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Effects of Water, Sanitation, Handwashing, and Nutritional Interventions on Environmental Enteric Dysfunction in Young Children: A Cluster-randomized, Controlled Trial in Rural Bangladesh

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Background. We hypothesized that drinking water, sanitation, handwashing (WSH), and nutritional interventions would improve environmental enteric dysfunction (EED), a potential contributor to stunting.

Methods. Within a subsample of a cluster-randomized, controlled trial in rural Bangladesh, we enrolled pregnant women in 4 arms: control, WSH, child nutrition counseling plus lipid-based nutrient supplements (N), and nutrition plus WSH (N+WSH). Among the birth cohort, we measured biomarkers of gut inflammation (myeloperoxidase, neopterin), permeability (alpha-1-antitrypsin, lactulose, mannitol), and repair (regenerating gene 1β) at median ages 3, 14, and 28 months. Analysis was intention-to-treat.

Results. We assessed 1512 children. At age 3 months, compared to controls, neopterin was reduced by nutrition (–0.21 log nmol/L; 95% confidence interval [CI], –.37, –.05) and N+WSH (–0.20 log nmol/L; 95% CI, –.34, –.06) interventions; similar reductions were observed at 14 months. At 3 months, all interventions reduced lactulose and mannitol (–0.60 to –0.69 log mmol/L). At 28 months, myeloperoxidase was elevated in the WSH and nutrition arms (0.23–0.27 log ng/mL) and lactulose was higher in the WSH arm (0.30 log mmol/L; 95% CI, .07, .53).

Conclusions. Reductions in permeability and inflammation at ages 3 and 14 months suggest that the interventions promoted healthy intestinal maturation; however, by 28 months, the WSH and nutrition arms showed elevated EED biomarkers. These results underscore the importance of developing a better understanding of EED pathophysiology and targeting interventions early in childhood, when they are likely to have the largest benefit to intestinal health.

Clinical Trials Registration. NCT01590095.

Keywords. environmental enteric dysfunction; environmental enteropathy; early childhood intervention; nutrition; water sanitation hygiene trial.

Globally, 151 million children aged <5 years are stunted [1]. Stunting is associated with impaired cognitive development [1]. If existing nutrition-specific interventions were implemented globally with 90% coverage, they would reduce stunting prevalence by only 20% [2]. Environmental enteric dysfunction (EED), a pervasive, small bowel dysfunction [3], has been widely hypothesized as a main contributor to stunting [4, 5]. A small, early study attributed 43% of growth faltering to EED [6]; however, more recent, large, multicohort studies suggest that available EED biomarkers are only weakly predictive of linear growth [7–10].

EED encompasses various facets of gut dysfunction including increased intestinal permeability and microbial translocation, local and systemic inflammation, enterocyte damage, and malabsorption [11, 12]. Because biopsies are not feasible in epidemiologic contexts, noninvasive biomarkers are used to measure EED [4, 7, 13]. Validated putative EED markers include measures of intestinal permeability (fecal alpha-1-antitrypsin and urinary lactulose and mannitol), inflammation (fecal myeloperoxidase and neopterin), and repair (fecal regenerating

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gene 1β [REG1B]) [7, 8, 14–17]. Although EED studies historically report the lactulose-to-mannitol (L:M) ratio, with a high ratio thought to indicate gut dysfunction, new evidence has led to questions about its interpretation and utility [17, 18].

EED begins early in infancy [11, 18, 19]. Suboptimal infant feeding practices that lead to nutrient deficiencies may contribute to its development by exacerbating intestinal inflammation, impairing intestinal repair, and increasing susceptibility to enteric infections [3]. Several studies have evaluated the effects of nutritional interventions on EED markers with mixed results [3, 20–28]. Trials that delivered vitamin A, alanyl-glutamine, or zinc improved gut function [3, 24]. Those that administered probiotics, glutamine, or fish oil reported no effect [3, 20]. In 2 trials, multiple micronutrient supplementation improved EED markers [21, 25], but another trial of albendazole and multiple micronutrients found no benefits [26]. There is some evidence that iron fortification may worsen EED markers in children [22, 27, 28].

Enteric pathogen transmission occurs via fecal–oral pathways: water, food, fomites, hands, and flies [29]. Recurrent exposure to fecal pathogens that results in gut microbiome dysbiosis is hypothesized to contribute to the development of EED [5, 13, 18, 30]. Drinking water, sanitation, and handwashing (WSH) interventions can prevent pathogen transmission through environmental contamination reduction [29]. However, there is scant evidence on whether WSH interventions would reduce EED. Our study in rural Bangladesh found that children who live with improved WSH have better growth and EED biomarkers than those with poor WSH [13]. One small study that evaluated the effect of handwashing on EED markers in Nepal and another study that assessed community WSH and deworming interventions in Timor–Leste reported no impact [31, 32].

If poor nutrition and fecal exposure contribute to EED, combined nutrition plus WSH (N+WSH) interventions may improve EED markers more than nutrition or WSH interventions alone. Within a randomized trial in rural Bangladesh, we determined whether improved nutrition, WSH, or N+WSH interventions improved EED markers (prespecified outcomes) among young children [33].

METHODS

Study Design

The cluster-randomized WASH Benefits trial was conducted in rural districts in Bangladesh [33]. The study design and rationale were published previously [34]. The EED substudy enrolled a subsample of clusters that was factorial and evenly balanced across the control, WSH, nutrition, and N+WSH arms (allocation ratio 1:1:1:1). Clusters were selected based on logistical feasibility for specimen collection and transport to a central laboratory. Study protocols were approved by human subjects committees at the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B), the University of California–Berkeley, and Stanford University.

Participants

We enrolled pregnant women in their first 2 trimesters and their in utero children. Primary caregivers provided written, informed consent. Additional enrollment criteria are listed in the Supplementary Materials.

Randomization

Clusters of 8 neighboring households with eligible women were formed. A minimum of 1 km buffer around each cluster was created to prevent spillover between clusters. Eight geographically adjacent clusters were block-randomized off site (B. F. A.) with a random number generator to the double-sized control arm or 1 of the 6 intervention arms (water, sanitation, handwashing, WSH, nutrition, N+WSH). Masking details are provided in the Supplementary Materials.

Procedures

Interventions were described previously [33]. Nutrition included lipid-based nutrient supplements for children aged 6–24 months and age-appropriate maternal and infant nutrition recommendations (pregnancy–24 months). WSH included drinking water treatment (chlorine tablets and safe water storage vessel), sanitation improvements (child potties, sani-scoops for feces disposal, and a double-pit latrine with a water seal), and handwashing (stations with soapy water near the latrine and kitchen). N+WSH included all interventions. Community health promoters visited intervention households at least once per week during the first 6 months and, subsequently, at least once every 2 weeks to promote recommended behaviors. Controls did not receive interventions or promoter visits. Intervention adherence was high (>80%) for all interventions [33].

Fecal alpha-1-antitrypsin, myeloperoxidase, neopterin, and REG1B were measured at ICDDR,B following enzyme-linked immunosorbent assay kit protocols (details in the Supplementary Materials). The coefficient of variation (CV) of these assays was 5.7%–8.8%.

Staff administered a lactulose and mannitol (LM) solution (250 mg/mL of lactulose and 50 mg/mL of mannitol) in a dose of 2 mL/kg to each child up to a maximum of 20 mL. A pre-LM single-void and a continuously pooled 5-hour post-LM urine specimen were collected. Urine specimens were analyzed for lactulose and mannitol recovery using high-performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry at Wagner College, Staten Island, New York (details are in the Supplementary Materials). The intraassay CV was 1.2% for mannitol and 6.8% for lactulose.

Outcomes

Prespecified outcome measures were fecal EED markers (REG1B [only at age 14 months], alpha-1-antitrypsin, myeloperoxidase, and neopterin) and urinary EED markers (lactulose, mannitol, and L:M ratio) measured shortly after birth (median age, 3 months), 1 year after intervention initiation (median age, 14 months), and 2 years after intervention initiation (median age, 28 months). Lipid-based nutrient supplements ended at age 24 months; WSH interventions ended 36 months after intervention initiation.

Statistical Analyses

The statistical analyses plan was published previously [34]. The preregistered analysis protocol and replication files for the EED substudy are available [35]. Analyses were performed using R statistical software, version 3.2.3.

The sample size for the EED study was 1500 children. Assuming a 2-sided type I error of 5%, 54 clusters enrolled per arm with 7 children measured per cluster, and a cluster-level intraclass correlation coefficient equal to 0.15, the study had 80% power to detect a difference of 0.26 standard deviations in standardized log EED biomarkers between any 2 arms.

Analyses were intention-to-treat. We compared intervention vs control arms and combined vs individual intervention arms (N+WSH vs nutrition and WSH) separately at median age 3 months, 14 months, and 28 months.

The analyses generally followed the same methods as described for the trial's primary outcomes (details in the Supplementary Materials) [34]. Randomization led to balance in observed covariates across arms; per our prespecified analysis plan, the primary analysis was unadjusted and used generalized linear models. Two secondary analyses included adjusting for child age and sex only and fully adjusting for child age, sex, and covariates associated with each outcome (likelihood ratio test P value < .20). We used inverse probability of censoring weighting (IPCW) to correct for potential bias due to informative censoring [36]. To control family-wise error rates, P values were Bonferroni corrected to account for multiple testing across 3 visits.

RESULTS

Study staff identified 13 279 pregnant women in their first or second trimester. Of these women, 5551 (in 720 clusters) were randomly allocated to 1 of the intervention or control arms between 31 May 2012 and 7 July 2013 (Supplementary Figure S1). The EED team visited 1645 children at age 3 months, 1978 children at age 14 months, and 2049 children at age 28 months in the control, nutrition, WSH, and N+WSH arms (Supplementary Figure S1). EED outcomes were measured in 1090 children (66%) at age 3.0 (interquartile range [IQR], 1.6–4.1) months, 1499 children (76%) at age 14.0 (IQR, 12.6–15.3) months, and 1512 children (74%) at age 28.1 (IQR, 26.8–29.3) months (Supplementary Figure S1). EED study household enrollment characteristics were balanced across all study arms (Table 1) and were similar to those from the overall trial (Supplementary Table S1) [33]. Characteristics were also balanced between individuals who had EED outcomes at age 3 months vs those who were lost to follow-up at ages 14 and 28 months (Supplementary Table S2).

Children's mean (± standard deviation) age was 3.0 (± 1.7) months at the first assessment, 14.0 (± 2.0) months 1 year after intervention initiation, and 28.1 (± 1.9) months 2 years after intervention initiation. At age 3 months, in the nutrition arms, 51%–55% of women reported exclusively breastfeeding their children in the prior 24 hours compared to 18% in the controls and 27% in the WSH arm. Pre-LM urinary sugar levels were only evaluated in a subset of children (n = 377) aged 14 months; mean concentrations of mannitol and lactulose were 0.06 mmol/L and 0.002 mmol/L, respectively. All biomarker distributions were right-skewed (Supplementary Table S3); therefore, we log-transformed them.

At age 3 months, neopterin (an inflammation marker) was lower in the WSH (−0.13 log nmol/L; 95% confidence interval [CI], −.26 to −.00), nutrition (−0.21 log nmol/L; 95% CI, −.37 to −.05), and N+WSH (−0.20 log nmol/L; 95% CI, −.34 to −.06) arms compared to controls (Figure 1, Table 2, Supplementary Figure S2, and Supplementary Table S4). When the children were aged 14 months, neopterin was lower in the nutrition (−0.19 log nmol/L; 95% CI, −.38 to −.01) and N+WSH arms (−0.18 log nmol/L; 95% CI, −.34 to −.02) compared to controls (Figure 1, Table 3, Supplementary Figure S2, and Supplementary Table S5). By age 28 months, no differences in neopterin were observed between any of the arms (Figure 1, Table 4, Supplementary Figure S2, and Supplementary Table S6).

At ages 3 months and 14 months, myeloperoxidase concentrations (measuring inflammation) were not different between arms (Figure 1, Tables 2 and 3, Supplementary Figure S2, and Supplementary Tables S4 and S5). At age 28 months, myeloperoxidase was higher in WSH (0.23 log ng/mL; 95% CI, .06 to .39) and nutrition (0.27 log ng/mL; 95% CI, .07 to .47) arms compared to controls (Figure 1, Table 4, Supplementary Figure S2, and Supplementary Table S6).

At age 3 months, lactulose, a marker for intestinal permeability, was lower in the nutrition (−0.63 log mmol/L; 95% CI, −.96 to −.29), WSH (−0.60 log mmol/L; 95% CI, −.91 to −.28), and N+WSH arms (−0.69 log mmol/L; 95% CI, −1.03 to −.36) compared to controls (Figure 1, Table 2, Supplementary Figure S2, and Supplementary Table S4). At age 14 months, none of the intervention arms had lower lactulose concentrations compared to controls (Figure 1, Table 3, Supplementary Figure S2, and Supplementary Table S5). At age 28 months, lactulose was higher in the WSH arm (0.30 log mmol/L; 95% CI, .07 to .53; Figure 1, Table 4, Supplementary Figure S2, and Supplementary Table S6).

Table 1. Enrollment Characteristics by Intervention Group

Characteristic	Control	WSH	Nutrition	Nutrition + WSH
No. of Compounds:	n = 454	n = 429	n = 450	n = 450
Maternal				
Age, y	23 (5)	24 (5)	24 (5)	24 (5)
Years of education	7 (3)	6 (3)	6 (4)	6 (3)
Paternal				
Years of education	5 (4)	5 (4)	5 (4)	5 (4)
Works in agriculture	104 (23%)	128 (29%)	148 (34%)	127 (28%)
Household				
Number of people	5 (2)	5 (2)	5 (2)	5 (2)
Has electricity	269 (60%)	278 (62%)	269 (62%)	272 (61%)
Has a cement floor	75 (17%)	55 (12%)	50 (11%)	53 (12%)
Acres of agricultural land owned	0 (0)	0 (0)	0 (0)	0 (0)
Drinking water				
Shallow tube well primary water source	329 (73%)	338 (76%)	309 (71%)	318 (71%)
Stored water observed at home	230 (51%)	200 (45%)	209 (48%)	229 (51%)
Reported treating water yesterday	1 (0%)	0 (0%)	0 (0%)	1 (0%)
Distance (minutes) to primary water source	1 (2)	1 (6)	1 (2)	1 (2)
Sanitation				
Reported daily open defecation				
Adult men	19 (4%)	30 (7%)	38 (9%)	38 (9%)
Adult women	12 (3%)	16 (4%)	23 (5%)	21 (5%)
Children: 8 to <15 y	9 (5%)	17 (8%)	13 (8%)	22 (11%)
Children: 3 to <8 y	65 (30%)	90 (37%)	90 (40%)	92 (37%)
Children: 0 to <3 y[a]	71 (72%)	73 (75%)	68 (80%)	79 (88%)
Latrine				
Owned[b]	271 (60%)	244 (55%)	234 (54%)	230 (51%)
Concrete slab	426 (97%)	401 (93%)	382 (93%)	399 (94%)
Functional water seal	157 (38%)	95 (26%)	114 (32%)	111 (31%)
Visible stool on slab or floor	197 (45%)	225 (54%)	210 (52%)	222 (53%)
Owned a child potty	37 (8%)	20 (4%)	27 (6%)	21 (5%)
Human feces observed in the house	25 (6%)	36 (8%)	41 (9%)	36 (8%)
Human feces observed in the child's play area	5 (1%)	4 (1%)	7 (2%)	6 (1%)
Handwashing location				
Within 6 steps of latrine				
Has water	84 (21%)	51 (13%)	38 (10%)	54 (13%)
Has soap	45 (11%)	32 (8%)	23 (6%)	27 (7%)
Within 6 steps of kitchen				
Has water	48 (12%)	40 (10%)	43 (11%)	42 (10%)
Has soap	18 (4%)	11 (3%)	19 (5%)	14 (3%)
Nutrition				
Household is food secure[c]	331 (74%)	298 (67%)	308 (71%)	317 (71%)

Abbreviation: WSH, drinking water, sanitation, handwashing.

Data are number (%) or mean (standard deviation). Percentages were estimated from slightly smaller denominators than those shown at the top of the table for the following variables due to missing values: mother's age, father's education, father works in agriculture, acres of land owned, open defecation, latrine has a concrete slab, latrine has a functional water seal, visible stool on latrine slab or floor, ownership of child potty, observed feces in the house or child's play area, handwashing variables.

[a]Open defecation does not include diaper disposal of feces.

[b]Households that do not own a latrine typically share a latrine with extended family members who live in the same compound.

[c]Assessed by the Household Food Insecurity Access Scale [37] .

At age 3 months, mannitol (a permeability marker) concentrations were lower in the nutrition (–0.64 to –0.67 log mmol/L), WSH, and N+WSH intervention arms (Figure 1, Table 2, Supplementary Figure S2, and Supplementary Table S4). At ages 14 months and 28 months, no differences in mannitol concentrations were observed between the intervention arms and controls (Figure 1, Tables 3 and 4, Supplementary Figure S2, and Supplementary Tables S5 and S6).

At age 3 months, none of the interventions impacted L:M (Figure 1, Table 2, Supplementary Figure S2, and Supplementary Table S4). At age 14 months, the WSH intervention arm had a lower L:M (difference, –0.24; 95% CI, –.43 to –.06; Figure 1, Table 3, Supplementary Figure S2, and Supplementary Table S5). At age 28 months, L:M was higher (difference, 0.18 to 0.20) in the WSH, nutrition, and N+WSH arms (Figure 1, Table 4, Supplementary Figure S2, and Supplementary Table S6).

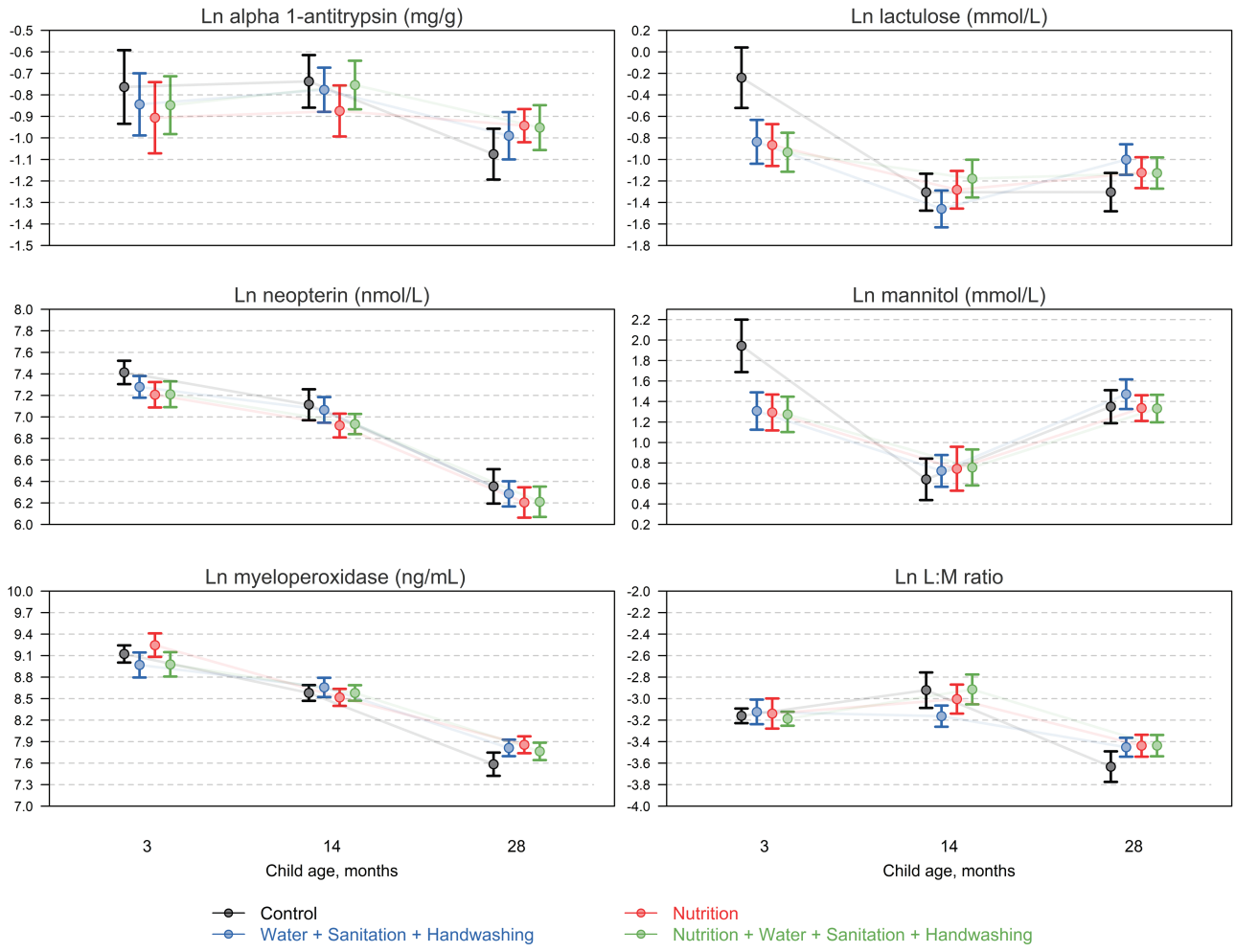

Figure 1. Means for log-transformed environmental enteric dysfunction biomarkers in each arm at child age 3 months, 14 months, and 28 months. Abbreviation: L:M, lactulose-to-mannitol.

Lactulose and mannitol percent recovery results at all 3 time points were similar to results for absolute concentrations (Supplementary Table S7). Forty-three percent of children at age 3 months, 34% of children at age 14 months, and 23% of children at age 28 months experienced at least 1 episode of urine loss due to urine bag leakage or fecal contamination. Repeating the analyses after excluding children with urine loss episodes yielded similar results (Supplementary Tables S8 and S9).

Alpha-1-antitrypsin concentrations (permeability marker) were not different between arms at age 3 months (Figure 1, Table 2, Supplementary Figure S2, and Supplementary Table S4) and 14 months (Table 3 and Supplementary Table S5). At age 28 months, alpha-1-antitrypsin was elevated in the nutrition arm (0.13 log mg/g; 95% CI, –.00 to .27; Figure 1, Table 4, Supplementary Figure S2, and Supplementary Table S6). At age 14 months, the REG1B marker for intestinal repair was not different between arms (Table 3 and Supplementary Table S5).

Overall, N+WSH interventions did not provide additional benefit or harm beyond combined WSH or nutrition-only interventions at ages 3 months, 14 months, and 28 months (Tables 2–4). Overall, unadjusted, adjusted, and IPCW analyses yielded similar estimates (Supplementary Tables S4–S6), suggesting little evidence of measured confounding. Supplementary Tables S10–S12 display unadjusted and adjusted *P* values.

DISCUSSION

Compared to controls, children in all intervention groups had lower intestinal permeability (lactulose and mannitol) and inflammation (neopterin) at age 3 months. Lower neopterin concentrations were sustained through age 14 months only in the nutrition and N+WSH groups; the WSH intervention group displayed a lower L:M ratio. Combined N+WSH interventions did not provide additional benefit beyond the WSH or nutrition-only interventions. Unexpectedly, by age 28 months, children in WSH and nutrition intervention arms had higher markers of intestinal inflammation (myeloperoxidase) and permeability (alpha-1-antitrypsin and lactulose) compared to controls.

EED likely begins soon after birth, as demonstrated by the early onset of severe intestinal inflammation at age 3 months

Table 2. Effect of Intervention on Environmental Enteric Dysfunction Measurements at Age 3 Months

Outcome, Arm	N	Mean	Standard Deviation	Difference from Control (95% Confidence Interval)	Difference from Nutrition	Difference from WSH
Ln myeloperoxidase (ng/mL)						
Control	200	9.12	1.03	…	…	…
WSH	229	8.97	1.10	–0.15 (–.36, .05)	…	…
Nutrition	207	9.25	1.13	0.12 (–.09, .33)	…	…
Nutrition + WSH	206	8.98	1.12	–0.15 (–.36, .07)	–0.27 (–0.46, –0.07)	0.01 (–0.21, 0.23)
Ln alpha-1-antitrypsin (mg/g)						
Control	200	–0.76	1.09	…	…	…
WSH	229	–0.84	1.01	–0.08 (–.30, .14)	…	…
Nutrition	206	–0.91	1.02	–0.14 (–.39, .10)	…	…
Nutrition + WSH	205	–0.85	0.85	–0.08 (–.33, .16)	0.06 (–0.16, 0.27)	–0.00 (–0.19, 0.18)
Ln neopterin (nmol/L)						
Control	200	7.41	0.62	…	…	…
WSH	229	7.28	0.72	–0.13 (–.26, –.00)	…	…
Nutrition	207	7.21	0.73	–0.21 (–.37, –.05)	…	…
Nutrition + WSH	207	7.21	0.70	–0.20 (–.34, –.06)	0.00 (–0.15, 0.16)	–0.07 (–0.21, 0.07)
Ln lactulose (mmol/L)						
Control	251	–0.24	1.16	…	…	…
WSH	268	–0.84	1.08	–0.60 (–.91, –.28)	…	…
Nutrition	255	–0.87	1.10	–0.63 (–.96, –.29)	…	…
Nutrition + WSH	259	–0.93	1.05	–0.69 (–1.03,–.36)	–0.07 (–0.29, 0.15)	–0.10 (–0.31, 0.11)
Ln mannitol (mmol/L)						
Control	251	1.94	1.04	…	…	…
WSH	268	1.31	1.01	–0.64 (–.92, –.35)	…	…
Nutrition	255	1.29	1.02	–0.65 (–.95, –.35)	…	…
Nutrition + WSH	259	1.28	0.97	–0.67 (–.99, –.35)	–0.02 (–0.20, 0.16)	–0.03 (–0.23, 0.16)
Ln lactulose-to-mannitol ratio						
Control	250	–3.16	0.51	…	…	…
WSH	267	–3.12	0.69	0.04 (–.09, .17)	…	…
Nutrition	255	–3.14	0.63	0.02 (–.14, .18)	…	…
Nutrition + WSH	259	–3.19	0.53	–0.03 (–.12, .06)	–0.05 (–0.21, 0.11)	–0.06 (–0.19, 0.06)

Confidence intervals were adjusted for clustered observations using robust standard errors.

Abbreviation: WSH, drinking water, sanitation, handwashing.

in the control group. At age 3 months, the nutrition, WSH, and N+WSH interventions had lower neopterin but not myeloperoxidase. Breast milk oligosaccharides could have attenuated inflammation or prevented enteropathogen attachment, resulting in a reduction in neopterin for children in the nutrition arms [38]. The WSH intervention may have reduced intestinal inflammation by limiting exposure to pathogens in drinking water and food; the trial found reductions in diarrhea [33] and *Escherichia coli* in stored drinking water and food given to young children [29]. Similar magnitudes of neopterin reduction were sustained through age 14 months in the nutrition and N+WSH arms but not the WSH-only arm. Increased child mobility at age 14 months combined with the high frequency of observed hand-mouthing behaviors could lead to higher pathogen exposure [39].

Persistent intestinal inflammation increases permeability and microbial translocation [11]. We assessed intestinal permeability using alpha-1-antitrypsin and the LM assay. Historically, mannitol, thought to diffuse through transcellular pathways and reflect absorptive surface area, was used to normalize lactulose measurements. However, new evidence, consistent with our findings, suggests that both lactulose and mannitol diffuse via paracellular pathways and reflect tight junction function; thus, we would expect interventions to reduce lactulose and mannitol permeability [17]. In accordance with most EED studies and our prespecified analyses plan, we have reported L:M results. However, this newly proposed pathway model obfuscates traditional interpretation of the L:M ratio, which is that a high L:M previously denoted gut dysfunction. However, in the new model, a low L:M could denote either normal or poor gut function because lactulose and mannitol are strongly correlated [17]. Therefore, we adopted the recommended approach of interpreting lactulose and mannitol separately [17].

At age 3 months, the nutritional interventions resulted in lower lactulose and mannitol but not alpha-1-antitrypsin. Higher rates of exclusive breastfeeding may have facilitated "gut closure," an essential intestinal maturation process in infants whereby the intestine transitions from high to selective

Table 3. Effect of Intervention on Environmental Enteric Dysfunction Measurements at Age 14 Months

Outcome, Arm	N	Mean	Standard Deviation	Difference from Control (95% Confidence Interval)	Difference from Nutrition	Difference from WSH
Ln myeloperoxidase (ng/mL)						
Control	331	8.58	1.06	…	…	…
WSH	373	8.66	1.04	0.08 (–.10, .25)	…	…
Nutrition	358	8.52	1.00	–0.06 (–.25, .12)		
Nutrition + WSH	365	8.58	1.01	–0.00 (–.15, .15)	0.06 (–0.10, 0.23)	–0.08 (–0.24, 0.08)
Ln alpha-1-antitrypsin (mg/g)						
Control	331	–0.74	0.98	…	…	…
WSH	374	–0.78	0.87	–0.04 (–.20, .12)	…	…
Nutrition	358	–0.87	0.96	–0.14 (–.30, .03)	…	…
Nutrition + WSH	365	–0.75	1.03	–0.02 (–.16, .13)	0.12 (–0.02, 0.26)	0.02 (–0.11, 0.16)
Ln neopterin (nmol/L)						
Control	331	7.11	0.95	…	…	…
WSH	374	7.07	0.99	–0.05 (–.25, .15)	…	…
Nutrition	358	6.92	0.98	–0.19 (–.38,–.01)	…	…
Nutrition + WSH	365	6.93	0.91	–0.18 (–.34, –.02)	0.01 (–0.11, 0.13)	–0.13 (–0.29, 0.03)
Ln regenerating gene 1β (µg/mL)						
Control	331	4.96	1.12	…	…	…
WSH	374	4.99	0.99	0.03 (–.13, .19)	…	…
Nutrition	357	4.93	1.05	–0.03 (–.20, .13)	…	…
Nutrition + WSH	364	5.01	0.88	0.05 (–.11, .21)	0.08 (–0.06, 0.22)	0.02 (–0.13, 0.17)
Ln lactulose (mmol/L)						
Control	332	–1.30	1.20	…	…	…
WSH	357	–1.46	1.20	–0.16 (–.39, .08)	…	…
Nutrition	342	–1.28	1.22	0.02 (–.21, .26)	…	…
Nutrition + WSH	356	–1.18	1.21	0.13 (–.11, .36)	0.10 (–0.11, 0.32)	0.28 (0.09, 0.47)
Ln mannitol (mmol/L)						
Control	332	0.64	1.22	…	…	…
WSH	357	0.72	1.11	0.08 (–.16, .32)	…	…
Nutrition	342	0.74	1.26	0.10 (–.16, .37)	…	…
Nutrition + WSH	356	0.76	1.26	0.12 (–.14, .37)	0.01 (–0.23, 0.26)	0.03 (–0.15, 0.22)
Ln lactulose-to-mannitol ratio						
Control	330	–2.92	0.93	…	…	…
WSH	356	–3.16	0.77	–0.24 (–.43, –.06)	…	…
Nutrition	342	–3.00	0.87	–0.08 (–.30, .14)	…	…
Nutrition + WSH	355	–2.91	0.92	0.01 (–.19, .20)	0.09 (–0.09, 0.27)	0.25 (0.08, 0.42)

Confidence intervals were adjusted for clustered observations using robust standard errors.

Abbreviation: WSH, drinking water, sanitation, handwashing.

permeability [40]. Breast milk transforming growth factors may contribute to this process via upregulation of tight junction proteins [38]. WSH interventions also lowered lactulose and mannitol concentrations at age 3 months, potentially via disruption of pathogen transmission pathways. Consistent with this pathway, WSH interventions reduced *E. coli* in water and food [29], *Giardia* infections [41], and diarrhea [33]. Although WSH reduced the L:M ratio at age 14 months, new evidence, as described above, obscures interpretation of the ratio [17]. Point estimate trends for alpha-1-antitrypsin, mannitol, and lactulose were similar, underscoring the internal consistency of the permeability measures.

Overall intervention effects declined over time and never achieved healthy reference levels [4, 7, 14]. Unexpectedly, at age 28 months, inflammation (myeloperoxidase) and permeability (alpha-1-antitrypsin and lactulose) were elevated in the arms that contained nutrition or WSH compared to controls. At age 3 months, interventions likely transformed a naive gut; higher rates of exclusive breastfeeding could have improved micronutrient status and WSH interventions could have limited pathogen exposure, both results leading to improved epithelial immunity [29, 33, 41]. Post-breastfeeding, as children aged and the frequency of insults increased [39], interventions could have been less effective, rendering healthy biomarker levels unattainable. Early improvements before age 14 months and later worsening of EED markers after age 14 months could indicate that the interventions delayed the onset of the peak deterioration in inflammation and barrier function. In the critical first 2 years of a

Table 4. Effect of Intervention on Environmental Enteric Dysfunction Measurements at Age 28 Months

Outcome, Arm	N	Mean	Standard Deviation	Difference from Control (95% Confidence Interval)	Difference from Nutrition	Difference from WSH
Ln myeloperoxidase (ng/mL)						
Control	338	7.59	1.16	…	…	…
WSH	392	7.81	1.05	0.23 (.06, .39)	…	…
Nutrition	360	7.86	1.04	0.27 (.07, .47)	…	…
Nutrition + WSH	395	7.77	1.18	0.18 (–.01, .36)	–0.09 (–0.26, 0.07)	–0.05 (–0.22, 0.12)
Ln alpha-1-antitrypsin (mg/g)						
Control	338	–1.08	1.13	…	…	…
WSH	392	–0.99	0.99	0.09 (–.06, .23)	…	…
Nutrition	360	–0.94	0.81	0.13 (–.00, .27)	…	…
Nutrition + WSH	395	–0.95	0.99	0.12 (–.03, .28)	–0.01 (–0.14, 0.12)	0.04 (–0.10, 0.17)
Ln neopterin (nmol/L)						
Control	338	6.35	1.18	…	…	…
WSH	392	6.28	1.02	–0.07 (–.25, .11)	…	…
Nutrition	360	6.20	1.14	–0.15 (–.38, .08)	…	…
Nutrition + WSH	395	6.21	1.12	–0.14 (–.33, .05)	0.01 (–0.17, 0.18)	–0.07 (–0.23, 0.08)
Ln lactulose (mmol/L)						
Control	330	–1.30	1.42	…	…	…
WSH	377	–1.00	0.97	0.30 (.07, .53)	…	…
Nutrition	349	–1.12	1.09	0.18 (–.04, .40)	…	…
Nutrition + WSH	391	–1.13	1.22	0.18 (–.04, .40)	–0.00 (–0.22, 0.21)	–0.13 (–0.34, 0.09)
Ln mannitol (mmol/L)						
Control	330	1.35	1.09	…	…	…
WSH	377	1.47	0.97	0.12 (–.08, .33)	…	…
Nutrition	349	1.34	0.96	–0.01 (–.20, .18)	…	…
Nutrition + WSH	391	1.33	1.01	–0.02 (–.22, .18)	–0.00 (–0.17, 0.16)	–0.14 (–0.33, 0.05)
Ln lactulose-to-mannitol ratio						
Control	330	–3.63	0.93	…	…	…
WSH	377	–3.45	0.63	0.18 (.02, .34)	…	…
Nutrition	349	–3.44	0.74	0.19 (.01, .38)	…	…
Nutrition + WSH	391	–3.44	0.75	0.20 (.02, .37)	0.00 (–0.13, 0.13)	0.02 (–0.11, 0.14)

Confidence intervals were adjusted for clustered observations using robust standard errors.

Abbreviation: WSH, drinking water, sanitation, handwashing.

child's life, a delay in the proinflammatory response that diverts energy toward the immune system and away from growth and development could have knock-on health benefits including reductions in acute and chronic disease risk later in life [42].

Although WSH interventions reduced EED markers at age 3 months, WSH alone did not improve growth; only children in the nutrition and N+WSH arms grew taller [33]. There are 2 potential interpretations of these results; first, that they do not support the decades-long hypothesis that EED is a main contributor to stunting [4–6] or, second, that the WSH interventions did not prevent pathogen exposure sufficiently to reduce EED enough to alter its impact on linear growth. A clearer understanding of EED's pathophysiology would aid development of preventative strategies. Available EED biomarkers are only weakly predictive of linear growth [7–10]. Because EED is a disorder that encompasses several aspects of intestinal dysfunction, development of a low-cost panel of EED biomarkers would enable more robust assessment of interventions.

The strengths of this trial include high adherence, sufficient statistical power to detect small effects, and masked statistical analyses independently replicated by 2 investigators. This trial was an efficacy study with higher intervention adherence than would be expected in a large-scale program, thereby increasing the chance to observe intervention effects.

The trial had limitations. Results from this low-income, rural setting in Bangladesh may not generalize to other populations, but widespread stunting and environmental contamination provided a relevant setting to test the EED hypothesis. Cluster selection based on logistical feasibility may also limit the generalizability of study findings to conditions that predominate in rural Bangladeshi hospitals that are within 2 hours travel time of larger towns. Additionally, diarrheal stools were not excluded from analysis. Diluted specimens potentially cause reductions in EED markers [19] and would bias our estimated intervention effects toward the null. Mannitol, which is present at low levels in some local foods, was not measured prior to LM dosing for all children and may affect post-LM mannitol recoveries [17]; therefore, intervention effects would be biased toward the null.

This study illustrates that intensive water, sanitation, hygiene, and nutritional interventions can reduce markers of EED among

infants who live in an environment with high levels of fecal contamination. The absence of a sustained effect or impact of WSH on linear growth highlights the importance of developing a better of understanding the pathophysiology and consequences of EED.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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