# Rationale, design, and methods: A randomized placebo-controlled trial of an immunomodulatory probiotic intervention for Veterans with PTSD

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## ABSTRACT

*Background:* United States military Veterans from recent conflicts are experiencing symptoms related to post-traumatic stress disorder (PTSD). Many Veterans are resistant to conventional health and mental health interventions (e.g., medication, psychotherapy). Alternative treatment approaches are needed. An underlying feature of PTSD is exaggerated inflammation, both peripherally and in the central nervous system. This inflammation is thought to play an important role in the vulnerability to, aggravation of, and persistence of PTSD symptoms. Therefore, an innovative intervention strategy would be the use of immunoregulatory/anti-inflammatory probiotics to reduce inflammation. Here we describe the rationale, design, and methods of a randomized placebo-controlled trial (RCT) of *Lactobacillus rhamnosus* GG (LGG; ATCC 53103) for posttraumatic stress disorder (PTSD).

*Methods:* This is a Phase IIa trial of LGG for United States military Veterans with PTSD, using a longitudinal, double-blind, randomized placebo-controlled design. The primary outcome measure is plasma concentration of high-sensitivity C-reactive protein.

*Conclusion:* Despite the fact that symptoms associated with PTSD can be disabling, individuals living with this trauma-related disorder have limited options in terms of evidence-based interventions. Recent research efforts aimed at highlighting the biological mechanisms of PTSD suggest that increased inflammation and altered autonomic nervous system activity may be treatment targets, and that immunoregulatory probiotics, such as LGG, have the potential to decrease trauma-induced inflammatory responses, as well as associated symptoms. This manuscript describes the best powered human subjects Phase IIa trial, to date, of a probiotic intervention for those living with PTSD.

## 1. Introduction

Since October of 2001, approximately 2.7 million troops have been deployed in the recent conflicts, with many reporting mental health concerns upon return [1,2]. Posttraumatic stress disorder (PTSD), a trauma-related disorder that can result from exposure to an event that poses actual or threatened death or injury, has become increasingly prevalent. Although the estimated lifetime prevalence of PTSD in the United States (US) adult population is approximately 8.7% [3], findings suggest that approximately 20% of returning Service Members meet criteria for PTSD or associated mental health conditions [4]. Chronic PTSD symptoms associated with disruptions in the regulation of emotional and cognitive processes, including hypervigilance, low frustration tolerance/irritability, and impaired decision-making [5], can pose significant challenges for Service Members and Veterans. Thus, there is an ever-increasing need for the identification of new treatment options that are implementable, safe, and non-stigmatizing.

Treatment guidelines for PTSD commonly center around forms of psychotherapy, such as exposure-based or cognitive restructuring interventions [6]. However, traditional treatments can be ineffective, with non-response rates in outcome studies often reaching as high as 50% [7]. Further, many Operation Enduring Freedom/Operation Iraqi Freedom/Operation New Dawn (OEF/OIF/OND) Veterans can be resistant to engaging in conventional mental health treatments [8], highlighting the importance of exploring alternative interventions. Use of complementary and alternative medicine (CAM), particularly for symptoms associated with stress/trauma, has quickly risen in the US. Interestingly, research suggests that Veterans with PTSD may be more accepting of CAM approaches than those without this condition [9]. Taken together, these data indicate the need to explore novel CAM intervention strategies that can target mechanisms underlying PTSD symptomology.

Recent research efforts aimed at elucidating potential biological mechanisms of PTSD have facilitated the development of such novel interventions. Psychological trauma is known to be associated with altered autonomic nervous system activity [10] and impaired regulation of the hypothalamic–pituitary–adrenal (HPA) axis and insufficient glucocorticoid-signaling [11,12]. Evidence for inflammatory drivers of trauma-related disorders has also been accumulating [13,14]. For example, increased circulating levels of interleukin (IL)-6 immediately following trauma exposure have been found to predict the later development of PTSD symptoms [15]. Although not confirmed in all studies, low-grade inflammation has been shown to be associated with PTSD and physiological stress responses, as indicated by elevated serum C-reactive protein (CRP), IL-1β, IL-6, IL-12, interferon gamma (INFγ) and tumor necrosis factor (TNF) [16–24]. In prospective studies, high baseline plasma concentrations of CRP, measured pre-deployment among Military personnel, were associated with an increased likelihood of having PTSD symptoms post-deployment, suggesting that inflammation prior to trauma exposure may predispose individuals to developing a trauma-related condition [25]. In line with this finding, machine learning approaches have demonstrated that among features assessed prior to deployment, biomarkers of inflammation, including CRP, were among the highest-ranking features predicting PTSD symptoms following deployment [26]. Gene expression profiles of peripheral blood mononuclear cells (PBMCs) 1- and 4-months following trauma distinguish trauma survivors with PTSD from those without PTSD, signifying that altered immune functional status following trauma exposure is also predictive of PTSD [18]. Moreover, individuals with PTSD show enhanced spontaneous secretion of IL-1β, IL-6, and TNF from PBMCs, which correlate with symptom severity [18,27]. Anti-inflammatory interventions have been successfully used among those with other psychiatric disorders [28–31]. Therefore, it is reasonable to suggest that anti-inflammatory or immunoregulatory agents may be a useful intervention for treating symptoms of PTSD.

The use of probiotics, or microorganisms deemed beneficial for human health, as a method for reducing inflammation has become a growing area of interest. *Lactobacillus rhamnosus* GG (LGG) is a gram-positive strain of the *Lactobacillus rhamnosus* species that is found in the human gut microbiome. LGG is immunoregulatory, increasing peripheral regulatory T cells (Treg) and anti-inflammatory cytokines including IL-10, and decreasing proinflammatory cytokines such as IL-6 [32,33]. It is one of the most widely used probiotic strains [34] and has been studied in relation to gastrointestinal health, stimulation of the immune response to improve vaccination efficacy, and prevention of allergic symptoms [35–39].

Accumulating evidence also suggests that probiotics with anti-inflammatory and immunoregulatory properties, such as LGG [32,33], have the potential to decrease trauma-induced inflammatory responses, while being highly accessible, low-cost, self-sustaining (e.g., portable) and, based on previous safety and tolerability trials, without significant side effects [40]. Moreover, in our recent pilot randomized placebo controlled trial among OEF/OIF Veterans with PTSD and mild traumatic brain injury (mTBI), supplementation with *Lactobacillus reuteri DSM 17938*, another probiotic with anti-inflammatory and immunoregulatory properties, was found to be feasible, acceptable, and safe [40], and was associated with decreases in plasma CRP that approached statistical significance, as well as decreases in an objective measure of distress (stress-induced increase in heart beats per minute [BPM]) during the Trier Social Stress Test [40], a biomarker of autonomic stress responsivity [41,42].

Based on previous research [14,22,23,43], we created a working model of PTSD, with an emphasis on immunomodulatory factors (see Fig. 1). The presented clinical trial is based on our overarching theses that: 1) elevated inflammation increases risk of developing PTSD; 2) chronic inflammation and impaired immunomodulation perpetuate symptoms among those with PTSD; 3) exacerbation of inflammation is associated with increased severity of PTSD symptoms; and, 4) administration of an anti-inflammatory/immunoregulatory probiotic is likely to influence an individual's gut microbial community, and decrease intestinal permeability (IP) and systemic inflammation, as well as dampen autonomic responsivity and PTSD symptoms.

The goal of this Phase IIa placebo-controlled randomized controlled trial (ClinicalTrials.gov Identifier: NCT04150380) is to investigate the effects of daily oral administration of LGG (ATCC 53103) in a cohort of OEF/OIF/OND Veterans with PTSD as assessed by the gold standard Clinician Administered PTSD Scale-5 (CAPS-5) [44] who also have Functional Bowel Disorders (FBD), including irritable bowel syndrome. Outcomes include plasma concentration of high-sensitivity CRP (mechanistic, primary), and PTSD symptom severity (clinical, exploratory) [45]. Additional biological signatures will be considered exploratory. The primary hypothesis is that those who receive LGG supplementation will respond with lower plasma levels of CRP as compared to those allocated to a placebo supplement. Exploratory hypotheses include, as compared to those allocated to placebo, those who receive LGG supplementation will respond with: 1) decreased PTSD symptoms; 2) increased abundance of LGG and community-level shifts (e.g., increased alpha diversity) in the gut microbiota (measured using RT-qPCR and DNA sequencing of the 16S rRNA gene, respectively), decreases in IP (decreased fatty acid binding protein 2 [FABP2] and D-amino acid oxidase [DAO]), increases in plasma concentrations of anti-inflammatory biomarkers (IL-10, IL-4), decreases in additional plasma biomarkers of inflammation (IL-6, IL-8, IFNγ, IL-1α, IL-1β, and IL-12p70), reduced stress responsivity (biological and psychological) during and after participation in the Cyberball task, and improved decision-making (measured by performance on the modified Iowa Gambling Test [mIGT]); and, 3) the effect of LGG supplementation on stress responsivity, decision-making, and PTSD symptom severity will be mediated by effects of LGG supplementation on the gut microbiota, IP, and plasma biomarkers of inflammation.
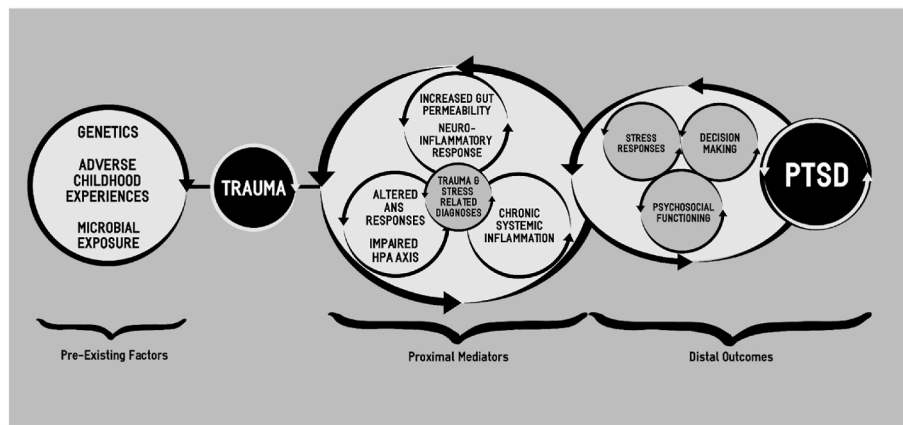
**Fig. 1.** Working model of PTSD with an emphasis on immunomodulatory factors in the etiology and pathophysiology of PTSD. The model includes the influence of pre-existing factors that contribute to individual variability in physiological and psychological responses to trauma, leading to development and persistence of PTSD in a subset of individuals. Abbreviations: ANS, autonomic nervous system; HPA, hypothalamic-pituitary-adrenal; PTSD, posttraumatic stress disorder.

## 2. Materials and methods

### 2.1. Study design

The described Phase IIa trial will examine the effects of supplementation with LGG, a probiotic with anti-inflammatory and immunoregulatory properties, using a longitudinal, double-blind, randomized placebo-controlled design. It is expected that up to 215 Veterans will participate in initial evaluation procedures, with 118 being randomized to one of two interventions (probiotic or placebo supplementation; see Consort Diagram, Fig. 2). Randomization will be stratified by sex. This study is being conducted according to the guidelines outlined in the Declaration of Helsinki and all procedures involving human participants were approved by the Colorado Multiple Institutional Review Board (COMIRB).

At Time 1 (T1, Initial Study Assessment), diagnoses of PTSD and functional bowel disorder (FBD) will be confirmed. A blood draw will be conducted to identify an individual's baseline CRP level for inclusion, based on plasma CRP concentration >1.0 mg/L, the clinical threshold for average risk of cardiovascular disease [46]. Between Time 1 and Time 2 (T2, Assessment and Randomization Visit), participants will keep a pre-supplementation daily diary of gastrointestinal (GI) symptoms for approximately 2 weeks. At T2, data will bei collected regarding group profile/potential confounding variable measures, psychological outcomes, and biological signatures. Between T2 and Time 3 (T3, End of Study Period Assessment), individuals will supplement with LGG or placebo (for 8±2 weeks), and measures of tolerability, safety, acceptability, and feasibility will be collected. Data regarding diet, sleep, exercise, and PTSD symptoms will also be collected (via phone or email surveys). At T3, data will be collected regarding psychological outcomes (e.g., PTSD symptoms), and biological signatures (e.g., inflammation), as well as potential covariates (see Fig. 3, Participant Flow Diagram).

### 2.2. Probiotic and placebo interventions

LGG ATCC strain 53103 (Chr. Hansen A/S, $1.0 \times 10^{10}$ colony-forming units (CFUs)/capsule) is being used in the proposed study. Participants will be randomized to one of two conditions in which they are instructed to consume one LGG or placebo supplementation capsule daily, for 8±2 weeks.

Through traditional and molecular methods, our team will conduct independent testing regarding the purity and viability of the probiotic upon receipt of the capsules and after the expected shelf life. After initial receipt, a randomized subsampling of 10 capsules (~0.5%) will be analyzed, and another 10 pills will be stored in the lab under the same conditions as recommended to the participants in the study. An identical process will be conducted for placebo pills to verify absence of LGG. The viability and CFUs of the probiotic and placebo will be confirmed against the manufacturer's documentation by culturing on *Lactobacillus* selection agar plates. Furthermore, the purity and quantity of the probiotic will be confirmed against the manufacturer's documentation by reverse transcription quantitative polymerase chain reaction (RT-qPCR).

### 2.3. Study population

Potential participants will be willing and eligible US military Veterans between the ages of 18 and 60 from the following populations: 1) those seeking outpatient mental health, rehabilitative, psychological, or other services within a VA Health Care System; 2) those in existing clinical and research databases; and 3) Veterans in the community not seeking care at the VA. Inclusion and exclusion criteria are presented in Table 1.

### 2.4. Measures and variable definitions

For a full list of measures and variable definitions, see Table 2.

### 2.5. Outcome measures

The primary mechanistic outcome for this study is plasma concentration of high-sensitivity CRP. Exploratory outcomes include: 1) PTSD symptom severity (PTSD Symptom Checklist for DSM-5; PCL-5) [47]; 2) type and abundance of LGG and community-level gut microbiota (measured using qRT-PCR and DNA sequencing of the 16S rRNA gene); 3) IP (FABP2 and DAO; 4) plasma concentrations of IL-10, IL-4, IL-6, IL-8, IFNγ, IL-1α, IL-1β, and IL-12; 5); biological and psychological stress responsivity during and after participation in the Cyberball task [48]; and, 6) decision-making (mIGT). All outcomes are listed in Table 2.

### 2.6. Safety monitoring

<u>Adverse Events from the Study Drug.</u> Participants will be asked about GI symptoms throughout the study as identified in procedures. Participants will be asked about new symptoms, adverse events and any new medications or supplements during the weekly check-in phone call using the Generic Assessment of Side Effects (GASE)/Generic Assessment of Side Effects – Probiotics (GASE-P).

<u>Suicide Risk Assessment and Management.</u> All research staff will also complete Veterans Affairs (VA) required research trainings. Study staff will contact the PI or her clinical designee if other concerning information regarding the participant's safety or the safety of others is identified during administration of the protocol. Assessors will have
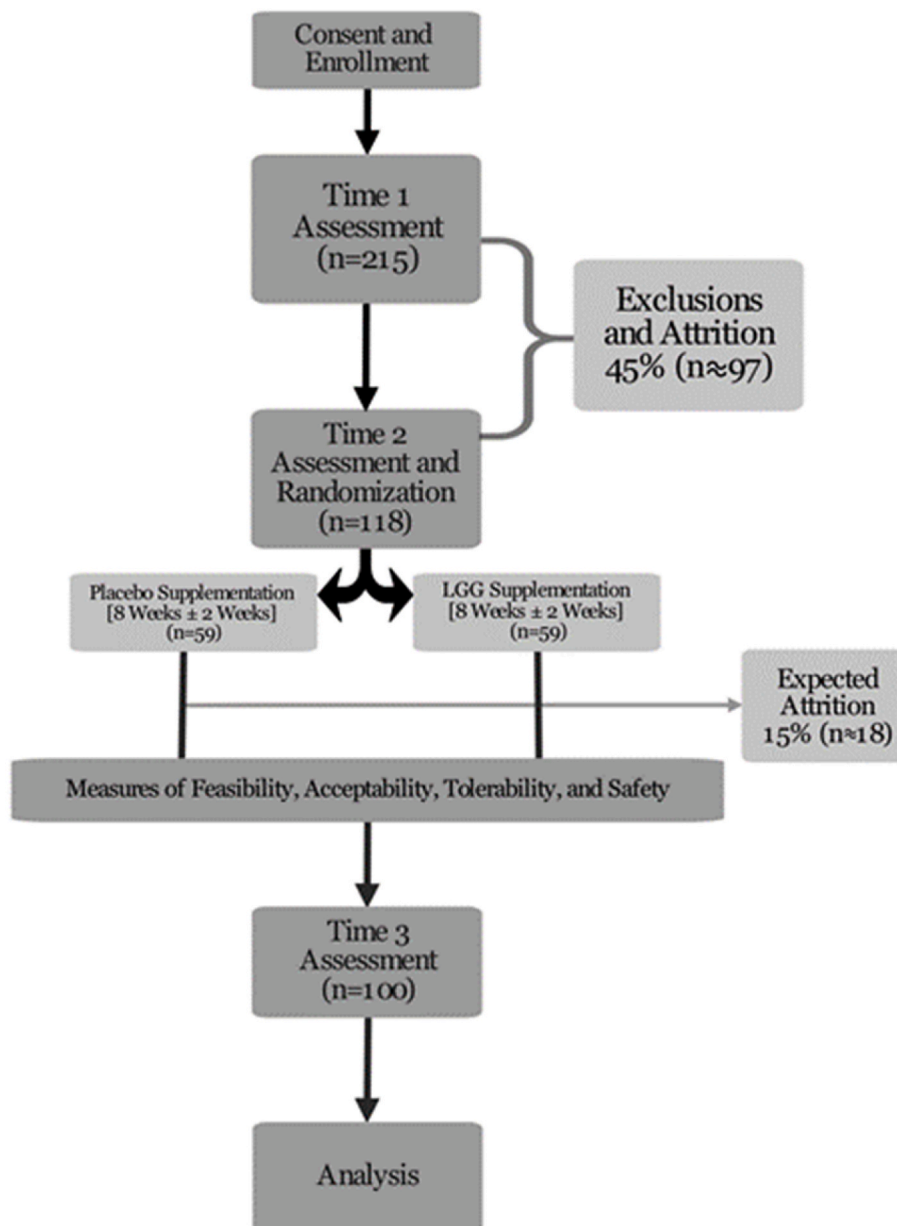
**Fig. 2.** Consort diagram. Abbreviations: LGG, *Lactobacillus rhamnosus* GG.

emergency contact information available at all times for the PI or designee, as well as crisis management and emergency services (e.g., VA National Veterans Crisis Line, Psychiatric Emergency Services, 911). If contacted, the PI or her clinical designee will conduct a more thorough risk evaluation and will initiate appropriate follow-up procedures.

For assessments conducted over the phone, when indicated, assessors will stay on the telephone with the participant until they are able to consult with the PI or clinical designee, determine next steps, and follow through with them. Prior to beginning the phone assessment, assessors will ask participants to provide the address of the location they are at while completing the phone assessment so that the assessor, after consulting with the PI or designated clinician, can direct emergency services to the participant's location when warranted. With respect to confidentiality, prior to participating in the study, informed consent documents will contain statements explaining mandatory reporting requirements for information regarding intention to harm self or others. In situations where confidentiality is breached due to threats to participants' or others' safety (e.g., suicide or homicide risk), precautions will

be taken to protect their privacy by limiting the information being disclosed to only the minimum information necessary to ensure the safety and welfare of participants and others affected.

Adverse Events. Significant adverse events are not expected. For this study, adverse events are defined as symptoms reported by the participant that are directly related to participation in the study. The study PIs will be notified of any complaints or reports of adverse events. Data and safety monitoring will be regularly reviewed by the study personnel and the Study PIs to ensure protocol adherence and participant safety. Any unexpected serious adverse events (uSAEs), defined as death, life threatening illness, suicide attempt, hospitalization or prolonged hospitalization, and persistent/significant disability, will be reported to COMIRB by the study PIs within 5 days of them being made aware. Other adverse events will be recorded and reported at continuing review. In addition, there is a Data Safety Monitor Board (DSMB) who will review data produced by the study team and will meet with the lead investigators and study statistician on a regular basis.
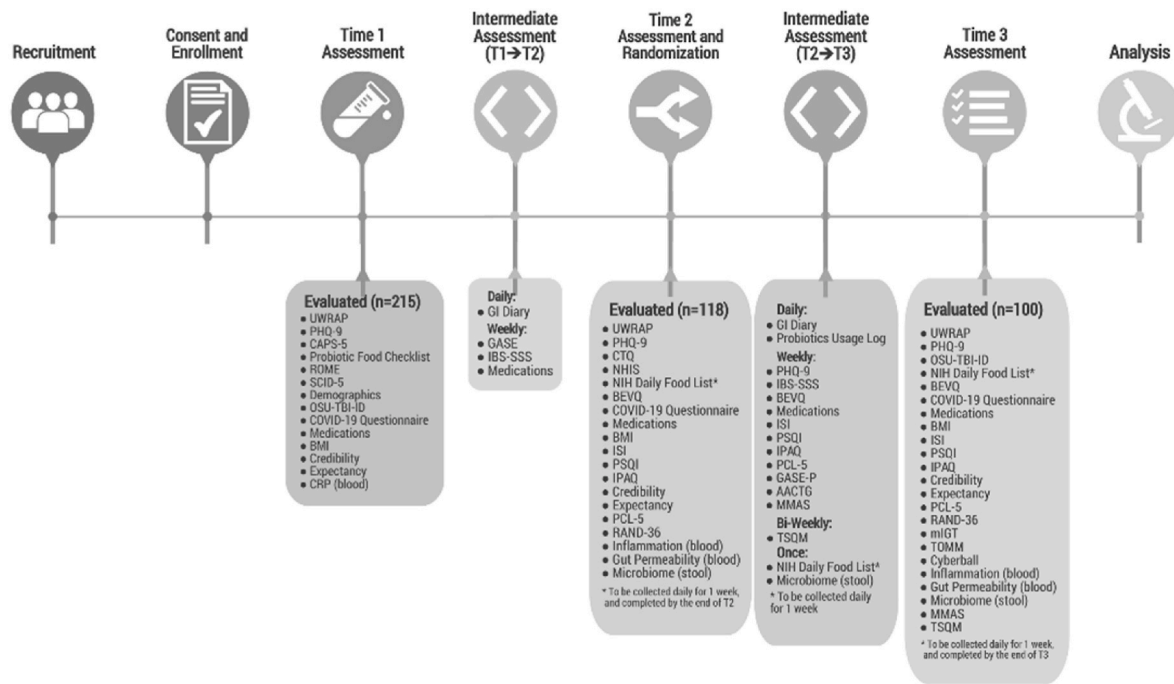
**Fig. 3.** Participant flow diagram. Abbreviations: AACTG, Adult AIDS Clinical Trials Group; BEVQ, Beverage Intake Questionnaire (BEVQ-15); BMI, body mass index; CAPS-5, Clinician Administered PTSD Scale-5; COVID-19, Coronavirus Disease 2019; CRP, C-reactive protein; CTQ, Childhood Trauma Questionnaire; GASE, Generic Assessment of Side Effects; GASE-P, Generic Assessment of Side Effects – Probiotics; GI, gastrointestinal; IBS-SSS, Irritable Bowel Syndrome Severity Scoring System; IPAQ, International Physical Activity Questionnaire; ISI, Insomnia Severity Index; mIGT, Modified Iowa Gambling Task; MMIS, Modified Morisky Medication-Taking Adherence Scale; NHIS, National Health Interview Survey (NHIS) - Chronic Conditions; NIH, National Institutes of Health; OSU-TBI-ID, Ohio State University Traumatic Brain Injury (TBI) Identification Method; PCL-5, Posttraumatic Stress Disorder Checklist for DSM-5; PHQ-9, Patient Health Questionnaire-9; PSQI, Pittsburgh Sleep Quality Index; RAND-36, 36-Item Short Form Survey (SF-36) - RAND Corporation; ROME, Rome IV Diagnostic Questionnaire for the Adult Functional GI Disorders; SCID-5, Structured Clinical Interview for DSM-5-Research Version (SCID-5 for DSM-5, Research Version; SCID-5-RV, Version 1.0.0); T1, Time 1 assessment; T2, Time 2 assessment; T3, Time 3 assessment; TOMM, Test of Memory and Malingering; TSQM, Modified Treatment Satisfaction Questionnaire; UWRAP, University of Washington Risk Assessment Protocol-Revised.

## 2.7. Study modifications during COVID-19 pandemic

Given challenges associated with in-person research due to the COVID-19 pandemic, the PIs made approved study modifications that allowed for continuance of the trial. Specifically, we modified T3 to include remote strategies both in terms of the stressor and strategy for measuring heart rate variability (HRV). This included switching from our original stressor, the Trier Social Stress Test [49] to Cyberball [48], which is a virtual ball-toss game that can be used for research on ostracism, social exclusion, or rejection. During Cyberball, the participant is told that they are playing a computerized ball tossing game with three other players over the internet: in reality, the other 'players' characters are controlled by the computer. After a few initial trials where the other players toss the ball to the participant, the other players then exclude the participant, only throwing to one another. This exclusionary experience has been found to be distressing to participants [50].

For HRV measurement, we will use the Firstbeat Bodyguard 2 (BG2) [51]. BG2 is a beat-to-beat heart rate monitoring device that is intended for short-term or long-term monitoring of heart rate variability and physical activity. The device records ECG with electrodes, processes the signal with an integrated algorithm and provides beat-to-beat R-to-R interval as an output with 1 ms resolution. The device easily attaches directly to the skin with two chest electrodes and starts recording data automatically. The BG2 has been designed for 24 h recordings. R-to-R timing data is downloaded from the BG2 to a computer for further analysis using standard HRV analysis software.

## 2.8. Data analysis

All analyses will use a two-sided test of hypotheses, with a significance level of 0.05 unless otherwise noted, and will be run in SAS v9.4 or higher, R v4.1.1 or higher, or QIIME2 2019.1 or higher. Group profile variables will be summarized as means and standard deviations (SD), medians and ranges, or *N* and percent and will be compared between those allocated to LGG supplementation and those allocated to placebo supplementation using *t*-tests, Wilcoxon rank-sum tests, chi-square tests and Fisher's exact tests, as appropriate. Group profile variables that are found to be significant at the $p < 0.10$ level and are considered as plausible potential confounders will be included as noted in the analysis plans below, although given randomization, the number meeting this criterion will be low. These may include demographics, history of TBI, childhood trauma, chronic health conditions, diet, beverage consumption, medications, BMI, insomnia severity, sleep duration and quality, and/or exercise. Data from the Test of Memory and Malingering (TOMM) [52,53] will be used to increase understanding regarding participants' ability to engage in mIGT testing. mIGT scores will not be used from those whose TOMM scores suggest poor effort/engagement.

The recruitment goal for this study is 59 participants per group. Anticipating a 15% attrition rate, we will have 50 participants per group for the final analysis. For the primary outcome of plasma concentration of CRP inflammatory biomarker, linear regression will be used to model the changes between T2 and T3, as a function of group, sex, the baseline value of CRP and any potential confounders identified as noted above. A similar analysis will be performed for the exploratory clinical outcome of PTSD symptoms (PCL-5 score). For the gut microbiome, linear regression will be used to model the changes between T2 and T3 both in alpha diversity, measured by Shannon Diversity Index, and in the gut

**Table 1**

Inclusion and exclusion criteria.

| Inclusion criteria |
| --- |
| History of at least one deployment in support of Operation Enduring Freedom/ Operation Iraqi Freedom/Operation New Dawn (OEF/OIF/OND) |
| Current diagnosis of PTSD per the Clinician Administered PTSD Scale-5 (CAPS-5) |
| Current diagnosis of a Functional Bowel Disorder including Irritable Bowel Syndrome [IBS]) by the ROME IV |
| CRP level of 1.0 mg/L or above at Time 1 |
| Medical clearance to participate by study providers |
| Age between 18 and 60 |
| Ability to provide informed consent |
| Willingness to abstain from probiotic supplements (pills, tablets, oils, foods, etc.) other than the investigational product provided until all study procedures are completed |
| Willingness to provide blood and stool samples |
| **Exclusion criteria** |
| Inability to adequately respond to questions regarding the informed consent procedure |
| Currently involved in the criminal justice system as a prisoner or ward of the state |
| Non-English speaking |
| Current (past month) alcohol or substance abuse or dependence, excluding marijuana |
| Lifetime history of bipolar disorder or psychosis or anxiety disorders |
| 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 |
| Receiving intravenous, intramuscular, systemic or oral antibiotics within the last month |
| Presence of central venous catheters (CVCs) |
| Gastrointestinal (GI) barriers as identified by the 2-week run-in period as determined by the study team |
| Participation in conflicting interventional research protocol |
| Vital signs outside of acceptable range, i.e., blood pressure >160/100, pulse >100 |
| Use of any of the following drugs within the last 6 months: antifungals, antivirals or antiparasitics (intravenous, intramuscular, or oral); oral, intravenous, intramuscular, or inhaled corticosteroids; cytokines or cytokine inhibitors; methotrexate or immunosuppressive cytotoxic agents |
| Acute disease at the time of enrollment (defer sampling until the participant recovers). Acute disease is defined as the presence of a moderate or severe illness with or without fever (e.g., oral temperature >100 °F) |
| Any medical condition deemed exclusionary by the Principal Investigators |
| History of cancer |
| Unstable dietary history as determined by the PIs (e.g., major changes in diet during the previous month, where the subject has eliminated or significantly increased a major food group in the diet) |
| Positive test for human immunodeficiency virus (HIV), Hepatitis B virus, or Hepatitis C virus |
| Any confirmed or suspected condition/state of immunosuppression or immunodeficiency (primary or acquired) including HIV infection, or those receiving immunosuppressive drugs or treatment including antineoplastic therapy, post-transplantation immunosuppressive therapy, and/or radiation therapy |
| 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 |
| Female who is pregnant or lactating |
| Treatment for or suspicion of ever having had toxic shock syndrome |
| History of moderate and/or severe traumatic brain injury |

microbiota community, quantified by Unweighted and Weighted Uni-Frac [54], pairwise distance between times for each individual, as a function of group, sex, the baseline value of the outcome, and potential confounders. Linear regression will also be used to model changes between T2 and T3 in IP (fatty acid binding protein 2 [FABP2], D-amino acid oxidase [DAO]), as well as plasma biomarkers of inflammation (IL-6, IL-10, IFNγ, IL-1α, IL-1β, IL-12p70, IL-8, and IL-4), and decision making as a function of group, sex, the baseline value of the outcome, and potential confounders. For stress outcomes from Cyberball (HRV, visual analog scale [VAS] and psychological stress measure [PSM]), linear regression will be used to model each outcome at T3 as a function of group. For each outcome measured at T2 and T3, the estimated mean difference in change between the groups will be reported with 95% confidence intervals (CI). For the stress outcomes measured only at T3 the estimated mean differences between the groups at T3 will be reported with 95% CIs. For the mediation analysis, we will use methods proposed by VanderWeele et al. for multiple mediators [55,56], examining whether the relationships between probiotic treatment and the

**Table 2**

Study measures/outcomes and variable definitions.

| Measure | Condition(s)/Factor(s) of Interest |
| --- | --- |
| Adult AIDS Clinical Trials Group (AACTG) | Supplement adherence |
| Beverage Intake Questionnaire (BEVQ-15) | Frequency/quantity of intake of beverages |
| Bristol Stool Scale | Number of daily bowel movements and consistency of stool |
| C-reactive protein (CRP)[a] | Inflammation |
| Clinician Administered PTSD Scale-5 (CAPS-5) | Current PTSD diagnosis |
| Childhood Trauma Questionnaire (CTQ) | Childhood abuse experiences |
| COVID-19 Questionnaire | Impact of COVID-19 pandemic and stressors related to the pandemic |
| Credibility Scale | Sense of supplementation credibility |
| D-amino acid oxidase (DAO)[b] | Intestinal permeability |
| Expectancy Scale | Expectations regarding supplementation |
| Fatty acid binding protein 2 (FABP2)[b] | Intestinal permeability |
| Generic Assessment of Side Effects (GASE) | Medication side effects |
| Generic Assessment of Side Effects – Probiotics (GASE-P) | Supplement side effects |
| Heart rate variability (HRV) | Physiological stress |
| Height and weight | Body Mass Index (BMI) |
| Interleukin (IL)-6, interferon gamma (IFNγ), tumor necrosis factor (TNF), IL-1α, IL-1β, IL-12p70, IL-8, IL-4, and IL-10[b] | Inflammation |
| Insomnia Severity Index (ISI) | Insomnia symptom severity |
| International Physical Activity Questionnaire (IPAQ) | Physical activity |
| Irritable Bowel Syndrome Severity Scoring System (IBS-SSS) | Severity and frequency of abdominal pain and abdominal distension, dissatisfaction with bowel habits, and interference with quality of life |
| Medications Log | Current medications and dosage |
| Modified Iowa Gambling Task (mIGT) | Decision making |
| Modified Morisky Medication-Taking Adherence Scale (MMAS) | Supplement adherence |
| Modified Treatment Satisfaction Questionnaire (TSQM v1.4) | Supplement side effects |
| National Health Interview Survey (NHIS) - Chronic Conditions | Chronic health symptoms |
| National Institutes of Health Daily Food List | Food intake |
| Ohio State University Traumatic Brain Injury (TBI) Identification Method (OSU TBI-ID) | Lifetime history of TBI |
| Patient Health Questionnaire-9 | Depression symptom severity |
| Pittsburgh Sleep Quality Index | Subjective sleep duration |
| Posttraumatic Stress Disorder Checklist for DSM-5 (PCL-5)[b] | PTSD symptom severity |
| Probiotic Food Check List | Consumption of probiotic-containing food products |
| Psychological Stress Measure (PSM)[b] | Perceived stress |
| Rocky Mountain MIRECC Demographic Form | Demographics |
| Rome IV Diagnostic Questionnaire for the Adult Functional GI Disorders | Digestive system disorders |
| Structured Clinical Interview for DSM-5-Research Version (SCID-5 for DSM-5, Research Version; SCID-5-RV, Version 1.0.0) | Axis I psychiatric disorders |
| Supplement Usage Log | Date, time, and amount of each probiotic dose |
| Test of Memory and Malingering (TOMM) | Memory |
| University of Washington Risk Assessment Protocol-Revised (UWRAP) | Risk assessment |
| Veterans RAND 36, 36-Item Short Form Survey (SF-36) - RAND Corporation | Health-related quality of life |
| Visual Analog Scale (VAS)[b] | Perceived stress |

[a] Primary outcome.
[b] Additional outcomes measures.

outcome measures (PTSD symptoms, stress response, decision making) are mediated by changes in the gut microbiome, IP, and inflammation.

Assuming a CRP standard deviation of 1.19 mg/L as observed in Brenner et al. [40], 50 participants per group provides 85% power to detect a difference in change between groups of 0.73 mg/L at the α = 0.05 level for the primary outcome.

## 3. Discussion

Military personnel's recent exposures in Iraq and Afghanistan have resulted in a new generation of Veterans with persistent symptoms of PTSD. Moreover, stressors associated with the COVID-19 pandemic have placed both patients [57] and health care providers [58] at risk for developing trauma-related disorders. As such, novel means of intervention are required. In particular, efficacious interventions that are portable, non-stigmatizing, and cost-effective, such as probiotics, would be welcome.

As highlighted above, nonresponse rates in outcomes studies of PTSD have been notably high [7]. Schottenenbauer et al. explored possible predictors of nonresponse and dropout and highlighted the writing of Hembree and Foa, which suggested that those who need therapy the most have the greatest likelihood of dropping out or not responding to care [59]. In response, some have suggested that a subset of individuals may benefit from pre-treatment (e.g., skills training) to decrease dropout. However, Hembree and Foa also noted that dropout appears to be at least in part related to the complexity of treatment [60]. An alternate means of addressing this challenge would be to introduce an intervention, such as probiotics, that could alter patterns of autonomic activity during exposure to stressful experiences [40]. This strategy may facilitate sustained treatment engagement, even during periods in which the content of material being discussed during sessions is distressing.

Moreover, efforts outlined as part of this clinical trial are aimed at increasing understanding regarding inflammation, the gut microbiome, autonomic functioning (i.e., HRV), and PTSD symptoms. Such knowledge is expected to highlight multiple potential interventions for PTSD, as well as other mental health-related conditions that involve inappropriate inflammation and dysregulated autonomic function, for example long-COVID [61]. Also of note, Reber et al. recently completed an animal study in which mice were treated with a heat-killed preparation of an immunoregulatory environmental microorganism, *Mycobacterium vaccae* NCTC 11659 [62]. Findings showed that animals that were treated with *M. vaccae* NCTC 11659 were significantly more resistant to stress-induced pathology, thereby suggesting that immunoregulatory microorganisms may also have a role in preventing trauma-based disorders [62].

## 4. Conclusions

In summary, the study outlined above is expected to provide low risk of bias data regarding the effects of LGG on multiple outcomes, including inflammation and PTSD symptoms. Given the increasing rate of PTSD, as well as current limitations of efficacious and frequently implemented treatments, these results are expected to provide evidence to inform alternate means of addressing the needs of the wide range of individuals living with or at risk for developing trauma-related conditions.

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## Declaration of interest

Dr. Lowry serves on the Scientific Advisory Board of Immodulon Therapeutics Ltd. and is a member of the faculty of the Integrative Psychiatry Institute, Boulder, Colorado. Dr. Brenner reports grants from the VA, DOD, NIH, and the State of Colorado, editorial renumeration from Wolters Kluwer, and royalties from the American Psychological Association and Oxford University Press. In addition, she consults with sports leagues via her university affiliation.

## Data availability

No data were used for the research described in the article.

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