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Randomized Control Trials

Efficacy of probiotic treatment as post-exposure prophylaxis for COVID-19: A double-blind, Placebo-Controlled Randomized trial

Paul E. Wischmeyer [i, *, 1], Helen Tang [a, 1], Yi Ren [b], Lauren Bohannon [b], Danting Jiang [c, d], Matthew Bergens [a], Zeni E. Ramirez [c, e], Tessa M. Andermann [f], Julia A. Messina [g], Julia A. Sung [f], David Jensen [a], Sin-Ho Jung [a], Alexandra Artica [b], Anne Britt [b], Amy Bush [b], Ernaya Johnson [b], Meagan V. Lew [b], Hilary Winthrop [b], Claudia Pamanes [b], Alessandro Racioppi [b], Aaron T. Zhao [h], Zihan Wan [a], Neeraj K. Surana [c, e], Anthony D. Sung [b]

[a] Duke University School of Medicine, Durham, NC, USA
[b] Division of Hematologic Malignancies and Cellular Therapy, Department of Medicine, Duke University, Durham, NC, USA
[c] Division of Infectious Diseases, Department of Pediatrics, Duke University, Durham, NC, USA
[d] Program in Computational Biology and Bioinformatics, Duke University, Durham, NC, USA
[e] Department of Molecular Genetics and Microbiology, Duke University, Durham, NC, USA
[f] Division of Infectious Diseases, Department of Medicine, University of North Carolina, Chapel Hill, NC, USA
[g] Division of Infectious Diseases, Department of Medicine, Duke University, Durham, NC, USA
[h] Duke University Trinity College of Arts & Sciences, Durham, NC, USA
[i] Duke University School of Medicine, Department of Anesthesiology, Durham, NC, USA

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SUMMARY

Background & aims: The COVID-19 pandemic continues to pose unprecedented challenges to worldwide health. While vaccines are effective, additional strategies to mitigate the spread/severity of COVID-19 continue to be needed. Emerging evidence suggests susceptibility to respiratory tract infections in healthy subjects can be reduced by probiotic interventions; thus, probiotics may be a low-risk, low-cost, and easily implementable modality to reduce risk of COVID-19.

Methods: In this initial study, we conducted a randomized, double-blind, placebo-controlled trial across the United States testing probiotic *Lacticaseibacillus rhamnosus* GG (LGG) as postexposure prophylaxis for COVID-19 in 182 participants who had household exposure to someone with confirmed COVID-19 diagnosed within $\leq$7 days. Participants were randomized to receive oral LGG or placebo for 28 days. The primary outcome was development of illness symptoms within 28 days of COVID-19 exposure. Stool was collected to evaluate microbiome changes.

Results: Intention-to-treat analysis showed LGG treatment led to a lower likelihood of developing illness symptoms versus placebo (26.4 % vs. 42.9 %, $p = 0.02$). Further, LGG was associated with a statistically significant reduction in COVID-19 diagnosis (log rank, $p = 0.049$) via time-to-event analysis. Overall incidence of COVID-19 diagnosis did not significantly differ between LGG and placebo groups (8.8 % vs. 15.4 %, $p = 0.17$).

Conclusions: This data suggests LGG is associated with prolonged time to COVID-19 infection, reduced incidence of illness symptoms, and gut microbiome changes when used as prophylaxis $\leq$7 days post-COVID-19 exposure, but not overall incidence. This initial work may inform future COVID-19 prevention studies worldwide, particularly in developing nations where *Lacticaseibacillus* probiotics have previously been utilized to reduce other non-COVID infectious-morbidity.

Trial registration: ClinicalTrials.gov, NCT04399252, Date: 22/05/2020. https://clinicaltrials.gov/ct2/show/NCT04399252.

* Corresponding author. Duke University, School of Medicine DUMC, Box 3094 Mail # 41 2301 Erwin Road, 5692 HAFS Durham, NC 27710, USA.
E-mail address: Paul.Wischmeyer@Duke.edu (P.E. Wischmeyer).
[1] These authors contributed equally to this work as Co-First Authors.

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1. Introduction

The Coronavirus Disease 2019 (COVID-19) pandemic, caused by severe acute respiratory syndrome coronavirus (SARS-CoV-2) infection, has significantly altered global public health, with over 259 million cases and 5.1 million deaths worldwide as of 29-November-2021 [1]. Despite the advent of highly effective vaccines against SARS-CoV-2, widespread implementation has been limited in many parts of the globe, with only 29.2 % of people in low-income countries receiving at least one vaccine dose [2]. Vaccine uptake also remains incomplete overall with only 69.9 % of the worlds population having received at least one dose of a COVID-19 vaccine [2]. Further, this limited uptake is true even in developed nations, with only 68 % of the U.S. population being fully vaccinated against COVID-19 [2]. Finally, immunity and protection provided by vaccines appears to wane over time [3]. Thus, additional safe, low-cost, rapidly implementable strategies to address the ongoing COVID-19 pandemic continue to be necessary.

One potential target for intervention is via manipulation of the gut microbiota using probiotics (ingested live bacteria), a well-described strategy to modulate the human immune system and inflammatory responses [4]. This is particularly true as recent data has shown probiotics can prevent respiratory tract infections (RTI's) in healthy adults and children [5–7]. Recent studies suggest that prophylaxis with *Lacticaseibacillus* species specifically can prevent the development of upper and lower RTIs in healthy subjects [5,6]; with one key large randomized controlled trial of 4556 full-term healthy infants randomized to *Lacticaseibacillus* synbiotic vs. placebo showing a 40 % reduction in sepsis or death (9.0 % vs. 5.4 %, $p < 0.001$), including a 34 % reduction in lower RTIs (6.1 % vs. 4.0 %, $p = 0.002$) [5]. These outcomes may be mediated by the effects of probiotics on the immune system and intestinal/lung barrier function via improved intestinal homeostasis, increased regulatory T-cells, normalization of protective mucin production, decreased pro-inflammatory cytokines, modulation of antiviral gene expression, and increased expression of TLRs [8–12].

These clinical and laboratory reports suggest a potent immunomodulatory role for probiotic therapies in preventing or attenuating respiratory infections, and increasing evidence suggests that gut microbiota affect COVID-19 transmission risk and symptom severity [13]. Further, recent studies have further shown that gut microbiota composition reflects disease severity and dysfunctional immune responses in patients with COVID-19(14). Thus, modulation of the gut microbiome via probiotics is a promising strategy for prophylaxis and mitigation of COVID-19(13, 14). Since March 2020, several trials have launched investigating the benefits of probiotics in both treatment and prevention of COVID-19 [13]. Among commercially-available probiotics, *Lacticaseibacillus rhamnosus* GG (LGG) is particularly encouraging given the success of *Lacticaseibacillus* strains in numerous *in vivo* studies and clinical trials in health subjects, as discussed above [5,6]. We therefore conducted a randomized, double-blind, placebo-controlled trial of LGG as post-exposure prophylaxis in exposed household contacts (individuals living with someone recently diagnosed with COVID-19). We hypothesized that LGG prophylaxis would decrease the incidence of symptoms (primary endpoint) and incidence and time to confirmed diagnosis of COVID-19 infection.

2. Methods

2.1. Trial design

Participants were randomized using a permuted block randomization technique to receive LGG or placebo in a 1:1 ratio. Both subjects and study coordinators are blinded to the intervention; the randomization key was generated by the study statistician and only the pharmacist dispensing the study product had access to the key. Due to the ongoing COVID-19 pandemic, the study was designed so that all procedures could be conducted remotely. Study product was delivered by mail, and follow-up was obtained through web-based surveys and telephone calls, with stool samples shipped back to the study center. This research was conducted under Food and Drug Administration Investigational New Drug Application 24777. The research protocol and all methods were approved by the Duke University Institutional Review Board (IRB Approval #:Pro00105674, first approved: 06/24/2020), registered on ClinicalTrials.gov (NCT04399252), and this was previously published. [15] All methods in the trial were carried our according to the guidelines and regulations set forth by the Duke University Institutional Review Board and the Declaration of Helsinki. All participants or their legal guardians provided documented informed consent, via REDCap electronic written consent form per IRB approval.

2.2. Study design and potential full study power calculations

This trial was an initial first study to evaluate the role of LGG in the prevention of COVID-19 in exposed home contacts. Given the potential for significant enrollment numbers during the early stage of the pandemic (pre-vaccine availability), we attempted to calculate a pre-study estimate of sample size. Although limited data was available for the then-new COVID-19 virus and subsequent variants, we used an assumption of an attack rate of 10.5 % in household contacts based on available CDC reports at time of trial design [16]. Based on this early data, our initial sample size calculation indicated that it potentially would require 1076 participants (538 per arm) via the chi-squared test with 1-sided alpha = 5 %, which would give us 80 % power to detect a 40 % reduction (estimated from data showing 30–50 % reduction in respiratory infections with LGG from other studies [5,17,18]) in the attack rate of COVID-19, from 10.5 to 6.3 %. To ensure patient and public involvement in study design, household contacts exposed to COVID-19 were involved in the development and conduct of this clinical trial protocol.

2.3. Subject population and recruitment

Eligibility criteria included: age $\geq$ one year; exposed household contact (EHC) of someone diagnosed with COVID-19 within the past seven days; willingness to not take any other probiotic while on LGG/placebo; and access to e-mail/internet to complete electronic consent and surveys. Exclusion criteria included: symptoms of COVID-19 at enrollment, including fever, respiratory symptoms (e.g. cough, dyspnea), GI symptoms, anosmia, ageusia; >seven days since index case of household contact had first positive COVID-19 test; taking hydroxychloroquine or remdesivir for any reason; enrolled in a COVID-19 prophylaxis study or receiving COVID-19 prophylaxis as standard of care, including vaccination; any medical condition that would prevent taking oral probiotics or increase risks associated with probiotics; unable to read and follow directions in English or Spanish; living outside of the United States of America; and prisoners and institutionalized individuals. Participants were recruited locally via telephone outreach from study coordinators who identified index cases via the Duke University Hospital Epic dashboard or nationally via flyers, advertisements, social media platforms (https://www.facebook.com/protectehc/), or our study website (https://www.protect-ehc.org/). After electronic consent and randomization, product was dispensed to participants via Federal Express overnight delivery.

2.4. Interventions

Participants took LGG or placebo once daily for 28 days starting from receipt of the blinded shipped study package (age < five, one capsule daily, age ≥ five, two capsules daily). LGG capsules, made by Culturelle (DSM), contained ten billion colony forming units of *L. rhamnosus* GG (ATCC 53103). The placebo capsules (DSM) contained 325 mg of microcrystalline cellulose, a food additive commonly used as a bulking agent in food preparation and vitamin supplements, and as a placebo in probiotic studies [19–21]. Both products and their foil packaging were visually indistinguishable.

2.5. Data collection

Data on demographics, medical history, household risks, and infection details of index patient were collected remotely upon enrollment via REDCap, an electronic platform that supports secure data capture for research [22]. Authors did not have access to information that could identify individual participants during or after data collection. Data on medications, adherence, COVID-19 exposures, symptoms, adverse events, and COVID-19-related events were collected throughout the study up to day 60. Participants who reported symptoms were queried for laboratory-confirmed COVID-19 infection via electronic health record review, surveys, and phone calls. Subjects self-collected stool using OMNIgene-gut collection kits, which were returned via mail for sequencing analysis.

2.6. Outcomes

The primary endpoint was the development of symptoms, including fever/chills, headache, muscle aches, runny nose, sore throat, cough, shortness of breath, nausea or vomiting, diarrhea, stomach upset or pain, excessive bloating or gas, constipation, loss of sense of smell, loss of sense of taste, rash, painful toes, or other symptoms as reported by participants. Secondary endpoints included: time to COVID-19 diagnosis; incidence of COVID-19 diagnosis, severity of symptoms; and duration of symptoms. In participants who reported diagnosis of COVID-19, we reviewed medical records for laboratory confirmation of the diagnosis as well as complications (e.g., need for hospitalization, intubation, mortality), when available. We investigated the incidence of these events through day 28 and through day 60.

2.7. Sequencing analysis

DNA from stool samples was extracted using the Qiagen PowerSoil DNA kit, and the V4 region of the 16S rRNA gene was PCR amplified and sequenced on the Illumina MiSeq platform as previously described [23]. After demultiplexing, DADA2 was used for quality control and to generate an amplicon sequence variant (ASV) count table with taxonomy assigned using the Silva v138.1 database [24,25]. The identity of *L. rhamnosus* was confirmed by blasting the sequence of the only ASV that is present in ≥10 % of the samples and assigned to *Lacticaseibacillus* at the genus level. Data analysis was performed using the R programming packages phyloseq (principal coordinate analysis), microbiome (centered log-ratio transformation of raw counts), MaAsLin2 (differential abundance analysis), and ggplot2 (visualization) [26–29]. Unpaired t-test was used to compare the level of *L. rhamnosus* in the two arms using the t.test function in base R. PERMANOVA testing was performed to assess for statistical significance in beta diversity (Bray–Curtis dissimilarity index) using the adonis function from the vegan R package [30,31].

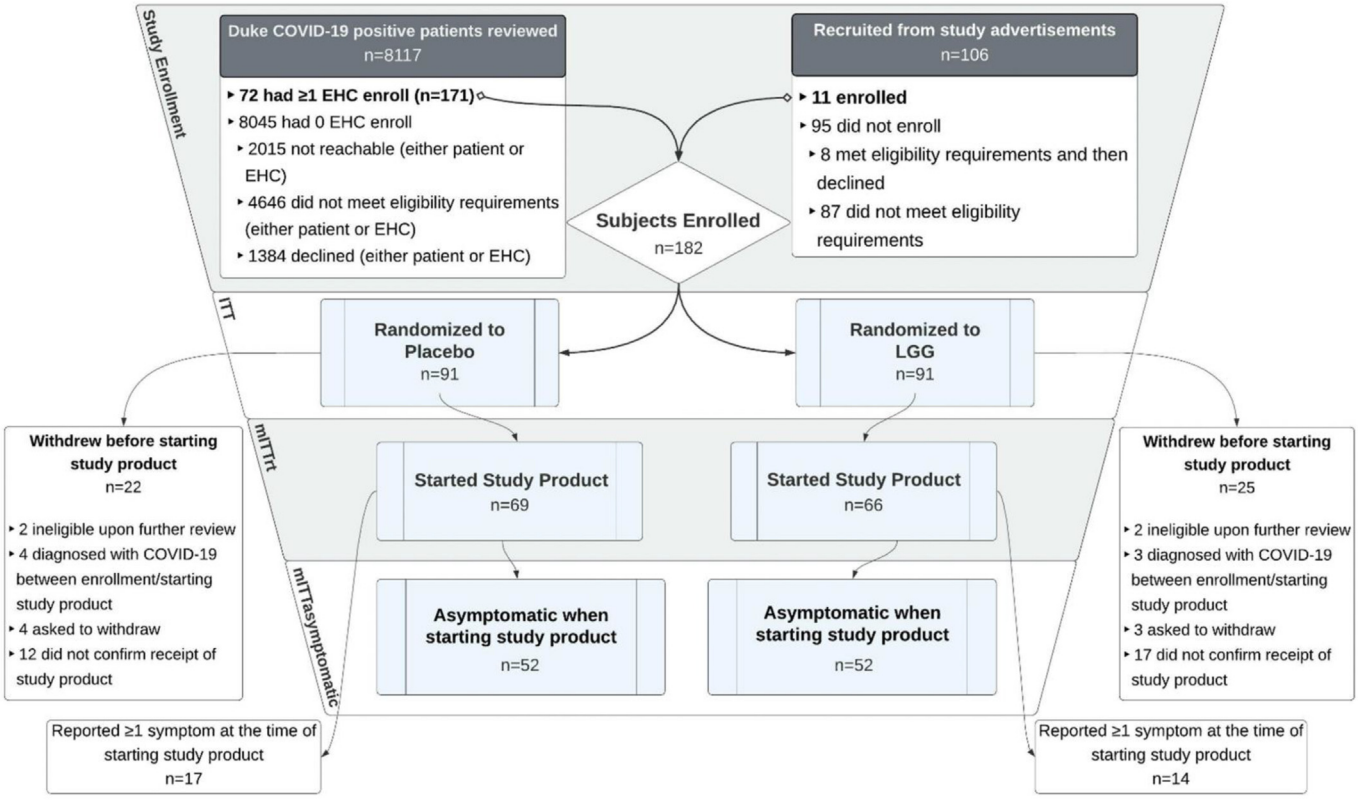


Fig. 1. CONSORT diagram. Flow diagram describing study design. EHC = exposed household contact.

2.8. Statistical analysis

Analyses were conducted via intention-to-treat (ITT) methodology, including all participants who were enrolled and randomized. Additionally, we performed pre-specified analysis with modified ITT methodology including all enrolled and randomized participants who confirmed physical receipt of the study product (mITTrt) as well as a pre-specified analysis that included enrolled and randomized participants who confirmed physical receipt of the study product and remained symptom free at the time of study product receipt (mITTasymptomatic). All analyses were performed in SAS 9.4 (SAS Institute, Cary, NC). Chi-squared tests were employed to test the differences in COVID-19 symptoms, laboratory-confirmed infections, and other categorical variables between the LGG and placebo arms. Student's t-tests were used to compare continuous variables such as symptom duration and adherence. Kaplan–Meier curves were constructed, and log-rank tests used to test the univariable differences in time-to-infection/symptom outcomes. Logistic regression modeling of day 28 symptoms was performed in mITTrt cohort to adjust for the potential confounding caused by age. To assess the effect of smoking and hypertension on LGG's effect on key COVID-19 outcomes we performed multi-variate analysis as follows. The outcome of symptoms (Yes/No) was regressed on treatment (LGG/placebo), while examining for confounding of smoking status (Yes/No) and history of hypertension (Yes/No) using logistic regression models for multivariate analysis. In addition, the Cox PH model was conducted to investigate the effects of treatment (LGG/placebo) on time to Covid-19 diagnosis, controlling for smoking status and history of hypertension.

3. Results

3.1. Participant characteristics

Enrollment began on June 24, 2020 and was stopped early on June 2, 2021, after the study team noted changes in recruitment patterns such that most individuals approached for the study had already been vaccinated and were therefore ineligible. During this period, 182 participants were enrolled and randomized (ITT). Of these, 135 confirmed that they physically received and started the study product and were considered to have received therapy (mITTrt); the other 47 participants did not respond to repeated queries. Of those 135, 31 participants reported development of symptoms prior to receiving study product; 104 remained asymptomatic at initiation of therapy (mITTasymptomatic) (Fig. 1). Please see specific descriptions of three analyzed treatment groups in Table 1.

The demographic characteristics of the ITT participants are displayed in Table 1; demographics of mITTrt and mITTasymptomatic analyses are available in Supplementary Tables S1a and S1b. Groups were evenly balanced other than the increased prevalence of smoking (14.3 % vs. 4.4 %) and hypertension in the placebo group (18.7 % vs. 5.5 %). There were no differences in employment in healthcare, recent visits to healthcare facilities, use of' probiotics or antibiotics prior to the start of the study, frequency of mask wearing, social distancing, and handwashing between groups (Supplementary Table S2a,b,c, all $p > 0.05$).

3.2. COVID-19 symptoms and infection

Participants randomized to LGG were significantly less likely to report any symptoms by day 28 (26.4 % vs. 42.9 %, $p = 0.02$, Table 2). No participants reported new symptoms after day 28. Participants receiving LGG had significantly prolonged time to onset of symptoms (log rank $p = 0.006$, Fig. 2a). There was no difference in the proportion of participants who reported specific symptoms in any of the analysis subgroups, though placebo recipients were more likely experience moderate to severe changes in taste perception (5.5 % vs. 0 %, $p = 0.02$, Supplementary Table S3).

Table 2
Demographic and Clinical Characteristics of Participants at Baseline, ITT analysis.

	LGG	Placebo	All Participants
	N = 91 (50 %)	N = 91 (50 %)	N = 182 (100 %)
Age group – no. (%)			
- <18	25 (27.5 %)	16 (17.6 %)	41 (22.5 %)
- 18-64	64 (70.3 %)	67 (73.6 %)	131 (72 %)
- > = 65	2 (2.2 %)	8 (8.8 %)	10 (5.5 %)
Female sex – no. (%)	60 (65.9 %)	55 (60.4 %)	115 (63.2 %)
Race – no. (%)			
- White	55 (60.4 %)	66 (72.5 %)	121 (66.5 %)
- Black	18 (19.8 %)	17 (18.7 %)	35 (19.2 %)
- Asian	2 (2.2 %)	2 (2.2 %)	4 (2.2 %)
- Other	11 (12.1 %)	4 (4.4 %)	15 (8.2 %)
- More Than One	4 (4.4 %)	1 (1.1 %)	5 (2.7 %)
- Not Reported	1 (1.1 %)	1 (1.1 %)	2 (1.1 %)
Hispanic Ethnicity – no. (%)	14 (15.4 %)	12 (13.2 %)	26 (14.3 %)
Comorbid Conditions – no. (%)			
- Current smoker	4 (4.4 %)	13 (14.3 %)	17 (9.3 %)
- Lung disease	0 (0 %)	2 (2.2 %)	2 (1.1 %)
- Allergies	13 (14.3 %)	25 (27.5 %)	38 (20.9 %)
- Cancer	1 (1.1 %)	4 (4.4 %)	5 (2.7 %)
- Hypertension	5 (5.5 %)	17 (18.7 %)	22 (12.1 %)
- Diabetes	2 (2.2 %)	5 (5.5 %)	7 (3.8 %)
- Heart disease/stroke	2 (2.2 %)	5 (5.5 %)	7 (3.8 %)
- Liver disease	0 (0 %)	1 (1.1 %)	1 (0.5 %)
- Currently pregnant	2 (2.2 %)	1 (1.1 %)	3 (1.6 %)
Antibiotic Use within past 30 days – no. (%)	4 (4.4 %)	6 (6.6 %)	10 (5.5 %)
Probiotic Use within past 30 days – no. (%)	5 (5.5 %)	6 (6.6 %)	11 (6 %)
Days from exposure to enrollment – median (IQR)	2 (1–3)	2 (1–2)	2 (1–2)
Days from exposure to study product start – median (IQR)	3.5 (2–5)	3 (2–4)	3 (2–4)
- 0	30 (33 %)	35 (38.5 %)	65 (35.7 %)
- 1	11 (12.1 %)	10 (11 %)	21 (11.5 %)
- 2	18 (19.8 %)	22 (24.2 %)	40 (22 %)
- 3	3 (3.3 %)	5 (5.5 %)	8 (4.4 %)
- 4	12 (13.2 %)	11 (12.1 %)	23 (12.6 %)
- Unknown	17 (18.7 %)	8 (8.8 %)	25 (13.7 %)

Table 1
Definitions of study groups used in analysis.

Study Group Name	Description of Included Participants
ITT: Intention to TreatIntention to Treat	All enrolled subjects (n = 182)
mITTrt: Modified Intention to Treat- Received/Started Study Treatment	All enrolled subjects who started study treatment with LGG (Probiotic) or Control (n = 135)
mITTasymptomatic: Modified Intention to Treat- Asymptomatic at Study Treatment Start	All enrolled subjects who were asymptomatic (reported no illness symptoms) when study treatment started (N = 104)

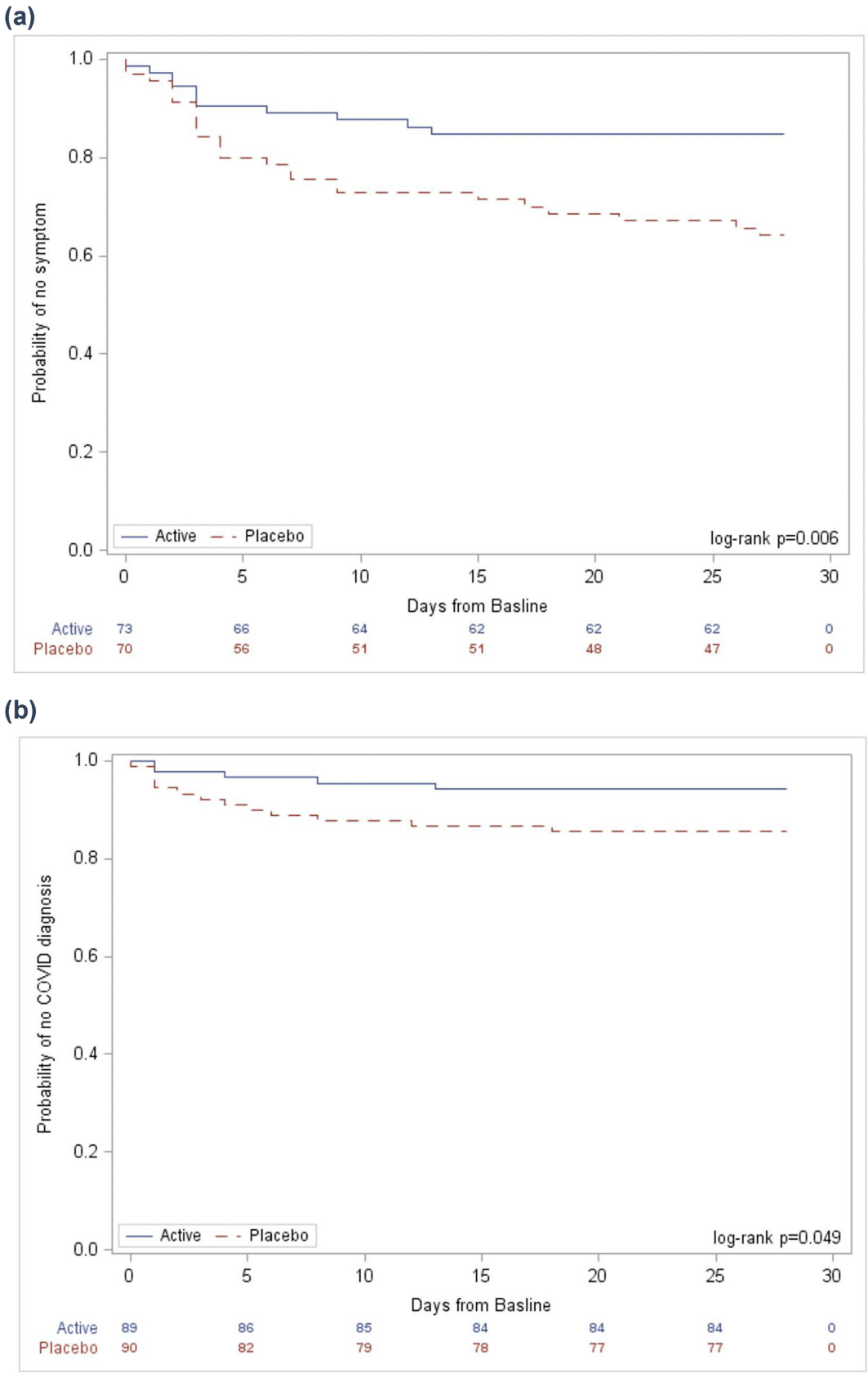


Fig. 2. a. Kaplan–Meier curves of times to event. Time to any symptoms (N = 143, event = 36, log rank p = 0.006). Participants receiving LGG had prolonged time to onset of any symptoms. Participants who had any symptoms at study start were excluded. Curves were right censored at D28. b. Kaplan–Meier curves of times to event. Time to reported laboratory-confirmed infection (N = 179, event = 18, log rank p = 0.049). Participants receiving LGG had prolonged time to reported laboratory-confirmed COVID-19 infection. Participants who reported laboratory-confirmed infection at study start were excluded. Curves were right censored at D28.

Table 3
Outcomes of LGG therapy for postexposure prophylaxis against COVID-19 at D28.

ITT	LGG	Placebo	All Participants	P-Value
	N = 91 (50 %)	N = 91 (50 %)	N = 182 (100 %)	
Any symptoms – no. (%)	24 (26.4 %)	39 (42.9 %)	63 (34.6 %)	0.02
Any moderate/severe symptoms – no. (%)	16 (17.6 %)	24 (26.4 %)	40 (22 %)	0.15
Symptom duration – median (IQR)	8 (4–17)	11 (4–22)	10 (4–21)	0.37
Reported COVID-19 Diagnosis – no. (%)	8 (8.8 %)	14 (15.4 %)	22 (12.1 %)	0.17
mITTrT	**N = 66 (48.9 %)**	**N = 69 (51.1 %)**	**N = 135 (100 %)**	
Any symptoms – no. (%)	24 (36.4 %)	39 (56.5 %)	63 (46.7 %)	0.02
Any moderate/severe symptoms – no. (%)	16 (24.2 %)	24 (34.8 %)	40 (29.6 %)	0.18
Symptom duration – median (IQR)	9.5 (6–18)	12 (4–27)	11 (5.5–22)	0.38
Reported COVID-19 Diagnosis – no. (%)	6 (9.1 %)	13 (18.8 %)	19 (14.1 %)	0.10
mITTasymptomatic	**N = 52 (50 %)**	**N = 52 (50 %)**	**N = 104 (100 %)**	
Any symptoms– no. (%)	14 (26.9 %)	25 (48.1 %)	39 (37.5 %)	0.03
Any moderate/severe symptoms – no. (%)	10 (19.2 %)	15 (28.8 %)	25 (24 %)	0.25
Symptom duration – median (IQR)	6.5 (5–9.5)	10 (4–18)	9 (4–12)	0.11
Reported COVID-19 Diagnosis – no. (%)	2 (3.8 %)	7 (13.5 %)	9 (8.7 %)	0.08

Of 77 symptomatic participants during the study period, 47 underwent testing under the care of their medical provider and 22 had laboratory confirmed COVID-19. Of these, 16 diagnoses were made by PCR testing and confirmed by electronic medical record review, and six were self-reported by participants after laboratory testing. While there was a trend to decreased COVID-19 incidence in participants randomized to LGG, this difference was not statistically significant (8.8 % vs. 15.4 %, p = 0.17, Table 3); however, time to COVID-19 diagnosis was significantly prolonged for LGG recipients (log rank p = 0.049, Fig. 2b). There were no hospitalizations or deaths among any participants. Similar findings were observed in the modified ITT analyses (Table 3, Supplementary Figs. 1 and 2), including a trend to a decreased incidence of COVID-19 in subjects not reporting symptoms at initiation of treatment (mITTasymptomatic, 3.8 % vs. 13.5 %, p = 0.08).

3.3. Sensitivity and multivariate analyses

Univariate sensitivity analysis by sex revealed no differences in development of COVID-19 symptoms or laboratory-confirmed infection (Supplementary Table S4). Univariate sensitivity analysis by age showed older participants were significantly more likely to report symptoms (age <18, 14.6 % vs. age 18–64, 38.9 %, vs. age ≥65, 60.0 %, p = 0.004) and have laboratory-confirmed infection (age <18, 12.2 % vs. age 18–64, 9.9 %, vs. age ≥65, 40.0 %, p = 0.02, Supplementary Table S5) at day 28. Multivariate logistic regression modeling revealed that age <18 was associated with significantly lower odds of developing symptoms by day 28 compared to the 18–64 age group (OR 0.29, 95%CI 0.1–0.82, p = 0.02); current smoking status was not associated with development of symptoms (OR 0.99, 95%CI 0.33–2.98, p = 0.98, Supplementary Table S6).

To evaluate for potential confounding of hypertension and smoking status multi-variate analysis of the occurrence of symptoms (Yes/No) was regressed on treatment (LGG/placebo), while examining for confounding of smoking status (Yes/No) and history of hypertension (Yes/No) using logistic regression models for multivariate analysis. Results demonstrate that participants who were randomized to LGG maintained a signal of statistical trends to having lower odds of being symptomatic, controlling for the effects of smoking status and hypertension in the ITT (p = 0.101), mITTrt (p = 0.079), and mITTasymptomatic group (p = 0.062). The effects of not having hypertension were also statistically significant for the presence of symptoms in the ITT (p = 0.029) and mITTrt (p = 0.026) group, but not the mITTasymptomatic group (p = 0.120). Smoking status had no effect on the occurrence of symptoms in multi-variate analysis (see Supplemental Tables S7a-c). A statistical trend to benefit of LGG on reducing COVID-19 symptoms was maintained in multivariate analysis controlling for smoking and hypertension status.

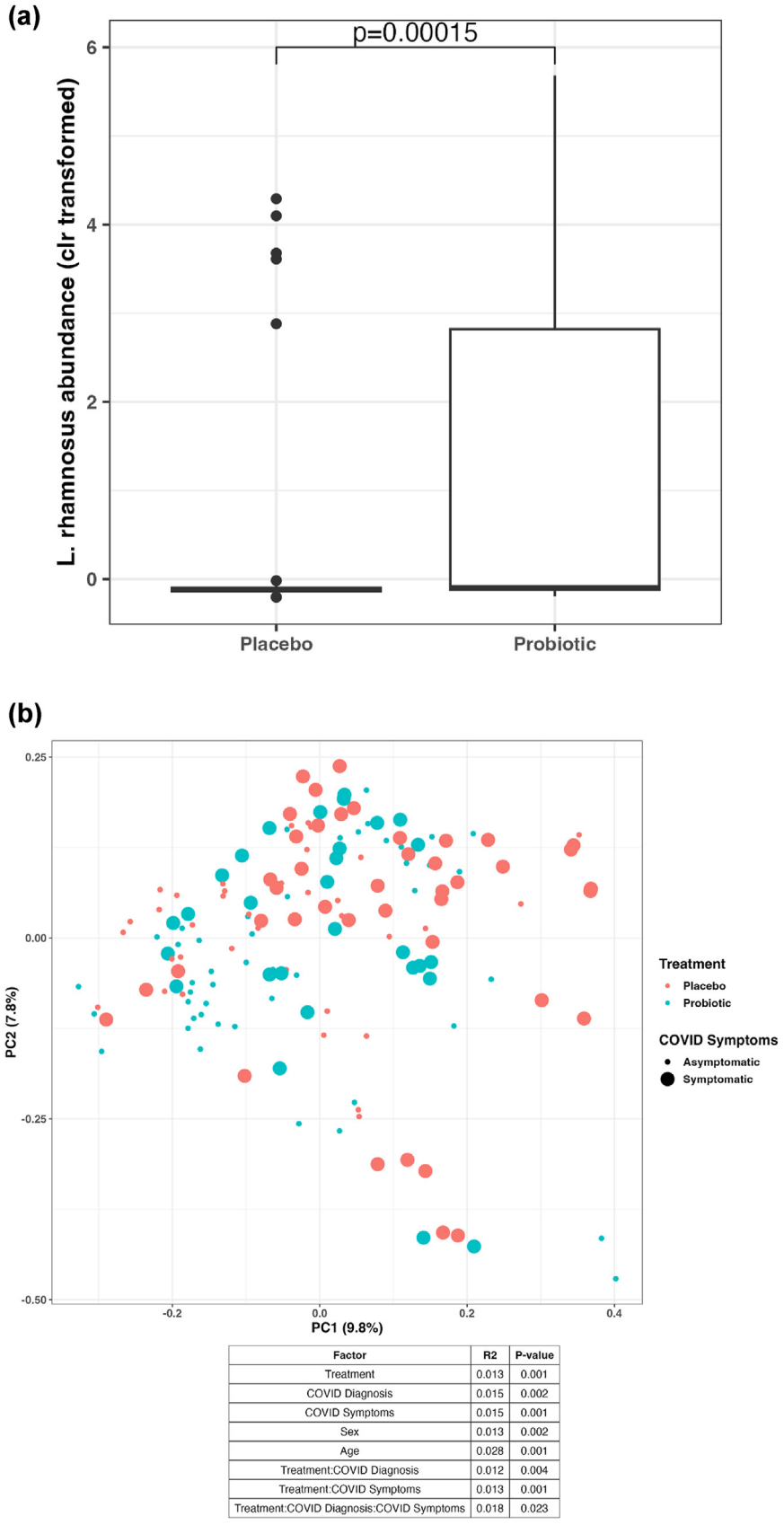
In addition, the Cox PH model was conducted to investigate the effects of treatment (LGG/placebo) on time to Covid-19 diagnosis, controlling for smoking status and history of hypertension. LGG treatment maintained a statistical trend to a prolonged time to reported laboratory-confirmed COVID-19 infection controlling for smoking and hypertension in the ITT (p = 0.134), mITTrt (p = 0.0997), and mITTasymptomatic group (p = 0.112). No statistical effect of either not smoking or not having hypertension (all p values > 0.778 for not smoking and all p values > 0.622 for no hypertension in all groups) was observed in prolonging time to COVID-19 in all groups (See Supplemental Tables S8. a-c). Thus, a statistical trend to benefit of LGG on prolonging time to COVID-19 diagnosis was maintained in multivariate analysis, with no effect of smoking or hypertension observed on this outcome.

3.4. Microbiome analyses

A total of 260 stool samples were received from 106 participants (all in the mITTrt group), with 85 day 7 samples and 70 day 28 samples. Participants who received LGG had a significantly greater abundance of *L. rhamnosus* compared to participants who received placebo (Fig. 3a). Although there was no difference in the α-diversity between participants who received placebo or probiotic (data not shown), there was a significant difference in the overall structure of the stool microbiota (i.e., β-diversity) (Fig. 3b; p = 0.001). Additionally, sex, age, and the presence of symptoms and a COVID-19 diagnosis significantly affected β-diversity, as did interactions between the treatment group, symptoms, and COVID-19 diagnosis. To determine specific bacterial taxa that are altered by oral administration of LGG, we used a mixed effect linear model with random effects from each individual participant, controlling for sex, age, symptoms, diagnosis, and timepoint. Interestingly, *L. rhamnosus* was identified as the only differentially abundant ASV between the two treatment arms.

3.5. Adherence and safety

Of 110 participants who reported at least one adherence time point, median adherence did not differ between LGG and placebo



Factor	R2	P-value
Treatment	0.013	0.001
COVID Diagnosis	0.015	0.002
COVID Symptoms	0.015	0.001
Sex	0.013	0.002
Age	0.028	0.001
Treatment:COVID Diagnosis	0.012	0.004
Treatment:COVID Symptoms	0.013	0.001
Treatment:COVID Diagnosis:COVID Symptoms	0.018	0.023

Fig. 3. a. Microbiome analyses of *L. rhamnosus*. Centered log-ratio (clr) transformed abundance of *L. rhamnosus* in participants who received placebo or probiotic. Data from day 7 and day 28 stool samples are depicted in a box and whisker plot. b. Microbial diversity principal coordinates analysis (PCoA) of Bray–Curtis distances of day 7 and day 28 samples. The treatment group and COVID-19 diagnosis status are highlighted in the plot. Statistical analysis was performed using PERMANOVA, with results shown in table.

groups (median 100 %, IQR 93–100 % vs. 100 %, IQR 93–100 %, p = 0.82, Supplementary Tables S9). Participants were unable to guess their randomization arm, suggesting that blinding was maintained. There was no significant difference in proportion of participants who attributed symptoms they experienced to LGG vs. placebo (8.8 % vs. 23.1 %, p = 0.32), though placebo recipients were more likely to stop the study product, temporarily or permanently, due to symptoms attributed to the study product (5.5 % vs. 0 %, p = 0.02, Supplementary Tables S9). These findings held true in mITTrt and mITTasymptomatic analyses (Supplementary Table (S9b, S9c).

4. Discussion

In this randomized, double-blind, placebo-controlled trial, we investigated the efficacy of the probiotic LGG as post-exposure prophylaxis against COVID-19. In our study of 182 enrolled participants, those randomized to LGG had fewer symptoms and prolonged time to development of COVID-19 compared to those receiving placebo; this finding held true in all three of our analyses (ITT, mITTrt, and mITTasymptomatic). Interestingly, the data suggest that placebo recipients were more likely to experience moderate to severe changes in taste perception— a relatively specific symptom for COVID-19. A similar trend was observed for changes in smell perception but did not reach statistical significance. Microbiome analyses confirmed that *L. rhamnosus* abundance was significantly increased in participants who received LGG compared to placebo, suggesting that participants were adherent with study therapy and that microbial community structure differentiated in response to probiotic treatment. It is important to note that the statistical trends of the primary outcome, incidence of symptoms, should be taken within context of the limitations of our study discussed below.

Our study has several limitations. First, we were limited in total sample size and time of study enrollment due to difficulty with recruitment during concurrent vaccine rollout, which increasingly limited the eligible population. Reaching the initial calculated sample size to complete the RCT became impractical as the population became increasingly vaccinated. We opted to conclude the study with the enrollment we had, as including both vaccinated and unvaccinated populations would have introduced significant bias. However, we have accounted for our sample size within our statistical analysis and acknowledge the overall limitations. Given the high transmissibility of newer viral strains and the potential for waning vaccine efficacy, future studies may consider including vaccinated individuals, especially as data suggest that probiotic administration improves vaccination efficacy against other viral pathogens, such as influenza [32]. Further, while allocation was blinded and randomized in a 1:1 fashion, participants in the placebo group had a small increased incidence of current smoking and hypertension at baseline, which are potential risk factors for the development of COVID-19 disease; however, smoking was not associated with the development of symptoms in our study in univariate analysis. To analyze for effects of hypertension and smoking status on key outcomes of symptoms and time to COVID-19 diagnosis multi-variate analysis was performed controlling for hypertension status and smoking status. Preservation of a statistical trend on the benefit of LGG on reducing symptoms in all groups was maintained in this multi-variate analysis. A statistical signal of hypertension status (no hypertension present) predicting fewer symptoms was observed only in the ITT and mITTrt groups. No statistical trend or effect of not smoking or not having hypertension present status was observed for increasing time to COVID-19 diagnosis in any group, and only treatment with LGG maintained a statistical trend to prolonged time to COVID-19 diagnosis when controlling for smoking and hypertension status. Thus, although full statistical significance at a $p < 0.05$ was not preserved for LGG's beneficial effects on reducing symptoms and prolonging time to COVID-19 diagnosis in multi-variate analysis controlling for smoking and hypertension status, statistical trends were preserved for LGG's benefits on both outcomes. Additionally, LGG and other probiotics may be associated with gastrointestinal side effects, potentially confounding our measurement of symptoms, although fewer GI side effects were noted in the probiotic group. Another limitation was the remote format, wherein the primary endpoint was self-reported symptoms rather than laboratory-confirmed infection; participants had inconsistent access to laboratory testing, with only 61 % of symptomatic participants ultimately undergoing testing. Another limitation is that data collection and outcomes were collected remotely due to the ongoing COVID-19 pandemic that necessitated remote operations. While these patients were not examined by physicians directly, this is mitigated by the fact that the primary endpoint, symptoms, depended on patient report, which we acknowledge as a potential risk in the reliability of remotely collected information.

In conclusion, COVID-19 continues to pose a unique and novel challenge to global health [4]. Ongoing research is showing a potentially significant role of the microbiome and dysbiosis in COVID-19 disease severity [14] and development of Long-Covid [33], thus studies evaluating the role of probiotics and other methods of microbiome optimization are urgently needed. In response to this need, we have conducted the first pilot double-blinded, randomized, placebo-controlled trial to evaluate the effect of prophylaxis with probiotic LGG on the development of COVID-19 symptoms in exposed household contacts. In this initial first trial, our study suggests that LGG is well-tolerated and is associated with prolonged time to development of COVID-19 infection, reduced symptomatic disease, and changes to gut microbiome structure. While there was a trend to decreased COVID-19 incidence in participants randomized to LGG, it was not statistically significant in our study. Further investigation of LGG probiotic intervention in larger randomized controlled trials is warranted, including comparison of pre-exposure vs. post-exposure prophylaxis with LGG probiotics in high-risk populations. It is key to note that vaccination should remain the first line in the prevention of COVID-19. Further investigation of probiotics is also warranted in vaccinated populations. Additionally, further research on probiotics is needed in severe COVID-19 infection where steroids and other data-driven interventions remain first line therapies. In conclusion, Our results lend credence to the notion that our symbiotic microbes may be valuable partners in the fight against COVID-19 and potentially other future pandemic diseases.

Author contributions

P.E.W.: conceptualisation, investigation, methodology, supervision, data analysis and interpretation, writing - review & editing (*co-first author and corresponding author).

H.T.: conceptualisation, investigation, methodology, figure and table creation, writing - original draft, writing - review & editing (*co-first author).

Y.R.: formal analysis, visualisation, data analysis and interpretation, figure and table creation, writing - review & editing.

L.B.: investigation, data collection, methodology, data curation, project administration, writing - review & editing.

D.Jiang: formal analysis, visualisation, writing - review & editing.

M.B.: writing - review & editing.

Z.E.R.: formal analysis, visualisation, writing - review & editing.

T.M.A.: methodology, writing - review & editing.
J.A.M.: methodology, writing - review & editing.
J.A.S.: conceptualisation, methodology, writing - review & editing.
D.Jensen: methodology, writing - review & editing.
S.H.J.: methodology, data analysis and interpretation, formal analysis, figure and table creation, writing - review & editing.
A.A.: investigation, data collection, writing - review & editing.
A.Britt: investigation, data collection, writing - review & editing.
A.Bush: investigation, data collection, writing - review & editing.
E.J.: investigation, data collection, writing - review & editing.
M.V.L.: investigation, data collection, writing - review & editing.
H.M.M.: investigation, data collection, writing - review & editing.
C.P.: investigation, data collection, writing - review & editing.
A.R.: investigation, data collection, writing - review & editing.
A.T.Z.: investigation, data collection, visualisation, writing - review & editing.
Z.W. - formal analysis, visualisation, data analysis and interpretation, figure and table creation, writing - review & editing.
N.K.S.: formal analysis, visualisation, writing - review & editing.
A.D.S.: conceptualisation, investigation, methodology, supervision, data analysis and interpretation, writing - review & editing.
All authors have read and reviewed manuscript.

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Conflict of interest

P.E.W. has received unrestricted gift funding from DSM/iHealth and has an investigator-initiated research grant from Abbott Inc. related to work focused on microbiome and probiotic research. P.E.W. has presented CME lectures for DSM on probiotic research. All other authors declare no competing interests. A.D.S has grants from Merck Sharpe & Dohme, consulting for Targazyme, and receipt of supplies from Clasado.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnu.2023.11.043.

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