ORIGINAL RESEARCH

Randomized Clinical Trial Examining the Impact of *Lactobacillus rhamnosus* GG Probiotic Supplementation on Cognitive Functioning in Middle-aged and Older Adults

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Purpose: The gut microbiome has been linked to cognitive function and appears to worsen with aging. Probiotic supplementation has been found to improve the health of the gut microbiome. As such, it is possible that probiotic supplementation may protect the aging brain. The current study examined the cognitive benefits of probiotic supplementation (*Lactobacillus rhamnosus* GG) in healthy middle-aged and older adults.

Materials and Methods: The study was a double-blind, placebo-controlled, randomized clinical trial. Two hundred community-dwelling adults aged 52–75 were enrolled (mean age=64.3, SD=5.52). A three-month intervention involved daily consumption of probiotic or placebo. Independent sample *t*-tests, chi-squared tests, and repeated measure ANOVAs compared groups and examined changes over time. Primary outcome was change in NIH Toolbox Total Cognition Score from baseline to follow-up.

Results: A total of 145 participants were examined in primary analyses (probiotic=77, placebo=68) and excluded persons due to discontinuation, low adherence, missing data, or outlier values. Established criteria (ie ≥1 subtest t-scores ≤35; n=19, n=23) were used to operationally define cognitive impairment. Repeated measures ANOVAs revealed that persons with cognitive impairment who consumed probiotics exhibited a greater total cognition score improvement than persons with cognitive impairment in the placebo group and cognitively intact persons in probiotic or placebo groups.

Conclusion: *Lactobacillus rhamnosus* GG probiotic supplementation was associated with improved cognitive performance in middle-aged and older adults with cognitive impairment. Probiotic supplementation may be a novel method for protecting cognitive health in aging.

Keywords: cognitive aging, dementia, microbiota, gastrointestinal microbiome, probiotics

Introduction
Cognitive Aging

Decline in mental abilities is normal with advancing age and coincides with changes in brain structure and function, including reductions in global and regional brain volume.[1–4] These changes are known to be mitigated by education[5] and physical activity,[6] though cognitive aging cannot be avoided.

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Gut Microbiota as Risk Factor for Cognitive Decline

There is increasing reason to believe that gut microbiota may be an important contributor to cognitive aging.[7,8] The term gut microbiota refers to the 10–100 trillion symbiotic microbial cells living in the human gut and the term gut microbiome refers to the catalog of their nucleic acids (DNA and RNA molecules).[9] Four major phyla of gut bacteria are present in mammals, namely: *Bacteroidetes*, *Proteobacteria*, *Actinobacteria*, and *Firmicutes*, identified through microbial DNA sequencing.[10] The primary microbial phyla in humans are *Firmicutes* and *Bacteroidetes* and comprise up to 90% of our gut microbiome.[11] Dysbiosis refers to a state of the gut microbiota in which the proportions of bacteria are atypical, resulting in a disease-promoting state.[12] Gut dysbiosis can be readily detected in both human and animal models, including the comparison of stool samples of lean with obese individuals or individuals who eat a healthy diet with those who eat a high fat/Western diet.[13,14] More importantly, gut dysbiosis has been associated with a number of medical conditions including inflammatory bowel disease (IBD),[15,16] cardiovascular disease,[17] metabolic disorders,[18,19] and autoimmune disorders,[20,21] each of which has been associated with poorer cognitive function.[22–25] Further, the aging process is associated with progressive decline in gut microbiota diversity and proportions of core microbiota.[26]

Gut-brain-microbiome Axis

The gut, the gut microbiota, and the brain form an interconnected system of processes and communication referred to as the gut-brain-microbiome axis. This complex system involves bidirectional signaling through several pathways, including immune responses,[27] the vagus nerve,[28] enteroendocrine cells,[29] and metabolites that influence the production of neurotransmitters.[30] These pathways provide signaling information among microbiota, the gut, and the brain regarding a wide range of processes, including inflammation and satiety, as well as complex behaviors like social isolation or repetitive movement.[31] The gut microbiota appears to influence the brain by altering axis signaling through bile acids,[7,32] inflammatory markers,[31,33] and metabolites.[31,34] These altered signals lead to changes in key neurochemical processes. Certain bile acids have been associated with brain volume, amyloid beta deposition,[7] and later development of Alzheimer's disease.[35] Short-chain fatty acid (SCFA) metabolites modulate neurotransmission impacting synthesis of noradrenaline and dopamine,[30] and also induce neuronal nerve activation.[34]

Can Probiotics Improve Cognitive Function?

Given the influence of the gut microbiome on the brain, modification of the gut microbiome through probiotic supplementation may protect against cognitive impairment. Probiotics are living microorganisms which, when administered, provide health benefit to the host.[36] Fermented foods can contain beneficial bacteria, which could be considered probiotics. Those include sauerkraut, pickles, yogurt, and miso,[37] though the ease and convenience of taking probiotic supplements containing larger proportions of bacteria appears to be more appealing for some individuals.[10]

A particularly promising strain of probiotic is *Lactobacillus rhamnosus* GG. This bacterium is known for its rapid growth, adhesive properties, and bile resistance[38] allowing it to remain in the gut longer and exert a greater influence than other strains, including protecting the gut lining.[39] Along the gut-brain-microbiome axis, strains of *Lactobacillus* probiotics have been associated with reduced inflammatory cytokines,[40] enhanced levels of cAMP response element binding protein (CREB) and brain derived neurotrophic factor (BDNF) in the hippocampus of rats,[41] and reduced kynurenine metabolites.[42] *Lactobacillus rhamnosus* GG itself has been found to protect intestinal epithelial cells[43] and reduce inflammatory markers such as interleukin-8 (IL-8).[44] It has also been found to improve metabolic factors including glucose tolerance, insulin-sensitivity, adiposity,[45] and inflammation.[46,47] Through these direct and indirect mechanisms, *Lactobacillus rhamnosus* GG may impact brain health and cognitive function. In fact, *Lactobacillus rhamnosus* GG supplementation has been associated with reduced anxiety-like,[48] obsessive compulsive disorder-like,[49] and depressive behaviors[50] in mouse models and has been associated with reduced risk of developing neuropsychiatric disorders in children.[51]

Although previous research has examined the role of *Lactobacillus rhamnosus* GG on physical and psychological outcomes, little is known about its potential impact on cognitive function. One RCT examined the possible cognitive benefits of eight weeks of *Lactobacillus rhamnosus* GG supplementation on cognitive function in young adult

males. No significant changes for inflammatory markers, stress-related anxiety behaviors, or performance on cognitive function were shown in that sample.[52] However, cognitive improvement in a healthy young sample may be unlikely due to range restriction and further investigation on the effects of *Lactobacillus rhamnosus* GG in samples of persons at risk for cognitive impairment is needed.

Current Study

The current study investigated whether *Lactobacillus rhamnosus* GG probiotic supplementation could be associated with improved cognitive function in community-dwelling middle-aged and older adults. Two hundred individuals were recruited into a double-blind RCT. Cognitive function was assessed at baseline and following three months of supplementation of either probiotic or placebo. It was hypothesized that *Lactobacillus rhamnosus* GG supplementation would be associated with improvements in cognitive performance both in persons with and without evidence of cognitive impairment.

Materials and Methods

All data was obtained in compliance with the regulations set forth by the Kent State University Institutional Review Board (approval no. #16-321) and was conducted in accordance with the Declaration of Helsinki. All participants were informed of the purpose of the study. Recruitment and data collection were partly financially supported by i-Health, Inc., a division of Royal DSM. All participants provided written consent acknowledging that any published work would not include identifying information. Participant data has been fully anonymized. All safety precautions (ie university physician oversight, adverse event reporting, eligibility screening for individuals at risk for physical discomfort/symptom exacerbation by probiotic use, participant written agreement to discuss with treating physician before participation) were determined prior to study onset, approved by the Kent State University Institutional Review Board, and adhered to throughout the project. Study methods have been described in detail previously.[53] Briefly, we conducted a parallel, double-blind, placebo controlled, RCT with a 1:1 allocation ratio. See Supplementary Table 1. The study was listed in advance of participant recruitment through clinicaltrials.gov (study no. NCT03080818). Two hundred healthy, middle-aged and older adults (aged 52–75) were recruited from the local community through fliers and advertisements. Sample size was predetermined through power analyses using G*Power 3.0.10 software.

Persons were excluded if they reported history of developmental, neurological, or severe psychiatric disorder, recent consumption of antibiotics, acid-blocking medication, prebiotic, or probiotic supplements, past alcohol or illicit drug dependence, history of severe heart, liver, or kidney problems, immunosuppression, or severe gastrointestinal conditions. Participants were randomized to a study group using a computerized number generator by the principal investigator (JG). The principal investigator was the only staff member aware of participant group assignment and prepared capsules for distribution in advance using unmarked containers. Other research staff, responsible for recruitment and study testing, and participants were blind to group assignment by using unmarked containers and restricted access to randomization documentation. To increase similarity between control and intervention procedures, all participants completed the same study protocol and placebo and probiotic capsules were identical in appearance and packaging.

Lactobacillus rhamnosus GG was selected for two reasons. Although previous research has encouraged the use of multi-strain probiotic supplementation,[54] it is unclear whether *Lactobacillus rhamnosus* GG as a single-strain or multi-strain confers greater benefit for physical and psychological outcomes.[55] Examining *Lactobacillus rhamnosus* GG in isolation also allowed examination of its independent effects on cognitive function, which would be difficult to accomplish in the presence of other probiotic strains. Intervention included Culturelle Vegetarian Capsules containing a 10 billion CFU blend of *Lactobacillus rhamnosus* GG (manufactured by iHealth, Inc., Cromwell, CT, USA) for the experimental group and Culturelle Placebo Veggie capsules containing microcrystalline cellulose for the control group. Participants were instructed to take two capsules daily.

For the study, participants completed telephone eligibility screening, baseline testing, two adherence visits, and follow-up testing. Study visits were conducted at a local retirement community in Northeastern Ohio to promote convenience . Participants were enrolled for approximately 90 days including baseline visit, adherence visits once a month for the following two months and follow-up visit. Baseline and follow-up visits involved brief medical interview, physical measures, computerized neuropsychological assessment using the NIH Toolbox Assessment of Neurological and Behavioral Function—Cognition battery, provision of capsules, and compensation. The NIH Toolbox Total Cognition

Score (ie composite index score based on age, sex, education, race, and mother's education reflecting performance on all cognitive subtests) was used as the primary outcome. NIH Toolbox was selected for its strong psychometric properties,[56,57] close association with traditional pencil-and-paper neuropsychological tests,[58] and ability to assess healthy samples like those found in the current study.

Adherence visits involved brief medical interview, count of nonconsumed capsules, provision of new capsules, and compensation. Study adherence was calculated by dividing self-reported number of capsules consumed by total number of capsules provided and multiplying that value by 100 to obtain a percentage. The primary outcome was the possible change in cognitive function at follow-up across persons randomized to probiotic vs placebo.

Data Analysis
Preliminary Analyses

All participants with missing data, low adherence (ie <80%), and/or incomplete study visits were excluded from analyses. Normality was assumed for variables with skewness <2.0 and kurtosis <6.0. Potential outliers were identified by examining boxplot graphs and clarified using the approach proposed by Iglewicz and Hoalgin[59] for each variable (ie creating $z_{modified}$ transformation scores and removing any values >3.5). Per protocol (PP) and intention-to-treat (ITT) analyses were conducted to determine any significant difference in dropout rates between probiotic and placebo groups.[60,61] To identify possible between-group differences between those who were retained or excluded, independent samples *t*-tests and chi-square d tests compared groups on age, education, sex, BMI, ethnicity, medical history (ie diabetes, hypertension, sleep apnea, anxiety/depression) and baseline NIH Toolbox scores. Finally, Petersen/Winblad criteria (ie one or more NIH Toolbox t scores at or below 35) was used to operationally define cognitive impairment using baseline test results including subtest scores and total composite score.

Hypothesis Testing

A 2 (probiotic vs placebo)×2 (cognitive impairment vs intact)×2 (baseline to follow-up) repeated measures ANOVA was used to identify possible changes in total cognition score over time by the cognitive group. This analysis was then repeated for each individual NIH Toolbox subtest score to clarify any significant omnibus test.

The effect of time, intervention group, and cognitive status were examined independently, as well as multiple interactions (ie time×group, time×cognitive status, time×-group×cognitive status).

To minimize potential impact of practice effects, reliable change indices (RCI; ie standardized z-scores) were calculated for individual subtest scores and total cognition score for each of the four final subgroups (ie probiotic vs placebo and intact vs impaired).[62] Steps for these calculations included: (1) calculation of standard error values for each cognition score using the square root of 1 minus the published reliability of each test,[61] (2) calculation of standard error of the difference values for each cognition score using the square root of two times the squared value of the standard error, and (3) calculation of the absolute value of change in each score divided by the standard error of the difference. RCIs (ie, z-scores reflecting standardized amount of change) calculated for subtest and total cognition scores with an absolute value greater than 1.96, reflecting less than 5% chance that scores were due to standard error,[62] were considered to reflect significant change.

Results
Data Cleaning

Recruitment began in May 2017 and ended in September 2019. From the original sample of 200 participants, data from 52 individuals were excluded from primary analyses due to discontinuation (n=15), missing cognitive data (n=28), missing medical history data (n=2), or low compliance (ie <80%; n=7). Reasons for study discontinuation included reported gastrointestinal symptoms (n=6), extenuating personal circumstances (n=1), starting antibiotics (n=1), abnormal liver enzyme panel (n=1), or lost to follow-up (n=6). See Figure 1. At the entire group level (n=148), no outliers for cognitive scores were identified (all $z_{modified}$ <3.5). When examining intervention groups separately ($n_{probiotic}$=78, $n_{placebo}$=70), one outlier was identified and removed from the probiotic group. When examining those with evidence of cognitive impairment (n=44), two outliers were identified and removed. See Table 1 for demographic and medical characteristics of retained study participants (n=145; $n_{probiotic}$=77, $n_{placebo}$=68).

Of the 55 participants excluded from primary analyses, all had complete data for age, education, ethnicity and gender while only 45 had complete data for BMI, 34 had complete data for cognitive subtests, and 32 had complete data for total cognition score. Incomplete cognitive data was due to
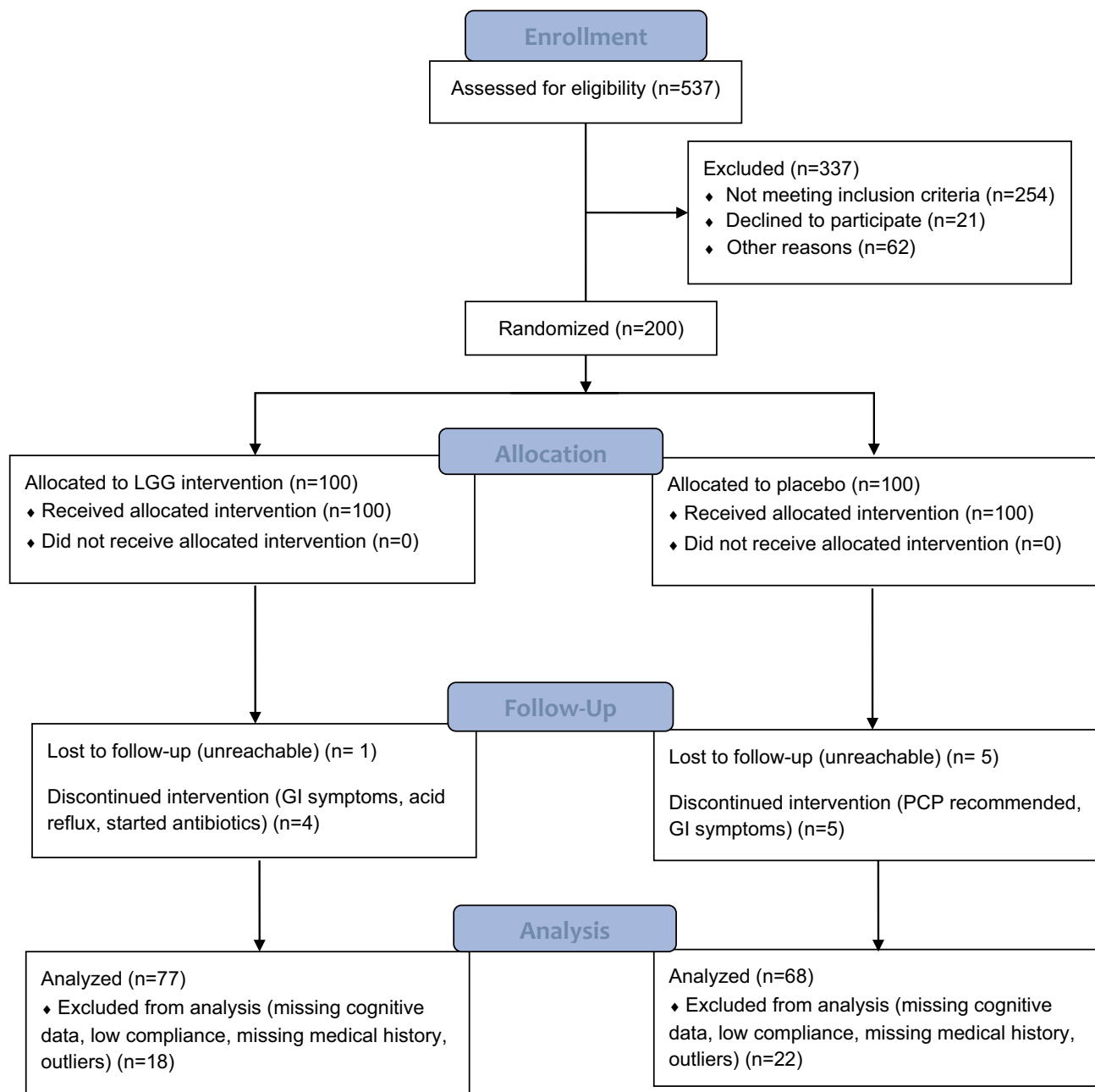
Figure 1 CONSORT (Consolidated Standards of Reporting Trials) flowchart. 2010 CONSORT Flow Diagram, Adapted from Schulz, KF, Altman DG, Moher D, for the CONSORT Group. CONSORT 2010 statement: updated guidelines for reporting parallel group randomized trials. *Obstet Gynecol.* 2010;115(5):1063–70.[63]
Abbreviations: LGG, *Lactobacillus rhamnosus* GG probiotic supplementation; GI, gastrointestinal; PCP, primary care physician.

a malfunction with a tablet used to administer testing. Excluded participants were not significantly different from included participants in demographic, medical, or cognitive function characteristics (all *p*>0.05; See Table 2).

Sample Characteristics
PP analysis showed that no differences in dropout rates emerged between probiotic (6%) and placebo groups (13%). This was confirmed through ITT analysis, which showed comparable dropout rates between probiotic (5%) and placebo groups (10%). These results suggest *Lactobacillus rhamnosus* GG was well-tolerated (*p*>0.05).

Within the probiotic supplementation group, participants that dropped out (M=13.0, SD=0.71) reported fewer years of education than those who completed the study (M=15.1, SD=2.59; t=4.95, df=13.2, *p*<0.01). No other differences emerged in demographic, medical, or cognitive variables (all *p*>0.05). Similarly, no differences

Table 1 Demographic, Medical, and Cognitive Characteristics by Study Group (n=145)

	Entire Sample (n=145)		Probiotic (n=76)		Placebo (n=69)	
	M/% (range)	SD	M/% (range)	SD	M/% (range)	SD
Demographics						
Age	64.4	5.44	64.6	5.58	64.1	5.32
BMI	27.9	6.56	27.5	6.88	28.3	6.21
Education	15.2	2.46	15.1	2.52	15.3	2.40
Gender (female)	59.3%	–	53.9%	–	65.2%	–
Ethnicity						
Caucasian	97.0%	–	100%	–	94.0%	–
Black	2.00%	–	0.00%	–	5.00%	–
Other	1.00%	–	0.00%	–	1.00%	–
Medical history						
Depression/anxiety	22.0%	–	19.7%	–	24.6%	–
Sleep apnea	9.66%	–	5.26%	–	14.5%	–
Diabetes	8.28%	–	6.58%	–	10.1%	–
Hypertension	27.6%	–	19.7%	–	36.2%	–
Cognition						
Picture	48.0 (28.0–84.0)	10.1	47.4 (29.0–78.0)	9.70	48.7 (28.0–84.0)	10.6
Flanker	43.1 (24.0–65.0)	6.94	43.7 (31.0–65.0)	6.60	42.4 (24.0–61.0)	7.28
Card sort	53.2 (29.0–81.0)	9.89	54.3 (35.0–75.0)	8.83	51.9 (29.0–81.0)	10.9
List sort	51.8 (28.0–73.0)	9.19	52.7 (28.0–70.0)	8.28	50.8 (33.0–73.0)	10.1
Pattern	47.9 (10.0–83.0)	13.1	48.6 (20.0–74.0)	11.8	47.2 (10.0–83.0)	14.5
Total	48.0 (27.0–78.0)	9.97	48.8 (27.0–76.0)	9.50	47.1 (27.0–78.0)	10.5

Abbreviations: Picture, picture sequence memory test; Flanker, flanker inhibitory control and attention test; Card sort, dimension change card sort test; List sort, list sorting working memory test; Pattern, pattern comparison processing speed test; Total, total cognition score.

emerged within the placebo group between those that dropped or remained (all $p>0.05$).

In the entire sample (n=145), there was a higher proportion of participants in the placebo group (36%) reporting hypertension than participants in the probiotic group (19%; $p=0.02$, Fisher's exact test). No differences emerged between probiotic and placebo groups for other variables of interest (all $p>0.05$; See Table 1). A significantly greater portion of participants with objective evidence of cognitive impairment (n=42) reported history of sleep apnea compared with cognitively intact participants (n=103). No other differences emerged between cognitively impaired and intact participants on medical or demographic characteristics (all $p>0.05$; See Table 3).

ITT analyses largely replicated PP analyses regarding baseline comparisons between intervention groups. For those with complete data, a significantly greater portion of participants in the placebo group had hypertension (40%) and sleep apnea (14%) than those in the probiotic group (23% and 6%). No significant differences in other demographic or medical characteristics emerged (all $p>0.05$).

It was noted that though no participants reported taking medications that met exclusion criteria for the current trial (eg antibiotics, prebiotics), number of self-reported medications and supplements ranged from 0 to 13 in the total sample; for participants with cognitive impairment, 13 participants in the probiotic group and 16 in the probiotic group reported taking medications beyond vitamins/supplements. The most common medications included statins, beta-blockers, metformin, antidepressants, anxiolytics, nonsteroidal anti-inflammatories, and pain medications. Groups did not differ on the prevalence of medications and it was not utilized in primary analyses.

Lactobacillus rhamnosus GG Improves Cognition in Persons with Cognitive Impairment

PP analyses using repeated measures ANOVA found a significant group by cognitive status by time interaction for total cognition score (F[1,141]=4.60, $p=0.03$, $\eta_p^2=0.03$); see Table 4. Though all groups improved from baseline to

Table 2 Comparing Participants Who Were Excluded or Included in the Study

	Excluded (n=55)		Compared with Entire Sample (n=145)					
	M/% (Range)	SD	t	df	p	χ^2	Fisher's
Demographics (n=55)								
Age	64.0	5.77	−0.40	198	0.69	–	–
BMI (n=50)	29.3	6.57	1.39	196	0.17	–	–
Education	15.2	2.40	0.12	198	0.91	–	–
Gender (female)	63.6%	–	–	–	–	–	0.63
Ethnicity							
Caucasian	96.0%	–	–	–	–	0.68	–
Black	4.00%	–	–	–	–	0.68	–
Other	0.00%	–	–	–	–	0.68	–
Medical history (n=45)							
Depression/anxiety	33.0%	–	–	–	–	–	0.12
Sleep apnea	8.89%	–	–	–	–	–	1.00
Diabetes	17.8%	–	–	–	–	–	0.10
Hypertension	42.2%	–	–	–	–	–	0.10
Cognition (n=34)							
Picture	47.6 (27.0–83.0)	12.4	−0.19	177	0.85	–	–
Flanker	42.1 (29.0–55.0)	6.72	−0.75	177	0.46	–	–
Card sort	53.4 (34.0–77.0)	10.5	0.25	177	0.80	–	–
List sort	51.1 (30.0–76.0)	9.56	−0.42	177	0.68	–	–
Pattern	45.4 (17.0–77.0)	16.2	−0.99	177	0.32	–	–
Total (n=32)	46.6 (23.0–76.0)	10.9	−0.72	171	0.47	–	–

Abbreviations: Picture, picture sequence memory test; Flanker, flanker inhibitory control and attention test; Card sort, dimension change card sort test; List sort, list sorting working memory test; Pattern, pattern comparison processing speed test; Total, total cognition score.

follow-up, participants with cognitive impairment in the probiotic group ($M_{baseline}$=38.7, $M_{follow-up}$=47.6) showed significantly greater improvement in total cognition score than participants with cognitive impairment in the placebo group ($M_{baseline}$=37.7, $M_{follow-up}$=42.4) and participants without cognitive impairment in the probiotic ($M_{baseline}$=52.1, $M_{follow-up}$=54.5) and placebo ($M_{baseline}$=51.8, $M_{follow-up}$=54.6) groups. When comparing change in total cognition score from baseline to follow-up to RCI estimates, it was discovered that change in total cognition score was reliable for participants with cognitive impairment in the probiotic group (RCI=2.07) but not for any other group (cognitive impairment-placebo, RCI=1.34; intact-probiotic RCI=0.54; intact-placebo, RCI=0.63). See Tables 5 and 6.

To clarify this improvement on the total cognition score, repeated measures ANOVA were performed for specific subtests from the NIH Toolbox. No group by cognitive status by time interaction emerged (p>0.05).

ITT analyses using the carry-forward method (ie, inserting baseline values for missing outcome values[63]) largely corroborated these findings. Repeated measures ANOVA (n=173)

showed a borderline significant group by time by cognitive status interaction (F[1,169]=3.90, p=0.05, η_p^2=0.02), such that impaired persons in the probiotic group ($M_{baseline}$=39, $M_{follow-up}$=46) showed greater improvement in cognitive performance than impaired persons in the placebo group ($M_{baseline}$=37, $M_{follow-up}$=42), intact persons in the probiotic group ($M_{baseline}$=53, $M_{follow-up}$=54), and intact persons in the placebo group ($M_{baseline}$=51, $M_{follow-up}$=54). No group by cognitive status by time interaction was identified (>0.05).

Discussion
Summary of Findings

The current study examined the possible cognitive benefits of *Lactobacillus rhamnosus* GG in a sample of healthy middle-aged and older adults. Results showed that *Lactobacillus rhamnosus* GG supplementation was associated with improvement in total cognition score in persons with objective evidence of cognitive impairment, though no such effect emerged in persons with intact cognitive function or those randomized to placebo. Several aspects of these findings warrant brief discussion.

Table 3 Demographic, Medical, and Cognitive Characteristics by Cognitive Status (n=145)

	Entire Sample (n=145)		Intact (n=103)		Impaired (n=42)	
	M/% (range)	SD	M/% (range)	SD	M/% (range)	SD
Demographics						
Age	64.4	5.44	64.5	5.48	64.0	5.41
BMI	27.9	6.56	28.1	6.85	27.4	5.83
Education	15.2	2.46	15.1	2.56	15.3	2.21
Gender (female)	59.3%	–	60.2%	–	57.1%	–
Ethnicity						
Caucasian	97.0%	–	98.0%	–	95.2%	–
Black	2.00%	–	1.00%	–	4.80%	–
Other	1.00%	–	1.00%	–	0.00%	–
Medical history						
Depression/anxiety	22.0%	–	18.6%	–	28.6%	–
Sleep apnea	9.66%	–	5.88%	–	19.0%	–
Diabetes	8.28%	–	8.82%	–	7.10%	–
Hypertension	27.6%	–	25.4%	–	33.3%	–
Cognition						
Picture	48.0 (28.0–84.0)	10.1	50.5 (36.0–84.0)	9.62	42.0 (28.0–67.0)	10.0
Flanker	43.1 (24.0–65.0)	6.94	45.1 (36.0–65.0)	6.49	38.2 (24.0–49.0)	5.54
Card sort	53.2 (29.0–81.0)	9.89	56.0 (38.0–81.0)	8.80	46.1 (29.0–67.0)	9.15
List sort	51.8 (28.0–73.0)	9.19	53.4 (37.0–73.0)	8.98	48.0 (28.0–69.0)	8.53
Pattern	47.9 (10.0–83.0)	13.1	52.4 (36.0–83.0)	10.4	38.2 (10.0–71.0)	13.0
Total	48.0 (27.0–78.0)	9.97	52.0 (37.0–78.0)	8.24	38.1 (27.0–55.0)	7.29

Abbreviations: Picture, picture sequence memory test; Flanker, flanker inhibitory control and attention test; Card sort, dimension change card sort test; List sort, list sorting working memory test; Pattern, pattern comparison processing speed test; Total, total cognition score.

Table 4 RM ANOVAs Examining Effect of Study Group and Cognitive Status on Total Cognition Score and Subtest Scores

Predictor	df$_{NUM}$	df$_{DEN}$	Epsilon	SS	F	p	η2
Total Cog.							
Time	1	141	1	1297.70	77.50	0.00**	0.36
Group	1	141	1	154.75	1.41	0.24	0.01
Time×group	1	141	1	51.81	3.10	0.08	0.21
Time×cognition	1	141	1	268.21	16.03	0.00**	0.10
Time×group×cognition	1	141	1	76.98	4.60	0.03*	0.03
Subtests							
Time	5	137	1	–	19.83	0.00**	0.42
Group	5	137	1	–	0.71	0.62	0.03
Time×group	5	137	1	–	1.07	0.38	0.04
Time×cognition	5	137	1	–	5.07	0.00**	0.16
Time×group×cognition	5	137	1	–	1.11	0.36	0.04

Notes: *$p<0.05$; **$p<0.01$.
Abbreviations: Total Cog., total cognition score RM ANOVA; Subtests, subtest scores RM ANOVA.

Test Improvement in Persons with Cognitive Dysfunction

The exact reason for finding cognitive benefits of *Lactobacillus rhamnosus* GG in persons with cognitive impairment, but not those with normal cognitive function,

is unclear. One possible explanation involves a limited capacity for improvement. At baseline, the total cognition score for the normal cognition subsample fell in the average range (M=48.0, SD=9.97). As noted above, past studies show that probiotic supplementation is associated with improved

Table 5 Demographic, Medical, and Cognitive Characteristics of Intervention by Cognitive Status Groups

| | Intact | | | | Impaired | | | |
| | Probiotic (n=57) | | Placebo (n=46) | | Probiotic (n=19) | | Placebo (n=23) | |
	M/% (range)	SD	M/% (range)	SD	M/% (range)	SD	M/% (range)	SD
Demographics								
Age	64.2	5.68	64.8	5.25	65.6	5.27	62.7	5.27
BMI	28.3	7.46	27.8	6.08	25.2*	4.09	29.2*	6.48
Education	15.2	2.65	15.1	2.48	14.8	2.14	15.7	2.25
Gender (female)	56.1%	–	65.2%	–	47.4%	–	65.2%	–
Ethnicity								
Caucasian	100%	–	96.0%	–	100%	–	91.3%	–
Black	0.00%	–	2.00%	–	0.00%	–	8.70%	–
Other	0.00%	–	2.00%	–	0.00%	–	0.00%	–
Medical history								
Depression/anxiety	15.8%	–	21.7%	–	31.6%	–	26.1%	–
Sleep apnea	3.50%	–	8.7%	–	10.5%	–	26.1%	–
Diabetes	7.00%	–	10.9%	–	5.30%	–	8.70%	–
Hypertension	19.3%	–	32.6%	–	21.1%	–	43.5%	–
Cognition								
Picture	49.6 (36.0–78.0)	8.61	51.5 (36.0–84.0)	9.72	40.7 (29.0–67.0)	9.96	43.0 (28.0–65.0)	10.1
Flanker	45.3 (36.0–65.0)	6.56	44.9 (36.0–61.0)	6.37	39.2 (31.0–47.0)	4.18	37.4 (24.0–49.0)	6.44
Card sort	56.4 (38.0–75.0)	8.03	55.6 (38.0–81.0)	9.54	48.1 (35.0–67.0)	8.38	44.5 (29.0–67.0)	9.62
List sort	54.5 (40.0–70.0)	7.59	52.0 (37.0–73.0)	10.5	47.5 (28.0–58.0)	8.24	48.4 (33.0–69.0)	8.92
Pattern	52.1 (36.0–74.0)	9.01	52.8 (36.0–83.0)	11.7	38.0 (20.0–68.0)	12.9	36.2 (10.0–71.0)	13.3
Total	52.1 (39.0–76.0)	7.37	51.8 (37.0–78.0)	8.66	38.7 (27.0–55.0)	8.10	37.7 (27.0–53.0)	6.70

Notes: *Significant difference (*p*<0.05) between intervention groups within cognitive status subgroup.
Abbreviations: Picture, picture sequence memory test; Flanker, flanker inhibitory control and attention test; Card sort, dimension change card sort test; List sort, list sorting working memory test; Pattern, pattern comparison processing speed test; Total, total cognition score.

Table 6 Baseline and Follow-up Cognitive Test Scores of Intervention by Cognitive Status Groups

| | Intact | | | | | | Impaired | | | | | |
| | Probiotic (n=57) | | | Placebo (n=46) | | | Probiotic (n=19) | | | Placebo (n=23) | | |
	Base M (SD)	Post M (SD)	F	Base M (SD)	Post M (SD)	F	Base M (SD)	Post M (SD)	F	Base M (SD)	Post M (SD)	F
Cognition												
Picture	49.6 (8.61)	52.7 (7.33)	8.9	51.5 (9.72)	54.3 (9.31)	3.3	40.7 (9.96)	52.0 (9.79)	34.9	43.0 (10.1)	47.9 (9.49)	14.9
Flanker	45.3 (6.56)	46.3 (6.67)	2.8	44.9 (6.37)	46.3 (6.84)	4.5	39.2 (4.18)	41.7 (6.18)	3.0	37.4 (6.44)	39.7 (7.57)	4.4
Card sort	56.4 (8.03)	56.6 (9.87)	0.0	55.6 (9.54)	55.9 (9.86)	0.1	48.1 (8.38)	54.4 (9.15)	10.5	44.5 (9.62)	48.8 (8.18)	7.7
List sort	54.5 (7.59)	54.4 (7.91)	0.0	52.0 (10.5)	52.5 (9.32)	0.0	47.5 (8.24)	49.1 (8.00)	1.1	48.4 (8.92)	48.9 (7.54)	0.1
Pattern	52.1 (9.01)	55.0 (10.1)	4.8	52.8 (11.7)	56.7 (11.0)	9.7	38.0 (12.9)	45.0 (14.4)	11.8	36.2 (13.3)	40.8 (15.1)	4.5
Total	52.1 (7.37)	54.5 (7.72)	8.4	51.8 (8.66)	54.6 (8.22)	11.5	38.7 (8.10)	47.6 (9.21)	46.9	37.7 (6.70)	42.4 (7.92)	17.0

Notes: Base, baseline; Post, follow-up; Picture, picture sequence memory test; Flanker, flanker inhibitory control and attention test; Card sort, dimension change card sort test; List sort, list sorting working memory test; Pattern, pattern comparison processing speed test; Total, total cognition score.

cognitive function in persons with baseline cognitive impairment[64,65] but, thus far, it has been found to have limited impact in persons with intact abilities.[66] Such findings suggest that probiotics may help to alleviate deficits in cognitive function (ie return to premorbid range) but may not be sufficient to improve test performance beyond that expected by an individual's preexisting genetic and biological capacity. Further examination of this possibility—especially clarification of benefits across the adult lifespan—is much needed.

Mechanisms for Cognitive Improvement from *Lactobacillus rhamnosus* GG

There are also several mechanistic pathways through which *Lactobacillus rhamnosus* GG probiotic supplementation may improve cognitive function in at-risk individuals. Along the gut-brain-microbiome axis, the gut microbiota and the brain engage in bidirectional signaling through bile acids,[7,32] metabolites,[34] and immune responses.[31] These means of signaling have been associated with a wide range of neurodegenerative disorders,[7,35] neuronal protein expression,[33] neurotransmission,[29] synaptic pruning,[67,68] and behavioral change.[69,70] As described previously, *Lactobacillus* strains in particular have been found to reduce inflammatory cytokines[40] and metabolites in humans,[42] and enhance levels of CREB and BDNF in rats.[41] As probiotics can modulate the composition and functionality of the gut microbiota, *Lactobacillus rhamnosus* GG could have improved these signaling markers as well—ultimately leading to better cognitive function.

It is also possible that *Lactobacillus rhamnosus* GG may improve cognitive function through indirect pathways. *Lactobacillus rhamnosus* GG specifically has been shown to improve glucose tolerance and insulin sensitivity[45] and reduce inflammatory responses.[46,71] As metabolic dysfunction[24,72,73] and inflammatory diseases[74–76] are associated with poor cognitive functioning, *Lactobacillus rhamnosus* GG may have indirectly improved cognitive health by reducing pathological responses associated with these conditions. Additional work is much needed to begin to clarify possible neuroprotective effects of *Lactobacillus rhamnosus* GG and other probiotics in aging adults.

Limitations

The current study is limited in several ways. Though concerted efforts were made, the exact number of days of supplementation differed slightly due to participant scheduling conflicts (M_{days}=92, SD=5.17). As previous research has shown that the gut microbiome can change quickly,[77] this variability may have subtle impact on study findings. Similarly, no gold standard for duration of probiotic supplementation has been established. Though the current study followed participants for a sufficient time to identify cognitive changes,[78] it is possible that supplementation could have differential cognitive effects at other timepoints, as past work has examined effects of supplementation from as little as three weeks to as long as six months.[64,79,80] As suggested above, much longer trials (eg 12–24 months) are also needed to clarify the possible protective effects of probiotic supplementation on cognitive decline in at-risk individuals. Relatedly, the absence of a no-treatment follow-up period in enrolled study participants limits the opportunity to clarify specific, acute benefits of *Lactobacillus rhamnosus* GG.

Another limitation is found in the use of self-report to identify psychiatric and neurodegenerative disorders. It is possible that participants denied such disorders to ensure eligibility for participation or may have been unaware that they met criteria for such diagnoses.

The observed practice effects also introduce a possible concern for the current study. Though previous research suggests that NIH Toolbox has limited practice effects,[56] probiotic and placebo groups both showed improvement on testing. Though the current study methodology (including use of RCI) helps to mitigate possible confound, the possibility of test-related issues or familiarity with testing itself,[81] cannot be fully ruled out. Similarly, minor differences were found between analyses that included all persons that fully adhered to study protocol vs those that did not or dropped out. This finding requires clarification in future studies, as explanations may include dose–response relationships for the cognitive benefits of probiotic supplementation or artifact due to the difficulties in adhering to study protocol in persons with cognitive dysfunction.[82]

A final limitation of the current study is the lack of data regarding changes in the gut microbiome during the study period. Past research concerning the effects of probiotics on cognitive and/or health outcomes have directly examined the microbiome using DNA sequencing.[10,83,84] As increases/decreases of certain microbial groups have been associated with changes in a variety of health and psychological outcomes (eg increased *Bacteroidetes* associated with increased inflammatory responses, increased *Lactobacillus* associated with reduced glucoregulatory markers);[69,85,86] DNA sequencing may help clarify the underlying mechanisms responsible for the observed cognitive gains. Stool samples have been collected in a subsample of participants, though are not available for analysis at the present time.

Conclusion

Persons with cognitive impairment who received *Lactobacillus rhamnosus* GG probiotic supplementation showed improvement on neuropsychological testing over a three-month period. These benefits emerged despite no difference in dropout rates between study arms, suggesting that

Lactobacillus rhamnosus GG was well-tolerated and had no deleterious impact on health, consistent with past work.[87–89] Should these findings be replicated in larger and clinical samples including persons with diagnosed neurodegenerative and neuropsychiatric conditions, probiotic supplementation may ultimately prove to be a low-risk and cost-effective approach to promote cognitive health in older adults.

Data Sharing Statement

Acknowledgment

Disclosure

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