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Effects of a strain-specific probiotic formulation on subjective sleep quality: a randomized, double-blind study

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Key words: probiotics; *Lactobacillus* spp.; microbiota; sleep quality

Abstract

The intestinal microbiota has been implicated in neurological function through the gut–brain axis, and specific probiotic strains have been proposed to influence neuroactive pathways relevant to sleep. This randomized, double-blind, study enrolled 60 otherwise healthy adults reporting poor subjective sleep quality. Sixty participants (mean age 22.0 ± 3.0 years) were randomized to receive either a probiotic formulation (*Lactobacillus rhamnosus* HN001, *L. rhamnosus* SP1, *L. acidophilus* LA1, *L. reuteri* LR92; total daily dose $6×10^9$ CFU) or a matched non-bacterial comparator for six weeks, followed by three-week washout period. Sleep quality was evaluated using the Pittsburgh Sleep Quality Index (PSQI) and the Insomnia Severity Index (ISI) at baseline (T0), 3 weeks (T1), 6 weeks (T2), and after a 3-week washout (T3). Compared with the matched non-bacterial formulation, participants receiving the probiotic formulation showed a significant improvement in subjective sleep quality at T2 and T3, as assessed by between-group non-parametric analyses. PSQI scores in the probiotic group decreased from 19.2 ± 3.1 at baseline to 7.5 ± 3.9 at week 6, with improvements exceeding the minimal

clinically important difference. Similar temporal patterns were observed for ISI scores. Benefits persisted through washout; however, the mechanisms underlying this persistence remain speculative. These findings suggest that supplementation with a targeted multi-strain *Lactobacillus* spp. formulation may provide additional benefits on perceived sleep quality when added to a matched non-bacterial formulation. Given the use of subjective sleep measures and the absence of microbiome or objective sleep assessments, further studies integrating polysomnography or actigraphy and microbiota profiling are required to confirm these results and clarify the underlying mechanisms.

Introduction

Chronic insomnia affects approximately 10-15% of the adult population worldwide, representing a significant public health concern.[1,2] Approximately 10% of adults suffer from insomnia disorder and another 20% experience occasional insomnia symptoms, with recent evidence pointing to gut-brain axis dysregulation as a potential contributing factor.[3]

The gut and brain communicate via the gut-brain axis, a bidirectional pathway involving neural, endocrine, and immune systems.[4-7] Research over the past decade has demonstrated an increasingly compelling relationship between gut microbiota composition and sleep regulation.[8,9] The scientific understanding of how gut microbiome influences sleep patterns has revealed several sophisticated biological mechanisms.

Studies have uncovered multiple pathways of gut-brain communication relevant to sleep regulation. Research suggests that gut bacteria may influence pathways related to neurotransmitters such as Gamma-Aminobutyric Acid (GABA) and serotonin,[10,11] which play essential roles in sleep-wake cycle regulation, while simultaneously influencing Hypothalamic-Pituitary-Adrenal (HPA) axis function.[12] The microbiome also exerts influence through modulation of immune-inflammatory pathways, generation of neuroactive metabolites, and regulation of vagal nerve signaling,[13-15] serving as a direct communication channel between gut and brain.

Modifying the microbial environment through probiotics may help improve sleep quality and circadian regulation. This hypothesis is supported by animal studies demonstrating beneficial effects of probiotics on sleep architecture and circadian rhythms.[16,17] Recent research shows that gut microbiota regulate insomnia-like behaviors via gut-brain metabolic axis, with microbial metabolites like butyrate promoting sleep by modulating orexin neuronal activity.[18]

Sleep quality has been shown to benefit from probiotic supplementation in human studies. Takada *et al.*[19] reported improved sleep quality in academic students during stress periods after consuming *Lactobacillus casei* strain Shirota for 8 weeks. A systematic review and meta-analysis of 15 randomized controlled trials showed significant reductions in Pittsburgh Sleep Quality Index (PSQI) scores at 4-6 weeks and 8-16 weeks, indicating improved sleep quality with probiotics.[20] Beneficial effects have also been reported in clinical populations with sleep disturbances.[21] Recent evidence further supports the role of gut microbiome in sleep quality and suggests dietary strategies for microbiota support in sleep disorders.[22] Probiotics, defined as live microorganisms conferring health benefits when administered in adequate amounts,[23] have emerged as potential therapeutic approaches for sleep disorders. Despite promising preclinical evidence, clinical studies investigating probiotic interventions for sleep disturbances remain limited.[24]

Our investigation focused on multiple dimensions of sleep health, examining a novel probiotic formulation containing targeted bacterial strains specifically selected for their potential to modulate sleep-related neurobiological pathways through gut-brain axis modulation.

Materials and Methods

Study design

This randomized, double-blind study enrolled 60 healthy adults divided into probiotic and matched non-bacterial comparator. Sleep quality and psychological parameters were assessed at four timepoints: baseline (T0), week 3 (T1), week 6 (T2), and after 3-week washout (T3). The probiotic supplement (PSICOBRAIN®, Bromatech, Milano, Italy) consisted of *L. rhamnosus* HN001 (1×10^9 CFU), *L. rhamnosus* SP1 ($<10^9$ CFU), *L. acidophilus* LA1 ($<10^9$ CFU), and *L. reuteri* LR92 ($<10^9$ CFU), delivering 3×10^9 CFU per capsule. These specific strains were selected based on documented sleep-related mechanisms and gut-brain axis modulation capabilities.

L. rhamnosus HN001 was selected based on its documented capacity to reduce cortisol levels and improve psychological well-being in clinical trials, demonstrating significant effects on stress-related sleep disturbances.[25] This strain has shown particular efficacy in modulating the hypothalamic-pituitary-adrenal axis, which directly impacts sleep quality and circadian rhythm regulation.

L. rhamnosus SP1 was chosen for its reported GABA–producing potential in experimental settings,[26] a primary inhibitory neurotransmitter essential for sleep initiation and maintenance. GABA-producing lactobacilli have been associated with pathways relevant to sleep–wake cycle regulation.

L. acidophilus LA1 was selected for its strong adherence to intestinal epithelium and documented influence on gut-brain axis signaling pathways.[27] This strain exhibits superior survival in gastric conditions and demonstrates enhanced interaction with enteroendocrine cells involved in circadian hormone production.

L. reuteri LR92 was included for its association with tryptophan metabolism and serotonin pathway modulation, both critical for circadian rhythm regulation and melatonin synthesis.[28] This strain has shown capacity to influence metabolic pathways directly related to sleep-wake cycle maintenance.

Participants consumed two capsules daily (total daily dose: 6×10^9 CFU), one with breakfast and one with lunch. The dosage was established based on successful clinical outcomes in previous probiotic sleep intervention studies using comparable CFU ranges, with demonstrated efficacy in improving sleep quality parameters.[19,21]

The complete formulation included specific strains, microcrystalline cellulose, gelatin (capsule), L-theanine, and *Eschscholtzia californica* Cham. (aqueous extract, aerial part). The comparator capsules contained the same excipients but without probiotic strains. Thus, the comparator matched the active product in non-bacterial components and sensory characteristics. To maintain study blindness, participants received standardized instructions for capsule consumption. If a dose was missed, participants were instructed to skip it and continue with the next scheduled dose.

Participants

The study enrolled 60 healthy adults (35 women and 25 men) aged 18-65 years with mean age 22.00±3.02 years; the majority of participants were aged between 19 and 29 years. All were university students or staff, with 85% holding bachelor's degree or higher. Exclusion criteria included psychiatric or neurological disorders, celiac disease, lactose intolerance, allergies, chronic conditions (irritable bowel syndrome, diabetes, ulcerative colitis), and antibiotic treatment within three months prior to study. From 71 screened individuals, 9 were excluded due to inclusion/exclusion criteria or unwillingness to participate. Of 62 randomized participants, 1 from probiotic group (treatment discontinuation) and 1 from comparator group (antibiotic intervention) were excluded. Sixty participants completed the study (Figure 1).

Two experimenters (FD, FB) conducted structured interviews to collect demographic information and assess physical activity levels. For women participants, additional screening addressed potential premenstrual syndrome to account for monthly fluctuations.

Stratified randomization using computer-generated lists ensured balanced allocation regarding sample size and physical activity levels between groups. Experimenters responsible for enrollment, randomization sequence generation, and intervention assignment remained blinded to group allocation, as did participants.

The study was conducted between June-September 2024 at Ecotekne campus, University of Salento, following Declaration of Helsinki provisions. The protocol was approved by institutional Ethics Committee of Palermo 1, Italy (Protocol no. 8 14/09/2022, Identifier NCT05565651). All participants provided written informed consent and could withdraw without providing reasons.

Sleep quality assessment

Sleep quality was evaluated using two validated instruments. The Pittsburgh Sleep Quality Index (PSQI)[29,30] examines sleep patterns over the previous month through 19 questions. Initial items gather data on sleep timing, onset latency, duration, and total sleep hours. Additional questions explore sleep quality aspects using appropriate rating scales. The tool generates seven component scores (including perceived quality, onset time, duration, medication use, daytime impact, and sleep efficiency) rated 0-3. The combined score (0-21) reflects overall sleep quality, with lower scores indicating better sleep patterns.

The Insomnia Severity Index (ISI)[31,32] was also administered. A composite score was computed and evaluated according to standard criteria (score >15 indicates pathological sleep disturbance). Both instruments assess subjective sleep quality based on participant self-reporting rather than objective physiological measurements.

E-Prime 2.0 software (Psychology Software Tools Inc., Sharpsburg, PA, USA) was used to present questionnaires and record responses. Each session lasted approximately 45 minutes.

Statistical analysis

Sample size computation was performed using G-Power 3.1 software[33] for a mixed-design Analysis of Variance (ANOVA; F-test within-between interaction) with two groups (probiotic vs. non-bacterial comparator) and four measurement timepoints (T0, T1, T2, T3). Based on previous probiotic sleep intervention studies showing medium effect sizes for PSQI improvements,[19,21] we assumed an a priori effect size of $f = 0.25$.[34] With α error probability set at 0.05 and desired power of 0.90, the calculated minimum sample size was 30 participants total (15 per group). To account for potential dropouts (estimated at 10-15% based on similar probiotic intervention studies), we recruited 60 participants (30 per group), ensuring adequate statistical power even with attrition. Post-hoc power analysis confirmed achieved power = 0.89, validating the adequacy of our sample size for detecting clinically meaningful differences in sleep quality measures.

Age and gender distribution were evaluated using independent sample t-test and chi-square analysis, respectively. The Shapiro-Wilk test ($p>0.05$) assessed normality of questionnaire responses. Given non-normal distribution, non-parametric statistical methods were employed. The data are presented as mean ± Standard Deviation (SD).

Changes in questionnaire scores across time intervals (T0, T1, T2, T3) were evaluated using Friedman test conducted separately for experimental and comparator. Post-hoc analyses employed Wilcoxon signed-rank test with Bonferroni correction, establishing adjusted significance threshold of $p<0.017$ for multiple comparisons. Between-group comparisons used Mann-Whitney U-test, with statistical significance set at $p<0.05$. The analysis was conducted using intention-to-treat approach.

Spearman correlation analyses examined the relationships between mood and sleep quality, conducted independently for each group.

Given the use of non-parametric methods, no formal group × time interaction test was performed; between-group differences at each time point were therefore assessed using Mann–Whitney U test.

Results

No significant differences ($p>0.05$) in clinical or demographic characteristics existed between groups at baseline. Groups did not differ by age [t-test = 1.062, p = 0.78] or gender distribution [$\chi^2 = 0.12$, p = 0.83]. Baseline demographic and clinical characteristics are summarized in Table 1. No significant baseline differences were found between groups on sleep quality questionnaires (Table 1).

Sleep quality

A significant time effect in the experimental group [$\chi^2 (3) = 11.14$, p = 0.009] was found. Between-group comparisons performed using the Mann–Whitney U-test revealed a significant improvement in the probiotic group compared with matched non-bacterial comparator at week 6 (T2) ($p < 0.001$), and this effect persisted after washout (T3) ($p < 0.001$). Post-hoc comparisons showed this effect was due to lower scores after 6 weeks of probiotic intake (T2) (mean ± SD, 7.5 ± 3.9) compared to baseline (T0) (mean ± SD, 19.2 ± 3.1) (Z = −2.820, p = 0.005, effect size = −0.470) indicating that improvements were significant in probiotic group compared with matched non-bacterial comparator (Figure 2). Detailed PSQI mean values and between-group comparisons at each timepoint are reported in Table 2. This p value was adjusted for multiple comparisons using Bonferroni correction. Changes in PSQI and ISI scores across all timepoints are illustrated in Figure 2.

Discussion

This study shows that a six-week multi-strain *Lactobacillus* spp. supplementation was associated with improved subjective sleep quality compared with a matched non-bacterial comparator. The observed clinical benefits may be associated with strain-specific properties of the administered *Lactobacillus* spp. strains, which may produce neuroactive compounds and modulate host-microbe interactions at the intestinal barrier.

The substantial improvement in perceived sleep quality (11.7-point PSQI reduction) suggests a possible association between the selected probiotic strains and pathways related to sleep–wake regulation.[34-36] *L. rhamnosus* strains have been reported to possess γ-aminobutyric acid (GABA)–producing potential in experimental settings.[26] Additionally, *L. reuteri* LR92 has been associated with pathways related to tryptophan metabolism in preclinical studies.[28] These strain-specific properties may have contributed to the observed improvements, although no direct mechanistic measurements were performed.

The persistence of sleep quality improvements during washout was observed; however, no conclusions can be drawn regarding microbial colonization or long-term ecosystem changes. However, it is important to note that these proposed mechanisms are hypothetical, as our study did not include microbiome analysis or measurement of microbial metabolites such as Short-Chain Fatty Acids (SCFAs) or neurotransmitters. The multi-strain approach likely provided synergistic effects, with different *Lactobacillus* species potentially occupying distinct ecological niches and contributing complementary metabolic functions relevant to sleep regulation.

While our selected *Lactobacillus* spp. strains showed beneficial effects, the individual contributions of *L. rhamnosus* HN001, *L. rhamnosus* SP1, *L. acidophilus* LA1, and *L. reuteri* LR92 to sleep outcomes require further investigation. Future studies should examine optimal strain combinations and dosing regimens, as well as the specific mechanisms by which each strain influences sleep parameters through targeted metabolomic and transcriptomic analyses. The strain-specific effects observed in our study highlight the importance of precision microbiome approaches rather than generic probiotic interventions for sleep disorders.[37-38] *L. acidophilus* LA1 and *L. rhamnosus* strains have demonstrated capacity to survive gastric transit and transiently interact with the intestinal environment.[22]

A major limitation of our study is the exclusive reliance on subjective sleep quality measures, which may be influenced by mood, expectancy, or matched non-bacterial comparator effects. The PSQI and

ISI assess perceived sleep quality based on participant self-reporting rather than objective physiological measurements such as polysomnography or actigraphy. Future studies should incorporate objective sleep measures to better understand how probiotic intervention affects specific sleep stages, sleep efficiency, and circadian rhythm markers.

Additional significant limitations include the absence of microbiome analysis, which would have provided direct evidence of microbial community changes and strain-specific colonization patterns. The study population consisted predominantly of young university students (mean age 22 years, 85% with university degrees), limiting generalizability to elderly populations or those with sleep comorbidities. Future research should incorporate 16S rRNA sequencing and metagenomic analysis to characterize microbiota composition changes induced by probiotic intervention, along with measurement of microbial metabolites.

The temporal dynamics of sleep improvement, with benefits becoming apparent by week 3 and reaching maximum effect by week 6, suggest that a longer exposure period may be required before measurable effects on perceived sleep quality become evident. The sustained improvements during washout may reflect unmeasured behavioral, expectancy-related, or contextual factors rather than durable biological changes.

While our study demonstrated significant improvements in PSQI scores, the relationship between specific sleep components and potential microbiota-mediated mechanisms suggests complex interplay requiring further investigation. The interaction between circadian rhythm regulation and overall sleep quality deserves particular attention in future microbiome research.[39]

Conclusions

This study provides evidence that multi-strain *Lactobacillus* spp. supplementation can improve subjective sleep quality. The observed improvements in perceived sleep parameters suggest a potential role for targeted probiotic formulations as adjunctive approaches in sleep-related research. The strain-specific effects of *L. rhamnosus* HN001, *L. rhamnosus* SP1, *L. acidophilus* LA1, and *L. reuteri* LR92 suggest that precision microbiome approaches may successfully influence sleep regulation, though the underlying mechanisms require further investigation with objective measures and microbiome analysis.

The therapeutic implications extend beyond symptom management, suggesting that probiotic interventions warrant further investigation alongside established sleep management strategies.

These findings provide a rationale for further investigation of targeted probiotic formulations as adjunctive approaches in sleep-related research, particularly in studies integrating objective sleep measures and microbiome profiling.

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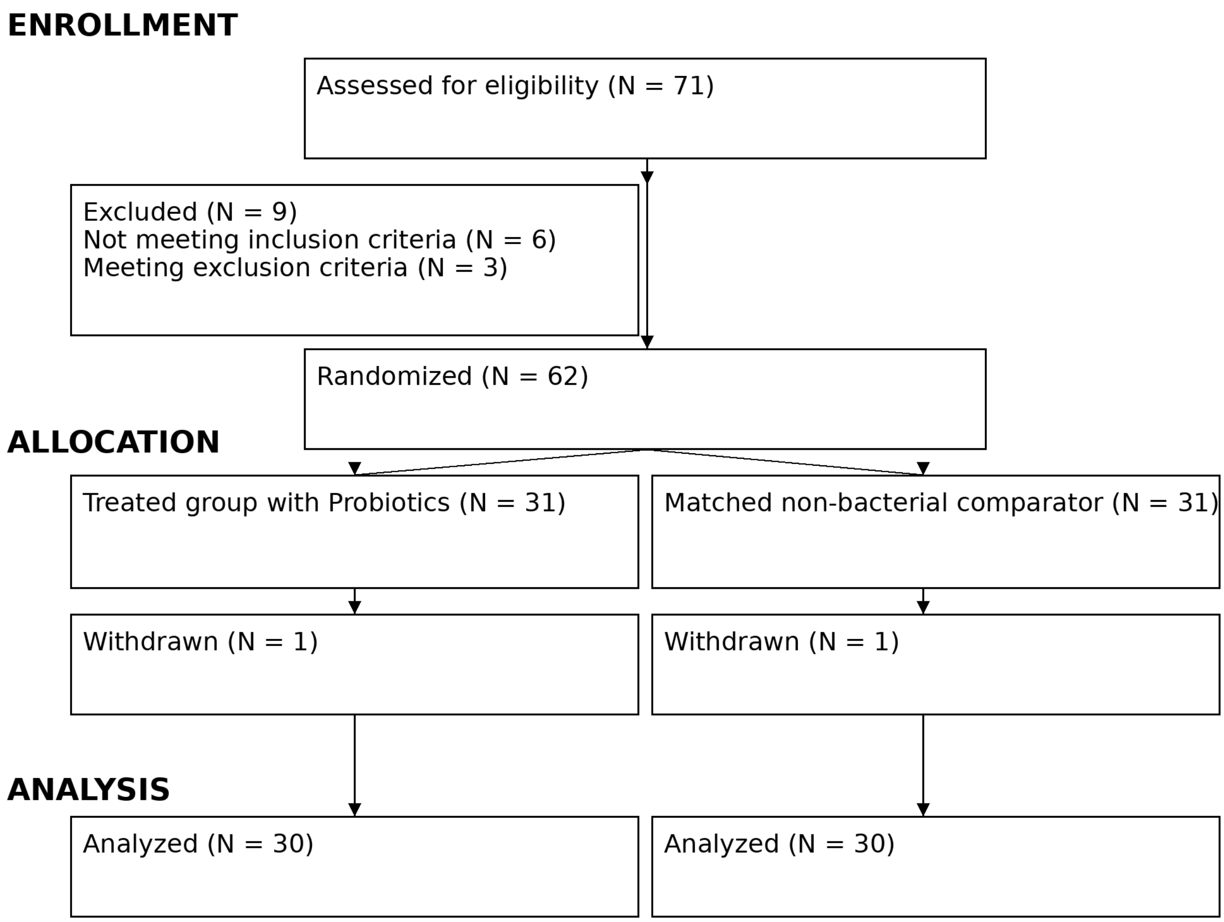


Figure 1. Flow diagram of participant enrollment, randomization, and analysis in the clinical trial evaluating the efficacy of a multi-strain *Lactobacillus* spp. probiotic on effects on sleep quality.

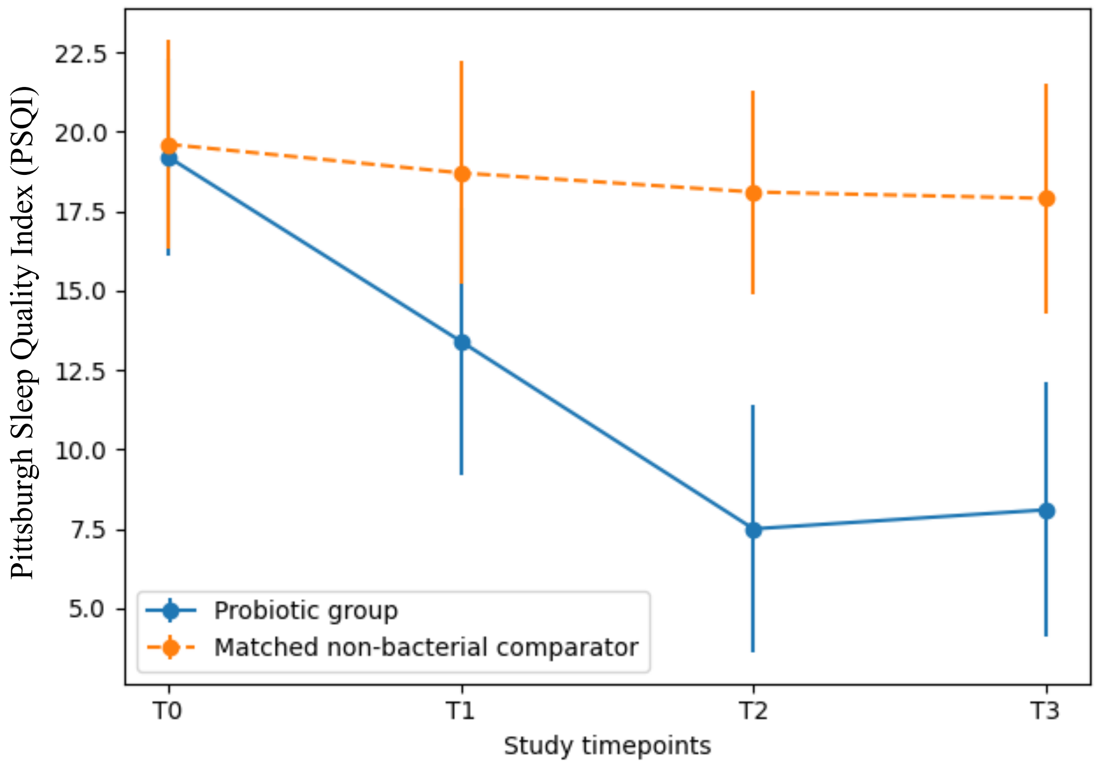
Table 1. Baseline demographic and clinical characteristics of participants at study entry (T0). No significant differences were observed between probiotic and matched non-bacterial comparator groups.

Characteristic	**Probiotic (n=30)**	**Matched non-bacterial comparator (n=30)**	**p value**
Age, years (mean±SD)	22.1±3.0	21.9±3.1	0.78
Women, n (%)	18 (60%)	17 (57%)	0.83
Men, n (%)	12 (40%)	13 (43%)	0.81
BMI, kg/m² (mean ± SD)	23.2 ± 2.7	23.0 ± 2.9	0.81
PSQI score (mean±SD)	19.2 ±3.1	19.6 (3.3)	0.72
ISI score (mean ± SD)	18.02± 2.41	18.11 ± 2.22	0.68

Body Mass Index (BMI); Pittsburgh Sleep Quality Index (PSQI); Insomnia Severity Index (ISI)

Table 2. Mean ± SD Pittsburgh Sleep Quality Index (PSQI) scores at baseline (T0), week 3 (T1), week 6 (T2), and after washout (T3) in probiotic and matched non-bacterial comparator. Within-group and between-group p values computed with Wilcoxon and Mann–Whitney tests, respectively; n.s., not significant.

Time point	**Probiotic (Mean ± SD)**	**Matched non-bacterial comparator (Mean ± SD)**	**p value (within-group)**	**p value (between-groups)**
T0	19.2 ± 3.1	19.6 ± 3.3	n.s.	n.s.
T1	13.4 ± 4.2	18.7 ± 3.5	0.045	0.031
T2	7.5 ± 3.9	18.1 ± 3.2	0.005	<0.001
T3	8.1 ± 4.0	17.9 ± 3.6	0.009	<0.001



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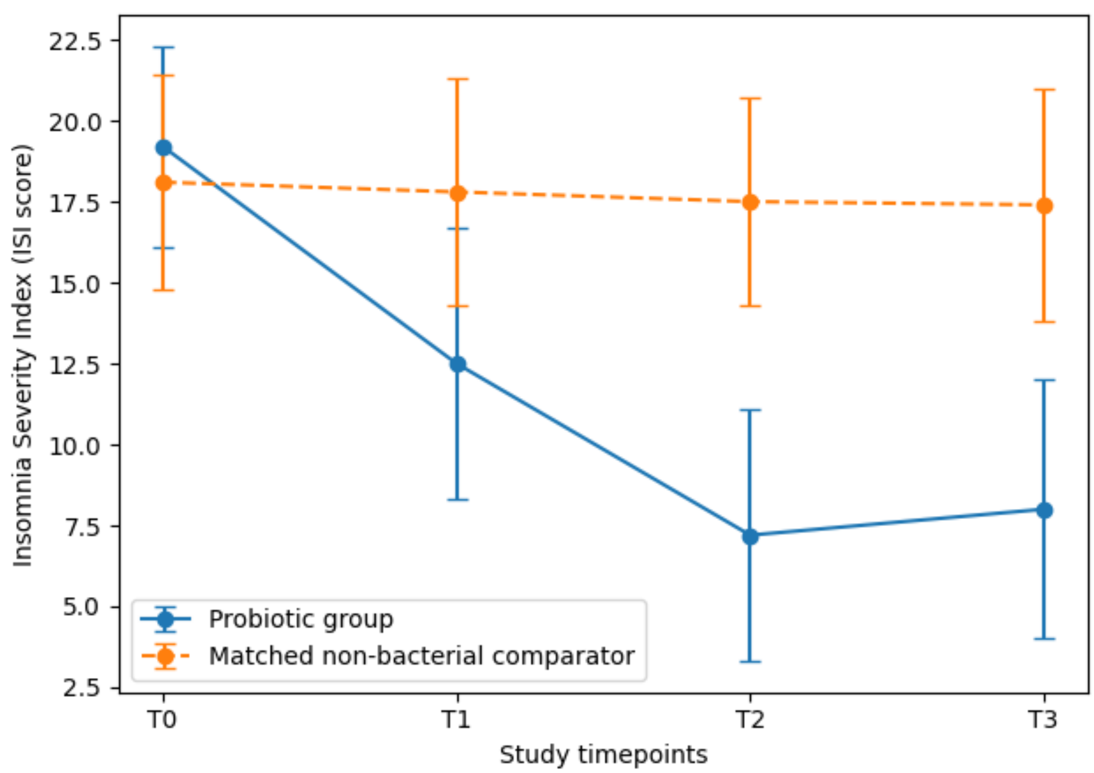

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Figure 2. Changes in subjective sleep quality during the study period. Mean ± Standard Deviation (SD) scores for (A) Pittsburgh Sleep Quality Index (PSQI) and (B) Insomnia Severity Index (ISI) at baseline

(T0), after 3 weeks of supplementation (T1), after 6 weeks of supplementation (T2), and after a 3-week washout period (T3) in the probiotic group and in the matched non-bacterial comparator group. Error bars represent standard deviations.

Contributions: all authors contributed equally to the present work.

Conflicts of interest: the authors declare no conflicts of interest.

Availability of data and materials: all data generated or analyzed during this study are included in this published article.

Ethics approval: the protocol was approved by institutional ethics committee of Palermo 1, Italy (Protocol no. 8 14/09/2022, Identifier NCT05565651).

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