COMIRB Protocol

COLORADO MULTIPLE INSTITUTIONAL REVIEW BOARD

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Project Title:	Acceptability and Feasibility of *L. reuteri* among Veterans: A Stress Resilience Model
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1. Aims/Hypotheses

Primary Aims:
To test the acceptability and feasibility of *Lactobacillus reuteri (L. reuteri)*, a probiotic dietary supplement for a cohort of Veterans with a history of mild traumatic brain injury (mTBI) and post traumatic stress disorder (PTSD).

Exploratory Aim: To collect data to identify potential biological signatures, including inflammatory makers and gut permeability among those with mTBI and PTSD.

Exploratory Hypotheses: Evaluation of supplementation requires a stepwise process in which initial aims are geared towards identifying biological signatures versus diagnosing, treating, mitigating or curing any conditions. Towards the end of identifying potential biological signatures, we hypothesize the following. 1) Participants who receive *L. reuteri* as a dietary supplement will have an attenuated **physiological stress response** to cognitive and emotional stressors as measured by lower heart rate, skin conductance, and body temperature when compared to those who received the placebo supplement. 2) Participants who receive *L. reuteri* as a dietary supplement will have attenuated **perceived psychological stress** to cognitive and emotional stressors as measured by lower scores on psychological correlates of acute stress when compared to scores from those who received the placebo supplement.

2. Background and Significance

There is now much evidence to suggest that modern man's lack of exposure to microorganisms in the environment is leading to a disordered and hyperactive immune system (Rook, 2009). The Old Friends Hypothesis (also known as the Hygiene Hypothesis) postulates that mammals co-evolved with an array of microorganisms that have become necessary for proper development of the immune system, particularly in quieting the immune response once it is no longer needed to eliminate a potential threat (Rook et al., 2013). For example, germ free rats have been shown to have poorly developed lymphoid systems and are highly susceptible to auto-inflammatory disorders (Kohashi et al, 1985). Similar findings have also been noted in humans, growing up with a hot-water tap in the home was found to increase the risk of developing Crohn's disease by five-hundred percent (Gent et al, 1994). Autoinflammatory diseases such as asthma have been shown to be significantly more prevalent in more sterile

urban environments as compared to the less sterile rural environments (Van Niekerk et al, 2006). Probiotics are one type of these commensal microorganisms that have a naturally occurring, symbiotic relationship with humans and other animals, exerting their beneficial effects through immunoregulatory pathways. One probiotic organism in particular, *Lactobacillus reuteri (L. reuteri),* has been shown to induce the proliferation of regulatory T-cells which produce the anti-inflammatory cytokine, IL-10 (Smits et al, 2005). This may explain the ability of this organism to treat disorders of inflammation as described by further evidence in the following sections. In recent years, there has been an increased interest in exploring and demonstrating clinical effects of commensal microbes such as probiotics on various systems within the body via probiotic supplementation.

A. Animal Studies

Animal studies have shown that manipulation of a host's microbiome via dietary supplementation can lead to changes in emotional and cognitive functions. Specifically, impaired gut colonization during development alters the physiological stress axis and may significantly contribute to an exaggerated stress response during adulthood, which, in turn, may contribute to mood disorders such as generalized anxiety disorder (Messaoudi et al., 2011). In addition to the influence the microbiota has on brain development, animal studies have also shown a more acute effect of gut bacteria on emotional behavior. Following infection with a pathogen (or injection of proinflammatory cytokines to mimic a physiological immune response) adaptive sickness behavior is elicited, which is similar to that of depression (Rook, 2009). Activation of the brain proinflammatory cytokine system is associated with decreases in general activity, eating, drinking, and social activity: behaviors all reminiscent of depression (Dantzer, 2001). Additionally, Lowry and colleagues see stress and immune function linked as rodents exposed to chronic mild stress for five weeks were found to have increased levels of the proinflammatory cytokine IL-1β and neurodegeneration in the hippocampus, one of the brain structures implicated in major depressive disorder (Bansar & Duman, 2007). *L. reuteri* has been shown to produce anti-inflammatory factors that could aid in quieting the immune response (Jones & Versalovic, 2009). Additionally *L. reuteri* colonization has been shown to reduce visceral pain in rodents (Kamiya et al, 2006).

B. Safety Trials in Humans

Mangalat et al. (2012) conducted a placebo-controlled, safety and tolerability study of *Lactobacillus reuteri* in healthy adults. At the end of a 2 month supplementation period, the authors concluded that *L. reuteri* is safe and well tolerated in adults as no severe adverse events and no significant differences in adverse events were reported relative to the control condition (Mangalat et al. 2012).

Safety and tolerance of *L. reuteri* was also tested in other studies. Recently, a research team found that no significant differences existed between an *L. reuteri* treated group and a placebo group when it came to hematology results and adverse event reporting (Jones et al. 2012). There have been numerous studies with vulnerable populations in which probiotics of the Lactobacillus genus have demonstrated evidence of safety. Some of these populations include: pregnant women, infants, the elderly, patients with rotavirus, adults with Crohn's disease, and malnourished children to name a few (Snydman, 2008; Sung et al., 2013). Not only have probiotics demonstrated safety in these populations, they have also alleviated symptoms of disease. For example, in a study of 61 cystic fibrosis patients, *L. reuteri* significantly reduced pulmonary exacerbations and upper respiratory infections in those with mild to moderate lung infections (Nardo, 2014).

C. Documented Probiotic Use

Probiotics have been used as a therapeutic agent in a variety of human diseases. *L. reuteri* has been found to be effective in shortening rotavirus-associated gastroenteritis (Shornikova et al, 1997). Further evidence supporting *L. reuteri*'s efficacy as an anti-inflammatory agent, supplementation was been found to reduce gingivitis in severe cases

(Krass et al, 2006). Interestingly, one study found *L. reuteri* supplementation aided in reducing gastrointestinal symptoms in children receiving therapy to eradicate *Helicobacter pylori* infections (Lionetti et al, 2006). Another study examined *L. reuteri* supplementation in healthy subjects and found the supplemented group to have significantly reduced work "sick-days" compared to placebo (Tubelius et al, 2005). *L. reuteri* seems to be effective both as a prophylactic supplement and as an anti-inflammatory interventional treatment.

As the interest in the gut microbiome and commensal microbes is relatively new, few studies exist examining their use closely in humans, and none exist looking specifically at the Veteran population. The compiled evidence in rodents suggests this supplement may improve cognition and ability to respond to stressful stimuli; furthermore, studies in humans show the supplement to be well tolerated. This study would allow for a closer examination of *L. reuteri* in a population that has yet to be examined, as well as examine the behavioral endpoint (biological signature) of response to acute stress that has yet to be considered in previous human studies.

3. Preliminary Studies/Progress Report

The proposed investigation is a pilot study intended to establish the acceptability and feasibility of an *L. reuteri* probiotic supplement in a population of Veterans. Exploratory data regarding biological signatures will also be collected.

4. Research Methods

A. Participants

Up to 200 participants will be recruited within the Eastern Colorado Health Care System (ECHCS).

Inclusion Criteria:

1. Veterans eligible to receive care by a VA provider
2. History of at least one deployment in support of OEF/OIF/OND
3. History of mTBI per the Ohio State University (OSU) TBI-ID with any endorsement of post concussive symptoms (PCS) associated with an mTBI, which occurred at least 6 months prior to the baseline assessment
4. Current symptoms in 3 or more of the following ICD-10 Post Concussive Symptom categories as measured by the Rivermead Post Concussive Symptom Questionnaire (RPCSQ; score of 2 or greater per symptom to qualify): a) headache, dizziness, malaise, fatigue, noise intolerance, b) irritability, depression, anxiety, emotional lability; c) subjective concentration, memory, or intellectual difficulties; and/or d) insomnia
5. Current diagnosis of PTSD per the Clinician Administered PTSD Scale-5 (CAPS-5)
6. Medical clearance by study physicians to participate in the protocol
7. Age between 18 and 50
8. Ability to provide informed consent
9. Willingness not to take probiotic supplements (pills, tablets, oils, etc.) other than the product provided in the clinical study until all study procedures are completed
10. Willingness to provide blood, as well as stool samples.

Exclusion Criteria

1. Inability to adequately respond to questions regarding the informed consent procedure
2. Currently involved in the criminal justice system as a prisoner or ward of the state
3. Current (past month) alcohol or substance abuse or dependence
4. Lifetime history of bipolar disorder or psychosis or anxiety disorders (excluding PTSD)

5. Consistent (e.g., 5x/week or greater) probiotic supplementation within the last month, including probiotic food products such as yogurt, as determined by phone screen interview and Probiotic Food Check List
6. Receiving antibiotics within the last month;
7. Receiving medications that interfere with gut motility (opiates, loperamide, stool softeners)
8. Presence of central venous catheters (CVCs)
9. Gastrointestinal (GI) barriers as identified by the 2-week run-in period as determined by the study team (e.g., daily GI discomfort with frequent diarrhea prior to supplementation)
10. Participation in conflicting interventional research protocol
11. Vital signs outside of acceptable range, i.e., blood pressure >160/100, oral temperature >100°F, pulse >100
12. Use of any of the following drugs within the last 6 months: systemic antibiotics, antifungals, antivirals or antiparasitics (intravenous, intramuscular, or oral); oral, intravenous, intramuscular, nasal or inhaled corticosteroids; cytokines or cytokine inhibitors; methotrexate or immunosuppressive cytotoxic agents
13. Acute disease at the time of enrollment (defer sampling until subject recovers). Acute disease is defined as the presence of a moderate or severe illness with or without fever.
14. Chronic, clinically significant (unresolved, requiring on-going medical management or medication) pulmonary, cardiovascular, gastrointestinal, hepatic or renal functional abnormality, as determined by medical history or physical examination other than irritable bowel syndrome (IBS)
15. History of cancer except for squamous or basal cell carcinomas of the skin that have been medically managed by local excision
16. Unstable dietary history as defined by major changes in diet during the previous month, where the subject has eliminated or significantly increased a major food group in the diet
17. Positive test for human immunodeficiency virus (HIV), Hepatitis B virus, or Hepatitis C virus
18. Any confirmed or suspected condition/state of immunosuppression or immunodeficiency (primary or acquired) including HIV infection
19. Major surgery of the GI tract, with the exception of cholecystectomy and appendectomy, in the past five years. Any major bowel resection at any time.
20. Regular urinary incontinence necessitating use of incontinence protection garments
21. Female who is pregnant or lactating
22. Treatment for or suspicion of ever having had toxic shock syndrome
23. Those receiving immunosuppressive drugs/medications (e.g. oral corticosteroids) or treatment including antineoplastic therapy, post-transplantation immunosuppressive therapy, and/or radiation therapy.

B. Recruitment

Dr. Brenner, Director of the Rocky Mountain MIRECC, will oversee all recruitment efforts. Recruitment will occur in the following ways: 1) mailing letters to research participants that have previously agreed to enrollment in the VISN 19 MIRECC Recruitment Database (as described in COMIRB 10-0554); 2) posting approved flyers around the ECHCS; and 3) informing health care staff about the current study and encouraging them to tell their patients about the study (interested Veterans will be encouraged to contact members of the study team).

We may retrieve names and addresses of potential participants from the VA database that have the ICD-9 and/or ICD-10 codes of interest for TBI (e.g., S06) and PTSD (e.g., F43.1). An initial invitation may be sent by U.S. mail from the PI to participate in the study, including a flyer, and a pre-stamped and addressed refusal response card. If the potential participant contacts the study team and expresses interest in participating, he/she may then be screened

for possible enrollment into the study. If the potential participant returns the refusal response card, that individual's name will be flagged as do not contact. The refusal response card will not include any PHI, nor the study name or Veteran's name. The refusal response card only states, "I do not wish to participate in this study". So that the research staff is able to identify a Veteran who would no longer like to be contacted, the refusal response card will include a unique identifier. One reminder letter may be sent if the participant has not expressed either interest or no interest in being contacted within 4 weeks.

All potential participants will convey interest directly to a member of the research team. Patients who convey interest to their provider will be directed to a member of the research team, who will speak with the potential participant to discuss study procedures and obtain informed consent.

Those who contact a member of the research team and are interested in participating will be screened for eligibility for the study. This screening process, which will be conducted by a member of the research team, will consist of a brief phone or in-person interview (at the convenience of the Veteran). Use of the study Screening Form (i.e. verbal consent) and HIPAA and consent waivers will be obtained to facilitate this process. If the potential participant does not meet study criteria, he/she will be informed of this immediately and will be thanked for their interest. Screening forms for these persons will not be destroyed per VA regulations. De-identified data already collected on the Screening Form may be used for comparative purposes only.

For those who pass the initial screening for the study and provide verbal consent, permission to contact a PCP or a licensed provider chosen by the study team will also be verbally obtained. A member of the research team will contact the provider either in person, via phone, and/or encrypted email. Veterans' potential participation in the study will be discussed with the provider, and the provider will be asked to complete a medical clearance form and indicate whether there are medical barriers to the Veteran participating in the study. If barriers are identified, the Veteran will not be eligible to participate. A research team member will contact the Veteran by phone to inform them of medical eligibility to participate in the study.

If the potential participant meets study criteria, is medically approved to participate, and is willing and able to commit to the study, he/she will be scheduled for an in-person appointment at the ECHCS to complete the informed consent process and the initial baseline procedures.

C. Consent

All members of the research team will have been trained in COMIRB procedures and will be under the direct supervision of Lisa Brenner, PhD. The nature of the study and potential risks and benefits will be discussed in a calm environment in a private office at the ECHCS and participants will have the opportunity to ask questions. After the informed consent document and purpose of the study are explained and provided to the participants and all questions are answered, the participants will be asked to answer six questions. These questions will ensure participants' ability to adequately provide informed consent. If participants are not able to adequately respond to these questions, they will be excluded from participation: 1) Why is this study being done? 2) What is the study asking you to do? 3) What are the risks/side effects of being in the study? 4) What are the benefits of being in the study? 5) Is the study voluntary? 6) What do you do if you have questions or possible side effects?

The informed consent document includes an option to be voluntarily included in a database of research participants for possible recruitment into VISN 19 MIRECC study protocols. On the study consent, participants are asked to check a box indicating whether they would like to be contacted to participate in future studies and to initial their choice. Willing participants, as determined by a "Yes" response and participant initials, will be given the option to sign a HIPAA B form and a separate consent document pertaining to the VISN 19 MIRECC Research database.

Participants will be provided with a copy of the study consent form, and will be assured that their decision regarding participation will in no way affect the care they receive from the VA nor their access to care although the consent process will be documented in the participant's electronic VA medical record.

Participants will be informed that the researcher will review their responses to the items regarding safety, and if necessary, will discuss these responses with them in an effort to ensure their safety. For participants who reveal clinically meaningful risk as indicated on the UWRAP, specifically if the participant rates suicide risk at or higher than a rating of 4 on the Pre-Assessment Risk Assessment (page 2 of the UWRAP), study personnel will review, and if necessary, will discuss these responses with them to ensure their safety. Responses may be also shared with the Veteran's treating clinician, if determined necessary by Dr. Brenner or her designee. Additional mental health interventions, such as escorting the Veteran to the Psychiatric Emergency Services will be determined at this time, by Dr. Brenner, her designee, or the treating clinician and appropriate notation will occur.

All researchers who will be interacting with study participants are trained in the area of risk assessment and management, and will be receiving supervision from Dr. Lisa Brenner, a licensed clinical psychologist, or her designee.

D. Re-Engagement of those Previously Excluded

Participants who have previously consented but were excluded due to C-Reactive Protein (CRP) < 3.0 mg/L, current major depressive disorder, and/or BMI less than 18 or greater than 35 will be given the option to re-consent into the study. A member of the research team will reach out to those participants by phone to explain that they are now eligible for the study. This visit may take place in person or over the phone, if all assessments were collected at Visit 1. The following measures will be re-administered: UWRAP, Neurobehavioral Symptom Inventory (NSI), Probiotic Food Checklist, Rivermead Post Concussive Symptom Questionnaire (RPCSQ), Pittsburgh/Epworth Sleep Scale, and Psychological Stress Measure-9 (PSM-9). If all measures were not completed during the initial visit 1- the participant may be asked to come for an in person visit to complete study measures. Individuals who are re-engaged will restart study procedures (e.g., Study Procedure Week) as outlined in Section G. Re-engagement will not require re-consenting or resigning HIPPA Waiver of Authorization forms.

E. Authorization

For recruitment, a waiver of consent and a HIPPA Waiver of Authorization will be obtained to allow members of the research team to view selected medical charts of patients who contact the PI or study coordinator and express interest in participating in the study. Review of the medical records allows members of the research team to screen for other criteria that would exclude participation (e.g., certain medical conditions or prescriptions).

For enrollment, an authorization to collect protected health information (PHI) will be obtained by a member of the research team (VA Authorization B: Enrollment into Research Form). A signed and dated copy of the release will be provided to the participant. All data in electronic format will be limited to data sets and stored in password protected spreadsheets on the VA shared drive, behind the VA firewall. Only members of the research team will have access to the limited data set.

The PI and/or Study Coordinator will maintain a master list of participant names, social security numbers, contact information (telephone numbers), and randomly assigned research identification numbers in a separate, password-protected, electronic file. All data in paper format or original documents containing sensitive information will be stored in locked filing cabinets within the MIRECC offices. Of note, the original signed informed consent forms and HIPAA authorization forms will be stored in locked filing cabinets separate from the rest of the paper

study data. Only the PI, VA co-Investigators, and Research Assistants on the protocol application will have access to the paper documents.

F. Study Design

The proposed study is a longitudinal, randomized, placebo-controlled, between groups design. The study is expected to provide feasibility and acceptability data regarding supplementation. Basic information regarding biological signatures will also be collected.

G. Study Procedures

In addition to the paragraphs below, please refer to the consort diagram on page 8.

Upon confirming eligibility and obtaining medical clearance, participants will complete the baseline assessment (Visit 1). History of mTBI and PTSD will be confirmed during Visit 1. A blood draw will be performed to confirm CRP levels. If CRP levels are not at the level necessary to participate in the study, participants will no longer be eligible to participate.

Between Visits 1 and 2, all participants will be asked to complete a daily diary of gastrointestinal (GI) symptoms (GI Symptom Diary including the Bristol Stool Scale). Participants reporting abnormal stool form (Bristol Stool Scale 1, 6, or 7) or frequency (>3 bowel movements per day or <3 bowel movements per week) or abdominal pain/discomfort on more than 2 days in a row will be excluded from further participation. This careful screening for gastrointestinal symptoms will be performed with the goal of facilitating evaluation of acceptability/feasibility (changes in GI symptoms pre- and post-supplementation). Participants will also be asked to provide a gut/fecal sample (the kit and instructions will be given to the participant at Visit 1) and send or bring this sample (per directions given to participant) to Visit 2.

After 2 weeks, participants will complete the Visit 2 assessment measures. At this point individuals will be randomized to one of two conditions, active or placebo group via random sampling by the research pharmacist. Half of participants will be given a probiotic oil solution and the other half will receive a placebo version of the oil solution. The study team will provide the participant with information regarding the supplement (e.g., How to take it? When to take it? How to note having taken it in their probiotic log?) and contact information regarding who to call if any questions arise during the trial regarding the supplements. Materials to track supplement usage and adverse events will be given to the participant during this meeting by the MIRECC Research Team.

Between Visit 2 and Visit 3, Veterans will independently complete daily GI Symptom Diary and Probiotic Usage Logs, and will speak with study staff by phone or in person one time per week to respond to questions (see measures table below). After the 8-week (+/- 2 weeks) supplementation intervention, participants will be invited back to the lab to complete the follow-up appointment (Visit 3). The Visit 3 assessment will occur within 2 weeks of the 8-week trial; that is, participants may complete the follow up assessment as early as 6 weeks and as late as 10 weeks post intervention initiation, depending on the research team and Veteran's scheduling capabilities. Participants will be asked to provide a gut/fecal sample (the kit and instructions will be given to the participant at Visit 2) and send or bring this sample (per directions given to participant) to Visit 3.

At Visit 3, Veterans will complete the final study visit. In addition to self-report measures, participants will complete the Trier Social Stress Task (TSST; see description below).

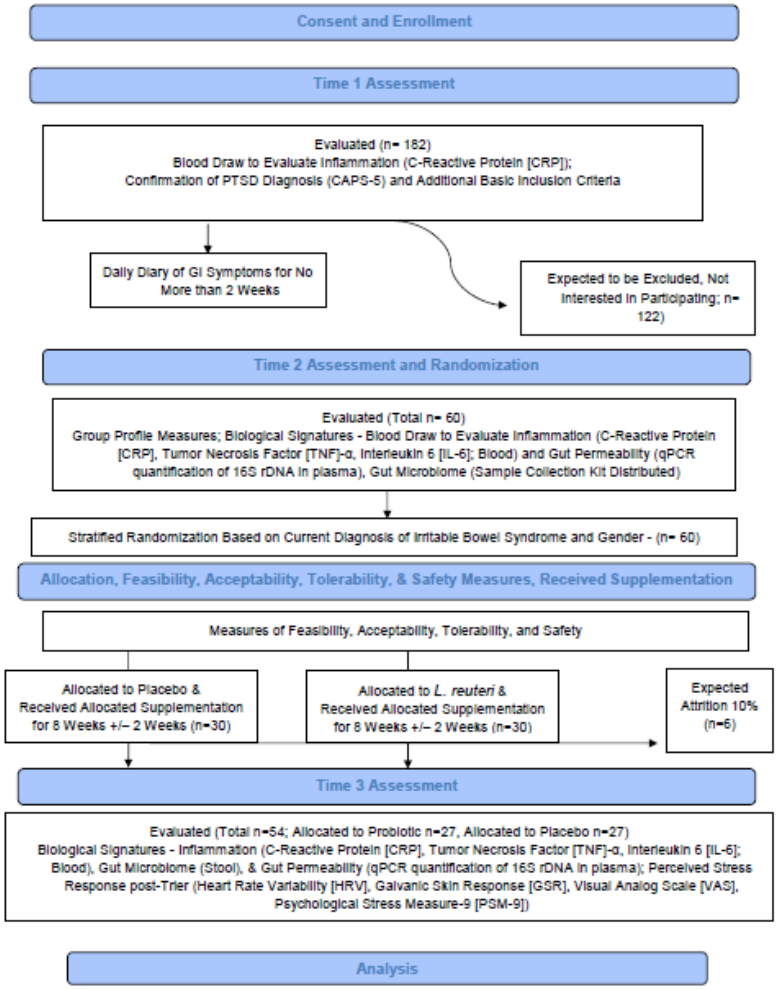
Physiological Biomarkers of Stress Response. Heart rate and skin conductance (e.g., galvanic skin response) will be measured continuously during task performance with a centrally placed remote heart rate monitor around the base of the sternum (heart rate) and the distal phalanges of the first and second fingers of the non-dominant hand (skin conductance). A sphygmomanometer will be used to measure blood pressure at 5-minute intervals throughout the task, by wrapping the cuff around the non-dominant arm. Signals from each of these stress

biomarkers will be recorded with an ADInstruments system, with appropriate bioamplifiers and software allowing for data acquisition in real time.

Psychological Markers of Stress Response (e.g. Perceived Stress). The individual's perception of stress will be assessed using a Distress Thermometer-Visual Analog Scale (VAS) for stress appraisal. This measure has been used reliably in the literature and by members of this team.

In addition to the procedures described above, phlebotomy of an antecubital vein to obtain 30 cc of blood will be performed. Blood will be drawn by certified technicians at Visits 1, 2 and 3. After centrifugation and separation of plasma, specimens will be coded and "blinded" and plasma will be stored in -80°C freezers at the Denver VA, and, once data collection has completed, all specimens will be securely delivered to University of Colorado Boulder Behavioral Neuroendocrinology Laboratory (WOC and Co-I Christopher Lowry). Data Transfer Agreements will be signed prior to the transport of any specimens. Blood samples will be analyzed and destroyed per VA policy. To minimize biohazard risk for others, the use of gloves, biohazard bags, and biohazard coolers will be assured, as well as documentation of relevant TMS training for all VA staff will be in place for every personnel handling blood or blood products.

In order to minimize the burden on participants, any study assessments that have been previously completed in a specified timeframe through participation in another MIRECC protocol may not be re-administered. Instead, assessment data will be shared as outlined in an internal standard operation procures (SOP) for sharing of assessment data.



Participation in the study will require 3 in person visits. In total, participation will be approximately 6 hours over the course of approximately 12 weeks. Participants will be compensated $25 for completion of each of the Visit 1, 2, and 3 appointments. Participants who are re-engaged (see Section D above) will also receive $25 for their participation in the additional visit. Participants will be paid $5/week for monitoring gastrointestinal (GI) symptoms including bowel movements and $5 for mailing/bringing in the stool sample between Visits 1 and 2 (for a total of up to $15). Individuals will be compensated $5/week for participating in the weekly calls/visits and $5 for keeping diaries between Visits 2 and 3, as well as $5 for sending mailing/bringing in the stool sample (for a total of up to $85). If the participant leaves the study early, or is no longer eligible, they will be paid only for the visits completed. Compensation for completion of study visits may occur in the form of cash, check, gift card or direct deposit. Supplements will be provided at no cost to the participants. Participants will be asked to bring the remainder of their supplement solution to the post-intervention appointment so that adherence to the suggested dosage can be verified. There will be no follow up visits or laboratory studies after the Visit 3 appointment associated with the proposed project, although medical records may be reviewed for up to two years prior and post participation.

Measure	**Format**	**Time (min)**	**Domain**	**Purpose**
Visit 1				
UWRAP	Interview	5	Risk Assessment	Safety
Rocky Mountain MIRECC Demographic Questionnaire	Questionnaire	5	Control Variables	Sample Characteristics
BMI	Weight and Height Measurement	5	BMI	Sample Characteristics
Clinician Administered PTSD Scale-5 (CAPS-5)	Interview	20	PTSD	Inclusion/Exclusion
Neurobehavioral Symptom Inventory (NSI)	Questionnaire	5	Symptom Evaluation	Sample Characteristics
Ohio State University TBI-ID (OSU-TBI-ID)	Interview	25	Lifetime TBI Identification	Inclusion/Exclusion
Probiotic Food Check List	Interview	5	Diet	Inclusion/Exclusion
Rivermead Post Concussive Symptom Questionnaire (RPCSQ)	Questionnaire	5	Symptom Evaluation	Sample Characteristics
Credibility/Expectancy Scales	Questionnaire	5	Credibility of Supplementation/ Expectancy	Primary Aim
Generic Assessment of Side Effects (GASE)	Questionnaire	5	Side Effects	Primary Aim
Rome III Diagnostic Questionnaire	Questionnaire	20	GI Symptoms	Sample Characteristics

Structured Clinical Interview for DSM-V (SCID-IV)	Interview	40	Psychiatric Evaluation	Inclusion/Exclusion; Sample Characteristics
Harvard Food Frequency Questionnaire Booklet	Questionnaire	20	Food Frequency	Sample Characteristics
Pittsburgh/Epworth Sleep Questionnaire	Questionnaire	5	Sleep	Sample Characteristics
Psychological Stress Measure 9 (PSM-9)	Questionnaire	5	Perceived Stress	Exploratory Aim
C-Reactive Protein (CRP)	Blood Draw	5	Inflammation	Sample Characteristics
qPCR quantification of 16S rDNA	Blood Draw	-	Gut Permeability	Exploratory Aim
Visit 1 Total Time: 175 min				
Up to 2 Weeks Between Visits 1 and 2				
Gastrointestinal (GI) Symptom Diary/Bristol Stool Scale (BSS)	Diary	5	GI Symptoms	Inclusion/Exclusion
Irritable Bowel Severity Scoring System (IBS-SSS)	Questionnaire	3	GI Symptoms	Primary Aim
Gut Microbiome	Gut/fecal sample	5	Microbiome	Exploratory Aim
Between Visits 1 and 2 Time: 13 min/week				
Visit 2 – Visit 2 Measures, Randomization & Intervention				
Credibility/Expectancy Scales	Questionnaire	5	Credibility of Supplementation/ Expectancy	Primary Aim
GASE	Questionnaire	5	Side Effects	Primary Aim
Psychological Stress Measure 9 (PSM-9)	Questionnaire	5	Perceived Stress	Exploratory Aim
Irritable Bowel Severity Scoring System (IBS-SSS)	Questionnaire	3	GI Symptoms	Primary Aim
CRP, Tumor Necrosis Factor (TNF)-α, Interleukin 6 (IL-6), IL-8	Blood Draw	5	Inflammation	Exploratory Aim
qPCR quantification of 16S rDNA	Blood Draw	-	Gut Permeability	Exploratory Aim
Visit 2 Total Time: 23 min				
Between Visits 2 and 3				
Probiotics Usage Log	Diary	5	Acceptability/ Feasibility	Primary Aim

GI Symptom Diary/ BSS	Diary	5	GI Symptoms	Inclusion/Exclusion
IBS-SSS	Questionnaire	3	GI Symptoms	Primary Aim
Generic Assessment of Side-Effects – Probiotics (GASE-P)	Questionnaire	5	Acceptability/ Feasibility	Primary Aim
Adult AIDS Clinical Trials Group (AACTG) Follow-up: Section G.	Questionnaire	3	Feasibility	Primary Aim
Modified Morisky Medication-Taking Adherence Scale (MMAS)	Questionnaire	3	Acceptability/ Feasibility	Primary Aim
Modified Treatment Satisfaction Questionnaire for Medication (TSQM)	Questionnaire	5	Acceptability	Primary Aim
Gut Microbiome	Gut/fecal sample	5	Microbiome	Exploratory Aim
Between Visits 2 and 3 Time: 34 min/week				
Visit 3				
UWRAP	Interview	5	Risk Assessment	Safety
Credibility/Expectancy Scales	Questionnaire	5	Credibility of Supplementation/ Expectancy	Primary Aim
GASE	Questionnaire	5	Side Effects	Primary Aim
GASE-P	Questionnaire	5	Acceptability/ Feasibility	Primary Aim
Physiological Biomarkers of Stress Response	Physiological Measurements	During Trier	Biological Measure of Stress Response	Exploratory Hypotheses
Visual Analog Scale (VAS)	Questionnaire	During Trier	Perceived Stress	Exploratory Hypotheses
Psychological Stress Measure 9 (PSM-9)	Questionnaire	5	Perceived Stress	Exploratory Hypotheses
Irritable Bowel Severity Scoring System (IBS-SSS)	Questionnaire	3	GI Symptoms	Primary Aim
AACTG	Questionnaire	3	Feasibility	Primary Aim
MMAS	Questionnaire	3	Acceptability/ Feasibility	Primary Aim
TSQM	Questionnaire	5	Acceptability	Primary Aim
CRP, TNF-α, IL-6, IL-8	Blood Draw	5	Inflammation	Exploratory Aim
qPCR quantification of 16S rDNA	Blood Draw	-	Gut Permeability	Exploratory Aim
Visit 3 Total Time: about 93 minutes				

Adult AIDS Clinical Trials Group (AACTG) Follow-up: Section G. The AACTG developed an adherence measure for HIV/AIDS patients receiving medication. This questionnaire has been used in at least six AACTG clinical trials and has been widely disseminated (Chesney et al., 2000). We will use only section G of this follow-up measure which queries why one might not take medication as directed (e.g. forgetfulness). This will lend to our primary aim assessing feasibility.

Clinician Administered PTSD Scale-5 (CAPS-5). The CAPS-5 (U.S. Department of Veterans Affairs, 2014) is a structured clinical interview used to determine PTSD diagnosis, using the Diagnostic and Statistical Manual of Mental Disorders-V criteria.

Credibility/Expectancy Scales. The scale that determines credibility (Shapiro, 1981) assesses participants' willingness to endorse the intervention, whether or not the intervention's effects could be generalized to similar situations, and willingness to recommend it to others. An expectancy questionnaire (Myers et al., 2008) assesses specific expectations regarding the intervention period. In the context of an efficacy RCT, this measure would be utilized as a potential cofounder.

Gastrointestinal (GI) Symptom Diary. Participants will be provided with a handout document to track their bowel movements for two weeks during between visits 2 and 3 of the study, and 8 weeks during the intervention. The participants will record their number of daily bowel movements and the consistency of their stool using the Bristol Stool Scale (Lewis & Heaton, 1997). This careful screening for gastrointestinal symptoms will be performed with the goal of isolating probiotic effects on emotional systems, rather than observing secondary changes due to potentially observable improvements in gastrointestinal symptoms.

Generic Assessment of Side Effects (GASE)/ Generic Assessment of Side Effects - Probiotics (GASE-P). GASE consists of 36 symptom descriptions organized by body parts (Reif et al., 2009). Participants are asked to rate if these "symptoms" were either "not present", "mild", "moderate", or "severe" in the past week. After making the rating, the participant is asked whether the symptom is related to current medication. GASE-P is identical to GASE expect it queries whether the side effect is related to their probiotic rather than their medication. The GASE will be administered at baseline; the GASE-P will be administered during weekly phone call check-ins, and at the follow up appointment. The GASE-P will include a question regarding whether any new medications or supplements have been started in the past week.

Harvard Food Frequency Questionnaire 2007 Booklet (Harvard FFQ). The Harvard FFQ (Willet et al., 2007) is a comprehensive 101-item semi-quantitative food frequency questionnaire that includes questions on specific foods, diet types, and supplements. Analysis of the questionnaire provides a wide range of macro- and micronutrient quantities. A number of studies have demonstrated fair to good validity and fair to good reproducibility depending upon nutrient of interest.

Irritable Bowel Severity Scoring System (IBS-SSS). The IBS-SSS (Francis et al., 2003) is a 5-item questionnaire used as a multidimensional measure of severity of abdominal pain, frequency of abdominal pain, severity of abdominal distension, dissatisfaction with bowel habits and interference with quality of life.

Modified Morisky Medication-Taking Adherence Scale (MMAS). The MMAS is available as a four or eight item questionnaire that is designed to test whether or not a subject adheres to taking a particular medication or supplement. Both the 4 and 8 item versions of the MMAS have been shown to demonstrate concurrent and predictive validity in regard to the measurement of patient adherence in clinical studies (Morisky et al., 1986, Morisky et al., 2008). Our modified scale simply alters the word medication to supplement, with permission from the author of the scale.

Modified Treatment Satisfaction Questionnaire (TSQM v1.4). The modified TSQM v1.4 is a nine-item questionnaire that is designed to measure side effects associated with the supplement itself and the ease of administration of the supplement. The TSQM has been found

to be a valid measure of the major components of satisfaction with a medication (Atkinson et al., 2004). We have modified the TSQM so that it may be applied to dietary supplements with the permission of the authors of the scale.

Neurobehavioral Symptom Inventory (NSI). The NSI (Cicerone & Kalmar, 1995) is a widely used measure of post concussive symptoms among military personnel and Veterans and is recommended by the Veterans Health Administration for screening and evaluation.

Ohio State University TBI Identification Method (OSU TBI). The OSU TBI-ID is a structured interview developed using recommendations from Centers for Disease Control (CDC) for the detection of history of exposure to TBI. It was designed to elicit self-reports of TBI occurring over a person's lifetime. In a study including 119 participants, the OSU TBI-ID was found to have high inter-rater reliability. From this study and another including 103 participants, the authors concluded that the results provide preliminary support for the reliability and validity of summary indices of lifetime history of TBI elicited via a structured interview (Corrigan and Bogner, 2007). This study will use the OSU TBI-ID to identify whether a participant has experienced a moderate or severe TBI that would exclude them from the study. For participants who are re-consented, we will inquire if there have been any injuries since their first assessment.

Pittsburgh/Epworth Sleep Questionnaire. Sleep, quality of sleep and sleepiness (Johns et al., 1991; Buysse et al., 1988) using a rating scale from 0-3 (0= would never doze to 3=high chance of dozing) with which to assess the chance of dozing in various situations. In addition, sleep quality and frequency of sleep disturbance will be assessed using a frequency chart ranging from "not during the past week" to "three or more times a week".

Probiotic Food Checklist. The Probiotics Checklist for food was created from a list of probiotic food products and supplements developed for a Scoping Review regarding probiotics and mental health. As the list was originally created with the rigor appropriate for a systematic literature search, it encompasses foods from a variety of regions and diets. The list is being developed into a checklist for this pilot study with the goal of inventorying the amount of probiotics already present in the participant's regular diet. The measure will query weather the participant has consumed a particular probiotic containing food product in the last month, and if so, how often in the past week and past month. If this checklist shows that a participant is consuming a significant amount of probiotics on a regular basis before study initiation, the participant may be excluded from the study or re-screened after 2 weeks (See "Exclusion Criteria").

Probiotics Usage Log. Participants will be provided with a handout to document the date, time, and amount of each probiotic dose. On this same sheet there will be space provided to document symptoms and their severity; definitions to indicate mild, moderate, or severe interference are provided. The document is modeled after the Dana-Farber Harvard Cancer Center (DF/HCC) Oral Chemotherapy Drug Diary.

Psychological Stress Measure 9 (PSM-9). The PSM-9 will assess the participant's perception for stress appraisal (Lemyre & Tessier, 1988 and 2003).

Rivermead Post Concussive Symptom Questionnaire (RPCSQ). The RPCSQ (King et al., 1995) is a brief measure of post concussive symptom severity. Individuals completing the measure are asked to compare any current symptoms to pre-injury levels. The measure has demonstrated good inter-rater and test-retest reliability and internal construct validity.

Rocky Mountain MIRECC Demographic Questionnaire. Information will be gathered on topics such as participant age, gender, race/ethnicity, education, period of military service, and combat exposure.

Rome III Diagnostic Questionnaire. The Rome III Diagnostic Questionnaire (Rome Foundation, 2009) classifies disorders of the digestive system.

Structured Clinical Interview for the Diagnostic and Statistical Manual of Mental Disorders -IV-TR Axis I Disorders, Research Version, Patient Edition With Psychotic Screen

(SCID-I/P W/ PSY SCREEN). The SCID (First et al., 2002) is a reliable and valid semi-structured interview used to diagnose Axis I psychiatric disorders in clinical and research settings

Trier Social Stress Task (TSST). Our procedures are entirely consistent with the extensive TSST literature and have been used routinely in Dr. Brenner's laboratory and by her collaborators at CU Boulder. Biological markers (e.g. blood pressure) will be collected. Specific procedures will be outlines in an internal SOP document.

Single question regarding perception of treatment type (probiotic or placebo): After all measures have been completed, the individual will be asked to state which type of supplement he/she received (probiotic or placebo) and this will be noted by researcher. The researcher will then inform the participant that they will receive a letter at the end of the study (upon completion of data collection and analysis) revealing which supplement they received during their participation in the study.

University of Washington Risk Assessment Protocol-Revised (UWRAP). The UWRAP (Reynolds, Lindenboim, Comtois, Murray, & Linehan, 2006) will be used to assess and address any potential risk associated with participating in the study. Participants will be asked to articulate pre-test potential stressors (Pre-Assessment Risk Assessment Questions). Post-test administration, a debriefing checklist and protocol will be initiated. Using results from the debriefing, members of the research team will be trained to evaluate responses and access additional assistance if necessary. The UWRAP has been recommended by National Institute of Mental Health, and has been used in over 20 years of research with potentially high-risk patients. The UWRAP will be utilized pre and post Visits 1 and 3.

Visual Analog Scale (VAS). Stress will be measured during the TSST using a VAS. This psychological stress marker will work in conjunction with the physiological stress markers described above.

Participants will also have blood samples collected to obtained information regarding:

Inflammation. We will use high sensitivity ELISA assays, conducted in duplicate wells, from R&D Systems to measure a biological signature of exaggerated inflammation. Biweekly assessment of baseline plasma hsCRP concentrations for determination of eligibility of subjects for treatment allocation will be done using R&D Systems hsCRP ELISA (Cat. No. DCRP00). Plasma concentrations of hsCRP, TNF-α (Cat. No. HSTA00D; R&D Systems), IL-6 (Cat. No. HS600B; R&D Systems), and IL-8 (Cat. No. HS800; R&D Systems) will be measured at Time 2 and Time 3 assessments. The baseline CRP assay may be conducted at the University of Colorado Hospital CTRC. The remaining assays will be conducted in the Lowry lab at CU-Boulder. However, if there are throughput challenges with obtaining results, initial or follow-up assays may be conducted at either lab. Both labs have extensive experience conducting ELISA assays for measurement of plasma cytokine concentrations in clinical samples.

Gut Permeability. Total quantity of bacterial cell copy numbers in plasma samples will provide measurement of gut permeability (Abad-Fernandez et al., 2013). Plasma will be separated from cells via chilled centrifugation. DNA extraction will be conducted with the Qiagen DNA EZI mini kit per manufacturer recommendations. Amplification and measurement of DNA in triplicate will be conducted with universal bacterial primers and SYBR® green qPCR supermix on the Applied Biosystems 7500 Real-Time PCR System. Standard curves will be created concurrently to plasma bacterial sample measures with purified *E. coli* DNA, concentrated over six orders of magnitude. Additional quality control measures will include melt curve analysis and verifying no amplification of negative controls.

5. Security, Risks, and Justifications

A. Protected Health Information (PHI)

PHI to be collected includes first and last name, date of birth, telephone number, and last 4 digits of social security number. This information is needed in order to accurately document each participant's study involvement into the electronic medical record. The participant will not be identified by name in any study reports, which will be used for research purposes only. The Denver VAMC Research Department may inspect the records of this study. COMIRB may inspect the PHI. Every effort will be made to keep the participants' personal medical data confidential. If, following screening or eligibility assessment, an individual is determined ineligible to participate, the only information to be kept will be non-PHI demographic descriptors for comparative purposes. These descriptors will include age range, gender, and race/ethnicity, in addition to their reason for exclusion.

B. Estimated duration of study

Each participant will complete approximately 4 hours of laboratory testing. The laboratory procedures will be completed over three visits to the ECHCS. The 2-week run in period is expected to take participants 15 minutes each week. The check-ins and usage logs are estimated to take 35 minutes per week. It is expected to take approximately 2 years to accrue and evaluate participants for the entire study.

C. Data Security and Storage

For the purposes of this research project a screening database and master record database will be developed as well as a limited data set. The screening database will contain subject identification, such as name, last 4 digits of the Social Security Number, and telephone number. Upon enrollment in the study, the participant and their identifying information will be added to the master record database. There will be no clinical data stored in either database. A unique identifier (UI) will be assigned to each of these records. Both databases will be password protected with only Dr. Brenner and Research Study Coordinator(s) knowing the password. Eligible participants' screening and verbal consent form will be kept with the participant's informed consent, HIPAA authorization, and medical clearance form per VA requirements. Ineligible participants' screening and verbal consent form and medical clearance form will be kept in a separate paper research folder. Screening and verbal consent forms for potential participants who were not eligible and/or interested in the study will remain de-identified and stored in a separate research folder, located in a locked filing cabinet in secure MIRECC offices within the Denver VAMC. Only authorized members of the research team will have access to paper documents. Forms containing PHI will be stored in a locked filing cabinet separate from the rest of the paper data.

A dataset including study variables and limited PHI (i.e., dates) obtained from either the measures or medical records will be stored on the VA file server. The UI from the master database will be used identify records in this limited data set. All data will be password-protected and stored within the VA firewall in a Microsoft Access database. The data can only be retrieved from within the VA network and only by members of the research team. These data will not be kept on laptops that do not meet the VA standards for encryption, anti-virus protection, and firewall security. Data will not leave the ECHCS campus except as an aggregate dataset. Paper data will be stored in locked file cabinets in a locked office.

Study data may also be managed using REDCap (Research Electronic Data Capture). REDCap is a secure web application approved by the VA Office of Information & Technology (OI&T), and designed to support data capture for research studies, providing user-friendly web-based case report forms, real-time data entry validation (e.g. for data types and range checks), audit trails and a de-identified data export mechanism to common statistical packages (SPSS, SAS, Stata, R/S-Plus). The system was developed by a multi-institutional consortium which includes the Department of Veteran's Affairs and was initiated at Vanderbilt University. The database is hosted on VA Informatics and Computing Infrastructure (VINCI) virtual servers.

REDCap is flexible enough to be used for a variety of types of research and provides an intuitive user interface for database design and data entry.

All data and statistical endeavors will be performed with assistance from the Denver MIRECC Data and Statistical Core. Procedures designed to maintain confidentiality will include formal training sessions for all study personnel in the importance of confidentiality and procedures to be followed, as well as formal mechanisms for limiting access to all information that can link data to individual participants.

After centrifugation and separation of plasma, specimens will be coded and "blinded" and plasma will be stored in -80°C freezers at the Denver VA, and, once data collection has completed, specimens will be securely delivered to University of Colorado Hospital CTRC or University of Colorado Boulder Behavioral Neuroendocrinology Laboratory (WOC and Co-I Christopher Lowry). Blood samples will be analyzed and destroyed per VA policy. To minimize biohazard risk for others, the use of gloves, biohazard bags, and biohazard coolers will be assured, as well as documentation of relevant TMS training for all VA staff will be in place for every personnel handling blood or blood products.

D. Risks

Veteran participants may become upset or frustrated during administration of the interviews or questionnaires due to the nature of the topics of the study. Although it is not believed that participating in this study places the participants at increased risk for suicide, the UWRAP will be utilized to assess and address potential risks with participating in the study. Participants will be informed that the researcher will review their responses to the items regarding safety, and if necessary, will discuss these responses with them in an effort to ensure their safety. For participants who reveal clinically meaningful risk as indicated on the UWRAP, specifically if the participant rates suicide risk at or higher than a rating of 4 on the Pre-Assessment Risk Assessment (page 2 of the UWRAP), study personnel will review, and if necessary, will discuss these responses with them to ensure their safety. Responses may be also shared with the Veteran's treating clinician, if determined necessary by Dr. Brenner or her designee. Additional mental health interventions, such as escorting the Veteran to the Psychiatric Emergency Services will be determined at this time, by Dr. Brenner, her designee, or the treating clinician and appropriate notation will occur.

All researchers who will be interacting with study participants are trained in the area of risk assessment and management, and will be receiving supervision from Dr. Lisa Brenner, a licensed clinical psychologist, or her designee.

Immediately following completion of study measures the participants will undergo a debriefing and post-assessment risk. The results of this assessment will be used to evaluate the participant's suicide risk. At a time deemed clinically appropriate, the PI or his/her designee will be notified if a participant is judged to be at either elevated (with respect to the participant's baseline level of risk) or imminent suicide risk. Responses may be also shared with the Veteran's treating clinician, if determined necessary by Dr. Brenner or her designee. Assessors will have emergency contact information available at all times for the PI or designee and emergency services (e.g. VA National Veterans Crisis Line). For assessments done over the phone, as clinically indicated, assessors will stay on the telephone with the participant until they are able to consult with the PI or designee, determine next steps, and follow-through with them. Assessors will ask participants to provide the assessor with the address of the location they are at while completing the phone assessment so that the assessor can direct emergency services as needed to the participant's location.

If a participant is judged by clinical impression to be at *imminent* suicide risk during the:

All visits (at the Denver VA facility):

- If the assessment occurs at the Rocky Mountain MIRECC, the participant will be escorted to Psychiatric Emergency Services, where s/he will be assessed for risk and potential need for hospitalization.

All visits (over the telephone):

- If the assessment occurs over the telephone, the assessor will immediately contact 911 to initiate an on-site rescue if such action is clinically indicated. As clinically indicated, the assessor may stay on the phone with the participant until emergency services arrive.

If, based on clinical impression, a patient appears to be at *elevated but not imminent* risk of suicide with respect to the participant's baseline level of risk during the:

All visits (at a Denver VA facility):

- The assessor will contact the PI or her designee to complete a risk assessment and facilitate the Veteran accessing additional resources (e.g., Psychiatric Emergency Services, Veteran's Crisis Line) as is clinically indicated.

All visits (over the telephone):

- The assessor will contact the PI or her designee to complete a risk assessment and facilitate the Veteran accessing additional resources (e.g., Psychiatric Emergency Services, Veteran's Crisis Line) as is clinically indicated.

Assessors and clinicians will receive training and supervision on suicide risk assessment and suicide crisis procedures. These procedures include reporting these incidents to the PI and initiating appropriate emergency response interventions as indicated. For any participants who demonstrate high levels of emotional distress, suicide, or self-harm risk, appropriate follow-up actions outlined above will be implemented. Members of the research team will be trained to assist participants in completing the protocol while minimizing psychological distress. Further, Dr. Brenner, a Licensed Clinical Psychologist, or her designee, will assist if difficulties arise. There are no other risks to completing study measures known to the investigators.

Although Lactobacilli are "generally regarded as safe" (GRAS), there is theoretical risk associated with their use. These theoretical risks include the potential for transmigration, negative impact on gastrointestinal physiology and function, and the potential for antibiotic resistance transfer within the gastrointestinal tract. These risks are indeed largely theoretical and, in cases where evidence is available, occur in immunocompromised individuals or in those with specific gastrointestinal diseases; such individuals are excluded in the present study (Snydman, 2008). Involvement in the study requires a medical clearance form be signed by each participant's VA health care provider and a 2-week run in period. Veterans with certain medical conditions will be excluded from the study (see Exclusion criteria). As this is an acceptability and feasibility pilot study, any potential adverse events will be closely monitored and documented.

In the unlikely event of prolonged bleeding during blood sampling, local pressure will be applied to achieve hemostasis. In the unlikely event of local infection, the subject will be advised to seek appropriate medical evaluation and treatment.

E. Benefits

There are no direct, guaranteed benefits to Veterans participating in this study. Increased knowledge regarding the potential benefits and feasibility of future trials using probiotic supplementation for Veterans will be obtained. Findings from this study will be shared with mental health and other health care communities through journal publications and conference presentations. Such knowledge may contribute to treatment options and inform future studies.

F. **Data Analysis**

As this study is a pilot study the primary outcomes to be tracked will speak to feasibility and the effects on Veterans health via the outcome measures. Specifically, outcome measures of interest will be evaluated to detect differences between active and placebo groups. All measures will be scored using standard methods for each to assess within- and between subject changes. *Missing Values:* In instances in which participants fail to complete occasional items on questionnaires, scale scores will be prorated. If participants omit numerous or all items on a particular measure, their scale scores will be set to missing values and they will be omitted from analyses involving that measure.

G. Safety Monitoring Plan

Veteran participants may have the potential to become frustrated, fatigued, or distressed during the assessments. Although it is not believed that participating in this study places the participants at significantly greater risk than daily living, the UWRAP will be utilized to assess and address potential risks with participating in the study. Immediately following completion of study measures, the participants will undergo a debriefing and post-assessment risk evaluation. If a participant's risk or another individual's risk is elevated at pre- or post- testing we will access additional assistance from Dr. Brenner or another MIRECC licensed mental health professional designated by Dr. Brenner, or Psychiatric Emergency Services.

Probiotics are safely being used in clinical practice as well as other research arenas and are readily available in many pharmacies and health food stores. Millions of people around the world consume probiotics daily in hopes they will confer a health benefit (Snydman, 2008). Side effects associated with probiotic use are being tracked throughout the study as the primary aim of this study is to document the acceptability and feasibility of probiotic use in the Veteran population. Further, participants are educated about the potential side effects associated with probiotic use before agreeing to participate. As such, minor side effects typically associated with probiotic use, such as flatulence, will not be formally submitted to the Colorado Multiple Institutional Review Board (COMIRB) as Adverse Events per say. Any significant or severe adverse events will be reported per COMIRB guidelines.

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