**Probiotics for Reduction of Exam Stress in Students (PRESS) Study**

**Rationale and Study Design**

**BACKGROUND**

There is increasing awareness of stress and the detrimental effect it has on both psychological wellbeing and physical health. This awareness has prompted interest in interventions that are designed to help people prevent stress, manage stress when it does occur and promote wellbeing. Many of these interventions in educational and employment settings include a focus on lifestyle improvements in exercise and diet.

The microbiota in the human gut are linked to the central nervous system, leading to a rapid increase in pre-clinical experiments suggesting that manipulation of the microbial composition with probiotics could enhance human health. Despite this, studies in human populations are required to investigate how supplementation with probiotics could improve wellbeing and health 1. Although previous studies have reported that probiotics can decrease negative psychological symptoms, the criticism of these studies has been small sample size [2].

We conducted a previous study with pregnant women in New Zealand and found that supplementation with *Lactobacillus rhamnosus* HN001 reduced scores on measures of postnatal depression and anxiety [3].

University students are a population who report stress, often associated with assessments and examinations. Japanese medical students supplemented with *Lactobacillus casei* (strain Shirota) had fewer gastrointestinal symptoms and cold and flu like symptoms during their examination period [4, 5].

**AIMS**

The overall aim of the PRESS Study is to investigate the effect of probiotic supplementation to reduce exam stress in university students. Specifically the study aims to examine whether supplementation with the probiotic *Lactobacillus rhamnosus* HN001:

1. Reduces the build-up of stress experienced by university students leading up to exams.
2. Reduces anxiety symptoms in students prior to exams.
3. Improves psychological wellbeing of students prior to exams.

**Strengths of the Study**

1. This will be the first study with a large enough sample size to detect a beneficial effect of probiotics for psychological symptoms of stress, anxiety and depression in students.
2. The probiotic *L. rhamnosus* HN001 has been shown to improve symptoms of anxiety and depression in pregnant women indicating that it is likely to have a beneficial effect for psychological wellbeing in this study.
3. The research processes have been trialled in university students demonstrating that students can be recruited, data collected and participants retained (details of the pilot trial below).

**STUDY DESIGN**

**The PRESS Pilot Trial**

The PRESS pilot trial was a small feasibility trial conducted in 120 first year university students between July and November 2019. The aim of this pilot study was to test research procedures (recruitment and online data collection) and inform a more accurate sample size calculation for the full PRESS trial proposed here.

Outcomes of the pilot trial showed that first year students are keen to participate in a trial of probiotics for reduction of stress, 120 students registered to participate in the study within the first 2.5 days of recruitment. Unfortunately, due to manufacturing difficulties Fonterra were not able to supply the capsules in time for the pilot study, several students expressed their disappointment about this indicating that they would have been willing to take the capsules if they had been available. At the end of the semester two days before their exam students were asked to complete the psychological stress and wellbeing questions again, 105 (87.5%) participants completed these end of study questions. Feedback and retention rates indicate that the online data collection platform worked well and was easy for students to use on their phone, tablet or computer.

**The Full PRESS Trial**

The PRESS Study is a randomised, double-blind, placebo-controlled trial.

**Recruitment of Participants**

The New Zealand university system operates on two semesters per year with examinations for a paper being held at the end of a semester. The PRESS Study will recruit 815 students enrolled in either the POPHEALTH 111 paper or the PSYCH 108 & 109 papers taken by first year students.

**Data Collection**

All consent and data collection is managed by an online web-interface so students can register for the study and complete the questionnaires using their mobile phone, tablet or computer. This online interface was trialled in 120 stage one university students in the PRESS pilot study with success.

**Exclusion criteria**

There are three exclusion criteria for the study. Participants will be excluded if they are:

* Currently taking a regular probiotic supplement
* Currently taking immunosuppressant’s e.g. chemotherapy
* Currently involved in another trial

**Sample Size**

A sample size for the full PRESS trial has been calculated based on the results from the pilot study. To give a 90% chance of detecting a difference of 1.6 points (0.25 SD) in perceived stress scale scores between the probiotic supplemented group and placebo group at the 5% level we need 326 participants in each arm of the study. This equals a sample size of 652. Allowing for 25% attrition we need to recruit a total of 815 students. A greater allowance for attrition than that seen in the PRESS pilot trial is required because the students in the pilot trial differ from those who will be recruited into the full PRESS trial in two main ways.

* The 120 participants who enrolled in the pilot trial registered within 2.5 days of the study being advertised and were particularly motivated to take part in the study. They are therefore not likely to be representative of the greater population of first year students.
* Participants for the pilot trial were recruited from a medical science paper and all of them had previously learnt about randomised trials. In the full PRESS study we will be recruiting students in the second week of university from a combination of population health and psychology papers.

**Measures**

*Stress*

The Perceived Stress Scale is a 10 item self-report questionnaire where participants rate their feelings of stress over the previous month. Higher scores on the PSS represent higher levels of perceived stress.

*Anxiety*

State Trait Anxiety Inventory 6 item version (STAI6): The STAI6 is a short 6 item scale derived from the longer STAI [6].

*Psychological Well-Being*

The World Health Organisation well-being index the WHO-5 is a five item, positively worded measure of psychological well-being. In contrast to the PSS and STAI6, higher scores in the WHO-5 represent better psychological wellbeing [7].

*Academic achievement (Baseline only)*

We will ask student’s permission to access their first and second semester exam results and results of the English language assessment that all students complete on entry to university (DELNA). In the PRESS pilot trial 97% of students gave permission for their grades to be accessed.

**Intervention**

Capsules containing the probiotic *Lactobacillus rhamnosus* HN001 (6×109 colony forming units) manufactured to pharmaceutical grade will be supplied by Fonterra. Placebo capsules identical in appearance and smell to the probiotic contain corn-derived matodextrin. Both probiotic and placebo capsules are lactose free and gluten free. The probiotic *L. rhamnosus* HN001 (6 X 109 cfu) has been safely used in previous studies conducted in New Zealand including in pregnant women [3] and infants [8].

*Intervention period*

Students will take one capsule a day from the time that they are enrolled in the study and supplied with the capsules until the day of their exam. The intervention period will be between 8-12 weeks but a minimum of 8 weeks.

**Post-intervention Questionnaires**

In addition to completing the PSS, STAI6 and WHO-5 again after the intervention participants will also answer the following questions.

1. How many times have you been unwell this semester e.g. with a cold flu, stomach bug)?
2. Have you been unwell due to illness in the last two weeks? Yes No
3. Please tell us how many capsules you have left?
4. Do you follow any of the following dietary plans/ways of eating?

Vegetarian

Vegan

Gluten Free

Dairy Free

Nut free

Paleo or high protein

Low fat

None of the above

**Koha/gift Vouchers**

Each participant receives a $10 Munchy Mart voucher for completing the baseline questionnaires. Participants receive a further $15 Munchy Mart voucher for completing the post-intervention questionnaires. Munchy Mart is similar to a large dairy or 7/11 store on campus that stocks a wide range of snacks and lunch food at student prices. Feedback from students was that Munchy Mart vouchers were appreciated and encouraged them to participate.

**Ethics**

An application has been made for ethics approval to the University of Auckland Human Participants Ethics Committee which will be considered in late November. Given the pilot trial received full ethical approval from this committee it is expected that ethical approval will obtained without complications.

**Trial registration**

The trial will be registered with the Australia and New Zealand Trials Registry

**Timeline**

Ethics: Prior to 28 February 2020

Recruitment period: 9 March 2020-3 April 2020

Randomisation: Occurs as participants sign-up

Distribution of capsules 16 March 2020-10 April 2020

Intervention period: enrolment – exam date approx. 13 June 2020

Final Report to Fonterra: 31 July 2020

Publication & presentation: 30 June2020-31 December 2021

**REFERENCES**

1. Molina-Torres G, Rodriguez-Arrastia M, Roman P, Sanchez-Labraca N, Cardona D (2019) Stress and the gut microbiota-brain axis. Behavioural Pharmacology. doi: 10.1097/FBP.0000000000000478

2. McKean J, Naug H, Nikbakht E, Amiet B, Colson N (2017) Probiotics and Subclinical Psychological Symptoms in Healthy Participants: A Systematic Review and Meta-Analysis. J Altern Complement Med. doi: 10.1089/acm.2016.0023 [doi]

3. Slykerman RF, Hood F, Wickens K, Thompson JMD, Barthow C, Murphy R, Kang J, Rowden J, Stone P, Crane J, Stanley T, Abels P, Purdie G, Maude R, Mitchell EA (2017) Effect of Lactobacillus rhamnosus HN001 in Pregnancy on Postpartum Symptoms of Depression and Anxiety: A Randomised Double-blind Placebo-controlled Trial. EBioMedicine. doi: 10.1016/j.ebiom.2017.09.013

4. Kato-Kataoka A, Nishida K, Takada M, Kawai M, Kikuchi-Hayakawa H, Suda K, Ishikawa H, Gondo Y, Shimizu K, Matsuki T, Kushiro A, Hoshi R, Watanabe O, Igarashi T, Miyazaki K, Kuwano Y, Rokutan K (2016) Fermented Milk Containing Lactobacillus casei Strain Shirota Preserves the Diversity of the Gut Microbiota and Relieves Abdominal Dysfunction in Healthy Medical Students Exposed to Academic Stress. Appl Environ Microbiol. doi: 10.1128/AEM.04134-15 [doi]

5. Takada M, Nishida K, Kataoka‐Kato A, Gondo Y, Ishikawa H, Suda K, Kawai M, Hoshi R, Watanabe O, Igarashi T, Kuwano Y, Miyazaki K, Rokutan K (2016) Probiotic Lactobacillus casei strain Shirota relieves stress‐associated symptoms by modulating the gut–brain interaction in human and animal models. Neurogastroenterology & Motility. doi: 10.1111/nmo.12804

6. Marteau TM, Bekker H (1992) The development of a six-item short-form of the state scale of the Spielberger State-Trait Anxiety Inventory (STAI). Br J Clin Psychol

7. Topp CW, Østergaard SD, Søndergaard S, Bech P (2015) The WHO-5 Well-Being Index: A Systematic Review of the Literature. Psychotherapy and Psychosomatics. doi: 10.1159/000376585

8. Wickens, Kristin, PhD|Black, Peter N., FRACP|Stanley, Thorsten V., FRCP|Mitchell, Edwin, FRACP, DSc|Fitzharris, Penny, FRACP|Tannock, Gerald W., PhD|Purdie, Gordon, BSc|Crane, Julian, FRACP (2008) A differential effect of 2 probiotics in the prevention of eczema and atopy: A double-blind, randomized, placebo-controlled trial. Journal of Allergy and Clinical Immunology, The. doi: 10.1016/j.jaci.2008.07.011