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Author Contributions:

All authors were involved in the conception and design of this review. CI and SK were responsible for collating manuscripts and retrieving data. CI conducted the analysis of the data. All authors contributed to the drafting and revising of the article, and the final approval of the published version of the manuscript.

Abstract

Inadequate sleep (i.e. duration and/or quality) is becoming increasingly recognized as a global public health issue. Interaction via the gut-brain axis suggests that modification of the gut microbial environment via supplementation with live microorganisms (probiotics) or non-viable microorganisms/microbial cell fractions (paraprobiotics) may improve sleep health. This systematic review and meta-analysis aimed to clarify the effect of consuming probiotics/paraprobiotics on subjective and objective sleep metrics. Online databases were searched from 1980 to October 2019 for studies involving adults who consumed probiotics or paraprobiotics in controlled trials, during which, changes in subjective and/or objective sleep parameters were examined. 14 studies (20 trials) were included in meta-analysis. Random effects meta-analyses indicated that probiotics/paraprobiotics supplementation significantly reduced Pittsburgh Sleep Quality Index (PSQI) score (i.e. improved sleep quality) relative to baseline (-0.78-points, 95% Confidence Interval (CI): 0.395-1.166; $p<0.001$). No significant effect was found for changes on other subjective sleep scales, nor objective parameters of sleep (efficiency/latency) measured using polysomnography or actigraphy. Subgroup analysis for PSQI data suggested that the magnitude of the effect was greater (although not statistically) in healthy participants than those with a medical condition, when treatment contained a single (rather than multiple) strain of probiotic bacteria, and when the duration of treatment was ≥8 weeks. Probiotics/paraprobiotics supplementation may have some efficacy in improving perceived sleep health, measured using the PSQI. While current evidence does not support a benefit of consuming probiotics/paraprobiotics when measured by other subjective sleep scales, nor objective measures of sleep; more studies using well-controlled, within-subject experimental designs are needed.

Key words: *probiotic bacteria; sleep; PSQI; sleep quality; sleep efficiency; sleep latency*

Introduction

Sleep is an essential biological function that plays an important role in physiological processes that promote physical recovery and repair, support neurological development, enhance cardiac, immune and metabolic functions, and improve cognition and mood [1,2]. Sufficient duration and adequate quality sleep is therefore necessary to support both mental and physical health; and overall quality of life [3]. Inadequate sleep has been associated with increased risk of developing chronic diseases such as obesity [4], type 2 diabetes [5,6], heart disease [7], some types of cancer [8] and mental illness – particularly, in later life [9].

Inadequate sleep (i.e. duration and/or quality) is increasingly being recognized as a global public health issue [3]; with alarmingly high rates of sleep disorders being reported and significant numbers of people complaining of sleep disturbances and/or suffering from insufficient sleep [2,3]. For instance, reports on population sleep health from many industrialized nations indicate that more than one-third of adults regularly experience inadequate sleep [10-12]. In addition to feelings of tiredness [13], the broader economic costs of inadequate sleep (i.e. related to increased health care costs, lost productivity, impaired decision making and cognitive performance, workplace absenteeism, road traffic accidents) are also high; estimated at ~$66 billion annually in Australia [14] and between $280 and $411 billion in the US [15]. A number of personal, social, behavioral, organizational and environmental factors have been reported to contribute to inadequate sleep [16-19]. While some are beyond our control (e.g. age associated decline in sleep quality and architecture [2,20]), many are directly associated with lifestyle behaviors that give rise to higher levels of stress, anxiety and depression; which can themselves disrupt sleep [21].

Sleep disturbances are typically characterized by a decrease in one's ability to initiate and maintain sleep, and by a reduced proportion of the deeper, more restorative sleep [2]. While measuring sleep and the impact of sleep disturbances has methodological challenges, several techniques are currently employed. Sleep duration and quality can be measured objectively under standardized laboratory

conditions or in the home, using polysomnography (PSG) [22] or wearable devices such as actigraphy monitors [23]. Many studies also measure sleep subjectively using validated tools such as the Pittsburgh Sleep Quality Index (PSQI) [24], Epworth Sleepiness Scale (ESS) [25], Oguri-Shirakawa-Azumi (OSA) sleep inventory [26], or questionnaires/visual analog scales querying individuals about sleep duration and/or quality [27]. Irrespective of the method employed, the premise of these assessments is to diagnose and characterize sleep issues and determine the effectiveness of treatment interventions.

Managing chronic sleep disorders may involve a combination of therapies, typically beginning with non-pharmacological strategies aimed at improving sleep hygiene; cognitive behavior therapies may also be used [28,29]. Alternatively, pharmacological agents (e.g. benzodiazepines, Z-drugs, melatonin agonists) may be employed. However, the efficacy of these treatments vary, establishment of correct dosing is challenging, and the risk of adverse side-effects may be high [30,31]. For these reasons, many individuals affected by sleep issues avoid using pharmacological treatments, especially as a long-term remedy [32,33]. Another alternative is the use of herbal and dietary supplements, which has received increased scientific attention in recent years [34,35]; likely because of their natural properties and the perceived absence of residual effects [31]. Supplements containing probiotics (i.e. live microorganisms which, when ingested in adequate amounts can provide health benefits to the host [36]) or "paraprobiotics" (i.e. non-viable microbial cells or crude cell extracts, which when administered in adequate amounts, confer a benefit on the consumer [37]) are one such example. With an extensive bidirectional communication network between the gastrointestinal tract and central nervous system (known as the "gut–brain axis") [38], modifying the microbial environment (by means of supplementing with probiotic bacteria), may benefit sleep. Indeed, alterations in the microbiome have been shown to influence neurotransmission of serotonin (increasing production of free tryptophan, and in turn increasing serotonin availability) in both the peripheral and central nervous system (CNS) [39]. While this may convey a positive impact on mood and psychological

well-being [39,40], it also has the potential to influence sleep [41] as serotonin is acetylated, then methylated to yield melatonin; the hormone important in helping regulate sleep/wake cycles [42]. In fact, Wong et al., [43] recently found that 6 weeks of probiotics supplementation in individuals with irritable bowel syndrome increased salivary melatonin levels, significantly so, in males.

Given the increased global prevalence and potential harms associated with insufficient sleep, exploring options to effectively optimize sleep quality and continuity are paramount. Therefore, the aim of this systematic review and meta-analysis was to clarify the effect of consuming probiotics or paraprobiotics on subjective and objective sleep metrics. Findings will provide a better understanding of the efficacy of probiotics/paraprobiotics to improve sleep duration and quality; thus, help inform recommendations regarding use of these supplements as an alternative or adjunct therapy for individuals suffering chronically from inadequate sleep.

Methods

This review was performed in accordance with specifications outlined in the Preferred Reporting Items for Systematic Reviews and Meta-Analysis Protocols PRISMA-P 2015 Statement [44] and registered at the International Prospective Register of Systematic Reviews (PROSPERO) (identification code: 149721) ahead of the formal study selection process.

Literature Search

Potential research studies were identified by searching the online databases PubMed (MEDLINE), Web of Science (via Thomas Reuters), Scopus and PsycINFO from 1980 until October 2019 using the terms probiotic* OR lactobacill* OR bifidobacter* OR saccharomyces* OR enterococcus* OR streptococcus* OR symbiotic* OR bacteria OR microbio* AND sleep* OR wakefulness OR tired* OR drows*. The star symbol (*) was used to capture the derivatives (by suffixation) of a search term. Limits were applied to database searches in PubMed, Web of Science and Scopus (i.e. limited to

articles published in 'English' language, conducted on 'Human' 'Adults', and 'Original Articles & Conference Proceedings') to reduce the capture of articles that did not meet the inclusion criteria (see 'Study Eligibility). Two investigators (C.I. and S.K.) independently screened the potential research studies to identify relevant texts. Initially, all irrelevant titles were discarded. The remaining articles were systematically screened for eligibility by abstract and full text, respectively. The decision to include or discard potential research studies was made between two investigators (C.I. and S.K.). Any discrepancies were resolved in consultation with a third investigator (D.M.). The reference lists of all included studies were hand searched for missing publications.

Study Eligibility

Studies were included if they: (1) were randomized controlled trials or quasi-experimental (non-randomized controlled trials), (2) included adults >18 years of age, (3) administered live bacteria (probiotics) or heat-inactivated/killed probiotic bacteria (paraprobiotics) as a daily oral supplement (e.g. capsules/tablets, fermented milk, yoghurt, powder sachets), (4) compared the treatment arm against a placebo control condition, and (4) had accessible full-text articles in English. Studies were not excluded from the review if the treatment was co-administered with other substances (e.g. theanine), but they were omitted from the meta-analysis if the effect of the probiotics or paraprobiotics could not be isolated (e.g. there was no "theanine-only" comparator condition). Studies were excluded from the review if sleep outcome data were limited to reports of number of sleep disturbances associated with a medical condition (e.g. rib fracture, cold-flu, bowel preparation for colonoscopy). Studies were also excluded from the meta-analysis (only) if sleep data were not adequately reported, i.e. neither the mean±standard deviation (SD) nor an appropriate effect size were reported or calculatable. In the event that data was not adequately reported, and the study was published within the previous 10 years (2009 – 2019), the corresponding author was contacted via email in an attempt to retrieve missing data. Where data were presented in graphical format only, a

web-based tool ('WebPlotDigitizer', https://apps.automeris.io/wpd/) was used to extract numerical values.

Several publications identified via the literature search conducted treatment vs. control comparisons at multiple times throughout the study. In these instances, the separate treatment durations were treated as individual investigations, termed *'trials'*. Separate trials derived from a single research study are denoted by the addition of letters (i.e. a – d) to the citation. Full details of the screening process are displayed in Figure 1.

INSERT FIGURE 1 HERE

Methodological Quality Assessment

Included studies were examined for methodological quality using the Rosendal Scale [45], which combines the PEDro scale [46], Jadad scoring system [47] and Delphi List [48] to assess a number of factors associated with the minimization of experimental bias in areas such as randomization, blinding, participant selection and data reporting (see Table II in [45]); and where excellent methodological quality is indicated by a Rosendal Score ≥60% [47]. Scoring was determined by dividing the number of 'yes' responses (denoted by a score of 1) by the total number of applicable items. Studies with Rosendal scores <50% are typically excluded from meta-analyses due to increased risk of experimental bias.

Data Extraction, Synthesis and Analysis

Relevant data were extracted in accordance with the Cochrane Handbook for Systematic Reviews of Interventions *'Checklist of Items to Consider in Data Collection or Data Extraction'* [49] and entered into a Microsoft Excel spreadsheet.

The effect of consuming probiotics/paraprobiotics on subjective and objective sleep parameters was quantified as the mean difference (MD) in the pre- vs. post-treatment change between the intervention and control groups. In general, this value was derived by: (1) calculating the Mean±SD within-group treatment effect (i.e. the pre- vs. post-intervention change) for the control and intervention conditions; and then (2) calculating the Mean±SD between-group treatment effect (i.e. the difference between these two change scores). The within-group standard deviation of change (SD_{Δ}) and the between-group pooled standard deviation of change (SD_{pooled}) were also derived in each respective step. As subjective sleep quality was assessed using a variety of different scales (i.e. ESS, mESS, VAS, OSA) the MD was later standardized against the SD_{pooled} as described in section 2.7 'Meta-analysis'). The Cochrane Handbook for Systematic Review of Interventions [49] was used as the guideline to perform statistical analysis.

Where the within-group SD_{Δ} was not reported directly, a *t*-statistic resulting from a paired *t*-test was used to estimate the value using the following equation (where *n* is number of participants [49]:

$$SD_{\Delta} = \frac{|\ MD\ |}{t\ \text{statistic}} \times \sqrt{n}$$

If an appropriate *t*-statistic was not reported, it was derived from an equivalent *p*-value. When $p < x$ was reported (where x is the numerical value for *p* reported), *p* was assumed to equal x; if only $p>0.05$ was reported the missing SD_{Δ} was imputed using the correlation coefficient (*r*) and the following equation [49]:

$$SD_{\Delta} = \sqrt{(SD_{baseline}^2 + SD_{final}^2) - (2 \times r \times SD_{baseline} \times SD_{final})}$$

r was approximated as the mean correlation coefficient calculated using published *t*-statistics (or *p*-values) resulting from paired *t*-tests and SD_{Δ}'s, as described by Higgins and Green [49]. Sensitivity analyses were performed to test the robustness of the imputed *r* (see section '*Sensitivity and Subgroup Analyses*').

One study [50] reported post-treatment data only. Hence, the SD_{Δ} was not required. Nonetheless, the between-group "treatment" effect was calculated and included in meta-analysis. Based on advice by Higgins and Green [49], there is no statistical reason why studies with change-from-baseline outcomes should not be combined in a meta-analysis with studies using post-intervention measurement outcomes when using an unstandardized (i.e. mean difference) method.

Where the between-group SD_{pooled} was not reported directly, it was calculated using the following equation [51]:

$$SD_{pooled} = \sqrt{\frac{(SD^2_{control} + SD^2_{intervention})}{2}}$$

Meta-analysis

Comprehensive Meta-Analysis (CMA) software, Version 3.3.070 was used to perform the meta-analysis of data using random-effect models. Statistical significance was attained if the 95% CI did not include zero. Heterogeneity was assessed with Cochran's Q and the I^2 index. Low, moderate and high heterogeneity was indicated by an I^2 value of 25, 50 and 75%, respectively [49]. A *p*-value <0.10 for Cochran's Q was used to indicate significant heterogeneity [52].

For meta-analysis of PSQI and objective (EEG, Actigraphy) sleep outcomes, the weighted mean treatment effect was calculated as the mean (unstandardized) difference between control and intervention groups. For trials reporting both global and sub-scores (i.e. sleep latency, duration, disturbance) of PSQI, only the global score was used for meta-analysis. Studies often report several

EEG and actigraphy sleep parameters; as such, meta-analysis was limited to data on sleep efficiency and latency outcomes. These are often regarded as important objective sleep parameters [53], and were the most commonly available objective parameters (reported in the same metric) across the studies employing EEG or actigraphy techniques in this review.

For other subjective sleep scale outcomes (i.e. ESS, mESS, VAS, OSA), the weighted mean treatment effect was calculated as the standardized mean difference (SMD) between control and intervention groups (i.e. an independent-groups Hedges' g effect) [54], where the MD was standardized against the pooled SD_{Δ} and corrected for bias due to small sample size. The magnitude of effect was defined in accordance with Cohen [51]: Hedges' $g \leq 0.2$ = small; 0.2–0.5 = medium; and ≥ 0.8 = large. Because the OSA sleep inventory is classified into five factors of sleep quality (i.e. sleepiness on rising, initiation and maintenance of sleep, dreaming, recovery from fatigue, and sleep length); in trials where the OSA sub-scores were also provided as a total score (i.e. OSA global) [55], only this value was used for meta-analysis.

All positive values indicate a beneficial effect of probiotics/paraprobiotics, irrespective of the measure used. Weighted mean difference and standardized mean difference estimates are presented as Mean±SEM. All other data are presented as Mean±SD.

Sensitivity and Subgroup Analysis

The influence of individual trials on an overall meta-analysis result was assessed in a one-out method, where the changes in heterogeneity and summary effect were assessed after excluding individual trials. The robustness of the meta-analysis to the imputed SD_{Δ} was assessed by calculating SD_{Δ} using different correlation coefficients (r = 0.2 and 0.8) and observing their influence on the summary effect and heterogeneity.

Subgroup analysis were performed to compare the effect of probiotics/paraprobiotics on sleep in healthy participants (i.e. with no disclosed medical conditions other than sleep issues) and those with

known medical conditions (i.e. cirrhosis patients who had recovered from an episode of hepatic encephalopathy, patients with IBS and depression). Furthermore, several previous reviews suggest that the health benefits of probiotics may increase when supplementation continues for ≥8 weeks [56-58]. To test this, trials with supplementation duration ≥8 weeks were compared with those with <8 weeks. A subgroup analysis of trials involving supplements containing a single vs. multiple strains of probiotic bacteria was also performed.

Results

Overview of Included Studies and Study Quality

Fifteen studies were included in the qualitative synthesis for the effect of consuming probiotics/paraprobiotics on subjective and objective sleep metrics. Of these, one study [59] was unable to be included in meta-analysis because the probiotic treatment was administered in combination with another constituent (i.e. theanine) that was not matched in the placebo condition. As such, 14 studies (a total of 20 trials; three studies [50,60,61] had two duration arms eligible and one study [55] had four duration arms) were eligible for inclusion in the meta-analysis. Of the 20 trials, 11 measured PSQI, eight measured other subjective sleep parameters, and seven measured sleep efficiency and/or sleep latency via EEG or actigraphy.

Fourteen of 15 studies included in the review employed a randomized design, while one conducted a comparative non-randomized pilot study [62]. Nine studies [43,50,55,60,61,63-66] employed a between-subjects parallel design, while six studies [59,62,67-70] followed a within-subjects crossover design. All but one study [67] specifically reported using a double-blinded protocol. The included studies yielded an average Rosendal Score of 75 ± 8% (Mean ± SD). The highest Rosendal Score of 87% was calculated for three studies [55,61,63] and all studies had good methodological quality with a Rosendal score ≥60% [47]. Hence, no studies were excluded from meta-analysis due

to increased risk of experimental bias. Complete results of the quality assessment are displayed in Supplementary Table S1.

Participants and Study Protocols

A summary of included investigations examining PSQI outcomes is presented in Table 1. Details of studies exploring other subjective sleep parameters are displayed in Table 2, and studies reporting on objective sleep parameters are presented in Table 3. Of the 15 studies included in the review, 10 reported using a single strain of probiotic bacteria [50,55,59,60,62,66-70], with three of these indicating use of paraprobiotics treatment [50,62,66] and one incorporating an additional ingredient (theanine) in the supplement that was not provided in the placebo condition [59]. Five studies used multiple strains of probiotic bacteria in their supplements [43,61,63-65]. The duration of supplementation varied from four days [68] to 24 weeks [63]. One study used yoghurt as the medium to deliver probiotic bacteria [65], while five studies used fermented milk [50,55,60,66,70] and the remainder provided supplementation via powder sachets [61,63,64,69], tablets [59,68] or capsules [43,62,67]. The dose of probiotic bacteria provided typically ranged between 1×10^9 CFU [67] to 9×10^{11} CFU [63], although details of probiotic bacteria dose were not specified in four studies [59,62,68,70].

INSERT TABLES 1, 2 & 3 HERE

The majority of studies (*n*=12) used healthy participants; specifically, five employed healthy university students enrolled in courses with a high-degree of stress (i.e. cadaver dissection, medical examination course) [50,55,60,66,69], three used healthy participants with reported sleep challenges/problems [59,62,68], one had healthy participants with symptoms of stress [64] and one employed healthy elderly (71 ± 6 y) participants [70]. Three studies enrolled patients with a known

medical condition; one employed patients with cirrhosis who had recovered from an episode of hepatic encephalopathy during the previous month [63], one had patients with diagnosed irritable bowel syndrome (IBS) and major depression [65], and one had patients with diagnosed IBS and sleep complaints [43]. Only five studies reported the baseline body mass index (BMI) of participants [50,55,60,65,67], and all except one [65] reported mean BMI <25 $kg \cdot m^{-2}$. Compliance to supplementation was only reported in four studies [55,60,63,65], with three of these indicating >95% compliance to intake [55,60,63], and one study indicating that 67% of the participants had 100% compliance to supplement intake [65]. All studies reported that supplementation was well-tolerated, with no indications of adverse events related to treatments.

Meta-analysis Results

Meta-analyses for the effect of consuming probiotics/ paraprobiotics on subjective and objective sleep metrics are presented in Figures 2 to 5.

The meta-analysis for the mean difference in Δ PSQI score (11 trials; n=452) indicated an overall significant improvement (i.e. greater reduction in score from baseline) of 0.78-points (95% CI: 0.395, 1.166; p<0.001) with probiotics/paraprobiotics supplementation (Figure 2). Low heterogeneity was observed (I^2 = 0%; ρ=0.584). The magnitude and significance of the overall meta-analysis result was stable during sensitivity analyses where trials were individually excluded (MD range: 0.703 to 0.920, 95% CI: 0.302 to 0.503, 1.103 to 1.336). This analysis used a mean correlation coefficient of 0.68 which was imputed using one reported SD_Δ [69] and four p-values [60,63,66,67]. Findings were comparable across different levels of correlation, suggesting the meta-analysis was robust to the imputed correlation coefficient (Supplementary Table S2).

INSERT FIGURE 2 HERE

The meta-analysis for the SMD in score derived from other subjective ratings scales (change from baseline) (8 trials; n=549) did not show evidence for an effect of consuming probiotics/paraprobiotics (Hedges g' = 0.081, 95% CI: -0.135, 0.297; p=0.462;) (Figure 3). Low to moderate heterogeneity was observed (I^2 = 36%; ρ=0.142). The magnitude and significance of the overall meta-analysis result was stable during sensitivity analyses where trials were individually excluded (SMD range: 0.022 to 0.122, 95% CI: -0.199 to -0.093, 0.244 to 0.361). This analysis used a mean correlation coefficient of 0.65 which was imputed using two reported SD_Δ's [55,64] and two p-values [63,65]. Findings were comparable across different levels of correlation (Supplementary Table S2).

INSERT FIGURE 3 HERE

Meta-analysis for the MD in sleep efficiency (MD = 0.019, 95% CI: -1.207, 1.244; p=0.976; 6 trials, 453 participants) (Figure 4) and sleep latency (MD = 0.693, 95% CI: -1.364, 2.750; p=0.509; 7 trials, 467 participants) (Figure 5) (change from baseline) also did not demonstrate evidence for an effect of consuming probiotics/paraprobiotics. The meta-analysis showed an overall low heterogeneity for both sleep efficiency (I^2 = 0%; ρ=0.979) and sleep latency (I^2 = 0%; ρ=0.496). The magnitude and significance of the overall meta-analysis results were relatively stable during sensitivity analyses where trials were sequentially removed (sleep efficiency = MD range: -0.170 to 0.128, 95% CI: -1.559 to -1.161, 1.219 to 1.450; sleep latency = MD range: 0.373 to 2.828, 95% CI: -1.743 to -0.265, 2.489 to 5.922). No correlation coefficient was imputed for these analyses as all [55] but one trial reported SD_Δ's, with the remaining trial providing p values [70].

INSERT FIGURES 4 & 5 HERE

Subgroup Analysis

The subgroup analysis based on health status of participants indicated a greater change for Δ PSQI scores in healthy participants (*n*=9) compared to those with a medical condition (*n*=2); although the difference between groups was not statistically significant (Supplementary Table S3). No differences were observed for other subjective scales based on subgroups of participant health status. However, the low number of trials in the subgroup for participants with a medical condition limits the interpretation of these findings. All trials providing data on objective sleep parameters involved healthy participants.

Results of subgroup analyses based on the number of probiotic strains (single vs. multiple) did not demonstrate any overall meaningful results; although there was a greater change for Δ PSQI scores when a single probiotic strain was provided (*n*=7). Nonetheless, the test for subgroup difference was not statistically significant (Supplementary Table S3). All trials providing data on objective sleep parameters administered supplements containing only a single strain of probiotic bacteria.

Similar results were observed for the intervention duration subgroup analyses. Although not statistically significant, changes for Δ PSQI scores, subjective sleep ratings and sleep efficiency were more pronounced when supplementation with probiotics/paraprobiotics was provided for ≥8 weeks. Sleep latency was significantly improved when supplementation of probiotics/paraprobiotics was provided for ≥8 weeks (*n*=4) compared to <8 weeks (*n*=3) (Supplementary Table S3).

Discussion

To our knowledge, this systematic review and meta-analysis is the first to coalesce the emerging evidence exploring the impact of consuming probiotics/paraprobiotics on subjective and objective sleep metrics. Results suggest that consumption of probiotics/paraprobiotics may improve subjective sleep quality, measured via the PSQI. However, we found no evidence (based on meta-analysis) for a benefit on sleep efficiency or sleep latency measured using PSG or actigraphy; nor was a beneficial

effect observed on sleep parameters measured using other subjective scales/questionnaires (e.g. OSA, ESS, VAS).

The overall weighted mean effect summary for the PSQI indicates that consumption of probiotics/paraprobiotics significantly improved (a mean reduction of 0.78-points) global PSQI score relative to baseline. The magnitude of the effect was greater (although not statistically so) in healthy participants than those with a diagnosed medical condition, when the treatment contained a single (rather than multiple) strain of probiotic bacteria, and when the duration of treatment was ≥8 weeks. However, the number of trials included in some subgroups was small; thus, limiting the conclusions that can be drawn from these results. It is also important to note that in six [50,60,66,69] of the nine trials involving healthy participants, an academic stress model was employed in the experimental design. That is, participants were university students undertaking exams or courses involving curriculum components (i.e. cadaver dissection) likely to induce high levels of stress and anxiety [71-73]. Exposure to psychological stress triggers the release of corticotropin-releasing factor (CRF), activating both the hypothalamus-pituitary-adrenal (HPA) axis and sympathetic nervous system (SNS); contributing to a decline in sleep quality [74,75]. Indeed, tension and stress have been highlighted as major predictors of poor sleep quality in college students [76]. Consumption of probiotic bacteria has been shown to attenuate stress-induced increases in glucocorticoids [77] and salivary cortisol [60,77]; thus, providing a potential mechanism for the improved PSQI scores identified in the aforementioned studies. Research in animal models also suggests that the ingestion of probiotic bacteria (e.g. *L. rhamnosus* (*JB-1*)) can modulate the GABAergic system, with transmission of stimulation from the intestine to the CNS via the vagus nerve, suppression of sympathetic nervous activity and enhancement of parasympathetic nervous activity; ultimately resulting in reduced stress-induced corticosterone and anxiety- and depression-related behaviors [78]. However, two studies that did not employ an academic stress model [61,67] also observed a positive effect of consuming probiotics (although not statistically different compared to control groups) on

PSQI score. In the study by Marotta et al. [61], a relatively large mean difference was observed after 6 weeks of supplementation compared to the overall mean effect found with the present meta-analysis. Positive correlations were also observed between PSQI scores and different aspects of mood (e.g. anxiety, depressive symptoms) [61]. Hence, consumption of probiotic bacteria may improve perceived sleep quality via enhancements to broader mood-related characteristics, irrespective of stress. Indeed, previous studies have demonstrated a strong relationship between sleep quality and broader aspects of mood [79]. Overall, the collective evidence suggests that individuals may perceive an improvement in sleep health after consuming probiotics/paraprobiotics. Nonetheless, it is important to recognize that while the PSQI is a reliable and valid assessment of self-reported sleep quality and sleep disturbance, like other self-reported inventories, it has limitations in that responses can be easily exaggerated or understated by individuals. As such, results of the present meta-analysis related to PSQI should be interpreted with this in mind. Indeed, the clinical significance of a 0.78-point reduction in global PSQI score is likely dependent on an individual's baseline sleep health relative to the threshold for diagnosis of a sleep disorder (i.e. PSQI >5) [24]. In the case of studies included in the present analysis, participants had a mean pre-treatment PSQI score of 5.59 (range: 4.57 [67] to 6.63 [63]); thus a 0.78-point reduction could be considered a clinically meaningful improvement in sleep quality. Nonetheless, in comparison to other forms of treatment, which have demonstrated greater efficacy (e.g. a recent meta-analysis indicated a weighted MD of 3.30 (SE 0.44) points on PSQI with cognitive behavioral therapy in persons with comorbid insomnia [80]), the impact of consuming probiotics/paraprobiotics supplements appears small.

Although the overall standardized mean effect was positive, meta-analysis demonstrated no significant benefit of consuming probiotics/paraprobiotics on changes observed with other subjective sleep-related scales (i.e. OSA, ESS, VAS). Unlike the PSQI, which provides a global measure of subjective sleep quality over the previous month, other subjective sleep scales often provide different outcomes that may be less sensitive to the effects of consuming supplements containing probiotic

bacteria. For example, the ESS (or modified version) used in three of the studies [43,63,65] gives an estimate of a more general characteristic; a person's 'average sleep propensity' rather than sleep quality per se [81]. Likewise the VAS used by Diop et al. [64] measured the severity of adverse sleeping symptoms in healthy volunteers with symptoms of stress. While the OSA sleep inventory is a popular method used in Japan to evaluate sleep quality [26], and has been suggested to have greater sensitivity at reflecting acute changes in sleep quality in an academic stress model [55,60], only two studies [55,68] employed this questionnaire; one of which did not provide a global OSA score [68]. Hence, it is important to acknowledge that more studies employing other subjective inventories are required before conclusions can be drawn about the utility of methods other than PSQI. At present, data suggest that the PSQI is sensitive to the effects of probiotics/paraprobiotics consumption on subjective sleep quality. Furthermore, employing a common measure allows for comparisons between investigations and is therefore encouraged for use in future studies.

The overall mean differences observed for the two objective sleep parameters meta-analyzed in this review (sleep efficiency and sleep latency) were small and positive, but non-significant. Hence, it appears that individuals may perceive a benefit of consuming probiotics/paraprobiotics on sleep quality (as previously mentioned with PSQI), yet this may not translate to improvements in objective sleep markers such as sleep efficiency and sleep latency, measured by PSG and actigraphy. One possible explanation for this could be that when using the PSQI, individuals rate their sleep quality retrospectively over the past month. On the other hand, objective measures are collected prospectively, and other than Yamamura et al. [70] who collected actigraphy data every day, PSG measures were only collected for three [55,68] or four [62] consecutive nights of the entire experimental protocol. Any night-to-night variation may therefore limit the ability to detect subtle changes in objective sleep parameters with probiotics/paraprobiotics consumption; especially when studies employ between-subject experimental designs with moderate (n = 14 – 60) sample sizes. Another possible reason may be that of the eight studies assessing sleep metrics with the PSQI, only

one [60] reported on the successfulness of participants blinding to the treatments. If blinding was not effective, one might expect to see changes in participants' subjective ratings of sleep quality, but not necessarily in objective sleep parameters. Of course, it is important to note that only a limited number of objective sleep outcomes were meta-analyzed in the present review. While studies often report a large number of other EEG and actigraphy parameters, meta-analysis was limited to the two parameters most frequently reported (in the same units of measure) across all of the studies using objective assessment techniques. Quantifying other EEG and actigraphy sleep-related outcomes may provide greater insight into the effect of consuming probiotics/paraprobiotics on sleep architecture and overall sleep health. However, there is a need for more studies, reporting these parameters in a standardized way to ensure appropriate summaries can be established.

It is important to acknowledge some limitations of the present review. While it is well-established that changes to sleep patterns occur as part of the normal aging process [20], we were unable to conduct sub-group analysis based on age. This was because several studies included participant populations with large age variations (e.g. participants in the study by Wong et al [43] had an age range of 20 – 76 y) and there is a lack of research conducted with older/elderly age groups (i.e. only one study investigated elderly (mean age = 71.4±5.7 y) participants [70]). Furthermore, there are potential sex differences in the effect of probiotics/paraprobiotics on sleep outcomes. For example, Wong et al [43] demonstrated that probiotics potentially alter the metabolism and production of endogenous melatonin differently between sexes (i.e. males had a significant increase in early morning systemic melatonin in response to probiotics supplementation, which was not observed in females). However, we were unable to conduct subgroup analysis based on gender because data reported in studies was typically aggregated and unable to be separated into gender specific data. Hence, more research exploring the influence of probiotics/paraprobiotics on sleep is needed, specifically focusing on gender and age group comparisons.

Overall, results of this systematic review and meta-analysis suggest that probiotics/paraprobiotics consumption can induce positive changes to perceived sleep health, measured using the PSQI; especially in healthy subjects (i.e. without medical comorbidities) and those who may have experienced periods of chronic inadequate sleep. While the current evidence suggests that consuming probiotics/paraprobiotics does not significantly influence responses on other subjective sleep scales; nor does it influence sleep efficiency and sleep latency measured objectively using PSG and actigraphy, the number of well-designed research investigations is presently limited. Hence, further research, specifically employing objective sleep outcome measures using well-controlled, within-subject experimental designs, and both homogenous and heterogenous populations are needed.

Conflict of interest

The authors declare that they have no conflict of interest.

Supplementary information is available at EJCN's website.

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Figures:

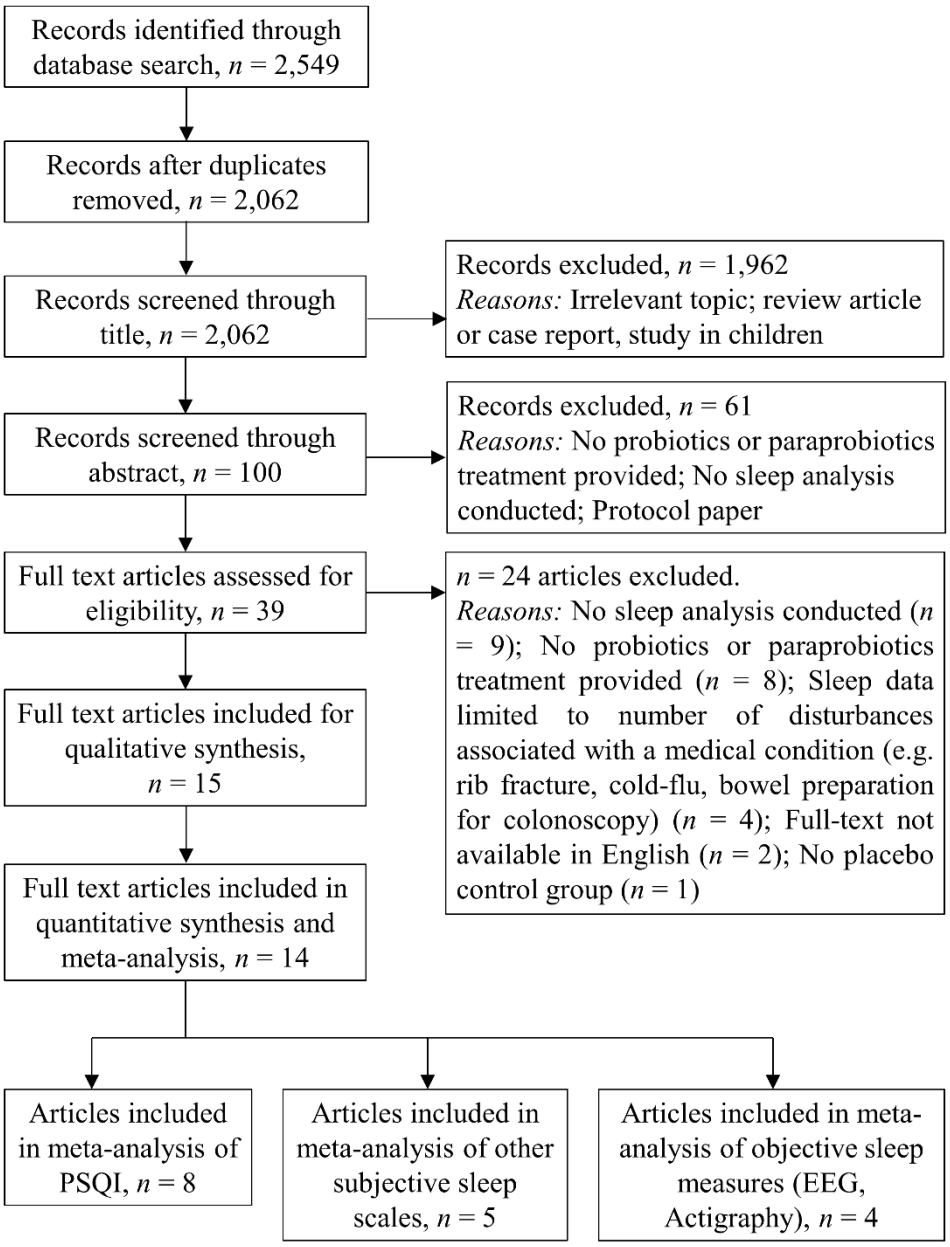

Figure. 1. PRISMA flow diagram (study selection methodology) for the systematic literature review on the effect of probiotics/paraprobiotics on subjective and objective sleep metrics.

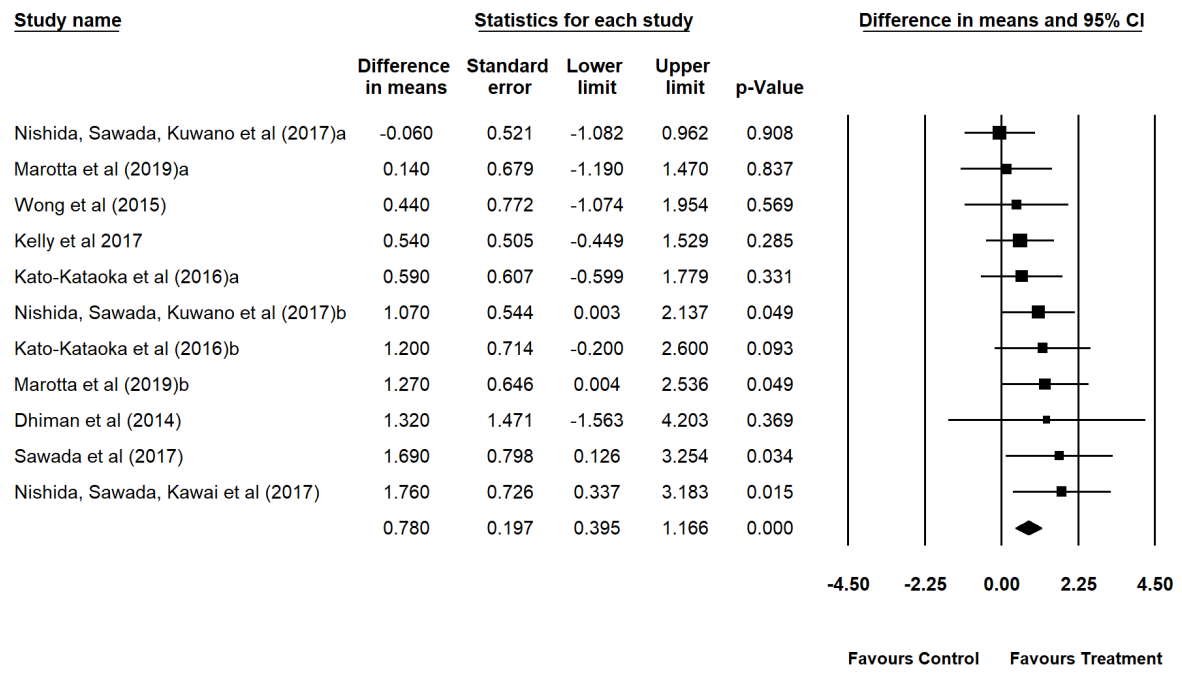

Figure 2. Forest plot displaying the effect of consuming probiotics/paraprobiotics treatment vs. placebo on Δ PSQI score. Size of the squares are proportional to the weight of the study. A positive mean difference indicates a beneficial effect of consuming probiotics/paraprobiotics.

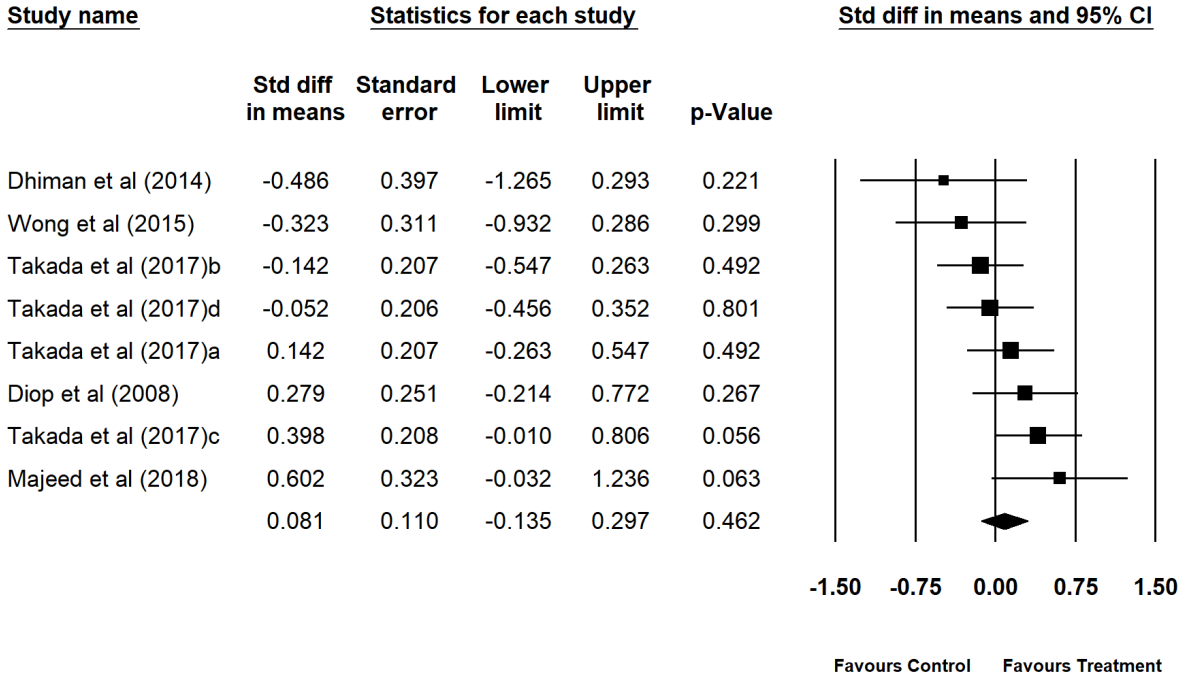

Figure 3. Forest plot displaying the effect of consuming probiotics/paraprobiotics vs. placebo on subjective sleep ratings (OSA, VAS, ESS, mESS). Size of the squares are proportional to the weight of the study. A positive effect estimate (Hedges' *g*) indicates a beneficial effect of consuming probiotics/paraprobiotics.

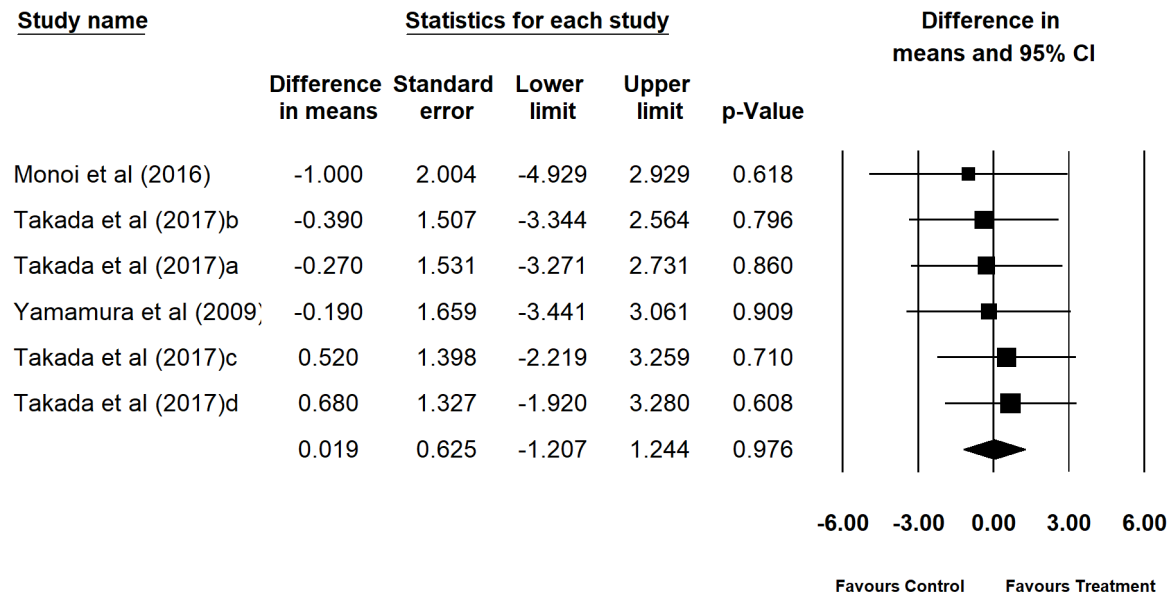

Figure 4. Forest plot displaying the effect of consuming probiotics/paraprobiotics vs. placebo on sleep efficiency (%) determined by objective measures (EEG and Actigraphy). Size of the squares are proportional to the weight of the study. A positive mean difference indicates a beneficial effect of consuming probiotics/paraprobiotics.

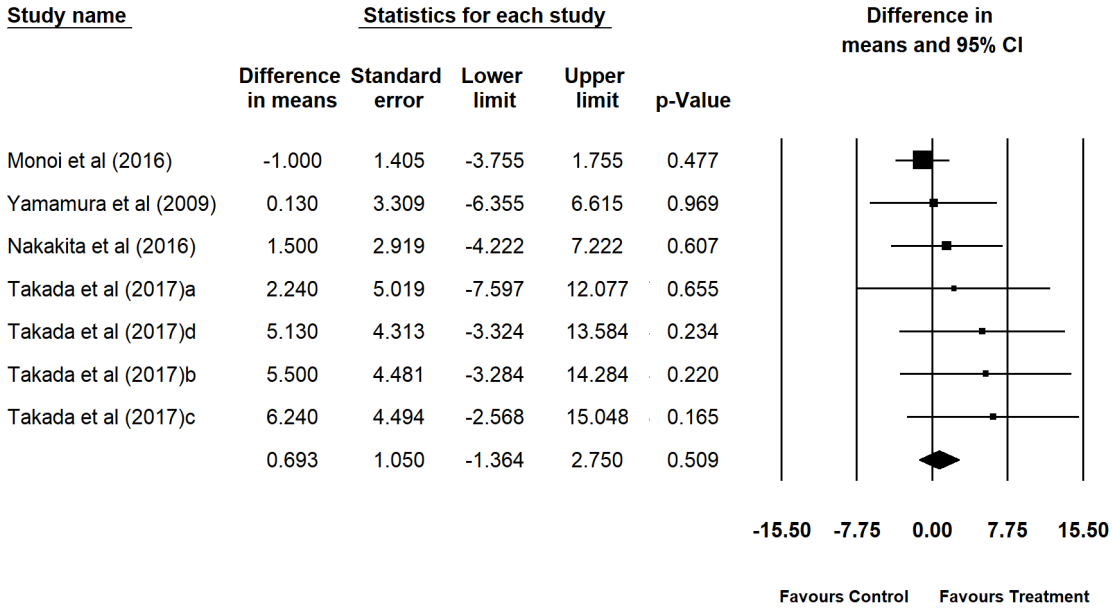

Figure 5. Forest plot displaying the effect of consuming probiotics/paraprobiotics vs. placebo on sleep latency (min) determined by objective measures (EEG and Actigraphy). Size of the squares are proportional to the weight of the study. A positive mean difference indicates a beneficial effect of consuming probiotics/paraprobiotics.

Table 1. Characteristics of studies that investigated the effects of probiotics/paraprobiotics on Δ PSQI score

Citation	Participants (n)	Age (y)	BMI	Participants Health Status	Study Design	Intervention Treatment	Probiotic Bacteria	CFU	Placebo Treatment	Duration	Mean Δ PSQI
Dhiman et al. (2014) [63], India	PLA: 11 PRO: 16	50.1 ± 9.8[a] 48.0 ± 11.4[a]	NS	Cirrhosis patients who had recovered from an episode of HE	BSD / DB	1 x VSL#3 Sachet Daily	*L. casei, L. plantarum, L. acidophilus, L. delbrueckii bulgaricus, B. longum, B. breve, B. infantis, Streptococcus thermophilus*	9×10^{11}	Sachets: corn flour	24 weeks	**+1.32**
Kato-Kataoka et al. (2016a – b) [60], Japan	PLA: 23 (12M) PRO: 24 (14M)	22.7 ± 0.4 23.0 ± 0.4	20.6 ± 0.5 21.0 ± 0.3	Healthy 4th year medical students undergoing promotion examination	BSD / DB	100 ml Fermented Milk Daily	*L. casei shirota*	1×10^{9} / ml	Milk: similar constituents	a: 6 weeks b: 8 weeks	**+0.59** **+1.20**
Kelly et al. (2017) [67], Ireland	29M	24.6 ± 4.0	24.6 ± 3.1	Healthy males	WSD / NS	1 x Capsule Daily	*L. rhamnosus*	1×10^{9}	Capsules: corn starch, magnesium stearate, silicon dioxide	4 weeks	**+0.54**
Marotta et al. (2019a - b) [61], Italy	PLA: 15 (10M) PRO: 18 (11M)	21.7 ± 2.2 21.6 ± 2.2	NS	Healthy participants	BSD / DB	1 x Sachet Daily	*L. fermentum, L. rhamnosus, L. plantarum, B. longum*	4×10^{9}	Sachets: maltodextrin	a: 3 weeks b: 6 weeks	**+0.14** **+1.27**
Nakagawa et al. (2018) [59], Japan	21 (11M)	53.6 ± 6.9	NS	Healthy participants who report problems with everyday sleep	WSD / DB	8 x Tablets Daily	*L. helveticus (+ theanine)*[c]	NS	Tablets: maltitol, lactose, calcium stearate, lactic acid, flavour, yeast extract	4 weeks	0.00
Nishida, Sawada, Kawai et al. (2017) [66], Japan	PLA: 16 (10M) PRO: 16 (11M)	21.3 ± 3.6 20.8 ± 1.6	NS	Healthy 2nd year undergraduate students	BSD / DB	200 ml Fermented Milk Daily	*heat-inactivated L. gasseri*[d]	1×10^{10}	Milk: similar constituents	5 weeks	**+1.76**
Nishida, Sawada, Kuwano et al. (2017a – b) [50], Japan	PLA: 35 (21M) PRO: 34 (19M)	25.1 ± 3.0 24.9 ± 1.8	20.8 ± 5.3 21.1 ± 2.3	Healthy 6th year medical students undergoing national examination for medical practitioners	BSD / DB	200 ml Fermented Milk Daily	*heat-inactivated L. gasseri*[d]	1×10^{10}	Milk: similar constituents	a: 6 weeks b: 12 weeks	**NA**[b] **NA**[b]
Sawada et al. (2017) [69], Japan	24M	NS	NS	Healthy male undergraduate students	WSD / DB	1 x Sachet Daily	*L. gasseri*	1×10^{10}	Powder: skim milk (20%), yeast extract (0.50%)	4 weeks	**+1.69**
Wong et al. (2015) [43], Singapore	PLA: 22 (11M) PRO: 20 (12M)	40.9 ± 16.5 53.4 ± 18.6	NS	Patients with IBS and sleep complaints	BSD / DB	4 x Capsules Daily	*L. casei, L. plantarum, L. acidophilus, L. delbrueckii bulgaricus, B. longum, B. breve, B. infantis, Streptococcus thermophilus*	1.125×10^{11}	Capsules: NFS	6 weeks	**+0.44**

Δ PSQI: Mean difference between treatments in global PSQI change from baseline score (note: more +ve values indicate greater efficacy of intervention treatment); PLA: Placebo treatment group; PRO: Probiotics treatment group; DB: Double-blinded; BSD: Between-subjects design; WSD: Within-subjects design; NS: Not specified; NA: Data not available; M: Male; Shaded trials were excluded from the meta-analysis. [a] SD calculated from 95%CI and *t*-distribution, age of participants is for entire group (n=64 in PLA and n=66 in PRO groups respectively) that commenced the study prior to attrition. [b] Baseline and change from baseline data were not provided in the paper and could not be obtained on contact with the corresponding author. Data available in the paper was limited to post treatment (placebo and probiotics) values only (mean, SD, sample size for each group), which were used to input data for meta-analysis. [c] Trial not included in meta-analysis because probiotic treatment was provided with other constituents (i.e. theanine) that were not matched in placebo condition. [d] Paraprobiotics treatment.

Table 2. Characteristics of studies that investigated the effect of probiotics/paraprobiotics on other subjective sleep parameters

Citation	Participants (n)	Age (y)	BMI	Participants Health Status	Study Design	Intervention Treatment	Probiotic Bacteria	CFU	Placebo Treatment	Duration	Subjective Scales	Δ Score
Dhiman et al. (2014) [63], India	PLA: 11 PRO: 16	50.1 ± 9.8[a] 48 ± 11.4[a]	NS	Cirrhosis patients who had recovered from an episode of HE	BSD / DB	1 x VSL#3 Sachet Daily	*L. casei, L. plantarum, L. acidophilus, L. delbrueckii bulgaricus, B. longum, B. breve, B. infantis, Streptococcus thermophilus*	9×10^{11}	Sachets: corn flour	24 weeks	**ESS**	**PLA: -0.87 ± 2.82** **PRO: 0.62 ± 3.07**
Diop et al. (2008) [64], France	PLA: 33 PRO: 31	38 ± 11[b]	NS	Healthy volunteers with symptoms of stress	BSD / DB	1 x Probio-Stick Daily	*L. acidophilus, B. longum*	3×10^{9}	Powder: NS	3 weeks	**VAS – adverse sleeping symptoms**	**PLA: -9.45 ± 2.37** **PRO: -13.46 ± 2.66**
Majeed et al. (2018) [65], India	PLA: 20 (3M) PRO: 20 (3M)	43.9 ± 9.9 40.4 ± 10.3	25.9 ± 4.5 25.4 ± 4.5	Patients with IBS and depression	BSD / DB	100 ml Yoghurt Daily	*L. bulgaricus, Streptococcus thermophilus*	2×10^{9}	Yoghurt: acidified milk (milk, skimmed milk, HFCS, sugar, pectin)	90 days	**mESS**	**PLA: -2.00 ± 6.29** **PRO: -6.10 ± 7.03**
Manoi et al. (2016) [68], Japan	60 (31M)	37.7 ± 9.1	NS	Healthy participants with poor sleep quality	WSD / DB	1 x Tablet Daily	*Sake yeast powder (Saccharomyces cerevisiae)*	NS	Tablets: crystalline cellulose, cyclodextrin, calcium CMC, HPMC, silicon dioxide, calcium stearate, glycerol, Sanfixbutter No. 24644, tartrazine	4 days	OSA – sleepiness on awakening	NA[d]
											OSA - onset and maintenance of sleep	NA[d]
											OSA - dreaming	NA[d]
											OSA - recovery from fatigue	NA[d]
											OSA - sleep duration	NA[d]
Nakagawa et al. (2018) [59], Japan	21 (11M)	53.6 ± 6.9	NS	Healthy participants who report problems with everyday sleep	WSD / DB	8 x Tablets Daily	*L. helveticus (+ theanine)*[e]	NS	Tablets: maltitol, lactose, calcium stearate, lactic acid, flavour, yeast extract	4 weeks	OSA – sleepiness on awakening	PLA: 1.70 ± 7.70 PRO: 3.80 ± 8.10
											OSA - onset and maintenance of sleep	PLA: 3.00 ± 10.30 PRO: 5.30 ± 7.40
											OSA - dreaming	PLA: 2.60 ± 8.70 PRO: 4.60 ± 8.50
											OSA - recovery from fatigue	PLA: 3.50 ± 6.10 PRO: 5.80 ± 6.40
											OSA - sleep duration	PLA: -1.00 ± 9.80 PRO: -0.40 ± 9.80
											VAS – awakening	PLA: -1.12 ± 1.54 PRO: -2.08 ± 2.10
											VAS onset of sleep	PLA: -0.88 ± 2.76 PRO: -1.07 ± 2.16
											VAS – sleep duration	PLA: -1.29 ± 2.39 PRO: -1.17 ± 2.20
Nakakita et al. (2016) [62], Japan	14M	54.2 ± 8.5[c]	NS	Healthy males who suffered sleep challenges	WSD / DB	1 x Capsule Daily	*heat-killed L. brevis*[f]	NS	Capsules: caramel pigment, powdered silica, calcium stearate, starch, cellulose	10 days	Sleep journal - quality of sleep	NA[d]
											Sleep journal - feeling of awakened during night	NA[d]
											Sleep journal - waking	NA[d]
											Sleep journal - feeling of soundness of sleep	NA[d]
											Sleep journal - sleep period time	NA[d]
											Sleep journal - drowsiness during following day	NA[d]
Takada et al. (2017a – d) [55], Japan	PLA: 46 (28M) PRO: 48 (27M)	22.6 ± 1.4 22.8 ± 1.4	20.5 ± 2.0 20.7 ± 2.1	Healthy 4th year medical students undergoing promotion examination	BSD / DB	100 ml Fermented Milk Daily	*L. casei shirota*	1×10^{9} / ml	Milk: similar constituents	a: 6 weeks	**OSA – global score**	**PLA: -5.06 ± 18.88** **PRO: -2.89 ± 17.29**
											OSA – sleepiness on awakening	PLA: -1.08 ± 4.20 PRO: 0.00 ± 6.01

Citation	Participants (n)	Age (y)	BMI	Participants Health Status	Study Design	Intervention Treatment	Probiotic Bacteria	CFU	Placebo Treatment	Duration	Subjective Scales	Δ Score
											OSA - onset and maintenance of sleep	PLA: -1.49 ± 3.96 PRO: -0.97 ± 4.17
											OSA - dreaming	PLA: 0.35 ± 6.29 PRO: -0.16 ± 6.05
											OSA - recovery from fatigue	PLA: -1.60 ± 4.79 PRO: -2.01 ± 6.40
											OSA - sleep duration	PLA: -1.14 ± 5.69 PRO: 0.18 ± 5.81
										b: 8 weeks	**OSA – global score**	**PLA: -9.67 ± 19.66** **PRO: -11.93 ± 17.85**
											OSA – sleepiness on awakening	PLA: -2.51 ± 3.84 PRO: -1.98 ± 5.40
											OSA - onset and maintenance of sleep	PLA: -0.34 ± 4.80 PRO: -1.75 ± 4.90
											OSA - dreaming	PLA: -1.15 ± 4.81 PRO: -2.00 ± 7.18
											OSA - recovery from fatigue	PLA: -2.50 ± 4.91 PRO: -4.61 ± 7.03
											OSA - sleep duration	PLA: -3.02 ± 6.06 PRO: -1.16 ± 6.43
										c: 9 weeks	**OSA – global score**	**PLA: -1.08 ± 18.88** **PRO: 5.33 ± 19.16**
											OSA – sleepiness on awakening	PLA: -0.60 ± 4.20 PRO: 2.00 ± 4.66
											OSA - onset and maintenance of sleep	PLA: -0.12 ± 5.28 PRO: 0.55 ± 3.43
											OSA - dreaming	PLA: -1.60 ± 6.04 PRO: -1.15 ± 6.93
											OSA - recovery from fatigue	PLA: 0.20 ± 4.67 PRO: 1.57 ± 4.89
											OSA - sleep duration	PLA: 0.93 ± 5.81 PRO: 2.61 ± 5.94
										d: 11 weeks	**OSA – global score**	**PLA: 2.17 ± 18.51** **PRO: 1.36 ± 18.48**
											OSA – sleepiness on awakening	PLA: 0.37 ± 5.04 PRO: 1.72 ± 5.27
											OSA - onset and maintenance of sleep	PLA: 0.80 ± 4.68 PRO: -0.04 ± 3.68
											OSA - dreaming	PLA: -1.09 ± 5.55 PRO: -1.80 ± 5.79
											OSA - recovery from fatigue	PLA: 1.26 ± 5.65 PRO: 0.79 ± 5.65
											OSA - sleep duration	PLA: 0.64 ± 5.57 PRO: 1.48 ± 5.20
Wong et al. (2015) [43], Singapore	PLA: 22 (11M) PRO: 20 (12M)	40.9 ± 16.5 53.4 ± 18.6	NS	Patients with IBS and sleep complaints	BSD / DB	4 x Capsules Daily	*L. casei, L. plantarum, L. acidophilus, L. delbrueckii bulgaricus, B. longum, B. breve, B. infantis, Streptococcus thermophilus*	1.125×10^{11}	Capsules: NFS	6 weeks	**ESS**	**PLA: -0.69 ± 5.72** **PRO: 0.65 ± 3.67**

Δ Score: Mean±SD change from baseline ratings; PLA: Placebo treatment group; PRO: Probiotics treatment group; DB: Double-blinded; BSD: Between-subjects design; WSD: Within-subjects design; NS: Not specified; NA: Data not available; M: Male; Shaded trials were excluded from the meta-analysis; Bolded data were only included in meta-analysis; ESS: Epworth Sleepiness Scale; mESS: Modified Epworth Sleepiness Scale; VAS: Visual analog scales; OSA: Oguri-Shirakawa-Azumi sleep inventory (MA version). [a] SD calculated from 95%CI and *t*-distribution, age of participants is for entire group (n=64 in PLA and n=66 in PRO groups respectively) that commenced the study prior to attrition. [b] Age of participants is for entire group (n=75) that commenced the study prior to attrition. [c] Age of participants is for entire group (n=17) that commenced the study prior to attrition. [d] Baseline and change from baseline data were not provided in the paper and could not be obtained on contact with the corresponding author. Data available in the paper was limited to post treatment (placebo and probiotics) values only, but these could not be used for meta-analysis because a standardized method was employed. [e] Trial not included in meta-analysis because probiotic treatment was provided with other constituents (i.e. theanine) that were not matched in placebo condition. [f] Paraprobiotics treatment.

Table 3. Characteristics of studies that investigated the effect of probiotics/paraprobiotics on objective sleep parameters (EEG or Actigraphy)

Citation	Participants (n)	Age (y)	BMI	Participants Health Status	Study Design	Intervention Treatment	Probiotic Bacteria	CFU	Placebo Treatment	Duration	Objective Outcome Measure	Δ Parameter
Manoi et al. (2016) [68], Japan	60 (31M)	37.7 ± 9.1	NS	Healthy participants with poor sleep quality	WSD / DB	1 x Tablet Daily	*Sake yeast powder (Saccharomyces cerevisiae)*	NS	Tablets: crystalline cellulose, cyclodextrin, calcium CMC, HPMC, silicon dioxide, calcium stearate, glycerol, Sanfixbutter No. 24644, tartrazine	4 days	EEG - Delta power during first NREM (x 105 μV^2)	NA
											EEG - Sleep latency (min)	**NA[a]**
											EEG - Sleep efficiency (%)	**NA[a]**
											EEG - Total sleep time (min)	NA
											EEG - Light NREM sleep (min)	NA
											EEG - Deep NREM sleep (min)	NA
											EEG - REM sleep (min)	NA
											EEG – Awaking (min)	NA
Nakagawa et al. (2018) [59], Japan	21 (11M)	53.6 ± 6.9	NS	Healthy participants who report problems with everyday sleep	WSD / DB	8 x Tablets Daily	*L. helveticus (+ theanine)*[b]	NS	Tablets: maltitol, lactose, calcium stearate, lactic acid, flavour, yeast extract	4 weeks	EEG - Sleep period time (min)	PLA: −18.76 ± 77.23 PRO: 1.26 ± 71.11
											EEG - Total sleep time (min)	PLA: −21.26 ± 70.69 PRO: 7.71 ± 70.60
											EEG - Sleep efficiency (%)	PLA: −0.70 ± 5.69 PRO: 2.13 ± 4.34
											EEG - Wake time after sleep onset (min)	PLA: 3.17 ± 23.52 PRO: −4.00 ± 13.26
											EEG - Rate of wake time after sleep onset (%)	PLA: 0.82 ± 5.97 PRO: −1.31 ± 3.52
											EEG - Sleep latency (min)	PLA: −0.43 ± 20.48 PRO: 2.64 ± 20.67
											EEG - REM latency (min)	PLA: 11.02 ± 33.50 PRO: −6.57 ± 32.95
Nakakita et al. (2016) [62], Japan	14M	54.2 ± 8.5[c]	NS	Healthy males who suffered sleep challenges	WSD / DB	1 x Capsule Daily	*heat-killed L. brevis*[c]	NS	Capsules: caramel pigment, powdered silica, calcium stearate, starch, cellulose	10 days	EEG – Time in bed (min)	NA
											EEG - Sleep latency (min)	**NA[a]**
											EEG - Sleep period time (min)	NA
											EEG - Waking during sleep (%)	NA
											EEG - Deep sleep (%)	NA
											EEG - Delta power value (μV^2/min)	NA
Nishida, Sawada, Kuwano et al. (2017a – b) [50], Japan	PLA: 35 (21M) PRO: 34 (19M)	25.1 ± 3.0 24.9 ± 1.8	20.8 ± 5.3 21.1 ± 2.3	Healthy 6th year medical students undergoing national examination for medical practitioners	BSD / DB	200 ml Fermented Milk Daily	*heat-inactivated L. gasseri*[c]	1×10^{10}	Milk: similar constituents	a: 6 weeks	EEG - Sleep latency (min)	NA
											EEG - Delta power during first sleep cycle (μV^2/min)	NA
											EEG - ratio of appearance of stage N3 in NREM sleep (%)	NA
											EEG - Awakenings in 2h before getting up (#)	NA
										b: 12 weeks	EEG - Sleep latency (min)	NA

Citation	Participants (n)	Age (y)	BMI	Participants Health Status	Study Design	Intervention Treatment	Probiotic Bacteria	CFU	Placebo Treatment	Duration	Objective Outcome Measure	Δ Parameter
											EEG - Delta power during first sleep cycle (μV^2/min)	NA
											EEG - ratio of appearance of stage N3 in NREM sleep (%)	NA
											EEG - Awakenings in 2h before getting up (#)	NA
Takada et al. (2017a – d) [55], Japan	PLA: 46 (28M) PRO: 48 (27M)	22.6 ± 1.4 22.8 ± 1.4	20.5 ± 2.0 20.7 ± 2.1	Healthy 4th year medical students undergoing promotion examination	BSD / DB	100 ml Fermented Milk Daily	*L. casei shirota*	1×10^9 / ml	Milk: similar constituents	a: 6 weeks	**EEG - Sleep latency (min)**	**PLA: 7.64 ± 24.17 PRO: 5.4 ± 24.47**
											EEG - Total sleep time (min)	PLA: -11.39 ± 89.52 PRO: -1.87 ± 112.44
											EEG - Sleep efficiency (%)	**PLA: -0.73 ± 7.37 PRO: -1 ± 7.47**
											EEG - Wake after sleep onset (%)	PLA: -0.53 ± 2.83 PRO: -0.02 ± 3.20
											EEG - N3 sleep (%)	PLA: -0.85 ± 7.84 PRO: 1.32 ± 4.91
											EEG - Delta power during first sleep cycle (%)	PLA: 4.44 ± 36.44 PRO: 18.40 ± 59.45
										b: 8 weeks	**EEG - Sleep latency (min)**	**PLA: 2.4 ± 22.08 PRO: -1.61 ± 21.49**
											EEG - Total sleep time (min)	PLA: -2.29 ± 92.46 PRO: 20.74 ± 104.19
											EEG - Sleep efficiency (%)	**PLA: -0.61 ± 7.42 PRO: -0.08 ± 6.09**
											EEG - Wake after sleep onset (%)	PLA: -5.98 ± 2.34 PRO: 0.57 ± 3.86
											EEG - N3 sleep (%)	PLA: -2.65 ± 5.62 PRO: 0.47 ± 5.29
											EEG - Delta power during first sleep cycle (%)	PLA: 0.05 ± 34.54 PRO: 12.74 ± 72.99
										c: 9 weeks	**EEG - Sleep latency (min)**	**PLA: 2.40 ± 5.20 PRO: 2.21 ± 6.46**
											EEG - Total sleep time (min)	PLA: -2.29 ± 9.92 PRO: -2.42 ± 13.24
											EEG - Sleep efficiency (%)	**PLA: -0.61 ± 1.03 PRO: -1.14 ± 14.62**
											EEG - Wake after sleep onset (%)	PLA: -5.98 ± 19.59 PRO: -6.97 ± 36.56
											EEG - N3 sleep (%)	PLA: -2.65 ± 5.62 PRO: 0.47 ± 5.29
											EEG - Delta power during first sleep cycle (%)	PLA: 0.05 ± 34.54 PRO: 12.74 ± 72.99
										d: 11 weeks	**EEG - Sleep latency (min)**	**PLA: 2.40 ± 5.20 PRO: 2.21 ± 6.46**
											EEG - Total sleep time (min)	PLA: -2.29 ± 9.92 PRO: -2.42 ± 13.24
											EEG - Sleep efficiency (%)	**PLA: -0.61 ± 1.03 PRO: -1.14 ± 14.62**
											EEG - Wake after sleep onset (%)	PLA: -5.98 ± 19.59 PRO: -6.97 ± 36.56

Citation	Participants (n)	Age (y)	BMI	Participants Health Status	Study Design	Intervention Treatment	Probiotic Bacteria	CFU	Placebo Treatment	Duration	Objective Outcome Measure	Δ Parameter
											EEG - N3 sleep (%)	PLA: -0.67 ± 6.73 PRO: 0.35 ± 4.83
											EEG - Delta power during first sleep cycle (%)	PLA: 0.26 ± 30.28 PRO: 6.19 ± 51.72
Yamamura et al. (2009) [70], Japan	29 (23M)	71.4 ± 5.7	NS	Healthy elderly subjects	WSD / DB	100 g Fermented Milk Daily	*L. helveticus*	NS	Artificially acidified milk	3 weeks	**Actigraphy - sleep efficacy (%)**	**PLA: -0.61 ± 1.03** **PRO: -1.14 ± 14.62**
											Actigraphy - sleep latency (min)	**PLA: -5.98 ± 19.59** **PRO: -6.97 ± 36.56**
											Actigraphy - wake episodes (#)	PLA: -0.61 ± 1.03 PRO: -1.14 ± 14.62
											Actigraphy - wake after sleep onset (min)	PLA: -5.98 ± 19.59 PRO: -6.97 ± 36.56

Δ Parameter: Mean difference between treatments in outcome change from baseline score; PLA: Placebo treatment group; PRO: Probiotics treatment group; DB: Double-blinded; BSD: Between-subjects design; WSD: Within-subjects design; NS: Not specified; NA: Data not available; M: Male; Shaded trials were excluded from the meta-analysis; Bolded data were only included in meta-analysis. [a] Baseline and change from baseline data were not provided in the paper and could not be obtained on contact with the corresponding author. Data available in the paper was limited to post treatment (placebo and probiotics) values only (mean, sample size, *p*-value for comparison between conditions), which were used to input data for meta-analysis. [b] Trial not included in meta-analysis because probiotic treatment was provided with other constituents (i.e. theanine) that were not matched in placebo condition. [c] Paraprobiotics treatment.