A randomized clinical trial examining the impact of LGG probiotic supplementation on psychological status in middle-aged and older adults

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A B S T R A C T

Cognitive decline is common in older adults and more than 5 million Americans suffer from Alzheimer's disease (AD). A number of physiological processes including systemic inflammation, excess adiposity, and impaired glucoregulation arise from a combination of genetic and behavioral factors and increase risk for developing AD or other forms of dementia. Recent research suggests that the gut microbiome may moderate these pathological processes and possibly influence cognitive outcomes. This paper reviews the methodology for a double-blind, randomized clinical trial examining the influence of Lactobacillus GG (LGG) probiotic supplementation on mood and cognitive functioning in middle-aged and older adults. Our two primary hypotheses include: 1) Participants randomized to the probiotic group will show greater improvements in psychological status compared to participants in the placebo group; 2) Participants randomized to the probiotic group will show greater improvements in executive functioning and processing speed, as evidenced through performance on neuropsychological testing, than participants in the placebo group. We anticipate these results will inform future efforts on the feasibility of LGG probiotic supplementation as an intervention for psychological status and cognitive functioning and further elucidate the link between the gut microbiome and cognitive health.

1. Introduction

It is proposed that 5.4 million Americans are affected by Alzheimer's disease (AD) and more than half of adults 71 or older may suffer some form of dementia [1,2]. Numerous physiological processes are known to increase risk of dementia, including systemic inflammation, impaired glucoregulation, excess adiposity, and a range of cardiovascular pathology [3–6]. In turn, these factors are the result of an array of genetic and behavioral contributors, including physical inactivity, poor diet, smoking tobacco, and high rates of alcohol consumption [7–10].

Recent research suggests that the gut microbiota may be another important contributor to accelerated cognitive decline. Animal studies have shown that the composition and manipulation of the gut microbiome are linked to mood [11], cognition [12], and behavior [13]. Recent work in humans has found that composition of the gut microbiome is associated with cognitive function in healthy middle-aged and older adults [14] and that manipulation through probiotic supplementation improves depressive and anxiety symptoms in healthy adults [15] and cognitive function in patients with HIV [16].

Taken in combination, these findings raise the possibility that

modification of the gut microbiota may also lead to improved neurological outcomes in healthy middle-aged and older adults. Our objective is to determine the potential impact of a 3-month trial of LGG probiotic supplementation on psychological status and cognitive performance in middle-aged and older adults. We hypothesize that: 1) Participants randomly assigned to probiotic supplementation will show greater improvements in psychological status, as measured by subtotal and total scores on standardized and well-validated mood questionnaires, from baseline to follow-up than the placebo control group; 2) probiotic supplementation will also be associated with improvements in executive functioning and processing speed, as measured by normative scores on the objective neuropsychological testing (i.e. NIH Toolbox Cognitive Battery), relative to placebo.

2. Methods

2.1. Participants

We plan to enroll 200 English-speaking, middle-aged and older adults (aged 55–75), recruited from the community using social media

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and local advertisements. Subjects will be excluded if they report history of neurological, developmental, or severe psychiatric disorder (e.g. dementia, stroke, schizophrenia); antibiotic use in the past 30 days; history of significant stomach or GI surgery (e.g. gastric bypass, cholecystectomy); history of alcohol or illicit drug dependence; history of severe heart, kidney, or liver problems (e.g. heart failure, liver failure); regular use of an acid-blocking medication in the past 30 days (i.e. proton pump inhibitor, H2 blocker); likely immunosuppression (e.g. chemotherapy treatment); regular use of other pre- or probiotic supplements in the past 30 days; or severe gastrointestinal conditions including celiac disease, Crohn's disease, or diverticulitis.

2.2. Randomization

Participants will be randomized to either the probiotic or placebo group using random number generation conducted by the principal investigator prior to study initiation.

2.3. Intervention

Intervention will include the provision of a Culturelle Gelatin Capsule containing an LGG blend (10 billion CFUs) including LGGb, LGGm, and LGGe (created by iHealth, Inc., Cromwell, CT) for the experimental group. For comparison, participants in the control group will receive a Culturelle A327 Placebo Veggie Capsule containing 250 mg of purified wood pulp as the filler and 75 mg of cellulose as the cap. All participants will be asked to consume two capsules per day.

2.4. Protocol overview

As part of the study, participants will engage in a telephone screening, baseline testing, two adherence visits, and follow up testing. Telephone screening will include a 10-min interview prior to enrollment to determine their eligibility, scheduling for all four study appointments, and a description of expectations for baseline testing. Baseline and follow up testing visits will be conducted at a nearby senior living community and last approximately 2 h (See Fig. 1). Baseline testing will include informed consent procedures and a health history review. Both baseline and follow up testing will involve blood draw, neuropsychological testing, completion of mood and health questionnaires, physical measures (i.e. heart rate, blood pressure, height, and weight), explanation and provision of saliva and stool sample kits, and provision of capsules. Saliva and stool sample kits will be required to be returned within 7 days of the testing visit. Adherence visits will last approximately 5 min and involve review of medical changes that may have occurred since the previous appointment and adherence information (see "Adherence" section). At each visit, participants will be provided with enough placebo or probiotic capsules (i.e. approximately 60) to last until their following appointment.

2.5. Neuropsychological assessment

The NIH Toolbox for the Assessment of Neurological and Behavioral Function – Cognition was developed to assess cognitive performance across the lifespan [17]. It was chosen due to its excellent reliability and validity and normative database [17]. It has minimal practice effects and reduces possible examiner bias through computerized assessment [17]. Memory tasks include the Picture Sequence Memory Test, which asks individuals to learn and remember a series of pictured objects and their actions on the screen, and the Auditory Verbal Learning Test, which assesses immediate recall by asking participants to learn and recall 15 words over three trials. Executive function/attention tasks include The Dimension Change Card Sort Test, which is similar to the Wisconsin Card Sort Task and assesses hypothesis testing and ability to change mental set, the Flanker Inhibitory Control and Attention Test, which is similar to the Erikson flanker task and requires participants

make rapid decisions about the direction of central stimuli (i.e. congruent or incongruent), and the List Sorting Working Memory Test, which asks individuals to attend to a string of both visual and auditory information and recite them back in pre-determined order. Processing speed will be measured by the Pattern Comparison Processing Speed Test which is a complex reaction time task and asks participants to quickly identify if the two visual patterns are the same or different. All tests will be delivered in the same sequence to participants in both groups and delivered through iPads under the supervision of research assistants. Assessment will last approximately 30 min.

2.6. Psychological status

All participants will be provided questionnaires assessing mood and psychological status at baseline and follow up. Participants will complete questionnaires during their downtime in testing visits and/or at home within 7 days of the testing visit. Mood questionnaires will include the State Trait Anxiety Inventory (STAI), Positive and Negative Affective Scale (PANAS), Warwick-Edinburgh Mental Well-being scale (WEMWBS), Connor-Davidson Resilience Scale-10 (CDRS-10), Rosenburg Self-Esteem Scale (RSES), Perceived Stress Scale (PSS), Center for Epidemiological Studies-Depression (CES-D), Difficulties in Emotion Regulation Scale (DERS), and Profile of Mood States Questionnaire (POMS).

2.7. Health status

During baseline testing, all participants will complete a brief health history interview with a member of the research team. The interview will include a check for eligibility criteria and a review of current and past health status.

Participants will also complete several questionnaires assessing health-related behaviors and outcomes. Questionnaires will include Gastrointestinal Symptom Rating Scale (GSRS), 36-item Short-Form Health Survey (SF-HS 36), International Physical Activity Questionnaire (IPAQ), a food-frequency questionnaire (FFQ) and the Pittsburgh Sleep Quality Index (PSQI).

2.8. Physical assessment

During baseline and follow up testing, all participants will be weighed using a Med-Weigh scale (capacity 600lb × 0.2lb) and height will be recorded using a TallTape metric secured to a wall. Participants will be asked to remove shoes and coats during weight and height measures. Weight will be taken three times for accuracy and final recorded weight will be determined by averaging the three numbers. Blood pressure and heart rate will be measured using a Datascope™ Accutorr Plus machine and conducted by a trained research assistant or the study coordinator. The cuff will be placed on the participants' left arm. Participants will be asked to keep both feet flat on the floor and sit up straight. Blood pressure and heart rate will be taken three times in succession and the dependent variable will be the average of the three readings.

2.9. Gut microbiome composition

DNA isolation from stool. DNA from stool will be isolated on the King Fisher Flex automated instrument (Thermo Fisher Scientific, Grand Island, NY) using the MagMAX™ DNA protocol. Briefly, stool samples will be transferred to sterile 2 ml tubes containing 200 mg of ≤106 µm glass beads (Sigma, St. Louis, MO) and 0.5 ml of lysis/binding buffer. Bead beating will be then carried out for 3 min in a Qiagen TissueLyser II at 30 Hz after which samples will be centrifuged at $21000 \times g$ for 3 min. Subsequently, 115 µl of supernatants will be transferred to MME-96 deep well plates followed by addition of magnetic bead mix and isopropanol. Finally, the sample plate will be immediately placed into
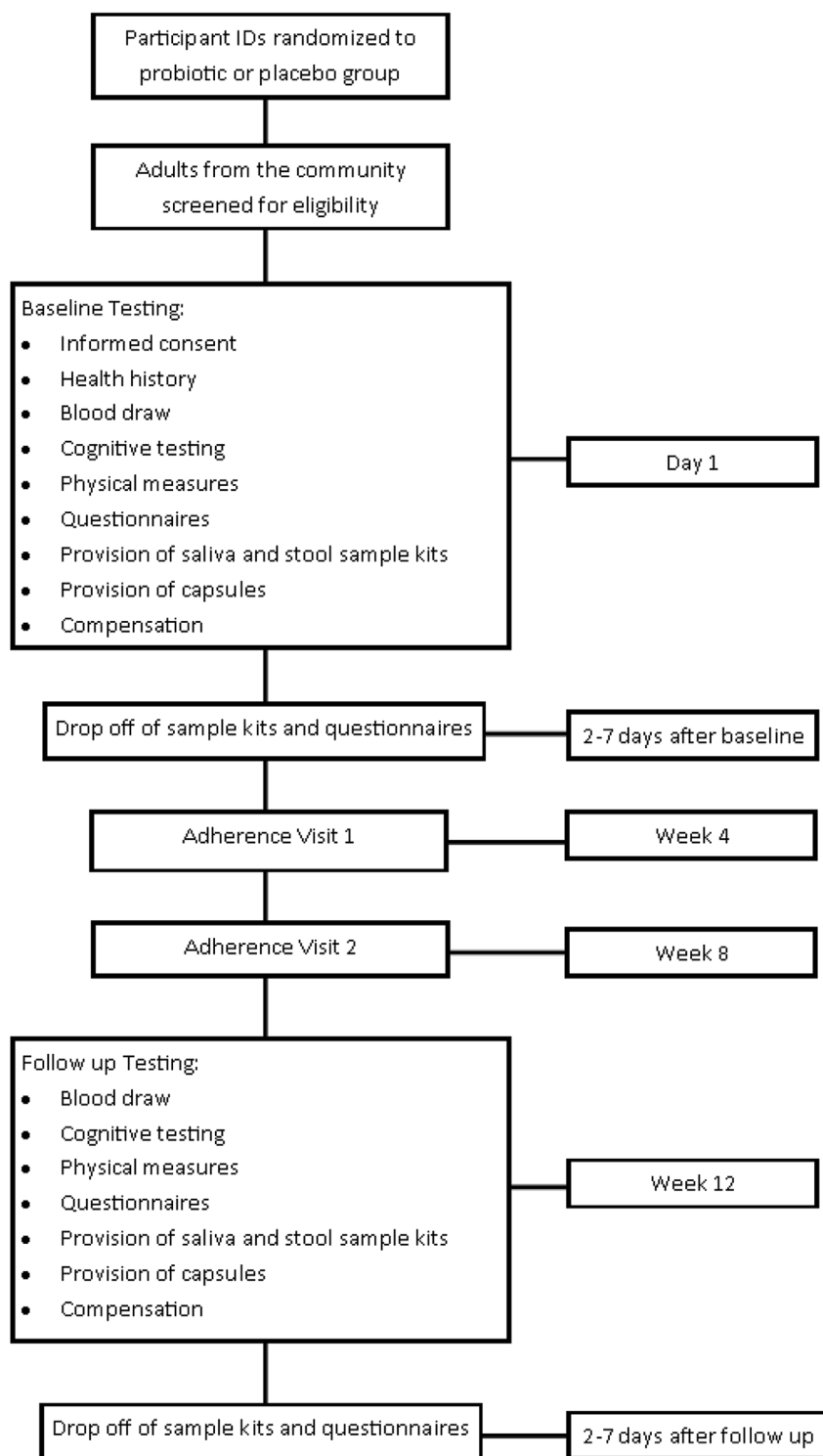
Fig. 1. Schedule of study activities.

the King Fisher Flex instrument along with two isopropanol-based and two ethanol-based washing solutions plates as well as an elution buffer plate, and the MME-96 processor script will be executed. Upon process completion, DNA will be stored in elution buffer at $-20\,°C$ prior to further processing.

DNA isolation from saliva. Saliva samples will be centrifuged at $21,000 \times g$ for 3 min and the supernatant will be collected by pipette and discarded. The pellet will be resuspended in 0.3 ml of Qiagen ATL buffer (Valencia, CA) and supplemented with 20 mg/ml lysozyme

(Thermo Fisher Scientific, Grand Island, NY). The suspension will be incubated at $37\,°C$ for 1 h with occasional agitation and then transferred to a new 2 ml tube containing 200 mg of $\leq 106\,\mu m$ glass beads (Sigma, St. Louis, MO). Bead beating will be carried out for 3 min in a Qiagen TissueLyser II at 30 Hz. Subsequently, 0.3 ml of Qiagen AL buffer containing Proteinase K (600IU/μl) will be added and samples will be incubated at $56\,°C$ for 1 h. DNA will be purified using a standard on-column purification method with Qiagen buffers AW1 and AW2 as washing agents and eluted in 10 mM Tris (pH 8.0).

16S rRNA amplicon sequencing. Total bacterial DNA will be amplified using primers targeting the V4 region of the 16S rRNA gene and overhang adapter sequences appended to the primer pair for compatibility with Illumina index and sequencing adapters[1]. Master mixes will use 2x KAPA HiFi HotStart Ready-mix (KAPA Biosystems, Wilmington, MA). Each 16S rRNA amplicon will be purified using AMPure XP reagent (Beckman Coulter, Indianapolis, IN). In the next step each sample will be amplified using a limited cycle PCR program, adding Illumina sequencing adapters and optional dual-index barcodes (index 1(i7) and index 2(i5)) (Illumina, San Diego, CA) to the amplicon target. The final libraries will be again purified using AMPure XP reagent, quantified and normalized prior to pooling. The DNA library pool will be then denatured with NaOH, diluted with hybridization buffer and heat denatured before loading on the MiSeq reagent cartridge and on the MiSeq instrument (Illumina).

Bioinformatic analysis of 16S rRNA amplicon sequencing data: Automated cluster generation and paired-end sequencing with dual reads will be performed. Data analysis will be carried out using the new version of QIIME (QIIME2). This new version has updated analytic tools, including ANCOM for identifying differentially abundant OTUs, diverse sequence count normalization techniques, and new quality control and OTU assignment tools, including vsearch, DADA2 and swarm. A combination of Unifrac significance, PCoA using Fast Unifrac [18], and network analysis [19,20] will be done to compare groups. Confounding factors (known factors impacting the gut microbiota) will be balanced across experimental groups. The control group will be carefully selected from the demographics of the population available for this study. A detailed dietary questionnaire will be administered to volunteer individuals and information from the questionnaire will be included in the metadata file. The strength and statistical significance of sample groupings using a distance matrix as the primary input will be evaluated by analysis of similarities (ANOSIM) and permutational multivariate analysis of variance (PERMANOVA) methods.

2.10. Laboratory markers

Participants will complete a fasting blood draw during baseline and follow up testing. Participants will be reminded in advance to fast for 12 h prior to testing. A trained nurse will conduct the blood draw. Samples will be placed in a Clay Adams® Brand Compact II Centrifuge immediately as appropriate after collection then placed on ice and delivered to a nearby hospital laboratory within 1.5 h of drawing. They will be analyzed for indicators of glycemic control (i.e. HbA1c, insulin), inflammation (i.e. Hs-CRP, IL6, TNFα), comprehensive metabolic panel (i.e. Albumin, BUN, calcium, carbon dioxide, chloride, creatinine, glucose, potassium, sodium, bilirubin, total protein, ALT, ALP, AST) and lipids (i.e. total cholesterol, HDL, LDL, VLDL). All analyses will be conducted using standard procedures through this CLIA-certified hospital laboratory.

Waking cortisol will be measured through saliva samples collected using Salivette sample kits following methodology used in a previous research study [21]. Participants will be instructed to collect the sample soon after waking and to not smoke, eat, drink, or brush their teeth before sample collection. Upon receiving the samples, researchers will store the saliva in a −80 °C freezer until shipped for analyses.

2.11. Reimbursement

Participants can be compensated up to $250 for their participation in the study. They will receive $100 at the end of both their baseline and follow up testing appointments and $25 at the end of each adherence visit. Compensation will be provided in the form of cash.

2.12. Adherence

Several measures will be taken to enhance participant adherence to capsule ingestion and appointment attendance. During baseline testing, researchers will instruct participants to take two capsules every day. Participants will be informed that they can take the capsules together or separately, with or without food, and at any time of day. Participants will receive a weekly email or text message encouraging adherence to the regimen and bi-weekly phone calls for the same purpose. Participants will also receive phone calls 48 h in advance of their appointments to encourage attendance.

Participant adherence to treatment and appointments will be tracked at each study visit. Researchers will record the number of returned capsules and ask participants about their adherence to the treatment over the past 7 days. Upon study completion, compliance will be calculated by dividing the number of capsules taken by the number of capsules provided. Data from participants whose intervention compliance is below 80% or did not attend study visits within previously determined time limits (i.e. ± 7 days) will be noted. We will conduct intention to treat analyses to examine potential between-group differences in adherence and study completion.

3. Data analyses

3.1. Power analysis

Estimated power for the proposed study is based on past studies which examined the association between gut microbiome composition cognition in healthy older adults [14] and the influence of probiotic supplementation on similar outcomes in patients with liver cirrhosis [22]. Initial studies on the possible benefits of probiotic supplementation show large effects in samples of depressed individuals (Cohen's d = 0.74 [23]) and persons identified as having high stress (d = 0.87 for depression, d = 1.13 for anxiety [24]). Researchers have also found emotional benefits in healthy samples, including reduced reactivity to sad mood after 4 weeks of supplementation (d = 0.28 [25]) and reduced depression after just 3 weeks (d = 0.16 [26]). Considering these findings, we anticipate a small to medium effect size benefit of probiotic supplementation on psychological status in the current study of healthy middle-aged and older adults. Using the power analysis program GPower version 3.0.10, we determined at the upper end of this possible effect (i.e. medium effect size; Cohen's d = 0.50), just 34 participants would be needed to obtain 80% power and 46 for 90% power. Using the most conservative estimate of possible benefits (i.e. small effect size; Cohen's d = 0.20), a total of 200 participants would be needed to obtain 80% power. These estimates were conducted using NIH Toolbox Composite Cognition Score to estimate power for cognitive function and the PSS and CES-D to estimate power for psychological status.

3.2. Analytic strategy

3.2.1. Preliminary analyses

Descriptive Statistics and Distribution: Data analysis will begin by examining the distribution of scores for all measures separately by group (i.e., probiotic vs. placebo) and combined across groups to evaluate the assumptions of normality and homogeneity of variance. Those measures that violate statistical assumptions of will be transformed as appropriate.

Influence of Attrition: Past studies of probiotic supplementation suggest high rates of adherence to protocol, though the age and health status of the proposed sample raise the possibility of greater than expected attrition or non-compliance. We will examine whether drop-out and missing data is missing not at random (MNAR) at follow-up and employ statistical correction as appropriate. More specifically, we will follow the guidelines proposed for clinical trials in previous research [27] and use a combination of selection and pattern mixture models. Sensitivity analyses will then be used to guide interpretation of robustness of treatment benefit.

3.2.2. Hypothesis testing

We hypothesize that probiotic supplementation will be associated with improvements in psychological status and cognitive function in middle-aged and older adults. A separate analysis will be conducted for each hypothesis. Mixed effect Model Repeat Measurement (MMRM) will be used to compare the probiotic and placebo groups across the follow-ups while controlling for baseline, as MMRM is an approach used for longitudinal designs which can also handle ignorable missing data better than other longitudinal approaches such as MANOVA or multiple imputation [28]. Dependent variables will include measures of affect, emotional regulation, and stress (i.e., PANAS, DERS, and PSS).

This analytic approach will then be repeated for cognitive function. MMRM will examine group differences in executive function and psychomotor speed (i.e. Dimension Change Card Sort Test, List Sorting Working Memory Task, Flanker Inhibitory Control and Attention Test, Pattern Comparison Processing Speed Task) across the follow-up assessments.

3.2.3. Exploratory analyses

Finally, to clarify possible mechanisms by which probiotic supplementation may lead to improved mood or cognitive function, this study will examine a series of variables as possible mediators. Specifically, mediation analyses will be conducted for both a direct and indirect effect of changes in the composition of the gut and oral microbiome as well as circulating markers of glycemic control and inflammation. Past work has shown these biomarkers are associated with cognitive function and may be important contributors. Mediation analyses will also be conducted for both a direct and indirect effect of changes in the composition of the gut and oral microbiome with number of days between baseline and follow-up visits and biospecimen drop-off visits. As microbiome composition can change quickly, it will be important to determine whether number of days between visits and biospecimen collection influences gut and oral microbiome composition analyses.

4. Discussion

This paper reviews the methodology for a randomized, placebo-controlled study examining the effects of LGG probiotic supplementation on mood and cognitive function in healthy middle-aged and older adults. Past work in both animal models and patient samples raises the possibility of the brain-gut-microbiome axis as an important contributor to both neurological and psychiatric outcomes [11,12,14]. Investigation of this possibility in a healthy sample will provide new insight into the potential benefits of LGG supplementation in this population. If hypotheses are confirmed and subsequently replicated in other studies, it would suggest LGG supplementation as a possible low-cost and low-risk preventive approach for brain-based conditions.

Similarly, examination of the mechanisms linking composition of the gut microbiome to cognitive function will also advance the literature in this area. LGG is posited to have a variety of characteristics that allow important benefits for the microbiome that transfer to human health. LGG is noted for its strong adhesive abilities leading to longer lasting presence and improved colonization of microbiota [29]. This adhesive ability also seems to promote close interactions between LGG and host cells which allows effector molecules to engage in immunomodulatory activities such as reducing pro-inflammatory markers such as interleukin 8 (IL-8 [29]). LGG may also have protective effects by secreting antimicrobials and proteins that protect intestinal epithelial cell growth [29]. Other work shows that LGG is associated with reduced insulin resistance in diet-induced obese mice [30] and modulation of the gut obesity levels of rats given a high-fat diet [31].

In turn, these processes are consistent with the many possible mechanisms by which composition of the gut microbiome may be linked to neurological outcomes [15,32], particularly reduced inflammation and improved glycemic control. A number of pro-inflammatory markers are known to predict cognitive decline and conditions like Alzheimer's

disease [33] and gut dysbiosis is associated with greater inflammation [34]. Similarly, both peripheral and central markers of insulin resistance are associated with cognitive decline and dementia [35] and probiotic supplementation has been shown to improve glycemic control [36]. The current study will provide preliminary insight into these possible pathways.

4.1. Future directions

Future research may benefit from using more detailed psychiatric interview to better exclude individuals with potentially confounding characteristics and determine possible subgroups of interest. Additional studies may also seek to obtain microbiome biospecimens within a smaller window of time (e.g. 12–24 h) following important study visits to better determine the potential effects of the intervention and better associate microbiome composition with outcomes of interest. Should LGG supplementation be associated with improvements in mood and cognitive function in the current study, it would encourage additional studies to clarify the extent to which this pattern reflects aging or pathological processes [37] and detailed examination of mechanisms using advanced neuroimaging, including both functional magnetic resonance imaging (fMRI), arterial spin labeling (ASL), and positron emission tomography (PET). Similarly, work is needed to better understand the influence of the gut microbiome on gene expression important for neurological outcomes such as Alzheimer's disease and accelerated cognitive decline, especially in light of the rapidly growing application of metabolomics in such conditions [38].

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.conctc.2018.11.006.

References

[1] Centers for Disease Control and Prevention, Promoting Health and Independence for an Aging Population: at a Glance 2017, (2017) Retrieved from: https://www.cdc.gov/chronicdisease/resources/publications/aag/alzheimers.htm.

[2] B.L. Plassman, K.M. Langa, G.G. Fisher, S.G. Heeringa, D.R. Weir, M.B. Ofstedal, … R.B. Wallace, Prevalence of dementia in the United States: the aging, demographics, and memory study, Neuroepidemiology 29 (1–2) (2007) 125–132.

[3] A.L. Marsland, P.J. Gianaros, D.C.-H. Kuan, L.K. Sheu, K. Krajina, S.B. Manuck, Brain morphology links systemic inflammation to cognitive function in midlife adults, Brain Behav. Immun. 48 (2015) 195–204.

[4] R. Ravona-Springer, A. Heymann, J. Schmeidler, E. Moshier, J. Godbold, M. Sano, … M.S. Beeri, Trajectories in glycemic control over time are associated with cognitive performance in elderly subjects with type 2 diabetes, PloS One 9 (6) (2014) e97384.

[5] J. Gunstad, A. Lhotsky, C.R. Wendell, L. Ferrucci, A.B. Zonderman, Longitudinal examination of obesity and cognitive function: results from the baltimore longitudinal study of aging, Neuroepidemiology 34 (4) (2010) 222–229.

[6] M.E. Mortby, R. Burns, A.L. Janke, P.S. Sachdev, K.J. Anstey, N. Cherbuin, Relating education, brain structure, and cognition: the role of cardiovascular disease risk factors, BioMed Res. Int. (2014) 271487 2014.

[7] F.W. Booth, C.K. Roberts, M.J. Laye, Lack of exercise is a major cause of chronic diseases, Comprehensive Physiology 2 (2) (2012) 1143–1211.

[8] C.S. Fox, S.H. Golden, C. Anderson, G.A. Bray, L.E. Burke, I.H. de Boer, … D.K. Vafiadis, Update on prevention of cardiovascular disease in adults with type 2 diabetes mellitus in light of recent evidence: a scientific statement from the American Heart Association and the American Diabetes Association, Diabetes Care 38 (9) (2015) 1777–1803.

[9] J.A. Ambrose, R.S. Barua, The pathophysiology of cigarette smoking and cardiovascular disease, J. Am. Coll. Cardiol. 43 (10) (2004) 1731–1737.

[10] M.R. Piano, Alcohol's effects on the cardiovascular system, Alcohol Res. Curr. Rev. 38 (2) (2017) 219–241.

[11] S.M. O'Mahony, N.P. Hyland, T.G. Dinan, J.F. Cryan, Maternal separation as a model of brain-gut axis dysfunction, Psychopharmacology (Berlin) 214 (2011) 71–88.

[12] M.G. Gareau, E. Wine, D.M. Rodrigues, J.H. Cho, M.T. Whary, D.J. Philpott, ... P.M. Sherman, Bacterial infection causes stress-induced memory dysfunction in mice, Gut 60 (2011) 307–317.

[13] R.D. Heijtz, S. Wang, F. Anuar, Y. Qian, B. Bjorkholm, A. Samuelsson, ... Y. Pettersson, Normal gut microbiota modulates brain development and behavior, Proc. Natl. Acad. Sci. Unit. States Am. 108 (2011) 3047–3052.

[14] L. Manderino, I. Carroll, M.A. Azcarate-Peril, A. Rochette, L. Heinberg, C. Peat, ... J. Gunstad, Preliminary evidence for an association between the composition of the gut microbiome and cognitive function in neurologically healthy older adults, J. Int. Neuropsychol. Soc. 23 (8) (2017) 700–705.

[15] M. Messaoudi, R. Lalonde, N. Violle, H. Javelot, D. Desor, A. Nejdi, ... J.M. Cazaubiel, Assessment of psychotropic-like properties of a probiotic formulation (Lactobacillus helveticus R0052 and Bifidobacterium longum R0175) in rats and human subjects, Br. J. Nutr. 105 (5) (2011) 755–764.

[16] G. Ceccarelli, M. Fratino, C. Selvaggi, N. Giustini, S. Serafino, I. Schietroma, ... G. d' Ettorre, A pilot study on the effects of probiotic supplementation on neuropsychological performance and microRNA-29a-c levels in antiretroviral-treated HIV-1-infected patients, Brain and Behavior 7 (8) (2017) e00756.

[17] R.K. Heaton, N. Akshoomoff, D. Tulsky, D. Mungas, S. Weintraub, S. Dikmen, ... R. Gershon, Reliability and validity of composite scores from the NIH Toolbox cognition Battery in adults, J. Int. Neuropsychol. Soc. 20 (6) (2015) 588–598.

[18] C. Lozupone, M. Hamady, R. Knight, UniFrac–an online tool for comparing microbial community diversity in a phylogenetic context, BMC Bioinf. 7 (2006) 371.

[19] R.E. Ley, M. Hamady, C. Lozupone, P.J. Turnbaugh, R.R. Ramey, J.S. Bircher, ... J.I. Gordon, Evolution of mammals and their gut microbes, Science 320 (2008) 1647–1651.

[20] R.E. Ley, C.A. Lozupone, M. Hamady, R. Knight, J.I. Gordon, Worlds within worlds: evolution of the vertebrate gut microbiota, Nat. Rev. Microbiol. 6 (2008) 776–788.

[21] M.L. Pacella, N. Feeny, L. Zoellner, D.L. Delahanty, The impact of PTSD treatment on the cortisol awakening response, Depress. Anxiety 31 (10) (2014) 862–869.

[22] J.S. Bajaj, J.M. Ridlon, P.B. Hylemon, L.R. Thacker, D.M. Heuman, S. Smith, ... P.M. Gillevet, Linkage of gut microbiome with cognition in hepatic encephalopathy, Am. J. Physiol. Gastrointest. Liver Physiol. 302 (1) (2011) G168–G175.

[23] G. Akkasheh, Z. Kashani-Poor, M. Tajabadi-Ebrahimi, P. Jafari, H. Akbari, M. Taghizadeh, ... A. Esmaillzadeh, Clinical and metabolic response to probiotic administration in patients with major depressive disorder: a randomized, double-blind, placebo-controlled trial, Nutrition 32 (3) (2016) 315–320.

[24] J. Gruenwald, H.J. Graubaum, A. Harde, Effect of a probiotic multivitamin compound on stress and exhaustion, Adv. Ther. 19 (3) (2002) 141–150.

[25] L. Steenbergen, R. Sellaro, S. van Hemert, J.A. Bosch, L.S. Colzato, A randomized controlled trial to test the effect of multispecies probiotics on cognitive reactivity to sad mood, Brain Behav. Immun. 48 (2015) 258–264.

[26] D. Benton, C. Williams, A. Brown, Impact of consuming a milk drink containing a probiotic on mood and cognition, Eur. J. Clin. Nutr. 61 (3) (2007) 355.

[27] J. Dziura, L. Post, Q. Zhao, Z. Fu, P. Peduzzi, Strategies for dealing with missing data in clinical trials: from design to analysis, Yale Journal of Biological Medicine 86 (3) (2013) 343–358.

[28] O. Siddiqui, MMRM versus MI in dealing with missing data—a comparison based on 25 NDA data sets, J. Biopharm. Stat. 21 (3) (2011) 423–436, https://doi.org/10.1080/10543401003777995.

[29] M.E. Segers, S. Lebeer, Towards a better understanding of Lactobacillus rhamnosus GG-host interactions, Microb. Cell Factories 13 (1) (2014) S7.

[30] K.Y. Park, B. Kim, C.K. Hyun, Lactobacillus rhamnosus GG reverses insulin resistance but does not block its onset in diet-induced obese mice, J. Microbiol. Biotechnol. 25 (5) (2015) 753–757 Ji et al., 2018.

[31] Y. Ji, S. Park, H. Park, E. Hwang, H. Shin, B. Pot, W.H. Holzapfel, Modulation of active gut microbiota by Lactobacillus rhamnosus GG in a diet induced obesity murine model, Front. Microbiol. (9) (2018).

[32] E. Sherwin, K.V. Sandhu, T.G. Dinan, J.F. Cryan, May the force be with you: the light and dark sides of the microbiota–gut–brain axis in neuropsychiatry, CNS Drugs 30 (11) (2016) 1019–1041.

[33] Z.S. Tan, A.S. Beiser, R.S. Vasan, R. Roubenoff, C.A. Dinarello, T.B. Harris, ... S. Seshadri, Inflammatory markers and the risk of alzheimer disease the framingham study, Neurology 68 (22) (2007) 1902–1908.

[34] H. Tilg, A. Kaser, Gut microbiome, obesity, and metabolic dysfunction, J. Clin. Invest. 121 (6) (2011) 2126–2132.

[35] S.E. Arnold, Z. Arvanitakis, S.L. Macauley-Rambach, A.M. Koenig, H.Y. Wang, R.S. Ahima, ... D.M. Nathan, Brain insulin resistance in type 2 diabetes and Alzheimer disease: concepts and conundrums, Nat. Rev. Neurol. 14 (3) (2018) 168–181.

[36] Z. Mazloom, A. Yousefinejad, M.H. Dabbaghmanesh, Effect of probiotics on lipid profile, glycemic control, insulin action, oxidative stress, and inflammatory markers in patients with type 2 diabetes: a clinical trial, Iran. J. Med. Sci. 38 (1) (2013) 38–43.

[37] J.D. Hoffman, I. Parikh, S.J. Green, R.P. Chlipala, R.P. Mohney, M. Keaton, ... A.L. Lin, Age drives distortion of brain metabolic, vascular and cognitive functions, and the gut microbiome, Front. Aging Neurosci. 9 (2017) 298.

[38] J.M. Wilkins, E. Trushina, Applications of metabolomics in Alzheimer's disease, Front. Neurol. 8 (2018) 719.