Effect of Probiotics on Amino Acid Absorption- Study Protocol

IRB Approval No.: 2022180

Date: 16/02/2023

Aim: This project aims to investigate the effect of probiotics on amino acid absorption after the consumption of a plant-protein beverage.

Study Design:

This study is a randomized crossover study to assess the amino acid concentration in the blood after the consumption of a plant protein beverage. The plant protein beverage is formulated and developed in SIT. The study will take place over a duration of 10 to 12 weeks, following the schedule depicted below in Figure 1. The study has already been approved by SIT-IRB.

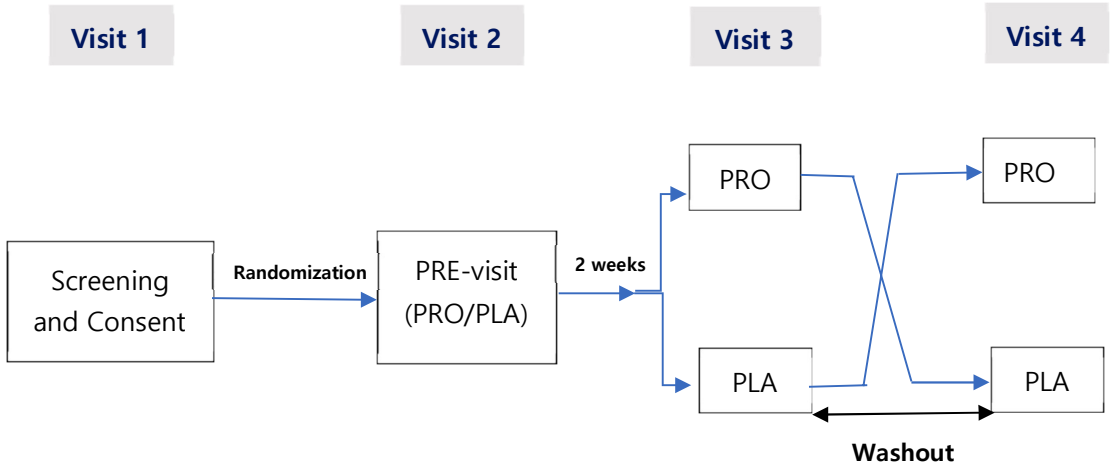

Figure 1: Schematic diagram of Study Visits

For this study, 20 healthy participants will be recruited. Inclusion criteria include healthy body weight (BMI 18.5-23kg/m^2), and lack of any underlying medical conditions. Exclusion criteria include any individuals who were treated for or diagnosed witha gastrointestinal, cardiac, respiratory, circulatory, musculoskeletal, metabolic, immune, autoimmune, haematological, neurological or endocrinological disorder, and having any form of food allergy/intolerances/avoidances, in particular allergies to eggs, soya, peanuts, tree nuts, crustaceans, and gluten. Participants are not allowed to consume any nutritional supplements known to affect measures of the current study for 8 weeks of supplementation and washout periods, including probiotics, prebiotics, synbiotics and/or digestive enzymes. Participants who unable to be weight stable defined as measured body mass deviating by 2% or more, and participants who were not willing to abstain from alcohol, nicotine, and caffeine for 12h prior to each study visit are also excluded.

Screening, Consent and Recruitment

Participants will be recruited using recruitment advertisements sent via mass email to SIT staff and students using email distribution lists. A briefing for all potential study subjects on the nature and purpose of the study, roles and responsibilities of the study subjects, study procedures, risk and benefits and other relevant information will be carried out. All potential subjects will be given a participant information sheet and informed consent form if they wish to participate in the study after the briefing. The PI will also be available for any clarification either through in-person or email.

Recruited participants will be provided a stool collection kit. Once stool sample is collected, participant will contact the study team for collection. The stool sample will be sent to AMILI's lab for analysis, for the prescription of personalized probiotics from among different probiotic cocktail formulations commercially

offered by AMILI.

PRE-visit

After informed consent, the study team will arrange for the participants to collect their personalized probiotics / placebo supplements and commence their supplementation period of 2 weeks before each study visit. Participants will be randomly assigned the probiotics or placebo supplementation arm using an online randomization software (https://www.randomizer.org/). Unblinding will only take place upon completion of all participants' study visit.

The PLA (placebo) capsules will consist of maltodextrin. The PRO (probiotics) supplements are commercially available products offered by AMILI. All study participants will complete the Lifestyle Questionnaire designed by AMILI and undergo a stool sample analysis to determine the which formulation of probiotics supplement they will receive. All participants will consume the PLA/PRO supplement capsules daily for 2 weeks.

Study Visits 1 and 2

At the end of each supplementation period, participants will report to SIT@Dover between 0800-1200 after an overnight 10-12h fast for their study visit (Visits 3 and 4 in Figure 1). There will be a washout period of 4 weeks between supplementations.

Both study visits will involve:

1. Anthropometric measurements.
   Weight and height will be measured and used to calculate Body Mass index. Body composition will be measured using bioimpedance analysis. Blood pressure and resting heart rate will also be taken. All measurements will be taken before blood draw.

2. Diet History
   A diet history will be performed at each study visit to obtain information about the usual dietary practices of participant. SIT Dietetics and Nutrition students will be engaged to assist in collecting this participant diet history information by the PI, Dr Verena Tan.

3. Serial blood draw
   Peripheral intravenous cannula will be inserted into antecubital vein for multiple blood sampling (5ml per collection). A baseline blood sample will be drawn at 0 min, after which participants will consume the beverage within 15 mins. Serial blood samples will be taken at 15, 30, 45, 60, 90, 120, 150 minutes after participant began consuming the plant protein beverage.
   After each 5 ml blood sample is drawn, the cannula will be flushed with 2 ml normal saline to ensure patency until the next blood draw time. Upon the completion of the blood draw and removal of cannula, the venipuncture site and overall condition of the participant will be examined before the participant leaves the SIT study site, to ensure that the participant is no longer bleeding and can stand up without dizziness.

4. Stool samples will be collected using a gut microbiome test kit provided by AMILI. Participants will submit their seconds and third stool samples on Study Visit 1 and 2 respectively.

Serial Blood Samples Analysis

Blood samples will be centrifuged at 1500xg for 15 min at 4˚C. The resulting serum/plasma will be aliquoted, and the resultant plasma stored at -80oC before analysis. Blood samples will be analyzed for amino acid profile as well as for glucose and insulin profile. After analysis, the samples will be disposed.

Stool Samples Analysis

A total of 3 stool samples will be collected from each participant to determine any changes in gut microbiome profile with probiotic/placebo supplementation. Stool samples will be stored in AMILI's laboratory prior to analysis and disposed post-analysis. Stool will be analyzed for microbiome diversity as well as functional pathways using shotgun metagenomic sequencing with the Illumina's DNA Library Preparation kit according to the manufacturer's recommended protocol. Differences in bacterial communities between pre-post samples from the same participants, and between groups with and without probiotic supplement after consumption of the plant-based protein will be compared. Taxonomic and functional profiles will also be generated.

Data Analysis Plan

Sample size was calculated based on a recent randomized, double-blind, multicenter crossover study. 15 healthy subjects showed 16- 26% increase in amino acid appearance in the blood following probiotic administration (Jäger et al., 2020). Assuming a drop-out rate of 20-30%, we plan to recruit 20 study subjects.

The area under the concentration vs. time curve (AUC) will be calculated for each of the 20 amino acids, as well as branched chain amino acids (BCAAs), essential amino acids (EAAs) and total amino acids, using the linear trapezoidal rule and using all available time points. AUC values will be comparedbetween conditions via paired t-tests. A p value of <0.05 will be considered as statistically significant.

References

Jäger, R., Zaragoza, J., Purpura, M., Iametti, S., Marengo, M., Tinsley, G. M., Anzalone, A. J., Oliver, J. M., Fiore, W., Biffi, A., Urbina, S., & Taylor, L. (2020). Probiotic Administration Increases Amino Acid Absorption from Plant Protein: a Placebo-Controlled, Randomized, Double-Blind, Multicenter, Crossover Study. Probiotics and Antimicrobial Proteins, 12(4), 1330–1339. https://doi.org/10.1007/s12602-020-09656-5