Probiotics (*Lactobacillus gasseri* KS-13, *Bifidobacterium bifidum* G9-1, and *Bifidobacterium longum* MM-2) improve rhinoconjunctivitis-specific quality of life in individuals with seasonal allergies: a double-blind, placebo-controlled, randomized trial[1,2]

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ABSTRACT

Background: Rhinoconjunctivitis-specific quality of life is often reduced during seasonal allergies. The Mini Rhinoconjunctivitis Quality of Life Questionnaire (MRQLQ) is a validated tool used to measure quality of life in people experiencing allergies (0 = not troubled to 6 = extremely troubled). Probiotics may improve quality of life during allergy season by increasing the percentage of regulatory T cells (Tregs) and inducing tolerance.

Objective: The objective of this study was to determine whether consuming *Lactobacillus gasseri* KS-13, *Bifidobacterium bifidum* G9-1, and *B. longum* MM-2 compared with placebo would result in beneficial effects on MRQLQ scores throughout allergy season in individuals who typically experience seasonal allergies. Secondary outcomes included changes in immune markers as part of a potential mechanism for changes in MRQLQ scores.

Design: In this double-blind, placebo-controlled, parallel, randomized clinical trial, 173 participants (mean ± SEM: age 27 ± 1 y) who self-identified as having seasonal allergies received either a probiotic (2 capsules/d, 1.5 billion colony-forming units/capsule) or placebo during spring allergy season for 8 wk. MRQLQ scores were collected weekly throughout the study. Fasting blood samples were taken from a subgroup (placebo, $n = 37$; probiotic, $n = 35$) at baseline and week 6 (predicted peak of pollen) to determine serum immunoglobulin (Ig) E concentrations and Treg percentages.

Results: The probiotic group reported an improvement in the MRQLQ global score from baseline to pollen peak (-0.68 ± 0.13) when compared with the placebo group (-0.19 ± 0.14; $P = 0.0092$). Both serum total IgE and the percentage of Tregs increased from baseline to week 6, but changes were not different between groups.

Conclusions: This combination probiotic improved rhinoconjunctivitis-specific quality of life during allergy season for healthy individuals with self-reported seasonal allergies; however, the associated mechanism is still unclear. This trial was registered at clinicaltrials.gov as NCT02349711. *Am J Clin Nutr* 2017;105:758–67.

Keywords: healthy adults, probiotics, seasonal allergies, allergic rhinitis, quality of life, *Lactobacillus gasseri*, *Bifidobacterium bifidum*, *Bifidobacterium longum*

INTRODUCTION

Medical practice often focuses on treating diseases and alleviating symptoms of disease, but quality of life is also an important aspect that is sometimes overlooked. Secondary effects of a disease or its consequent treatment can negatively impact well-being and the ability of an individual to continue a daily routine, diminishing quality of life. Allergic rhinitis, commonly known as seasonal allergies or hay fever, has been associated with a lack of sleep, reduced productivity at work or school, emotional distress, and embarrassment (1). A steady increase in the prevalence of allergic diseases has been observed over the past 50 y (2). Current medications for allergies may have undesirable side effects depending on the individual (e.g., dry mouth, drowsiness, sleeplessness) (3), some of which may affect quality of life. It is of interest to continue to search for alternatives.

A recent meta-analysis reports that probiotics have potential to improve quality of life in people experiencing allergies, but more high-quality studies are required to confirm this (4). Evidence for the effects of probiotics on allergies varies widely based on the bacterial strain(s) used for the intervention, delivery vehicle (yogurt, capsule, milk, etc.), duration of intervention, subject characteristics, and method of quantifying efficacy. A highly cited, validated tool to measure improvements in rhinoconjunctivitis-specific quality of life is the Rhinoconjunctivitis Quality of Life Questionnaire (RQLQ)[6] (1).

[1] Supported by Wakunaga of America Co., Ltd. and USDA National Institute of Food and Agriculture Hatch Project number FLA-FOS-005129.

[2] Supplemental Figure 1 is available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.

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[6] Abbreviations used: GSRS, Gastrointestinal Symptom Rating Scale; MRQLQ, Mini Rhinoconjunctivitis Quality of Life Questionnaire; OTU, operational taxonomic unit; PE, phycoerythrin; RQLQ, Rhinoconjunctivitis Quality of Life Questionnaire; Treg, regulatory T cell.

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Costa et al. (5) demonstrated an improvement in ocular symptoms and quality of life (RQLQ scores) in allergic rhinitis patients supplemented with capsules of *Lactobacillus paracasei* LP33 for 5 wk. Peng and Hsu (6) and Wang et al. (7) showed improvement in quality of life in 2 different studies both using a pediatric-adapted version of the RQLQ in children supplemented for 30 d with either capsules of heat-killed LP33 or fermented milk containing live LP33.

The probiotic used in this study (*L. gasseri* KS-13, *Bifidobacterium bifidum* G9-1, and *B. longum* MM-2), when previously consumed by older adults, resulted in increased in vitro–stimulated peripheral blood mononuclear cell production of IL-10, a cytokine important in induction of tolerance to allergens (8). An animal study using only one of these strains (*B. bifidum* G9-1) demonstrated potential clinical benefit (lower nose-rubbing and intranasal pressure compared with the control) (9). Although *B. bifidum* G9-1 has improved allergy-related outcomes in animals, only the combination of the 3 strains has shown immune-related benefit in humans (8). These data suggest that this combination of strains has the potential to improve rhinoconjunctivitis-specific quality of life for individuals experiencing allergies. Based on these findings, the objective of this study was to determine whether individuals who self-identify with seasonal allergies and consume a daily dose of *L. gasseri* KS-13, *B. bifidum* G9-1, and *B. longum* MM-2 have improved quality of life scores by using the validated (10) miniature version of the RQLQ (MRQLQ) when compared with those consuming the placebo.

METHODS

Participants

The study population was recruited (**Figure 1**) from a community in Florida. Trained study coordinators received consent from healthy male and female adults between the ages of 18 and 60 y. Participants were included if they *1*) self-identified as having seasonal allergies and would typically receive a global score of ≥2 on the MRQLQ during peak allergy season, *2*) were willing and able to provide informed consent in English, *3*) were willing and able to maintain their regular level of physical activity and diet for the 8-wk study, and *4*) were willing to discontinue consumption of fermented foods, probiotics (e.g., yogurts with live, active cultures or supplements) or immune-enhancing supplements (e.g., Echinacea or fish oil). Participants were excluded if they *1*) typically used allergy medications, including nasal sprays, ≥5 d/wk during allergy season, *2*) received allergy shots, *3*) were pregnant at the time of enrollment or were attempting to get pregnant, *4*) were taking any systemic corticosteroids, androgens (such as testosterone), or large doses of anti-inflammatory drugs (i.e., aspirin in doses >600 mg/d) on a regular basis at the time of enrollment, *5*) were being treated for or had any of the following physician-diagnosed diseases or conditions: HIV; immune modulating diseases (autoimmune disease, hepatitis, cancer, etc.); kidney disease; pancreatitis; pulmonary disease; hepatic or biliary disease; or gastrointestinal diseases or conditions, such as diverticulitis, ulcerative colitis, Crohn's disease, Celiac disease, short bowel disease, ileostomy, or colostomy, but not including gastroesophageal reflux disease, *6*) had a central venous catheter, or *7*) if they had received chemotherapy or other immune-suppressing therapy within the previous year.

This study was conducted in accordance with the guidelines laid down in the Declaration of Helsinki of 1975 as revised in 1983, and all study-related procedures were approved and monitored by the University of Florida Institutional Review Board.

Study capsules

The study capsules contained either the probiotic or a placebo in a gelatin casing and were identical in appearance and mass (350 mg). For the probiotic, each capsule contained 1.2 billion CFU of *L. gasseri* KS-13, 0.15 billion CFU of *B. bifidum* G9-1, and 0.15 billion CFU of *B. longum* MM-2 for a total of 1.5 billion CFU/capsule before expiration. For the placebo, each capsule contained 348 mg potato starch. One capsule was to be taken at the end of the morning meal and one at the end of the evening meal. The capsules were manufactured and supplied by Wakunaga of America Co., Ltd.

Study design and questionnaires

For this prospective, double-blind, parallel study, participants were randomly assigned to their study group over a 5-d period by using sealed envelopes administered by study coordinators. A member of the department not involved in the study generated the randomization scheme using a random-number generator available in Excel (Microsoft) and prepared the randomization envelopes. Participants were randomly assigned to receive either the probiotic or placebo capsules for 8 wk during spring allergy season (March 2015 to May 2015; the predicted peak of pollen counts was estimated to be early-to-mid April, or week 6 of the study). All participants and researchers or study coordinators were blinded throughout the entire study. The study was unblinded after statistical analyses were completed.

Participants were instructed to complete daily and weekly questionnaires for the entire 8 wk of the study. The primary outcome, rhinoconjunctivitis-specific quality of life, was measured by using the global score from the previously validated (10) MRQLQ. This tool produces a global score (calculated by averaging 14 items that are valued on a 7-point scale: 0 = not troubled to 6 = extremely troubled) and domain scores (calculated by averaging the 2 or 3 items in each domain category). Differences in domain scores between groups were included as secondary outcomes. Domains address how affected an individual was because of nose and eye symptoms with regard to activities (at home and at work, recreational activities, and/or sleep), practical problems (the need to rub nose and eyes and/or the need for repeated nose blowing), nose symptoms (sneezing, having a stuffy or blocked nose, and/or having a runny nose), eye symptoms (itchy eyes, sore eyes, and/or watery eyes), and other symptoms (tiredness or fatigue, thirst, and/or feeling irritable). Weekly questionnaires, which inquired about the previous week, began on the first day of the study and continued weekly thereafter. Weekly questionnaires included the MRQLQ and the Gastrointestinal Symptom Rating Scale (GSRS) (11), which records gastrointestinal symptoms (diarrhea, constipation, abdominal pain, indigestion, and reflux) on a rating scale (1 = no discomfort at all to 7 = very severe discomfort). Differences in GSRS symptom scores between groups were included as secondary outcomes, because probiotics have been established to

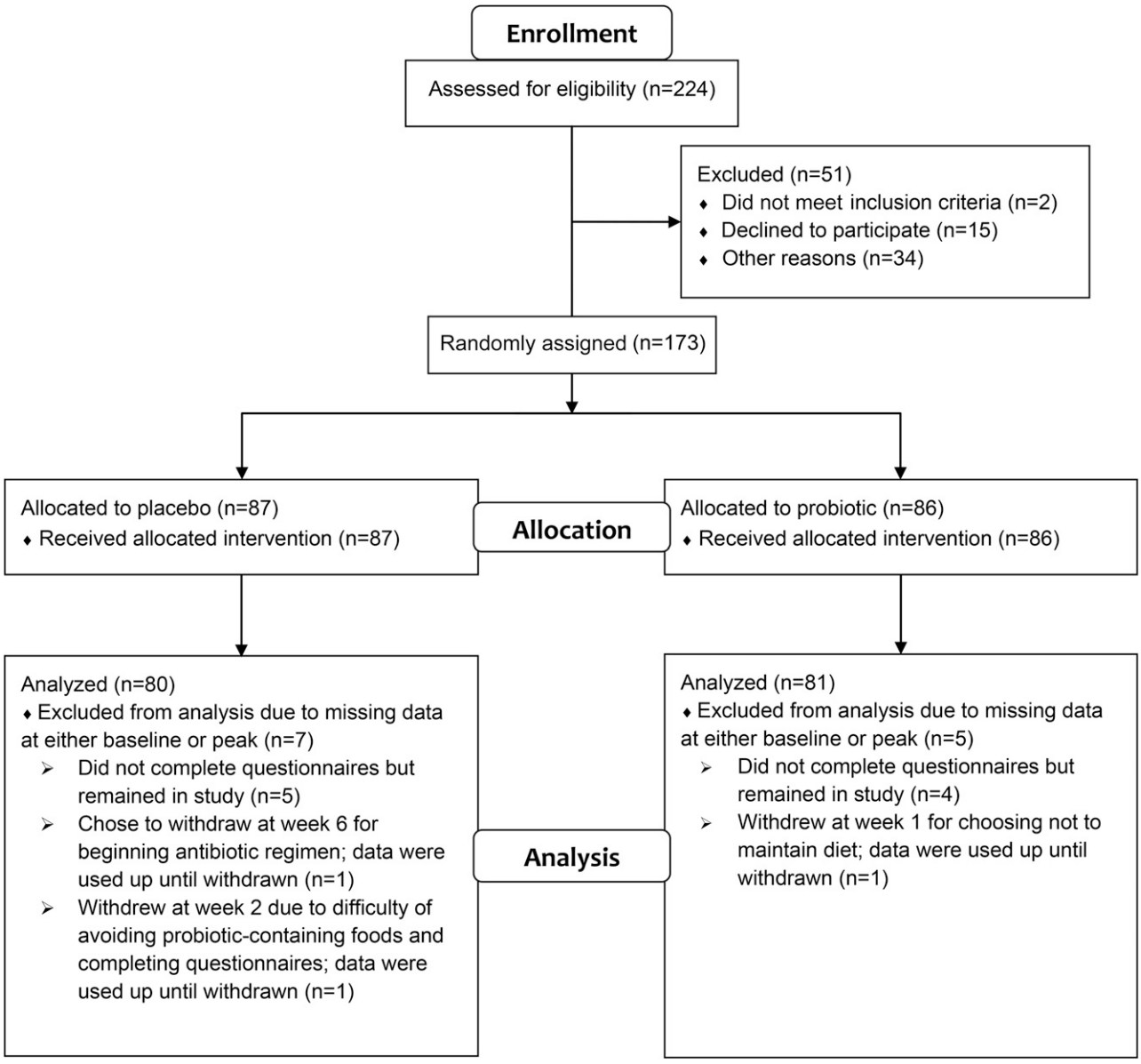

FIGURE 1 Participant flow diagram. The number of participants analyzed refers to those included in the primary outcome analysis. Participants were analyzed on an intent-to-treat basis.

modulate gastrointestinal function. Daily questionnaires began on the first day of the study (day of random assignment and receipt of capsules) and recorded intake of the study capsules, time spent outdoors converted from time to a categorical variable (2 categories: <30 or ≥ 30 min/d), symptoms they were experiencing (other than allergy or gastrointestinal symptoms), visits to a physician, and medication use including allergy medications. Questionnaires were administered online (Qualtrics, LLC); paper copies of questionnaires were available for participants (n = 2) who did not have Internet access. Study coordinators tracked daily questionnaire completion and supplement consumption. If questionnaires had not been completed or supplements not consumed for 3 d in a row, study coordinators contacted participants to inquire why and provide strategies for improving compliance when appropriate. Study coordinators evaluated adverse events daily.

A subgroup of participants (placebo, $n = 37$; probiotic, $n = 35$) who had already consented to participate in the main study consented to an additional portion of the study in which they provided blood and stool samples at baseline (before random assignment) and at week 6 (± 5 d) of the study to assess immune parameters and microbiota composition.

Sample collection and laboratory analyses

Characterization of bacterial DNA in stool samples by 16S rRNA sequencing

Participants collected stool samples in commode specimen collectors (Fisher Scientific) and delivered the samples on ice to the study laboratory within 4 h of defecation. Within 6 h of defecation, samples were homogenized in a sterile plastic bag, aliquotted into bead-beater tubes and stored at -70°C. A standard

protocol was used to extract DNA from samples (QIAamp DNA Mini Kits; Qiagen) with the addition of a bead-beating step (12). Methods for 16S rRNA sequencing have been previously described in detail (13). Briefly, samples were amplified by PCR by using uniquely barcoded primers. Successful amplification was verified by gel electrophoresis. On amplification, samples were pooled and sequenced (MiSeq; Illumina). Sequence reads were processed into operational taxonomic units (OTUs) at similarity levels of 95% and 98% and analyzed for differences in various microbiota diversity measures between baseline and week 6 in probiotic and placebo groups by using an in-house pipeline.

Flow cytometry to quantify regulatory T cells

Fasting whole-blood samples were collected at the study site between the hours of 0700 and 1030 in tubes containing sodium heparin. Samples were kept at room temperature in the dark on a mixer (Clay Adams Nutator) and used for assays within 24 h. Samples were incubated for 45 min at 4°C in the dark with antibodies [human anti-CD3-phycoerythrin (PE), anti-CD4-fluorescein isothiocyanate, and anti-CD25-allophycocyanin or mouse IgG1 κ-PE, IgG2b κ-fluorescein isothiocyanate, IgG1 κ-allophycocyanin, and IgG1 κ-(PE-Cyanine7) for isotype controls; eBioscience]. A set of fluorescence-minus-one controls was created for the purpose of analyzing flow cytometry data. A set of controls was also created by using compensation beads (eBioscience). Red blood cells were lysed (eBioscience), and samples were washed 3 times with flow stain buffer [PBS (0.09% sodium azide) and 2% FBS albumin]. Remaining cells were permeated and incubated for 1 h at 4°C in the dark. Cells were washed with permeabilization buffer (3% FBS; eBioscience) 3 times. Anti-Foxp3-PE (eBioscience) was then added to the appropriate samples and incubated for 1 h at 4°C in the dark. Cells were washed 2 more times with permeabilization buffer and resuspended in flow stain buffer. Counting beads (eBioscience) were added to the samples containing all antibodies to identify regulatory T cells (Tregs). Samples were analyzed on a flow cytometer (BD Accuri C6; BD Biosciences), and gates were set for total lymphocytes, $CD3^+$ cells (T cells), $CD4^+$ T cells, and $CD4^+CD25^+Foxp3^+$ cells (Tregs) by using the accompanying software (BD Accuri C6 Software, version 1.0.264.21; BD Biosciences).

Quantification of serum total IgE and IL-10 concentrations

Fasting whole-blood samples collected in silicone-coated tubes were allowed 30 min to clot over ice and were centrifuged at $1300 \times g$ for 10 min at room temperature. Serum was aliquotted into tubes and frozen at −70°C until used for assays. Concentrations of serum total IgE and IL-10 were quantified via ELISA (eBioscience) by using the recommended protocols. Ninety-six–well plates were analyzed with a spectrophotometer (SpectraMax 340PC384; Molecular Devices) and accompanying software (SoftMax Pro, version 5.4; Molecular Devices). Readings at 570 nm were subtracted from readings at 450 nm.

Statistical methods

Sample size calculation

When controlling for as-needed allergy medication use, a total of 84 participants/group was calculated as needed to see a 0.40 difference in the global MRQLQ score with a SD of 0.82 (1) at peak allergy season between participants who received the probiotic and those who received the placebo with 95% confidence, 80% power, and a 20% attrition rate. A previous study of the effect of a probiotic on immune parameters during allergy season reported a significant difference of 14 pg/mL in serum concentrations of IL-10 between probiotic and placebo groups (14). To obtain this difference, it was calculated that 23 participants/subgroup would be required to see a 14-pg/mL difference between probiotic and placebo groups with 95% confidence and 80% power. It was determined that a total of 30 participants/subgroup would be appropriate for exploratory advanced bioinformatics analyses of fecal samples to determine microbial populations and diversity. To account for attrition, 72 total participants were enrolled in the substudy.

Questionnaire analyses

To verify that the peak of allergy season occurred during the study period, pollen indexes from the local newspaper (15) were recorded beginning 2 wk before the start of the study and ending on the final day of the study (**Supplemental Figure 1**). It was expected that the peak of allergy symptoms in the participants would correspond with the peak of pollen counts as observed in some cases (16). The peak of pollen counts was determined to be the 8 d of highest pollen indexes (around weeks 4 and 5 of the study). Because the peak period occurred over parts of 2 wk and weekly questionnaires for the prior week were completed by participants, each participant's MRQLQ scores for weeks 4 and 5 were combined into a single weighted average with weights equal to the proportion of peak time for which the weekly MRQLQ score was recorded.

Analysis of all MRQLQ outcomes used a general linear model. The change in MRQLQ scores from baseline to peak was analyzed as the primary outcome instead of comparing peak values because of sex differences between groups at baseline. The model for MRQLQ global and domain scores (placebo, $n = 80$; probiotic, $n = 81$) included intervention, several covariates, and all interactions. Covariates in the full model included sex and a 2-category time-spent-outdoors indicator during the peak and the week before peak. Because the categorical time spent outdoors was recorded daily, the modal score was calculated for each week and used in the modeling as a covariate. Nonsignificant covariates were removed hierarchically beginning with interactions with the largest P values.

Analysis of GSRS scores used a general linear mixed model with a random effect of subject to account for the repeated weekly observations. The full model for GSRS outcomes (placebo, $n = 87$; probiotic, $n = 86$) included week and sex and all interactions between these 2 covariates and the intervention. Nonsignificant covariates were removed hierarchically beginning with interactions with the largest P values. Week was retained in all final models of GSRS outcomes, regardless of significant contribution. Residuals for all models were checked for normality and homoscedasticity. Weekly GSRS syndrome scores were log-transformed for analysis to meet the assumptions of normality and homogeneity of variance. Post hoc tests with the use of the Holm-Tukey method for multiple comparisons were conducted on GSRS outcomes that had significant intervention effects.

Allergy medications were not included as a covariate in any model because only 10 participants (placebo, $n = 6$; probiotic, $n = 4$) reported taking allergy medications on any given day during the peak.

Immune variable analyses

A general linear model was used to analyze the change in mean serum total IgE (placebo, $n = 37$; probiotic, $n = 35$) between baseline and week 6. IgE values were log-transformed for analysis to meet the assumptions of normality and homogeneity of variance. Variance was found to be heterogeneous even after transformation (chi-square test, $P = 0.0013$) with larger residual variance for those reporting more time outdoors than those reporting less time (0.071 compared with 0.007, respectively). Hence, a heterogeneous variance model was used. Intervention, categorical time spent outdoors during the peak and the week before peak, and their interactions were included in the full model for IgE. Nonsignificant covariates were removed hierarchically beginning with interactions with the largest P values.

A general linear mixed model was used to analyze percentages of $CD3^+$ cells (T cells), $CD4^+$ T cells, and Tregs out of total lymphocytes (placebo, $n = 35$; probiotic, $n = 35$). Percentages were analyzed with intervention, time point (baseline, week 6), and the interaction between intervention and time point as covariates in the model. A random effect of subject was included to account for the repeated observations on each subject.

Serum IL-10 concentrations were below the limit of detection for many of the samples, so statistical analysis was not performed for this outcome.

Data were analyzed on an intent-to-treat basis. Unless stated otherwise, data are reported by using the model least squares means ± SEMs by using a type I error rate of 0.05. All statistical models were analyzed by using SAS version 9.4.

Microbiota analyses

Two participants were excluded from the microbiota analysis because they did not provide the second stool sample. Seven samples (from 6 different participants) were excluded because there were not enough OTUs in the samples to be analyzed. The Quantitative Insights into Microbial Ecology (QIIME) package was used for microbiota analyses. Data were sorted into 4 categories for analyses: placebo and probiotic groups at baseline and placebo and probiotic groups at week 6 (predicted peak). Rarefaction curves displaying the number of unique OTUs by increasing number of sequences per sample were created for each of the 4 categories. Chao1 rarefaction diversity, a measure of α diversity, was calculated between baseline and week 6 for each intervention group. Principal component analysis was conducted to visualize differences within each intervention group between the 2 time points. UniFrac distance, a measure of β diversity, was calculated between baseline and week 6 for both groups. Shannon diversity indexes were calculated for each sample by using the total number of OTUs per sample and the relative volume of each OTU. Proportions of phyla were analyzed by using t tests between the percentages of each phylum at baseline and week 6. To determine whether specific OTUs changed in prevalence throughout the study, a z score was calculated for the differences in proportions of participants who had an OTU present between 2 time points. A z score of >1.96 or <-1.96 indicated significance ($P < 0.05$). The aim of the microbiota analyses was to generate, rather than test, hypotheses regarding relation between microbial OTUs and the primary endpoint of this study. P values were thus not corrected for the multiple comparisons.

RESULTS

Participant characteristics and compliance

Baseline demographic characteristics were not different between the 2 intervention groups in either the main study (**Table 1**) or the substudy (data not shown). Compliance measures for capsule intake and completion of daily questionnaires were relatively high and did not differ between the 2 groups for both the main study (Table 1) and the substudy (data not shown). Two participants withdrew from the placebo group, and 1 participant withdrew from the probiotic group after random assignment (Figure 1). Two participants, 1 from each intervention group, developed skin rashes within week 1 of supplementation and elected to stop taking the capsules; they both remained in the study and continued questionnaires, reporting no capsule consumption. No adverse events could be attributed to the probiotic supplement.

All participants in the substudy provided initial blood and stool samples. One participant was unable to come to the study site during the week of the second blood draw and did not provide a second blood sample. Two participants (1 from each group) were unable to provide second stool samples.

Blinding efficacy

Blinding was assessed at the week 6 visit. Of the participants who had the placebo, 46% guessed placebo and 54% guessed probiotic; of the participants who had the probiotic, 59% guessed placebo and 41% guessed probiotic. Blinding was determined to be effective based on a contingency table analysis ($P = 0.125$).

TABLE 1
Demographic characteristics and compliance by study group[1]

	Placebo ($n = 87$)	Probiotic ($n = 86$)
Sex, *n* (%)		
M	22 (25)	32 (37)
F	65 (75)	54 (63)
Age, y	27.6 ± 1.4[2]	26.0 ± 1.2
Race, *n* (%)		
Asian	11 (13)	13 (15)
Black/African American	4 (5)	6 (7)
Other	4 (5)	6 (7)
White	68 (78)	61 (71)
BMI, kg/m^2	25.0 ± 0.6	25.6 ± 0.6
Days of correct capsule intake,[3] %	86.9 ± 1.8	87.9 ± 1.8
Daily questionnaires completed, %	95.4 ± 1.2	95.5 ± 1.2

[1] Data were analyzed for incidental differences between groups by using contingency tables or 2-sample t tests as appropriate. None of the outcomes were significantly different.

[2] Mean ± SEM (all such values).

[3] Blank responses on questionnaires were considered "0" for consumption for that day.

Questionnaire data

Rhinoconjunctivitis-specific quality of life

Between-group comparisons revealed that the difference in the global scores between baseline and peak for the probiotic group was greater than that of the placebo group ($P = 0.0092$; **Figure 2**A). Additionally, within this same statistical model, the difference in global score reported in the probiotic group was different from 0 ($P < 0.0001$) whereas the difference reported in the placebo group was not ($P = 0.1624$). This indicates that the observed difference between intervention groups was due to an improvement in rhinoconjunctivitis-specific quality of life only in the probiotic group that was not observed with the placebo. MRQLQ scores were highest at baseline (probiotic, 1.71 ± 0.12; placebo, 1.93 ± 0.13) rather than being highest at the peak of pollen indexes as originally expected.

Differences from baseline to peak between groups were different for activity ($P = 0.0203$), nose symptom ($P = 0.0144$), other symptom ($P = 0.0090$), and practical problem ($P = 0.0409$) domain scores (Figure 2B–E). The changes in eye symptom domain scores were not significantly different between the probiotic and placebo groups ($P = 0.1774$; Figure 2F). Furthermore, the probiotic group reported a decrease in symptom scores whereas the placebo group did not for activity (probiotic, $P = 0.0002$; placebo, $P = 0.6283$), nose symptom (probiotic, $P < 0.0001$; placebo, $P = 0.5594$), and other symptom (probiotic, $P < 0.0001$; placebo, $P = 0.2212$) scores. The practical problem domain scores decreased in both groups ($P < 0.05$), but not to the same degree in the placebo group as in the probiotic group (Figure 2E).

Although there was no difference in baseline MRQLQ score between intervention groups ($P = 0.1834$), there was an effect of sex on the global MRQLQ score. Women, regardless of intervention group, reported a decrease in global score from baseline to peak (-0.71 ± 0.11, $P < 0.0001$), whereas men did not (-0.16 ± 0.17, $P = 0.3354$). There were no statistically significant interactions between sex and intervention. Results for all domain scores were

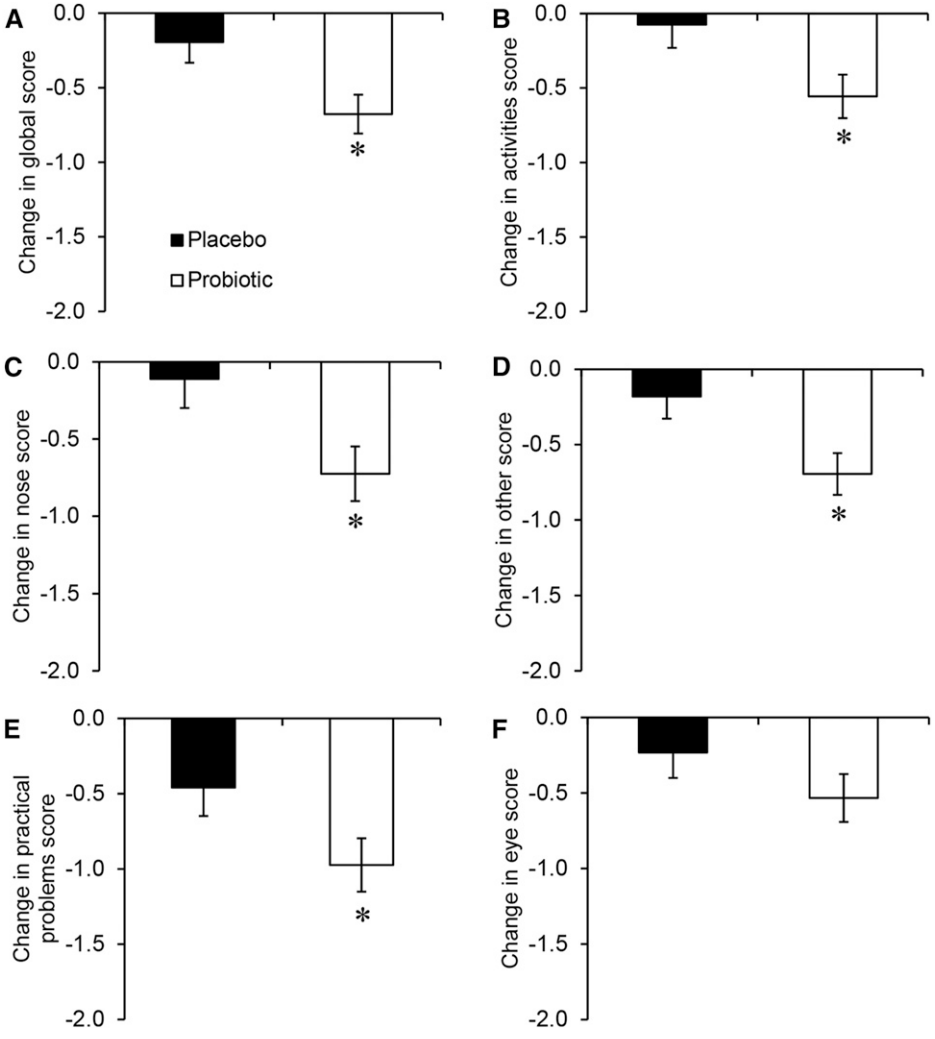

FIGURE 2 Changes in global (A), activity domain (B), nose symptom domain (C), other symptom domain (D), practical problem domain (E), and eye symptom domain (F) Mini Rhinoconjunctivitis Quality of Life Questionnaire scores (0 = not troubled, 6 = extremely troubled) represented as baseline subtracted from peak (the 8 d of highest pollen indexes) for participants consuming the placebo ($n = 80$) or the probiotic ($n = 81$). Values are least square means ± SEMs. A general linear model was used to analyze scores. Intervention, sex, time spent outdoors during the peak and the week before peak, and their interactions with the intervention were included as covariates in the full model. Nonsignificant covariates were removed hierarchically beginning with interactions with the largest P values. The final model included intervention and sex. *$P < 0.05$ compared with placebo.

similar for women across both intervention groups reporting decreases from baseline ($P < 0.0001$) and men reporting no change from baseline ($P > 0.05$) with no interaction between sex and the interventions.

Gastrointestinal function

Based on the weekly GSRS scores, there was a significant interaction between the intervention and study week for constipation ($P = 0.0041$). Overall, constipation symptom scores were low (i.e., <2, which equates to "slight discomfort") but were significantly lower in the probiotic group at weeks 3, 4, 6, and 7 than in the placebo group at those weeks (**Figure 3**). There was no effect of the intervention or interaction between the intervention and the week of the study for the other GSRS symptom scores. Sex was retained only in the model for abdominal pain symptom scores ($P = 0.0293$); however, there was no interaction between sex and intervention.

Immune markers

The difference in mean serum total IgE from baseline to week 6 was not significantly different between the probiotic and placebo groups. However, across intervention groups, mean IgE increased from baseline to week 6 (log-transformed means: 5.79 ± 0.12 ng/mL at baseline compared with 5.85 ± 0.12 ng/mL at week 6, $P = 0.0327$). Tregs as a percentage of total lymphocytes increased from baseline (4.3% ± 0.2%) to week 6 (4.8% ± 0.1%, $P = 0.0089$) but were not different between intervention groups. There were no differences in any other T cell phenotypes between groups or between time points (data not shown).

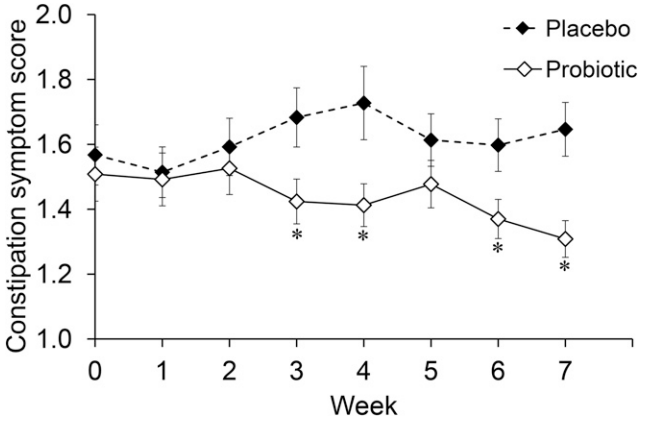

FIGURE 3 Constipation symptom scores from the Gastrointestinal Symptom Rating Scale (1 = no discomfort at all, 7 = very severe discomfort) for participants consuming the placebo ($n = 87$) and probiotic ($n = 86$). The constipation syndrome score includes constipation, hard stools, and the feeling of incomplete evacuation. Values were log-transformed for analysis in a general linear mixed model and presented as untransformed means ± SEMs. Intervention, week, sex, and their interactions with the intervention were included in the full model. Nonsignificant covariates were removed hierarchically beginning with interactions with the largest P values. The final model included intervention, week, and the interaction between intervention and week. The interaction between intervention and week was significant ($P = 0.0041$). *$P < 0.05$ compared with placebo, calculated by using the post hoc Holm-Tukey method for multiple comparisons.

Microbiota profile

The α and β diversity indexes used to measure representative intestinal bacterial phyla did not differ between groups. Proportions of phyla at each time point did not differ within intervention groups, indicating no significant changes during the intervention. Two phyla, Bacteroidetes and Firmicutes, typically dominated in all samples.

An OTU corresponding to *L. gasseri* increased in prevalence from baseline to week 6 in the probiotic group (1 of 33 compared with 22 of 34; z score = −7.083) although it had a low prevalence throughout the study in the placebo group (5 of 34 compared with 3 of 32; z score = 0.663; **Figure 4**). This confirmed compliance of the participants and that the probiotics were present in the intestine of the appropriate group. An OTU corresponding to *Streptococcus sanguinis*, a strain that is part of normal oral flora but known to be associated with endocarditis, increased in prevalence in the placebo group (2 of 34 compared with 9 of 32; z score = −2.488) but not in the probiotic group (7 of 33 compared with 3 of 34; z score = 1.457; Figure 4). An OTU corresponding to *Escherichia coli* decreased in prevalence in the probiotic group (22 of 33 compared with 13 of 34; z score = 2.449) but did not change in the placebo group (19 of 34 compared with 22 of 32; z score = −1.080; Figure 4). This same pattern was seen with an OTU corresponding to *Haemophilus parainfluenzae* (probiotic: 13 of 33 compared with 4 of 34; z score = 2.753; placebo: 6 of 34 compared with 10 of 32; z score = −1.290l; Figure 4). Both *E. coli* and *H. parainfluenzae* belong to the class Gammaproteobacteria, a class that is generally thought to be more pathogenic when present in the intestinal tract. An OTU corresponding to a species of *Faecalibacterium* increased in prevalence in the probiotic group (1 of 33 compared with 6 of 34; z score = −2.039) but not in the placebo group (3 of 34 compared with 5 of 32; z score = −0.840; Figure 4). Interestingly, more changes in prevalence of OTUs occurred in the probiotic than in the placebo group, suggesting that the probiotic mediated changes in intestinal microbial profiles.

DISCUSSION

Self-reported rhinoconjunctivitis-specific quality of life as indicated by the MRQLQ global score improved in healthy individuals consuming a daily probiotic combination compared with a placebo during allergy season. Additional improvements were seen in symptom-related aspects of allergies, indicated by MRQLQ domain scores. Because this study was placebo-controlled and well blinded and the probiotic was confirmed to be present in the stool of the probiotic group, the differences can likely be attributed to 1 or the combination of the 3 probiotic strains used in the intervention. To our knowledge, this is the first randomized, placebo-controlled trial addressing the clinical relevance of this particular combination of 3 probiotic strains on quality of life related to self-identified seasonal allergies.

An important consideration is whether the improvement in quality of life seen in the probiotic group is clinically relevant. MRQLQ scores were relatively low throughout the study and did not actually reach the inclusion criteria cutoff of ≥2. However, these reported values are consistent with a previous study during spring allergy season that enrolled participants with a 2-y

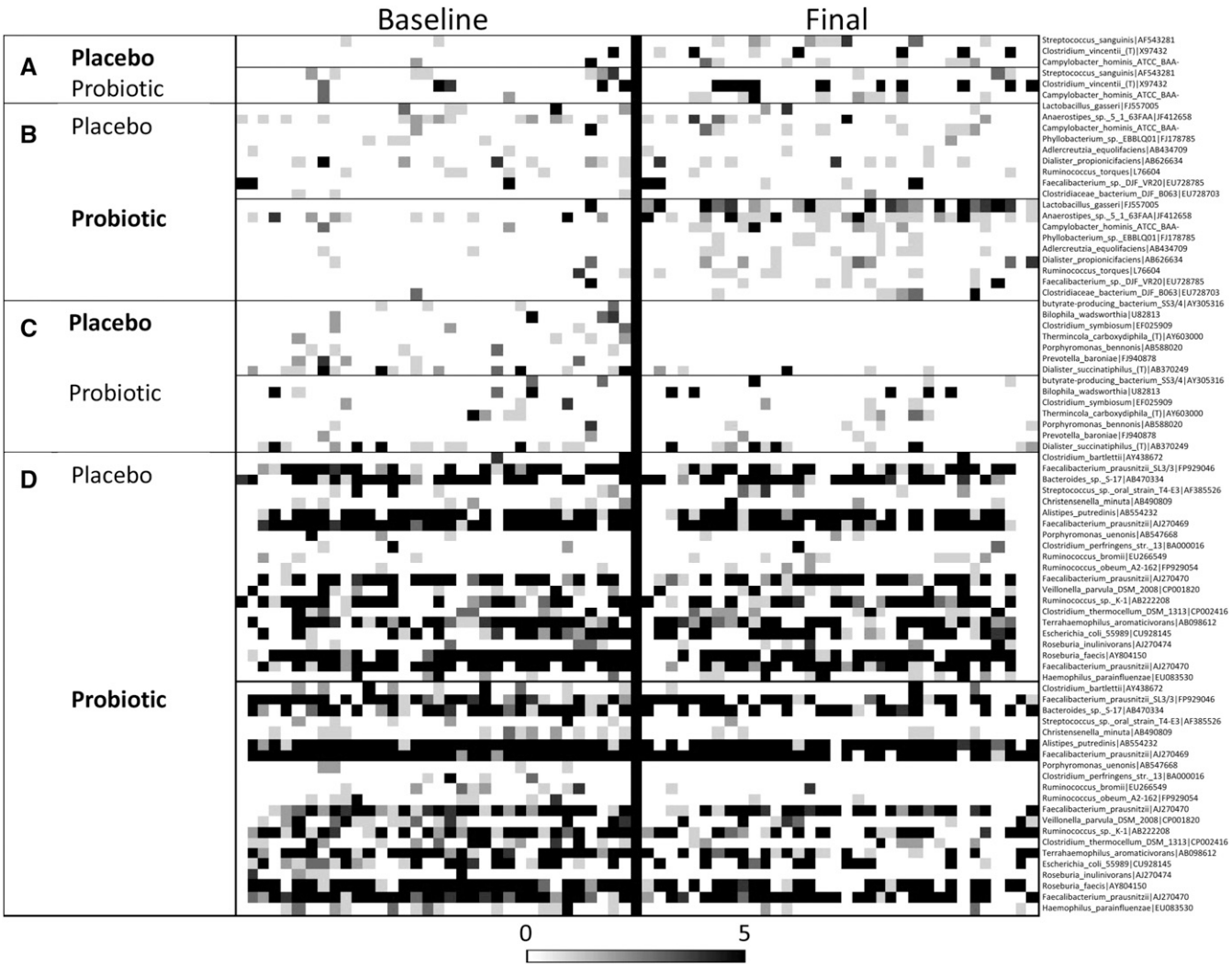

FIGURE 4 Changes in prevalence (determined by z scores) of specific fecal OTUs were detected more often in the probiotic group than in the placebo group. Bold text indicates the group that had changes in OTU prevalence from week 0 to week 6; the other group is included for comparison of the same OTUs. (A) OTUs that increased in the placebo group. (B) OTUs that increased in the probiotic group. (C) OTUs that decreased in the placebo group. (D) OTUs that decreased in the probiotic group. Placebo at week 0, $n = 34$; at week 6, $n = 32$. Probiotic at week 0, $n = 33$; at week 6, $n = 34$. OTU, operational taxonomic unit.

clinical history of allergic rhinitis, a positive grass-pollen skin prick test, and detectable serum grass pollen–specific IgE (16), indicating that MRQLQ values in this range are not atypical of people diagnosed with clinical allergic rhinitis. Additionally, a validation study of the MRQLQ established a minimally important difference of 0.70 (10). According to a global rating scale, this difference corresponded to between "a little better" and "somewhat better" (17). The global score decreased both statistically and by 0.70 from baseline to peak in the probiotic group, whereas in the placebo group it did not even decrease statistically, suggesting that the decrease in the probiotic group was clinically relevant.

Although immune effects of probiotics are thought to be strain-specific, Tregs, IL-10, and IgE were measured because of their implication in previous studies related to probiotics and immune modulation. An in vitro experiment showed that *L. gasseri* SBT2055 interacts with intestinal dendritic cells, resulting in production of TGF-β and IL-10 (18). These 2 cytokines maintain Treg function (19, 20) and are in turn produced by Tregs, creating a suppressive environment that purportedly induces peripheral tolerance (21). In the context of allergies, in vitro studies with human bronchial mucosal cells have indicated a heightened T-helper 2 cytokine response in allergic individuals that contributes to antibody class-switching to IgE and therefore higher IgE concentrations (22), and IL-10 is thought to inhibit T- helper 2 cytokine production (23). IL-10 has also been shown to directly inhibit IgE-mediated activation of mast cells in vitro (24). Although IL-10 is central to this mechanism and was proposed to explain the anticipated difference in allergy symptoms between intervention groups, serum concentrations of IL-10 were too low to accurately measure in participants from this study. A better indicator of IL-10 concentration as related to this mechanism may have been to measure intracellular concentrations of IL-10. Of note, the participants in this study who spent $\geq$30 min outdoors each day had a larger variation in serum total IgE than those participants who spent $<$30 min outdoors each day. This suggests a relation between IgE and exposure to allergens, although this may or may not be directly related to

allergy symptoms. Serum IgE has traditionally been used as a diagnostic tool for allergy, but the true clinical relevance of IgE alone without additional clinical indicators has been called into question, as some individuals produce IgE in response to allergens but are asymptomatic (25). Also, IgE concentrations may be altered in tissues rather than in circulation. Although studies in mice have demonstrated increased Tregs in mesenteric lymph nodes that are thought to migrate to tissues and alter systemic immune balance (26), only circulating Tregs are available for sampling from healthy humans, and percentages of circulating Tregs also may not accurately reflect changes in tissues. IgE and Tregs were not different between intervention groups; however, the observed increases from baseline to week 6 indicate immune stimulation, likely confirming exposure of the participants to allergens.

The most pronounced differences between the intervention groups that may explain differences in MRQLQ scores were seen in the fecal microbiota profiles. Observational studies have reported low prevalence of *F. prausnitzii* in atopic children (27) as well as higher prevalence of *E. coli* in infants that later developed eczema (28). In the current analysis, the OTUs corresponding to *E. coli* that decreased and to a species of *Faecalibacterium* that increased in prevalence in the probiotic group indicate a beneficial shift in the overall profile of intestinal microorganisms. Although we did not measure metabolites in this study, the probiotic strains present in this supplement have previously been shown in vitro to produce anti-inflammatory metabolites such as short-chain fatty acids (29), which are thought to be beneficial (30). Other mechanistic targets known to affect immune balance might include intestinal secretory IgA (18, 31) and/or reduced gut microbial translocation (32). Also, the fact that the constipation syndrome score (syndrome score includes constipation, hard stools, and the feeling of incomplete evacuation) was lower in the probiotic group supports the idea that the probiotic group indeed experienced changes in intestinal microbiota.

There are some limitations of this study that should be considered. This study was originally designed to begin before the start of spring allergy season to allow time for changes in intestinal microbiota and systemic immunity before exposure to allergens, and it was thus hypothesized that MRQLQ scores of the probiotic group would be maintained whereas those from the placebo group would increase to follow pollen counts. However, the highest reported levels of allergy troubles (indicated by MRQLQ score) occurred at the beginning of the study (baseline global MRQLQ: 1.81 ± 0.09). Other studies have shown that clinical symptoms do not necessarily correlate with the peak of pollen (16, 33) as was originally expected in this study. It is unknown whether a larger difference would have been seen between intervention groups if the study began before allergy season. The peak used for analyses was designated based on pollen indexes (15) rather than actual pollen counts of the surrounding area, because that was the only data available. Screening of participants by serum total IgE would have provided a quantitative, nonsubjective criterion for inclusion into the study; however, as stated above, IgE alone can mistakenly identify someone who is allergic but asymptomatic. Also, enrollment in this study occurred in the winter before spring allergy season, and it was unknown if serum IgE taken at that time would accurately identify individuals allergic to springtime pollen because IgE specific to an allergen has a half-life of 2 d (34). Skin prick tests, although commonly used to identify allergic individuals, involve selection of a specific allergen. Because the probiotic was expected to provide benefit by broadly modulating immune mechanisms rather than targeting a specific allergen (as immunotherapy does), selection of participants based on a single or only a few allergens was also not appropriate for this study. For these reasons, screening for allergic participants was done by using a subjective, self-identifying questionnaire. Self-reported outcomes capture the individual's own perspective on his or her health, which drives the decision to continue to use a treatment or not. In this way, a self-identifying screening process was deemed appropriate to assess clinical benefit of this intervention. Finally, people who regularly used allergy medications ($\geq$5 d/wk) or received immunotherapy to treat allergies were excluded from this study, because those medications may mask the more subtle effects of a probiotic. These criteria likely excluded individuals who have more severe seasonal allergies, thus limiting the generalizability of the results.

It is plausible that probiotics, as commensal organisms, may serve a greater role in preventing allergies earlier in life when the immune system is still developing (35). Our study demonstrates a potential benefit for healthy adults with self-identified seasonal allergies when the probiotic is administered starting at the greatest level of allergy symptoms. Prophylactic administration of the probiotic might potentiate the beneficial effects observed in this study. Future research should focus on the molecular mechanism by which probiotics modulate immune function. If elucidated, this information may lead to a more complete understanding of the role of commensal microorganisms in developing and maintaining immune balance.

The authors' responsibilities were as follows——JCD-W, TC, VM, and BL-H: designed the research; JCD-W, CN, CCR, AMB, CTR, AF, and BL-H: conducted the research; JCD-W, MU, SW, and MCC: performed the statistical analysis; JCD-W, VM, MCC, and BL-H: wrote the manuscript; BL-H: had primary responsibility for the final content; and all authors: read and approved the final manuscript. None of the authors reported a conflict of interest related to the study.

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