COVER PAGE

OFFICIAL TITLE: Probiotics, Immune Function, and the Brain in Alcohol Consumers

NCT NUMBER: NCT05232682

DOCUMENT DATE: October 25, 2022

PART III. RESEARCH DESIGN & METHODS

THE BLUE TEXT IN THE FOLLOWING SECTIONS IS A GUIDE TO ENSURE ALL RELEVANT INFORMATION IS INCLUDED IN YOUR APPLICATION. YOU MAY DELETE THE BLUE TEXT BEFORE SUBMISSION

1. **Introduction and Background.** *In reviewing the protocol, the IRB must consider the rationale for the study and the importance of the knowledge that may reasonably be expected to result.*

   Animal models and clinical studies show preliminary support for probiotic supplementation to ameliorate negative physiological consequences of heavy drinking, specifically inflammation. However, previous clinical trials have focused on alcohol-related complications, usually alcoholic liver disease. Such studies have been conducted in controlled settings with supervised abstinence from alcohol. As a result, it is not known whether probiotics improve alcohol-related inflammation in the absence of liver disease or in individuals who continue to drink heavily. The latter factor is important, as many individuals who currently are not seeking to cut down on drinking may nevertheless be willing to consume a dietary supplement to improve their health. In summary, probiotic therapy may be an acceptable intervention to reduce alcohol-related tissue injury in individuals not seeking to change their drinking behavior, but no clinical studies to date have addressed this population. This study will investigate effects of probiotics on inflammation in healthy heavy drinkers who currently are not seeking to change their alcohol use (i.e., are non-treatment-seeking).

2. **Specific Aims and Study Objectives.** *The IRB must evaluate the objectives of the research in order to determine whether the risks to participants are reasonable in relation to the importance of the knowledge that may be gained.*

This pilot project is a brief, open-label clinical trial of probiotics as an intervention to reduce systemic and neural inflammation in heavy drinkers. Heavy drinkers who do not yet show significant signs of liver disease also may stand to benefit from probiotics, but no clinical trials to date have addressed this population. This study will recruit 15 non-treatment-seeking heavy drinkers to complete an open-label within-subjects trial. Aim 1 is to demonstrate proof-of-concept for beneficial effects of probiotic use on inflammatory processes. Aim 2 is to examine effects of probiotic use on brain metabolites correlated with neuroinflammation using magnetic resonance spectroscopy. Aim 3 is to gather preliminary data on acceptability and feasibility of the probiotic intervention in non-treatment-seeking heavy drinkers.

Aim 1: Demonstrate proof-of-concept for beneficial effects of probiotic use on inflammatory processes, as reflected in plasma markers of microbial translocation, immune activation, systemic inflammation, and liver function.

Aim 2: Examine effects of probiotic use on brain metabolites correlated with inflammation using magnetic resonance imaging (MRI).

Aim 3: Gather preliminary data on acceptability and feasibility of the probiotic intervention in the specific population of interest, i.e., non-treatment-seeking heavy drinkers.

If your study ONLY involves the use of identifiable secondary data / biospecimens, including coded data from which you may be able to ascertain participant identity, skip to PART V. Otherwise, please continue to next page.

Brown University IRB Original Approval: 10/21/2021
Brown University IRB Amendment Approval: 10/25/2022

3. **Materials, Methods and Analysis.** *The study design, methods and procedures must be adequately described in order for the IRB to understand all activities in which human subjects will participate. The IRB must also be able to differentiate those procedures that are performed for research purposes from those that are performed for routine care or evaluation.*

The study will enroll participants to complete a brief open-label clinical trial of a probiotic dietary supplement. Individuals who provide informed consent and are eligible for participation will attend three study visits. At Visit 1, the research team will collect data on demographics, substance use, and medical history. Eligibility will be determined using the criteria listed below. Blood samples will be taken by a licensed healthcare provider. Eligible participants will complete a one-hour magnetic resonance imaging (MRI) scan, to occur on a separate day due to MRI scheduling constraints. Participants will receive a 30-day supply of the probiotic supplement with dosing and storage instructions. Participants will be instructed to take the standard dosage of 2 probiotic capsules per day. Participants will be asked to respond to a daily query from the research team confirming that they have taken their daily probiotic dose. Visit 2 will be scheduled 15 days (plus or minus 5 days) after the initiation of the probiotic supplement. The research team will collect blood samples and assess alcohol consumption since Visit 1, protocol adherence, and potential side effects. Participants will attend Visit 3 upon completion of the probiotic supplement, to occur 30 days (plus or minus 5 days) after initiation. Data will be collected as described for Visit 2, plus participants will complete a final one-hour MRI scan. Visit 4 will take place 1-10 months after completion of the study probiotic as a "washout" time point. A blood sample and substance use assessment will be collected at Visit 4. Appointments will take place at the Center for Alcohol and Addiction Studies lab space in the School of Public Health, and MRI scans will be conducted at Brown's Magnetic Resonance Imaging Facility (MRF).

Data to be collected at each session is described below.

- Visit 1 (Baseline Screening & MRI): Breath alcohol content (BrAC) will be measured using a handheld digital sensor to confirm BrAC of .00 g% at the time informed consent is obtained. Age, weight, pregnancy status, urine drug metabolites, recent alcohol use, treatment-seeking status, MRI safety/compatibility, safety for blood draw, basic medical history, and medication use will be assessed per Eligibility Criteria listed in Section 4. The research team will check the individual's state-issued photo ID to verify age and identity. Height and weight will be measured using a standard scale. Urine samples will be collected to test for presence of drug metabolites. Urine samples from participants who are able to become pregnant will be tested for pregnancy hormones. Basic demographic information, social history, and health-related data will be collected on questionnaires. Questionnaires on alcohol use and related symptoms (Alcohol Use Disorders Identification Test; AUDIT) will be administered. Blood samples will be taken for testing of biomarkers per the research aims. The Timeline Followback (TLFB) measure will be used to assess alcohol use in the past 30 days. Current or recent seeking of treatment for alcohol or drug use will be assessed. Participants will be interviewed for history of major medical conditions and current medication usage. Because it is necessary to confirm eligibility prior to MRI scanning and because advance notice is needed for MRI scheduling, participants will be asked to return on a separate day to complete a one-hour MRI scan of the brain at Brown's MRI Research Facility. Participants who are able to become pregnant will be given a urine pregnancy test prior to MRI scanning. BrAC of .00 g% will be confirmed prior to the MRI scan. Eligible participants will be given a 30-day supply of the probiotic supplement with dosing and storage instructions. The research team will collect contact information and request permission to send daily emails to confirm participants' adherence to the study protocol.
- Visit 2: Participants will complete questionnaires on side effects and adherence to the study protocol. Blood samples will be taken for testing of biomarkers. BrAC of .00 g% will be confirmed prior to study procedures.
- Visit 3 (Day 30 of Probiotic Intervention): The TLFB will be used to assess alcohol use since Visit 1. Side effects, adherence data, and blood samples will be collected as before. Participants who are able to become pregnant will be given a urine pregnancy test prior to MRI scanning. BrAC of .00 g% will be confirmed prior to the MRI scan. Participants will complete a one-hour MRI scan. They will be debriefed about the study and given the opportunity to ask questions at study completion.

- Visit 4: A post-assessment visit will be conducted 1 to 10 months after completion of the probiotic. The Timeline Followback will be used to assess alcohol use in the past 30 days. A blood sample will be taken.

Probiotic Supplement Storage and Dispensing:

- The probiotic product will be stored in a locked cabinet in the PI's office until the time of dispensing to the participant. Participants eligible for the study will receive a 30-day supply with dosing and storage instructions per the manufacturer. Any unused probiotic products at the end of the study will be disposed of by the PI by placing the unused product in a waste bin, or taken home by study staff. This product is not a controlled substance or considered hazardous with regard to storage or disposal.

Procedures for a Positive Breath Alcohol Reading (BrAC):
In the event that a participant has a positive BrAC reading, the visit will be rescheduled for another date. If a participant has a repeated positive BrAC reading, the participant will be withdrawn from the study. If a participant's BrAC exceeds the legal limit for operating a motor vehicle (>.08%) at any visit, the person will be asked to wait at the research site until the BrAC reading is under .08%. The person will be offered a rideshare for transportation home at study expense.

4. **Participant Population.** *In order to approve research, the IRB must determine that the selection of participants is equitable and reasonably related to the purpose and aims of the research. The IRB must also consider whether adequate safeguards are in place to minimize any risks that are unique to vulnerable populations. To make this determination, the IRB must review all methods and materials used to contact and recruit potential participants, including letters, flyers, emails, etc.*

Human participants will be healthy, non-treatment-seeking adult heavy drinkers recruited from the community. Participants will not be asked or encouraged to change their alcohol use as part of the study. Up to 100 individuals will be enrolled to obtain a final sample of 15 individuals to complete the study. The sample size is appropriate for a pilot study intended to generate preliminary data for future full-scale work. Full eligibility criteria are listed below.

Inclusion Criteria:
1) 18-64 years of age;
2) Able to speak and read English well enough to complete study procedures;
3) Meets NIAAA guidelines for heavy drinking in the past 30 days, defined as at least one of the following:
   A) at least 4 heavy drinking episodes (≥4 drinks for women, ≥5 drinks for men on a given day);
   B) at least 2 heavy drinking weeks (>7 drinks for women, >14 drinks for men per week).

Exclusion Criteria:
4) Chronic disease requiring daily use of medication;
5) Seeking or receiving treatment for alcohol/drug use, with exception of smoking cessation;
6) Self-reported history of liver disease;
7) Antibiotic or probiotic use in past 1 month, wherein probiotic use pertains only to supplements and not to foods that contain probiotics such as yogurt, kefir, kimchi, etc.;
8) Positive urine test for amphetamine, cocaine, methamphetamine, opioids, or benzodiazepines (cannabis use will be assessed but is not an exclusion criterion);
9) History of fainting, weakness, infection, excessive bruising, or extreme distress from blood draw;
10) Safety contraindication for MRI (e.g., ferromagnetic implant in the body, claustrophobia);
11) History of head trauma with loss of consciousness >10 min;
12) For people able to become pregnant: Pregnant, breastfeeding, or not using effective birth control;
13) Unable to complete the study visits due to time or scheduling constraints;
14) Weight <110 lbs (due to blood draw guidelines).
15) Conditions of immunodeficiency, such as HIV infection, primary immune deficiency, or taking immune-suppressant medications.

Vulnerable Populations: Vulnerable populations (e.g., fetuses, pregnant women, prisoners, children) will **not** be included in this study.

5. Recruitment Methods

Participants will be recruited from the general community using print or online advertisements (Google), flyers posted in public venues, listservs (Brown University listservs), postings on social media (Facebook), Today@Brown postings, and online bulletin boards (Craigslist). Contact information will be provided for follow-up. Interested individuals who contact the study will complete a brief (10-minute) telephone pre-screening interview administered by research staff. Individuals will be prescreened by phone to assess key eligibility criteria and interest in participation. Information collected from the prescreening interview will not be retained for research purposes. Before beginning the interview, staff will confirm that the caller is in a private location. Ineligible individuals will be informed of their ineligibility and thanked for their interest. Eligible individuals will be scheduled for Visit 1. The PI and Research Assistant will complete all applicable training in human subjects research protections prior to recruiting participants. The study will be advertised as not providing treatment or medication, such that the probiotic supplement should not be confused with clinical care.

6. Compensation / Reimbursement

Participants will be compensated for their time and effort as follows: $50 for Visit 1 screening (approximately 2.5 hours) and $50 for Visit 1 MRI (approximately 1.75 hours); $30 for Visit 2 (approximately 1.5 hours); $80 for Visit 3 (approximately 3 hours); $1 per day for each day the participant responds to emails about adherence (maximum $30); $40 for Visit 4 (approximately 1.5 hours). Participants found not eligible to complete the study at Visit 1 will be given compensation prorated at $20/hour. Participants will be offered reimbursement for parking and/or transportation as needed. Payments will be made by electronic gift card, specifically an Amazon gift card. The compensation rate of approximately $20/hour is appropriate for the time, effort, and activities involved in participation. Participants will be paid at the completion of each visit. For the daily response compensation, participants will be paid at the end of the 30-day period for the total of days that they responded.

7. Potential Research Risks / Discomforts to Participants.

In order to approve the research, the IRB must consider the risks posed to participants by the research and any efforts to mitigate those risks. The IRB needs to determine that the risks have been both minimized and are reasonable in relation to the anticipated benefits to participants, as well as to the importance of the knowledge that may be gained. The IRB will also consider whether the informed consent process provides potential participants with an accurate and fair description of the risks or discomforts.

1. Risk of side effects from probiotic supplementation: The study will utilize a commercially available probiotic supplement product (Seed DS-01™ Daily Synbiotic) that has an excellent safety and tolerability profile. The most common side effects of probiotics are abdominal bloating and/or gas, which typically dissipate after the first few days of use. The Seed DS-01™ Daily Synbiotic undergoes rigorous quality control testing for contaminants (e.g., heavy metals, pesticides) and allergens (e.g., nuts, gluten, milk) and is the subject of ongoing independent clinical trials for irritable bowel syndrome and post-antibiotic recovery. The product is vegan, preservative-free, and free of common allergens (soy, dairy, gluten). Probiotic supplements are not regulated by the FDA or subject to IND requirements.

2. Risks of MRI scan: MRI has no known side effects and does not involve radiation. However, there are several contraindications for MRI scan. The first is metallic materials within the body, such as aneurysm clip, bullet or shrapnel fragment, ear implant, certain prosthetic devices, heart pacemaker, or artificial heart valve. Participants will be thoroughly screened, first by the researcher and again by the MRI technician, to prevent

admission of individuals with these contraindications to the MRI suite. The second contraindication is pregnancy, as risks to the fetus are unknown. Pregnancy tests will be administered to all participants able to become pregnant at the baseline assessment and again on the days of MRI scanning. Aside from medical contraindications, there is risk of subjective discomfort from being in a small space or from noise emitted by the scanner. Participants will be given a call button to hold during the MRI so that they can communicate distress to the MRI technician and research staff, and procedures can be discontinued if indicated. Participants will be required to wear earplugs to reduce exposure to noise from the scanner.

3. Psychological discomfort associated with assessment: Participants will be advised during the informed consent process that they may choose not to answer any particular question, that their verbal and written responses are confidential, and that they are free to withdraw from the study at any time. All data collection procedures will be performed in private rooms reserved for these purposes.

4. Breach of confidentiality of personal identifiable information (PII): Several steps will be taken to protect against loss of confidentiality. All data will be collected and stored using technology that employs the highest industry standards for security. Data will be stored in de-identified, password-protected files on Brown University's secure servers. Participant data and samples will be identified using a unique study ID code not related to their PII. Participants' personal identifying information will be kept in a separate location from their response data. At the conclusion of the study, the file that contains links of personal identifiers with unique study IDs will be destroyed by the study PI. Biological samples will be identified with the participant's study ID and will not be associated with PII. With these precautions, loss of confidentiality is considered very unlikely; however, participants will be informed of the potential risk at the time they provide consent.

5. Risks associated with blood draw procedures. During the informed consent process, participants will be advised of the necessity of obtaining blood samples throughout the trial. The study will exclude individuals who report physical or psychological discomfort caused by blood draw or the sight of blood, or who report history of excessive bruising, fainting, or weakness following standard blood draw procedures. Blood draw procedures will follow World Health Organization guidelines for safe and well-tolerated procedures to minimize risk of infection or other complications. A licensed research nurse will collect the blood samples. Risk reduction strategies are to use sterile, single-use implements; surface disinfectant; proper hand hygiene; application of 70% isopropyl alcohol to the blood draw site; minimal vein probing; application of pressure after drawing blood; and non-latex, vinyl gloves.

6. Risk of coercion from compensation received for participation: The risk of coercion from compensation for participation is low, as the total amount of compensation is modest. The hourly compensation rate for the study sessions translates to approximately $20/hour, which is consistent with rates offered by similar studies in the Brown University area and is intended to offset the effort required of participants outside of study sessions (i.e., daily adherence to the probiotic protocol).

7. Risk of adverse reaction to the probiotic supplement: In the unlikely event that a participant reports adverse reaction to the probiotic beyond the mild side effects noted in #1 above, the participant will be instructed to discontinue the probiotic and will be withdrawn from the study.

Standard of Care: There is no established "standard of care" for healthy heavy drinkers who are not seeking treatment. The probiotic supplement does not replace or otherwise alter any clinical care that participants would receive. At the conclusion of the study, participants will be given an NIAAA pamphlet on the risks of heavy drinking.

DSMP/DSMB: The DSMP is attached and contains information on the DSMB.

8. **Potential Benefits of the Research. NOTE: Compensation for participation is not a benefit and should not be included in this section.** *In order to approve this research, the IRB must determine that the potential benefits to research participants are reasonable in relation to the potential risks. Very often, research at Brown does not include potential direct benefits to participants, but may only benefit society as a whole by helping researchers.*

Some individuals experience a noticeable benefit in health when using probiotics. Many subjects in this study are unlikely to benefit directly from participation. Benefits to society are expected to accrue through generation of scientific knowledge about effects of probiotics on inflammation in heavy drinkers.

PART IV. INFORMED CONSENT

Informed consent is a *process*, not just a form. The IRB must ensure the informed consent process clearly discloses and facilitates the understanding of all information needed to make an informed decision to participate while promoting the voluntariness of participation.

Please use the Brown consent / assent templates and related guidance on the HRPP Forms & Templates page to develop your consent forms.

1. **Describe the informed consent process, including:**

Informed consent will be obtained at Visit 1. BrAC of .00 g% will be confirmed at the time that consent is obtained. The nature of the study will be described verbally and in writing. Potential participants will be informed that study participation is voluntary and that they have the right to terminate study procedures at any time. Participants will be informed that the alternative is to not participate. Written informed consent will be obtained by the PI or trained Research Assistant. The Research Assistant will receive one-on-one training from the PI in consent procedures. The names and contact information of the PI (Dr. Monnig) and study staff will be provided on the consent document in case questions arise. Participants will receive a copy of the signed consent document, and the original form will be kept in a locked filing cabinet.

2. **Facilitate Understanding**

Participants will be encouraged to ask questions to clarify the nature of the study and to ensure understanding. Participants who decide to withdraw from the study at any point prior to completion will be paid for their participation up to that point.

3. **Documentation**

Participants will indicate informed consent by signing the consent form.

4. **Additional Considerations**

STATISTICAL ANALYSIS PLAN

The goal of this analysis was to observe the effect of the probiotic on plasma concentrations of sCD14, sCD163, LBP, IL-6, IL-8, IL-10, MCP-1, and TNF-α. Of the 16 eligible participants who initiated the probiotic, 14 participants completed the probiotic course, and two were lost to follow-up prior to completion. Ten participants returned after the washout period for Visit 4. Descriptive statistics were obtained on demographic characteristics and drinking history. All collected datapoints were considered for analysis regardless of probiotic completion or loss to follow-up. Biomarker concentrations greater than three standard deviations from the mean were excluded to limit the impact of statistical outliers on the analysis. Regarding undetectable biomarker values, sCD14 had 3.7% missing data (n=2), IL-6 had 35% missing data (n=19), IL-10 had 5.6% missing data (n=3), LBP had 1.8% missing data (n=1), and MCP-1 had 5.6% missing data (n=3). There were no undetectable biomarker values for IL-8, sCD163, or TNF-α. Regarding outlier exclusions, IL-6 had one outlier value, IL-8 had two outlier values, LBP had one outlier value, sCD163 had one outlier value, and TNF-α had one outlier value. Distributions for sCD14, IL-10, and MCP had no outlier values.

Linear mixed models were used to assess changes in biomarkers over the study period. Models included the effect of alcohol use variables (assessed at Visit 1, Visit 3, and Visit 4) as time-varying covariates, specifically, number of heavy drinking days, total number of drinking days in, total number of standard drinks, and days since last drink. Self-reported alcohol use variables were treated as continuous. Self-reported drinking variables were assessed for collinearity and included in the final model if they met an acceptable variance inflation factor (VIF) <10. Total number of standard drinks was dropped from the final model due to high VIF. Despite prior evidence for sex effects on alcohol consumption and its impacts, there were too few females to test for this effect, and sex was not included in models. Models were specified to identify participant as the subject variable and visit number (1, 3, and 4) as the repeated measure, and with fixed slope and intercept. An autoregressive variance-covariance structure was selected for optimal model fit based on the Akaike Information Criterion (AIC). In the primary model (Model 1), the trajectory of biomarker concentrations before, during, and after probiotic use were examined, with alcohol variables (number of heavy drinking days, number of drinking days, and days since last drinks) included as time-varying covariates. In Model 1, biomarker

concentrations below the threshold of detectability were treated as missing data in analyses. Of note, maximum likelihood estimation as applied in the linear mixed model approach is able to handle missing data without excluding cases listwise or imputing data, thus making full use of the available data and minimizing bias in parameter estimates (Baraldi & Enders, 2010). To assess for non-linear changes in biomarker concentrations, a secondary model (Model 2) was tested to include a quadratic effect of time for the biomarker outcomes. Finally, a sensitivity analysis model (Model 3) was tested to assess for the effect of data missing at random. In Model 3, data below the detectability threshold of the assay (i.e., missing) were imputed at half the value of the threshold per the manufacturer. All analyses in this paper were conducted using SAS software, version 9.4 for Windows (SAS Institute, Cary NC.)