Title: The human gut microbiome and sleep across adulthood: associations and therapeutic potential.

Authors: Jacob Cavon[1,2,#], Melissa Basso[3,4,#], Kathrin Cohen Kadosh[3,*], Sean M. Gibbons[1,2,5-7*]

Affiliations: [1] Institute for Systems Biology, Seattle, WA 98109, USA; [2] Molecular Engineering Graduate Program, University of Washington, Seattle, WA 98195, USA; [3] School of Psychology, Faculty of Health and Medical Sciences, University of Surrey, Guildford GU2 7HX, United Kingdom; [4] School of Biosciences and Medicine, Faculty of Health and Medical Sciences, University of Surrey, Guildford, Surrey GU2 7XH, UK, [5] Department of Bioengineering, University of Washington, Seattle, WA 98195, USA; [6] Department of Genome Sciences, University of Washington, Seattle, WA 98195, USA; [7] eScience Institute, University of Washington, Seattle, WA 98195, USA; [#] equal contributions; [*] correspondence can be addressed to k.cohenkadosh@surrey.ac.uk and sgibbons@isbscience.org

Abstract

Sleep is an essential homeostatic process that undergoes dynamic changes throughout the lifespan, with distinct life stages predisposed to specific sleep pathologies. Similarly, the gut microbiome also varies with age, with different signatures associated with health and disease in the latest decades of life. Emerging research has shown significant cross-talk between the gut microbiota and the brain through several pathways, suggesting the microbiota may influence sleep, though the specific mechanisms remain to be elucidated. Here, we critically examine the existing literature on the potential impacts of the gut microbiome on sleep and how this relationship varies across adulthood. We suggest that age-related shifts in gut microbiome composition and immune function may, in part, drive age-related changes in sleep. We conclude with an outlook on the therapeutic potential of microbiome-targeted interventions aimed at improving sleep across adulthood, particularly for individuals experiencing high stress or with sleep complaints.

Introduction

Sleep is an essential biological process encompassing behavioral, experiential, and physiological dimensions (Andrillon & Oudiette, 2023). Disrupted sleep results in adverse health outcomes (Nayak & Anilkumar, 2024; Patel *et al.*, 2024), and prolonged sleep deprivation leads to death (Vaccaro *et al.*, 2020). Both external and internal factors, including psychological stress, influence sleep, with emerging research showing a connection between the gut microbiome and sleep (Matenchuk, Mandhane & Kozyrskyj, 2020; Han, Yuan & Zhang, 2022; Wang *et al.*, 2022; Dos Santos & Galiè, 2024). Importantly, improving sleep quality across the lifespan offers significant health benefits and reduces mortality rates (Ramar *et al.*, 2021).

Sleep and the gut microbiome are strongly linked in the first few months of life, with gut microbiome profiles and sleep/wake patterns predicting behavioral development (Schoch *et al.*, 2022). Indeed, evidence suggests that optimizing sleep via the gut, starting in early life and extending into adulthood, may provide long-lasting benefits (Schoch *et al.*, 2022). For example, targeting the gut microbiome with pre- and probiotic interventions appears to be effective in supporting sleep in adults (Nishida *et al.*, 2019; Fei *et al.*, 2023; Santamarina *et al.*, 2024). Given the lifelong interplay of lifestyle, stress, environmental, and physiological factors that can affect sleep (Li, Vitiello & Gooneratne, 2018; Yang *et al.*, 2018; Silva-Costa *et al.*, 2021), understanding how sleep can be improved throughout the lifespan remains crucial. In the present review, we first provide a brief overview of how sleep, the gut microbiome, and the immune system change across adulthood, highlighting key future research directions that could further our mechanistic understanding of the gut-sleep relationship. Although the microbiome-sleep relationship appears to be bidirectional (Sen *et al.*, 2021), we focus primarily on how the gut microbiome influences sleep, rather than vice versa, with the aim of advancing microbiome-based therapies to improve sleep. We discuss observational studies examining the gut microbiome and sleep of young, middle-aged, and older adults, and expand on this discussion

with intervention studies showing the potential of pre- and probiotic interventions in supporting sleep quality.

Quality sleep is characterized by sufficient duration, lack of disruption, and absence of sleep disorder (Ramar *et al.*, 2021), and faces different threats throughout adulthood. Early adulthood (late teens to mid-forties) is marked by substantial developmental milestones including the establishment of autonomy, career paths, and intimate relationships, all of which shape evolving life structure (Yahya Aktu, 2017). However, the competing demands of work and family roles can disrupt sleep patterns. Gao et al. reported that 85% of 534 surveyed students slept less than seven hours per night, indicating a substantial burden of suboptimal sleep during early adulthood (Gao *et al.*, 2022). Work-family conflicts are associated with sleep complaints (Silva-Costa *et al.*, 2021), and, along with high job-stress, increased insomnia risk (Yang *et al.*, 2018). Stress disrupts normal sleep, with the extent of disruption varying based on individual sleep reactivity to stress (Kalmbach, Anderson & Drake, 2018). Interestingly, 72.85% of Gao et al. participants reported frequent insomnia alongside gastrointestinal symptoms, and consistent sleep schedules appear a critical predictor of gut microbiome health (Kado, 2024). In older adults, polypharmacy and multimorbidity increase the prevalence of sleep disorders (Li *et al.*, 2018; Kim, Elkhadem & Duffy, 2022). Additionally, the sleep of older adults tends to be shorter and more easily disrupted compared to younger adults (Ohayon *et al.*, 2004), with the timing of these sleep changes coinciding with age-related shifts in gut microbiome composition (Ghosh, Shanahan & O'Toole, 2022a).

Unlike the genome, the microbiome's composition and function can be modulated through dietary, prebiotic, and probiotic interventions (Rothschild *et al.*, 2018; Hitch *et al.*, 2022; Ross *et al.*, 2024). Although our understanding of the gut microbiome's functional outputs is still emerging, it is increasingly clear that these microbial products are critical for human health (Postler & Ghosh, 2017; Krautkramer, Fan & Bäckhed, 2021; Zheng, Cai & Hao, 2022; Zhang *et al.*, 2023). The human gut microbiome produces thousands of bioactive compounds, like

neurotransmitters, organic acids, lipid vesicles, and proteins, that influence the host immune system, interact with host receptors, and circulate throughout the body (Postler & Ghosh, 2017; Cryan *et al.*, 2019). One study found that the human gut microbiome significantly contributes to cross-sectional variation in 44% of measured blood metabolites, independently of host genetics (Diener *et al.*, 2022). Microbial metabolism of amino acids (e.g., tryptophan), has been linked to numerous central nervous system (CNS) functions, including sleep (Gao *et al.*, 2020). Indeed, research on the gut-brain-axis has revealed multiple communication routes between microbial outputs and the CNS, including vagal afferents, immune responses, and the hypothalamic-pituitary-adrenal (HPA) axis, giving rise to the concept of “psychobiotics,” or pre/probiotic interventions supporting brain health (Cryan *et al.*, 2019). These findings suggest that the gut microbiome may influence sleep through a variety of biological pathways, making it a promising target for interventions that improve sleep quality.

Quantifying Sleep

Several tools are used to quantify physiological and neurological parameters of sleep. Physiologically, sleep is divided into different stages - rapid eye movement (REM) and non-REM (NREM) stages 1, 2, and 3 - each characterized by specific brain waveforms recorded by scalp **electroencephalogram (EEG)**. Changes in muscle tone, eye movement, heart and breathing rates, and blood oxygen levels also characterize these sleep stages, and can be monitored simultaneously alongside EEG with polysomnography (PSG) (Andrillon & Oudiette, 2023; Nayak & Anilkumar, 2024; Patel *et al.*, 2024). Sleep cycles through the different sleep stages over the course of a night, and this pattern of sleep stages is known as sleep architecture (Nayak & Anilkumar, 2024), where alterations are associated with a variety of health and disease indicators (Ujma & Bódizs, 2024). Delta power, a measure of delta wave activity derived from EEGs using Fourier analysis, serves as an indicator of homeostatic sleep drive, and it strongly correlates with NREM sleep intensity and duration (Davis *et al.*, 2011).

Actigraphs - wrist-worn devices monitoring movement - are another objective sleep assessment tool. These devices estimate total sleep time, number of wake events, wake after sleep onset (WASO), sleep efficiency (the ratio of total sleep time to time spent in bed), and sleep latency (Martin & Hakim, 2011). Actigraphy offers a key advantage over gold-standard EEG or PSG by enabling continuous at-home sleep assessment (Martin & Hakim, 2011). Limitations include overestimation of total sleep time due to reliance on movement, and less accuracy in estimating sleep latency than EEG, particularly in individuals with sleep disorders (Martin & Hakim, 2011). Compared to PSG, clinical-grade actiwatches overestimate total sleep time and sleep efficiency for shorter nights, while underestimating sleep latency and WASO, particularly when these measures are large (Chinoy *et al.*, 2021).

Beyond objective measurements, validated questionnaires like the **Pittsburgh Sleep Quality Index (PSQI)** and **Epworth Sleepiness Scale (ESS)** are widely used to assess perceived sleep quality and daytime sleepiness, respectively, in both clinical and research settings (Fabbri *et al.*, 2021). These questionnaires measure sleep parameters distinct from each other (Mondal *et al.*, 2013) and from those measured by objective tools (Buysse *et al.*, 2008), highlighting the importance of utilizing both tools for a comprehensive assessment (Fabbri *et al.*, 2021).

Sleep, the gut microbiome, and the immune system across the lifespan

The gut microbiome is in a dynamic, reciprocal relationship with the host immune system, starting from birth (Fig. 1) (Postler & Ghosh, 2017; Wernroth *et al.*, 2022). Early gut microbial colonization is essential for normal immune development, influenced by factors such as delivery mode, birth weight, and antibiotics (Wernroth *et al.*, 2022). Colonization disruptions before 2-3 years of age have been linked to an increased risk of chronic immune diseases later in life (Wernroth *et al.*, 2022). Similarly, early life sets the stage for future sleep quality, with one study showing that sleep disturbances at 16-years-old predicted sleep disturbances as late as 42-

years-old, independently from depression (Dregan & Armstrong, 2010). Further, the gut microbiomes of children with sleep-disordered breathing show a pro-inflammatory signature (Collado *et al.*, 2019; Valentini *et al.*, 2020). Together, these findings point to early life as a critical window that shapes future sleep, marked by the concurrent maturation of the gut microbiome, immune system, and sleep.

The gut-immune-sleep connection remains relevant beyond early life. In young to middle-aged adults (18-45), psychological stress strongly contributes to sleep disruptions (Kalmbach *et al.*, 2018), and insomnia is frequently reported alongside gastrointestinal symptoms (Gao *et al.*, 2022). Psychological stress increases stress-related hormones hyperactivating the HPA axis, and triggering a systemic cascade of pro-inflammatory cytokines like TNF-α and IL-6. This inflammatory cascade downregulates tight junctions in the gut lining and blood-brain barrier, and facilitates gut dysbiosis and LPS translocation (Liu, Wang & Jiang, 2017b; Geng *et al.*, 2020). These changes can disrupt sleep by amplifying inflammation and stress responses in a feedback loop (Fig. 1). For instance, LPS can elevate TNF-α by binding to Toll-like receptor 4 on macrophages, which further stimulates the release of corticotropin-releasing factor in the hypothalamus where sleep-regulatory circuits reside (Liu *et al.*, 2017b; Geng *et al.*, 2020). Microbial metabolites such as short-chain fatty acids (SCFAs) also play a critical role in balancing pro- and anti-inflammatory cytokines (Vinolo *et al.*, 2011). In turn, the immune system can shape the gut microbiome through inflammation, lumenal redox disruption, secreted antibodies, and antimicrobial peptide production (Bosco & Noti, 2021).

Dysregulation of the host immune system can occur in otherwise healthy older adults (Franceschi *et al.*, 2018; Nagpal *et al.*, 2018). So-called “inflammaging” is a chronic, low-grade inflammatory state marked by age-associated increases in systemic levels of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6 (Franceschi *et al.*, 2018; Nagpal *et al.*, 2018). Coinciding with the timing of the onset of inflammaging signatures, the gut microbiome shifts, as early as 40-50-years-old, depending on the host’s health (Fig. 1) (Bosco & Noti, 2021;

Wilmanski, Gibbons & Price, 2022). Changes to sleep architecture are also occurring through this period and do not stabilize until around 60-years-old (Ohayon *et al.*, 2004). Age-associated changes in the gut microbiome can be partitioned into healthy versus unhealthy, disease-associated aging trajectories (Fig. 1). On one hand, healthy gut microbiome aging signatures are associated with disease-independent health metrics, and include increasing beta-diversity uniqueness (a measurement of inter-individual microbiome difference) (Wilmanski *et al.*, 2021; Ghosh, Shanahan & O'Toole, 2022b), and a decline in core gram-negative anaerobes, like *Bacteroides* and *Prevotella* (Wilmanski *et al.*, 2021). On the other hand, signatures of unhealthy gut microbiome aging are broadly characterized by increased abundances of pathobionts and a maintenance of certain core taxa that tend to dominate in younger individuals (Biagi *et al.*, 2016; Wilmanski *et al.*, 2021; Ghosh *et al.*, 2022a). Unhealthy gut microbiome aging may contribute to inflammaging (Bosco & Noti, 2021), and although this relationship is not clear, work in animal models has shown that gut dysbiosis induces inflammaging signatures (Clark *et al.*, 2015; Thevaranjan *et al.*, 2017).

Interestingly, many cytokines play important roles in sleep regulation (Krueger, 2008). In particular, nearly three decades of preclinical research establishes TNF-α as a sleep-relevant molecule that acts directly on sleep regulatory circuits in the brain (Rockstrom *et al.*, 2018). Human studies mirror preclinical findings on the role of TNF-α in sleep regulation. For example, in HIV+ men, who frequently present with fragmented sleep and fatigue, nocturnal serum TNF-α levels positively correlate with delta power, and this correlation becomes weaker with infection progression (Darko *et al.*, 1995). Further, TNF-α inhibitor treatment in obstructive sleep apnea (OSA) patients greatly reduced excessive daytime sleepiness (Vgontzas *et al.*, 2004), and treating rheumatoid arthritis patients with recombinant TNFR improved their percentage of N2 sleep, total sleep time, and sleep efficiency (Detert *et al.*, 2016).

Although more work is needed to characterize chronically elevated levels of systemic TNF-α on sleep (Rockstrom *et al.*, 2018), several studies show that systemic TNF-α can affect

sleep. TNF-α can cross the blood-brain barrier in rodents (Gutierrez, Banks & Kastin, 1993), and systemic administration of TNF-α induces brain TNF-α expression that disrupts sleep (Kubota *et al.*, 2001; Zielinski *et al.*, 2013). These effects are reduced by subdiaphragmatic vagotomy, highlighting the role of vagal afferents (Kubota *et al.*, 2001; Zielinski *et al.*, 2013). Importantly, the gut microbiome can influence systemic cytokine levels, such as TNF-α (Mossad & Blank, 2021), and impact microglia activity in the brain, although the detailed mechanisms remain unclear (Wang *et al.*, 2018). Additionally, gut microbes can produce neurotransmitters, such as serotonin, GABA, dopamine, and histamine, which are implicated in sleep regulation, though how these molecules interact with the CNS from the gut to influence sleep remains poorly understood (Wang *et al.*, 2022; Lin *et al.*, 2024). As reviewed in Lin et al. and Z. Wang et al., gut serotonin may signal to the brain via serotonergic vagal afferents, but how this signaling connects to sleep-regulatory circuits is unclear. Similarly, GABA-producing microbes, like *Lactobacilli*, have shown promise in improving sleep quality, yet the mechanisms underpinning these effects remain elusive. Microbially-produced dopamine and histamine may influence sleep by interacting with sleep-regulatory circuits, potentially through systemic circulation to the brain via the hepatoportal system, highlighting the need for targeted mechanistic research to clarify these pathways (Wang *et al.*, 2022; Lin *et al.*, 2024).

Gut-Sleep Observational Studies Across Adulthood

Observational studies in young to middle-aged adults (18-45) report alterations to the gut microbiome associated with different sleep parameters. For example, one study showed that insomniac adults had decreased F/B ratio (ratio of the relative abundances of the Firmicutes and Bacteroidetes phyla), alpha-diversity (measures of intra-individual microbiome diversity), and beta-diversity (measures of inter-individual differences in microbiome composition) compared to healthy adults (Liu et al. 2019). Similarly, poor sleep quality (PSQI) has been associated with a lower F/B ratio (Grosicki *et al.*, 2020), and lower F/B ratios have been linked

to intestinal inflammation (Stojanov, Berlec & Štrukelj, 2020). An actigraphy study found that sleep efficiency, fewer WASO events, and total sleep time were positively associated with alpha-diversity (Smith *et al.*, 2019). Smith et al. found that time in bed and total sleep time were positively associated with IL-6, and that IL-6 was positively associated with alpha-diversity. Consistent with gut-immune-sleep interplay, both Smith et al. and Grosicki et al. found that specific Lachnospiraceae genera, including *Blautia, Oribacterium,* and *Ruminococcus*, were associated with sleep quality, though the directionality of these associations varies across studies (Table 1) (Smith *et al.*, 2019; Grosicki *et al.*, 2020). Notably, Lachnospiraceae contains major butyrate-producers (Singh *et al.*, 2023), and butyrate has anti-inflammatory effects (Siddiqui & Cresci, 2021a). Although most butyrate is metabolized in the colon, some can enter systemic circulation through the hepatoportal vein where it can then cross the blood-brain barrier (Siddiqui & Cresci, 2021a). Butyrate can directly inhibit histone deacetylases and downregulate NF-κB signaling in various cell types, including intestinal and immune cells, which allows it to modulate production of pro-inflammatory cytokines, such as TNF-α (Siddiqui & Cresci, 2021a). Furthermore, butyrate can activate G-protein coupled receptors (GPCRs) including GPR41, GPR43, and GPR109A (Siddiqui & Cresci, 2021a). These GPCRs are distributed across several cell types and tissues where they regulate immune responses and maintain intestinal homeostasis (Siddiqui & Cresci, 2021a).

Levels of butyrate may be influenced by sleep (e.g. through temporally aberrant production due to eating during times when humans normally sleep, like in the case of shift workers), and butyrate itself may directly influence sleep. For instance, in healthy students, sleep deprivation (SD) and sleep restriction (SR) disrupted butyrate-producing bacteria, including taxa within the Lachnospiraceae family and the *Faecalibacterium* genus, reducing butyrate levels by 53.1% in SD and 30.7% in SR (Gao *et al.*, 2022). Interestingly, SD mice that were fed butyrate were protected from adverse gut microbiome and intestinal changes (Gao *et al.*, 2022). When butyrate was administered to healthy mice, NREM sleep increased by 50%

and 70%, respectively, depending on oral versus intraportal administration (Szentirmai *et al.*, 2019). One study in humans reported that sodium-butyrate supplementation improved PSQI scores, decreased inflammatory biomarkers, and upregulated circadian-clock genes in patients with ulcerative colitis (Firoozi *et al.*, 2024). These studies suggest that while SD and SR can adversely alter the gut microbiome, butyrate supplementation can protect against these changes and promote better sleep, possibly by modulating TNF-α signalling in the brain via entering systemic circulation through the hepatoportal system. Further research is needed to elucidate the specific mechanisms involved.

Observational studies in older adults (45+) with OSA highlight specific gut bacterial taxa with different age-dependent effects. These studies offer insights into the interplay between the gut microbiome, immune system, and sleep, given that OSA has immunological components (Nadeem *et al.*, 2013). One study found that OSA was associated with several taxa including depletion of *Alistipes* and *Ruminococcus gnavus*, along with enrichment of *Coprococcus* (Lu *et al.*, 2022). Similarly, Baldanzi et al. showed that Bacteroidales (including the genus *Alistipes*) negatively correlated with two OSA parameters: the percentage of time asleep with oxygen saturation below 90% (T90) and the number of times per hour that oxygen saturation falls at least 4% below baseline (ODI). In contrast, *Coprococcus comes*, *R. gnavus*, and *Collinsella aerofaciens* positively correlated with both T90 and ODI (Baldanzi *et al.*, 2023). *Coprococcus* is a core butyrate-producing genus, typically associated with health in youth, and is depleted with age (Ghosh *et al.*, 2022a). Interestingly, the decline of *Coprococcus* in older healthy adults is typically less severe than in those with health issues (Ghosh *et al.*, 2022a), indicating that there might be an optimal level of *Coprococcus* for healthy aging. Alternatively, the enrichment of *Coprococcus* observed in the OSA studies (Lu *et al.*, 2022; Baldanzi *et al.*, 2023) may result from the sleep disorder itself, rather than underpinning its etiology. More work is needed to understand the roles *Coprococcus* plays in host sleep quality with age. Similarly, the role of *R. gnavus* in host physiology is complex, as shown by its conflicting associations with OSA (Table

1) (Lu *et al.*, 2022; Baldanzi *et al.*, 2023), and links to both anti- and pro-inflammatory effects (Crost *et al.*, 2023). In the context of Crohn's disease, *R. gnavus* produces a pro-inflammatory polysaccharide that induces TNF-α expression in dendritic cells (Henke *et al.*, 2019). In contrast, another study found that *R. gnavus* was positively associated with longevity, suggesting it may have beneficial health effects (Wang *et al.*, 2019). The odds ratio increased significantly after controlling for smoking, BMI, food preference, and alcohol consumption, which suggest that these lifestyle factors may dampen the effect of *R. gnavus* on longevity (Wang *et al.*, 2019). Further studies that consider *R. gnavus* within the broader context of the gut microbiome ecology, host physiology, and environmental factors will need to be conducted to understand its contradictory roles.

The presence/absence of bacterial taxa and their directionality of associations with sleep are inconsistent across studies. For instance, the directionality of associations of Lachnospiraceae genera are opposite between the Smith et al. and Grosicki et al. studies, and there are differences in taxa across the OSA studies (Table 1). These discrepancies may reflect distinct pathological mechanisms or symptoms of different sleep conditions (i.e. insomnia vs. OSA), or may be due to differences in study design (i.e. cross-sectional vs. case-control), cohort size (e.g. Baldanzi et al. study had N=4045, whereas other studies had around N=50 or fewer), demographics (e.g. Smith et al. focused exclusively on younger male adults), health status (e.g. Lu et al. looked at hypertensive patients with OSA), taxonomic resolution, or stool sample collection and analysis protocols. Furthermore, different sleep outcome measurements (such as the use of PSQI vs. actigraphy and differing definitions of OSA), different covariate adjustments, and an lack of multiple test corrections in most studies might contribute to the heterogeneity of findings. Nonetheless, these studies highlight significant associations between the gut microbiome, immune system, and sleep across adulthood, and underscore butyrate and specific bacterial taxa as promising targets for gut-based interventions to improve sleep, warranting further investigation (Fig. 1).

Probiotic effects on sleep in young and middle-aged adults (18-45)

Several studies have used EEG alongside stress and subjective sleep measurements to specifically examine the effects of *Lactobacillus* probiotics on sleep in young to middle-aged adults (18-45 years old). For example, a 4-week administration of *Lactobacillus gasseri* CP2305 prevented sleep disturbances in medical students during exams, reduced afternoon cortisol levels, inhibited *Enterobacteriaceae*, and showed trends toward *Veillonella* suppression and increased *Lactobacillus* abundance (Sawada *et al.*, 2017). Better PSQI scores were also found following a 24-week intervention with heat-inactivated *L. gasseri* CP2305, along with less stress-induced reductions in *Bifidobacterium* and an attenuated rise in *Streptococcus*. The probiotic also increased EEG delta power ratio (the delta power of the first sleep cycle to the delta power of the total sleep period) and shortened sleep latency of the first N3 stage and WASO, with no effects on total REM and NREM sleep times (Nishida *et al.*, 2019). Similar findings were reported following an 11-week intervention with *L. casei* strain Shirota YIT 9029 in students under stress, where the probiotic significantly suppressed sleep latency and N3 sleep reduction, increased delta power by 20%, but did not affect EEG-sleep total time and efficiency. The probiotic improved self-reported “sleepiness on rising” and “sleep length” factors of the Oguri-Shirakawa-Azumi sleep quality assessment (Takada *et al.*, 2017). These studies show that *Lactobacillus* probiotics may improve sleep in young to middle-aged adults by attenuating stress-induced sleep disruptions, possibly by reducing stress-induced perturbations of the gut microbiome, and by increasing NREM sleep, without a significant impact on REM sleep.

Pre- and probiotic effects on sleep in older adults (45+)

In a recent study, overweight, older adult volunteers took a nutraceutical blend with or without milk thistle extract (Santamarina *et al.*, 2024). Depletion of *Bacteroides* and enrichment of *Collinsella*, *Lachnospiraceae Ruminococcus*, *Faecalibacterium*, and *Alistipes onderdonkii* were

314 associated with improvement in daytime sleepiness (ESS). Additionally, both groups had less
315 inflammatory profiles at the endpoint, with decreases in blood IL-6/IL-10 ratio and increases in
316 IL-4 compared to their respective pretreatment baselines. The milk thistle group showed a
317 decrease in blood TNF-α compared to pretreatment baseline (Santamarina *et al.*, 2024).
318 Another prebiotic study found that a preparation of traditional Chinese medicine plant granules
319 ameliorated perimenopausal insomnia (PSQI). The treatment group's endpoint gut microbiomes
320 were depleted in *Ruminococcus*, *Blautia obeum*, *Roseburia faecis, Prevotella copri,* and
321 *Fusicatenibacter saccharivorans*, and enriched in *Bacteroides*, *Faecalibacterium*,
322 *Bifidobacterium*, and *Lactobacillus*. The change in abundance of *Ruminococcus* and
323 *Bacteroides* is opposite to that observed by Santamarina et al., possibly highlighting sex-specific
324 differences that warrant further investigation. However, both studies found positive effects of
325 *Faecalibacterium* on improving sleep, in terms of PSQI and ESS scores (Table 1), possibly due
326 to *Faecalibacterium*'s role as a core butyrate producer (Vital, Karch & Pieper, 2017).
327 Considering the Santamarina et al. study in light of the observational OSA findings
328 above (Table 1) (Lu *et al.*, 2022; Baldanzi *et al.*, 2023), where Bacteroidales (including the
329 genus *Alistipes*) were depleted in older adult OSA patients, it appears that *Alistipes* may play a
330 favorable role in improving daytime sleepiness. Promising results in mice show that oral
331 administration of *Alistipes onderdonkii* can sufficiently repress systemic TNF-α expression to
332 allow mismatched skin engraftment (Li *et al.*, 2023), hinting at its positive effects on sleep
333 through TNF-α regulation. Additionally, *Alistipes* has been positively associated with longevity in
334 centenarians in East China (Wang *et al.*, 2019), and older adults living in longevity villages in
335 South Korea (Park *et al.*, 2015). Both *Faecalibacterium* and *Alistipes* are associated with
336 healthy aging, with findings from these prebiotic and OSA studies suggesting they may improve
337 sleep quality and support healthy aging trajectories in older adults, possibly mediated by the
338 immune system. However, further investigation of *Alistipes* is required given its association with
339 depression (Parker *et al.*, 2020). It may prove useful to identify the metabolic underpinnings of

these taxon-phenotype associations in order to apply them effectively across different contexts.

Bacterial probiotics have been shown to improve sleep in older adults (45+), particularly in individuals with sleep complaints or a sleep-related condition. Murakami et al. showed that a *Bifidobacterium adolescentis* probiotic increased total sleep time, time spent in REM sleep, and daytime wakefulness in healthy Japanese men and women with sleep complaints (Murakami *et al.*, 2024). Similarly, Ben Othman et al. found that daytime sleepiness (ESS) decreased in a study of predominantly female, obese patients on a low-carb diet given a prebiotic intervention of carob beans or a probiotic cocktail, but not in those on the low-carb diet alone (Ben Othman *et al.*, 2023). Kikuchi-Hayakawa et al. reported that *Lacticaseibacillus paracasei* Strain Shirota fermented milk improved daytime sleepiness (EEG) in individuals with sleep complaints (Kikuchi-Hayakawa *et al.*, 2023), while Yamamura et al. found that *Lactobacillus helveticus* fermented milk improved sleep efficiency and reduced waking episodes in healthy older adults (Yamamura *et al.*, 2009). Finally, a study in older adults with mild cognitive impairment found that a probiotic mixture of 18 different strains from the genera *Lactobacillus*, *Lactococcus*, and *Bifidobacterium* significantly improved the sleep quality, latency, duration, and disorder components of the PSQI (Fei *et al.*, 2023). These sleep improvements were accompanied by better gastrointestinal health, with greater abundances of several taxa including *Blautia*, *Coprococcus*, L*. Ruminococcus,* and Lachnospiraceae observed in the treatment group compared to controls. Additionally, the probiotic group showed an increase in serum BDNF, an anti-inflammatory protein that downregulates NF-κB, which is a key player in TNF-α signalling (Liu *et al.*, 2017a) in brain microglia (Charlton *et al.*, 2023).

Lactic acid bacterial probiotics have been shown to exert immunomodulatory effects that restore immune homeostasis in the host (Mazziotta *et al.*, 2023), and above, we see several instances of their positive effects on sleep improvement. Notably, Fei et al. found both increases in anti-inflammatory BDNF and enrichment of several butyrate-producers associated with improved PSQI in their probiotic intervention trial. Santamarina et al. found decreased TNF-α

and enrichment of *Faecalibacterium* alongside improved ESS in the milk thistle group of their prebiotic study. Together, these intervention studies complement the observational studies, highlighting gut-immune-sleep interplay.

As in the observational studies, heterogeneity in findings can be attributed to differences in sample size, demographics, study duration, treatments, dosages, placebo controls, outcome metrics, and study designs. Furthermore, few studies collected both gut microbiome sequencing and sleep data (Table 1), limiting insights into how gut interventions change the microbiome to affect sleep. Most studies reported improvements in self-assessed sleep (e.g. PSQI or ESS), while those using objective measures like EEG were constrained by small sample sizes and limited recordings per participant. The high costs and logistical challenges of collecting objective sleep data likely contribute to these studies being underpowered to detect microbiome-related effects. Despite these limitations, intervention studies show promising results (Fig. 1), but further research is needed to clarify the causal mechanisms by which these pre- and probiotics improve sleep.

Conclusions and Future Perspectives

In the present review, we show that poor sleep quality in observational cohorts is generally associated with lower gut microbiome alpha-diversity and perturbations in butyrate-producing bacteria, particularly taxa within the Lachnospiraceae family (Smith *et al.*, 2019; Grosicki *et al.*, 2020; Gao *et al.*, 2022). In animal models, butyrate supplementation promotes sleep and protects against the detrimental consequences of poor sleep (Szentirmai *et al.*, 2019; Gao *et al.*, 2022), possibly through its immunomodulatory actions (Chang *et al.*, 2014; Siddiqui & Cresci, 2021b). In most of the pre- and probiotic intervention trials reviewed here, participants were often experiencing some form of stress (Sawada *et al.*, 2017; Takada *et al.*, 2017; Nishida *et al.*, 2019), disrupted sleep (Kikuchi-Hayakawa *et al.*, 2023; Murakami *et al.*, 2024), or some other sleep-related condition (Yang *et al.*, 2021; Ben Othman *et al.*, 2023; Fei *et al.*, 2023). In

392 contrast, Yamamura et al.'s study (Yamamura *et al.*, 2009) was conducted in healthy older
393 adults without sleep complaints or disorders. Yamamura et al. reported weak effects from the
394 probiotic, in terms of sleep improvement, suggesting there may be a "ceiling effect" in
395 populations with optimal sleep (Yamamura *et al.*, 2009). Overall, it seems prebiotics and
396 probiotics may be helpful in mitigating the impact of psychological stress on sleep, or correcting
397 an underlying biological dysregulation of systems that impact sleep, like the immune or
398 endocrine signaling.

399 Although both human observational and intervention studies show promising results,
400 many microbiome-sleep associations are unclear or contradictory when integrated across
401 studies. This may be due, in part, to the context dependencies of microbiota-sleep interactions,
402 especially those associated with age, diet, and lifestyle. Future studies should include a
403 demographically diverse mixture of young, middle-aged, and older adults to tease apart this
404 heterogeneity. Furthermore, to better understand the mechanisms by which bacterial taxa affect
405 sleep, it may be necessary to look beyond compositional shifts and consider the metabolic
406 outputs of the gut microbiome (Wilmanski *et al.*, 2022). More research is needed to identify the
407 specific microbial metabolites, such as butyrate, that influence sleep, as well as their
408 mechanisms of action. While cytokines, such as TNF-α, may mediate some of the effects of the
409 gut microbiome on sleep quality, there are also likely many unknown microbially-derived
410 molecules that interact with host pathways. Indeed, many microbially-derived molecules, such
411 as N-acyl amides, SCFAs, tryptophan derivatives, and secondary bile acids are known to
412 interact with host GPCRs expressed by intestinal, immune, and neuronal cells (Aleti, Troyer &
413 Hong, 2023). However, about one-third of human GPCRs remain orphan receptors, while
414 numerous microbial metabolites either remain functionally uncharacterized or undiscovered
415 (Postler & Ghosh, 2017; Aleti *et al.*, 2023). To fully unravel these interactions and their role in
416 gut-brain crosstalk in the context of sleep, work that integrates blood metabolomics, blood
417 proteomics, gut microbiome sequencing, and sleep assessment is needed. One approach

would be to leverage large, deeply phenotyped human cohorts for hypothesis generation (Melamud *et al.*, 2020). These datasets offer integrated multi-omic, longitudinal data that enable high-powered statistical analyses to detect bacterial taxa and metabolic outputs associated with phenotypes of interest (Melamud *et al.*, 2020). Associations identified in human cohorts can then be used to generate mechanistic hypotheses for preclinical experiments, where the implicated bacterial taxa or bacterial consortia can be cultured *in vitro* to examine their metabolic outputs (Rudi & Zhao, 2021). The metabolites, or the relevant taxa, can then be introduced into animal models to assess their effects on sleep.

In terms of sleep assessment in humans, future studies should more often strive to include objective sleep measures. Indeed, effective microbiome-mediated therapies for sleep might require precise targeting of sleep architecture, where improper alterations to the gut may have harmful collateral consequences. For example, a study of healthy older adults (60+) found that those with sleep latency over 30 minutes, sleep efficiency below 80%, or a REM sleep percentage outside a range of 16-25% showed a nearly two-fold higher risk of all-cause mortality, even after controlling for age, sex, and medical burden (Dew *et al.*, 2003). Additionally, chronotype and circadian misalignment (e.g. social jet lag) should be accounted for in experimental designs, as this could affect sleep, while both being affected by and affecting the gut microbiome (Bermingham *et al.*, 2023; Yue *et al.*, 2023). Importantly, advancements in wearable technologies now enable high-throughput, continuous monitoring of sleep and activity. Several consumer wearable devices perform as well as, or even better than, clinical-grade actiwatches at recording sleep/wake measures when benchmarked against the gold-standard PSG (Chinoy *et al.*, 2021). In addition to their accuracy, consumer wearable devices are significantly more cost-effective than EEG, PSG, and clinical-grade actiwatches (Martin & Hakim, 2011; Zambotti *et al.*, 2019). These technological advancements will make larger longitudinal studies capturing objective sleep measures more feasible.

Recognizing the technical, ethical, and cost challenges of microbiome (and, more broadly, multi-omic) research underscores key challenges that should be carefully navigated going forward. Standardizing gut microbiome (and multi-omic) sample collection and storage is essential to minimize biases, such as changes in stool microbial composition that can occur without prompt freezing or the use of chemical preservatives (Kim *et al.*, 2017). Bias can also be introduced from sample contamination and batch effects due to the use of different kits or reagents across experiments (Kim *et al.*, 2017). Additionally, microbiome sequencing can reveal sensitive personal information, like host genetic material in fecal metagenomes, highlighting the need for robust privacy protections similar to those implemented in genomic research (Ejtahed, Parsa & Larijani, 2023). Furthermore, human interventions demand careful oversight, as they may have unanticipated effects on participants and their broader microbiomes, raising safety and community impact concerns (Ejtahed *et al.*, 2023). Finally, we should focus on applying expensive microbiome (and multi-omic) research more equitably to populations outside the developed world so that the resulting medical advances can benefit all of humankind (Abdill, Adamowicz & Blekhman, 2022). Carefully navigating these challenges will enable responsible advancement of gut-sleep axis research and reveal opportunities for therapeutic interventions.

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Declaration of Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

There are no new data associated with this article.

Author Contributions

JC and MB: conceptualization, investigation, methodology, visualization, writing – original draft.

KCK and SMG: supervision, resources, project administration, and writing – review & editing.

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477 **Figure and Table Captions**

478 **Figure 1.** Graphical summary of key points across the review. a) Key points from discussion on

479 interplay between sleep, the immune system, and the gut microbiome across different life

480 stages. b) Key points from the review of observational studies and intervention trials across
481 adulthood.

Table 1. Summaries of the studies evaluated in the Observational and Pre- and Probiotic sections of this review. Studies were found through a PubMed search for (gut microbio* AND sleep), (prebiotic AND sleep), and (probiotic AND sleep). Observational studies examining the gut microbiome and sleep (or sleep disorders) and pre- and probiotic intervention studies targeting sleep (or sleep disorders) were included. To qualify, studies needed to use actigraphy, EEG/PSG, PSQI, or ESS as sleep outcome measures. Studies were organized into lifestage bins (18–45 and 45+) based on sample mean age, with those near bin cutoffs (within 5 years) included only if the standard deviation was less than 5 years. For intervention studies in the 18–45 bin, only those investigating stress alongside sleep were included. The table rows are sorted by sample age in ascending order, with observational studies listed first followed by intervention studies.

Study reference	Study type	Sample Characteristics	Age	Lifestage	Treatment	Significant taxa findings	Sleep outcomes	Other relevant notes/findings
Smith et al., 2019	Observational, cross-sectional	Healthy young male adults (N = 26)	22.19 ± 3.11, mean ± SD	18-45	N/A	Associations with increasing sleep efficiency: ↑ Bacteroidetes ↑ Firmicutes ↑ Lachnospiranaceae ND3007 ↓ Lachnospiranaceae, Lachnospiraceae UCG-004 ↓ Blautia ↓ Oribacterium Associations with decreasing number of awakenings: ↑ Erysipelotricheaceae ↑ Holdemania ↑ Brevibacterium ↑ Corynebacterium ↑ Holdemania ↑ Dermabacter ↑ Neisseria ↑ Sutterella ↑ Actinobacteria ↓ Coprococcus ↓ Parasutterella ↓ Citrobacter Associations with increasing TST: ↑ Lachnospiraceae ↓ Blautia ↓ Lachnospiraceae (UCG-004) ↓ OribacteriumAssociations with increasing sleep efficiency: ↑ Bacteroidetes ↑ Firmicutes ↑ Lachnospiranaceae ND3007 ↓ Lachnospiranaceae, Lachnospiraceae UCG-004 ↓ Blautia ↓ Oribacterium Associations with decreasing number of awakenings: ↑ Erysipelotricheaceae ↑ Holdemania ↑ Brevibacterium ↑ Corynebacterium ↑ Holdemania ↑ Dermabacter ↑ Neisseria ↑ Sutterella ↑ Actinobacteria ↓ Coprococcus ↓ Parasutterella ↓ Citrobacter Associations with increasing TST: ↑ Lachnospiraceae ↓ Blautia ↓ Lachnospiraceae (UCG-004) ↓ Oribacterium	N/A	sleep efficiency, sleep time and/or fewer awakenings associated with alpha diversity and IL-6, no associations with cortisol. IL-6 associated with increased alpha diversity.sleep efficiency, sleep time and/or fewer awakenings associated with alpha diversity and IL-6, no associations with cortisol. IL-6 associated with increased alpha diversity.

Grosicki et al., 2020	Observational, cross-sectional	Healthy young adults (N = 28)	29.8 ± 10.4, mean ± SD	18-45	N/A	*Associations with improved self-reported sleep quality (PSQI):* ↑ *Lachnospiraceae* ↑ *Blautia* ↑ *Ruminococcus* ↓ *Bacteroidetes* ↓ *PrevotellaAssociations with improved self-reported sleep quality (PSQI):* ↑ *Lachnospiraceae* ↑ *Blautia* ↑ *Ruminococcus* ↓ *Bacteroidetes* ↓ *Prevotella*	N/A	↑ Shannon diversity and F/B ratio. In subjects with Prevotella abundance ≥ 2%, Prevotella explains 25.6% of PSQI variance.↑ Shannon diversity and F/B ratio. In subjects with Prevotella abundance ≥ 2%, Prevotella explains 25.6% of PSQI variance.
B. Liu et al., 2019	Observational, case-control	Insomniac adults and healthy controls (N = 20)	Controls: 26.10 ± 1.85, mean ± SD Insomnia: 33.00 ± 6.90Controls: 26.10 ± 1.85, mean ± SD Insomnia: 33.00 ± 6.90	18-45	N/A	Comparing insomnia group to controls: ↑ Bacteroidetes ↑ Bacteroides ↓ Firmicutes ↓ Proteobacteria ↓ Clostridiales Associations with increasing Bacteroides: ↑ sleep latency ↑ insomniac symptoms ↓ self-reported sleep quality (PSQI) ↓sleep efficiency Associations with increasing Clostridiales: ↑ self-reported sleep quality (PSQI) ↓ self-reported daytime sleepiness (ESS) ↓ insomniac symptoms ↓ REM latencyComparing insomnia group to controls: ↑ Bacteroidetes ↑ Bacteroides ↓ Firmicutes ↓ Proteobacteria ↓ Clostridiales Associations with increasing Bacteroides: ↑ sleep latency ↑ insomniac symptoms ↓ self-reported sleep quality (PSQI) ↓sleep efficiency Associations with increasing Clostridiales: ↑ self-reported sleep quality (PSQI) ↓ self-reported daytime sleepiness (ESS) ↓ insomniac symptoms ↓ REM latency	N/A	↓ Chao1 and PD indices (INS) ↓ F/B ratio (INS) 87 bacteria biomarkers distinguish between insomniacs and controls 67.13% of gut microbiome variance explained by clinical sleep parameters↓ Chao1 and PD indices (INS) ↓ F/B ratio (INS) 87 bacteria biomarkers distinguish between insomniacs and controls 67.13% of gut microbiome variance explained by clinical sleep parameters
Lu et al., 2022	Observational, case-control	Hypertensive OSA patients and hypertensive controls w/o OSA (N = 52)	Group A (controls): 51.73 ± 11.09, mean ± SD Group B (mild OSA): 51.12 ± 10.76 Group C (moderate to severe OSA): 52.35 ± 10.43Group A (controls): 51.73 ± 11.09, mean ± SD	45+	N/A	*Comparing OSA patients to non-OSA participants:* ↓ *Alistipes* ↓ *Eubacterium coprostanoligenes* ↓ *Ruminococcus gnavus* ↓ *Blautia* ↓ *Roseburia* ↑ *Coprococcus* ↑ *Megamonas* ↑ *Lactobacillus* ↑ *MegasphaeraComparing OSA patients to non-OSA participants:* ↓ *Alistipes* ↓ *Eubacterium coprostanoligenes* ↓ *Ruminococcus gnavus* ↓ *Blautia* ↓ *Roseburia* ↑ *Coprococcus* ↑ *Megamonas* ↑ *Lactobacillus* ↑ *Megasphaera*	N/A	Lower Shannon diversity in OSA patients.

			Group B (mild OSA): 51.12 ± 10.76 Group C (moderate to severe OSA): 52.35 ± 10.43					
Baldanzi et al., 2024	Observational, cross-sectional	Swedish CArdioPulmonary bioImage Study (SCAPIS) (N = 4,045)	57.7 (53.9 - 61.4), mean (IQR)	45+	N/A	*Associations with increasing T90 (the percentage of time asleep with oxygen saturation below 90%) and ODI:* *↓ Bacteroidales* *↓ Eubacteriales* *↑ Coprococcus comes* *↑ Collinsella aerofaciens* *↑ Ruminococcus gnavus* *↑ Blautia obeum* *↑ Mediterraneibacter glycyrrhizinilyticusAssociations with increasing T90 (the percentage of time asleep with oxygen saturation below 90%) and ODI:* *↓ Bacteroidales* *↓ Eubacteriales* *↑ Coprococcus comes* *↑ Collinsella aerofaciens* *↑ Ruminococcus gnavus* *↑ Blautia obeum* *↑ Mediterraneibacter glycyrrhizinilyticus*	N/A	Various microbial metabolic pathways up/down-regulated.
Sawada et al., 2017	Probiotic randomized controlled trial (RCT), cross-over	Healthy male students under stress (N = 24)	N/A	18-45	4-week Lactobacillus gasseri CP2305 (1x10^10 CFU)	Change in within-group Enterobacteriaceae abundance from pretreatment to after treatment was greatly inhibited in probiotic group compared to control group. Change in within-group Enterobacteriaceae abundance from pretreatment to after treatment was greatly inhibited in probiotic group compared to control group.	Decreased self-reported sleep disturbances (PSQI), improved self-reported sleep quality (PSQI)	Probiotic decreased salivary cortisol.
Takada et al., 2017	Probiotic RCT	Healthy medical students under stress (N = 94)	Placebo: 22.6 ± 0.2, mean ± SD Treatment: 22.8 ± 0.2Placebo: 22.6 ± 0.2, mean ± SD Treatment: 22.8 ± 0.2	18-45	11-week L. casei Shirota YIT 9029 (1x10^9 CFU/mL)	N/A	Improved self-reported sleep length, decreased EEG-N3 sleep reduction*, increased 20% Delta power, decreased self-reported sleepiness on rising*, decreased EEG-sleep latency *remain statistically significant after multiple testing correction (Bonferroni) Improved self-reported sleep length, decreased	N/A

							EEG-N3 sleep reduction*, increased 20% Delta power, decreased self-reported sleepiness on rising*, decreased EEG-sleep latency *remain statistically significant after multiple testing correction (Bonferroni)	
T. Gao et al., 2022	Intervention trial	Healthy college students (N = 22)	Total sample: 23.6 ± 2.01, mean ± SDTotal sample: 23.6 ± 2.01, mean ± SD	18-45	Sleep Deprivation (24h) Sleep Restriction (<7h for 7d)Sleep Deprivation (24h) Sleep Restriction (<7h for 7d)	Enrichment/depletion comparing posttreatment to pretreatment within Sleep Deprivation group (SD1 vs SD0): ↑ Firmicutes ↑ Proteobacteria ↑ Dialister ↑ Agathobacter ↓ Bacteroidetes ↓ Actinobacteria ↓ Bacteroides ↓ Faecalibacterium Enrichment/depletion comparing posttreatment to pretreatment within Sleep Restriction group (SR1 vs SR0): ↑ Firmicutes ↑ Bacteroides ↑ Megaonas ↑ Subdoligranulum ↑ Agathobacter ↑ Dialister ↑ Escherichia-Shigella ↓ Bacteroidetes ↓ Faecalibacterium ↓ Prevotella-9 ↓ Acidaminococcus ↓ BifidobacteriumEnrichment/depletion comparing posttreatment to pretreatment within Sleep Deprivation group (SD1 vs SD0): ↑ Firmicutes ↑ Proteobacteria ↑ Dialister ↑ Agathobacter ↓ Bacteroidetes ↓ Actinobacteria ↓ Bacteroides ↓ Faecalibacterium Enrichment/depletion comparing posttreatment to pretreatment within Sleep Restriction group (SR1 vs SR0): ↑ Firmicutes ↑ Bacteroides ↑ Megaonas ↑ Subdoligranulum ↑ Agathobacter ↑ Dialister ↑ Escherichia-Shigella ↓ Bacteroidetes ↓ Faecalibacterium ↓ Prevotella-9 ↓ Acidaminococcus ↓ Bifidobacterium	Decreased deep sleep, increased light sleep	↑ alpha diversity (SD only) ↓53.1 (SD) and 30.7(SR)% of butyrate. ↓ F/B ratio.↑ alpha diversity (SD only) ↓53.1 (SD) and 30.7(SR)% of butyrate. ↓ F/B ratio.

Nishida et al., 2019	Probiotic RCT	Healthy medical students under stress (N = 60)	Placebo: 25.3 ± 0.6, mean ± SD Treatment: 24.9 ± 0.5Placebo: 25.3 ± 0.6, mean ± SD Treatment: 24.9 ± 0.5	18-45	24-week heat-inactivated Lactobacillus gasseri CP2305 (1x10^10 bacterial cells)	Mitigation of Bifidobacterium reduction and Streptococcus increase seen in the placebo group.Mitigation of Bifidobacterium reduction and Streptococcus increase seen in the placebo group.	Improved self-reported sleep quality (PSQI), increased delta power ratio of first sleep cycle, decreased sleep latency of first N3 stage, decreased WASO	Probiotic decreased salivary CgA levels, no effect for salivary cortisol.
Firoozi et al., 2024	Postbiotic RCT	Active ulcerative colitis patients (N = 36)	Placebo: 38.16 ± 12.38, mean ± SD Treatment: 41.6 ± 10.95Placebo: 38.16 ± 12.38, mean ± SD Treatment: 41.6 ± 10.95	18-45	12-week sodium-butyrate (600 mg/kg)	N/A	Improved self-reported sleep quality (PSQI)	↓ fecal calprotectin ↓ CRP ↑ circadian clock genes expression (CRY1, CRY2, PER1, BMAL1).↓ fecal calprotectin ↓ CRP ↑ circadian clock genes expression (CRY1, CRY2, PER1, BMAL1).
Yang et al., 2021	Prebiotic controlled trial	Perimenopausal women with insomnia and healthy spouses control (N = 26)	Control: 50.28 ± 6.84, mean ± SD Treatment: 50.19 ± 6.25Control: 50.28 ± 6.84, mean ± SD Treatment: 50.19 ± 6.25	45+	Traditional Chinese medicine granules: Semen platycladi Semen ziziphi spinosae Asparaginase Radix Ophiopogonis Dried radix rehmanniae Angelica sinensis Ginseng Radix scrophulariae Salvia miltiorrhiza Radix platycodi Poria cocos Polygala amflra Fructus schizandrae Traditional Chinese medicine granules: Semen platycladi Semen ziziphi spinosae Asparaginase Radix Ophiopogonis Dried radix rehmanniae Angelica sinensis Ginseng	*Enrichment/depletion comparing posttreatment to pretreatment within treatment group:* ↑ *Faecalibacterium prausnitzii* ↑ *Bacteroides* ↑ *Bifidobacterium* ↑ *Lactobacillus* ↓ *Blautia obeum* ↓ *Roseburia faecis* ↓ *Ruminococcus* ↓ *Prevotella copri* ↓ *Fusicatenibacter saccharivorans*Enrichment/depletion comparing posttreatment to pretreatment within treatment group: ↑ *Faecalibacterium prausnitzii* ↑ *Bacteroides* ↑ *Bifidobacterium* ↑ *Lactobacillus* ↓ *Blautia obeum* ↓ *Roseburia faecis* ↓ *Ruminococcus* ↓ *Prevotella copri* ↓ *Fusicatenibacter saccharivorans*	Improved self-reported sleep quality (PSQI) compared to baseline.	N/A

					Radix scrophulariae Salvia miltiorrhiza Radix platycodi Poria cocos Polygala amflra Fructus schizandrae			
Santamarina et al., 2024	Prebiotic randomized trial	Overweight adults (N = 41)	NSupple group: 56 ± 6, mean ± SD NSupple Silybum (milk thistle) group: 57 ± 5NSupple group: 56 ± 6, mean ± SD NSupple Silybum (milk thistle) group: 57 ± 5	45+	NSupple Group: zinc 1% magnesium 1% fructooligosaccharide 45% selenomethionine 0.01% galactooligosaccharide 10% tixosil 5% 1.3/1.6-(β-glycosidic bonds) yeast β-glucans (Saccharomyces cerevisiae) 6%. NSuppleSilybum (milk thistle) Group: Same neutraceutical as NSupple group plus Silybum marianum (3.11% of seed extract).	*Associations with improved self-reported daytime sleepiness (ESS) (within-group multiple linear regression):* *NSupple Group:* ↑ *Collinsella* ↑ *L. Ruminococcus* ↓ *Bacteroides* *NSuppleSilybum (milk thistle) Group:* ↑ *Faecalibacterium* ↑ *Alistipes onderdonkiiAssociations with improved self-reported daytime sleepiness (ESS) (within-group multiple linear regression):* *NSupple Group:* ↑ *Collinsella* ↑ *L. Ruminococcus* ↓ *Bacteroides* *NSuppleSilybum (milk thistle) Group:* ↑ *Faecalibacterium* ↑ *Alistipes onderdonkii*	Improved self-reported daytime sleepiness (ESS) and improved self-reported sleep quality (PSQI sleep quality and sleep latency components) compared to baselines.	NSupple and NSuppleSilybum groups: Decreased IL-6/IL-10 ratio compared to baseline. NSuppleSilybum group: Decreased TNF-α compared to baseline.NSuppl e and NSuppleSilybum groups: Decreased IL-6/IL-10 ratio compared to baseline. NSuppleSilybum group: Decreased TNF-α compared to baseline.

					6%. NSuppleSilybum (milk thistle) Group: Same neutraceutical as NSupple group plus Silybum marianum (3.11% of seed extract).			
Kikuchi-Hayakawa et al., 2023	Probiotic RCT, cross-over	Healthy adults with sleep complaints (N = 12)	Placebo first: 47.2 ± 7.8, mean ± SD Treatment first: 45.5 ± 5.9Placebo first: 47.2 ± 7.8, mean ± SD Treatment first: 45.5 ± 5.9	45+	4-week Lacticaseibacillus paracasei fermented milk (1x10^11 CFU)4-week Lacticaseibacillus paracasei fermented milk (1x10^11 CFU)	N/A	Less daytime drowsiness (lower theta power in EEG), and higher daytime attention (reported by a non-standard questionnaire) compared to controls. Overall findings: probiotic increased total sleep time, time in bed, REM sleep, and wakefulness (measured by EEG) compared to controls. Stress subgroup analysis: participants w/ above average salivary amylase given probiotic had increased time in bed and decreased awakening in last 2 hours of sleep.Overall findings: probiotic increased total sleep time, time in bed, REM sleep, and wakefulness (measured by EEG) compared to controls.	Small sample size, no statistically significant findings with PSQI. Probiotic improved mood scores compared to controls.
Murakami et al., 2024	Probiotic RCT	Healthy adults with sleep complaints (N = 126)	Placebo: 46.7 ± 7.3, mean ± SD Treatment: 46.1 ± 7.0Placebo: 46.7 ± 7.3, mean ± SD Treatment: 46.1 ± 7.0	45+	*4-week Bifidobacterium adolescentis (>1x10^11 bacterial cells / 4 pills)4-week Bifidobacterium adolescentis (>1x10^11 bacterial cells / 4 pills)*	N/A		

							to controls. Stress subgroup analysis: participants w/ above average salivary amylase given probiotic had increased time in bed and decreased awakening in last 2 hours of sleep.	
Ben Othman et al., 2023	Pre- and probiotic 3-arm RCT	Obese adults (predominantly female) (N = 45)	Total sample: 48.73 ± 7.7, mean ± SDTotal sample: 48.73 ± 7.7, mean ± SD	45+	*Control: Low-calorie diet* *Prebiotic: Control diet + 2 carob beans/day* *Probiotic: Control diet + probiotic mixture (10.109 CFU/capsule/day): Bifidobacterium longum Lactobacillus helveticus Lactococcus lactis Streptococcus thermophilus*Control: Low-calorie diet* *Prebiotic: Control diet + 2 carob beans/day* *Probiotic: Control diet + probiotic mixture (10.109 CFU/capsule/day): Bifidobacterium longum Lactobacillus helveticus Lactococcus lactis Streptococcus thermophilus*	N/A	Improved self-reported daytime sleepiness (ESS) compared to baseline in both pre- and probiotic groups.	Depression and stress improved compared to baseline in all groups.
Yamamura et al., 2009	Probiotic RCT, cross-over	Healthy older adults (N = 25)	Placebo first: 70.6 ± 5.65, mean ± SD Treatment first: 72.14 ± 5.67	45+	3-week Lactobacillus helveticus fermented milk	N/A	Increased sleep efficiency and decreased number of awakenings (actigraphy) compared to baseline.	Individuals with worse baseline sleep show greater improvement in sleep compared to those with better baseline sleep.

Fei et al., 2023	Probiotic RCT	Older adults with mild cognitive impairment (N = 42)	Placebo: 75.30 ± 9.75, mean ± SD Treatment: 76.40 ± 9.61	45+	*12-week Probiotic mixture (>2x10^10 CFU/g): Lactobacillus plantarum BioF-228 Lactococcus lactis BioF-224 Bifidobacterium lactis CP-9 Lactobacillus rhamnosus Bv-77 Lactobacillus johnsonii MH-68 Lactobacillus paracasei MP137 Lactobacillus salivarius AP-32 Lactobacillus acidophilus TYCA06 Lactococcus lactis LY-66 Bifidobacterium lactis HNO19 Lactobacillus rhamnosus HNO01 Lactobacillus paracasei GL-156 Bifidobacterium animalis BB-115 Lactobacillus casei CS-773 Lactobacillus reuteri TSR332 Lactobacillus fermentum TSF331 Bifidobacterium infantis BLI-02 Lactobacillus plantarum CN2018*	*Probiotic group compared to placebo group:* ↑ *Blautia* ↑ *Lachnospiraceae* ↑ *Muribaculaceae* ↑ *Haemophilus* ↑ *Coprococcus* ↑ *Ruminococcus* ↑ *Anaerostipes* ↑ *Erysipelotrichaceae* ↑ *Prevotellaceae* ↑ *Pantoea*	Improved self-reported sleep quality (PSQI sleep quality, time to fall asleep, and sleep duration components) comparing probiotic group to placebo control group.	Probiotic increased serum BDNF levels and improved gastrointestinal symptoms compared to controls.

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