests that subclinical intestinal damage may be ubiquitous in LMICs and enteric recovery may be delayed.

Evidence for the relationship between I-FABP and child growth is equivocal in the literature. We showed that plasma I-FABP at follow-up was negatively associated with HAZ at follow-up in multivariate linear regression modeling. Similar results were observed in Peruvian children 5–12 mo of age, where I-FABP concentrations at baseline were significantly higher in children with stunting (46). However, a study on Zimbabwean infants showed inconsistent results regarding I-FABP, noting that plasma I-FABP concentrations were associated with increased length-age *z* score velocity in children 1–3 mo old but finding the opposite in children 6–12 mo old (47). Although findings have been mixed in previous studies, our results support the use of plasma I-FAPB concentrations in assessing growth outcomes and their potential utility in combination with other biomarkers.

In concert with our primary focus, this study explored other potential determinants of young child growth in the Haitian context, revealing some key factors: breastfeeding frequency, dietary intake of ASFs, quality of drinking water, and the duration of diarrhea. Given the complex interactions of infection, bowel function and integrity, and nutritional status, it is paramount to assess these covariates as they are likely confounders in the association between plasma biomarkers and growth outcomes. Specifically, drinking water from a public pump resulted in an adverse change in WHZ. Poor access to water is common in Haiti, where 31% of people have access only to water contaminated with *Escherichia coli* (48, 49). Consumption of contaminated drinking water has been associated with poor growth and EED, aligning with our results (50).

Some limitations were present in this study. First, as a case-control observational study, we were limited in the conclusions that we could draw from the findings. However, measures were taken to reduce bias and confounding in regression modeling and the longitudinal design. The number of samples assayed for plasma DHA, choline, and betaine was also reduced due to budget changes during COVID-19. Despite this, we examined trends in those with acute diarrhea and the relationship between intestinal damage and nutritional biomarkers on growth within a community setting in Cap-Haitien, Haiti. This study served as preliminary data for a large randomized controlled trial, *Grandi Byen*, ongoing to test the efficacy of a parenting and nutrition intervention on a full range of outcomes, including biomarkers assessed here (51).

The findings from this case-control study showed high plasma I-FABP in both groups with significantly higher concentrations in those with acute diarrheal illness, evincing the rampant malnutrition and enteric damage present in young Haitian children. This evidence also suggests a role for DHA in association with growth outcomes, and the overall low concentrations across the sample population point to likely dietary deficiencies. Our results provide formative data highlighting the absolute necessity of these underappreciated nutrients in childhood diarrheal illness and malnutrition.

Acknowledgments

We thank our field team, including nurses Bernadine Joseph, Jeanise Borgella, Solange Cheristin, and Eugénie Geffrard, and our laboratory technician Saintano Asse, for all the fantastic work interacting with the mother-child dyads and collecting the data.

The authors' responsibilities were as follows—FMK, LLI, FJMP: designed the study; SJLD: oversaw data collection and had hands-on conduction of the research; JND: oversaw the investigation, performed the ELISA testing on plasma and stool samples, performed the statistical analysis, wrote the manuscript, and is primarily responsible for the final content; XJ: conducted the quantitative choline analysis; NW, EG: worked on statistical analysis and the writing of the manuscript; and all authors: read and approved the final manuscript.

Data Availability

Data described in the manuscript, code book, and analytic code will be available upon request.

References

1. Levine MM, Kotloff KL, Nataro JP, Muhsen K. The Global Enteric Multicenter Study (GEMS): impetus, rationale, and genesis. Clin Infect Dis 2012;55 Suppl 4:S215–24.
2. Institute for Health Metrics and Evaluation. Haiti profile. Seattle (WA): University of Washington; 2021.
3. Institut Haïtien de l'Enfance, ICF. Haïti, Enquête Mortalité, Morbidité et Utilisation des Services (EMMUS-VI 2016–2017). Pétion-Ville (Haïti) and Rockville (MD): Institut Haïtien de l'Enfance and ICF; 2018.
4. Tickell KD, Sharmin R, Deichsel EL, Lamberti LM, Walson JL, Faruque ASG, et al. The effect of acute malnutrition on enteric pathogens, moderate-to-severe diarrhoea, and associated mortality in the Global Enteric Multicenter Study cohort: a post-hoc analysis. Lancet Glob Health 2020;8(2):e215–24.
5. Ordiz MI, Shaikh N, Trehan I, Maleta K, Stauber J, Shulman R, et al. Environmental enteric dysfunction is associated with poor linear growth and can be identified by host fecal mRNAs. J Pediatr Gastroenterol Nutr 2016;63(5):453–9.
6. Harper KM, Mutasa M, Prendergast AJ, Humphrey J, Manges AR. Environmental enteric dysfunction pathways and child stunting: a systematic review. PLoS Negl Trop Dis 2018;12(1):e0006205.
7. MAL-ED Network Investigators. Relationship between growth and illness, enteropathogens and dietary intakes in the first 2 years of life: findings from the MAL-ED birth cohort study. BMJ Glob Health 2017;2:e000370.
8. Shapiro MJ, Downs SM, Swartz HJ, Parker M, Quelhas D, Kreis K, et al. A systematic review investigating the relation between animal-source food consumption and stunting in children aged 6–60 months in low and middle-income countries. Adv Nutr 2019;10(5):827–47.
9. Black RE, Victora CG, Walker SP, Bhutta ZA, Christian P, de Onis M, et al. Maternal and child undernutrition and overweight in low-income and middle-income countries. Lancet North Am Ed 2013;382(9890):427–51.
10. Iannotti LL, Lutter CK, Waters WF, Gallegos Riofrío CA, Malo C, Reinhart G, et al. Eggs early in complementary feeding increase choline pathway biomarkers and DHA: a randomized controlled trial in Ecuador. Am J Clin Nutr 2017;106(6):1482–9.

11. Iannotti LL, Lutter CK, Stewart CP, Gallegos Riofrío CA, Malo C, Reinhart G, et al. Eggs in early complementary feeding and child growth: a randomized controlled trial. Pediatrics 2017;140(1):e20163459.
12. Alves da Silva AV, de Castro Oliveira SB, Di Rienzi SC, Brown-Steinke K, Dehan LM, Rood JK, et al. Murine methyl donor deficiency impairs early growth in association with dysmorphic small intestinal crypts and reduced gut microbial community diversity. Curr Dev Nutr 2019; 3(1):nzy070.
13. Ibrahim MK, Zambruni M, Melby CL, Melby PC. Impact of childhood malnutrition on host defense and infection. Clin Microbiol Rev 2017;30(4):919–71.
14. Durkin LA, Childs CE, Calder PC. Omega-3 polyunsaturated fatty acids and the intestinal epithelium—a review. Foods 2021;10(1):199.
15. Bragg MG, Prado EL, Stewart CP. Choline and docosahexaenoic acid during the first 1000 days and children's health and development in low- and middle-income countries. Nutr Rev 2022;80(4):656–76.
16. Zeisel SH, da Costa KA. Choline: an essential nutrient for public health. Nutr Rev 2009;67(11):615–23.
17. Calder PC. Docosahexaenoic acid. Ann Nutr Metab 2016;69 Suppl 1:7–21.
18. Adjepong M, Pickens CA, Jain R, Harris WS, Annan RA, Fenton JI. Association of whole blood n-6 fatty acids with stunting in 2-to-6-year-old Northern Ghanaian children: a cross-sectional study. PLoS One 2018;13(3):e0193301.
19. World Health Organization. Diarrhoea: why children are still dying and what can be done. New York (NY): United Nations Children's Fund; 2009.
20. Harris PA, Taylor R, Minor BL, Elliott V, Fernandez M, O'Neal L, et al. The REDCap Consortium: building an international community of software platform partners. J Biomed Inform 2019;95:103208.
21. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)—a metadata-driven methodology and workflow process for providing translational research informatics support. J Biomed Inform 2009;42(2):377–81.
22. de Onis M, Onyango AW, Van den Broeck J, Chumlea WC, Martorell R. Measurement and standardization protocols for anthropometry used in the construction of a new international growth reference. Food Nutr Bull 2004;25(1 Suppl 1):S27–36.
23. WHO Multicentre Growth Reference Study. WHO child growth standards: length, height for-age, weightfor-age, weight-for-length and body mass index-for age. Methods and development. Geneva (Switzerland): World Health Organization; 2006.
24. World Health Organization. Training course on child growth assessment. Geneva (Switzerland): World Health Organization; 2008.
25. World Health Organization. WHO Anthro for personal computers, version 3.2.2, 2011: software for assessing growth and development of the world's children. Geneva (Switzerland): World Health Organization; 2010.
26. World Health Organization. WHO guidelines on drawing blood: best practices in phlebotomy. Geneva (Switzerland): World Health Organization; 2010.
27. Office of the Vice Chancellor for Research. Metabolomics Facility [Internet]. St Louis (MO): Washington University in St Louis; [cited 2022]. Available from: https://research.wustl.edu/core-facilities/metabolomics-facility/
28. Li Z, Vance DE. Phosphatidylcholine and choline homeostasis. J Lipid Res 2008;49(6):1187–94.
29. Semba RD, Zhang P, Gonzalez-Freire M, Moaddel R, Trehan I, Maleta KM, et al. The association of serum choline with linear growth failure in young children from rural Malawi. Am J Clin Nutr 2016;104(1):191–7.
30. Adriaanse MP, Tack GJ, Passos VL, Damoiseaux JG, Schreurs MW, van Wijck K, et al. Serum I-FABP as marker for enterocyte damage in coeliac disease and its relation to villous atrophy and circulating autoantibodies. Aliment Pharmacol Ther 2013;37(4):482–90.
31. Prata MM, Havt A, Bolick DT, Pinkerton R, Lima A, Guerrant RL. Comparisons between myeloperoxidase, lactoferrin, calprotectin and lipocalin-2, as fecal biomarkers of intestinal inflammation in malnourished children. J Transl Sci 2016;2:134–9.
32. Iannotti LL, Dulience SJ, Green J, Joseph S, François J, Anténor ML, et al. Linear growth increased in young children in an urban slum of Haiti: a randomized controlled trial of a lipid-based nutrient supplement. Am J Clin Nutr 2014;99(1):198–208.
33. Miller TM, Abdel-Maksoud MF, Crane LA, Marcus AC, Byers TE. Effects of social approval bias on self-reported fruit and vegetable consumption: a randomized controlled trial. Nutr J 2008;7(1):18.
34. Hebert JR, Hurley TG, Peterson KE, Resnicow K, Thompson FE, Yaroch AL, et al. Social desirability trait influences on self-reported dietary measures among diverse participants in a multicenter multiple risk factor trial. J Nutr 2008;138(1):226S–34S.
35. Glickman ME, Rao SR, Schultz MR. False discovery rate control is a recommended alternative to Bonferroni-type adjustments in health studies. J Clin Epidemiol 2014;67(8):850–7.
36. Glaser C, Demmelmair H, Sausenthaler S, Herbarth O, Heinrich J, Koletzko B. Fatty acid composition of serum glycerophospholipids in children. J Pediatr 2010;157(5):826–31.e1.
37. Shokry E, Sadiq K, Soofi S, Habib A, Bhutto N, Rizvi A, et al. Impact of treatment with RUTF on plasma lipid profiles of severely malnourished Pakistani children. Nutrients 2020;12(7):2163.
38. Yaméogo CW, Cichon B, Fabiansen C, Rytter MJH, Faurholt-Jepsen D, Stark KD, et al. Correlates of whole-blood polyunsaturated fatty acids among young children with moderate acute malnutrition. Nutr J 2017; 16(1):44.
39. Arab L. Biomarkers of fat and fatty acid intake. J Nutr 2003;133(3): 925S–32S.
40. Sun Q, Ma J, Campos H, Hankinson SE, Hu FB. Comparison between plasma and erythrocyte fatty acid content as biomarkers of fatty acid intake in US women. Am J Clin Nutr 2007;86(1):74–81.
41. Calder PC. Omega-3 fatty acids and inflammatory processes. Nutrients 2010;2(3):355–74.
42. Attia S, Feenstra M, Swain N, Cuesta M, Bandsma RHJ. Starved guts: morphologic and functional intestinal changes in malnutrition. J Pediatr Gastroenterol Nutr 2017;65(5):491–5.
43. Patterson GT, Manthi D, Osuna F, Muia A, Olack B, Mbuchi M, et al. Environmental, metabolic, and inflammatory factors converge in the pathogenesis of moderate acute malnutrition in children: an observational cohort study. Am J Trop Med Hyg 2021;104(5):1877–88.
44. Prendergast AJ, Rukobo S, Chasekwa B, Mutasa K, Ntozini R, Mbuya MN, et al. Stunting is characterized by chronic inflammation in Zimbabwean infants. PLoS One 2014;9(2):e86928.
45. Derikx JP, Vreugdenhil AC, Van den Neucker AM, Grootjans J, van Bijnen AA, Damoiseaux JG, et al. A pilot study on the noninvasive evaluation of intestinal damage in celiac disease using I-FABP and L-FABP. J Clin Gastroenterol 2009;43(8):727–33.
46. Zambruni M, Ochoa TJ, Somasunderam A, Cabada MM, Morales ML, Mitreva M, et al. Stunting is preceded by intestinal mucosal damage and microbiome changes and is associated with systemic inflammation in a cohort of Peruvian infants. Am J Trop Med Hyg 2019;101(5):1009–17.
47. Mutasa K, Ntozini R, Mbuya MNN, Rukobo S, Govha M, Majo FD, et al. Biomarkers of environmental enteric dysfunction are not consistently associated with linear growth velocity in rural Zimbabwean infants. Am J Clin Nutr 2021;113(5):1185–98.
48. Gelting R, Bliss K, Patrick M, Lockhart G, Handzel T. Water, sanitation and hygiene in Haiti: past, present, and future. Am J Trop Med Hyg 2013;89(4):665–70.
49. Rogers-Brown J, Johnson R, Smith D, Ramsey-White K. A pilot study to examine the disparities in water quality between predominantly Haitian neighborhoods and dominican neighborhoods in two cities in the Dominican republic. Int J Environ Res Public Health 2015;13(1):39.
50. Lauer JM, Duggan CP, Ausman LM, Griffiths JK, Webb P, Bashaasha B, et al. Unsafe drinking water is associated with environmental enteric dysfunction and poor growth outcomes in young children in rural southwestern Uganda. Am J Trop Med Hyg 2018;99(6):1606–12.
51. PL K. Grandi Byen—supporting child growth and development through integrated, responsive parenting, nutrition and hygiene: study protocol for a randomized controlled trial. BMC Pediatr 2022;22(1):54.