Title

Association between Age, Weight, and Dose and Clinical Response to Probiotics in Children with Acute Gastroenteritis

Journal

Journal of Nutrition, 151(1)

ISSN

0022-3166

Authors

Schnadower, David
Sapien, Robert E
Casper, T Charles
et al.

Publication Date

2021

DOI

10.1093/jn/nxaa313

Peer reviewed

The Journal of Nutrition
Nutrition and Disease

Association between Age, Weight, and Dose and Clinical Response to Probiotics in Children with Acute Gastroenteritis

David Schnadower,[1] Robert E Sapien,[2] T Charles Casper,[3] Cheryl Vance,[4] Phillip I Tarr,[5] Karen J O'Connell,[6] Adam C Levine,[7] Cindy G Roskind,[8] Alexander J Rogers,[9] Seema R Bhatt,[1] Prashant Mahajan,[9] Elizabeth C Powell,[10] Cody S. Olsen,[3] Marc H Gorelick,[11] J Michael Dean,[3] and Stephen B Freedman[12] for the Pediatric Emergency Care Applied Research Network (PECARN) Probiotics Study

[1]Division of Emergency Medicine, Cincinnati Children's Hospital Medical Center, Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, OH, USA; [2]Department of Emergency Medicine, University of New Mexico, Albuquerque, NM, USA; [3]Department of Pediatrics, University of Utah, Salt Lake City, UT, USA; [4]Departments of Emergency Medicine and Pediatrics, University of California, Davis, School of Medicine, Sacramento, CA, USA; [5]Division of Gastroenterology, Hepatology, & Nutrition, Department of Pediatrics, Washington University in St. Louis School of Medicine, St. Louis, MO, USA; [6]Division of Emergency Medicine, Children's National Health System, Department of Pediatrics and Emergency Medicine, The George Washington School of Medicine and Health Sciences, Washington, DC, USA; [7]Department of Emergency Medicine, Rhode Island Hospital/Hasbro Children's Hospital and Brown University, Providence, RI, USA; [8]Division of Emergency Medicine, Department of Pediatrics, Columbia University College of Physicians & Surgeons, New York, NY, USA; [9]Departments of Emergency Medicine and Pediatrics, University of Michigan, Ann Arbor, MI, USA; [10]Division of Emergency Medicine, Department of Pediatrics, Ann & Robert H Lurie Children's Hospital, Northwestern University Feinberg School of Medicine, Chicago, IL, USA; [11]Central Administration, Children's Minnesota, Minneapolis, MN, USA; and [12]Sections of Pediatric Emergency Medicine and Gastroenterology, Department of Pediatrics, Alberta Children's Hospital, Alberta Children's Hospital Research Institute, Cumming School of Medicine, University of Calgary, Calgary, Alberta, Canada

ABSTRACT

Background: Gastroenteritis is a common and impactful disease in childhood. Probiotics are often used to treat acute gastroenteritis (AGE); however, in a large multicenter randomized controlled trial (RCT) in 971 children, *Lactobacillus rhamnosus* GG (LGG) was no better than placebo in improving patient outcomes.

Objectives: We sought to determine whether the effect of LGG is associated with age, weight *z* score and weight percentile adjusted for age and sex, or dose per kilogram administered.

Methods: This was a preplanned secondary analysis of a multicenter double-blind RCT of LGG 1×10^{10} CFU twice daily for 5 d or placebo in children 3–48 mo of age with AGE. Our primary outcome was moderate to severe gastroenteritis. Secondary outcomes included diarrhea and vomiting frequency and duration, chronic diarrhea, and side effects. We used multivariable linear and nonlinear models testing for interaction effects to assess outcomes by age, weight *z* score and weight percentile adjusted for age and sex, and dose per kilogram of LGG received.

Results: A total of 813 children (84%) were included in the analysis; 413 received placebo and 400 LGG. Baseline characteristics were similar between treatment groups. There were no differential interaction effects across ranges of age (*P*-interaction = 0.32), adjusted weight *z* score (*P*-interaction = 0.43), adjusted weight percentile (*P*-interaction = 0.45), or dose per kilogram of LGG received (*P*-interaction = 0.28) for the primary outcome. Whereas we found a statistical association favoring placebo at the extremes of adjusted weight *z* scores for the number of vomiting episodes (*P*-interaction = 0.02) and vomiting duration (*P*-interaction = 0.0475), there were no statistically significant differences in other secondary outcome measures (all *P*-interactions > 0.05).

Conclusions: LGG does not improve outcomes in children with AGE regardless of the age, adjusted weight *z* score, and adjusted weight percentile of participants, or the probiotic dose per kilogram received. These results further strengthen the conclusions of low risk of bias clinical trials which demonstrate that LGG provides no clinical benefit in children with AGE. This trial was registered at clinicaltrials.gov as NCT01773967. *J Nutr* 2021;151:65–72.

Keywords: probiotic, pediatric, gastroenteritis, emergency medicine, randomized controlled trial, secondary analysis

Manuscript received May 22, 2020. Initial review completed June 25, 2020. Revision accepted September 17, 2020.
First published online December 3, 2020; doi: https://doi.org/10.1093/jn/nxaa313.

Introduction

Acute gastroenteritis (AGE) is a common and burdensome condition that affects millions of children worldwide each year (1). Treatment strategies are limited to symptomatic management, prevention and treatment of dehydration, and infection control (2). Despite weak evidence, probiotics (3), defined as live bacteria that are beneficial to the host, are commonly used to treat AGE in children (4–8).

We recently demonstrated, in a double-blind multicenter randomized controlled trial (RCT) of 971 children 3–48 mo of age presenting to 1 of 10 participating US pediatric emergency departments (EDs) with AGE, that a 5-d course of *Lactobacillus rhamnosus* GG (LGG) was not more effective than placebo in improving AGE outcomes independently of the duration of symptoms, use of antibiotics in the 14 d preceding enrollment, and type of enteric pathogen identified (9). The total dose of LGG used in this study was based on prior clinical trials and is recommended by the manufacturer (8, 10, 11). Unlike most pediatric medications which employ weight-dependent dosing (12), all participants in this trial received the same total dose (1×10^{10} CFU twice daily). Given the lack of benefit observed in this large trial which conflicts with positive earlier results (8), it is important to explore potential explanations such as *1*) dose per kilogram (expressed as CFU/kg): prior pediatric studies have alluded to a dose–response relation with better results associated with larger total doses, up to a ceiling of 1×10^{10} CFU/dose (8, 11, 13, 14); *2*) age: the intestinal microbial environment varies with age, with the infant intestinal microbiome beginning to converge on an adult community structure at ∼3 y of age (15); and *3*) weight: there are no data regarding the differential response to LGG in patients with gastroenteritis according to their age- and sex-adjusted weight *z* score and weight percentile, which we employed as surrogate markers of nutritional status.

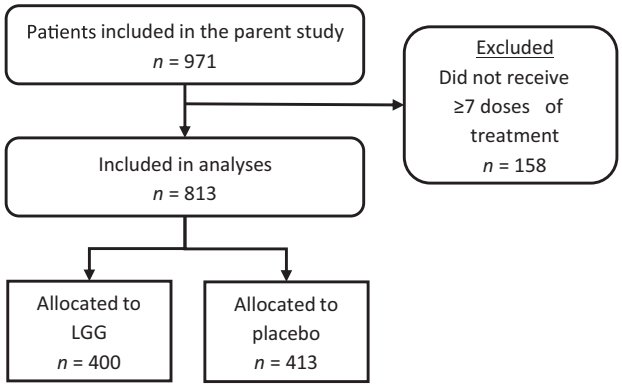

FIGURE 1 Participant flow diagram. LGG, *Lactobacillus rhamnosus* GG.

Given the wide natural variation in participants' ages and adjusted weights, our study offers an ideal opportunity to test the hypothesis whether the effect of LGG administered to children 3–48 mo of age presenting to the ED with AGE varies based on the host's age, weight *z* score and weight percentile adjusted for age and sex, or dose per kilogram received in CFU per kilogram.

Methods

Ethical approval

All participating institutions obtained ethics approval from their respective Institutional Review Boards.

Study design

This was a preplanned analysis of the multicenter Pediatric Emergency Care Applied Research Network (PECARN) Probiotic Study, for which detailed methods have been previously published (9, 16). Briefly, the trial was a prospective, randomized, parallel-group, double-blind trial (NCT01773967) that included 971 children 3–48 mo of age with AGE who presented to 10 US pediatric EDs between July 2014 and June 2017.

Participants received a 5-d course of 1×10^{10} CFU of LGG or a placebo that was of similar appearance and taste by sprinkling the contents of the assigned capsule into 20 mL of liquid maintained at room temperature. Eligible participants had ≥3 watery stools per day, with or without vomiting, for <7 d and were diagnosed by the ED physician as having AGE. Children were excluded if they or their direct caregivers had risk factors for bacteremia (i.e., immunocompromised,

Supported by Eunice Kennedy Shriver National Institute of Child Health and Human Development grant R01HD071915 (to DS); the Washington University Biobank Core, supported by National Institute of Diabetes and Digestive and Kidney Diseases of the NIH grant P30DK052574 (to PIT); and the Alberta Children's Hospital Foundation Professorship in Child Health and Wellness (to SBF). PECARN is supported by the Health Resources and Services Administration of the US Department of Health and Human Services, and the Emergency Medical Services for Children program through DCC-University of Utah grant U03MC00008, GLEMSCRN-Nationwide Children's Hospital grant U03MC00003, HOMERUN-Cincinnati Children's Hospital Medical Center grant U03MC22684, PEMNEWS-Columbia University Medical Center grant U03MC00007, PRIME-University of California at Davis Medical Center grant U03MC00001, SW NODE-University of Arizona Health Sciences Center grant U03MC28845, WBCARN-Children's National Medical Center grant U03MC00006, and CHaMP-Medical College of Wisconsin grant H3MC26201.
Author disclosures: All authors have completed the Unified Competing Interest form (available on request from the corresponding author) and declare the following: DS has received in-kind (study drug and placebo) from iHealth Inc. and is supported in part by the Richard and Barbara Ruddy Endowed Chair in Emergency Medicine at Cincinnati Children's Hospital Medical Center. PIT is a consultant to, a holder of equity in, and a member of the Scientific Advisory Board of MediBeacon Inc., which is developing technology to test intestinal permeability in people. He is also a co-inventor on a patent that might generate royalties on this technology in the future. He is also a consultant to Takeda Pharmaceuticals on childhood intestinal disorders. SBF has received in-kind (study drug and placebo) from Institut Lallemand Inc., Novartis, and GlaxoSmithKline. He also provides consulting services to Takeda Pharmaceutical Company. All other authors report no conflicts of interest.
DS affirms that the article is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as registered have been explained. Patients or the public were not involved in the design, conduct, reporting, or dissemination of our research. The product and placebo were provided in-kind by iHealth, the distributors of Culturelle in the United States; however, iHealth did not contribute financially to the trial or to the investigators, and their employees did not have access to the trial data. Personnel at iHealth had no role in the design or conduct of the trial; in the collection, management, analysis, or interpretation of the data; in the preparation of the manuscript; or in the decision to submit the manuscript for publication.
Data Availability: The data described in the article, code book, and analytic code will be made publicly and freely available upon request after June 2022 at www.pecarn.org.
Supplemental Tables 1 and 2 are 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/jn/.
Address correspondence to DS (e-mail: david.schnadower@cchmc.org).
Abbreviations used: AGE, acute gastroenteritis; ED, emergency department; LGG, *Lactobacillus rhamnosus* GG; MVS, Modified Vesikari Scale; ORS, oral rehydration solution; PECARN, Pediatric Emergency Care Applied Research Network; QIC, quasi-likelihood under the independence model criterion; RCT, randomized controlled trial.

TABLE 1 Baseline characteristics by treatment group[1]

	Treatment received		
	Placebo ($n = 413$)	LGG ($n = 400$)	*P* value
Age, mo			0.388[2]
0 to <12	138 (33.4)	132 (33.0)	
12 to <24	154 (37.3)	134 (33.5)	
24 to <36	78 (18.9)	78 (19.5)	
36 to <48	43 (10.4)	56 (14.0)	
Mean body weight, kg	11.3 ± 3.0*	11.2 ± 2.9*	0.973[3]
Body weight *z* score			0.071[2]
<−2	15 (3.6)	5 (1.3)	
−2 to <0	147 (35.6)	159 (39.9)	
0 to <2	217 (52.5)	210 (52.8)	
≥2	34 (8.2)	24 (6.0)	
Weight-for-age <25th percentile	113 (27.4)	120 (30.2)	0.380[3]
LGG dose, billion CFU/kg body weight			
Placebo	413 (100.0)	0 (0.0)	
<0.75	0 (0.0)	97 (24.4)	
0.75 to <1.0	0 (0.0)	142 (35.7)	
1.0 to <1.25	0 (0.0)	110 (27.6)	
≥1.25	0 (0.0)	49 (12.3)	
Baseline MVS, score	11.7 ± 2.9*	11.6 ± 2.9*	0.789[3]
Antibiotics in the 14 d before enrollment	31 (7.6)	33 (8.3)	0.708[2]
Antibiotics at/after enrollment	44 (10.7)	56 (14.0)	0.146[2]
Enrollment season			0.879[2]
Winter	97 (23.5)	95 (23.8)	
Spring	135 (32.7)	132 (33.0)	
Summer	105 (25.4)	93 (23.3)	
Fall	76 (18.4)	80 (20.0)	
Multiplex PCR results (stool)	333 (80.6)	320 (80.0)	0.821[2]
Negative	144 (43.2)	133 (41.6)	0.664[2]
Norovirus GI/GII	64 (19.2)	68 (21.3)	0.518[2]
Rotavirus A	55 (16.5)	63 (19.7)	0.292[2]
Adenovirus 40/41	35 (10.5)	19 (5.9)	0.034[2]
Clostridium difficile, Toxin A/B	30 (9.0)	19 (5.9)	0.136[2]
Shigella	13 (3.9)	21 (6.6)	0.126[2]
Campylobacter	3 (0.9)	5 (1.6)	0.442[2]
Salmonella	3 (0.9)	3 (0.9)	0.961[2]
Enterotoxigenic *E. coli* (ETEC) LT/ST	3 (0.9)	2 (0.6)	0.686[2]
Stx-producing *E. coli* (STEC) stx1/stx2	4 (1.2)	3 (0.9)	0.744[2]
Giardia	2 (0.6)	2 (0.6)	0.968[2]
Cryptosporidium	1 (0.3)	0 (0.0)	0.327[2]
Entamoeba histolytica	0 (0.0)	1 (0.3)	0.307[2]
Vibrio cholerae	1 (0.3)	0 (0.0)	0.327[2]

[1] Values are means ± SDs or *n* (%) unless otherwise indicated. Missing data are as follows: weight, $n = 2$; dose, $n = 2$; baseline MVS score, $n = 12$; antibiotics before enrollment, $n = 4$. LGG, *Lactobacillus rhamnosus* GG; LT/ST, temperature labile/stable; MVS, Modified Vesikari Scale; stx, Shiga toxin. *All values are frequencies (%) except weight and baseline severity score which are expressed as mean (SD).
[2] Chi-square test.
[3] Wilcoxon's rank sum test of association.

used systemic steroids in the past 6 mo, presence of an indwelling catheter, known structural heart disease, history of prematurity when younger than 6 mo at enrollment) or a chronic gastrointestinal disorder (e.g., inflammatory bowel disease). Additional exclusion criteria were presence of pancreatitis; bilious emesis; hematochezia; known allergy to LGG; microcrystalline cellulose or erythromycin, clindamycin, and β-lactam antibiotics (because they may be required to treat an invasive infection caused by LGG); or if their caregivers spoke neither English nor Spanish. Because we were interested in the dose-response to LGG, for this secondary analysis we only included those participants who self-reported receiving ≥7 of the 10 treatment doses, which was our a priori definition of adherence in the trial. Follow-up surveys were completed daily by email or phone for 5 d and again at day 14 and 1 mo after enrollment.

Study outcomes

The primary outcome was moderate to severe AGE, defined as having a postenrollment Modified Vesikari Scale (MVS) score ≥9 and calculated on day 14 based on the responses to the daily and day 14 surveys. The MVS is a validated global AGE severity score that includes diarrhea frequency and duration, vomiting frequency and duration, maximal height of fever, health care resource use, and treatments received. Scores range from 0 to 20, with higher scores indicating more severe disease (17, 18).

Secondary outcomes included the maximal number of diarrhea and vomiting episodes per 24-h period, diarrhea and vomiting duration, development of chronic diarrhea defined as persistence of diarrhea ≥7 d after randomization, and side effects. They were recorded during all daily surveys and again at the 14-d surveys.

TABLE 2 Results from statistical models testing for a differential treatment effect of LGG compared with placebo[1]

Outcome	Covariate			
	Age, mo	Weight *z* score adjusted for age and sex	<25th percentile weight for age and sex	Dose/kg in billion CFU/kg
Moderate–severe acute gastroenteritis (MVS ≥9)	0.3286[2]	0.4288[2]	0.4519[2]	0.2768[3]
Chronic diarrhea	0.1358[2]	0.1384[2]	0.1753[2]	0.8522[2]
Diarrhea duration, d	0.2069[2]	0.3577[2]	0.6534[2]	0.1028[4]
Diarrhea episodes, *n*	0.6389[2]	0.2989[3]	0.4051[2]	0.1085[3]
Vomit duration,[5] d	0.3495[3]	0.0475[3]	0.9034[2]	0.0793[3]
Vomit episodes,[5] *n*	0.1813[3]	0.0208[6]	0.7780[2]	0.1586[6]

[1] LGG, $n = 400$; Placebo, $n = 413$. Missing data as follows: MVS, $n = 32$; vomit duration, $n = 16$; all others, $n = 0$. Significant *P* values are evidence that the effect of LGG on the outcome depends on the covariate. *P*-interaction values are *P* values from score tests of interactions between the treatment and the covariate in a model of the specified outcome. In quadratic, cubic, or log-linear models, *P* values are from a score test comparing with a model with no interactive effects. All models adjusted for baseline duration of symptoms, baseline MVS, dehydration, multiplex PCR (positive compared with negative compared with not tested), and season as fixed effects, and enrolling clinical site using generalized estimating equations and compound symmetry correlation. Logistic models were fit to MVS and chronic diarrhea outcomes; negative binomial models were used for other outcomes. Models were chosen based on the lowest QIC. When the QIC was ≥1 point lower for a more complex model, the more complex model was selected. Otherwise, the less complex model was selected. Only linear models were applicable for <25th percentile weight for age. Log-linear models were not considered for weight *z* score adjusted for age due to subzero values. LGG, *Lactobacillus rhamnosus* GG; MVS, Modified Vesikari Scale; QIC, quasi-likelihood under the independence model criterion.
[2] Linear model.
[3] Cubic model.
[4] Log-linear model.
[5] Analysis is limited to 397 children with ≥3 vomiting episodes within the 24 h before enrollment.
[6] Quadratic model.

Only children with ≥3 vomiting episodes in the 24 h before enrollment were included in analyses of vomit duration and number of vomiting episodes. Side effects were defined as the occurrence of a priori identified specific symptoms reported within 1 mo of enrollment: bloating, gas, intestinal rumbling, diarrhea, visible blood in stool, abdominal pain, abdominal cramps, nausea, vomiting, loss of appetite, heartburn, constipation, skin rash, diaper rash, fever, nasal congestion, runny nose, sore throat, cough, headache, muscle aches, chills, or weakness. We reported side effects according to the International Conference on Harmonization guideline for Good Clinical Practice (19).

Definitions

z Score units are the number of SDs a subject's actual weight deviates from their expected weight based on their age and sex. Weight *z* scores of children <24 mo of age were adjusted for age and sex using WHO growth charts per standard recommendations (20–22). Weight *z* scores for children 24–48 mo of age, and weight percentiles for all enrolled children, were calculated using CDC growth charts (23). We chose to categorize weight percentiles into quartiles to enable the creation of groups that received the highest (lowest quartile) and lowest (highest quartile) dose per kilogram in terms of billion CFU per kilogram. Those assigned to the placebo group had a dose per kilogram of 0; for all others, it was 10 divided by the child's weight in kilograms because each active pill has a total of 10 billion CFU. Dehydration was evaluated using the clinical dehydration scale score which is a validated scale which includes the following clinical examination findings: general appearance, eyes, mucous membranes, and tears (24). Seasonality at randomization was categorized as spring (1 March–31 May), summer (1 June–31 August), autumn (1 September–30 November), and winter (1 December–28/29 February).

Statistical analysis

Categorical data are presented as proportions, and continuous variables as means ± SDs or medians (IQRs) for normally distributed and nonnormally distributed data, respectively. We performed univariate comparisons using chi-square and Wilcoxon's rank sum tests as appropriate. We investigated potential differential treatment effects between outcomes and the following characteristics: *1*) age in months, *2*) weight *z* score adjusted for age and sex, *3*) weight percentile adjusted for age and sex, and *4*) dose per kilogram in billion CFU per kilogram. We adjusted analyses for baseline duration of symptoms (<48 compared with ≥48 h), given that efficacy of probiotics may be subject to symptom duration before treatment initiation (25, 26); MVS score at the time of ED presentation; clinical dehydration scale score; season; multiplex PCR (positive compared with negative compared with not tested); and enrolling clinical site. Associations of age, weight *z* score and weight percentile adjusted for age and sex, and dose per kilogram with the study primary and secondary outcomes were explored through multivariable generalized estimating equations models adjusting for correlation within enrolling clinical site. The differential effect of treatment across age, weight, and dose per kilogram was tested using interaction effects to allow the treatment effect estimate to differ across values of age, weight, and dose per kilogram. The main objective of this study was accomplished by testing for nonzero interaction effects, i.e., whether the effect of the treatment differed by age, adjusted weight *z* score and adjusted percentile, and/or dose per kilogram.

In order to allow for nonlinear relations, we fit linear, quadratic, and cubic models for age, adjusted weight *z* score and weight percentile, and dose per kilogram, and log-linear models for age and dose per kilogram. For each outcome/covariate combination, we used the quasi-likelihood under the independence model criterion (QIC) statistic to select the model that best fit the data (27). The linear model was the default when the QIC of an alternate model was <1 unit lower; otherwise, we chose the model with the lowest QIC. We used SAS software version 9.4 (SAS Institute Inc.) for all analyses.

Results

Of 971 randomly assigned participants, 813 (83.8%) received ≥7 capsules and were included in this secondary analysis (**Figure 1**). There were 413 patients allocated to placebo and 400 to LGG. Completion of daily follow-up surveys for the first 5 d, 14 d, and 1 mo was achieved in 813 (100%), 808 (99.4%), and 805 (99.0%) of the participants, respectively. There were no significant differences between groups in baseline disease severity (baseline MVS), age, *z* score, weight percentiles, or stool pathogens except for a higher proportion of adenovirus infections in the placebo group (10.5% compared with 5.9%, $P = 0.034$) (**Table 1**). LGG dose per kilogram administered ranged from 0.46 to 2.13 billion CFU/kg.

Primary outcome

The proportion of patients with moderate to severe gastroenteritis postenrollment was 11.7% (95 of 813). There were no significant differences in the MVS score during the 2-wk

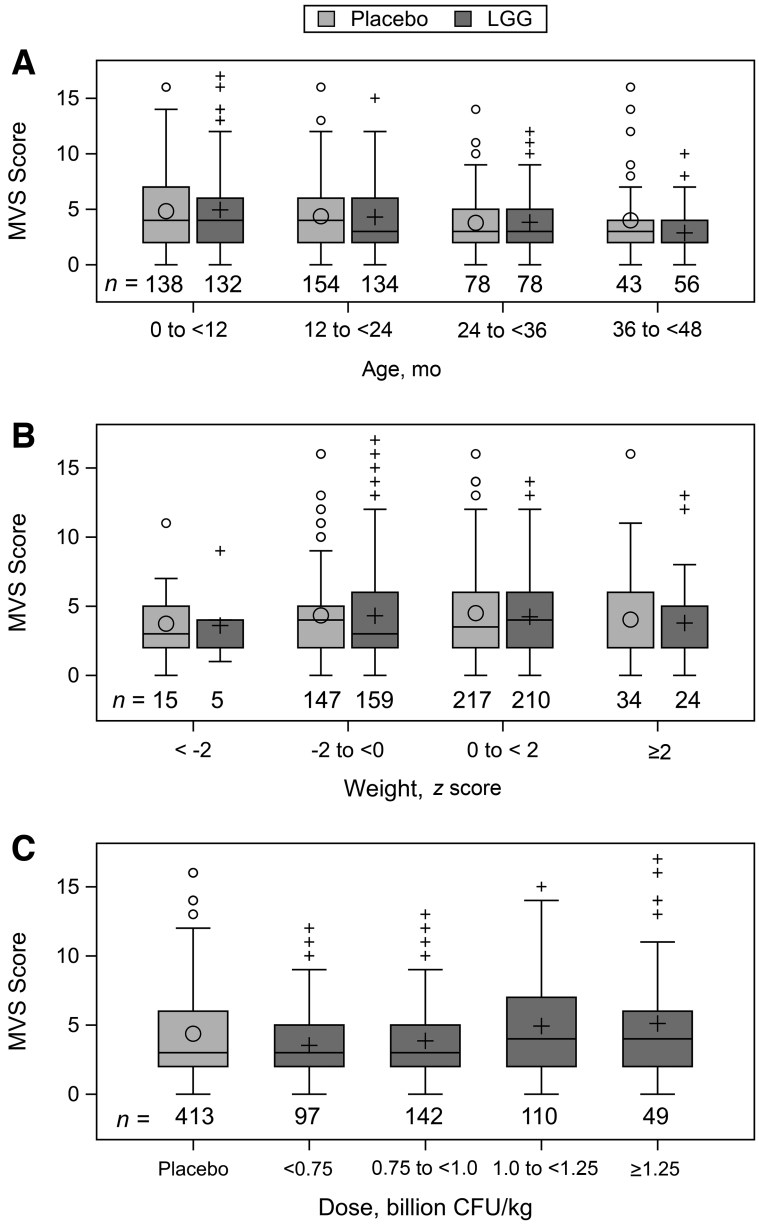

FIGURE 2 Age, age- and sex-adjusted weight *z* score, and treatment dose per kilogram had no effect on the outcome at 14-d follow-up. Boxplot graphs of the 14-d MVS score plotted against (A) age, (B) weight *z* scores adjusted for age and sex, and (C) LGG dose per kilogram (CFU/kg). LGG, $n = 400$; Placebo, $n = 413$. LGG, *Lactobacillus rhamnosus* GG; MVS, Modified Vesikari Scale; +, treatment arm; O, placebo arm.

follow-up period between participants receiving LGG and those receiving placebo by age group (*P*-interaction = 0.35), adjusted weight *z* score (*P*-interaction = 0.39), dose per kilogram ($P = 0.26$) (**Table 2**, **Figure 2**), or adjusted weight percentile (*P*-interaction = 0.41) (Table 2).

Secondary outcomes

Overall, the median postenrollment duration of diarrhea and vomiting was 2.1 d (IQR: 1.0–3.7 d) and 0.0 d (IQR: 0.0–0.7 d), respectively. We found no differences between the participants receiving LGG and those receiving placebo in diarrhea severity and duration, chronic diarrhea, or side effects across age groups, adjusted weight *z* score or weight percentile, or dose per kilogram (all *P*-interactions > 0.05) (Table 2). We found evidence of associations at the extremes of the distribution of adjusted weight *z* score for the duration of vomiting (cubic model, *P*-interaction = 0.0475) and the number of vomiting episodes with a quadratic (U-shaped) relation (*P*-interaction = 0.02) (Table 2, **Figure 3**). Better outcomes were observed for patients administered placebo with weight *z* scores <-1 or >1, whereas those with *z* scores close to 0 demonstrated little difference between LGG and placebo. There was no clear benefit of LGG for any value across the range of weight *z* scores.

The most common side effects across group categories were cough (7%–21%), runny nose (9%–17%), and loss of appetite (9%–17%), but we found no differences in side effects between LGG or placebo within age group categories (**Supplemental Table 1**) or weight percentile categories (**Supplemental Table 2**).

Discussion

In this preplanned secondary analysis of a large RCT that compared the administration of LGG 1×10^{10} CFU twice a day

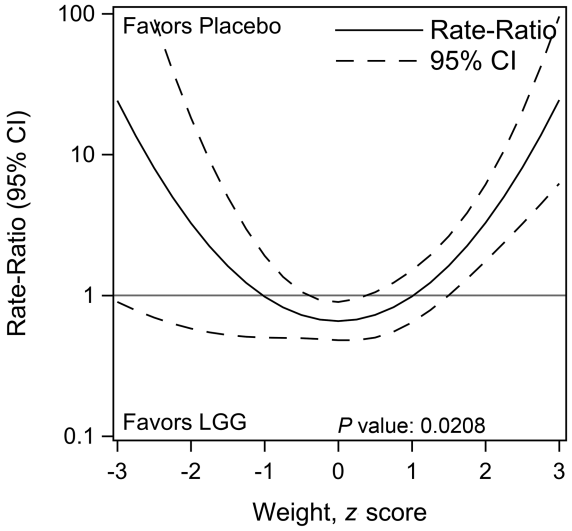

FIGURE 3 Differential treatment effect of weight and LGG compared with placebo on number of vomiting episodes. A nonlinear relation was identified among weight *z* scores, treatment, and the number of vomiting episodes. This figure shows the rate-ratio and 95% CI estimated from the best model comparing LGG with placebo across values of weight *z* scores. The model contained terms for treatment, weight *z* score, weight *z* score-squared, and interaction terms between treatment and the age terms along with terms for duration of symptoms, baseline Modified Vesikari Scale score, dehydration score, multiplex PCR (positive compared with negative compared with not tested), season, and enrolling clinical site. LGG, $n = 400$; Placebo, $n = 413$. LGG, *Lactobacillus rhamnosus* GG.

with placebo in children 3–48 mo of age with AGE, we found no associations between participants' age, adjusted weight *z* score, adjusted weight percentile, or dose per kilogram administered and most gastroenteritis outcomes.

Although we found a treatment effect favoring placebo at the extremes of standardized weight for the number of vomiting episodes and vomit duration, these results should be viewed with caution. First, the results are sensitive to the choice of model and our assessment of statistical significance assumes that only that final model was evaluated. Second, the significance levels of multiple hypotheses were not controlled for using multiple comparison approaches. Finally, these effects were not observed throughout the spectrum of standardized weights or in any other models.

Prior literature suggests a possible dose–response relation with greater benefits associated with higher doses per kilogram of LGG. A systematic review of LGG in AGE reported decreased effectiveness at lower total dose ($<1 \times 10^9$ CFU twice daily) compared with higher total dose, which has led to the recommendation to use higher dosing regimens (i.e., $\geq 1 \times 10^{10}$ CFU twice daily), which is the capsule content we used in our trial (8). A 3-arm RCT of 559 hospitalized Indian children with AGE reported a benefit associated with the consumption of LGG-supplemented oral rehydration solution (ORS) compared with ORS supplemented with placebo, but no added benefit was found associated with use of higher total doses (i.e., 1×10^{12} CFU) compared with lower total doses (i.e., 1×10^{10} CFU) (11). In this study the lack of dose–response relation may be explained by the use of relatively high (i.e., $>1 \times 10^{10}$ CFU twice daily) total doses in both groups suggesting a ceiling effect. In another study which employed an open-label design and recruited 23 children with rotavirus infection, there was decreased fecal rotavirus concentration after 3 d of treatment in children receiving higher total doses of LGG (6×10^8 CFU LGG/d $\times$ 3 d) compared with those receiving lower total doses (2×10^8 CFU LGG/d $\times$ 3 d) and controls. However, both total doses employed were actually lower than our study intervention total dose (13). Lastly, in a study quantifying gut colonization by LGG in infants consuming an LGG-supplemented formula, the authors reported similar grades of gut colonization across 3 logs of total dose of LGG (1×10^8 CFU compared with 1×10^9 CFU, compared with 1×10^{10} CFU) (14). These data, combined with our results, suggest that the total dose we used in our study was adequate. The lack of beneficial response to LGG in our trial was unrelated to the choice of total dose and a higher total dose would have been unlikely to yield any additional benefits. Furthermore, given the weight differences in our population, the range of dose per kilogram (as expressed by CFU/kg) was 4-fold, which we believe would be potentially large enough to detect a dose-response trend if present.

To the best of our knowledge, no prior study has explored the clinical effects of LGG in children with AGE based on the host's age, or adjusted weight *z* scores and percentiles. We were interested in assessing patient age as a potential factor in response to probiotics because the gut microbiome matures around the age of 3 y (15), and 12% of our patients were 3 y of age or older, which should have allowed us to detect an age trend if present. We also assessed the response according to nutritional status using 2 different proxies: adjusted weight *z* scores and adjusted weight percentiles. We analyzed both measures to ensure consistency of our results. This was an important consideration given there are differences in microbial gut environments according to nutritional states (28, 29). Moreover, a recent randomized trial showed no benefit of a combination probiotic in severely malnourished children in Uganda (30). In our study, however, >90% of patients had adjusted weight *z* scores between −2 and 2, limiting our ability to extrapolate to more malnourished or obese populations.

The fact that we used a previously recommended preparation, and that our data show no convincing differential effect or trend according to age, weight, or dose per kilogram received, further supports our findings that LGG does not confer a beneficial clinical effect when administered to children with AGE in US EDs. Other explanations for this lack of effectiveness may include the fact that responsiveness may vary according to indigenous and individual microbiota and gene-expression profiles (31, 32). Furthermore, physiologic and microbiome analyses are needed to shed more light on this topic and to identify the presence or absence of benefits that might not have achieved clinical relevance.

Our study's strengths include a large sample, a geographically diverse population, excellent follow-up rates, a systematic measurement of outcomes, stool pathogen determination, and independent verification of probiotic bacterial counts. We were limited, however, by the fact that we did not measure participant length/height, which would have allowed us to calculate weight/length *z* scores and BMI, which would provide a more complete nutritional status assessment, and to analyze dose based on body surface area in addition. Our findings, however, did not identify any of the exposures of interest as explanations for the lack of beneficial effect of LGG reported in our study. We believe this study is important because, along with another recent RCT (33), it questions the benefit of probiotics in children with AGE and highlights the need for high-quality studies before recommending their use (34).

We conclude that LGG 1×10^{10} CFU twice a day is not effective in improving AGE symptoms in children 3–48 mo of age and that this lack of effect is independent of participants' age, weight (adjusted for age and sex), and the dose per kilogram employed in the trial.

Acknowledgments

We thank Ms. Viani Dickey and Ms. Carla Hall-Moore at Washington University; Ms. Sheila Mason and Dr. Richard Buller for stool testing; Dr. Carey-Ann Burnham, Dr. Erik Dubberke, and Ms. Tiffany Hink for *L. rhamnosus* GG sample testing all at Washington University; and the members of the data and safety monitoring board [Dr. David Reboussin (Chair of the DSMB, Wake Forest School of Meidicne), Dr. Anupam Kharbanda (Children's Hospitals and Clinics of Minnesota), Dr. Oscar Gomez (University of Buffalo), Dr. George Fuchs (University of Kentucky), and Dr. Robert Shulman (Baylor College of Medicine)]. The authors' responsibilities were as follows—DS: had primary responsibility for the final content; DS, SBF, and PIT: conceptualized and designed the study and drafted, reviewed, and revised the manuscript; TCC and CSO: performed all statistical analyses; RES, CV, KJO, ACL, CGR, AJR, SRB, PM, ECP, MHG, and JMD: collected data and reviewed the manuscript for important intellectual content; and all authors: agree to be accountable for all aspects of the work and read and approved the final manuscript.

References

1. Walker CLF, Rudan I, Liu L, Nair H, Theodoratou E, Bhutta ZA, O'Brien KL, Campbell H, Black RE. Global burden of childhood pneumonia and diarrhoea. Lancet 2013;381(9875):1405–16.
2. Freedman SB, Pasichnyk D, Black KJ, Fitzpatrick E, Gouin S, Milne A, Hartling L, Pediatric Emergency Research Canada Gastroenteritis Study Group. Gastroenteritis therapies in developed countries: systematic review and meta-analysis. PLoS One 2015;10(6):e0128754.
3. Schnadower D, Finkelstein Y, Freedman SB. Ondansetron and probiotics in the management of pediatric acute gastroenteritis in developed countries. Curr Opin Gastroenterol 2015;31(1):1–6.
4. Allen SJ, Martinez EG, Gregorio GV, Dans LF. Probiotics for treating acute infectious diarrhoea. Cochrane Database Syst Rev 2010(11):CD003048.
5. Guandalini S. Probiotics for prevention and treatment of diarrhea. J Clin Gastroenterol 2011;45:S149–53.
6. Guarino A, Ashkenazi S, Gendrel D, Lo Vecchio A, Shamir R, Szajewska H. European Society for Pediatric Gastroenterology, Hepatology, and Nutrition/European Society for Pediatric Infectious Diseases evidence-based guidelines for the management of acute gastroenteritis in children in Europe: update 2014. J Pediatr Gastroenterol Nutr 2014;59(1):132–52.
7. Szajewska H, Setty M, Mrukowicz J, Guandalini S. Probiotics in gastrointestinal diseases in children: hard and not-so-hard evidence of efficacy. J Pediatr Gastroenterol Nutr 2006;42(5):454–75.
8. Szajewska H, Skórka A, Ruszczyński M, Gieruszczak-Białek D. Meta-analysis: *Lactobacillus* GG for treating acute gastroenteritis in children – updated analysis of randomised controlled trials. Aliment Pharmacol Ther 2013;38(5):467–76.
9. Schnadower D, Tarr PI, Casper TC, Gorelick MH, Dean JM, O'Connell KJ, Mahajan P, Levine AC, Bhatt SR, Roskind CG, et al. *Lactobacillus rhamnosus* GG versus placebo for acute gastroenteritis in children. N Engl J Med 2018;379(21):2002–14.
10. Basu S, Chatterjee M, Ganguly S, Chandra PK. Efficacy of *Lactobacillus rhamnosus* GG in acute watery diarrhoea of Indian children: a randomised controlled trial. J Paediatr Child Health 2007;43(12):837–42.
11. Basu S, Paul DK, Ganguly S, Chatterjee M, Chandra PK. Efficacy of high-dose *Lactobacillus rhamnosus* GG in controlling acute watery diarrhea in Indian children: a randomized controlled trial. J Clin Gastroenterol 2009;43(3):208–13.
12. JCI. Joint Commission International accreditation standards for hospitals. 6th ed. Oak Brook (IL): Joint Commission International; 2016.
13. Fang S-B, Lee H-C, Hu J-J, Hou S-Y, Liu H-L, Fang H-W. Dose-dependent effect of *Lactobacillus rhamnosus* on quantitative reduction of faecal rotavirus shedding in children. J Trop Pediatr 2009;55(5):297–301.
14. Petschow BW, Figueroa R, Harris CL, Beck LB, Ziegler E, Goldin B. Effects of feeding an infant formula containing Lactobacillus GG on the colonization of the intestine: a dose-response study in healthy infants. J Clin Gastroenterol 2005;39(9):786–90.
15. Yatsunenko T, Rey FE, Manary MJ, Trehan I, Dominguez-Bello MG, Contreras M, Magris M, Hidalgo G, Baldassano RN, Anokhin AP, et al. Human gut microbiome viewed across age and geography. Nature 2012;486(7402):222–7.
16. Schnadower D, Tarr PI, Casper TC, Gorelick MH, Dean MJ, O'Connell KJ, Mahajan P, Chun TH, Bhatt SR, Roskind CG, et al. Randomised controlled trial of *Lactobacillus rhamnosus* (LGG) versus placebo in children presenting to the emergency department with acute gastroenteritis: the PECARN probiotic study protocol. BMJ Open 2017;7(9):e018115.
17. Freedman SB, Eltorky M, Gorelick M, Pediatric Emergency Research Canada Gastroenteritis Study Group. Evaluation of a gastroenteritis severity score for use in outpatient settings. Pediatrics 2010;125(6):e1278–85.
18. Schnadower D, Tarr PI, Gorelick MH, O'Connell K, Roskind CG, Powell EC, Rao J, Bhatt S, Freedman SB. Validation of the modified Vesikari score in children with gastroenteritis in 5 US emergency departments. J Pediatr Gastroenterol Nutr 2013;57(4):514–9.
19. FDA. 16 May, 2018. [Internet]. 2018 [cited 2018 Nov 5]. Available from: https://www.fda.gov/scienceresearch/specialtopics/runningclinicaltrials/guidancesinformationsheetsandnotices/ucm219488.htm.
20. WHO Multicentre Growth Reference Study Group. WHO Child Growth Standards based on length/height, weight and age. Acta Paediatr Suppl 2006;450:76–85.
21. Grummer-Strawn LM, Reinold C, Krebs NF, Centers for Disease Control and Prevention (CDC). Use of World Health Organization and CDC growth charts for children aged 0–59 months in the United States. MMWR Recomm Rep 2010;59(RR-9):1–15.
22. WHO Multicentre Growth Reference Study Group. WHO Child Growth Standards: length/height-for-age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age: methods and development. Geneva: WHO; 2006.
23. CDC. 2000 CDC growth charts for the United States: methods and development. Hyattsville (MD): Department of Health and Human Services, CDC, National Center for Health Statistics; 2002.
24. Bailey B, Gravel J, Goldman RD, Friedman JN, Parkin PC. External validation of the clinical dehydration scale for children with acute gastroenteritis. Acad Emerg Med 2010;17(6):583–8.
25. Nixon AF, Cunningham SJ, Cohen HW, Crain EF. The effect of *Lactobacillus* GG on acute diarrheal illness in the pediatric emergency department. Pediatr Emerg Care 2012;28(10):1048–51.
26. Szajewska H, Guarino A, Hojsak I, Indrio F, Kolacek S, Shamir R, Vandenplas Y, Weizman Z. Use of probiotics for management of acute gastroenteritis: a position paper by the ESPGHAN Working Group for Probiotics and Prebiotics. J Pediatr Gastroenterol Nutr 2014;58(4):531–9.
27. Pan W. Akaike's information criterion in generalized estimating equations. Biometrics 2001;57(1):120–5.
28. de Clercq NC, Groen AK, Romijn JA, Nieuwdorp M. Gut microbiota in obesity and undernutrition. Adv Nutr 2016;7(6):1080–9.
29. Million M, Diallo A, Raoult D. Gut microbiota and malnutrition. Microb Pathog 2017;106:127–38.
30. Grenov B, Namusoke H, Lanyero B, Nabukeera-Barungi N, Ritz C, Molgaard C, Friis H, Michaelsen KF. Effect of probiotics on diarrhea in children with severe acute malnutrition: a randomized controlled study in Uganda. J Pediatr Gastroenterol Nutr 2017;64(3):396–403.
31. Suez J, Zmora N, Zilberman-Schapira G, Mor U, Dori-Bachash M, Bashiardes S, Zur M, Regev-Lehavi D, Ben-Zeev Brik R, Federici S, et al. Post-antibiotic gut mucosal microbiome reconstitution is impaired by

probiotics and improved by autologous FMT. Cell 2018;174(6):1406–23.

32. Zmora N, Zilberman-Schapira G, Suez J, Mor U, Dori-Bachash M, Bashiardes S, Kotler E, Zur M, Regev-Lehavi D, Brik RB, et al. Personalized gut mucosal colonization resistance to empiric probiotics is associated with unique host and microbiome features. Cell 2018;174(6):1388–405.
33. Freedman SB, Williamson-Urquhart S, Farion KJ, Gouin S, Willan AR, Poonai N, Hurley K, Sherman PM, Finkelstein Y, Lee BE, et al. Multicenter trial of a combination probiotic for children with gastroenteritis. N Engl J Med 2018;379(21):2015–26.
34. LaMont JT. Probiotics for children with gastroenteritis. N Engl J Med 2018;379(21):2076–7.