1-2024

Multi-strain probiotic improves subjective sleep quality with no impact on body composition, hemodynamics, and physical activity

Chad Kerksick

Jessca Moon

Kylie Walden

Anthony Hagele

L. E. Allen

See next page for additional authors

Authors

Chad Kerksick, Jessca Moon, Kylie Walden, Anthony Hagele, L. E. Allen, Connor J. Gaige, Joesi M. Krieger, Ralf Jäger, Marco Pane, and Petey Mumford

BRILL
WAGENINGEN
ACADEMIC

BENEFICIAL MICROBES (2024) DOI:10.1163/18762891-BJA00002

Beneficial Microbes
brill.com/bm

RESEARCH ARTICLE

Multi-strain probiotic improves subjective sleep quality with no impact on body composition, hemodynamics, and physical activity

C.M. Kerksick[1], J.M. Moon[1], K.E. Walden[1], A.M. Hagele[1], L.E. Allen[1], C.J. Gaige[1], J.M. Krieger[1], R. Jäger[2], M. Pane[3] and P. Mumford[1]*

[1]Exercise and Performance Nutrition Laboratory, College of Science, Technology and Health, Department of Kinesiology, Lindenwood University, St. Charles, MO 63301, USA; [2]Increnovo, LLC, Milwaukee, WI, USA; [3]Probiotical Research srl, Novara, Italy; *ckerksick@lindenwood.edu

Received 13 October 2023 | Accepted 17 December 2023 | Published online 31 January 2024

Abstract

The objective of the study was to examine the impact of a multi-strain probiotic (MSP) on sleep, physical activity, and body composition changes. We used a randomised, double-blind, placebo-controlled approach with 70 healthy men and women (31.0 ± 9.5 years, 173.0 ± 10.4 cm, 73.9 ± 13.8 kg, 24.6 ± 3.5 kg/m^2) supplemented daily with MSP (4×10^9 live cells *Limosilactobacillus fermentum* LF16, *Lacticaseibacillus rhamnosus* LR06, *Lactiplantibacillus plantarum* LP01, and *Bifidobacterium longum* 04; Probiotical S.p.A., Novara, Italy) or placebo (PLA). In response to supplementation (after 0, 2, 4, and 6 weeks of supplementation) and 3 weeks after stopping supplementation, participants had subjective (Pittsburgh Sleep Quality Index, PSQI) and objective sleep indicators, body composition, daily physical activity and resting hemodynamics assessed. Subjective sleep quality indicators using the PSQI (sleep latency, sleep disturbance, and global PSQI score) improved ($P < 0.05$) at various time points with MSP supplementation. Systolic blood pressure in PLA increased ($P < 0.05$) after 6 weeks of supplementation with no change in MSP. No changes ($P > 0.05$) in sleep (hours asleep, minutes awake, number of times awake) or physical activity (step count, minutes of sedentary activity, total active minutes) metrics assessed by the wearable device were observed. Additionally, no changes in resting heart rate, diastolic blood pressure, and body composition were discerned. In conclusion, MSP supplementation improved the subjective ability to fall asleep faster and disturbances experienced during sleep, which resulted in improved overall sleep quality as assessed by the PSQI. No differences in other sleep indicators, physical activity, hemodynamics, and body composition were observed during or following MSP supplementation.
Registered at clinicaltrials.gov: NCT05343533

Keywords

probiotic – gut-brain – quality – latency – onset – steps

1 Introduction

The existence of the gut-brain axis has been well-established and recognised as a bidirectional pathway in which the nervous, endocrine, and immune systems communicate back and forth with the intestinal microbiota of the gastrointestinal tract (GIT) (Grenham *et al.*, 2011; Mayer, 2011; Mayer *et al.*, 2014). Through this

Published with license by Koninklijke Brill NV | DOI: 10.1163/18762891-BJA00002
 | ISSN: 1876-2883 (print) 1876-2891 (online)

pathway, the GIT microbiota's bacteria, intestinal neurons, neurotransmitters, and gut hormones are interconnected. The GIT bacteria signals the brain to activate intestinal neurons, leading to the production of neurotransmitters and gut hormones, which help maintain homeostasis within a host (Ahmed *et al.*, 2022; Muller *et al.*, 2020). As the GIT alone has been estimated to have ten times more microorganisms than that of human cells in our bodies (Grenham *et al.*, 2011). Consequently, disruptions within the GIT can easily occur, inhibiting the gut-brain axis (Mayer, 2011). With these factors being identified, research has focused on specific interventions to help combat potential disruptions, improve and/or maintain the gut-brain axis communication, and support the GIT microbiota to ensure signalling of all components are sufficient (Bagga *et al.*, 2018a; Marotta *et al.*, 2019; Tillisch *et al.*, 2013).

Notably, probiotics have been investigated as a significant focal point for GIT microbiota interventions. Defined as live microorganisms that, when administered in adequate amounts, confer a health benefit on the host (Colin Hill, 2014), probiotics have been shown to significantly impact the microbial environment in the GIT. In particular, a 2018 study conducted by Bagga *et al.* (2018b) saw that four weeks of supplementation with a multi-strain probiotic in 45 healthy adults changed GIT microbiome profiles as seen through stool samples. Changes in brain activation patterns in response to emotional memory and decision-making tasks were also seen through functional magnetic resonance imaging (fMRI). Tillisch *et al.* (2013) also used fMRI to conduct a randomised, double-blind study in healthy females (n = 36) to investigate the effects of supplementation with a fermented milk product with probiotics (FMPP). After four weeks of FMPP consumption, brain activity was improved in the brain regions that control the central processing of emotion and sensation. Additionally, through focusing specifically on different aspects of mood, a six-week study that involved supplementation with a probiotic mixture in healthy adults (n = 38) resulted in significant improvements in overall mood and sleep quality, with reductions in a depressive mood state, anger, and fatigue (Marotta *et al.*, 2019).

As research has shown that probiotics significantly affect the GIT microbiota and the bidirectional pathway of the gut-brain axis to improve various aspects of brain activation and mood, different factors associated with this bidirectional pathway that may be impacted by probiotics have been brought into question. Specifically, parameters of sleep and sleep quality in general have been investigated as to whether probiotics could improve the duration, quality, latency, and efficiency of sleep. For example, Takada *et al.* (2017) saw that 11 weeks of supplementation with 1×10^9 cfu/ml of *Lactobacillus casei* strain Shirota YIT 9029 (LcS) probiotic fermented milk in healthy adults (n = 94) positively improved subjective scores for sleepiness, sleep length, and sleep latency. Sleep was also investigated in a 2021 study in 20 healthy male adults, where after eight weeks of supplementation with 1×10^9 cfu/day of *Bifidobacterium longum* 1714 significant improvements in overall sleep quality and duration of sleep were observed using the Pittsburgh Sleep Quality Index (PSQI) (Moloney *et al.*, 2021). As briefly mentioned earlier, Marotta *et al.* (2019) also examined the beneficial aspects of probiotic supplementation on sleep and administered six weeks of supplementation with 4×10^9 cfu/Active Fluorescent Unit (AFU) of 2.5 g freeze-dried powder of the probiotic mixture containing *Lactobacillus fermentum* LF16 (DSM 26956), *L. rhamnosus* LR06 (DSM 21981), *L. plantarum* LP01 (LMG P-21021), and *Bifidobacterium longum* BL04 (DSM 23233). Using the PSQI, improvements in sleep quality were seen along with improved sleep quality being correlated with improved anxiety/depressive symptoms, fatigue, anger, confusion, and vigour. Although examining sleep, mood, and brain functioning in relation to the beneficial effect probiotics have on the gut-brain axis are at the forefront of investigations and research studies, there are background factors that warrant discussion.

Levels of physical activity and body composition can also prove to be important factors to focus on regarding the gut-brain axis and probiotic supplementation. To date, limited research has explored the effect of probiotic supplementation on physical activity levels and changes in body composition in conjunction with sleep assessment. Briefly, Quero *et al.* (2021) supplemented young, sedentary and professional soccer players with a mixture of probiotic strains (*Bifidobacterium lactis* CBP-001010, *Lactobacillus rhamnosus* CNCM I-4036, and *Bifidobacterium longum* ES1) and the prebiotic fructooligosaccharides for four weeks and evaluated changes in inflammatory, immune, and stress biomarkers in addition to other questions and assessments related to physical and mental health. The more physically active group (the professional soccer players) experienced greater improvements in physical activity and sleep quality along with perceived levels of health, stress, and anxiety. Furthermore, differential responses were observed in IL-1β, CRH, and dopamine that were specific to each group. While more research is needed to explore the interaction between probiotics and phys-

ical activity levels, the links between mood, sleep, and brain functioning on physical activity and body composition have undergone more research. Motivation, for example, an aspect of mood and is a significant factor that can impact the physical activity level of an individual, which can extend to impact physical and mental health in addition to body composition (Frederick *et al.*, 1996; Mayer, 2011). Motivation may also be an indicator of how well the GIT microbiota and brain are communicating via the bidirectional pathway (Ahmed *et al.*, 2022; Mayer, 2011). Ensuring the gut-brain axis has efficient bidirectional communication can help to improve motivation specifically, but also mood, sleep, and brain functioning.

Although there is a strong foundation for research concerning probiotic supplementation on gastrointestinal function, immunity, and certain aspects of the gut-brain axis, less research is available that has explored the potential efficacy for probiotics to modulate sleep, physical activity, and body composition. Due to the need for research in this area, the purpose of this study was to investigate the efficacy of supplementing with a multi-strain probiotic on changes in sleep, physical activity, and body composition.

2 Materials and methods

Overview of research design

A randomised, double-blind, placebo-controlled, parallel group study was employed to evaluate the ability of a multi-strain probiotic to influence sleep quality and quantity, physical activity, body composition and hemodynamics. The primary outcomes for this investigation were the sleep quality and quantity while the outcomes related to physical activity, body composition, and hemodynamics were secondary outcomes. Healthy men and women between the ages of 18-50 years were assigned to one of two supplementation groups. They either received a multi-strain probiotic (1×10^9 live cells of each of the four strains: *Limosilactobacillus fermentum* LF16 (DSM 26956), *Lacticaseibacillus rhamnosus* LR06 (DSM 21981), *Lactiplantibacillus plantarum* LP01 (LMG P-21021), and *Bifidobacterium longum* 04 (DSM 23233); Bifizen™, Probiotical S.p.A., Novara, Italy) or a maltodextrin placebo (PLA). Prior to study engagement, all participants signed an IRB-approved informed consent document (Lindenwood University: IRB-19-212, approval date: 7/17/19, conformed to the standards set by the latest revision of the Declaration of Helsinki), completed a health history questionnaire, and answered a series of questions to determine study eligibility.

TABLE 1 Overview of research design

Week	Pre	0	2	4	6	9
Visit	1	2	3	4	5	6
Review and sign consent	X					
Answer study questions	X					
Food/fluid log		X	X	X	X	X
Take assigned supplement			X	X	X	
DEXA		X			X	X
Pittsburgh Sleep Quality Index		X	X	X	X	X
Wearable sleep metrics		X	X	X	X	X
Hemodynamics	X	X	X	X	X	X
Wearable physical activity		X	X	X	X	X
Adverse event monitoring		X	X	X	X	X

Participants were scheduled for four identical study visits between 06:00-10:00 h while attempts were made to standardise visit timing across the investigation. For all visits, subjects were instructed to undergo an 8-10 h fast (except water) and abstain from strenuous physical activity 48 hours prior to each visit. All participants supplemented for six weeks followed by a three-week washout period post-supplementation. As seen in Table 1, all participants completed 2-day food records and were evaluated for changes in sleep parameters, body composition, hemodynamics, and physical activity levels after 0, 2, 4, and 6 weeks of supplement administration and 3 weeks after supplementation ceased. This study protocol and design was registered on Clinicaltrials.gov on April 25, 2022, as NCT05343533. Outcomes related to self-assessed parameters of mood, anxiety, and personality have been published previously (Walden *et al.*, 2023). The study design was powered, and sample size was determined based upon the required changes needed to investigate the impact of supplementation on self-assessed parameters of mood. Based upon previous data of Marotta *et al.* (2019) an *a priori* sample size evaluation indicated that if a moderate effect size was realised ($d = 0.5$), a sample size of 28-33 participants would be needed to detect statistical significance with an assumed alpha (α) level of 0.05 and estimated power ($1 - \beta$) of 0.80.

Study participants

A total of 70 healthy men and women (31.0 ± 9.5 years, 173.0 ± 10.4 cm, 73.9 ± 13.8 kg, 24.6 ± 3.5 kg/m^2) successfully completed the study protocol. A Consolidation Standards of Reporting Trials (CONSORT) diagram is provided in Figure 1. Participants were included in

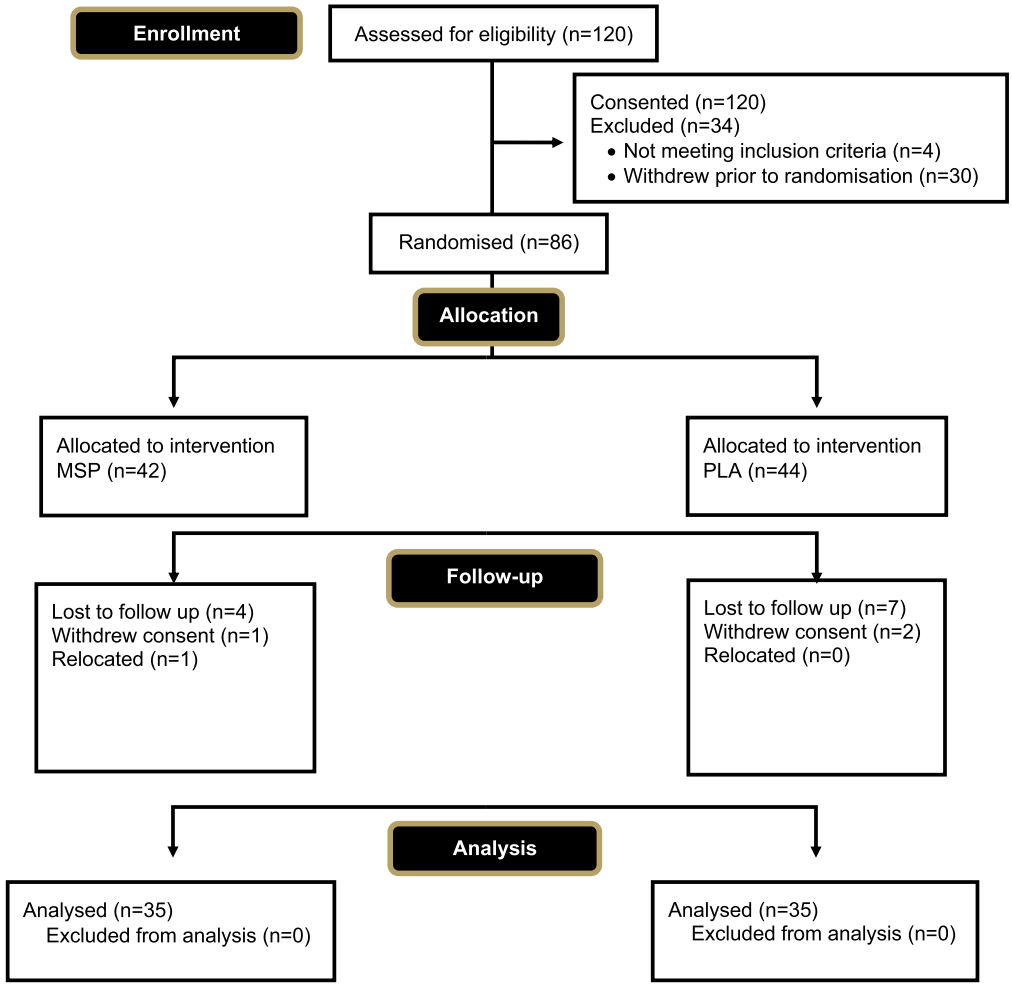

FIGURE 1 CONSORT diagram.

the study if they were between the ages of 18-50 years, had a body mass index (BMI) between 18.5-32 kg/m^2 (if BMI was 30-32 kg/m^2, participant was required to have body fat percentage < 25% for men and <35% for women for inclusion), were weight stable for the past three months (<5% variation in body mass), and deemed healthy through completion of a health history questionnaire. Participants were excluded if they were diagnosed or were being treated for any cardiac, respiratory, endocrine, psychiatric, musculoskeletal, renal, hepatic, neuromuscular or metabolic disease or disorder that precluded safe participation or would contraindicate quality control over the collected data, were diagnosed with or being treated for celiac disease, lactose intolerance, digestive insufficiencies, or other gastrointestinal complications such as irritable bowel syndrome, ulcerative colitis, etc., reported being a current smoker or had quit within the past six months, reported having used any illicit or recreational drugs including anabolic steroids within the past 30 days, reported the intake of any prescription or over-the-counter medications (i.e. antibiotics) that may impact study outcomes, reported the current use of any dietary supplements known to impact digestion or sleep quality for the past 30 days, reported taking a probiotic within the past 30 days, had been actively trying to lose weight, or were currently following a ketogenic or low carbohydrate diet within the past 30 days. Further, antibiotic use at any point in the study protocol led to removal from the study. Women who were pregnant, lactating, or indicated during screening they were actively trying to become pregnant were excluded from the study.

Procedures

Baseline demographics and hemodynamics

Body mass was determined using a digital scale (Tanita BWB-627A, Tokyo, Japan) and recorded to the nearest ± 0.1 kg. Height was measured using a standard wall-mounted stadiometer (Tanita, HR-200) and recorded to the nearest ± 0.5 cm. Both measurements were made after participants voided their bladders and removed their shoes. Participants then rested quietly for approx-

imately 10 minutes before measuring resting heart rate and blood pressure (Omron BP785, Omron Corporation, Kyoto, Japan).

Diet assessment and control

Study participants completed the automated self-administered 24-h dietary assessment tool (ASA24; https://epi.grants.cancer.gov/asa24/). Healthy Eating Index values were computed from the completed food records. The Healthy Eating Index is a measure of diet quality that can be used to assess compliance with the Dietary Guidelines for Americans. From the collected food intake information, 13 different food categories (seafood and plant proteins, fatty acids, refined grains, sodium added sugars, saturated fats, total fruits, whole fruits, total vegetables, greens and beans, whole grains, dairy, and total protein foods) are populated and the amount of food and fluid consumed that aligns with that food category is evaluated against the recommended intake (https://epi.grants.cancer.gov/hei/interpret-visualise-hei-scores.html). Each category is then assigned a percentage and from there a Healthy Eating Index composite score is determined. Radar plots have been generated to evaluate the difference in Healthy Eating Index values between groups and to evaluate the extent to which composite Healthy Eating Index values changed across time.

Body composition

Body composition was obtained using dual-energy X-ray absorptiometry (DEXA) after 0 and 6 weeks of supplementation as well as three weeks after stopping supplementation to evaluate changes in fat mass, fat-free mass, and percent body fat. Calibration procedures were performed before each testing session and all scans were completed using a Hologic QDR Discovery A (Hologic, Bedford, MA, USA) and analysed using the accompanying software (Hologic APEX Software, Version 4.5.3). The test-retest reliability (ICC and CV) of these procedures has previously been determined to be 0.99% and 1.26% for DEXA fat and 0.99% and 0.75% for DEXA fat-free mass (data not shown). All results were calculated using the NHANES correction factor.

Sleep quality and quantity

The Pittsburgh Sleep Quality Index (PSQI) is a validated self-report questionnaire that subjectively evaluates various aspects of sleep quality over the past month (Buysse *et al.*, 1989). The PSQI has 19 questions that inquire about usual bedtime, time to fall asleep, waking time, hours slept per night, and other aspects of sleep quality. Each item is weighted on 0-3 scale, whereby a score of 3 reflects the negative extreme on the scale. All scores are grouped in seven component scores (sleep duration, use of sleeping medications, sleep disturbances, subjective sleep quality, habitual sleep efficiency, daytime dysfunction, and sleep latency) and all component scores can be added together to yield a global score. Qualitatively, a global sum of 5 or greater indicates poor sleep quality. In addition to the PSQI, participants were outfitted with a wrist-based accelerometer (Fitbit Inspire 2, Fitbit, San Francisco, CA, USA) to quantitively and objectively evaluate additional sleep metrics (average total minutes slept, number of times restless, number of times awake, total minutes awake or restless).

Physical activity

Physical activity was not controlled throughout this study protocol. Eligible participants were instructed to maintain physical activity status throughout the study protocol, and to inform research staff if their physical activity habits changed throughout the study protocol. Further, wrist-based accelerometers (FitBit Inspire 2) were worn for one week prior to beginning the supplementation intervention and for the entire duration of the study. Weekly averages of step counts, minutes of sedentary activity, and total activity minutes per day were recorded and evaluated.

Supplementation protocol

Using a randomised, double-blind, placebo-controlled, parallel group fashion, participants were assigned to ingest a single capsule of either a maltodextrin placebo (PLA) or a mixture of four probiotic strains (MSP). Each probiotic dose was delivered in capsules containing a 1×10^9 live cells dose of each of the following strains (total daily dose of 4×10^9 live cells): *Limosilactobacillus fermentum* LF16 (DSM 26956), *Lacticaseibacillus rhamnosus* LR06 (DSM21981), *Lactiplantibacillus plantarum* LP01 (LMG P-21021), and *Bifidobacterium longum* 04 (DSM 23233) (Probiotical S.p.A., Novara, Italy). Capsules for the placebo and probiotic strain were identical in color, shape, size, and transparency and were packaged into identical, bottled, containers that contained the same number of capsules. Participants were instructed to consume each dose at approximately the same time each day with 240-360 ml of water and within two hours of consuming a meal. Product stability was monitored across the whole study up to 24 months at three different temperature conditions (refrigerated at 5 °C, Zone II at 25 °C and 65% relative humidity and Zone IVb

at 30 °C and 75% relative humidity) confirming the threshold values of 4×10^9 live cells at the end of the 24 months program (Walden *et al.*, 2023).

Adverse event reporting

Adverse events were collected via spontaneous reporting by the participants, clinical evaluation, interaction of a research team member with a participant, or through review of a participant's research file throughout the entire duration of the protocol. This data has been previously reported (Walden *et al.*, 2023).

Statistical analysis

Before any statistical tests were performed, raw data was screened for data entry and organisation errors, and then analysed for normality, skewness, and kurtosis. Any non-normally distributed value was normalised, if possible, using log-transformations. All data is reported as mean ± standard deviation. For all statistical tests, data was considered statistically significant when the probability of type I error is 0.05 or less. A trend or a tendency for change was determined when the probability of type I error was $P = 0.051– \leq 0.10$. Parametric data was analysed using mixed factorial ANOVA (group × time) with repeated measures on time. When significant group × time interactions were found, delta values (from baseline) were calculated and assessed using independent t-tests. Normality was not assessed on the calculated delta values. Main effects of time were analysed using single-factor ANOVA with repeated measures on time, and pairwise comparisons were evaluated using Bonferroni corrections. Non-parametric data was first assessed using a Friedman test for K-related samples, and if significant ($P < 0.05$), follow-up assessments were completed using a Wilcoxon signed-rank test between each baseline score and each subsequent follow-up timepoint. Following this approach, the Friedman test was used to evaluate the presence of changes over time for sleep latency, subjective sleep, sleep duration, sleep efficiency, sleep medications, sleep disturbance, daytime dysfunction, and global PSQI scores. Wilcoxon signed rank tests were used to evaluate changes between timepoints within a given condition for sleep latency and global PSQI scores. Bonferroni corrections were applied to evaluate pairwise comparisons (0.05 / # of comparisons being made). Between-group differences were evaluated at individual timepoints using Mann-Whitney U for subjective sleep, sleep duration, sleep efficiency, sleep medications, sleep disturbance, daytime dysfunction, and global PSQI scores. All analyses were completed using Microsoft Excel and the Statistical Package for the Social Sciences (v27; SPSS Inc., Chicago, IL, USA).

3 Results

Confirmation of probiotic viability

As reported previously (Walden *et al.*, 2023), the finished supplementation product was analysed (Biolab srl., Novara, Italy) via flow cytometry (ISO 19344, 2015: IDF 232: 2015) upon batch release which resulted in a cell count of $>4 \times 10^9$ Active Fluorescent Unit (AFU)/g and plate count method as cfu (Internal Method 014-06).

Supplementation compliance

Compliance to the supplementation protocol was calculated and reported herein. Participants assigned to the MSP condition reported compliance between 76.2-100.0% (mean ± standard deviation (SD): 93.7 ± 7.3%) while compliance in the PLA group ranged from 71.4-100.0% (mean ± SD: 94.6 ± 7.1%).

Adverse event reporting

Participants self-reported adverse events throughout the study protocol. Twelve adverse events were reported in MSP (constipation, n = 1; tired in morning, n = 1, flatulence, n = 4, bloating, n = 3; lower back pain, n = 2; lower abdominal pain, n = 1) while 46 adverse events were reported by participants assigned to the PLA condition (constipation, n = 3; tired in morning, n = 1, burping, n = 3; flatulence, n = 6, bloating, n = 4; lower back pain, n = 2; lower abdominal pain, n = 1; acid reflux, n = 4; irritated oesophagus, n = 2; skin irritation, n = 13, dry eyes, n = 4, fatigue, n = 2, and increased need to use bathroom, n = 1). All reported adverse events, independent of supplement assignment, were mild or moderate in severity. No serious adverse events were reported.

Dietary intake

Of the 70 participants who completed the current study, 60 of them provided suitable dietary data and this data has been presented previously (Walden *et al.*, 2023). Four additional participants provided suitable baseline dietary data but did not provide a suitable follow-up measure. For these four cases, missing data was replaced by carrying the baseline value forward. No differences were identified between groups for energy (MSP: 1,891 ± 569 kcals/day vs PLA: 2,050 ± 618 kcals/day, $P = 0.27$), protein (MSP: 87.2 ± 31.8 g/day vs PLA: 97.2 ± 37.6 g/day, $P = 0.23$), fat (MSP: 77.1 ± 26.2 g/day vs PLA: 88.5 ± 29.6

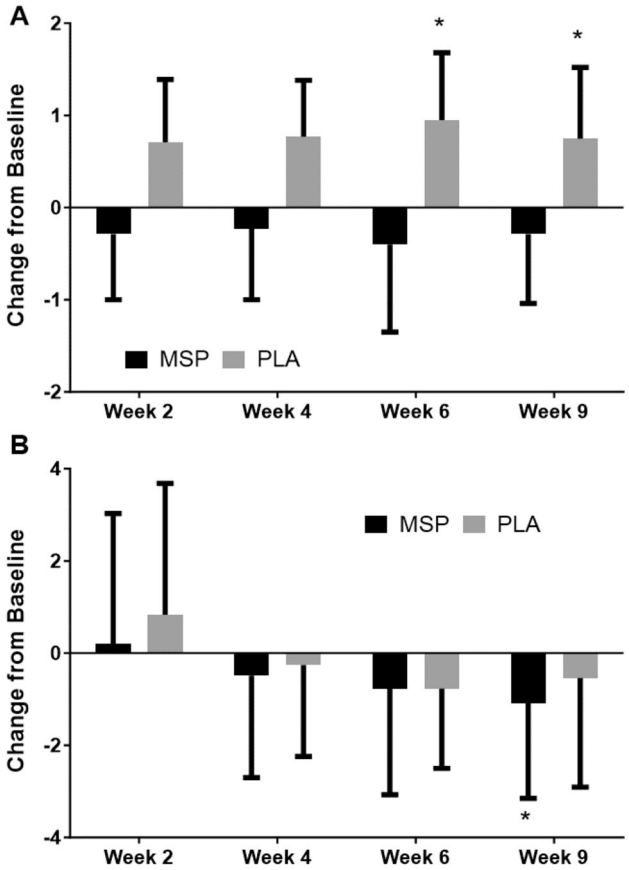

FIGURE 2 (A) Changes in PSQI Sleep Latency scores from baseline. (B) Changes in Global PSQI scores from baseline. * = Significantly different from baseline ($P < 0.05$). Data is presented as change scores from baseline ± standard deviation.

g/day, $P = 0.09$), and carbohydrates (MSP: 212.3 ± 66.2 g/day vs PLA: 213.0 ± 76.7 g/day, $P = 0.97$) throughout the study. In addition, composite Healthy Eating Index (HEI) scores were computed as part of the ASA24 and are illustrated as a radar plot in Figure 2.

Baseline characteristics

Table 2 provides age, height, body mass, percent body fat, and BMI values at baseline for all participants (n = 70), men (n = 35), and women (n = 35). No differences were present prior to supplementation.

Hemodynamics

All hemodynamic data can be found in Table 3. Resting heart rate values indicated no changes in the group × time interaction ($P = 0.913$, $\eta_p^2 = 0.004$), group effect ($P = 0.724$, $\eta_p^2 = 0.002$), or time effect ($P = 0.980$, $\eta_p^2 = 0.002$). A statistically significant group × time interaction was observed for the changes in systolic blood pressure ($P = 0.040$, $\eta_p^2 = 0.036$) while no statistically significant changes were observed for the main effect for group ($P = 0.347$, $\eta_p^2 = 0.013$), or time ($P = 0.568$, $\eta_p^2 = 0.011$). Change scores from baseline were computed and revealed the changes in PLA were statistically different than the changes observed in MPS after 6 weeks of supplementation ($P = 0.006$) and three weeks after stopping supplementation ($P = 0.08$). Diastolic blood pressure values indicated no changes in the group × time interaction ($P = 0.742$, $\eta_p^2 = 0.007$), group effect ($P = 0.420$, $\eta_p^2 = 0.010$), or time effect ($P = 0.521$, $\eta_p^2 = 0.012$).

Body mass and body composition

Changes in body mass indicated no significant group × time interaction ($P = 0.513$, $\eta_p^2 = 0.012$), further, the main effects for group ($P = 0.213$, $\eta_p^2 = 0.023$) and time ($P = 0.311$, $\eta_p^2 = 0.017$). Body composition was also assessed and can be found in Table 4. Changes in fat mass indicated no significant group × time interaction ($P = 0.266$, $\eta_p^2 = 0.019$) or group effect ($P = 0.509$, $\eta_p^2 = 0.006$), but did demonstrate a significant main effect of time ($P = 0.014$, $\eta_p^2 = 0.067$). Lean mass did not exhibit a statistically significant group × time interaction ($P = 0.383$, $\eta_p^2 = 0.014$) or group effect ($P = 0.240$, $\eta_p^2 = 0.020$), but did yield a significant main effect of time ($P = 0.007$, $\eta_p^2 = 0.078$). Fat-free mass did not exhibit a statistically significant group × time interaction ($P = 0.359$, $\eta_p^2 = 0.015$) or group effect ($P = 0.252$, $\eta_p^2 = 0.019$), but did yield a significant main effect of time ($P = 0.006$, $\eta_p^2 = 0.081$). Body fat percentage did not exhibit a statistically significant group × time interaction ($P = 0.206$, $\eta_p^2 = 0.023$) or group effect ($P = 0.955$, $\eta_p^2 = 0.000$), but did yield a significant main effect of time ($P = 0.011$, $\eta_p^2 = 0.070$). Lean: fat ratio did not exhibit a statistically significant group × time interaction ($P = 0.404$, $\eta_p^2 = 0.013$) or group effect ($P = 0.522$, $\eta_p^2 = 0.006$), while the main effect of time tended to change ($P = 0.081$, $\eta_p^2 = 0.037$).

Perceived sleep and wearable sleep outcomes

As seen in Table 5, perceived sleep parameters were evaluated using the Pittsburgh Sleep Quality Index. Within the PSQI, seven subscales and a global score were analysed. No changes across time were observed within each group using the Friedman test for subjective sleep (MSP, $P = 0.668$; PLA, $P = 0.195$), sleep duration (MSP, $P = 0.668$; PLA, $P = 0.195$), sleep efficiency (MSP, $P = 0.668$; PLA, $P = 0.195$), and sleep medications (MSP, $P = 0.668$; PLA, $P = 0.195$). Between-group differences were evaluated at each timepoint using Mann-Whitney U, which revealed no significant differences for subjective sleep, sleep duration, sleep efficiency, and use of sleep medications at any timepoint (all $P > 0.05$).

TABLE 2 Baseline characteristics[1]

		Group	Mean	(SD)	Minimum	Maximum	*P*-value
All subjects (n = 70)	Age	MSP	29.7	(9.0)	18	47	0.25
		PLA	32.3	(10.0)	18	50	
	Height (cm)	MSP	171.8	(10.6)	152	193	0.35
		PLA	174.2	(10.2)	148	195	
	Weight (kg)	MSP	71.9	(13.9)	50.6	107.5	0.23
		PLA	75.9	(13.6)	46.5	104.7	
	DXA % fat	MSP	26.5	(5.8)	13.2	37.3	0.88
		PLA	26.8	(8.3)	14.4	45.9	
	BMI (kg/m^2)	MSP	24.3	(3.5)	18.8	32.5	0.41
		PLA	25.0	(3.5)	19.4	32.3	
Females (n = 35)	Age	MSP	28.0	(8.9)	18	47	0.03
		PLA	35.3	(10.2)	20	50	
	Height (cm)	MSP	165.7	(7.7)	152	181	0.50
		PLA	167.6	(8.5)	148	178.5	
	Weight (kg)	MSP	64.6	(9.7)	50.6	82.1	0.37
		PLA	67.8	(11.2)	46.5	88.9	
	DXA % fat	MSP	29.2	(4.9)	21.5	37.3	0.05
		PLA	33.0	(6.5)	22.5	45.9	
	BMI (kg/m^2)	MSP	23.6	(3.8)	18.9	31.9	0.68
		PLA	24.1	(3.3)	19.4	31.8	
Males (n = 35)	Age	MSP	31.7	(9.0)	19	47	0.57
		PLA	29.8	(9.6)	18	48	
	Height (cm)	MSP	179.1	(9.0)	160	193	0.83
		PLA	179.7	(8.2)	156.5	195	
	Weight (kg)	MSP	80.6	(13.3)	59.5	107.5	0.62
		PLA	82.8	(11.8)	64	104.7	
	DXA % fat	MSP	23.4	(5.4)	13.2	34.1	0.32
		PLA	21.6	(5.5)	14.4	34.2	
	BMI (kg/m^2)	MSP	25.1	(3.1)	20.5	31.4	0.60
		PLA	25.7	(3.5)	19.9	31.0	

[1] Data is presented as mean (standard deviation (SD)); BMI = body mass index; MSP = multi-strain probiotic; PLA = placebo; DXA = dual-energy x-ray absorptiometry; *P*-value as assessed by independent t-tests.

For the PSQI – Sleep Latency scores, a notable main effect of time was identified for MSP ($P = 0.009$). In contrast, the PLA group showed no significant change ($P = 0.744$). In the MSP group, the PSQI – Sleep Latency scores decreased after 6 weeks of supplementation ($P = 0.005$) and remained lower three weeks after supplementation ended ($P = 0.012$) compared to baseline values. No between-group differences were found for PSQI – Sleep Latency scores at any timepoint (all $P > 0.05$). No main effect of time was observed for PSQI – Sleep Disturbance for either group (MSP, $P = 0.343$; PLA, $P = 0.480$) or Daytime Dysfunction (MSP, $P = 0.688$; PLA, $P = 0.133$) while no between-condition differences (all $P > 0.05$) were observed between conditions for either variables at any timepoint. Global PSQI scores indicated a tendency for MSP scores ($P = 0.055$) to be different across time while no changes were observed for PLA ($P = 0.154$). Wilcoxon signed-rank tests with Bonferroni corrections applied for multiple comparison indicated a tendency for PSQI global scores to be different from baseline after 2 weeks of supplementation ($P = 0.07$) and six weeks after supplementation ($P = 0.07$) while statistically significant differences were observed three weeks after stopping supplementation (week 9, $P = 0.005$). No changes within the PLA group were observed across time (all *P*-values > 0.05). Mann-Whitney U tests indicated no between-group differences at all time points (all *P*-values > 0.05).

TABLE 3 Hemodynamic parameters after 0, 2, 4, and 6 weeks of supplementation as well as 3 weeks after stopping supplementation (week 9)[1]

	Group	Week 0	Week 2	Week 4	Week 6	Week 9		*P*-value
Resting heart rate	MSP	64.6 (11.5)	63.8 (10.6)	64.3 (11.7)	64.3 (9.5)	63.5 (10.0)	Group (G)	0.72
(beats/min)	PLA	64.5 (10.0)	64.4 (10.0)	65.2 (9.4)	64.7 (9.5)	65.3 (10.1)	Time (T)	0.97
							G × T	0.89
Diastolic blood	MSP	71.8 (9.8)	71.0 (9.2)	71.2 (9.9)	72.1 (10.3)	71.3 (10.1)	Group (G)	0.42
pressure (mm Hg)	PLA	71.6 (11.3)	72.9 (10.0)	72.4 (10.8)	74.8 (8.7)	73.3 (8.1)	Time (T)	0.52
							G × T	0.74
Systolic blood	MSP	112.7 (12.7)	111.3 (13.0)	111.4 (12.4)	109.8 (14.6)	111.4 (13.1)	Group (G)	0.35
pressure (mm Hg)	PLA	111.4 (14.8)	111.3 (10.8)	114.4 (11.4)	116.2 (12.0)	115.1 (12.4)	Time (T)	0.57
							G × T	0.04

[1]MSP = multi-strain probiotic; PLA = placebo. Data is presented as mean (standard deviation).

TABLE 4 Body composition data after 0 and 6 weeks of supplementation as well as 3 weeks after stopping supplementation (week 9)[1]

	Group	Week 0	Week 6	Week 9		*P*-value
Fat mass (kg)	MSP	19.4 (6.1)	19.7 (6.1)	19.8 (6.2)	Group (G)	0.51
	PLA	20.6 (7.50	20.5 (7.7)	21.1 (8.2)	Time (T)	0.02
					G × T	0.26
Lean mass (kg)	MSP	50.9 (10.4)	50.7 (10.0)	50.3 (9.7)	Group (G)	0.24
	PLA	53.8 (11.8)	54.1 (11.6)	53.1 (11.0)	Time (T)	0.008
					G × T	0.37
Fat-free mass (kg)	MSP	53.4 (10.8)	53.3 (10.4)	52.9 (10.1)	Group (G)	0.25
	PLA	56.4 (12.2)	56.7 (12.0)	55.7 (11.4)	Time (T)	0.007
					G × T	0.34
Fat %	MSP	26.5 (5.8)	26.8 (5.8)	27.0 (5.9)	Group (G)	0.96
	PLA	26.8 (8.3)	26.5 (8.2)	27.3 (8.5)	Time (T)	0.01
					G × T	0.21
Lean: Fat	MSP	2.8 (0.9)	2.8 (0.9)	2.8 (0.9)	Group (G)	0.52
	PLA	3.0 (1.3)	3.0 (1.3)	2.9 (1.3)	Time (T)	0.08
					G × T	0.39

[1]Data is presented as mean (standard deviation). W0 = Week 0 of supplementation (baseline); W6 = Week 6 of supplementation; W9 = 9 weeks after supplementation which was equivalent to three weeks after stopping supplementation. MSP = multi-strain probiotic; PLA = placebo.

In addition to PSQI, sleep was assessed using a wearable sleep tracking device and this data can be found in Table 6. No significant group × time interaction (P = 0.449, η_p^2 = 0.012), main effect of time (P = 0.389, η_p^2 = 0.014), or main effect of group (P = 0.086, η_p^2 = 0.043) was observed for total hours of sleep per day. No significant group × time interaction (P = 0.388, η_p^2 = 0.014), main effect of time (P = 0.273, η_p^2 = 0.019), or main effect of group (P = 0.830, η_p^2 = 0.001) was observed for minutes awake per day. A significant group × gender × time interaction was observed (P = 0.009, η_p^2 = 0.071) for number of times awake. In males, the group × time interaction was statistically significant between conditions (P = 0.030, η_p^2 = 0.110) while no main effect of time (P = 0.445, η_p^2 = 0.023) or group was observed (P = 0.431, η_p^2 = 0.021). The significant interaction was further decomposed by calculating the changes from baseline and comparing the differences between each supplemental condition in the males. Using this approach, a significant difference in the numbers of times males reported waking up from the start of the supplementation protocol was different (P = 0.025, Mean difference: 1.85 ± 0.78, 95% confidence interval: 0.25, 3.44). An identical approach was taken to analyse changes in

TABLE 5 Pittsburgh Sleep Quality Index (PSQI) after 0, 2, 4, and 6 weeks of supplementation as well as 3 weeks after stopping supplementation (week 9)[1]

	Group	Week 0	Week 2	Week 4	Week 6	Week 9	Friedman (*P*-value)
Subjective sleep quality	MSP	1.14 (0.65)	1.11 (0.58)	1.03 (0.71)	1.11 (0.76)	1.03 (0.71)	0.67
	PLA	1.18 (0.57)	1.03 (0.66)	1.09 (0.70)	0.94 (0.59)	1.00 (0.54)	0.20
Sleep latency	MSP	1.17 (0.98)	0.91 (0.85)	0.97 (0.79)	0.74 (0.82)*	0.80 (0.93)*	0.01
	PLA	0.91 (0.98)	0.83 (0.95)	0.80 (0.87)	0.71 (0.86)	0.86 (0.91)	0.74
Sleep duration	MSP	0.77 (0.60)	0.74 (0.70)	0.77 (0.69)	0.83 (0.86)	0.71 (0.79)	0.83
	PLA	0.71 (0.62)	0.89 (0.72)	0.80 (0.72)	0.77 (0.73)	0.74 (0.70)	0.23
Sleep efficiency	MSP	0.54 (0.78)	0.46 (0.82)	0.43 (0.70)	0.46 (0.82)	0.37 (0.65)	0.74
	PLA	0.38 (0.64)	0.60 (0.69)	0.60 (0.74)	0.46 (0.70)	0.60 (0.69)	0.16
Sleep disturbances	MSP	0.86 (0.36)	0.80 (0.41)	0.89 (0.47)	0.80 (0.41)	0.77 (0.43)	0.34
	PLA	0.97 (0.45)	0.97 (0.17)#	0.89 (0.32)	0.94 (0.34)	0.86 (0.36)	0.48
Sleep medication	MSP	0.26 (0.61)	0.09 (0.28)	0.14 (0.36)	0.09 (0.28)	0.09 (0.37)	0.24
	PLA	0.41 (0.91)	0.31 (0.80)	0.31 (0.72)	0.31 (0.83)	0.26 (0.66)	0.61
Daytime dysfunction	MSP	0.69 (0.68)	0.66 (0.64)	0.71 (0.75)	0.63 (0.65)	0.57 (0.65)	0.69
	PLA	0.85 (0.73)	0.77 (0.73)	0.74 (0.66)	0.57 (0.61)*	0.63 (0.65)	0.13
Global score	MSP	5.43 (2.4)	4.77 (2.3)	4.94 (2.6)	4.65 (2.7)	4.34 (2.5)*	0.06
	PLA	5.41 (2.9)	5.40 (2.5)	5.22 (2.7)	4.71 (2.4)	4.94 (2.5)#	0.15

[1] MSP = multi-strain probiotic; PLA = placebo. Data is presented as mean (standard deviation). * Indicates differences of specified time point from respective W0 (baseline) value using a Wilcoxon Signed Rank Test ($P < 0.05$). # Indicates difference between groups using Mann-Whitney U at specified time point ($P < 0.05$).

females whereby the group × time interaction tended to be different between conditions ($P = 0.087$, $\eta_p^2 = 0.071$) while no main effect of time ($P = 0.244$, $\eta_p^2 = 0.041$) or group was observed ($P = 0.734$, $\eta_p^2 = 0.004$). However, our *a priori* statistical approach was to evaluate differences in a mixed gender cohort. When this is completed and both genders were collapsed, no significant group × time interaction ($P = 0.698$, $\eta_p^2 = 0.006$), main effect of time ($P = 0.241$, $\eta_p^2 = 0.021$), or main effect of group ($P = 0.739$, $\eta_p^2 = 0.002$) was observed for number of times awake. Additionally, no significant group × time interaction ($P = 0.198$, $\eta_p^2 = 0.024$), main effect of time ($P = 0.258$, $\eta_p^2 = 0.020$), or main effect of group ($P = 0.080$, $\eta_p^2 = 0.045$) was found for the total time spent in bed each day.

Physical activity

All physical activity data can be found in Table 6. A significant main effect of time was observed for total calories burned as calories expended decreased across the study protocol ($P < 0.001$, $\eta_p^2 = 0.400$). No significant group × time interaction ($P = 0.274$, $\eta_p^2 = 0.021$) or main effect of group was observed ($P = 0.469$, $\eta_p^2 = 0.009$). A significant main effect of time was observed for step count as the number of steps accumulated in all participants decreased across the study protocol ($P < 0.001$, = $\eta_p^2 = 0.179$), however, no significant group × time interaction ($P = 0.478$, $\eta_p^2 = 0.011$) or main effect of group was observed ($P = 0.521$, $\eta_p^2 = 0.006$). No significant group × time interaction ($P = 0.787$, $\eta_p^2 = 0.004$), main effect of time ($P = 0.086$, $\eta_p^2 = 0.040$), or main effect of group ($P = 0.762$, $\eta_p^2 = 0.002$) was observed for minutes of sedentary activity. A significant main effect of time was observed demonstrating that total active minutes accumulated per day decreased in all participants across the study protocol ($P < 0.001$, $\eta_p^2 = 0.205$), however, no significant group × time interaction ($P = 0.113$, $\eta_p^2 = 0.036$) or main effect of group was observed ($P = 0.212$, $\eta_p^2 = 0.026$).

4 Discussion

In this investigation, we sought to examine the presence of any changes in indicators of sleep quality and quantity, physical activity, hemodynamics, and body composition in a mixed gender cohort of healthy adults after supplementing with a multi-strain probiotic preparation for six weeks and also three weeks after ceasing supplementation. The primary findings from this

TABLE 6 Wearable sleep and physical activity data after 0 and 6 weeks of supplementation as well as 3 weeks after stopping supplementation (week 9)[1]

	Group	Week 0	Week 6	Week 9		*P*-value
Sleep data						
Hours asleep	MSP	6.8 (1.0)	6.9 (0.9)	7.0 (1.0)	Group (G)	0.09
	PLA	6.5 (1.5)	6.3 (1.4)	6.6 (1.4)	Time (T)	0.39
					G × T	0.45
Minutes awake	MSP	24.2 (15.5)	29.7 (22.6)	31.5 (24.1)	Group (G)	0.83
	PLA	29.7 (29.1)	28.0 (24.2)	31.2 (34.4)	Time (T)	0.27
					G × T	0.37
Number of times awake	MSP	5.1 (8.9)	5.6 (9.8)	5.2 (9.1)	Group (G)	0.74
	PLA	3.9 (6.8)	5.1 (8.4)	4.8 (8.8)	Time (T)	0.24
					G × T	0.70
Total time in bed (h)	MSP	7.2 (1.1)	7.5 (0.9)	7.5 (0.9)	Group (G)	0.08
	PLA	7.0 (1.4)	6.8 (1.3)	7.1 (1.1)	Time (T)	0.26
					G × T	0.20
Physical activity data						
Total calories burned	MSP	2,481 (494)	2,416 (518)	2,108 (471)	Group (G)	0.47
	PLA	2,494 (499)	2,526 (504)	2,241 (475)	Time (T)	<0.001
					G × T	0.27
Step count	MSP	9,683 (3605)	9,188 (4284)	7,601 (4049)	Group (G)	0.52
	PLA	9,765 (4136)	10,218 (4387)	8,179 (3966)	Time (T)	<0.001
					G × T	0.48
Minutes of sedentary activity	MSP	721 (97)	728 (181)	689 (218)	Group (G)	0.76
	PLA	698 (115)	735 (145)	674 (228)	Time (T)	0.09
					G × T	0.79
Total active minutes per day	MSP	291 (73)	288 (80)	230 (76)	Group (G)	0.21
	PLA	298 (85)	306 (90)	275 (92)	Time (T)	<0.001
					G × T	0.11

[1] Data is presented as mean (standard deviation). MSP = multi-strain probiotic; PLA = placebo.

investigation indicate that probiotic supplementation improved perceived global sleep scores, sleep latency, and sleep disturbance scores as assessed by the Pittsburgh Sleep Quality Index whereas daytime dysfunction ratings were improved in the placebo group. Additionally, a trend was observed ($P = 0.086$, $\eta_p^2 = 0.043$) for total hours of sleep per day recorded by the wearable device whereby greater levels of sleep tended to be greater across all timepoints in the MSP group when compared to PLA. No changes were identified in either group for physical activity and body composition variables while hemodynamic evaluation demonstrated an increase in systolic blood pressure in the placebo group with no concomitant change in the probiotic group. Additionally, no changes in heart rate or diastolic blood pressure were observed in either group. The observed improvements in sleep outcomes align with previous studies involving probiotic supplementation in healthy (Marotta *et al.*, 2019; Takada *et al.*, 2017), acutely stressed (Kato-Kataoka *et al.*, 2016; Sawada *et al.*, 2017), and individuals with chronic fatigue (Jackson *et al.*, 2015) and chronic pain (Kato-Kataoka *et al.*, 2016). The results communicated by Marotta *et al.* (2019) are particularly helpful as they used an identical multi-strain probiotic combination and supplementation regimen as the present study (1×10^9 live cells of each of the four strains: *Limosilactobacillus fermentum* LF16 (DSM 26956), *Lacticaseibacillus rhamnosus* LR06 (DSM 21981), *Lactiplantibacillus plantarum* LP01 (LMG P-21021), and *Bifidobacterium longum* 04 (DSM 23233) and also showed improvements in global sleep scores as assessed by the Pittsburgh Sleep Quality Index. Additionally, Sawada and colleagues (Sawada *et al.*, 2017) supplemented 24 medical students for four weeks in a crossover manner with either a placebo or 1×10^{10} cfu of *Lactobacillus gasseri* CP2305. They reported significant

reductions in the global PSQI score and sleep disturbance ratings like what was observed in the present study. A recent study published by Boehme *et al.* (2023) had 45 healthy adults supplement with a probiotic (*Bifidobacterium longum* NCC3001, 1×10^{10} cfu/day) for six weeks and evaluated changes in stress, sleep quality, and depression. Probiotic supplementation reduced perceived stress while also significantly improving subjective sleep quality ratings measured using the PSQI to a greater degree when compared to a placebo. To summarise their results, these authors pointed to the ability of probiotic strains to positively impact mental health in healthy participants, an outcome that is reinforced by the established and known relationship between sleep quality and mood (Lastella *et al.*, 2014) and align well with our previously reported data that highlighted improvements in mood, depression, and anxiety (Walden *et al.*, 2023).

MSP supplementation did not impact how much physical activity was completed by our study participants. Clear links have been established between psychological health and physical activity status in healthy, non-clinical populations (Peluso and Guerra de Andrade, 2005; Rebar *et al.*, 2015), which may in part be due to the known impact of physical activity on the bacterial communities found in the microbiome (Dziewiecka *et al.*, 2022). Currently, limited data is available that has examined any ability of probiotics to influence physical activity. A 2022 study by Akhgarjand *et al.* (2022) reported that 12 weeks of supplementation with two different probiotic strains in 90 patients diagnosed with Alzheimer's disease improved physical activity levels greater than what was observed in a placebo in one but not both physical activity scales. Two key differences from this study and the present study were the psychological health of the study participants and the length of supplementation (12 weeks vs 6 weeks). Regardless, more research is needed to fully explore the ability of probiotic supplementation to impact physical activity. Another key difference in terms of physical activity was the type of assessment. The previous work of Akhgarjand *et al.* (2022) used validated questionnaires that evaluated the quantity of activities of daily living that were completed while the present study utilised a commercially available wrist-based fitness tracker. However, many other studies have been published using these types of trackers to evaluate changes in physical activity (Brickwood *et al.*, 2019; Ferguson *et al.*, 2022) thus it remains more likely that MSP supplementation either did not produce a measurable impact in our healthy cohort or the supplementation period was not long enough (or both).

Results from the present study failed to identify any ability of the MSP to influence changes in body composition. Other studies have evaluated the ability of probiotics to impact body composition and obesity status with these studies demonstrating conflicting outcomes regarding the ability of probiotics to mitigate body mass and body fat changes. For example, Osterberg *et al.* (2015) had 20 non-obese males supplement with either a probiotic (VSL#3) or a placebo for four weeks while consuming a high fat, hypercaloric diet. Supplementation with the probiotic effectively offset the observed gains in fat mass and body mass that was seen in those participants consuming a placebo. Coman *et al.* (2022) also reported that probiotic use was responsible for improved weight loss, body mass index, and waist circumference values supplementing for 90 days with *L. plantarum* IMC 510 (1.5×10^{10} bacterial cells) in 19 overweight and obese volunteers while no changes were observed with placebo supplementation. Two other studies, one by Dechelotte *et al.* (2021) reported that supplementing with the probiotic *Hafnia alvei* HA4597 (100 billion bacteria per day) in combination with a hypocaloric diet over a 12-week period led to a significantly greater number of people who lost at least 3% of their body mass than the placebo group (54.9% vs 41.4%) and another by Cho *et al.* (2022) reported greater weight loss when a probiotic was added to a reduced calorie diet in 100 healthy obese and overweight subjects after 12 weeks. In contrast, not all studies have demonstrated the ability of a probiotic to positively influence body mass loss or fat loss. Choi *et al.* (2023) supplemented 152 overweight adults for 12 weeks with either a placebo or *L. rhamnosus* HA-114 (1×10^{10} cfu/day) and reported no difference in weight loss or fat loss in the probiotic group when compared to the placebo. While previous results do conflict as to whether probiotics can be considered successful weight loss agents, key considerations from the present study and these other studies include the length of supplementation and the inclusion of a hypocaloric diet. To this point, all studies which reported an enhanced potential for weight loss with probiotic use were at least 90 days in duration while our study only lasted for six weeks. Moreover, optimal practices for weight loss studies should include a hypocaloric diet to be followed alongside any supplementation as this is considered 'best practice' to achieve weight loss (Miller *et al.*, 1997).

Findings from the present study also suggested improvements in systolic blood pressure secondary to MSP

supplementation. Similar results were reported by Mahboobi *et al.* (2014) who recruited 60 prediabetic patients to supplement with either a placebo or a probiotic for 8 weeks. Moreover, a meta-analysis by Khalesi *et al.* (2014) summarised the probiotic literature and also reported that probiotic administration exerts a modest ability to improve blood pressure. Hemodynamic outcomes were considered a secondary outcome in our investigation and this would be the first time these outcomes have been reported after supplementing with this combination of probiotic strains. As such, any underlying mechanisms as to why blood pressure levels would be improved are currently unknown. Future research should further examine the potential ability of probiotics to positively impact observed blood pressure levels.

Considering the outcomes presented, some limitations of our study should be acknowledged. First, while dietary patterns have been previously shown to impact the bacterial communities present in the microbiome (Beam *et al.*, 2021; Bibbo *et al.*, 2016; Singh *et al.*, 2017), participants were not required to follow or abide by any specific type of dietary pattern throughout the study protocol. Evaluation of the dietary intake of our participants did determine that no changes in energy or macronutrient intake occurred throughout the study protocol. Moreover, Healthy Eating Index values were calculated and determined that there were no differences in the proportion of various food groups consumed by each group occurred across the study protocol (Walden *et al.*, 2023). These findings limit the possibility that foods containing prebiotics and synbiotics were consumed in varying amounts between the groups. One of the primary underlying mechanisms to explain the changes we observed in sleep quality implies that MSP supplementation altered the composition of the intestinal microbiota which impacted the production of various biomarkers that may have impacted sleep, physical activity, and body composition changes. As such, collecting blood and stool samples would have provided objective outcomes to combine with our subjective assessments to foster a deeper understanding of any underlying mechanistic reasons for our observed outcomes.

5 Conclusions

In conclusion, results from this study indicate that a six-week regimen of a MSP consisting of four probiotic strains (*Limosilactobacillus fermentum* LF16 (DSM 26956), *Lacticaseibacillus rhamnosus* LR06 (DSM 21981), *Lactiplantibacillus plantarum* LP01 (LMG P-21021), and *Bifidobacterium longum* 04 (DSM 23233 providing a total dosage of 4×10^9 live cells/day) in a cohort of 70 healthy men and women improved subjective ability to fall asleep and daytime dysfunction, which resulted in improved in overall sleep quality.

Acknowledgements

The authors would like to thank Angela Amoruso from Probiotical Research srl, in Novara, Italy for their efforts and support in this project. Your patience and professionalism have helped to make this project a success. In addition, the authors would like to thank the study participants for their commitment to this protocol.

Authors' contribution

Conception or design of the study: CMK, RJ, MP. Acquisition of data / data collection: KEW, AMH, JMK, JMM, CJG, LEA, PWM, CMK. Analysis and interpretation of data: CMK, PWM, AMH. Drafting work and/or providing critical edits: all authors. Approved final version and agree to be held accountable for its contents: all authors.

Conflict of interest

RJ is scientific advisor to Ashland. For this part, RJ assisted with study design and was not involved in collecting or analysing the data. MP is an employee of Probiotical, the sponsor of the study. All other authors report no conflicts of interest.

Data availability

The datasets used and/or analysed during the current study are available from the corresponding author upon reasonable request.

Funding

This study was co-sponsored by Probiotical S.p.A. (Italy) and Ashland (USA). The sponsors had no role in collecting the data, analysing the data, interpreting the results, or preparing the manuscript.

References

Ahmed, H., Leyrolle, Q., Koistinen, V., Karkkainen, O., Laye, S., Delzenne, N. and Hanhineva, K., 2022. Microbiota-derived metabolites as drivers of gut-brain communication. Gut Microbes 14: 2102878. https://doi.org/10.1080/19490976.2022.2102878

Akhgarjand, C., Vahabi, Z., Shab-Bidar, S., Etesam, F. and Djafarian, K., 2022. Effects of probiotic supplements on cognition, anxiety, and physical activity in subjects with mild and moderate Alzheimer's disease: a randomized, double-blind, and placebo-controlled study. Frontiers in Aging Neuroscience 14: 1032494. https://doi.org/10.3389/fnagi.2022.1032494

Bagga, D., Reichert, J.L., Koschutnig, K., Aigner, C.S., Holzer, P., Koskinen, K., Moissl-Eichinger, C. and Schopf, V., 2018a. Probiotics drive gut microbiome triggering emotional brain signatures. Gut Microbes 9: 486-496. https://doi.org/10.1080/19490976.2018.1460015

Bagga, D., Reichert, J.L., Koschutnig, K., Aigner, C.S., Holzer, P., Koskinen, K., Moissl-Eichinger, C. and Schöpf, V., 2018b. Probiotics drive gut microbiome triggering emotional brain signatures. Gut Microbes 9: 486-496.

Beam, A., Clinger, E. and Hao, L., 2021. Effect of diet and dietary components on the composition of the gut microbiota. Nutrients 13: 2795. https://doi.org/10.3390/nu13082795

Bibbo, S., Ianiro, G., Giorgio, V., Scaldaferri, F., Masucci, L., Gasbarrini, A. and Cammarota, G., 2016. The role of diet on gut microbiota composition. European Review for Medical and Pharmacological Sciences 20: 4742-4749.

Boehme, M., Remond-Derbez, N., Lerond, C., Lavalle, L., Keddani, S., Steinmann, M., Rytz, A., Dalile, B., Verbeke, K., Van Oudenhove, L., Steiner, P., Berger, B., Vicario, M., Bergonzelli, G., Colombo Mottaz, S. and Hudry, J., 2023. *Bifidobacterium longum* subsp. *longum* reduces perceived psychological stress in healthy adults: an exploratory clinical trial. Nutrients 15: 3122. https://doi.org/10.3390/nu15143122

Brickwood, K.J., Watson, G., O'Brien, J. and Williams, A.D., 2019. Consumer-based wearable activity trackers increase physical activity participation: systematic review and meta-analysis. JMIR Mhealth Uhealth 7: e11819. https://doi.org/10.2196/11819

Buysse, D.J., Reynolds, C.F., 3rd, Monk, T.H., Berman, S.R. and Kupfer, D.J., 1989. The Pittsburgh sleep quality index: a new instrument for psychiatric practice and research. Psychiatry Research 28: 193-213. https://doi.org/10.1016/0165-1781(89)90047-4

Cho, Y.G., Yang, Y.J., Yoon, Y.S., Lee, E.S., Lee, J.H., Jeong, Y. and Kang, C.H., 2022. Effect of MED-02 containing two probiotic strains, *Limosilactobacillus fermentum* MG4231 and MG4244, on body fat reduction in overweight or obese subjects: a randomized, multicenter, double-blind, placebo-controlled study. Nutrients 14: 3583. https://doi.org/10.3390/nu14173583

Choi, B.S., Brunelle, L., Pilon, G., Cautela, B.G., Tompkins, T.A., Drapeau, V., Marette, A. and Tremblay, A., 2023. Lacticaseibacillus rhamnosus HA-114 improves eating behaviors and mood-related factors in adults with overweight during weight loss: a randomized controlled trial. Nutritional Neuroscience 26: 667-679. https://doi.org/10.1080/1028415X.2022.2081288

Coman, M.M., Miorelli, L., Micioni Di Bonaventura, M.V., Cifani, C., Salvesi, C., Amedei, A., Silvi, S. and Verdenelli, M.C., 2022. Effects of probiotic Lactiplantibacillus plantarum IMC 510 supplementation on metabolic factors in otherwise healthy overweight and obese individuals. Journal of Applied Microbiology 133: 1956-1968. https://doi.org/10.1111/jam.15703

Dechelotte, P., Breton, J., Trotin-Picolo, C., Grube, B., Erlenbeck, C., Bothe, G., Fetissov, S.O. and Lambert, G., 2021. The probiotic strain *H. alvei* HA4597((R)) improves weight loss in overweight subjects under moderate hypocaloric diet: a proof-of-concept, multicenter randomized, double-blind placebo-controlled study. Nutrients 13: 1902. https://doi.org/10.3390/nu13061902

Dziewiecka, H., Buttar, H.S., Kasperska, A., Ostapiuk-Karolczuk, J., Domagalska, M., Cichon, J. and Skarpanska-Stejnborn, A., 2022. Physical activity induced alterations of gut microbiota in humans: a systematic review. BMC Sports Science and Medical Rehabilitation 14: 122. https://doi.org/10.1186/s13102-022-00513-2

Ferguson, T., Olds, T., Curtis, R., Blake, H., Crozier, A.J., Dankiw, K., Dumuid, D., Kasai, D., O'Connor, E., Virgara, R. and Maher, C., 2022. Effectiveness of wearable activity trackers to increase physical activity and improve health: a systematic review of systematic reviews and meta-analyses. The Lancet Digital Health 4: e615-e626. https://doi.org/10.1016/S2589-7500(22)00111-X

Frederick, C.M., Morrison, C. and Manning, T., 1996. Motivation to participate, exercise affect, and outcome behaviors toward physical activity. Perceptual and Motor Skills 82: 691-701. https://doi.org/10.2466/pms.1996.82.2.691

Grenham, S., Clarke, G., Cryan, J. and Dinan, T., 2011. Brain-gut-microbe communication in health and disease. Frontiers in Physiology 2: 94. https://doi.org/10.3389/fphys.2011.00094

Hill, C., Guarner, F., Reid, G., Gibson, G.R., Merenstein, D.J., Pot, B., Morelli, L., Canani, R.B., Flint, H.J., Salminen, S., Calder, P.C. and Sanders, M.E., 2014. The international scientific association for probiotics and prebiotics consensus

statement on the scope and appropriate use of the term probiotic. Nature Reviews Gastroenterology and Hepatology 11: 506-514.

Jackson, M.L., Butt, H., Ball, M., Lewis, D.P. and Bruck, D., 2015. Sleep quality and the treatment of intestinal microbiota imbalance in chronic fatigue syndrome: a pilot study. Sleep Science 8: 124-133. https://doi.org/10.1016/j.slsci.2015.10.001

Kato-Kataoka, A., Nishida, K., Takada, M., Suda, K., Kawai, M., Shimizu, K., Kushiro, A., Hoshi, R., Watanabe, O., Igarashi, T., Miyazaki, K., Kuwano, Y. and Rokutan, K., 2016. Fermented milk containing *Lactobacillus casei* strain Shirota prevents the onset of physical symptoms in medical students under academic examination stress. Beneficial Microbes 7: 153-156. https://doi.org/10.3920/BM2015.0100

Khalesi, S., Sun, J., Buys, N. and Jayasinghe, R., 2014. Effect of probiotics on blood pressure: a systematic review and meta-analysis of randomized, controlled trials. Hypertension 64: 897-903. https://doi.org/10.1161/HYPERTENSIONAHA.114.03469

Lastella, M., Lovell, G.P. and Sargent, C., 2014. Athletes' precompetitive sleep behaviour and its relationship with subsequent precompetitive mood and performance. European Journal of Sport Science 14(Suppl. 1): S123-S130. https://doi.org/10.1080/17461391.2012.660505

Mahboobi, S., Iraj, B., Maghsoudi, Z., Feizi, A., Ghiasvand, R., Askari, G. and Maayeshi, N., 2014. The effects of probiotic supplementation on markers of blood lipids, and blood pressure in patients with prediabetes: a randomized clinical trial. International Journal of Preventive Medicine 5: 1239-1246.

Marotta, A., Sarno, E., Del Casale, A., Pane, M., Mogna, L., Amoruso, A., Felis, G.E. and Fiorio, M., 2019. Effects of probiotics on cognitive reactivity, mood, and sleep quality. Frontiers in Psychiatry 10: 164. https://doi.org/10.3389/fpsyt.2019.00164

Mayer, E.A., 2011. Gut feelings: the emerging biology of gut-brain communication. Nature Reviews: Neuroscience 12: 453-466. https://doi.org/10.1038/nrn3071

Mayer, E.A., Knight, R., Mazmanian, S.K., Cryan, J.F. and Tillisch, K., 2014. Gut microbes and the brain: paradigm shift in neuroscience. Journal of Neuroscience 34: 15490-15496.

Miller, W.C., Koceja, D.M. and Hamilton, E.J., 1997. A meta-analysis of the past 25 years of weight loss research using diet, exercise or diet plus exercise intervention. International Journal of Obesity and Related Metabolic Disorders 21: 941-947.

Moloney, G.M., Long-Smith, C.M., Murphy, A., Dorland, D., Hojabri, S.F., Ramirez, L.O., Marin, D.C., Bastiaanssen, T.F., Cusack, A.-M. and Berding, K., 2021. Improvements in sleep indices during exam stress due to consumption of a *Bifidobacterium longum*. Brain, Behavior, & Immunity-Health 10: 100174.

Muller, P.A., Schneeberger, M., Matheis, F., Wang, P., Kerner, Z., Ilanges, A., Pellegrino, K., Del Marmol, J., Castro, T.B.R., Furuichi, M., Perkins, M., Han, W., Rao, A., Pickard, A.J., Cross, J.R., Honda, K., de Araujo, I. and Mucida, D., 2020. Microbiota modulate sympathetic neurons via a gut-brain circuit. Nature 583: 441-446. https://doi.org/10.1038/s41586-020-2474-7

Osterberg, K.L., Boutagy, N.E., McMillan, R.P., Stevens, J.R., Frisard, M.I., Kavanaugh, J.W., Davy, B.M., Davy, K.P. and Hulver, M.W., 2015. Probiotic supplementation attenuates increases in body mass and fat mass during high-fat diet in healthy young adults. Obesity 23: 2364-2370. https://doi.org/10.1002/oby.21230

Peluso, M.A. and Guerra de Andrade, L.H., 2005. Physical activity and mental health: the association between exercise and mood. Clinics 60: 61-70. https://doi.org/10.1590/s1807-59322005000100012

Quero, C.D., Manonelles, P., Fernandez, M., Abellan-Aynes, O., Lopez-Plaza, D., Andreu-Caravaca, L., Hinchado, M.D., Galvez, I. and Ortega, E., 2021. Differential health effects on inflammatory, immunological and stress parameters in professional soccer players and sedentary individuals after consuming a synbiotic. a triple-blinded, randomized, placebo-controlled pilot study. Nutrients 13: 1321. https://doi.org/10.3390/nu13041321

Rebar, A.L., Stanton, R., Geard, D., Short, C., Duncan, M.J. and Vandelanotte, C., 2015. A meta-meta-analysis of the effect of physical activity on depression and anxiety in non-clinical adult populations. Health Psychology Review 9: 366-378. https://doi.org/10.1080/17437199.2015.1022901

Sawada, D., Kawai, T., Nishida, K., Kuwano, Y., Fujiwara, S. and Rokutan, K., 2017. Daily intake of *Lactobacillus gasseri* CP2305 improves mental, physical, and sleep quality among Japanese medical students enrolled in a cadaver dissection course. Journal of Functional Foods 31: 188-197. https://doi.org/10.1016/j.jff.2017.01.042

Singh, R.K., Chang, H.W., Yan, D., Lee, K.M., Ucmak, D., Wong, K., Abrouk, M., Farahnik, B., Nakamura, M., Zhu, T.H., Bhutani, T. and Liao, W., 2017. Influence of diet on the gut microbiome and implications for human health. Journal of Translational Medicine 15: 73. https://doi.org/10.1186/s12967-017-1175-y

Takada, M., Nishida, K., Gondo, Y., Kikuchi-Hayakawa, H., Ishikawa, H., Suda, K., Kawai, M., Hoshi, R., Kuwano, Y., Miyazaki, K. and Rokutan, K., 2017. Beneficial effects of *Lactobacillus casei* strain Shirota on academic stress-induced sleep disturbance in healthy adults: a double-

blind, randomised, placebo-controlled trial. Beneficial Microbes 8: 153-162. https://doi.org/10.3920/BM2016.0150

Tillisch, K., Labus, J., Kilpatrick, L., Jiang, Z., Stains, J., Ebrat, B., Guyonnet, D., Legrain-Raspaud, S., Trotin, B., Naliboff, B. and Mayer, E.A., 2013. Consumption of fermented milk product with probiotic modulates brain activity. Gastroenterology 144: 1394-1401, 1401.e1-1404.e4. https://doi.org/10.1053/j.gastro.2013.02.043

Walden, K.E., Moon, J.M., Hagele, A.M., Allen, L.E., Gaige, C.J., Krieger, J.M., Jager, R., Mumford, P.W., Pane, M. and Kerksick, C.M., 2023. A randomized controlled trial to examine the impact of a multi-strain probiotic on self-reported indicators of depression, anxiety, mood, and associated biomarkers. Frontiers in Nutrition 10: 1219313. https://doi.org/10.3389/fnut.2023.1219313