

# Probiotics for Reduction of Examination Stress in Students (PRESS) Study: A Randomized, Double-blind, Placebo-Controlled Trial of the Probiotic *Lactobacillus rhamnosus* HN001

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## ABSTRACT

**Background:** Studies suggest that bioactive compounds such as probiotics may positively influence psychological health. This study aimed to determine whether supplementation with the probiotic *Lactobacillus rhamnosus* HN001 reduced stress and improve psychological wellbeing in university students sitting examinations.

**Methods:** The study was a randomized, double-blind, placebo-controlled design, in which 483 undergraduate students received either the probiotic *L. rhamnosus* HN001, or placebo, daily during a university semester. Students completed measures of stress, anxiety, and psychological wellbeing at baseline and post-intervention before examinations. T-tests compared the change in psychological outcomes between groups.

**Results:** Of the 483 students, 391 (81.0%) completed the post-intervention questions. There was no significant difference between the probiotic and placebo supplemented groups in psychological health outcomes. The COVID-19 pandemic restrictions may have influenced the typical trajectory of stress leading up to examinations.

**Conclusion:** These findings further reinforce the difficulty of translating preclinical evidence into probiotic mediated health improvements for humans.

**Keywords:** Probiotics; Stress; Anxiety; Psychological health; Randomised controlled trial

## INTRODUCTION

The microbiota in the human gut is a colony of microbes (bacteria, viruses, and fungi) that play an essential role in physiological and biochemical processes in the body. The microbiota-gut-brain axis refers to multidirectional signaling pathways that communicate between the microbes in the gut and the central nervous system, including the Hypothalamic-Pituitary-Adrenal (HPA) axis, which modulates stress response and the immune system, both pathways linked to the experience of stress, anxiety, and depression [1-3].

Disruption to the gut microbial balance influences the biochemical metabolites produced in the gut, thereby altering neurotransmitter synthesis and modulating regulation of the HPA axis [4]. Stress, in turn, can alter the balance of microbiota in the gut. A recent review concluded that although there is substantial evidence from preclinical studies showing the gut microbiota influence the physiological stress response, further work in human populations

is needed to realize the potential benefits of positively influencing the gut microbiota to manage stress [5].

Promising preclinical studies using mice have suggested that probiotics (defined as live microorganisms that, when ingested in sufficient quantity, confer a health benefit) may improve the mental health of human participants by enhancing the gastrointestinal microbial environment. In one of the foundational studies in the field, germ-free mice, who have no commensal microbiota, displayed exaggerated responses to stress which improved with probiotic supplementation [6]. Understanding the therapeutic role of probiotics in humans is far less well elucidated, in part due to the incredible complexity of human physiology and psychology. A systematic review of the evidence for the benefit of probiotics on subclinical symptoms of psychological stress concluded that probiotic supplementation could reduce depression, anxiety, and perceived stress in healthy volunteers. However, small sample sizes

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were a limitation of most of these studies [7].

Conversely, in their systematic review and meta-analysis of 10 randomized controlled probiotic trials for depression and anxiety, [8] suggested there was limited evidence that probiotics could reduce depression and anxiety. Six of the reviewed studies were in healthy volunteers. Of those, two studies found statistically significant improvements in depression or anxiety attributable to probiotic supplementation [8]. The need to translate preclinical research into therapeutic benefits for people with psychiatric or stress-related conditions remains [9,10]. With the prevalence of stress and its impact on physical and psychological health, probiotic supplementation trials are critical to advancing the potential utility of probiotics in humans.

University students experience increased stress associated with examinations. A study of Japanese medical students found that stress increased over eight weeks before a national examination peaking the day before and then decreased to baseline two weeks later. The same study found that those students supplemented with *Lactobacillus casei* (strain *Shirota*) had fewer gastrointestinal, cold, or flu-like symptoms [11,12]. A study of stressed American university students reported similar results, those supplemented with *Bifidobacterium bifidum* reported fewer days of cold and flu symptoms and more healthy days than those who received a placebo [13]. A previous study of 155 university students randomly allocated students to receive either a multispecies probiotic containing *Lactobacillus casei* DN 114001, *Lactobacillus delbrueckii* subspecies *Bulgaricus*, and *Streptococcus salivarius* subspecies *Thermophilus* or placebo daily for six weeks which included three weeks leading up to examinations and three weeks during the examination period. They found that scores on the State Trait Anxiety Inventory (STAI) increased for all students, but there were no significant differences between probiotic and placebo groups. However, the probiotic supplemented group showed a statistically significant increase in markers of immune function [14].

To date, there has not been a large study of the effect of probiotic supplementation for psychological symptoms of stress, anxiety, and mental wellbeing in university students. Previously, in the Probiotics in Pregnancy (PIP) Study, 423 pregnant women were randomly assigned to receive *L. rhamnosus* HN001 or placebo daily from enrolment at 14-16 weeks gestation until six months postpartum. Mothers supplemented with *Lactobacillus rhamnosus* HN001 had significantly lower postnatal depression and anxiety scores than the placebo group. Furthermore, supplementation with *L. rhamnosus* HN001 improved anxiety and depression in all women, not only those who had high baseline levels of depression or anxiety [15]. In addition, the HN001 strain was associated with improved mental wellbeing in a population of pre-diabetic adults during a calorie-restricted diet [16], suggesting that probiotic supplementation with the HN001 strain may be beneficial for psychological wellbeing. Furthermore, this benefit may confer to all participants, not simply those who already have high levels of stress, anxiety, or depression.

This study aimed to investigate whether supplementation with the probiotic *Lactobacillus rhamnosus* HN001 reduced the build-up of stress, reduced symptoms of anxiety, and improved psychological well-being in university students leading up to examinations.

## MATERIALS AND METHODS

The study design was a randomized, double-blind, placebo-controlled trial.

## Participants

The New Zealand university system operates on two semesters per year, with examinations for a paper held at the end of a semester. Participants were undergraduate students at the University of Auckland enrolled in semester one of 2020. Exclusion criteria were: Currently taking a regular probiotic supplement, taking immunosuppressants, e.g., chemotherapy, or current participation in another research trial.

## Data collection

All consent and data collection was through an online web interface. Students registered for the study and completed the questionnaires using their mobile phones, tablet, or computer. Fonterra Co-operative Group Limited (FCGL) managed the randomization schedule and was not involved with the recruitment, collection of data, or analysis of results. Both participants and the researchers were blind to the randomization schedule. As students completed baseline consent and registration information, they were assigned the following available sequential study number and provided with the corresponding bottle of capsules according to the study number.

## Intervention

Fonterra Co-operative Group Limited (FCGL) supplied capsules containing the probiotic *Lactobacillus rhamnosus* HN001 (6 × 10<sup>9</sup> colony forming units) manufactured to pharmaceutical grade. Placebo capsules identical in appearance and smell to the probiotic contain corn-derived maltodextrin. Both probiotics and placebo capsules are lactose-free and gluten-free. Previous studies have safely used the probiotic *L. rhamnosus* HN001 (6 × 10<sup>9</sup> cfu) in previous studies conducted in New Zealand, including in pregnant women [15] and infants [17].

Instructions to students were to take one capsule a day from when they enrolled in the study and received the capsules until two days before the commencement of university examinations for the semester.

## Measures

**Stress:** The Perceived Stress Scale (PSS) is a 10 item questionnaire that asks about stress and coping in the previous month. Scores range from 0-40, with higher scores being indicative of higher levels of stress. Scores from 0-13 represent low stress, 14-26 indicate moderate stress, and 27-40 indicate high stress.

**Anxiety:** The State Trait Anxiety Inventory 6 (STAI6) is a short 6 item scale validated as an anxiety screening questionnaire based on the more extended State Trait Anxiety Inventory (STAI6) [18]. Clinically significant anxiety was defined as a score above a cut-off of score >15.

**Psychological well-being:** The World Health Organisation-Five (WHO-5) Well-Being Index (WBI) is a five-item, positively worded measure of psychological wellbeing with scores ranging from 0 to 25. Higher scores represent better wellbeing. Scores of 13 or lower indicate low levels of psychological wellbeing. A systematic review of the WHO-5 concluded that it was a widely used and sensitive measure of depression [19].

## Statistical analysis

Intent-to-treat analysis was conducted in Statistical Analysis System (SAS) 9.4 using a two-sample t-test. Change in stress, anxiety, and psychological wellbeing was calculated by subtracting post-intervention scores from baseline scores for each of the three measures. The findings are reported according to the consort statement.

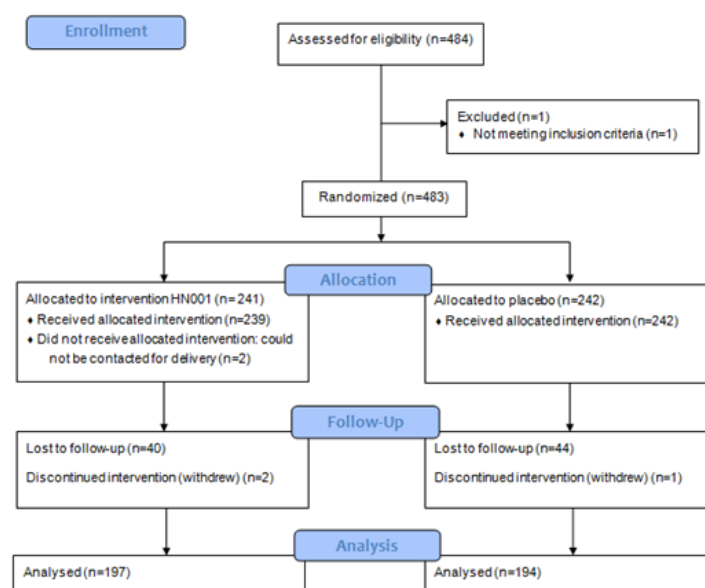
## RESULTS

Of the 483 participants initially enrolled in the trial, 391(81.0%) completed the end-of-intervention questions. Figure 1 shows the consort flow diagram for the trial. There was no significant difference between respondents and non-respondents to the end

of intervention questions in the intervention group ( $p=0.66$ ), sex ( $p=0.91$ ), ethnicity ( $p=0.51$ ), study paper ( $p=0.65$ ), or year of study ( $p=0.42$ ) (Figure 1).

The probiotic supplemented, and placebo groups did not significantly differ in demographic factors or measures of psychological health (Table 1).

Table 2 shows the outcome measure results according to study group assignment. Overall there were no significant differences between groups for measures of stress, anxiety, or psychological wellbeing. For the group as a whole there was a significant improvement in stress (mean change=1.70; SED=6.10,  $p<0.0001$ ), anxiety (mean change=0.87; SD=6.88,  $p=0.001$ ) and psychological wellbeing scores (mean change=0.49; SD=4.49,  $p=0.03$ ) between baseline and post-intervention (Table 2).



**Figure 1:** CONSORT flow diagram showing recruitment and group assignment.

**Table 1:** Participant characteristics by intervention group.

	Placebo N (%)	HN001 N (%)	p-value
Sex			
Male	60(24.8)	57(23.7)	0.71
Female	179(74.0)	184(76.4)	
Unspecified	3(1.2)	0(0.0)	
Ethnicity			
European	110(45.5)	106(44.0)	0.98
Maori	19(7.9)	19(7.9)	
Pacific	11(4.5)	9(3.7)	
Asian	85(35.1)	89(36.9)	
Other	17(7.0)	18(7.5)	
Study paper			
Medicine	41(17.4)	36(15.9)	10 (39.1)
Medical Science	5(2.1)	8(3.5)	
Population Health	91(38.6)	89(39.2)	
Psychology	68(28.8)	77(33.9)	
Other	31(13.1)	17(7.5)	
Study year			
First year	134(56.8)	144(63.4)	0.14
Second or third year	102(43.2)	83(36.6)	
Baseline scores (n=483)	Mean (SD)	Mean (SD)	p-value

Stress	20.4(6.2)	20.4(6.2)	0.87
Anxiety	54.2(5.9)	54.0(5.6)	0.72
Wellbeing	12.8(4.3)	13.0(4.3)	0.74
Post-intervention scores (n=391)	Mean (SD)	Mean (SD)	p-value
Stress	18.9(6.1)	18.5(7.0)	0.57
Anxiety	53.2(5.7)	53.7(6.0)	0.37
Wellbeing	13.3(4.4)	13.4(4.7)	0.73

Note: SD: Standard Deviation.

**Table 2:** Mean change in stress, anxiety and psychological wellbeing scores between baseline to post-intervention for the probiotic and placebo groups.

	Placebo	HN001	p-value
	Mean change in score (SD)		
Stress	1.6(6.0)	1.8(6.3)	0.79
Anxiety	1.1(7.1)	0.6(6.7)	0.48
Wellbeing	-0.4(4.5)	-0.6(4.5)	0.73

Note: SD: Standard Deviation.

## DISCUSSION

In this randomized, double-blind, placebo-controlled trial of the probiotic *Lactobacillus rhamnosus* HN001, we found no significant difference in stress, anxiety, and psychological wellbeing in university students between the placebo and probiotic intervention groups. Although there is promising evidence that probiotic supplementation may improve the stress-induced suppressed immune function in students, these previous trials have also not shown a significant difference in psychological symptoms of stress, anxiety, or depression in university participants [12,13,14,20]. In a previous trial of *L. rhamnosus* JB-1 in healthy volunteers, there was no significant difference between placebo and probiotic groups on stress, depression, and anxiety or physiological measures, including HPA-axis function, neurocognitive and inflammatory markers, despite preclinical evidence in a mouse model showing promising results for this strain of probiotic. This finding highlights the difficulties in translating promising preclinical evidence into human populations [21].

It is well understood in the probiotic field that benefits for health conditions associated with probiotics are strain-specific [21,22]. While the HN001 strain did not benefit psychological outcomes in our study where other strains may have, we have previously demonstrated that the HN001 strain significantly lower depression and anxiety scores in postpartum mothers [15]. Furthermore, a recent trial showed improvements in psychological wellbeing associated with HN001 probiotic supplementation in pre-diabetic adults on an intermittent fasting diet [16].

The potential impact of COVID-19 on this research requires discussion. The university semester this study was conducted in was interrupted by government-led restrictions that forced the closure of the university campus and a complete shift to online learning, studying, and assessment for the university campus semester. The COVID-19 pandemic is often assumed to increase stress and anxiety; however, the opposite was true for our cohort of university students, and this may have influenced our study results. New Zealand has been one of the most successful countries worldwide in containing and eliminating COVID-19. During the university semester of this study, New Zealand moved from alert level 4 (complete lockdown) to alert level 1 (no restrictions except on international travel). Our lockdown was one of the most restrictive in the western world. Stay at home instructions asked people to restrict outings to essential personal movement, travel

was severely limited, gatherings were cancelled, and all public venues closed, businesses were closed except for essential services, and all educational facilities were closed. Many students returned home where they may have been more supported by family.

Furthermore, students may have experienced a reduction in stress before their examinations due to a significant improvement in eliminating COVID-19 from the community that resulted in a shift down to alert level 1 during the semester. In addition, examinations for students were online and uninvgilated and this may also have resulted in a reduction of stress for students leading up to examinations in direct contrast to previously reported increase in pre-examination stress in studies conducted before COVID-19 [12,23]. Literature indicates that some students find online examinations less stressful [24,25].

Young adult university students in our sample may have had reduced adherence to the daily capsule. Adherence to medication is low in young adults compared with older age groups, even in the presence of chronic illness [26]. In addition, the COVID-19 lockdown announcement in New Zealand occurred at the beginning of week four of the university semester. Many students left Auckland to return to other parts of New Zealand for the lockdown, and it is possible that this may have interrupted adherence to daily capsule intake.

## CONCLUSION

In this large double-blind, randomized placebo-controlled trial of the probiotic *L. rhamnosus* HN001 in university students, we found no significant difference in stress, anxiety, or psychological wellbeing before end-of-semester examinations. The COVID-19 lockdown may have impacted any potential benefit of probiotics as psychological wellbeing in the students improved contrary to previous reports that suggest wellbeing typically deteriorates before examinations. Our study further highlights the difficulty of realizing the potential benefit of probiotics for different health outcomes and indicates that further research across populations and probiotic species is required.

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to be involved in the study during a semester affected by COVID-19.

## FUNDING

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## ETHICAL APPROVAL

The study received full ethical approval from the University of Auckland Human Participants Ethics Committee: Reference 023964.

## TRIAL REGISTRATION

The trial was prospectively registered with the Australia and New Zealand Trials Registry ACTRN12620000275965.

## DISCLOSURE

No author has any conflict of interest.

## DATA AVAILABILITY

Raw data from this research cannot be made available due to ethical approval requirements.

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