ACTIVATED PROBIOTICS®

Results from the Biome Lift™ Study

6th July 2023

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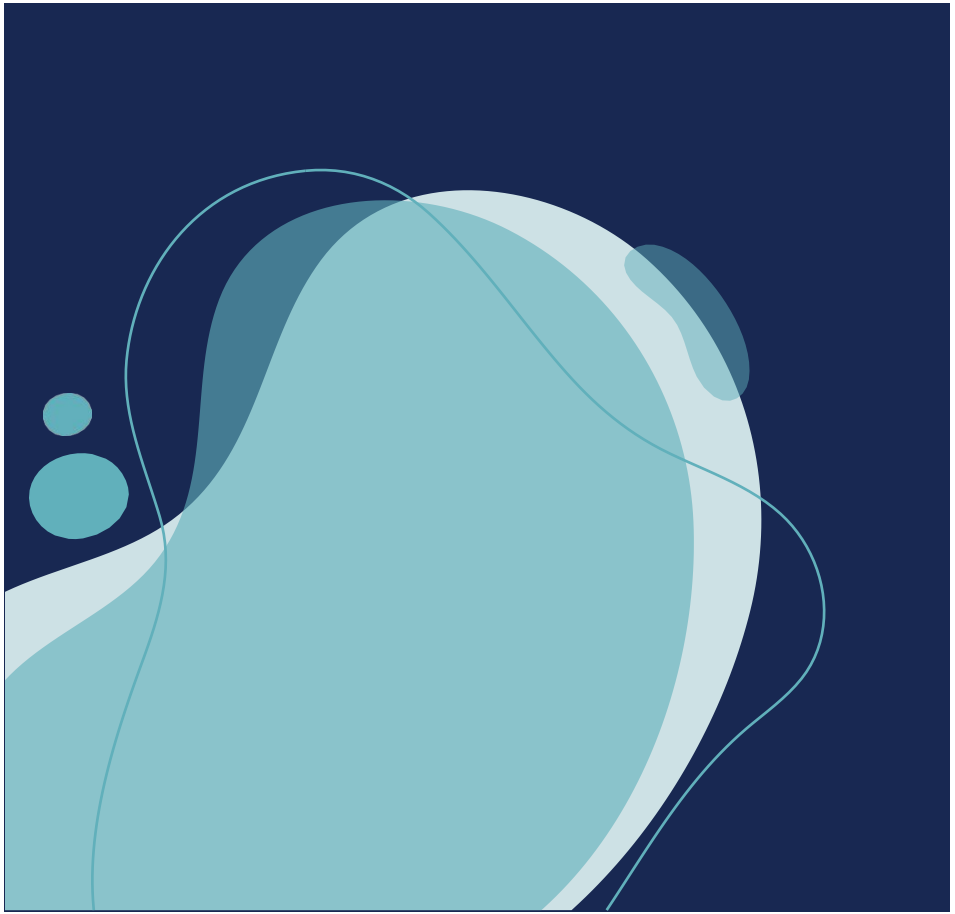

Practitioner-only probiotics

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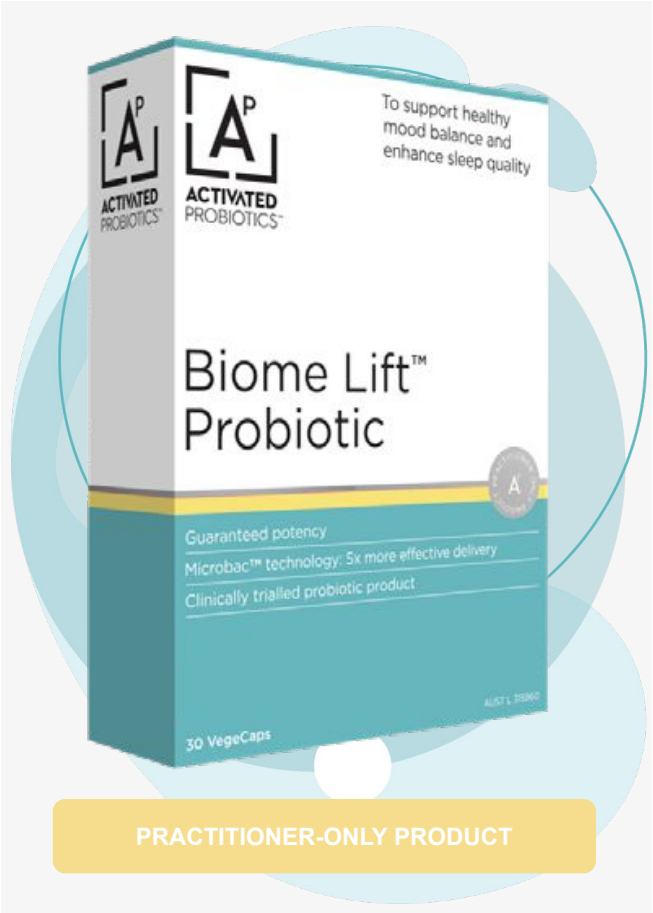
Biome Lift Probiotic



Biome Lift™ Probiotic

To support healthy mood balance and enhance sleep quality

- May help reduce cognitive fatigue
- Clinically trialled formulation
- Microbac™ technology: 5x more effective delivery
- Guaranteed potency

FORMULATION

Lactobacillus plantarum LP01 (LMG P-21021)	1 BLB*
Lactobacillus rhamnosus LR06 (DSM 21981)	1 BLB*
Bifidobacterium longum 04 (DSM 23233)	1 BLB*
Lactobacillus fermentum LF16 (DSM 26956)	1 BLB*
Total live bacteria	4 BLB*

*BLB = Billion Live Bacteria

Gut-brain axis

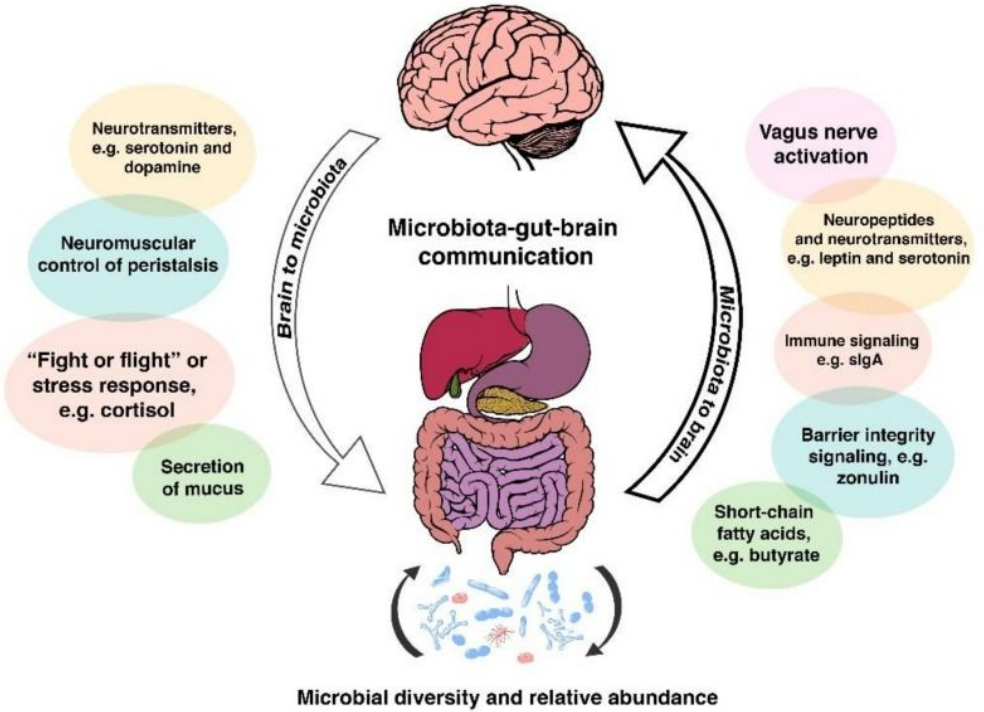
The gut-brain axis refers to the bidirectional communication between the gut and the brain.

It involves complex interrelationships between many biological systems, including:

- The gastrointestinal system
- The nervous system
- The immune system
- The endocrine system
- The circulatory system

Of recent interest is **the gut-microbiota-brain axis** which considers the coordinating and modulatory role of the ‘microbial system’.

This concept encompasses the direct and indirect communication between gut bacteria and the brain through the human’s biological channels.



Toribio-Mateas, M. (2018). Harnessing the Power of Microbiome Assessment Tools as Part of Neuroprotective Nutrition and Lifestyle Medicine Interventions. Microorganisms, 6(2), 35.

Previous studies

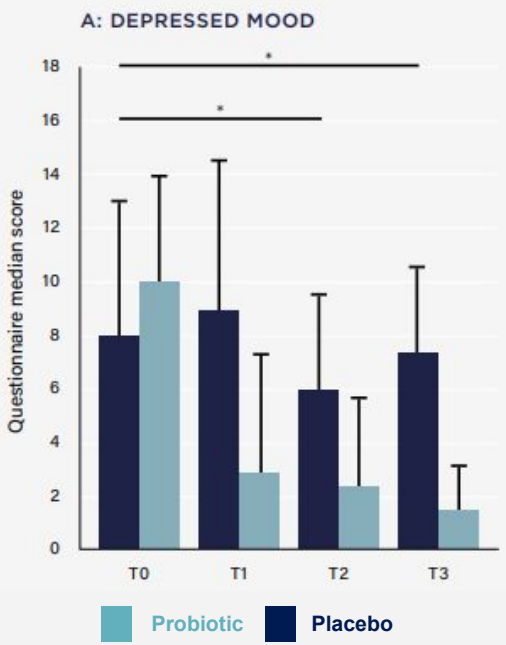
Results of the first study

First trial examining the effects of the formulation in Biome Lift Probiotic-

Marotta A, Sarno E, Casale A Del, Pane M, Mogna L, Amoruso A, et al.
Effects of probiotics on cognitive reactivity, mood, and sleep quality.
Front Psychiatry. 2019;10(164):1–11.

Results from the Profile of Mood State and Pittsburgh Sleep Quality Index found that using this probiotic formula in a healthy population significantly improved:

- Sleep quality and mood
- Depressive feelings
- Anger-hostility
- Fatigue



Median Profile of Mood States (POMS) depression subscale score from the participants in the probiotic group and the placebo group. Error bars represent 95% confidence intervals. Asterisks indicate within-group significant differences (Bonferroni corrected p<0.017).

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Results of the second study

Second trial examining the effects of the formulation in Biome Lift Probiotic-

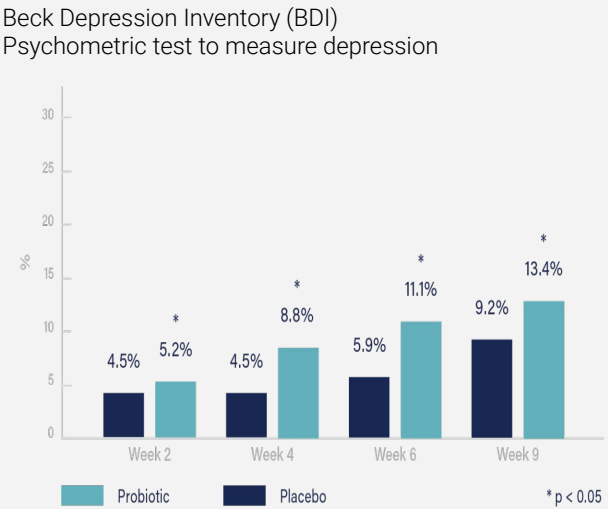
C. Kerksick, R. Jäger, et al. **Multi-strain probiotic supplementation increases sleep quality, mood and neurotransmitter levels.**

Positive findings for improved depressive mood and symptoms of anxiety using:

- Beck Depression Inventory
- Leiden Index of Depression-Sensitivity
- Life Orientation Test
- State Trait Anxiety Inventory

Confirmed Marotta's previous findings of improved sleep quality using Pittsburgh Sleep Quality Index.

Found increases in serum serotonin levels.



The Biome Lift Study

Biome Australia Limited has received the pre-publication results of the Biome Lift™ Probiotic clinical trial taking place at La Trobe University in Melbourne.

These findings are the culmination of a strategic research partnership with La Trobe University and help to strengthen the existing clinical evidence of efficacy for Biome Lift™ Probiotic.

The hypothesis

The study hypothesized that the probiotic experimental group would experience a reduction in the severity of depressive symptoms compared to the control group receiving a placebo, and that this would be mediated by beneficial changes in gut microbiota composition and a consequent production of precursors of neuroactive substances that impact oxidation and inflammatory pathways (demonstrated by improved levels of inflammation and oxidative stress markers).

Study aims and outcome measures

Primary aim: to assess the efficacy of the Biome Lift™ Probiotic compared with a placebo in reducing the severity of depressive symptoms in patients with subthreshold depression.

The primary outcome measure: the subjective assessment of depression using the following validated questionnaire: Beck Depression Inventory 2nd edition (BDI-II; self-report, brief specifically measures characteristic attitudes and symptoms of depression) [4 timepoints at pre, mid, post and follow-up]

Secondary aims were to assess:
i) the efficacy of the Biome Lift™ Probiotic compared with a placebo in delivering an improvement in quality of life and other measures of psychosocial health (i.e., anxiety, stress, mood) in subjects with subthreshold depression;
ii) the effects of the Biome Lift™ Probiotic on blood and saliva biomarkers of inflammatory, immune and stress response;
iii) the effects of the Biome Lift™ Probiotic on gut microbiota composition and function;
iv) the effects of the Biome Lift™ Probiotic on anthropometric and body composition markers; and,
v) the effects of the Biome Lift™ Probiotic on gastrointestinal health (gut symptom severity).

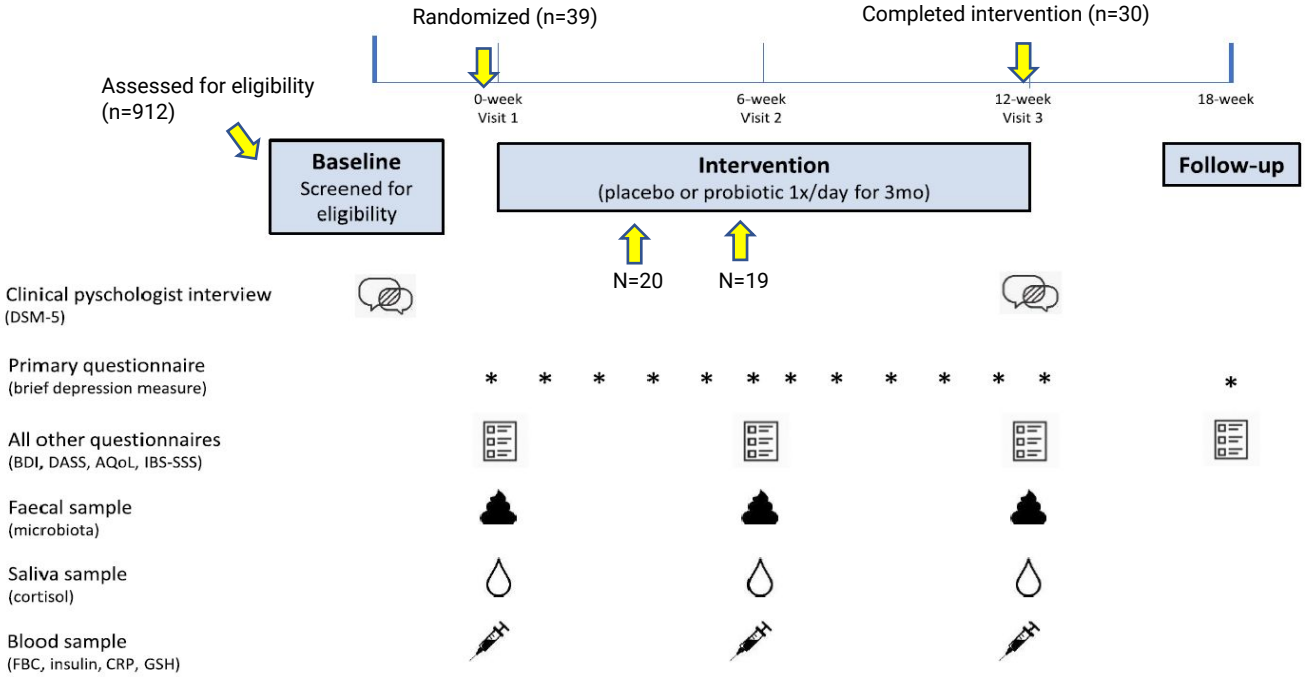
Study aims and outcome measures

The secondary outcome measures are as followed:

1. Subjective and clinical assessment of depression, anxiety, mood and QoL, using the following validated questionnaires:
   - Patient Health Questionnaire (PH9; self-report, brief specifically measures depression and has been shown to be responsive to intervention) [weekly]
   - Structured Clinical Interview for DSM-5 (SCID 5; clinically administered; depression symptoms) [pre and post only]
   - Hospital Anxiety and Depression Scale (HADS; clinically administered; anxiety and depression) [4 timepoints at pre, mid, post and follow-up]
   - Depression, Anxiety and Stress Scale (DASS-21; self-report; depression, anxiety and stress severity ratings) [4 timepoints at pre, mid, post and follow-up]
   - Assessment of Quality of Life (AQOL-8; self-report; health-related quality of life) [4 timepoints at pre, mid, post and follow-up]
   - Perceived Stress Scale (PSS; self-report; stress perception measurement) [4 timepoints at pre, mid, post and follow-up]
   - Covid-19 impact tool (self-report; stress perception measurement) [4 timepoints at pre, mid, post and follow-up]
2. A range of inflammatory, immune and metabolic biomarkers assessed via blood samples including: fasting plasma glucose, serum insulin, HOMA-IR, hs-CRP and total GSH
3. Gut microbiota composition changes assessed via fecal samples testing for microbiota genome and functional predictions of gut microbiota [3 timepoints at pre, mid and post]
4. Salivary cortisol as a biomarker of stress assessed via saliva sample collection [3 timepoints at pre, mid and post]
5. Subjective assessment of gastrointestinal health using the following validated questionnaire: Irritable bowel syndrome-severity scoring system (IBS-SSS; self-report; gut symptom severity) [4 timepoints at pre, mid, post and follow-up]

Study design

The study was a double-blind randomised parallel group clinical trial of probiotic supplementation versus placebo in a 1:1 ratio in patients diagnosed with subthreshold depression. The probiotic or placebo supplementation was taken daily for 3-months.



Definition of subthreshold depression

Interested participants had to meet the diagnosis of subthreshold depression, via a screening interview with the study psychologist, using the Structured Clinical Interview for DSM-5 (SCID 5).

Sub-threshold depression is defined as:

- Exhibiting at least 1 of the 2 core symptoms* for depression, as well as one other symptom^, but not meeting the 5 or more symptoms required for a diagnosis of unipolar major depression, albeit, in general 2 to 4 symptoms of those seen in a depressive episode are present); and,
- Who are not undergoing any pharmacological or psychological treatment.

According to the Diagnostic and Statistical Manual of Mental Disorders (DSM-5), symptoms for subthreshold depression must be experienced during a period of at least 2 weeks before diagnosis.

*Core Symptoms
1. Depressed mood most of the day, nearly every day, as indicated by either subjective report (e.g., feels sad, empty, hopeless) or observation made by others (e.g., appears tearful);
2. Markedly diminished interest or pleasure in all or almost all activities most of the day, nearly every day (as indicated by either subjective account or observation made by others);

^Other Symptoms
3. Significant weight loss when not dieting or weight gain (e.g., a change of more than 5% of body weight in a month), or decrease or increase in appetite nearly every day;
4. Insomnia or hypersomnia nearly every day;
5. Psychomotor agitation or retardation nearly every day (observable by others, not merely subjective feelings of restlessness or being slowed down);
6. Fatigue or loss of energy nearly every day;
7. Feeling of worthlessness or excessive or inappropriate guilt (which may be delusional) nearly every day (not merely self-reproach or guilt about being sick);
8. Diminished ability to think or concentrate, or indecisiveness, nearly every day (either by subjective account or as observed by others);
9. Recurrent thoughts of death (not just fear of dying), recurrent suicidal ideation without a specific plan, or a suicide attempt or a specific plan for committing suicide.

Inclusion criteria

Inclusion Criteria

- Male and female adults
- Aged between 18 and 65 y
- Not in the underweight category (BMI ≥18.5 kg/m^2)
- Meet the criteria for subthreshold depression
- Have not been taking antidepressants /other medications acting on the CNS /psychological treatment (eg, cognitive behavioral therapy) for at least 6 weeks
- Are able to understand and thus correctly fill in the questionnaires required as part of the study protocol.

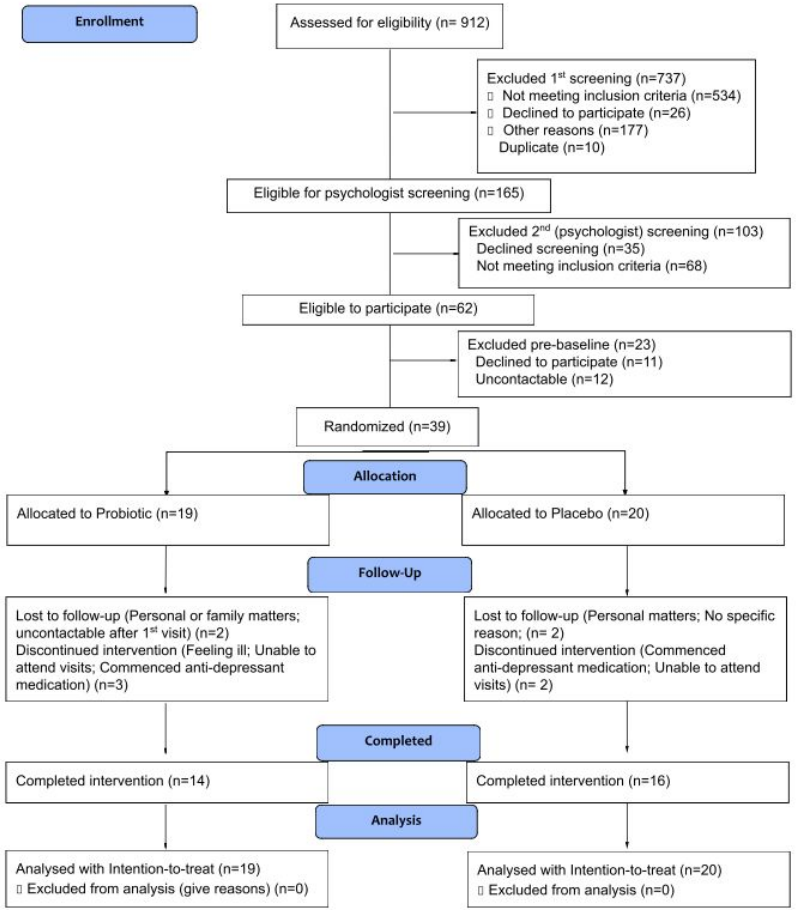
Exclusion Criteria

- Suicidal ideation, with or without a specific plan
- Comorbidity with substance use disorders or organic mental disorders
- Neurological conditions affecting the brain, other central functions or any other mental health condition (i.e., psychosis, eating disorder)
- Organic comorbidities (i.e., gastrointestinal, or autoimmune or chronic inflammatory conditions)
- Nutritional supplement use (vitamins, minerals or antioxidants)
- Antibiotic use in the six weeks before the start of the trial participation
- Pregnant or breastfeeding.

Study participant flow diagram

Figure 2 presents the CONSORT flow diagram of study participants.

Figure 2. Study participant flow diagram (CONSORT)



Baseline characteristics

Table 2 summarises the descriptive characteristics of study participants, in terms of socio-demographics and anthropometrics, in the total sample (n=39) and by treatment arm.

The non-existence of differences in almost all of the examined socio-demographic and anthropometric indices presented in Table 2, indicates homogeneity of the study sample at baseline. This is pivotal in RCTs, since homogeneity of the study sample reflects the validity of randomization, which increases the quality of the study.

Table 2. Baseline differences in demographic and anthropometric indices between treatment arms.

	Placebo (n=20)	**Probiotic (n=19)**	*P*-value*
	Mean (SD)	Mean (SD)	
Age (Years)	41.8 (11.4)	42.9 (15.8)	0.804
Weight (Kg)	83.4 (21.3)	76.1 (18.5)	0.257
Height (cm)	170.1 (8.3)	166.3 (10.1)	0.207
BMI (kg/m^2)	28.8 (6.6)	27.6 (6.4)	0.568
	n (%)	n (%)	
BMI categories			0.413
Normal weight	6 (30.0)	9 (47.4)	
Overweight	7 (35.0)	3 (15.8)	
Obese	7 (35.0)	7 (36.8)	
Gender			0.365
Males	8 (40.0)	5 (26.3)	
Females	12 (60.0)	14 (73.7)	
Educational level			**0.024**
Apprentice/Trade	2 (10.0)	0 (0.0)	
Secondary school	0 (0.0)	4 (21.1)	
Certificate/Diploma	7 (35.0)	2 (10.5)	
Bachelor's degree	8 (40.0)	6 (31.6)	
Postgraduate (Master's/PhD)	**3 (15.0)** *	**7 (36.9)** *	
Employment status			0.443
Unemployed	3 (15.0)	1 (5.3)	
Casual employment	4 (20.0)	4 (21.1)	
Part-time employment	5 (25.0)	2 (10.5)	
Full-time employment	8 (40.0)	11 (57.9)	
Retired	0 (0.0)	1 (5.3)	
Place of birth			0.763
Australian/New Zealander	16 (80.0)	15 (78.9)	
European	1 (5.0)	2 (10.5)	
Asian	3 (15.0)	2 (10.5)	
Region of residence			0.064
Urban	20 (100.0)	16 (84.2)	
Rural	0 (0.0)	3 (15.8)	

Data are presented as mean and standard deviation (SD) or as frequencies (n) and percentages (%)

*: Derived from Student's T-test for continues variables and from chi-square test for categorical variables.

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Pre-publication results

Results summary

Results from the self-report questionnaires provide evidence on the effectiveness of the Biome Lift intervention in reducing the severity of depressive symptoms in patients with subthreshold depression. According to these findings, although there were no differences observed between the two groups, there were several within-group changes that were primarily in favour of the probiotic group.

In a follow-up subgroup of the original cohort, improvements observed in the probiotic group in terms of depressive and anxiety symptoms (i.e., through the greater reductions seen in BDI HADS-A, HADS-D total scores) at the end of the intervention, also remained 6 weeks post-intervention.

The blood results clearly indicate a reduction in inflammation (i.e., through the reduction in hs-CRP levels) in the probiotic arm, as well as an improvement in brain antioxidant capacity (i.e., through the increases observed in total glutathione levels) both at 6 and 12 weeks of treatment.

The saliva results clearly indicate a reduction in stress levels (i.e., through the reduction in 30 minutes post-awakening cortisol and cortisol awakening response levels) in the probiotic arm, compared to the placebo after 12 weeks.

According to the faecal analysis, there were no differences or trends observed between timepoints or treatment arms for gut microbiome diversity measures.

The non-existence of between-group differences or within-group changes in anthropometric indices during the intervention is a positive finding, since this means that there is no confounding effect of weight status on the study outcomes.

The non-existence of between-group differences or within-group changes for the vast majority of dietary intake indices during the intervention is a positive finding, since this means that they had confounding effect on the study outcomes. The significant findings observed for energy, protein and especially dietary fibre (which has a prebiotic effect) might need to be taken into consideration on future statistical analyses of study outcomes.

Questionnaire results

Table 5 summarises the within-group changes and between-group differences in the examined depression, mood, anxiety, stress and quality of life scores that were assessed throughout the 12-month intervention period, via the administered self-report questionnaires. The figures on slides 20-25 present the results from the questionnaires that demonstrated statistically significant within-group changes only, except for the total HADS score.

The results presented in Table 5 provide evidence on the effectiveness of the Biome Lift intervention in reducing the severity of depressive symptoms in patients with subthreshold depression. According to these findings, although there were no differences observed between the two groups, there were several within-group changes that were primarily in favour of the probiotic group. This is a common finding stemming from other relevant small-scale studies that examine similar depressive symptoms related outcomes. In addition, our study, but also other similar studies, indicate a potential “placebo effect” on the examined outcomes. This is something that is expected, considering the subjective nature of the data collected through the administered questionnaires and the fact that people tend to report feeling better when receiving a treatment, even if this is just a placebo.

Table 5. Changes in the severity of depressive symptoms, other measures of psychosocial health and quality of life in people diagnosed with subthreshold depression receiving either a probiotic food supplement (n=19) or a placebo (n=20), from baseline to 6 and 12 weeks of intervention.

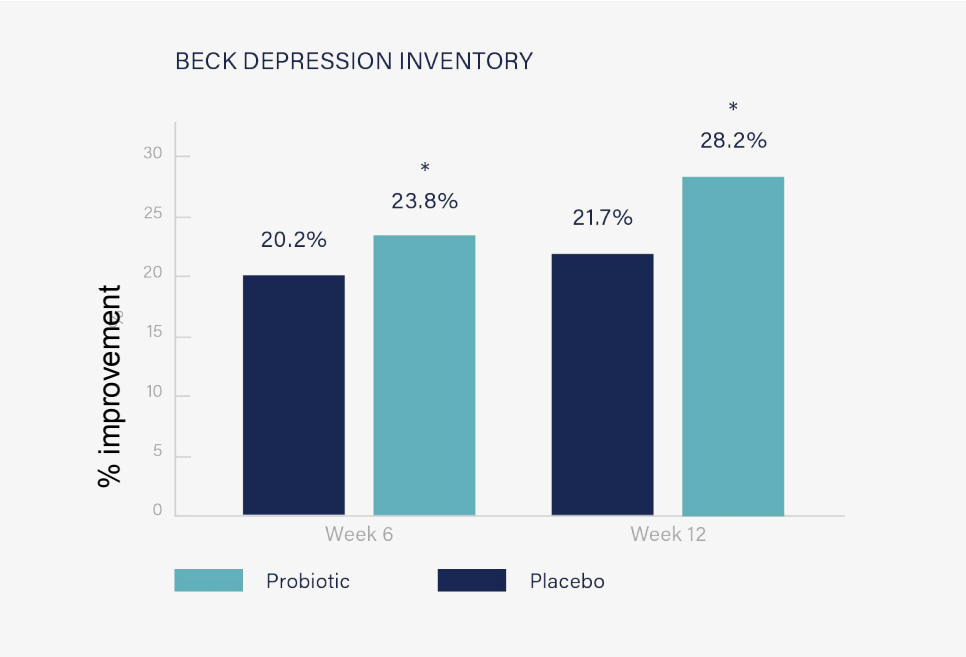
	Baseline	6-week Follow-up	6-Week Change	12-Week Follow-up	12-Week Change
	Mean (SD)	Mean (SD)	Mean (95% CI)	Mean (SD)	Mean (95% CI)
BDI total score					
Placebo	23.8 (9.7)	19.0 (9.1)	-4.8 (-10.3; 0.6)	17.2 (6.8)	**-6.6 (-12.1; -1.1)**
Probiotic	23.4 (9.3)	16.9 (8.2)	**-6.5 (-12.3; -0.7)**	15.8 (9.2)	**-7.6 (-13.4; -1.8)**
P-value†	0.889	0.450	0.451*	0.592	0.747*
PHQ total score					
Placebo	10.3 (4.6)	7.9 (3.8)	**-2.5 (-4.9; -0.006)**	7.1 (3.0)	**-3.2 (-5.7; -0.7)**
Probiotic	11.9 (4.6)	7.6 (4.3)	**-4.2 (-7.1; -1.3)**	7.8 (4.4)	**-4.1 (-7.0; -1.2)**
P-value†	0.299	0.856	0.235*	0.602	0.583*
HADS-A total score					
Placebo	**7.7 (3.1)**	6.9 (3.2)	-0.8 (-2.9; 1.2)	6.7 (3.5)	-1.0 (-3.0; 1.0)
Probiotic	**11.4 (3.6)**	8.6 (3.4)	**-2.8 (-5.2; -0.4)**	8.7 (4.0)	**-2.7 (-5.1; -0.3)**
P-value†	**0.001**	0.106	0.059*	0.094	0.231*
HADS-D total score					
Placebo	7.4 (2.8)	5.8 (3.5)	-1.6 (-3.6; 0.3)	6.6 (2.9)	-0.8 (-2.7; 1.2)
Probiotic	8.6 (3.9)	5.6 (3.0)	**-3.0 (-5.4; -0.7)**	6.1 (3.8)	**-2.5 (-4.9; -0.2)**
P-value†	0.268	0.854	0.166*	0.636	0.096*
HADS total score					
Placebo	**15.1 (4.5)**	12.7 (5.1)	-2.4 (-5.5; 0.7)	13.4 (5.0)	-1.7 (-4.8; 1.4)
Probiotic	**20.0 (6.8)**	14.4 (5.6)	**-5.6 (-9.8; -1.5)**	15.1 (6.6)	**-4.9 (-9.1 -0.8)**
P-value†	**0.011**	0.320	0.072*	0.367	0.133*
DASS-D total score					
Placebo	7.4 (4.4)	6.0 (4.0)	-1.4 (-3.9; 1.0)	5.2 (2.9)	-2.2 (-4.7; 0.2)
Probiotic	7.6 (3.8)	5.4 (2.5)	**-2.2 (-4.3; -0.04)**	5.9 (3.5)	-1.7 (-3.9; 0.4)
P-value†	0.861	0.624	0.451*	0.489	0.706*
DASS-A total score					
Placebo	3.8 (2.4)	2.7 (1.9)	-1.2 (-2.5; 0.2)	2.9 (2.2)	-0.9 (-2.3; 0.5)
Probiotic	4.4 (3.4)	3.8 (2.5)	-0.6 (-2.6; 1.4)	4.4 (3.2)	-0.01 (-2.0; 1.9)
P-value†	0.548	0.115	0.563*	0.097	0.423*
DASS-S total score					
Placebo	7.6 (3.2)	6.2 (2.8)	-1.4 (-3.4; 0.5)	5.5 (3.1)	**-2.1 (-4.0; -0.1)**
Probiotic	8.6 (4.2)	6.9 (4.2)	-1.7 (-4.3; 1.0)	6.8 (3.9)	-1.7 (-4.4; 0.9)
P-value†	0.416	0.518	0.841*	0.255	0.804*
PSS total score					
Placebo	20.5 (4.4)	19.2 (5.9)	-1.3 (-4.4; 1.7)	19.5 (4.1)	-1.0 (-4.1; 2.1)
Probiotic	21.4 (3.7)	20.4 (2.5)	-1.0 (-2.9; 0.9)	21.3 (2.4)	-0.06 (-2.0; 1.8)
P-value†	0.532	0.416	0.808*	0.118	0.485*
AQoL total score					
Placebo	81.4 (12.0)	77.6 (14.0)	-3.8 (-11.8; 4.1)	76.9 (11.7)	-4.5 (-12.5; 3.5)
Probiotic	84.2 (16.3)	77.6 (13.7)	-6.6 (-16.9; 3.7)	77.8 (17.4)	-6.4 (-16.8; 3.9)
P-value†	0.542	0.986	0.499*	0.859	0.661*

*: Treatment x Time interaction effect; †: Between-groups’ differences in mean values at baseline, 6 and 12 weeks, as well as in 6- and 12-wekk changes from baseline (Treatment effect)

Note: Decreases in the scores are indicative of improvements in the relevant depressive symptoms, mood, anxiety, stress, and quality of life that each score measures.

Questionnaire-Based Assessment Tools

BECK DEPRESSION INVENTORY 2nd EDITION



The Beck Depression Inventory 2nd edition (BDI-II) is designed to assess presence and severity of depressive symptoms.

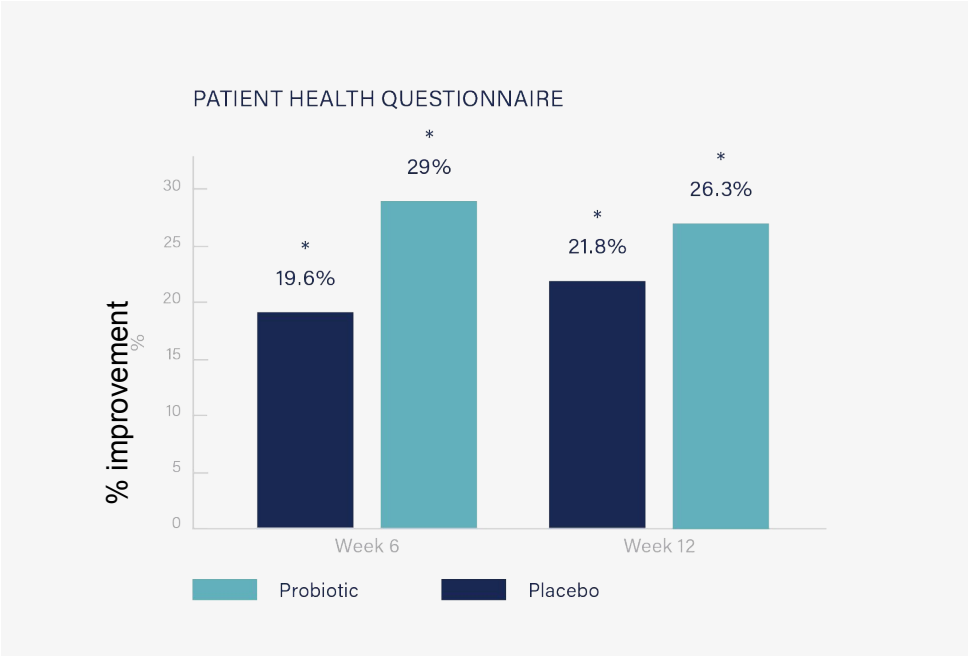
Figure 1: Changes in mean (95% CI) of BDI total scores *$p < 0.05$, significant within-group change from baseline to follow-up.

The probiotic group significantly decreased their BDI total score by -6.5 (95% CI -12.3; -0.7) and -7.6 (95% CI -13.4; -1.8) at 6 and 12 weeks of intervention respectively.

A significant decrease of -6.6 (95% CI -12.1; -1.1) was also observed in the placebo group, but only at the end of the 12-week intervention.

Questionnaire-Based Assessment Tools

PATIENT HEALTH QUESTIONNAIRE 9



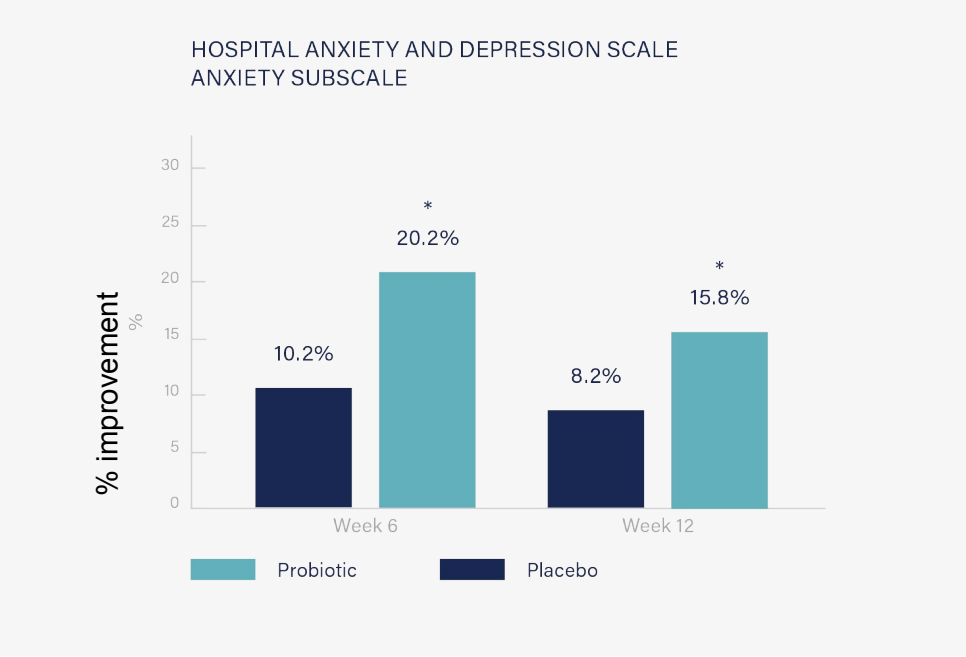
The Patient Health Questionnaire (PH9) is a self-report questionnaire that specifically measures depression.

Figure 2: Changes in mean (95% CI) PHQ total scores *$p < 0.05$, significant within-group change from baseline to follow-up.

The PHQ total score decreased significantly by -4.2 (95% CI -7.1; -1.3) and -4.1 (95% CI -7.0; -1.2) in the probiotic group and by -2.5 (95% CI -4.9; -0.006) and -3.2 (95% CI -5.7; -0.7) in the placebo group after 6 and 12 weeks of intervention respectively.

Questionnaire-Based Assessment Tools

HADS ANXIETY SUBSCALE (HADS-A)



The Hospital Anxiety and Depression Scale (HADS) measures anxiety and depression.

Figure 3: Changes in mean (95% CI) HADS-A total scores *$p < 0.05$, significant within-group change from baseline to follow-up.

The HADS-A total score decreased significantly only in the probiotic group by -2.8 (95% CI -5.2; -0.4) and -2.7 (95% CI -5.1; -0.3) at 6 and 12 weeks of intervention respectively.

The decrease in the HADS-A score for the probiotic group was borderline significantly higher compared to the decreases observed in the placebo group (P=0.059).

Questionnaire-Based Assessment Tools

HADS DEPRESSION SUBSCALE (HADS-D)

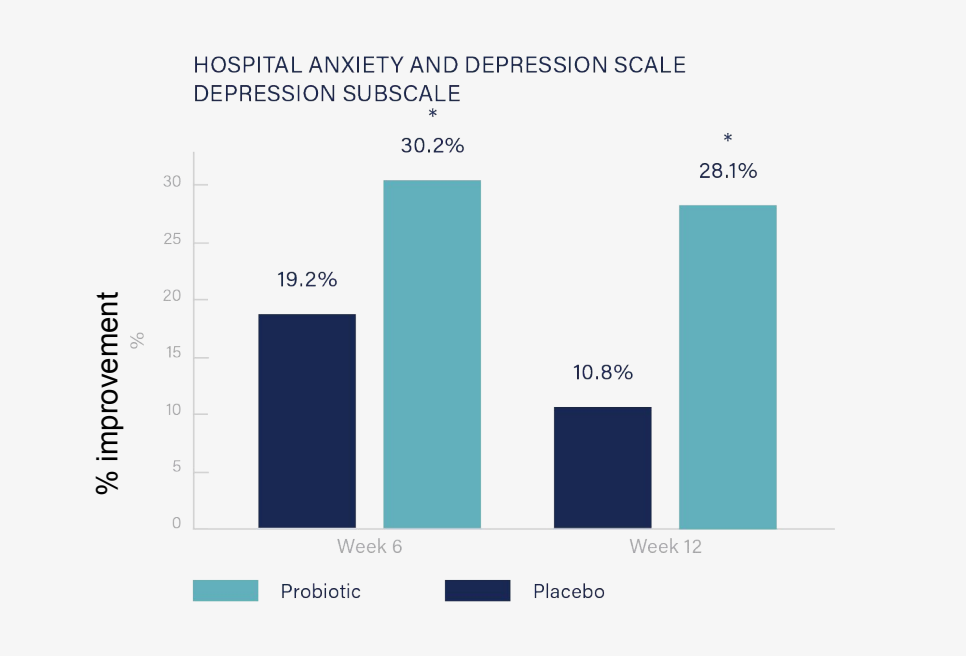
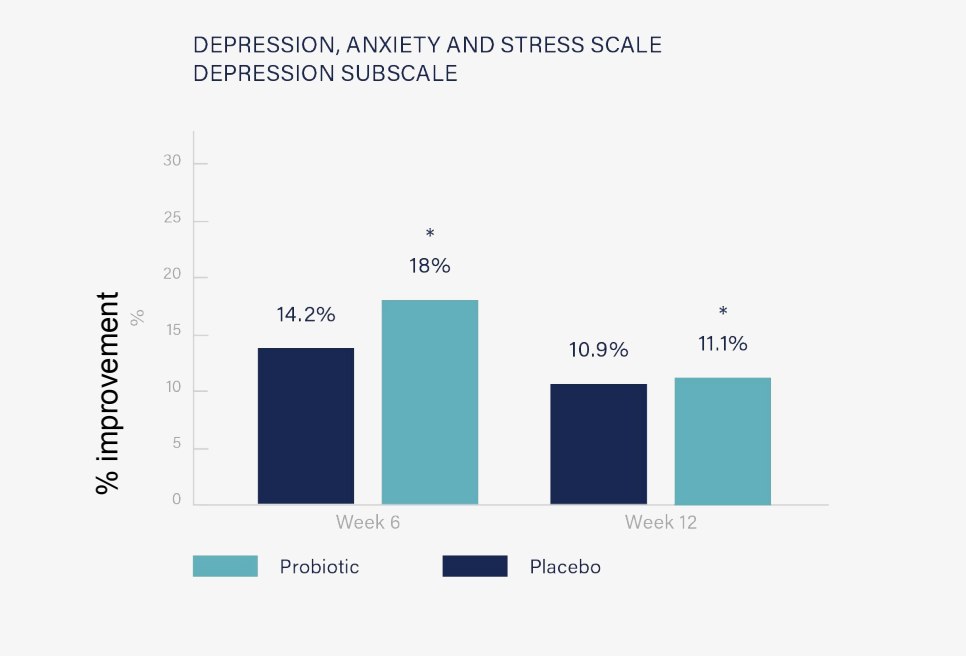


Figure 4: Changes in mean (95% CI) HADS-D total scores *$p < 0.05$, significant within-group change from baseline to follow-up.

The HADS-D total score decreased significantly only in the probiotic group by -3.0 (95% CI -5.4; -0.7) and -2.5 (-4.9; -0.2) at 6 and 12 weeks of intervention respectively.

Questionnaire-Based Assessment Tools

DASS-21 DEPRESSION SUBSCALE (DASS-D)



The Depression, Anxiety and Stress Scale (DASS-21) provides separate severity ratings for depression, anxiety and stress.

Figure 5: Changes in mean (95% CI) DASS-D total scores *$p < 0.05$, significant within-group change from baseline to follow-up.

The DASS-D total score also decreased significantly in the probiotic group by -2.2 (95% CI -4.3; -0.04), at the 6 weeks of intervention mark

Questionnaire-Based Assessment Tools

DASS-21 STRESS SUBSCALE (DASS-S)

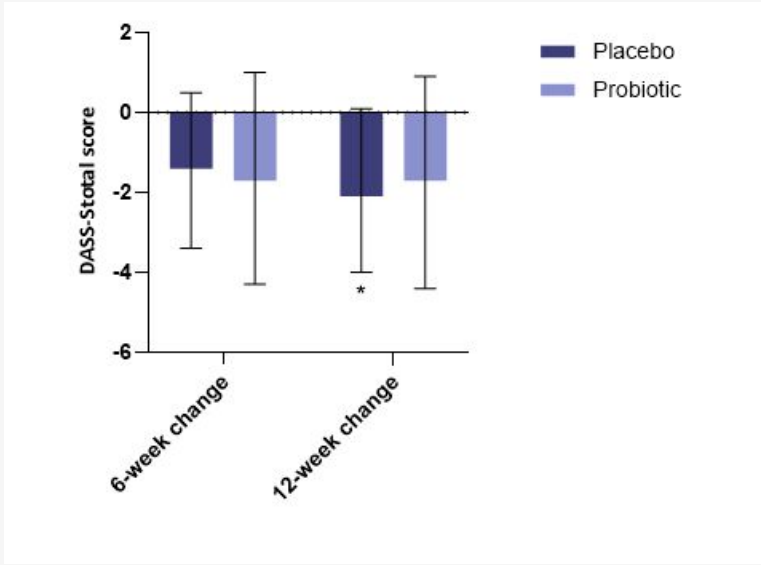


Figure 6: Changes in mean (95% CI) DASS-S total scores
*$p < 0.05$, significant within-group change from baseline to follow-up.

The DASS-S total score decreased significantly only in the placebo group by -2.1 (95% CI -4.0; -0.1) after the 12-week intervention

Anthropometrics data

Table 3 illustrates the changes observed in body weight and body mass index from baseline to the follow-up points (i.e., at 6 and 12 weeks), the changes within each treatment arm as well as the differences between them.

As per the results in Table 3 and considering that changes (especially increases) in weight status/body size can be associated with changes in mood, anxiety and stress levels, the non-existence of between-group differences or within-group changes in anthropometric indices during the intervention is a positive finding, since this means that there is no confounding effect of weight status on the study outcomes.

Table 3. Changes in anthropometrics measurements of people diagnosed with subthreshold depression receiving either a probiotic food supplement (n=19) or a placebo (n=20), from baseline to 6 and 12 weeks of intervention.

	Baseline	6-week Follow-up	6-Week Change	12-Week Follow-up	12-Week Change
	Mean (SD)	Mean (SD)	Mean (95% CI)	Mean (SD)	Mean (95% CI)
Body weight (Kg)					
Placebo	83.4 (21.3)	84.7 (21.5)	1.2 (-12.0; 14.5)	85.0 (19.9)	1.6 (-11.6; 14.8)
Probiotic	76.1 (18.5)	76.5 (17.7)	0.4 (-11.4; 12.2)	77.0 (18.3)	0.9 (-10.8; 12.8)
P-value†	0.257	0.202	0.660*	0.200	0.768*
Body Mass Index (Kg/m^2)					
Placebo	28.8 (6.6)	29.2 (6.9)	0.5 (-3.6; 4.5)	29.2 (5.7)	0.5 (-3.6; 4.6)
Probiotic	27.6 (6.4)	27.7 (6.1)	0.2 (-3.9; 4.3)	28.0 (6.4)	0.4 (-3.7; 4.5)
P-value†	0.568	0.477	0.687*	0.513	0.913*

*: Treatment x Time interaction effect

†: Between-groups' differences in mean values at baseline, 6 and 12 weeks, as well as in 6- and 12-week changes from baseline (Treatment effect)

Dietary intake data

The within-group changes observed in dietary energy and macronutrients' intake from baseline to follow-up time points, as well as the between-group differences are summarised in Table 4.

A 24-hour food recall diary was used to assess significant changes in diet intake across the study period. According to the data in Table 4 and as dietary intake can also affect gut microbiota composition, the non-existence of between-group differences or within-group changes for the vast majority of dietary intake indices during the intervention is a positive finding, since this means that there is no confounding effect of dietary intake (at least for all those nutrients where no significant changes/differences were reported) on the study outcomes. However, the significant findings observed for energy, protein and especially dietary fibre (which has a prebiotic effect) might need to be taken into consideration on future statistical analyses of study outcomes.

Table 4. Changes in dietary intake of people diagnosed with subthreshold depression receiving either a probiotic food supplement (n=19) or a placebo (n=20), from baseline to 6 and 12 weeks of intervention.

	Baseline	**6-week Follow-up**	**6-Week Change**	**12-Week Follow-up**	**12-Week Change**
	Mean (SD)	Mean (SD)	Mean (95% CI)	Mean (SD)	Mean (95% CI)
Energy Intake (KJ/day)					
Placebo	8942.8 (2537.5)	7259.3 (1901.8)	**-1683.4 (-3080.0; -286.8)**	7475.2 (2130.1)	**-1467.5 (-2864.01; -70.9)**
Probiotic	7733.4 (2081.5)	7613.6 (1189.5)	-119.8 (-1221.1; 981.4)	7645.5 (1688.6)	-87.9 (-1189.2; 1013.4
P-value†	0.113	0.493	**0.050***	0.784	0.113*
Protein Intake (% KJ)					
Placebo	19.7 (5.2)	19.7 (4.7)	0.01 (-3.0; 3.0)	18.9 (4.4)	-0.7 (-3.8; 2.3)
Probiotic	19.3 (4.1)	18.5 (3.0)	-0.8 (-3.1; 1.5)	16.8 (3.4)	**-2.5 (-4.8; -0.7)**
P-value†	0.806	0.361	0.590*	0.109	0.419*
Carbohydrates Intake (% KJ)					
Placebo	39.3 (10.5)	38.6 (7.2)	-0.7 (-6.2; 4.8)	37.7 (8.2)	-1.5 (-7.1; 4.0)
Probiotic	37.7 (5.5)	39.1 (7.3)	1.4 (-2.8; 5.6)	41.1 (6.4)	3.4 (-0.7; 7.6)
P-value†	0.555	0.830	0.503*	0.160	0.111*
Total Fibre Intake (% KJ)					
Placebo	2.1 (0.6)	**2.0 (0.8)**	-0.1 (-0.6; 0.3)	2.1 (0.9)	-0.1 (-0.6; 0.4)
Probiotic	2.3 (0.9)	**2.8 (1.4)**	0.4 (-0.3; 1.2)	2.7 (1.2)	0.3 (-0.5; 1.1)
P-value†	0.446	**0.045**	0.151*	0.079	0.237*
Total Fat Intake (% KJ)					
Placebo	34.9 (6.6)	34.4 (5.9)	-0.5 (-5.1; 4.2)	35.8 (9.1)	0.9 (-3.7; 5.6)
Probiotic	35.7 (6.2)	35.1 (5.7)	-0.6 (-4.3; 3.1)	36.7 (5.2)	1.0 (-2.7; 4.8)
P-value†	0.689	0.731	0.931*	0.705	0.976*
Saturated Fat Intake (% KJ)					
Placebo	13.1 (3.5)	12.7 (2.8)	-0.4 (-5.1; 4.2)	13.1 (2.5)	-0.01 (-1.9; 1.9)
Probiotic	13.4 (3.9)	12.4 (2.7)	-1.0 (-3.2; 1.2)	14.0 (3.4)	0.6 (-1.6; 2.8)
P-value†	0.797	0.742	0.588*	0.327	0.623*
Monounsaturated Fat Intake (% KJ)					
Placebo	13.3 (3.8)	13.4 (3.8)	0.2 (-2.9; 3.2)	14.1 (6.4)	-0.8 (-2.2; 3.9)
Probiotic	13.8 (3.8)	13.8 (3.7)	-0.01 (-2.3; 2.3)	14.0 (3.0)	0.2 (-2.1; 2.5)
P-value†	0.687	0.793	0.906*	0.929	0.667*
Polyunsaturated Fat Intake (% KJ)					
Placebo	5.6 (1.7)	5.3 (1.5)	-0.3 (-1.6; 0.9)	5.6 (2.7)	-0.05 (-1.3; 1.2)
Probiotic	5.4 (2.0)	5.4 (2.0)	-0.01 (-1.3; 1.3)	5.6 (2.1)	0.2 (-1.1; 1.5)
P-value†	0.715	0.851	0.755*	0.956	0.755*
Alcohol Intake (% KJ)					
Placebo	3.1 (3.0)	4.9 (6.8)	1.8 (-1.4; 5.1)	3.9 (4.7)	0.8 (-2.4; 4.0)
Probiotic	4.1 (5.3)	3.9 (3.4)	-0.2 (-2.8; 2.4)	4.1 (3.0)	-0.02 (-2.6; 2.6)
P-value†	0.445	0.577	0.239*	0.843	0.633*

*: Treatment x Time interaction effect

†: Between-groups' differences in mean values at baseline, 6 and 12 weeks, as well as in 6- and 12-wekk changes from baseline (Treatment effect)

6 week follow-up

Table 6 presents the within-group changes and between-group differences in the examined depression, mood, anxiety, stress, and quality of life scores from baseline to the 18-week follow-up time-point (i.e., 6 weeks post-intervention).

The results presented in Table 6 indicate that the improvements observed in the probiotic group in terms of depressive and anxiety symptoms (i.e., through the greater reductions seen in BDI HADS-A, HADS-D total scores) at the end of the intervention, also remained 6 weeks post-intervention. As this post-intervention survey was optional, data was received from 10 and 9 participants in the probiotic and the placebo group. Despite these numbers and the fact that an intention-to-treat analyses could not be performed, these significant changes are very important since they are indicative of the longer-term effectiveness of the probiotic supplement. Although, not statistically significant and with the only exception of the DASS-A, the changes (reductions) in all other scores were also in favour of the probiotic group.

Table 6. Changes in the severity of depressive symptoms, other measures of psychosocial health and quality of life in people diagnosed with subthreshold depression receiving either a probiotic food supplement (n=10) or a placebo (n=9), from baseline to the 18-week follow-up (6 weeks post-intervention).

	Baseline	18-Week Follow-up (6 weeks post-intervention)	18-Week Change
	Mean (SD)	Mean (SD)	Mean (95% CI)
BDI total score			
Placebo	20.9 (7.5)	18.8 (10.8)	-2.1 (-8.9; 4.7)
Probiotic	22.8 (10.1)	15.3 (12.4)	**-7.5 (-15.0; -0.005)**
P-value†	0.650	0.525	0.245*
PHQ total score			
Placebo	9.3 (3.7)	8.3 (4.2)	-1.0 (-5.4; 3.4)
Probiotic	12.3 (5.1)	10.0 (7.4)	-2.3 (-6.1; 1.4)
P-value†	0.171	0.563	0.611*
HADS-A total score			
Placebo	6.3 (2.1)	8.3 (4.4)	2.0 (-1.0; 5.0)
Probiotic	10.5 (3.2)	8.1 (5.3)	-2.4 (-5.6; 0.8)
P-value†	**0.004**	0.919	**0.037***
HADS-D total score			
Placebo	7.7 (2.3)	7.7 (4.0)	0.0 (-3.0; 3.0)
Probiotic	8.8 (4.1)	5.2 (4.9)	**-3.6 (-6.4; -0.8)**
P-value†	0.479	0.250	0.062*
HADS total score			
Placebo	**14.0 (2.7)**	16.0 (6.7)	2.0 (-3.5; 7.5)
Probiotic	**19.3 (7.0)**	13.3 (9.6)	**-6.0 (-11.7 -0.3)**
P-value†	**0.048**	0.491	**0.035***
DASS-D total score			
Placebo	7.7 (4.2)	7.4 (5.1)	-0.3 (-2.7; 2.3)
Probiotic	7.9 (4.5)	6.0 (5.4)	-1.9 (-6.3; 2.5)
P-value†	0.909	0.558	0.474*
DASS-A total score			
Placebo	2.7 (2.2)	2.8 (2.0)	0.1 (-0.9; 1.1)
Probiotic	3.4 (3.4)	4.0 (4.8)	0.6 (-1.2; 2.4)
P-value†	0.591	0.486	0.610*
DASS-S total score			
Placebo	6.8 (2.3)	7.0 (2.9)	0.2 (-1.6; 2.0)
Probiotic	9.1 (4.3)	7.3 (6.4)	-1.8 (-5.5; 1.9)
P-value†	0.167	0.899	0.294*
PSS total score			
Placebo	19.2 (4.8)	20.6 (3.0)	1.4 (-1.5; 4.1)
Probiotic	22.2 (3.5)	21.4 (3.3)	-1.2 (-5.5; 3.5)
P-value†	0.138	0.560	0.324*
AQoL total score			
Placebo	82.7 (12.2)	76.2 (16.8)	-6.4 (-16.2; 3.3)
Probiotic	87.5 (17.7)	76.6 (24.9)	-10.9 (-27.3; 5.5)
P-value†	0.502	0.970	0.614*

*: Treatment x Time interaction effect; †: Between-groups' differences in mean values at baseline, 18 weeks, as well as in 18-week changes from baseline (Treatment effect).

Note: Decreases in the scores are indicative of improvements in the relevant depressive symptoms, mood, anxiety, stress, and quality of life that each score measures.

Blood analysis

Table 7 presents the within-group changes and between-group differences in fasting plasma glucose, serum insulin, HOMA-IR (i.e. a biomarker of insulin resistance), serum high-sensitivity CRP (i.e., a marker of inflammation) and total glutathione concentrations (i.e., a peptide with antioxidant/protective properties, especially in brain cells).

The results presented in Table 7 clearly indicate a reduction in inflammation (i.e., through the reduction in hs-CRP levels) in the probiotic arm, as well as an improvement in brain antioxidant capacity (i.e., through the increases observed in total glutathione levels) both at 6 and 12 weeks of treatment. The significant differences that were observed between the probiotic compared to the placebo arm further support the anti-inflammatory and antioxidant properties (especially in the brain cells) of the probiotic supplement, which also "back-up" the favourable changes observed in the depression and anxiety scores.

Table 7. Changes in biochemical markers of glycemic profile, inflammation and oxidative stress in people diagnosed with subthreshold depression receiving either a probiotic food supplement (n=19) or a placebo (n=19), from baseline to 6 and 12 weeks of intervention.

	Baseline	6-week Follow-up	6-Week Change	12-Week Follow-up	12-Week Change
	Mean (SD)	Mean (SD)	Mean (95% CI)	Mean (SD)	Mean (95% CI)
Fasting Plasma Glucose (nmol/L)					
Placebo	6.3 (4.3)	6.3 (3.9)	-0.01 (-0.78; 0.77)	6.4 (4.8)	0.1 (-1.3; 1.5)
Probiotic	6.2 (3.3)	5.3 (3.2)	**-0.9 (-1.5; -0.2)**	4.4 (2.9)	**-1.8 (-2.8; -0.7)**
P-value†	0.941	0.422	0.082		**0.028**
Serum Insulin (µIU/mL)					
Placebo	9.7 (8.0)	9.5 (6.0)	-0.2 (-4.8; 4.5)	9.5 (6.8)	-0.2 (-4.4; 4.0)
Probiotic	9.1 (5.2)	8.5 (6.2)	-0.6 (-4.6; 3.3)	8.6 (7.6)	-0.5 (-5.5; 4.4)
P-value†	0.803	0.598	0.864*	0.701	0.909*
HOMA-IR					
Placebo	3.2 (3.6)	2.6 (2.0)	-0.6 (-2.0; 0.9)	2.7 (3.0)	-0.4 (-1.6; 0.7)
Probiotic	2.6 (2.1)	2.6 (3.4)	0.01 (-1.3; 1.3)	2.0 (2.6)	-0.6 (-1.9; 0.8)
P-value†	0.540	0.982	0.528		0.858
hs-CRP (ng/mL)					
Placebo	6720.8 (1190.7)	6682.5 (1600.5)	-38.3 (-459.8; 383.2)	7286.2 (1205.8)	565.4 (-297.4; 1428.3)
Probiotic	6931.0 (1517.2)	6108.4 (1540.0)	**-822.6 (-1528.2; -116.9)**	5976.4 (1408.3)	-954.6 (-1914.9; 5.69)
P-value†	0.638	0.267	**0.053***	**0.004**	**0.018***
Total GSH (ng/dL)					
Placebo	7.7 (3.8)	8.7 (2.8)	1.1 (-1.1; 3.4)	9.3 (4.7)	1.6 (-0.8; 4.1)
Probiotic	8.8 (4.1)	13.8 (5.0)	**5.0 (1.9; 8.1)**	14.2 (8.9)	**5.4 (0.1; 10.8)**
P-value†	0.392	**<0.001**	**0.044***	**0.040**	0.185*

hs-CRP: High sensitivity C-Reactive Protein; GSH: Total Glutathione

*: Treatment x Time interaction effect; †: Between-groups' differences in mean values at baseline, 6 and 12 weeks, as well as in 6- and 12-week changes from baseline (Treatment effect)

Saliva analysis

Table 8 summarizes the within-group changes and between-group differences in saliva cortisol levels (i.e., as stress biomarker) at awakening (S1), 30-minutes post-awakening (S2) and in the evening at 2030 hours (S3), as well as in the Cortisol Awakening Response (CAR), which represents the normally occurring increase in cortisol levels after awakening and has been positively related to stress.

The results presented in Table 8 clearly indicate a reduction in stress levels (i.e., through the reduction in S2 cortisol and CAR levels) in the probiotic arm, compared to the placebo at the end of the 12-week intervention period. These findings also support the favourable changes observed in the depression and anxiety scores coming from the questionnaires.

Table 8. Changes in saliva cortisol levels at awakening (S1), 30-minutes post-awakening (S2) and in the evening at 2030 hours (S3) in people diagnosed with subthreshold depression receiving either a probiotic food supplement (n=18) or a placebo (n=20), from baseline to 6 and 12 weeks of intervention.

	Baseline	6-week Follow-up	6-Week Change	12-Week Follow-up	12-Week Change
	Mean (SD)	Mean (SD)	Mean (95% CI)	Mean (SD)	Mean (95% CI)
Saliva cortisol levels S1 (µg/dL)					
Placebo	0.36 (0.20)	0.31 (0.26)	-0.05 (-0.19; 0.09)	0.42 (0.26)	0.06 (-0.09; 0.22)
Probiotic	0.34 (0.20)	0.42 (0.22)	0.08 (-0.09; 0.24)	0.39 (0.24)	0.05 (-0.10; 0.20)
P-value†	0.772	0.158	0.208*	0.703	0.908*
Saliva cortisol levels S2 (µg/dL)					
Placebo	0.48 (0.37)	0.49 (0.34)	0.01 (-0.21; 0.23)	0.59 (0.40)	0.11 (-0.07; 0.29)
Probiotic	0.53 (0.31)	0.51 (0.43)	-0.02 (-0.29; 0.26)	0.35 (0.24)	-0.18 (-0.40; 0.04)
P-value†	0.650	0.871	0.853*	**0.033**	**0.039***
Saliva cortisol levels S3 (µg/dL)					
Placebo	0.18 (0.22)	0.14 (0.17)	-0.04 (-0.15; 0.06)	**0.18 (0.17)**	-0.002 (-0.10; 0.10)
Probiotic	0.13 (0.18)	0.13 (0.16)	0.001 (-0.12; 0.12)	**0.09 (0.10)**	-0.04 (-0.15; 0.07)
P-value†	0.451	0.916	0.558*	**0.066**	0.589*
Cortisol Awakening Response (µg/dL)					
Placebo	0.11 (0.34)	0.18 (0.34)	0.07 (-0.15; 0.29)	**0.16 (0.25)**	0.05 (-0.10; 0.21)
Probiotic	0.18 (0.35)	0.08 (0.44)	-0.10 (-0.40; 0.21)	**-0.04 (0.17)**	**-0.22 (-0.40; -0.04)**
P-value†	0.534	0.469	0.360*	**0.006**	**0.020***

S1: saliva cortisol levels at awakening; S2: saliva cortisol levels 30-minutes post-awakening; S3: : saliva cortisol levels in the evening at 20:30 hours; CAR: Cortisol Awakening Response (i.e., Saliva cortisol levels 30-minutes post-awakening - Saliva cortisol levels at awakening).
*: Treatment x Time interaction effect; †: Between-groups' differences in mean values at baseline, 6 and 12 weeks, as well as in 6- and 12-week changes from baseline (Treatment effect)

Faecal analysis

Figures 10 and 11 graphically present the gut microbiome diversity measures by timepoint (i.e., baseline, 6-week and 12-week follow-up) and treatment arm (i.e., probiotic and placebo) respectively.

According to these figures there were no differences or trends observed between timepoints or treatment arms. The effects of the Biome Lift™ Probiotic on gut microbiota composition and function were a secondary aim of the study, and these results suggest that the improvements observed in depression and anxiety scores may not be mediated by changes to the gut microbiota.

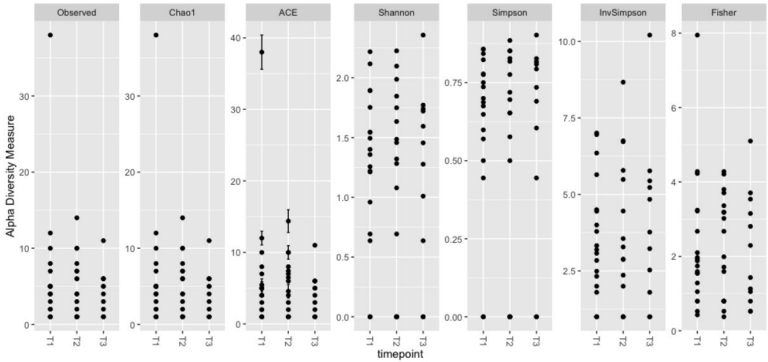


Figure 10. Gut microbiome diversity at baseline (T1), 6-week (T2) and 12-week (T3) follow-up time points of measurements.

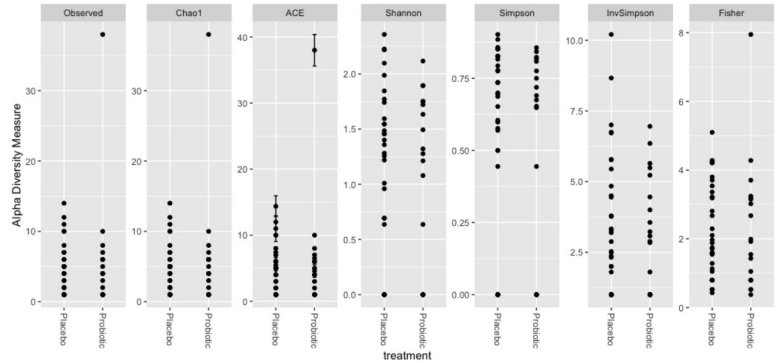


Figure 11. Gut microbiome diversity in the placebo and the intervention group.

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