Thesis

CORRELATION BETWEEN SLEEP QUALITY AND THE GUT MICROBIOME

submitted by
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Graz, 19 June 2024

Declaration of Academic Integrity

I confirm that this thesis results from my independent scholarly work. I also confirm that quotations and paraphrases are clearly indicated when information from the source of other authors (in essays, articles, scientific works, reviews, reports, and on the website) is used. I have become acquainted with and confirm my agreement with the Medical University’s rules about plagiarism.

Graz,19.06.2024 *Nataliya Pilyayeva eh.*

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Many thanks also to my family, whose support I could count on anytime.I also thank my colleagues for their moral support at that time.

Abstract in German

Es wurde eine Internetsuche nach Artikeln mit den Schlüsselwörtern „Schlafstörung“, „Schlaflosigkeit“ und „Mikrobiom“ durchgeführt. Basierend auf einer Rezension zu diesen Wörtern habe ich eine Einleitung meines Artikels und Hauptthemen für die weitere Suche im Internet verfasst. Dann wurde eine Internetrecherche in PubMed mit einer Kombination der Wörter „Schlafstörung“ + „Mikrobiom“, „Schlaflosigkeit“ + „Mikrobiom“, „Schlafstörung“ + „Mikrobiota“, „Schlaflosigkeit“ + „Mikrobiota“ durchgeführt für die Berichte der letzten klinischen Studien zu diesem Thema. Auf Basis der Suchergebnisse wurde einleitend eine Hypothese formuliert. Es wurden fast 80 Artikel gefunden, von denen nur 5 klinische Studien zum Einfluss von Mikrobiom-Interventionen auf den Schlaf beschrieben.

Schlafstörungen (insbesondere Schlaflosigkeit) stehen in einem wechselseitigen Zusammenhang mit dem Darmmikrobiom. Gehirn-Darm-Mikrobiom-Achse ist ein wirksames Instrument zur Beurteilung der Zusammenhänge zwischen Schlaflosigkeit und Veränderungen des Darmmikrobioms und kann verwendet werden, um Veränderungen im menschlichen Zustand vorherzusagen und bei bereits untersuchten Veränderungen eine Korrektur des Darmmikrobioms auszuwählen. Gehirn-Darm-Mikrobiom-Achse hat aufgrund der multifaktoriellen Natur dieser Wechselbeziehungen und individuellen Merkmale des Organismus und des Darmmikrobioms in jedem Fall Einschränkungen bei der Beurteilung quantitativer und qualitativer Veränderungen. Nicht-pharmakologische Behandlungen wie Ernährung, Änderung des Lebensstils und die Verwendung von Prä- und Probiotika gelten als perspektivische Möglichkeit zur Behandlung von Schlaflosigkeit und zur Korrektur von Veränderungen des Darmmikrobioms. Viele Studien, die sich mit dem Darmmikrobiom, Schlaflosigkeit, ihren Zusammenhängen und Korrekturmöglichkeiten befassten, konzentrierten sich nur auf mehrere Themen. Sie liefern kein

vollständiges Bild der Veränderungen im Mikrobiom, im Darm und im gesamten Organismus.

Aus diesem Grund wurde eine zusätzliche Suche mit den Wörtern „Probiotika“, „Schlaflosigkeit/Schlafstörung“, „Präbiotika“ und „Schlaflosigkeit/Schlafstörung“ durchgeführt, um die Suchergebnisse zu verbessern. Die Ergebnisse der Internetrecherche wurden präsentiert und diskutiert, wobei die wichtigsten Fragen und Probleme zum aktuellen Stand dieses Themas formuliert wurden. Es hat mein Verständnis des Problems und meine Sicht auf Einschränkungen dargelegt. Es wurden Ansätze für neue potenzielle klinische Studien im Bereich „Behandlung von Schlafstörungen durch Mikrobiomintervention“ und weitere Trends dieser Themenentwicklung vorgeschlagen.

Aufgrund der Durchsicht der Ergebnisse glaube ich, dass es zum jetzigen Zeitpunkt eine Anhäufung von Forschungserfahrungen und Wissen zu diesem Thema gibt. Eine erfolgreiche und evidenzbasierte probiotische Formel, die sowohl auf Darmmikrobiom -Ebene wirken als auch den Schlaf und/oder die kognitive Leistungsfähigkeit verbessern und Stress und Depressionen sowohl bei akuter als auch bei chronischer Schlaflosigkeit reduzieren würde, wurde noch nicht identifiziert. Aus diesem Grund ist es sinnvoll, mit placebokontrollierten, doppelblinden Designs fortzufahren. Es ist möglich, dass unterschiedliche Probiotika zur Korrektur unterschiedlicher Schlafstörungen angeboten werden.

Zukünftige Forschung sollte sich auf die Untersuchung des Mikrobioms (Darmmikrobiota, Darmmetaboliten und Darmzustand – Anzeichen von Durchlässigkeit und Entzündung) und die Untersuchung des menschlichen Körpers (Cortisolspiegel, Serumzytokine und Wohlbefinden der Patienten*innen) wie mindestens zu Beginn und nach der Behandlung konzentrieren. Schlaflosigkeit, Stress, Depression, Angstzustände und der Magen-Darm-Status können mithilfe von Standardfragebögen beurteilt werden. Es wurde vorgeschlagen, leichte Schlaflosigkeit mit

leichten Symptomen von Reizdarmsyndrom, Depression und Angstzuständen zu untersuchen und dabei eine Mischung aus mehreren Stämmen als Probiotikum für mindestens 4 Wochen zu verwenden. Es bestehen weiterhin Probleme mit der Stabilität positiver Veränderungen der Darmmikrobiota nach der Behandlung.

Abstract in English

It has performed an Internet search of articles using the keywords “sleep disorder,” “insomnia,” and “microbiome.” Based on a review related to these words, I wrote an introduction to my article and the main issues for further internet research. Then, it has been done Internet exploration in PubMed with a combination of the words “sleep disorder” +” microbiome,” “insomnia” + “microbiome,” “sleep disorder” + “microbiota,” “insomnia” + “microbiota” looking for the reports of last clinical studies for this topic. Based on the search results, an introduction and a hypothesis were formulated. Nearly 80 articles were found, where only 5 described clinical studies related to the impact of microbiome intervention on sleep.

Sleep disorders (particularly insomnia) have a reciprocal interrelation with gut microbiomes. Brain-gut-microbiome axis (BGMA) is a potent tool for assessments of interrelationships of insomnia with gut microbiome alterations, and it may be used to predict changes in the human condition and choose gut microbiome correction in case of already studied alterations. BGMA has limitations in assessing quantitative and qualitative changes in each case due to the multifactorial nature of these interrelations and individual features of the organism and gut microbiome. Non-pharmacological treatment such as diet, change of lifestyle, and usage of pre-and probiotics is considered a perspective way for Insomnia treatment and gut microbiome (GM) alterations corrections. Many studies that researched gut microbiome, insomnia, their interrelation, and ways of correction focused only on several issues. They do not provide a complete picture of changes in the microbiome, gut, and entire organism.

That is why an additional search using the words “probiotics,” “insomnia/sleep disorder,” “prebiotics,” and “insomnia/ sleep disorder” was performed to improve search results. Results of Internet searches were presented in tables and discussed with the formulation of main issues and problems on the current stage of this

topic realization. It has given my understanding of the problem and my view on limitations. It has been proposed approaches to new potential clinical trials in the field of “treatment of sleep disorder through microbiome intervention” and further trends of this topic development.

Based on the review of the results, I believe that at this stage, there is an accumulation of research experience and knowledge on this topic. A successful and evidence-based probiotic formula that would work both at the GM level and would improve sleep and/or cognitive performance and reduce stress and depression in both acute and chronic insomnia has not yet been identified. This is why it makes sense to continue with placebo-controlled, double-blind designs. It is possible that different probiotics will be offered to correct various sleep disorders.

Further investigations should be focused on microbiome exploration (gut microbiota, gut metabolites, and gut condition - signs of permeability and inflammation) and human organism examination (level of cortisol, serum cytokines, and well-being of the subject) at the baseline of research and after the treatment. Insomnia, stress, depression, anxiety, and gastrointestinal status may be assessed using standard questionnaires. It was proposed to investigate mild insomnia with mild symptoms of bowel disturbances, depression, and anxiety by administering a multi-strain mixture as a probiotic for at least four weeks. Issues with the stability of positive gut microbiota changes after the treatment are still open.

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List of Abbreviations

5-HT	5-hydroxytryptamine (tryptophan derivate)
AID	Acute insomnia disorder
ANS	Autonomic nervous system
BA	Bile acids
BC	Before Christ
BDNF	Brain-derived neurotrophic factor
BGMA	Brain-gut-microbiome axis
BZD	Benzodiazepines
BRB	Barbiturates
CANTAB	Digital assessments of cognitive function.
CAR	Cortisol awakening response
CBT-I	Cognitive behavioral therapy focuses on insomnia.
CFU	Colony-forming unit
CID	Chronic insomnia disorder
CNS	Central nervous system
CR	Circadian rhythm
DA	Dopamine
DM2	Diabetes mellitus 2
DSM 5	Diagnostic and Statistical Manual of Mental Disorders (5 Edition)
ECCs	Enterochromaffin cells
EEG	Electroencephalography
EMG	Electromyography
ENS	Enteric nervous system
EOG	Electrooculography
ESS	Epworth sleepiness scale
F/B ratio	The ratio of *Firmicutes/Bacteroidetes*
FMT	Fecal microbiota transplantation
GABA	Gamma-aminobutyric acid
GIT	Gastrointestinal tract
GM	Gut microbiome
HPA	Hypothalamic pituitary adrenal axis

IBD	Inflammatory bowel disease
IBS	Irritable bowel syndrome
IL	Interleukin
ISI	Insomnia severity index
LAB	*Lactic acid bacteria*
LcS	*Lactobacillus casei Shirota*
LPS	Lipopolysaccharide
MDD	Major depressive disorder
MRI	Magnetic resonance imaging
NE	Norepinephrine
NREM	Non-rapid eye movement sleep
OS	Oligosaccharides
OSA	Obstructive sleep apnea
PSG	Polysomnography
PSQI	Pittsburgh Sleep Quality Index
PTSD	Post-traumatic stress disorder
REM	Rapid eye movement
sBA	secondary bile acids
SCFA	Short-chain fatty acids
SCN	Supra chiasmic nuclei
SD	Sleep deprivation
SD-I	Sleep Disturbances, Insomnia
SE	Sleep efficacy
SF	Sleep fragmentation
SL	Sleep latency
TNF	Tumor necrosis factor
TST	Total sleep time
VN	Vagus nerve
WASO	Wakefulness after sleep onset
QoS	Sleep quality, Quality of Sleep

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Introduction

1.1 Insomnia

1.1.1 Sleep definition, regulation, stage

1) There are several definitions of the word “sleep.” According to Oxford and Cambridge dictionaries, sleep is “the natural state of rest in which your eyes are closed, your body is not active, and your mind is not conscious.” (2) “It is a condition of body and mind that typically recurs for several hours every night, in which the eyes are closed, the postural muscles relaxed, the activity of the brain altered, and consciousness of the surroundings practically suspended.” (3). Britannica gives the following definition: “a normal, reversible, recurrent state of reduced responsiveness to external stimulation that is accompanied by complex and predictable changes in physiology.” (4) According to Harvard Medical School, sleep is an easily reversible condition of decreased activity and diminished response to outer stimuli of organisms with specific postures, for example lying with closed eyes. (5).

Despite the immobility and lack of body reactions on the majority of external impulses, sleep is an active process. According to brain activity wave rhythm, which can be recorded on electroencephalography (EEG)and correspondent specific eye movement registered on electrooculography (EOG) sleep may be divided into the awake stage, rapid eye movement (REM) stage, and non-rapid eye movement (NREM) stage. Body changes according to the stage. So, during non-REM sleep, body temperature reduces, for approximately 0,5 Celsius, breathing, heart rate, and blood pressure decrease in contrast to increasing breathing, heart rate, and blood pressure during the REM stage. Intense dreaming occurs primarily during REM sleep, whereas digestion, tissue recovery, and growth become more active during all stages of sleep.

Why do we need to sleep? There is no single hypothesis that can provide a full and all-encompassing answer to this question. But there are at least four ones that give us the possibility to understand this process better. First is adaptive or evolutionary theory. Briefly, it states, that only those survived, who can sleep, as a result of natural selection. Next is energy conservation theory, which declares that at definite moments when food search is inefficient, the body should diminish energy consumption to save it for the active part of life. Restoration theory focuses on the statement that the body should have time to heal itself and to restore exhausted balance. It has some evidence because muscle, cell recovery, protein, and hormone biosynthesis, which are important for vitality, occur primarily during sleep. The most contemporary brain plasticity hypothesis supposed a connection between rest and the ability of the brain to adapt to external situations, where sleep enhances brain plasticity, which impacts learning capacity and routine issues resolving.

The process of sleep, as well as awake is quite complex. It is known that multiple brain areas and several neural circuits working together are involved into sleep and wake.

The hypothalamus contains groups of neurons working as control centers, impacting sleep, awakening. In the lateral part of this structure are nerve cells, containing orexin-receptors, and in the tuberomammillary nucleus are histamine-producing cells, which are responsible for arousal promotion (6) Within the hypothalamus lies the suprachiasmatic nucleus (SCN), which obtains signals directly from the vision organs about the light and, in the daytime, controls the behavior related to the day-night rhythm.

Other brain structures such as the brain stem including the pons, midbrain, and medulla, exchange information with the hypothalamus to control body`s wake/sleep transitions. They are responsible for relaxing muscles, body posture, and limb movements, preventing body reactions during dreams. Gamma-

aminobutyric acid (GABA)-neurons are in the preoptic and parafacial regions of the brain. GABA reduces the activity of awakening the brain`s centers during NREM sleep. Cholinergic nerve cells, which are located in the tegmental/pedunculopontine nuclei are responsible for the start of quick eye movement phase.

The thalamus acts as an information relay from the sensorial neurons to the cortex and correspondently for interpretation and storing received information (short/long-term memory transition). It is not active for a significant part of sleep (during N1-2-3REM) and can send signals to the cortex, such as images, sounds, and other sensations only during the REM stage that creates dreams. In this stage, the brain structure called the amygdala, responsible for emotions, also becomes active.

The pineal gland, connected with the SCN, is responsible for the melatonin secretion that acts as a sleep inductor when the quantity of light exposed to the body is diminished.

The basal forebrain stimulates the transition of sleep and arousal by releasing compound adenosine, regulating energy exchange in cells, and supporting sleep, while the midbrain works only on awakening.

Obtained from sensorial organs information can change sleep/awake status, using the not only hypothalamic-pituitary-adrenal (HPA) axis but also immune (cytokines), endocrine (cortisol, epinephrine), and neural channels, signals from other organs (gut, skin) (7, 8).

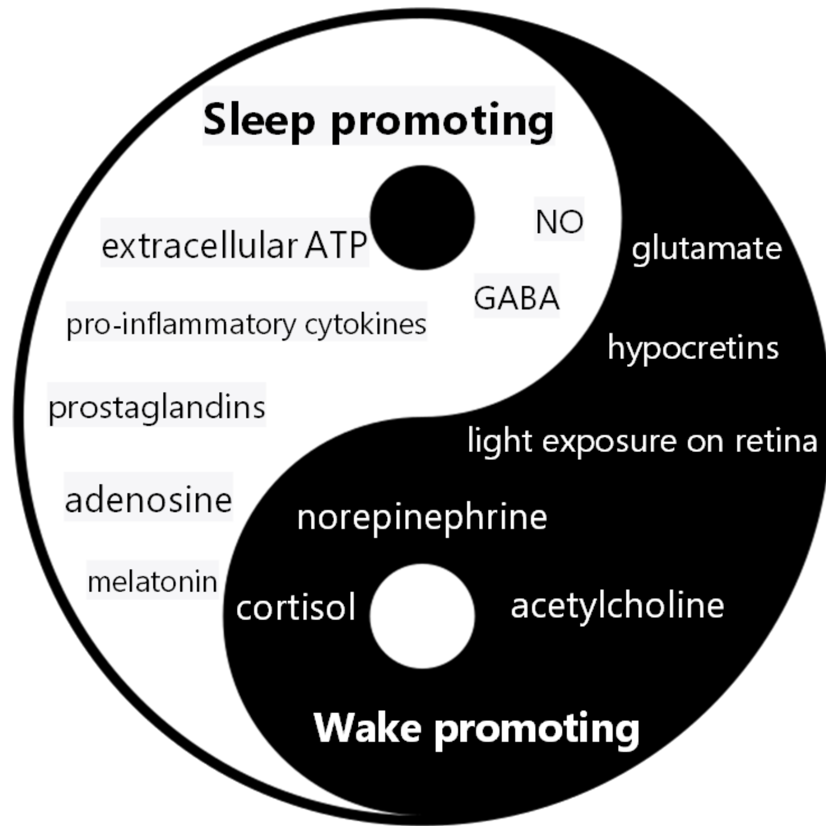


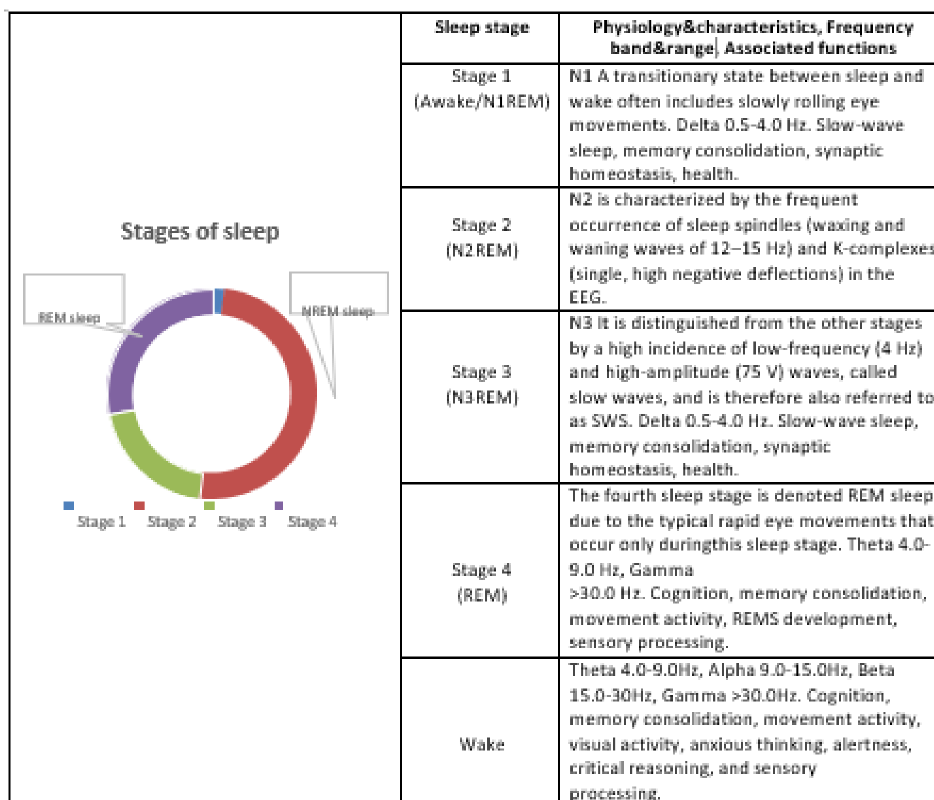
Figure 1. Substances that determined sleep and wakefulness.

Approximately one-third of our lives pass in sleep conditions. Our body requires sleep to maintain proper body functions physiological and psychological status. This is programmed for humans to recover their multisystem balance. The following collaborating systems determine the timing of the Sleep-Awake transition: the internal biological clock also called the circadian rhythm (CR) or process C, and the sleep-wake homeostatic process S. The Circadian clock is controlled by the SCN of the hypothalamus by receiving visual irritations by light exposure during defined hours. This is a central clock (9, 10). The time of circadian C and homeostatic S processed turnover in SCN lasts nearly twenty-four hours.The CR also provides control over the emotional status, thermoregulation, and secretion of releasing and growth factors and thus regulates the endocrinology and other cell mediators' status. This central clock works from the hypothalamus-periphery signals, synchronizing the peripheral clocks, which are

connected with nutritional and activity states (10). However, peripheral and central clocks may be desynchronized due to the acting of external stimuli, for example, by increasing physical activities or food intake in an inappropriate day phase, which can be a trigger for the launch of a variety of pathological pathways, resulting in psychological and physiological disturbances including metabolic disorders. The inventionof electric light, shifting work, and the lag jet greatly impacted the CR or sleep-awake cycle and normal sleep physiology.

Up to 1920, scientists considered sleep as an inactive state of the CNS (brain rest); the invention of EEG allowed to record brain activity and changed the approach to sleep, showing that it is a dynamic process where the brain is active and it manages all processes. Sleep studies using EEGs and other tools that can record and quantitively define ‘deep" or "slow wave" sleep according to eye movements, muscle, and brain activity would discover 2 kinds of sleep: REM and NREM. (TABLE1)

Table 1. Sleep stages/brain activity.



Sleep stage	Physiology&characteristics, Frequency band&range, Associated functions
Stage 1 (Awake/N1REM)	N1 A transitionary state between sleep and wake often includes slowly rolling eye movements. Delta 0.5-4.0 Hz. Slow-wave sleep, memory consolidation, synaptic homeostasis, health.
Stage 2 (N2REM)	N2 is characterized by the frequent occurrence of sleep spindles (waxing and waning waves of 12–15 Hz) and K-complexes (single, high negative deflections) in the EEG.
Stage 3 (N3REM)	N3 It is distinguished from the other stages by a high incidence of low-frequency (4 Hz) and high-amplitude (75 V) waves, called slow waves, and is therefore also referred to as SWS. Delta 0.5-4.0 Hz. Slow-wave sleep, memory consolidation, synaptic homeostasis, health.
Stage 4 (REM)	The fourth sleep stage is denoted REM sleep due to the typical rapid eye movements that occur only duringthis sleep stage. Theta 4.0-9.0 Hz, Gamma >30.0 Hz. Cognition, memory consolidation, movement activity, REMS development, sensory processing.
Wake	Theta 4.0-9.0Hz, Alpha 9.0-15.0Hz, Beta 15.0-30Hz, Gamma >30.0Hz. Cognition, memory consolidation, movement activity, visual activity, anxious thinking, alertness, critical reasoning, and sensory processing.

The relationship between NREM and REM sleep is approximately 4 to 1; thus, the REM phase is up to 25% of total sleep time (TST) in humans. The consequence of N1-4REM/REM phases is called Sleep architecture. The typical time of the first NREM/REM sleep cycle is around 70 – 110 minutes, while the average time of the next cycles lasts as a rule shorter. On average, 3-5 cycles pass per night, taking 6-9 hours of TST. Besides, napping (or daytime sleep for 30 min-1.5 hours) as an accepted practice in several countries also increased TST. So, it is impossible to define the time required for enough sleep due to its individuality. Required time can range from 4.5 to 10.5 hours per day; the most important is the feeling of sleep satisfaction.

Different factors can affect sleep architecture. Age, gender, total time of recent rest or arousal, together with the setting of an individual's internal clock (synchronizing), actions before sleep, for example, physical activity, experiencing distress, and interacting with external physicochemical factors and existing comorbidities

may become triggers of sleep disorders.

1.1.2. Sleep disorders

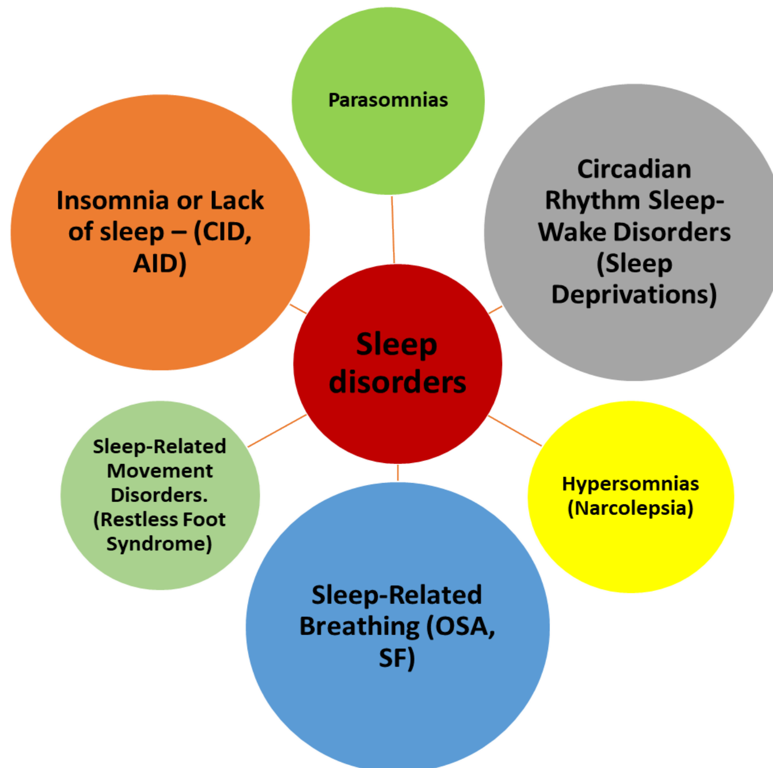


Figure 2. Structure of sleep disturbances according to Manual of Mental Disorders, 5th Edition.

Insomnia definition, incidence

Lack of sleep or insomnia is known from the ancient ages when Aristotle wrote "On sleep and sleeplessness" (350 BC), and Dionysius described the death of thetyrant of Heraclea due to sleep apnea (360 BC). Sleep disorder complaints comefrom 15 to 50% of the general population (11, 12), and frequency has been increasing during the last decades (13), particularly among women (14) and older people (15).

Lack of sleep can lead to diminishing workability, fatigue, weakness, decrease in mood, loss of motivation, anxiety, depression, cognitive impairment, memory deterioration, and a row of somatic issues (pain exacerbation, digestive problems, body

temperature increasing, and so on). Lack of sleep may be objective (TST decreasing) and subjective (sleep time is enough, but SE is low). The 5th edition of the Mental Disorders Guideline (DSM-5) (1) defines insomnia disorder diagnosis on the basis of a patient`s complaints related to initiation or maintenance of sleep disturbances, early awake or interrupted non-restorative sleep with impairment of day activity that lasts for 3 days in a week during 3 months or more. Diagnostic criteria of sleep disorder (DSM-5) group A defines difficulties initiating sleep (or sleep deprivation – SD), difficulties in sleep maintenance by frequent sleep awakening (or sleep fragmentation - SF), early morning awakeningwith inability to return to sleep (related more to CR), B criteria related to acute insomnia due to distress or social impairment, C, D, E related to chronic insomnia (three night per week, at least three month and adequate opportunity to sleep), F, G, H criteria excluded obstructive sleep apnea - OSA, narcolepsy, CR or sleep-awakening disorders (F), substance-abuse(G) and coexistent mental disorder (H) insomnia.

Due to the diagnosis being based on subjects feeling impairment in daily functioning, objective assessment such as polysomnography (PSG), actigraphy, EEG, EOG, and electromyography (EMG) should be supplied with subjective such a sleep diaries, a Pittsburgh sleep quality index (PSQI) questionnaire and/or assessment of sleep quality (QoS), cognitive/mental/psychiatric manifestations, tiredness and life quality, with focus on insomnia severity index (ISI) (16).

According to Spielman`s 3P sleeplessness model, there are following predisposing (or increasing vulnerability) factors of insomnia (genetic, such as the family history of insomnia, neurobiological such as advancing age, female sex; and environmental factors, such as lifestyle and stress or worrying), precipitating (or triggering) factors (stressful life events, medication, drug use, illness, jet lag, or a psychiatric condition such as an episode of depression) and perpetuating (or maintaining) factors

(maladaptive behavior, worry about disease, inappropriate sleep patterns, excessive napping or spending long periods in bed trying to fall asleep) were proposed for insomnia progression (17).

1.1.3 Review of Insomnia mechanisms

To explain the insomnia-psychopathology connection, it has been proposed to follow mechanisms focusing on the emergence or aggravating problems of mental status:

- A significant genetic correlation was found between sleeplessness and depression-anxiety in large genome-wide association studies (18), and a genetic relation between insomnia and post-traumatic stress disorder (PTSD) was also confirmed (19). In schizophrenia trials, any correlation between sleeplessness and genome was reported, it was found the link to schizophrenia with long sleep duration (18)
- Stress system (HPA) abnormality has been found in some mental disorders, such as bipolar disorders and major depressive disorder (MDD) (20, 21), where insomnia is related to cortisol reactivity. It has been suggested approaches to clarify results obtained in neuropsychiatric studies for cortisol reactivity (exhausting of neurotransmitters, HPA-neuro immune activation, neurotrophic factor dysfunction), but there is no common approach that is able to explain the entire combination found in the studies (22). Excessive HPA activation can induce sensitive sleep or arousal in case of lack of rest. It can lead to a rising level of HPA basal activity. (23).
- Sleep disruption during the restless REM sleep phase is characterized by a high arousal threshold and awake-like activity on EEG, accompanied by REM in different directions and muscle atony. Awake in this stage of sleep, which was reported in insomnia and PTSD subjects, may impact the emotional processing of daytime experiences (24).
- Magnetic resonance imaging showed that restless REM sleep causes amygdala disturbance. It may be the cause of depression

(25). In other neuroimaging studies, researchers reported an association of abnormaldegrees in the insula with anxiety levels and depression symptoms contribution (26). The reduced connectivity in the part of the brain (left superior parietal gyrus) has also been reported in subjects with chronic insomnia disturbances (CID). (27)

- Misbalance in neurotransmitters and the neuropeptide system can cause problems at the neurobiological level and impact sleep continuation. It has been described that sleep architecture was disrupted due toaltered the balance in the aminergic and cholinergic systems (24); over-activation of the orexin signaling system may increase wake periods, which leads to insomnia and can also affect mental health (28).
- CR disorders lead to sleep-wake cycle disturbances that are associated with insomnia and mental health deviation. Circadian light exposition on the eye retina and skin is important for the normal work of the biological clock, which relates to emotional regulation and projects directly to brain limbic structures (29).
- Besides genetic, stress, emotional/behavioral, brain structure or neuro metabolites abnormalities and environmental/circadian mechanisms, there are also gut microbiome (GM) composition mechanisms in scientific focus review. So some associations have been reported for GM composition, SD-I, and manifestations of psychiatric disturbances (30): first of all GM has an influence on digestive, neuroendocrine, and immune systems and also is able to impact sleep and psychical health (31); second important fact is that the most completed explanation of majority listed above mechanisms (HPA axis, metabolite misbalance, CR disturbances and brain structure connectivity abnormality) can be described and explained, through proposed for GM mechanisms which are in tight interrelation with stress and CRs. Eventhough this hypothesis does not consider genetic mechanisms, it covers all other predisposing, precipitating, and perpetuating factors.

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1.2. Microbiome

1.2.1 Definition of human microbiome/gut microbiota

It has been realized that despite the Human genome project being completed in 2003, decoding only the human genome is insufficient to understand human biology. Many microorganisms living on and within the human bodymay significantly affect human life throughout their life cycle. This realization started the “second genome project” – the Human microbiome project. (32)

Two terms are usually used in scientific articles: microbiome and microbiota. Bothappeared at the beginning of the 21st century and could be confused with each other. “Microbiota” is related to the bacteria located in a particular host`s area, for example, gut or vaginal microbiota. „Microbiome” defines structural elements, metabolites, and bacterial, fungal, and viral populations, which is more extensive than microbiota because it also includes microbiota environment. (33, 34)

Just as organs, the human microbiome develops during the human’s life: its composition relies upon the host`s genetics (temperature, acidity, passage, permeability), lifestyle, place of residence, and current health condition, and microbial composition after gestation depends on the way the baby is delivered, feeding method, family environment. During infancy, the GM is subject to strong changes; it develops and stabilizes over time. At early 2-4 years, the GM becomes in terms of composition and variety similar to that in adults. (35).

1.2.2. Gut microbiota composition

The structure of the GM in a healthy man is not constant; it has a significant variety depending on location, human genetics, race/ethnicity, daytime, geography, dietary habits, age, lifestyle (drug intake, physical activity, stress), potential or current diseases

(7, 13, 31, 34, 36-39). Every body part has unique microbiota (skin, oral cavity, vagina, gut). Still, it is considered that GM has a crucial (most important) role in preserving human health (40).

The human GM, called also the "forgotten organ," is variable andunique for every organism. More than a thousand kinds of living microorganisms are in the GIT with an amount near 10^{14} or 1 kg weight. Almost 90% of the presented strains are part of *phyla Firmicutes* or *Bacteroidetes*; the others up to 10% are Actinobacteria, Cyanobacteria, Fusobacteria, Proteobacteria, and *Verrucomicrobia* (41-43). There are also some fungi: *Candida, Saccharomyces, Malassezia,* and *Cladosporium* (44), which are 0.001– 0.1% of the entire microbiome and contain ~10^{9} microorganisms (45),or nearly million / gram in the feces (46). To the above-described organisms, the GM contains *Archaea*, *Phages*, *Viruses*, and *Protozoa*, which live in symbiosis with the host. It contains a considerable amount of chemical compounds called metabolome, digested and indigestible proteins, triglycerides and oligo- and polysaccharides, active compounds of plants, animal tissues(and products of its fermentation), bioactive components of fatty acids, amino acids, carbohydrates, cytokines, and other active substances, which are products not only host metabolism (like bile acids (BA)) but also the product of microbiota metabolism (lactate, ammonia, sulfhydryl, methane, carbon dioxide so on. Metabolome has an impact on microbiota (living microorganisms), supporting or suppressing some strains and helping digest food, and impacting the gut, blood (when products are absorbed), and other organs of the human (brain, liver, immune organs). Besides, mucus of gut epithelium can also be considered a part of the microbiome because it provides gut protection and impact absorption.

1.2.3 Gut microbiota function

The microbiome can impact the human digestive. So, GM helps to induce the breakdown of nutrients and drugs; it generates vitamins and provides defense from pathogens and immune

response. The microbiome recovers itself when influenced by outer factors. It synthesizes precursors of hormones and balances the variety of substrates that reach the blood. The GM can also impact the other organs and systems, producing active compounds, that is able to control the HPA (31, 34).

Different stimuli may disturb GM and influence its diversity, abundance, and, as a result, its properties. Most often, these include the following: lifestyle and region, dietary habits and drug intake, and stressogenic situations. Clinical trials have demonstrated that GM could be a predisposing factor to Alzheimer's disease (AD), Parkinson's disease (PD), MDD, and other neurological and psychiatric diseases development or progression. (38). Decreased chewing of food, lack of saliva or changes in its properties, changes in the passage time of the food in the gastrointestinal tract (GIT), and accordingly, the time of digestion can affect the GM, causing dysbiosis that leads to disease development. (34, 37).

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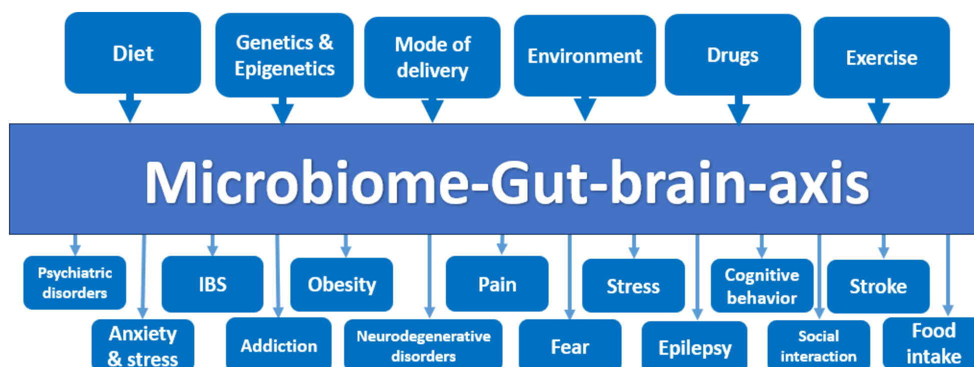


Figure 3: "Factors impacting GM and human`s condition appearing due to microbiome alteration."

Microorganisms and circadian genes can impact one another. The GM`s characteristics are associated with the human's CR. Emotional, physical, or physiological stress influences the GM composition. The inflammation in GIT is also connected with

insomnia, metabolic disease, CR disturbances. (38, 39). To have a more potent tool for establishing interrelation between the GM and the brain, the concept of the Brain-Gut-Microbiome Axis “BGMA” describes most ways/mechanisms for these two organs' relationship and is strongly supported by animal trials reported during the past decade. (47-54) To describe all reciprocal interactions and associations between the microbiome, intestine as a separate organ, and human brain, the term BGMA has been invented. BGMA describes the interrelations between the GM and gut, generating new approaches to inflammatory bowel disease (IBD), IBS, Crown disease, human overall conditions and comorbidity (metabolic syndrome, diabetes mellitus (DM2), obesity), pain perception, and nervous system disturbances (fibromyalgia, chronic fatigue syndrome, depression, Alzheimer, dipolar disorders, so on), however, we will be focusing onsleep disorder due to this review topic limitations.

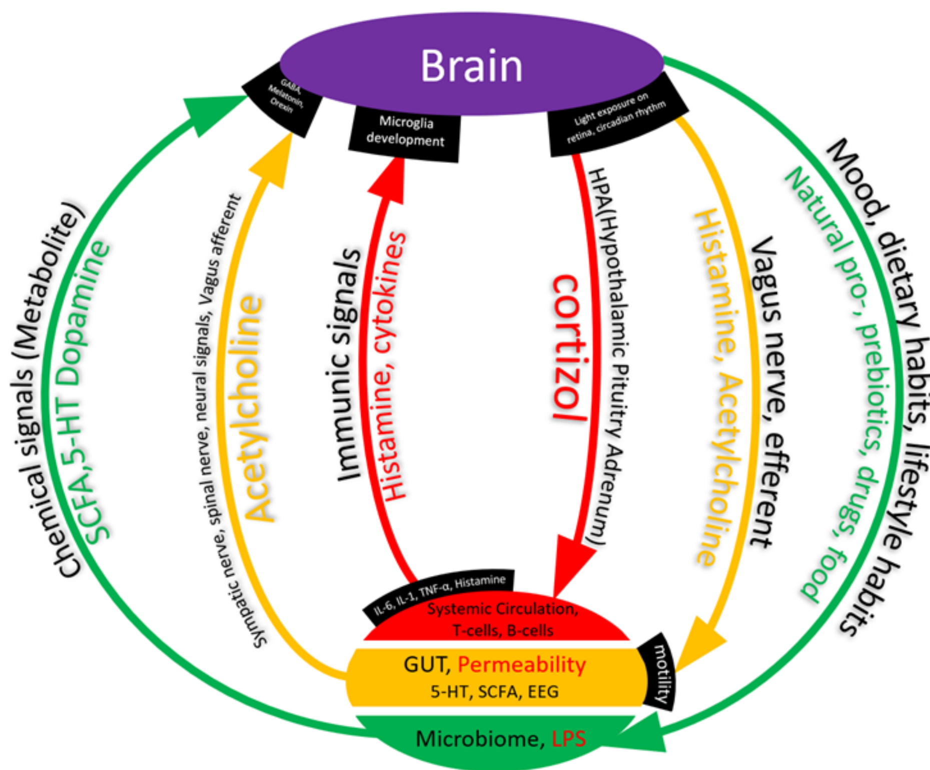


Figure 4. BGMA axis for sleep/insomnia.

1.3. BGMA

In the beginning, a lot of experimental animal models were suggested for studying the influence of GM on BGMA: antimicrobial drugs administration (55), fecal microbial transplantation (FMT) (55, 56), GM colonization (57, 58) and cultured GM (59), using of probiotic (60). These models had limitations, but they demonstrated that the disturbances of GM have a huge impact on reaction to stress, and it is able to be normalized by conventional microbiota colonization. Later investigations were focused on neurochemicals, such as brain-derived neurotrophic factor (BDNF) concentration (61, 62), diminished serotonin (5-HT) receptor 1A expression in hippocamp (62), and enlarged synthesis of monoamine in striata (63). They confirmed that the microbiome has a different and powerful influence on CNS phenotypes. Furtheranimal experiments have provided evidence that GM has an impact on anxiety (60, 62), depression (60, 64), nociceptive response (65, 66), CR (39, 67), and sleep behavior (39, 68). Despite acknowledging the limitations of these experiments, they supposed that GM is significant for neurogenesis and neurodevelopment (69) and gave the basis for the transition of these findings for human adults.

Within this BGMA, it has considered three ways which create a bidirectional informational flow:

1.3.1 Immunoregulatory pathway

This pathway describes GM interaction with immune cells and how it affects gut and brain by levels of cytokines or microglia-mediated effects and by the activity of the HPA. It is essential when some commensal and virulent gut microorganisms via lipopolysaccharide (LPS) cause inflammation or irritation of the intestine, increasing its permeability to toxins, cytokines, and other inflammatory mediators. Research on biomarkers shows that emotional disturbances have associations with GIT disturbances demonstrated by disruption of gut barrier integrity (70). In this

situation, cortisol suppresses cytokines production by immune cells. Cytokines' low levels cause bacterial cell formation (71), whereas increased levels can cause sleep disturbances (72) and circadian rhythm affection (73).

Patients who suffered from sleeplessness demonstrated IL-6 increasing in comparison with healthy ones (74). Gut microbiota influences CNS through resident immune cells, especially microglia (75). Anomalous heterogeneity of the nasal microbiome, especially genus Moraxella and elevated IL-6, was observed in sleepless subjects. (76). In animal studies, stimulation of the immune system happens due to the deliberation of sleep-regulatory compounds as tumor necrosis factor (TNF), interleukin-1, and bacterial breakdown products that improved NREM3, the deepest sleep (77).

1.3.2 CNS-ENS (enteric nervous system) pathway

Vagus nerve (VN)-gut microbiota- ENS-pathway – modulation of VN activity on the gut and then on microbiota or microbiota can affect the ENS, which provides a signal to the brain by afferent fibers.

Animal studies provided anatomical evidence, confirming that the GM affects sensory neurons of the myenteric plexus in the GIT, which are responsible for the regulation of the gut`s motility and hormone release. The ENS and VN are connected through synapses, forming GM-ENS-VN-Brain transmission. (78) VN impacts mucus, and gastric acid secretion and peristalsis through efferent neurons, gut and antimicrobial peptide production, epithelial fluid maintenance, and intestinal permeability (79).

Afferent signals from the GM go through the ENS on VN that are represented by inflammatory mediators, components of the diet, metabolites of bacteria, and regulatory intestinal peptides. The fact, that GM might directly activate neurons was also provided in some studies (80). The combination of the HPA-axis and GM-ENS-VN-brain pathway creates a tremendous and complex network for interrelation intestine with CNS and backward, providing human

physiological homeostasis regulation (81). This communication is mediated by cortisol and hypothalamic axis hormones, impulses from the CNS/VN, and cytokines release. Trough vagal regulation occurs the transmission of information from gut to the central nervous system and backward (47-49). Using BGMA is able to determine the role of neural regulation in physiological processes such as food consumption and satiety, fat, carbohydrate, and mineral turnover (50). With BGMA it has been demonstrated the influence of human behavior through the CNS-VN-ENS, so it has been proven that a decrease in the activity of the regulatory pathways relates to a higher risk of developing stress. (51).

1.3.3 Neuroendocrine/enteroendocrine pathway

This pathway works through direct and indirect signaling by chemical transmitters - metabolites produced or modified by GM (acetic, propionic, butyric acids, defined as short-chain fatty acids (SCFA), bile acids (BA), some of the active substances synthesized by bacteria and are predators of neurotransmitters may be exposed by the levels of GM. Enteroendocrine cells (EECs) are nearly one percent of the amount of all GIT epithelial cells. EECs are essential for supporting intestinal functions because of their production of signaling substances (82). It is known twelves kinds of EECs that contain various combinations of molecules. So-called L cells produce regulatory peptides that impact insulin/glucagon balance after food intake (83). A row of receptors, which are responsible for hunger/satiety control, was found on L cells. They can be triggered by products of microbial decay and vital activity, as well as bilic and short-chain fatty acids. (84). ECCs are responsible for producing of majority of tryptophan derivates (5-HT) in the body. 5-HT activates nerve receptors in the GIT and relates a variety of gut functions, such as electrolyte outbreak, motility, reactions of pain, and inflammation (85). An example of bidirectional BGMA interrelations is produced by the ECCs in GIT of tryptophan derivates, of which 5% are stored in the brain, and 95% are in enteric neurons and

chromaffin cells. This production is regulated by short-chain fatty and secondary bile acids synthesized by *Clostridial/Corynebacterium*, which are able to increase their effort when the food supply of tryptophan is high (86). When ECCs in GIT produce enough tryptophan derivates, they signal through synapse-like connections to afferent neurons of ANS and ANS are able to start deliberation of tryptophan derivates into the intestine to impact GM (87, 88).

GM representatives can produce by themselves or influence their synthesis/metabolism of all essential amino and short-chain fatty acids such as tyrosine derivates (adrenalin, norepinephrine (NE), dopamine (DA)), glutamate derivates (gamma-aminobutyric (GABA), gamma-oxybutyric acid), tryptophan derivates (5-HT, serotonin, and melatonin), histidine derivates (histamine), SCFA and Phosphatidylcholine derivates (butyric acid itself, acetylcholine) so on. Production of the serotonin precursor tryptophan and glutamate have waking-promoting functions (89). For example, serotonin is generated by *Escherichia*, *Candida, Enterococcus,* and *Streptococcus* (90), GABA is produced by *Bifidobacterium* and *Lactobacillus* (90-92), DA is generated by *Bacillus* and *Serratia*, NE is synthesized by *Escherichia* and *Saccharomyces,* acetylcholine is generated by *Lactobacillus*(77).

1.3.3.1 Tryptophan and 5-HT derivates (serotonin, melatonin)

Serotonin, a well-studied neurotransmitter in depressive illness, is involved in the control of sleep-wake architecture. Its amount in the intestine is dependent on GM, which was confirmed by an animal study that compared levels of germ-free and convectional mice (93). Serotonin can shorten NREMS length at rest time and reduce the amplitude of behavioral CR (94).

Melatonin is important in regulating the sleep-wake cycle. It is produced not only in the skin and pineal gland but also in the intestines. Postprandial production of melatonin in GIT can be

hundreds of times greater compared with pineal gland secretion. (95). Melatonin promotes TST, reduces SF (96), and relieves sleep initiation (or re-set it in shift workers or jet lag) (97). A decrease in melatonin levels that accompanies inflammation processes negatively affects sleep. (98). Melatonin is taken as a dietary supplement by people suffering from insomnia. (99).

1.3.3.2 Glutamate derivates (GABA, orexin)

Although glutamate itself has no direct effects on sleep, it can cause a feeling of satiety and indirectly impact CR-supporting process S (homeostasis). Glutamate derivates gamma-oxy-butyrate and gamma-amino-butyrate are recognized as sleep promotors and may be considered a significant factor in BGMA. Despite only oxy-butyric acid is able to penetrate through the hemato-encephalic barrier, we are talking about GABA action when meaning both (gamma-oxy-butyric acid and gamma-amino-butyric acid). GABA has evidence to reduce sleep latency (SL), improve sleep efficiency (SE) in subjects with CID, and alleviate sleep disturbances initiated by caffeine. It can prevent anxiety and can balance mood (60). It can be produced by row species in GM, particularly the *Lactobacillus* and *Bifidobacterium* (100). *Limosilactobacillus reuteri* and *Bifidobacterium breve* by producing GABA, impacted QoS, and improved sleep latency (SL) and sleep time (TST) in students-acute insomnia trials. (101)

Although Orexin A is not glutamate derivate, it is a neuropeptide, named after the “orexis,” translated as “appetite”, thanks to the potential of orexin, like glutamate, to initiate food consumption (initiation of homeostasis phase S in CR) (102). Initially, it was described as a regulator of eating search patterns. Further studies confirmed that lack of orexin/orexin–receptor may lead to narcolepsy in researched animals. So, orexin is essential for sleep regulation. (102). It was found in the CNS and in the intestine, for example, the EEC of the gut, where it can be produced. (103, 104). Clinical trials have confirmed that “daridorexant”, which is an

orexin-receptor antagonist, has benefits for insomnia treatment. The studies supposed orexin produced in EEC of the gut has an impact on sleep through BGMA (103-105).

1.3.3.3 Tyrosine derivates (adrenalin, NE, DA)

All three derivates have a potent impact on the sleep-wake cycle, as wake-promoting compounds. Although some amount of all these compounds are produced by GM, nonetheless, their basic source and actions (NREM to REM sleep phase transitions for DA and awake for NE and adrenaline) are being implemented in the ventral tegmental area of the hypothalamus by the cholinergic neurons and in lateral hypothalamus by orexin neurons. The key marker here is DA/NE antagonist “adenosin”, which is increased in the mentioned-above brain areas during long-term activity and is decreased after sleep. “Adenosine” is proposed as a sleep-inducing agent. There is no evidence of GM's direct impact on this pathway, however, the indirect impact may be through HPA and cortisol (28, 61, 81).

1.3.3.4 Other (histidine derivates, SCFA, BA,)

Histamine has also been reported that GM can synthesize histamine (for example, by *M. morganii* and *L. vaginalis*). High levels of these bacteria in the intestine are correlated with asthma grade (106). It acts also through immune (local inflammation) afferent and efferent neural pathways on the sleep/arousal. Histamine H1 receptor antagonist LY2624803 was proposed as an insomnia treatment alternative for clinical trials (107).

Both SCFA and secondary bile acids (sBA) act as gut anti-inflammatory agents. SCFAs are one of the most critical chemicals signaling microbiome metabolites, which are synthesized from carbohydrates by many species of GM. Short-chain fatty acids enhance the mucosal barrier, by stimulating mucus secretion (108, 109) and decreasing intestinal permeability by impacting on the tight junction assembly (110, 111). They can modulate immune cells themselves (112), including the inflammatory reactions and neutrophil chemotaxis (113), by suppressing of mononuclear cytokine production (114), and through T-cell (reg/helper) differentiation regulation (115, 116). All listed above SCFA's actions improve the overall

intestinal condition. When the level of SCFAs is decreased, it can diminish sleep duration. (117). Butyrate can regulate gene transcription and have an antidepressant effect in mice, impacting blood mononuclear cell differentiation and activating. (118). Preclinical animal trials demonstrated that SCFA intake leads to clock gene synchronization (67) and may also affect peripheral clock adaptation. (119). It has been found that there is a strong positive correlation between fecal propionic acid quantity and increased sleep duration in infants and between high propionate and decreasing sleep entrance in adults who suffer from CID (120). An increased content in GM of butyrate-producents also has a positive correlation with improved QoS(121).

Some intestinal L cells express surface receptor so-called G-protein-coupled-Bilic-Acid receptors, which is activated mainly by sBA and strongly influenced by microbial activity (122). Bilic Acids provide their antibacterial actions directly due to membrane-solubilizing capability (123). Deficits in gut of BA concentration correlate with inflammation and, as a result, bacterial overgrowth, and intestinal epithelial damage(124). Some studies report that secondary BAs are responsible for overexpression of the clock genes (125).

1.4. Sleep disturbances and gut microbiota.

1.4.1 Biodiversity & *Firmicutes/Bacteroidetes* ratio (F/B ratio*).*

It has been reported that between SE and GM diversity exists a direct correlation (126, 127). A higher amount of *Ruminococcus* and *Blautia* (*Firmicutes*) and a reduced amount of *Prevotella (Bacteroidetes)* presenting in the individuals GM was associated with better QoS, suggesting that QoS and the F/B ratio has a strong direct correlation (121). Results of observational studies on healthy volunteers revealed that the content of *Lentisphaerae* and *Verrucomicrobia* in GM has an inverse correlation to the QoS test`s scores of insomnia subjects. It is supposed that species are related to improved QoS (128). F/B ratio has doubled in healthy subjects' GM content after two nights of sleep deprivation during laboratory trials (129). In another study, the subjects with CID demonstrated a GM disturbance (microbial variety decreasing) and an increase in pyridoxal-

phosphate catabolism and folate production. The same study showed diminished inflammatory precursors` synthesis and decreased the F/B ratio for the insomnia group in comparison with the control group (130). These data are in contrast with the results of a previous study (129) that reported about increased F/B ratio after sleep deprivation or restriction. Moreover, in the study (130), *Bacteroides* and *Clostridiales* were defined as marker microorganisms, allowing the recognition of subjects with CID among all subjects. A study comparing healthy volunteers and acute and chronic insomnia groups confirmed that the sleepless subjects' GM was decreased in bacterial richness and variety. It has an exhausting of anaerobes and SCFA-synthesizing microorganisms. For this study, the following species were defined as markers for acute sleeplessness: Lachnospira, while *Faecalibacterium* and *Blautia* were defined as marker for CID (131). It was confirmed in other studies that phylum *Bacteroidetes* was inversely correlated with wakefulness after sleep onset (WASO), SD, and SF compared with the healthy group (127).

However, a variety of cross-sectional research reported that worked in search of correlations between sleep and microbiome are inconclusive as to whether QoS is the reason or result of GM disturbances (132-134); for example, the SR during the five-night study did not demonstrate any remarkable influence on GM (133); moreover, studies that researched the correlation between sleep/wake short-term alteration and GM composition reported about the absence of significant of GM profile changes among the patients.

Chronic disturbances of human CRs, sleep loss, and depression influence the GM metabolism. Long-term disturbances can promote GM variety disturbances to bacterial dysbiosis, which is observed first of all in diminishing the quantity of *Lactobacillaceae* bacteria and the abundance growth of *Enterococci*, *Bacteroides,* the *Ruminococcaceae,* and the *Lachnospiraceae* families populations (67, 135, 136).

1.4.2 Influence of inflammation and dysbiosis

Intestinal microorganisms can decrease gut permeability by reforming tight junctions in protective epithelial layers that cause the

breakdown of the intestinal barrier and entering harmful bacterial metabolites into mesenteric lymph nodes, initiating inflammatory immune reactions, stimulating VN and spinal afferent nerves (60, 137). This is one of the possible mechanisms for creating inflammatory reactions by GM dysbiosis. It is accompanied by indirect nervous system involvement, which can lead to depressive symptoms development, CID deterioration, and be a trigger for metabolic disease (138). Gut microorganisms via LPS cause inflammation or irritation of the intestine, increasing its permeability to toxins, cytokines, and other inflammatory mediators. There are 2 key intestinal markers for gut inflammation to distinguish it from irritative bowel: zonulin and fecal calprotectin. First (zonulin) is known as a protein that impacts intercellular tight junctions, leading to a spontaneous flow of antigens derived from the dietary products of bacteria. A high level of intestinal zonulin altered intestinal permeability can trigger bowel inflammation (139). The second (fecal calprotectin) is known as a neutrophilic Ca- and Zn-binding protein. Discovering calprotectin in feces is evidence of the movement of neutrophils inside the gut caused by increased gut permeability and inflammation. Calprotectin concentration in feces correlated with the level of inflammation and may be used to distinguish IBS (lower concentration of fecal calprotectin) from IBD (high concentration of fecal calprotectin) (140).

1.4.3 Influence of mental illnesses depression/anxiety/cognitive function

It was defined that subjects with different psycho-emotional disturbances had a diminishing number of *Bacteroidetes* and increased amounts of *Actinobacteria* and *Firmicutes* in GM (141). Increasing Shannon index level for GM was also a marker for depressed subjects compared with control. It has been found other prevalence of GM composition in another study: so *Firmicutes* amount was essentially decreased but *Actinobacteria, Bacteroidetes*, and *Proteobacteria* were in higher amounts. *Faecalibacterium* species correlated with healthy individuals, and the lesser, the greater symptoms of depression were discovered in patients. (142). One more trial demonstrated a decrease of *Lactobacilli* and *Bifidobacteria* in GM

of depressed subjects (143).

1.4.4 Data of latest research focused on GM and insomnia relationship

The research on the association of GM composition in MDD subjects and QoS revealed that *Actinomycineae* and *Porphyromonadaceae* increasing prevalence in the MDD group together with relatively decreasing family *Prevotellaceae* and at the genus level increased *Alistipes, Bacteroides,* and *Parabacteroides* together with *Eggerthella* and *Prevotella* diminishing. However, it has been reported that QoS is associated with *Coprococcus* and *Intestinibacter*, but not related to depression`s symptoms gravity. (144).

A clinical trial published in 2021 comparing GM in healthy control with acute and chronic insomnia showed that at the phylum level, *Actinobacteria* was overrepresented in subjects with chronic insomnia compared with control; in contrast, *Firmicutes* were decreased in the GM of subjects with acute insomnia compared with control group of subjects. Patients with CID had decreased *Feacalibacterium, Prevotella*, and *Roseburia* and increased *Blautia* and *Eubacterium* compared with healthy control; *Bacteroides* was increased and *Lachnospira* decreased in subjects with acute insomnia compared with healthy control (131).

Comparison GM (metabolite and microorganism profile) of subjectswith insomnia (PSQI more than 11) against healthy control (2022) defined genera *Fusicatenibacter, Gemmiger* as main species in sleeplessness; however, families *Ruminococcaceae, Bacteroidaceae* were diminished, but *Prevotelaceae* became higher (145).

A study of “functional connectivity strength” in CID related to QoS and GM diversity (2022) demonstrated that in Chronic insomnia subjects *Bacteroides* were in abundance, as well as *Blautia, Faecalibacterium, Prevotella*. They discovered a significant correlation of *Intestinibacter*, *Feacalicoccus, Lachnospiraceae* and alterations of brain activity in the area of the left superior parietal

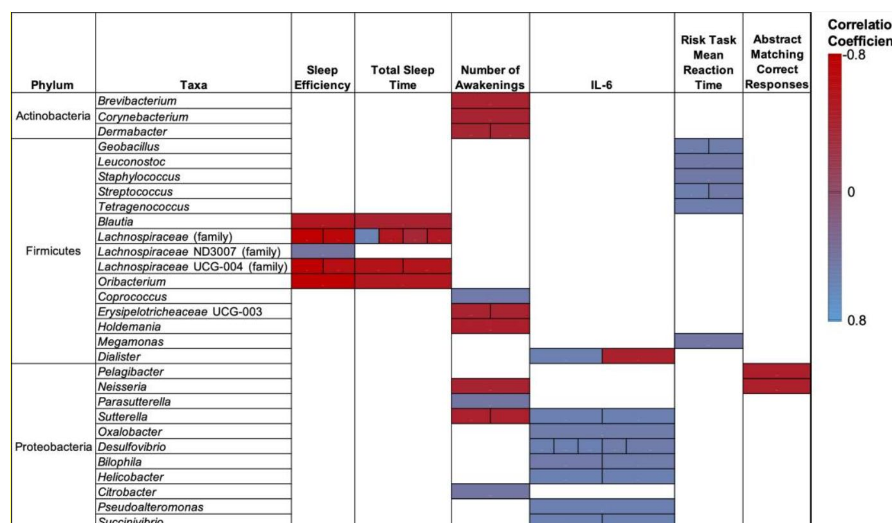
gyrus in CID subjects. *Alloprevotella* and *Feacalicoccus* have a correlation with an evaluation of emotional status and QoS. *Intestinibacter* correlated only with QoS (27).

Another trial considered the interplay of brain function, microbiome, and insomnia revealed a negative effect of *Lactobacillus* on mood and the left fusiform gyrus in the brain. The same study reported a positive correlation between the genus *Ruminococcus gnavus* and *Erysipelatoclostridiuum* in GM ofinsomnia subjects and a negative correlation with *Coprobacter* (146).

Research results on the microbiome connection with insomnia and cognitiveperformance (2022) showed that *Bacteroides* were the dominant taxon for insomnia subjects. In contrast, *Firmicutes* and *Proteobacteria* were predominant in the control group. *Lachnoclostridii* were associated with higher SE and cognitive performance (37)

Interesting data about microbiota's impact on sleep has been demonstrated in work (127). Results are provided in the tables below:

Table 2: Assessment of correlation between bacterial species and the sleep parameters, IL-6. This Table is from an article (127)



Phylum	Taxa	Sleep Efficiency	Total Sleep Time	Number of Awakenings	IL-6	Risk Task Mean Reaction Time	Abstract Matching Correct Responses
Actinobacteria	*Brevibacterium*						
	Corynebacterium						
	Dermabacter						
Firmicutes	*Geobacillus*						
	Leuconostoc						
	Staphylococcus						
	Streptococcus						
	Tetragenococcus						
	Blautia						
	Lachnospiraceae (family)						
	Lachnospiraceae ND3007 (family)						
	Lachnospiraceae UCG-004 (family)						
	Oribacterium						
	Coprococcus						
	Erysipelotricheaceae UCG-003						
	Holdemania						
	Megamonas						
	Dialister						
Proteobacteria	*Pelagibacter*						
	Neisseria						
	Parasutterella						
	Sutterella						
	Oxalobacter						
	Desulfovibrio						
	Bilophila						
	Helicobacter						
	Citrobacter						
	Pseudoalteromonas						
	Succinivibrio						

The authors' main idea is that *Bacteroidetes* and *Firmicutes* abundance have a direct correlation with SE. Still, *Bacteroidetes* alone are inversely correlated with SF, WASO, and an abundance

of the *Actinobacteria* has an inverse correlation with the arousal episodes. It has been obtained contrary results, enlarged *Actinobacteria* amount is directly correlated to better QoS, but *Lachnospiraceae* are inversely correlated with SE and TST.

The abundance of various producing GABA species has a positive correlation with QoS (e.g., higher SE, lower WASO, lower number of awakenings), bacteria producing serotonin (particularly taxa *Corynebacterium)* play an essential role in promoting sleep through the BGMA, *Corynebacterium*, and *Brevibacterium* has an inverse correlation with the frequency of arousals by producing somnogenic glutamate. *Lachnospiraceae* family producing SCFA , including *Blautia, Oribacterium, and*, *Coprococcus* inversely related to QoS. It was reported a role IL-6 is an initiating sleep factor, which positively correlated with microbiome diversity and TST. However, in other sources, high serum concentrations of IL-6 are usually connected with inappropriate QoS.

Due to the high difference in results, further studies will be aggregated in thetable to demonstrate and analyze the tendency for Microbiome changes and,according to the summary, propose some corrections and ways of control forsleep and microbiome status.

1.5. Short review of GM correction intervention.

1.5.1 Lifestyle, dietary habits – gut microbiome

Intervention into Gut microbiota, possible treatment, and further trends:

Contemporary sleeplessness therapy has definite long-term risks and limited effectiveness. Sleeping medications are usually selected, due to their quick action. These medications have sedative or hypnotic effects and have been used by many people for many years, but all these medications have adverse side effects. The most common are tiredness, dependency, and disturbances in the activity of the brain (21). It may be due to genetic predisposition or be caused by mental disorders or sleep alteration effects of

prescribed psychopharmacology. Another possibility to improve sleep is the effective correction of insomnia co-morbidities, which is a reason for sleep disturbances (for example, PTSD, bipolar and mood disorders) (147). So, in cases when insomnia appears due to a mental disorder or as an aggravation of pain-induced or mentally impacted disorder - the primary focus should be on psychiatric disease treatment; sleep improvement is like a bonus to successful therapy of primary disease. For insomnia treatment, the first line is cognitive behavioral therapy (CBT-I) (148). However, the efficacy of this kind of therapy is variable, especially for insomnia with a comorbid psychiatric disorder, and varied from 30 to 60% (strong respondents and partial respondents (149). Besides, it is time-consuming, and only 60% of respondents with insomnia started CBT-I have completed the course. Even in the case of primary insomnia and completion of CBT-I, there has been nearly 80% re-appearance of insomnia in 16 months of Follow-up (150). Patients often find it requires a lot of while, is complex, and is less successful than drug therapy. According to source data, the number of unsuccessful cases of CBT-I before successful results varied from 35 to 45%. (21). One of the reasons may be that CBT-I works with perpetuating factors of insomnia. Still, less with predisposing and precipitating, so inflammation and metabolite misbalance are not impacting and can lead to insomnia return.

Sedative and hypnotic (barbiturates (BRB) and benzodiazepines (BZD)) are commonly used for moderate insomnia treatment. They work as agonists of γ-aminobutyric acid -A – BZD and even can be GABA-mimetics (high dosage BRB)

Other medications are serotonin (5-HT) receptor agents (other anxiolytics like buspirone, Z-drugs) or selective agonist melatonin receptor (Ramelteon), antihistamines (Diphenhydramine, Doxylamine). They have a row of adverse events, like diarrhea, headache, loss of dreams/altered dreams, sleepiness, and appetite changes, that a little bit decrease their benefit and limit their use. Besides, hypnotics can also cause IQ and memory deterioration,

driving impairment/motor vehicle accidents, dependency/withdrawal, partial arousal from sleep, emotional and mood disorders, falls, infection, fractures, and risks of death. (151)

Moreover, insomnia Medications can alter gut microbiota and, as a result, dysbiosis and secondary adverse effects related to this dysbiosis. So, Nitrazepam intake stimulates *E. coli*(152), and it can induce teratogenicity (153)

Intake of Flunitrazepam can lead to GM alterations with pathogenic bacteria predomination such as *Aeromonas, Paracoccus, Shewanella* which are related to *Proteobacteria*. (154)

GM can stimulate medication metabolism, diminishing sedative effects or leading to toxicity and high adverse events levels. (155)

Recent studies demonstrated that long-term sedative and hypnotic use for insomnia treatment can increase the risk of carcinogenesis(11). Another study (156) reported that the short-term psychopharmacological treatment for MDD, emotional disorder, and CID, can facilitate the microbiota recovery to a healthier condition in old subjects. *Bacteroides* and *Parabacteroides*, involved in the tryptophan turnover, were increased for groups receiving medications for mental health status. Researchers supposed that the GM remodeling and derivates of tryptophan, produced by GM, may explain a patient's anxiety, depression, and insomnia remission.

For psychotropic medications, bidirectional interaction between drugs and the GM is described in (157)

A new approach to insomnia treatment is the impact on GM to normalize microbiota balance and diminish precipitating factors of insomnia. There are the following approaches to GM Intervention: diet and dietary Habits can significantly impact GM; however, like a lifestyle change and CBT-I, diet and dietary habits change is complicated for the patient task and requires high internal motivation, time, and external support.

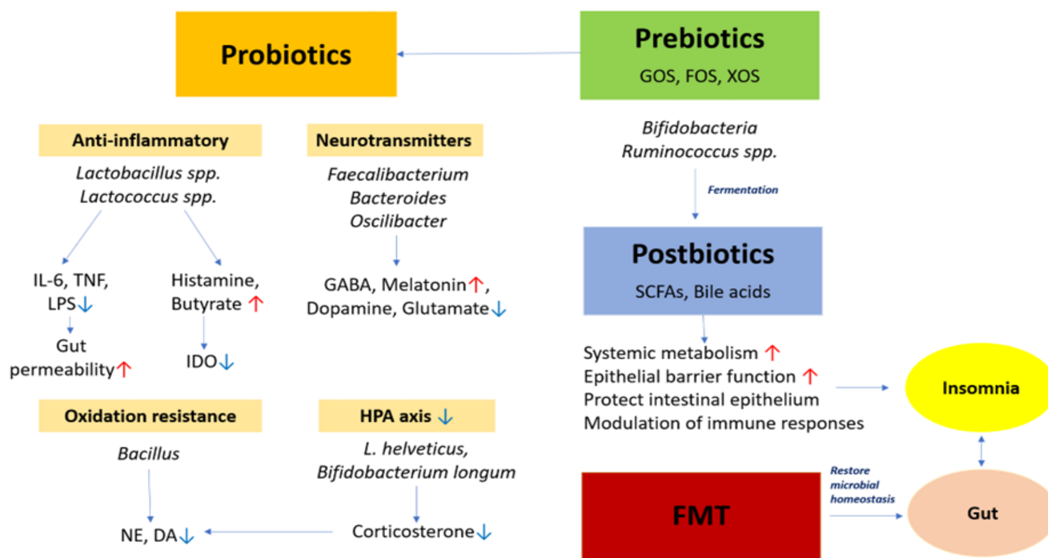


Figure 5. Insomnia therapy based on BGMA mechanism. This picture is adapted from (158)

It has been discovered that pro-, pre-, postbiotics and FMT may generate beneficial effects on insomnia. Probiotics provide their effect through ananti-inflammatory effect on the gut (by SCFA and sBA), decreasing LPS and gut permeability, modulation of neurotransmitters (especially GABA and melatonin) resistant to oxidation, and impact on HPA axis (lowering cortisol) – CR. SCFA is produced by bacterial fermentation of prebiotics, impacts on clock genes (CR), stimulates immunity (BDNF and microglia growth), and antagonistic pro-inflammatory bacteria in the gut.

Postbiotics improve epithelial barrier function, human microbiota regulation, and modulation of immune response and systemic metabolism, especially though theENS-CNS 5-HT system decreases DA, Glutamate, and Histamine.

Prebiotics were introduced in 1995 by Gisbson and Robenfroid. According to their definition, prebiotics is “a non-digestive food component that benefits the host by specifically promoting the proliferation and activity of a restricted number of bacteria in the colon and improves human health.” Another synonym that has been proposed for prebiotics is “bifidogenic.”

They are non-viable components that stimulate the development of useful microbiota at the same time, suppress noxious bacteria; thus, they are able to impact the GM. Prebiotics could enhance species as well as *Bifidobacteria Lactobacilli*, which are usually used as probiotics in amount and vitality, serving them as food. Plant-derived dietary supplements commonly used as prebiotics include inulin, gums, fructo-oligosaccharides (fructo-OS), and fiber. The pharmaceutical industry purifies oligosaccharides (OS) and offers prepared inulin, lactulose, mannan-OS, fructo-OS, xylo-OS, and fructan as therapeutic prebiotics along or as a combination with probiotics. (159)

1.5.2 Probiotics, symbiotics

Probiotics – the word means "for life" from the Greeks. The term was used initially in 1954 by Ferdinand Vergin. Probiotics are defined as "nonpathogenic living organisms which, if administered in adequate amounts, confer ahealth benefit on the host.

Evidence-based studied species that can convert dysbiosis to normal gut microbiota. Although the mechanism by which probiotics benefit the host cell is unknown,they do so. Probiotics can alter gut pH and boost the immune system.

Additionally, they may be used to treat various medical conditions, such as IBD. This facilitates the colonization of bacteria in the gut. (160). The most often used genera as probiotics are *Bifidobacterium, Lactobacilli,* and *Saccharomyces*. It has been used to treat IBS, IBD, Crohn's disease, and dysbiosis caused by *Helicobacter Pylori, Clostridium dificile*, and *Escherichia coli*. Symbiotics refer to food ingredients or dietary supplements combining pro- and prebiotics in the form of synergism. It is the most perspective formulation for soft impact on GM in scope of efficacy and anticipated effect on Microbiota and human health.

One advantage is that several strains of helpful microorganisms are used as probiotics (with different metabolic profiles), and they are supported by selective food for these microorganisms, which can

help their colonization in the gut. It will be discussed furtherin the article row of research with prebiotics, probiotics, and symbiotics to assess their role in GM modification and impact on sleep.

1.5.3 FMT

FMT is the administration of a solution of fecal composition from a healthy donor into the intestinal tract of a recipient to directly alter the recipient's GM and confer a health benefit. The feces are prepared by mixing with water or normal saline, followed by a filtration step to remove particulate matter.

The mixture can be administered through a nasogastric tube, nasojejunal tube, esophagogastroduodenoscopy, colonoscopy, or retention enema. FMT is utilized in patients with difficult-to-treat or resistant *Clostridium difficile* infection in clinical practice.Meanwhile, FMT has also shown promising results in the treatment of IBD, IBS, metabolic diseases, anorexia nervosa, malignancies, autoimmune diseases, multiple sclerosis, cancer, cardiovascular diseases, and neuropsychiatric disorders. (161)

2. Hypothesis formulation

Based on contemporary knowledge in the field of sleep disturbance, GM condition, and understanding of its interplay, it has been performed a literature search of formulation (prebiotic and/or probiotic) that has a positive impact on GM and normalized sleep. Considering the fact that sleep disorders are differing (chronic insomnia (lack of sleep), CR disturbances (sleep initiation difficulties), and OSA (SF) and also taking into account that these disorders accompany often central obesity, metabolic syndrome, or/and age with or without bowelmanifestation like IBD/IBS. The purpose of the review is to define the following aspects:

1. Review articles related to how different food supplementals, diets, and prebiotics impact GM.
2. Analyze what probiotics were used for GM intervention and what results were achieved.
3. Combine the results according to known mechanisms of impact of GM on SD-I. For this purpose, it makes sense to organize strains according to their ability to secrete key metabolites/active compounds or their ability to cause desirable changes in the host, which can impact Sleep.
4. Review probiotics clinical studies related to sleep disorders. This review will be organized according to microbial strains but subdivided into two periods 2014-2019 and 2020-2023 to understand what was changed in approach to these types of studies.
5. In the discussion part, some thoughts related to further investigations and further trends of SD-I and GM development will be proposed regarding the rationale of group homogeneity (inclusion/exclusion criteria), primary and secondary endpoints (examination and its objective assessment) proposed intervention (and comparators if applicable) and observation time.

3. Methods and Materials

The materials of this review are articles found in PUBMED. The topic of interest was insomnia and microbiome. Its interplay, relationships, and modern conception of the MGB axis establish the most obvious evidence-based relations articles (reviews, systematic reviews, and animal and clinical studies). The first search was done using the keywords" insomnia" and "microbiota"; further, we also used the words "sleep disturbances" instead of insomnia and "microbiota" instead of "microbiome." Counting the focus of conception, the most prominent articles were used as a cross reference, and relevant references were identified and added to the reference list. Some of the articles were not included in analyses due to limitations to the topic of my search. Reviews of clinical studies where sleep disorders or microbiome (or microbiome components such as microbiota, metabolome, virome, fungiome, or intestinal components such as intestinal epithelium) were not in focus have been excluded or have been used only as a reference in the introduction part. Additional search has been done for "insomnia/sleep disorders" and "prebiotics" "insomnia/sleep disorders" and "Probiotics" to add articles containing clinical studies for this topic.

Some results of clinical studies related to subjects' GM intervention such as diet, food supplemental, prebiotics, probiotics consumption, and its impact on GM and SD-I are going to be presented in tables with discussion only of the primary considerations due to the massive size of data and variety of found relation (only impact on strains, metabolites influenced on Sleep will be selected).

4. Results with graphs

4.1 Review of studies with prebiotics

First, because food due to its constituents, which are potentially prebiotic can significantly impact GM, affecting human homeostasis through biochemical and fermentative breakdown of food bolus, which occurs during the process of digestion and bacterial fermentation of food, it makes sense to present consolidated results of the impact of dietary habits on GM.

Table 3. Changes in gut microbiota, mucus layer, and immunity depending on different types of diet. This Table is adapted from (162, 163)

Diet/Typ of bacteria	Vegan/Vegetarian diet	Gluten-free Diet	Ketogenic Diet	Low Fodmap Diet	Western Diet	Mediterranean Diet
Gut microbiota variations						
Actinobacteria	↓ Bifidobacteria	↑ Bifidobacteria	↓ Bifidobacteria	↓ Bifidobacteria	↓ Bifidobacteria	↑ Bifidobacteria
		↓Corobacteriacea				
Firmicutes	↑Clostridium clostridiof	↓Vellionellaceae	↓Eubacteriun rectale	↓Ruminococcus gnav	↑Ruminococcus torques	↑Lactobacillus
	↑F. prausnitzii	↓Ruminococcus bromii	↓Dialister	↓Clostridium	↓Roseburia	↓Clostridium
	↓Clostridium cluster XI	↓Roseburia	↓Firmicutes	↓F.prausnitzii	↓Eubacteriun rectale	↑Lachnospiraceae
		↑Veillonellaceae			↓Ruminococcus bromii	
		↑Clostridiaceae			↓Lactobacillus	
		↓Lactobacillus				
		↓Clostridium Museburense				
		↓F.prausnitzii				
Proteobacteria	↑Klebsiella pneumonia	↑Enterobacteria (E.coli)	↑Enterobacteria (E.coli)		↑Enterobacteria	↓Enterobacteria
	↓Bilophila		↑Desulfovibrio spp		↑Bilophila	
Bacteroidetes	↑Bacteroides/Prevotella		↑Parabacteroides		↑Alistipes	↑Bacteroidetes
	↑B.thetaiotaomicron		↑Bacteroidetes		↓Prevotella	
	↑Bacteroidetes				↑Bacteroides	
Verrumicrobia			↑Akkermansia	↑Akkermansia	↑Akkermansia	
Overall microbiota change	Microbiota diversity	↓Healthy bacteria abundance	↓Total bacteria abundance and diversity	↓Total bacteria abundance	↓Microbiota diversity	↑Microbiota diversity and stability
Mucus layer			↓		↓	
SCFAs		↓	↓		↓	
Epithelial barrier			↓		↓	
Systemic circulation	Antimicrobial and anti-inflammatory effects?	Decrease of immunomodulatory role via cytokine modulation: IL-10, TNFα, IFNγ	Gut bacteria enhance hippocampal γ-aminobutyric acid and glutamate levels	Antimicrobial and anti-inflammatory effects?	Pro-inflammatory IL-17, TNFα, IFNγ	Anti-Inflammatory IL-10, IL-22

As it has been described in the table, diet can change not only GM but also protective gut properties and immune system. For example, a Western diet leads to a compromised mucus layer of the gut, with or without increased permeability, and results in a “leaky gut” and “unhealthy” GM because a diet contains a small quantity of fibers, excess fat, and refined carbohydrates. Gut alteration relates to major Depression and decreased QoS. Whereas Mediterranean high in fibers, polyphenols, and unsaturated fatty acids promotes good microbiome taxes that can metabolize food to anti-inflammatory SCFA, reducing depression and improving QoS (164)

The richness and variety of products, as well as the search for an exquisite unique taste, allows not only the use of raw or thermally processed vegetables containing natural prebiotics but also

increasing the level of prebiotics through pre-fermentation of products of plant and animal origin, increasing the level of such carbohydrates as psyllium, galactomannan, arabinose-OSs, lactose, undigestible starch and dietary fibers which stimulates forming of the beneficial GM. (165-168)

The concept of prebiotics as a resistant ingredient that stimulates the amount and vitality selectively of helpful bacteria in the gut, improving human health, has evolved over time. It led to the vast number of food products; compounds were defined as prebiotics: first were resistant OS (NDO): inulin, polydextrose, lactulose, fructo-OS, galacto-OS. Then xilo-OS, isomalt-OS were added as emergent bifidogenic. Later maltodextrin and hydrogenated OS (mannitol, sorbitol) were added as those food ingredients, which have confirmed effects to improve health and GM. Now it is considered resistant starch-rich whole grains and partially fermented cellulose, β-glucan. They reveal their potential to generate short-chain fatty acids and may considered bifidogenic (169)

Currently, all prebiotics are differed by source (from seed, milk, vegetable, soybean, wheat, honey, rye), chemical compounds, types, extraction method, and polymerization degree (3, 10 or more sugars in the chain), but that is the most important by function (or action on GM): Stimulation both *Lactic acid bacteria* (LAB*)* and *Bifidobacteria*, Selective stimulation of LAB, butyrate production, modulation of microbial diversity(170). Prebiotic action estimation should include three phases of clinical studies: digestion, fermentation, and analysis (171, 172) As a result following parameters are evaluated: which bacteria are stimulated, which SCFAs are synthesized and how microbiota profile is changed in terms of abundance and/or diversity of helpful strain in fecal lavage (173, 174).

The list of benefits of using prebiotics and their potential application for gut and human health is wide. It may be cholesterol removal, cancer prevention, gut maintenance, and immune reaction

modulation (170). The following impacts of prebiotics are essential for the review: QoS improvement and gut maintenance that can be reached through SCFA production, GABA production, and anti-inflammatory action per BGMA.

In this term the most perspective prebiotics related to galacto-OS/fructo-OS, which are produced by milk fermentation by strains *Lactobacilli (L.) acidophilus, bulgaricus and rhamnosus,* combined with *Bifidobacterium lactis,* and in some cases with *Str. thermophiles (175, 176)*

Fructo-OS and maltodextrin derived from Soy demonstrated improvement of the usage of simple carbohydrates with germination stimulation of *L. acidophilus, casei* and*, Bifidobacterii* decreasing gut alteration and preventing of colon inflammation (177).

The stimulation by the inulin of growth of *Faecalibacterium prausnitzii* , *Bifidobacterium adolescentis*, has demonstrated a food value on the GM (178) and for children, it not only prevents or at least decreases bowel inflammation (IL6+SCFA action) but also improves QoS (GABA action) (179)

Most of the reviewed prebiotics studies are focused on food digestion, fermentation, analyses of metabolite and microbiota profiles, and then the impact of GIT changes, which is accepted as a prebiotics assessment methodology in the industry. The main accent is what strains are stimulated and how they impact the metabolome. Human health is considered only through the prism of GIT condition and metabolome and microbiota profile because prebiotics mainly do not act on the gut or organisms themselves but only stimulate the growth of selected beneficial bacteria.

That is why it makes sense to use prebiotics only as supplemental to selected beneficial microbial strains that positively impact QoS, GM, and metabolome.

Separate words should be said about other active compound such as Polyphenols, Flavonoids, and other active ingredients (alkaloids, oil, ethers, organic acid), which are related to tees, medical plants, wine, and other herbal/mushroom food products.

They are not classic prebiotics but can impact the GM (metabolome and microbiota) and human health, particularly mental health and sleep, through BGMA. At least several articles and reviews were presented in PubMed for the last three years (13, 180-182). Despite these articles and reviews containing interesting data about a variety of clinical trials that demonstrated the impact of active compounds of herbal products on GM and insomnia itself or by BGMA, these data are out of the scope of the review and considered non-relevant. The same approach was used for the study, where only one metabolite or psychoactive/sleep initiative compound (melatonin, SCFA, or orexin receptor antagonist "daridorexant" or H1-antagonists) was prescribed for insomnia treatment.

Due to most prebiotics studies focused first on microbiome changes and only then on host changes, it makes sense that search results be presented according to accepted industry/research requirements:

	Microbial diversity	Bifido-bacteria	Lacto- bacilli	Bacte- roides	Alistipes	Bilophila	Clostri-dia	Ose-buria	Eubacterium Rectale	Faecali bacterium	Akker- mania	Rumi-nococcus	References
Animal protein	↑	↓↑		↓↑	↑	↑	↑	↓	↓↑				(1, 11, 16)
Whey protein extract	↑	↑	↑	↓			↓						(12)
Pea protein extract	↑	↑	↑										(13)
High saturated fat			↓	↑		↑	↑			↑			(7, 36)
High un-saturated fat		↑	↑								↑		(7, 14)
FOS Sucrose/ Fructan		↑		↓↑						↑		↓	(15, 26)
GOS/Lactose		↑	↓	↓			↓						(18)
XOS		↑	↑				↑		↑				(29, 30)
Artificial sweeteners		↓	↓	↑			↓						(19, 28)
Fiber as prebiotics	↑	↑	↑				↓						(20-22)
Resistant starch	↑	↑	↑					↑	↑			↑	(20, 21, 23)
Polyphenols		↑	↑	↓			↓						(15, 27)

Table 4. Effect of food/prebiotic content on GM

Results of a search in PubMed articles with prebiotics and insomnia gave 76 entries, where nearly 20 are out of topic, about 15 are reviews included prebiotics and probiotics, and the same amount is focused on GM, metabolites, ten related animal studies, and prebiotics preparations, last ten articles related to eczema,

psoriasis, postoperative pain relief, IBD, DM2 (183), infants (184), (185) fibromyalgia syndrome (186) where the quality of life and sleep improvement was explained by diminishing serum cortisol level and decreasing of bowel inflammation by SCFA production and anti-inflammatory cytokines (IL-6). In conclusion, further research should be focused on microbial strains' sleep-associated metabolites and active compounds involved in sleep and acting through BGMA.

4.2 Review of studies with probiotics.

Focus has been done on search studies where probiotics were administered to alleviate insomnia; however, an integrative search showed that there were not many articles related to Probiotics and sleep disorder/insomnia. Nearly 70 entries of words “probiotic” and “sleep” or “insomnia” or “sleep disorder” were found.

All processes that are responsible for probiotics' useful actions are not yet clear, but it looks like depend on strain and a variety of factors: several - to antagonism to pathogenic bacteria through the production of suppressing agents, others - to intestinal mucosa affinity, permeability or the gut epithelial layer improvement, last ones - from the immune response stimulation.

Before reviewing clinical research found in PubMed, it is worth separating this multifactorial task (to define the good combination of microbial strain for alleviation of insomnia on subtasks/separate blocks:

1. When use of probiotics is effective? (list of disorders and possible mechanisms)
2. Which microbial strains are beneficial and for what situations? (list of strain and evidence-based actions on gut and host)
3. What metabolites produce each beneficial strain, and should this strain be suppressed or stimulated? (What are strains, which produces metabolites/active compound effects on sleep/awake)
4. What comorbidities has insomnia? And if it will be treated, how it will impact on sleep? (MDD, stress, anxiety, metabolic

syndrome/obesity, IBS, age, cognitive performance)

5. What demonstrated current clinical studies, and what are the following steps to provide the best care for subjects with insomnia?

As has been written above, the term “probiotics” appeared in the last century, and it was initially related to bacteria administration as a way to prevent “dysbiosis” by substitution of pathogenic bacteria with helpful ones. So, initially, probiotics were used to interrupt dysbiosis development in cases, where subjects have risk factors such as acute GIT disturbances, long-term antibacterial treatment, etc., and as agents allowing to convert dysbiosis to eubiosis. The multiple studies gave evidence-based confirmation of the positive actions of selected strains of bacteria on GIT disturbances caused by pathogenic microflora diarrhea (187), connected with antibacterial treatment diarrhea (188), *Clostridium difficile* and *Helicobacter pylori* infections (189), or by gut inflammation: IBD(190), chronic constipation (191), necrotizing enterocolitis(192), celiac diseases(193). This approach was based on the conception of reshaping/rebalancing intestinal microbiota, improving intestinal absorption, increasing gut barrier function, decreasing intestinal inflammation, and modulating the immune response by suppressing/replacing harmful bacteria and recovery of the gut mucus layer, intestinal epithelium, and stimulation anti-inflammatory cytokines. Probiotics proved their effectiveness for listed GIT disorders and this kind of treatment is understandable and still actual.

Later indications for probiotics administration were updated for pathological overweight therapy(194), metabolic syndrome(195), DM2-related neuropathy(196), mild and moderate depression(197, 198), anorexia nervosa(199), multiple sclerosis, Alzheimer's disease, Parkinson disease, autism spectrum(200), schizophrenia, reducing carcinogenesis (201) and cardiovascular diseases treatment. This approach was based on the microbiome’s development and bidirectional BGMA conception, and despite

promising results, it is being researched now.

Last study data about GM and the interplay of resident microflora with neuropsychology, immunology, and biochemistry have started to play a very crucial role in further investigational search and treatment modes development. The caloric food intake and its content (diversity, balance, and presence of natural pre- and probiotics) have a great impact on the intestinal bacterial composition (microbiota), gut active compounds (metabolome), and its component genes (microbiome). New challenges for probiotics applications become the SARS-CoV-2 coronavirus pandemic (202) and insomnia.

Let`s consider in more detail the most studied strains of human resident microbes, which can be used as probiotics, and their actions on the gut, microbiota, and host. Probiotics include the following: LAB, *Bifidobacteria* (203), yeast like *Saccharomyces boulardii*, or *Propionibacteria*, *Bacillus spp*., and selected strains of *Enterococci* and *Escherichia coli* (204).

The results of my PubMed search are presented in the table below:

Table 5. Microbial strains used as probiotics/postbiotics their impact on the host with proposed by the author's mechanism.

Strain used as probiotic/Postbiotics	Study/Articles references	Result	Possible Mechanism
Escherichia coli strain Nissle 1917	(204)	improves the intestinal barrier	GABA regulation
Streptococcus thermophilus	Uremia, Colorectal cancer prophylaxis (205, 206)	anti-inflammatory and antiproliferative activities (207)	Pro-/anti-inflammatory cytokine secretion ratios (IL-1β/IL-10, IL-6/IL-10, and TNF-α/IL-10)
Saccharomyces boulardii	IBS subjects (208)		facilitating the production of lactic acid and group B vitamins
Akkermansia muciniphila	Obesity, Metabolic syndrome (145, 209)	reverse atherosclerotic injuries (they degrade phosphatidylcholine)	gut barrier restoration, and reduce metabolic endotoxemia-induced inflammation
Bacteroides uniformis CECT 7771	(210)	improvement in lipid profiles, leptin, and glucose level	increase in TNF-α production after LPS stimulation
Bacteroides spp	Ulcerative colitis (211, 212)	Activation CD4 + T cells, but it has potential in colorectal carcinogenesis	recognize and decompose of complex carbohydrates, such as xylan, mannan, xyloglucan or starch to SCFA; GABA-producing genus
Bifidobacterium bifidum G9-1	(213)	suppress the production of specific immunoglobulin E and to promote the IgA response	producer of Bc vitamin-folates
Bacillus subtilis	(214)	anti-diarrhea therapies	producer enzymes, such as a-amylase, arabinase, cellulase, b-glucanacase, and DNase.
L. rhamnosus, L. plantarum, L. casei, L. paracasei, L. salivarius, and L. acidophilus	(21)	produce vitamins, enzymes, acetic and lactic acids, some species can convert lactic acid to SCFA	regulation of intestinal inflammation and immune responses, decreasing the pH of the colon, and inhibiting pathogen growth. Secretion GABA
Clostridium spp (Clostridium leptum and coccoides)	IBD and allergy (215)	Promote the generation of TH17 cells	Tregs inducers in the colon
Faecalibacterium prausnitzii	(131, 216)	anti-inflammatory properties	able to produce butyrate and many SCFAs
Lactobacillus brevis BJ20	(217)		Can convert glutamic acid to GABA

LAB and *Bifidobacterii* can regulate intestinal inflammation and immune responses. They are considered as vitamins, enzymes, SCFA, and lactate producers; some species can convert lactic acid to SCFA (205) and secrete GABA (21); they also lower the colon's pH, inhibiting pathogen growth. *Bifidobacteria* as dominant strain for breastfed babies, are able to reduce the IgE and to increase the IgA response; they are the leading folate producer. They cause a reduction in depression and anxiety and a diminishing of serum cortisol levels in subclinical stress studies (206). Bacteroides, also known as the GABA-producing genus, can affect QoS. (156). They also can decompose complex

carbohydrates, such as xylan, mannan, xyloglucan, or starch, to SCFA. *Faecalibacterium prausnitzii*, with confirmed inflammation-reduce effects (207), can synthesize butyric acid (131), which has a big potential for further Chronic Insomnia research.

It has been reviewed all microbial strains proposed for GM modulation, but the focus was on those that can impact sleep through one of the BGMA pathways: Immune (through anti-inflammatory cytokines and SCFA or HPA axis, decreasing cortisol), metabolic (increasing GABA/Glutamate or decreasing DA, NE, or modulating Serotonin and derivates 5-HT, melatonin)

The next step that obviously is needed for planning and assessment of probiotics' impact on sleep considering data on the production potential of neurotransmitters or neuro-hormones by different strains (gut metabolome modulation). It has already been partially discussed in the introduction part and during strains and their action review, but it should be consolidated and summarized.

To consider the possible profile of produced metabolite and their impact on sleep when one or several strains are proposed for the clinical studies for a sleep disorder as non-pharmacological treatment and obviously that mixture should be selected with strains working through different metabolite pathways such as SCFA, GABA, melatonin, acetyl carnitine, avoiding producer of catecholamine’s, serotonin, histamine.

Table 6. Main metabolites related to sleep, associated strains, and studies.

Metabolite	Strain	Study	
Dopamine and norepinephrine	*Saccharomyces, Proteus vulgaris, B. subtilis, Bacillus mycoides, Serratia marcescens*	(77, 219)	impacts on the awakening process/delay sleep onset
Serotonin precursor, tryptophan	*Bifidobacterium infantis, Candida, Enterococcus, Escherichia, and Streptococcus, Lactobacillus casei*	(90, 220, 221)	modulation of sleep-wake architecture, depression
Melatonin, serotonin, 5-hydroxytryptophan, and N-acetylserotonin	*B. amyloliquefaciens*	MDD (98)	Melatonin increased TST, reduces SF, and relieves sleep initiation
GABA	L. *plantarum DSM 19,463, Enterococcus faecium BS5, Oscillibacter, LAB, Acetobacter aceti, L. fructivorans, Acetobactersp., Leuconostocspp., L. delbrueckii,L. fermentum, L. kefiranofaciens,Candida famata, Bacteroides and Candida krusei*(27, 145, 146, 213)	(220)	alleviate anxiety, depression, and stress scale scores
		(222)	improve short-time working memory, cognitive functions, visual-spatial and abstraction properties
SCFA	*Prevotella amnii, Prevotella buccalis, Prevotella timonensis, and Prevotella colorans, Genus Coprobacter, Alloprevotella and Lachnospiraceae, B. thetaiotaomicron or Bacteroides ovatus, Dorea spp* (27, 145, 146, 213)		diminishing gut inflammation and permeability
Butyrate (as GABA-like compound properties)	Genus *Fusicatenibacter, Oscillibacter, Roseburia, Faecalibacterium, Blautia, Coprococcus* were defined as strain-producers of butyrate (131, 145, 156)	(127, 223, 224)	sleep-inducing signal molecule, which enhances sleep alleviating CR disturbances
Acetylcarnitine	genus *Fusicatenibacter*	(145)	regulates sleep rhythm and quality
Cortisol (serum, salivatory)	Lactobacillus casei (220), Lactobacillus rhamnosus (225), Bifidobacterium breve (226)	(198)	Diminishing of stress and acute cortisol, which impact awakening/SF
IL-6 serum	Lactobacillus rhamnosus (225), Lactobacillus reuteri, Bifidobacterium adolescentis (227)	(127, 198)	

So, it makes sense to select beneficial microbial strains, producing metabolites or compounds positively effecting sleep initiation or support (serotonin/melatonin are sleep initiation, GABA/glutamate – is sleep support) or suppressing awakening compounds (such as cortisol or DA, NE) combining it with strains causing anti-inflammatory effect on the gut (SCFA producers or stimulators of IL-6 production) for further review. However, all these metabolites or compounds have an impact not only on sleep but also on gut, mood, and cognitive status. It may be worth considering the use of these strains for the relief of several conditions associated with SD-I.

Following results related to Insomnia and different co-morbidities. Is each separate co-morbidity consequence of Insomnia or reason or there are two independent diseases? Comorbidity and Insomnia have the same mechanism of appearance or different? Should this comorbidity be excluded from potential insomnia studies or not?

The simplest approach is the following: If comorbidity is related to somatic pathologies like DM2 and metabolic syndrome, obesity, cardiovascular diseases, cancer, Infectious diseases, or GIT disorders, such co-morbidity should be excluded. The same approach should be used for major psychiatry and neurology; schizophrenia, alcohol and drug addiction as well as Alzheimer's, Parkinson's diseases should not be included. The reason is the following: as it has been written above treatment of basic psychiatric disorder can alleviate insomnia symptoms, so, moderate and severe conditions should be excluded and treated by standard approach, whereas mild diseases or subclinical manifestations can be investigated.

IBS as a manifestation of dysbiosis, stress, subclinical or mild forms of depression, anxiety, autism, light form of dementia, and obesity may be included but reported separately (after stratification of all recruited subjects).

Let`s consider this approach an example of MDD: sleeplessness is one of the key symptoms for subjects with MDD and was recently considered a secondary manifestation of depression. Currently, a row of long-term trials has defined sleeplessness only as a predisposing factor for the onset and progression of MDD among patients of all ages (208).

MDD and CID are significantly connected with CR (31). It can be proved by the fact that patients suffering from MDD reported decreasing in severity in the evening and increasing in the first half of day. The frequency of depression manifestation is reliably higher in shift workers compared with those who work only in the daytime (209)

The impact of GM can be clarified not only by comorbid neuroendocrine and immune reactions but also by the representation of clock genes that influence both circadian rhythm and reaction to stress. Gut dysbiosis can cause not only sleeplessness but also mood disorders, emotional disturbances, and bipolar disorders; moreover, all these conditions have bi-directional interplay with sleeplessness (182).

The search of clinical studies related to Probiotics and insomnia (or sleep) in PubMed gave a list of 24 studies. (please refer to supplemental table) Some subjects were healthy men or men with symptoms of depression, anxiety, stress, autism, or anorexia nervosa. In other words, insomnia was considered as a manifestation symptom of depression, anxiety or other diseases related to Psychiatry.

The first part of the search results is related to articles published in 2014-2019. These studies were focused on acute stress. GM was not studied (in most of the studies), but serum or salivatory cortisol was proposed as a marker of acute stress and point of action for CR alteration. It was mainly studies with healthy volunteers and with one rarely two strains of genus *Lactobacillus* or *Bifidobacterium* as a probiotic, combining it with prebiotic or another supplement and comparing with a placebo. However, in 2018/2019 studies using a mixture of microbial strains began with GM, brain connectivity, and metabolites exploration at the baseline and last visits of the trial.

Table 7. Probiotics studies 2016-2019 researched insomnia

Bifidobacterium longum 1714	Allen AP. 2016 (230)	healthy men with acute stress	stress has decreased, memory improved (significantly less incorrect answers)
L. casei, L. acidophilus, L.paracasei, B. lactis W51, W52, L. salivarius, Lactococcus lactis, L. plantarum, B. bifidum,	Baga D et all 2018 (231, 232)	healthy men and women	Magnetic resonance imaging (MRI) brain connectivity study: results indicate an influence of probiotic administration on not only emotional processes, but going beyond extending into higher order cognitive processes.
Lactobacillus plantarum PS128	Liu Y-W et al 2019 (199)	children with autism	Improvement in autism symptoms, primarily those associated with disruptive and rule-breaking behaviors and hyperactivity/impulsivity
L. helveticus R0052 (n=145) Bifidobacterium bifidum R0071 (n=142) Bifidobacterium infantis R0033 (n=147)	Culpepper et al. 2016 (233)	healthy men and women with acute stress	negative correlation between self-reported stress and sleep for all groups (P=0.0259); less sleep affected stress levels to a lesser extent in B. bifidum group compared with other groups. The lower DS scores may have contributed to a reduction in overall stress in that self-reported levels of stress were also lower with B. bifidum R0071. These effects were not observed with *L. helveticus R0052* or *B. infantis R0033* confirming the effects of probiotics are strain specific.
Lactobacillus gasseri CP2305	Nishida et al., 2019 (234)	healthy men and women	Reduction PSQI scores after intervention, compared with placebo treatment (P=0.041); reduction SL of 1st NREM3 sleep (P<0.05) and reduction waking time after sleep onset (P<0.05) after intervention, compared with placebo treatment
Lactobacillus fermentum LF16, Lactobacillus rhamnosus LR06, Lactobacillus. plantarum LP01, Bifidobacterium longum BL04	Marotta et al. 2019 (235)	healthy men and women	Reduction mean PSQI scores after intervention, compared with baseline (P=0.005); reduction POMS subscale 'fatigue' after intervention, compared with baseline levels (P=0.008)
Lactobacillus helveticus LBH MIKI-020,+ theanine	Nakagawa et al. 2018 (236)	healthy men and women with reported sleep problems	SE improved in probiotic group at follow-up compared with baseline (P=0.036), and compared with placebo group (P=0.0342); no between-group differences for OSA/PSQI; OSA scores for 'sleepiness on awakening', 'onset and maintenance of sleep', 'dreaming and recovery from fatigue' improved in probiotic group at follow-up compared with baseline (P<0.05); placebo group showed improved 'recovery from fatigue' (P=0.0156); PSQI global score reduced in probiotic group at follow-up compared with baseline (P=0.0046)
Lactobacillus rhamnosus JB-1	Kelly et al., 2017 (225)	healthy men with acute stress	PSQI scores after intervention slightly reduced compared with baseline (P=0.07); no differences for sleep quality between treatment groups. Lactic acid bacteria-containing food improved the quality of sleep in this study use to lowering of deep body temperature and possible involving gene-clock mechanism of circadian rhythm
postbiotic *Lactobacillus gasseri CP2305*	Nishida et al., 2017b (237)	healthy men and women with acute stress	Reduction of mean PSQI scores (P=0.028), sleep quality (P=0.033) and duration (P=0.029) in intervention compared with placebo group; reduction SL in probiotic group compared with placebo group (P<0.001); increasing NREM3 sleep fraction within total NREM sleep (P<0.001) and reduction awakenings in 2 h before waking up (P<0.001) in probiotic group compared with placebo group
postbiotic *Lactobacillus gasseri CP2305*	Nishida et al., 2017a (238)	healthy men and women with acute stress	Reduction of mean PSQI scores after intervention, compared with baseline (P=0.038); stratification by gender showed reduced PSQI scores in men only (P=0.004) Sleep Quality was improved in probiotic group
LcS YIT9029	Takada et al., 2017 (239)	healthy men and women with acute stress	OSA scores decreased for 'sleepiness when waking up' (P<0.05) and higher for 'sleep length' (P<0.01) in probiotic group compared with control group; SL prolongation suppressed (P<0.05) and reduction of NREM3 sleep prevented (P<0.01) in probiotic group compared with control group; daily consumption of *Lactobacillus casei Shirota* (LcS) may help to maintain sleep quality during a period of increasing stress.
LcSYIT9029	Kato-Kataoka et al., 2016 (220)	healthy men and women with acute stress	no differences in PSQI scores over time between placebo and intervention groups; salivary cortisol and plasma L-tryptophan were essentially increased in only the placebo group (P<0.05) after stress.
Lactobacillus brevis SBC8803	Nakakita et al., 2016 (240)	Subjects with slight insomnia symptoms + acute stress	Quality of waking improved after intervention compared with control treatment (P=0.047); no differences in EEG measurements and AIS scores between intervention and control treatment; a beneficial effect on sleep due to consumption of heat-killed L. brevis SBC8803 was found in subjects with slightly challenged sleep.

The next studies dated 2020-2023 focused not only on GM metabolites but also focusing on immune profile and gut condition. Subjects enrolled have mild psychiatric symptoms (MDD, anorexia nervosa, autism, or cognitive impairment) and very good subjective self-examination by questionnaires and objective examination (WASO, TST, SE) by remote devices. It is also interesting that the share of Strain Mixture increased; besides Lactobacillus and

Bifidobacterium, *Bacilli, Streptococci*, or *Lactococci* were added to some strain mixtures, so the amount of pathway involved in impact also increased (immunes with IL6, serotonin modulation, BDNF associated so on). Correspondently, not only Quality of Life and QoS parameters were assessed but also depression, anxiety, thinking ability and somatic manifestations like gut irritation/inflammation. All clinical studies are placebo-controlled. A new correlation between metabolites/strains/examination findings and interrelations between changes/results are in the research focus.

Table 8. Probiotics studies 2020-2023 related to insomnia.

Bacillus subtilis PXN 21, B. bifidum PXN 23, B. breve PXN 25, B. infantis PXN 27, B. longum PXN 30, L. acidophilus PXN 35, L. delbrueckii ssp. bulgaricus PXN 39, L. casei PXN 37, L. plantarum PXN 47, L. rhamnosus PXN 54, L. helveticus PXN 45, L. salivarius PXN 57, Lactococcus lactis ssp. lactis PXN 63, Streptococcus thermophilus PXN 66*	Baião R et al. 2022 (241)	subjects with moderate depression	Reduction (−50% from baseline, $p < 0.05$, n = 35) depression scores on the PHQ-9, but these findings did not correlate with the changes in emotional processing. No effects of treatment on the other objective sleep variables including SL.
Lacticaseibacillus paracasei Lpc-37	Sanna M et al. 2023 (242)	healthy students	improvement in sleep-disturbance scores
Str. Thermophiles, B. breve, B. longum, B. lactis, L. acidophilus, L. plantarum, L. paracasei, L. helveticus	Schaub AC et al. 2022 (243)	subjects with mild depression	improvement depressive symptoms and maintains healthy enterotypes, species richness and increases specific health related bacterial taxa
Bifidobacterium breve	Lan, Y at al 2023 (226)	subjects with stress-induced insomnia	improvement sleep quality, reduce PSQI results, decrease wakening at night related to excessive cortisol level
Lactobacillus plantarum HEAL9+ S-adenosylmethionine SAMe)	Saccarello A et al 2020 (244)	subjects with mild-to-moderate depression	significant improvement symptoms of depression, anxiety, and cognitive and somatic components
Bacillus coagulans, L. rhamnosus, B. lactis, L. plantarum, B. breve, B. infantis + glutamine	Venkataraman R et al 2021 (218)	Healthy students	Reduction depression and anxiety stress scale and serum cortisol levels from the baseline in probiotic group
L. reuteri NK33, Bifidobacterium adolescentis NK98	Lee, H.J. et al. 2021 (227)	healthy adults with subclinical symptoms of depression, anxiety, and insomnia	reductions in both the PSQI and ISI scores at the final visit, improvements in the PSQI (21/63 [33.3%] vs. 9/59 [15.3%], p = 0.021) and ISI scores [18/63 (28.6%) vs. 7/59 (11.9%), p = 0.022], decrease in serum interleukin 6
L. casei, L. acidophilus, L.paracasei, B. lactis W51, W52, L. salivarius, Lactococcus lactis, L. plantarum, B. bifidum,	Gröbner EM et al 2022 (198)	Subjects with anorexia nervosa	Subjects weight increased, eating symptoms disorder were relieved, improved comorbid psychopathology including depression, anxiety, and compulsions
Bifidobacterium longum	Moloney et al., 2021 (245)	healthy men with chronic stress	No differences in PSQI, improvement sleep duration after intervention
Lactobacillus plantarum PS128	Ho, Y.-T. et al. 2021 (21)	Chronic insomnia; perimenopausal women, PSQI > 5	less frequent awakening and arousal, and decreased high-frequency brain wave activity
Lactobacillus plantarum JYLP-326	Zhu R et al, 2023 (246)	anxious students, HAMA>8	alleviation the physiological disorders and states of subjects facing chronic stress, including anxiety and depression as well as insomnia

Research of Probiotics mixtures (199, 206, 210-213) were described above and looks definitely better comparing with single strain research, because provide good description of Microbiota, Metabolome and in some cases immune, serum cortisol level together with cognitive, depression and anxiety scoring and insomnia assessment.

5. Discussion

Sleep parameter assessment and tool.

As described above, QoS depends on host multifactorial parameters predisposing, precipitating, and perpetuating. As has already been discussed, CBT-I is quite effective in alleviating insomnia, decreasing the influence of perpetuating (maintaining) factors such as maladaptive behavior and poor sleep factors, and may also help diminish the impact of such precipitating (triggering) factors like depression and PTSD.

Probably, GM has a comparatively restricted impact on perpetuating factors, but it has a significant impact on predisposing (vulnerability) (neurobiological and environmental BGMA-neurotransmitters / endocrine and CNS/ENS pathways) and precipitating (triggering) factors, for example, comorbid illness such as obesity and metabolic disease (through BGMA immunologic pathway), psychiatric conditions such as mood disorder MDD, anxiety, bipolar disorders and decreasing of related medication intake (through BGMA-neurotransmitters /endocrine and CNS/ENS pathways).

It means that CBT-I accompanied by GM correction/intervention may have the highest potential for insomnia treatment theoretically due to its cover of all 3P factors that cause sleep disorder, but this combination is out of the window of this review; we are focusing more on GM intervention as therapy that improves QoS. So, the first limitations for prospective studies will be defined as sleep disorders related to lack of Sleep or insomnia, including CR disturbances and sleep apnea, but excluding Hypersomnia, Parasomnias, and sleep-related movement disorder. We also should exclude major psychiatry, such as schizophrenia, Alzheimer's, epilepsy, and neurology, because these are subject to separate review/investigation.

There are large genome-wide association trials found and

proved direct genetic dependency CID with MDD, psycho-emotional stress traits (18), and PTSD (19). Genetic factors have, as usual, a significant impact on SD-I manifestation and severity in humans, and the estimated gene impact on sleeplessness was assessed from nearly 40% for men to 60% for women (214). However, considering that in 30 % of cases, insomnia is accompanied by depression, anxiety, grief, and/or cognitive impairment, it makes no sense to exclude subjects who have light symptoms of this statement. However, to have the possibility to separate these subjects, it is worth using questionnaires that provide results of assessment for MDD and anxiety. Research related to acute insomnia, caused by anticipated exams in students or caused by stress events or SD during the 2-5 days demonstrated the absence of significant evidence-based GM alterations (132-134) or showed unpredictable changes in GM composition, which do not confirm by studies with the same design (37, 131, 215). Therefore, it makes sense to use relevant assessment. However, sleep disorders related to CR disturbances and consequent SD caused by shiftwork should left the focus of perspective research.

Regarding the sleep parameter assessment, there are two options: objective (instrumental) sleep examination (such as PSG, EEG, EMG, EOG, and brain MRI) or subjective (using questionnaires) sleep examination (such as - PSQI, ISI, Epworth Sleepiness Scale (ESS). Despite some investigation, it declared that PSQI and ISI data do not provide an accurate assessment as objective tools combined with CANTAB (37) To assess Sleep Efficacy, WASO, TST, PSQI, and ISI are still potent tools for insomnia-grade definition and QoS, especially for light to moderate insomnia. It is also justified to use PSQI and/or ISI combined with questionnaires for stress anxiety, quality of life, and or GIT quality.

Limitations related to insomnia and a variety of factors related to co-morbidity (psychiatric, somatic, intestinal), habits, lifestyle, gender, age, and medication intake.

Predisposing (vulnerability) factors that significantly impact both sleep disorder and GM are genetics, age, gender, and lifestyle (dietary habits and physical activity).

For example, high cortisol concentration due to CID may empower the HPA axis effect, as a result, subjects with CID expressed increased cortisol awakening response (CAR) compared with the general population, a higher metabolism rate, and hyperthermia (216). There is an evidence-based correlation between pathological overweight, GIT disturbances, and psycho-emotional condition. (217, 218). However, the accent of the last studies was a combination of inherited genetic predisposing factors with dietary habits (219) and GM condition (alterations) (220) that demonstrate evidence-based interconnections between insomnia/comorbidity(IBD, MDD) and dietary habits or GM signatures. This means that despite genetics having a strong impact on insomnia and it is out of focus of our review, limitations, which are strongly correlated with such genetic factors as obesity/metabolic syndrome, IBD should be, if it is possible, excluded from analyses or at least their impact on potential study results should diminish bias, created by these comorbidities.

Regarding the condition of the digestive system, there are two possibilities to assess the presence of inflammation and severity: first is relying on information reported in medical history and the subject`s questionnaire prepared for GIT assessment, and second to have objective data, for example, colonoscopy or assessment specific markers for gut inflammation of gut permeability. For example, fecal calprotectin is a potent marker of GIT inflammation allowing us to distinguish inflammatory bowel disease from irritable bowel syndrome. So, IBD may be excluded in the presence of fecal calprotectin, but the development of IBS may be assessed when GM alteration occurs. Assessment of zonulin level allows us to investigate intestinal permeability and make a conclusion about gut inflammation and leak out, in the result of GM alteration and immune pathway of BGMA triggering at the end of the study. It may be a

marker of Bowel Infection at the study start to exclude intestinal infections. The definition of hair cortisol concentrations may be useful for the long-term cortisol assessment to exclude DM2 (by normal hair cortisol) and inherited insomnia related to CAR (genetically increased level of cortisol). Even if these markers will be found in the subject's samples, it allows for the formation of a homogenous group stratifying the subjects per marker level.

It is well known that women have sleeplessness 1.5 times more frequently than men (14), so subsequently they have a higher predisposal rate for MDD, psycho-emotional disturbances, and cardiovascular diseases. However, it has not been reported gender-related differences in GM, metabolome, BGMA mechanisms, or insomnia in women, which is why it has no significant impact on potential clinical trial results, in comparison, for example, with BMI/gut or psychiatric co-morbidities. However, comparative results anyway should be stratified by gender.

At age 50 the following changes were reported in sleep architecture: (16) earlier sleep start and stop times (shift of time), (1) more time required for sleep begin (onset time), (11) shorter sleep duration, (12) more shallow sleep with frequent arousals (SF), (13) greater sensitivity to external stimuli and a correspondingly high likelihood of awakening (fragile sleep), (36) diminished quantity of N3REM sleep, (7) more prolonged light stages of sleep (N1REM and N2REM) (14) the shortening and diminishing sleep cycles, and (15) nighttime wake-up times become more frequent (221-223).

With age, the need for daytime sleep increases: if adults aged 55–64 years have a tenth of the cases of sleep, then at the age of 75–84 years, this figure is already a quarter(224). The following concomitant pathologies contribute to the tendency of older people to nap and drowsiness chronic pain syndrome, psycho-emotional disorders, CID, nocturia, and pollakiuria. (224, 225).

That is why it makes sense to stratify subjects by age 18-65 years and more than 65 years due to increased sleep onset and SF and decreased TST and SL for subjects 65 + years.

The following limitations should be related to medication use. To provide evidence-based results of the potential study, all medication related to systemic antibiotics, prokinetics, and other pre- and probiotics should be excluded at least four weeks before the study to escape GM changes related to medication intake. The same limitation may be accepted medication impacted on sleep or psychological status to prevent impact on quality of life or sleep assessment (or its intake at least should be controlled to create correspondent subject group stratification).

Potential subjects somatic, GIT, psychiatry parameters assessment – to formulate a homogenous group.

In other words, to create a homogenous group of insomnia subjects, it should be mild to moderate insomnia per PSQI or ISI started at least four weeks before enrollment without significant somatic or psychiatry co-morbidity, without taking of medication with impact on GM or sedative-hypnotic with age 18-65 year In. The group should be stratified by gender, presence of GIT symptoms (with fecal calprotectin control as a marker to distinguish IBD from IBS and zonulin control to confirm gut inflammation) + hair cortisol to stratify long-time high level of cortisol (as a stimulant of awakening and marker of DM2/metabolic syndrome)

Insomnia and such parameters as TST, sleep onset, SL, and SE may be either objective (PSG, EEG, EMG, EOG) or subjective (PSQI, ISI, ESS questionnaires) method assessed.
Level of depression, stress, anxiety, and cognitive performance (if it is needed) may be assessed using validated questionnaires.

Gastrointestinal status may be assessed using visual methods or using specific markers like calprotectin and/or zonulin. GIT quality of life and QoS should be evaluated using validated questionnaires.

Metabolome assessment.

As described above, the following metabolites have the greatest impact on sleep: GABA as a sleep promotor, DA and

serotonin as awakening inductors, and SCFA and sBA as clock-gene regulators of CR and anti-inflammatory substances (sleep promotors). So not only active metabolite, but also its precursors, derivates, and breakdown products such as 5-HT, serotonin, kynurenine, Indolamine 2,3 / tryptophan 2,3 dioxygenase, for tryptophan pathway should be defined in Bowel lavage.

Zonulin and calprotectin should be defined as markers of gut inflammation and permeability at the study beginning of the study and as objective markers of gut inflammation decreasing at the end of the study.

Definition of cortisol, IL-2, TNF-alfa, IL-6, and IL-1 levels in blood serum may be useful for inflammatory pathway definition at the beginning and at the end of the study. Cortisol hair level may provide information about genetically higher level of cortisol (in hereditary insomnia) or to confirm diabetes/metabolic disease.

Microbiota composition

In spite of evidence that alfa- and beta-diversity of microbiota is significant for gut condition and also have a positive impact on sleep, neurological and psychological human status, no clear vision of what is normal GM composition, quantitative/qualitative definitions, markers for differentiation between altered and normal microbiota, mechanism of microbiota influence on sleep/depression/cognitive function is proposed, but still need clarifications and evidence-based confirmation for particular cases. The significance of the F/B ratio is controversial for sleep/depression/cognitive function. The prevalence of strains in GM composition and their impact on gut health/human health and sleep should be further investigated for beneficial strain composition and their association with metabolites.

Sleep disorders (particularly insomnia) have a reciprocal interrelation with gut microbiomes. BGMA is a potent tool for assessments of interrelationships of insomnia with GM alterations, and it may be used to predict changes in the human condition and

choose GM correction in case of already studied alterations. BGMA has limitations in assessing quantitative and qualitative changes in each case due to the multifactorial nature of these interrelations and individual features of the organism and gut microbiome. Non-pharmacological treatment such as diet, change of lifestyle, and usage of pre-and probiotics is considered a perspective way for insomnia treatment and GM alterations corrections. Many studies that researched gut microbiome, insomnia, their interrelation, and ways of correction focused only on several issues. They do not provide a complete picture of changes in the microbiome, gut, and entire organism. Further investigations should be focused on microbiome exploration (GM, gut metabolites, and gut condition - signs of permeability and inflammation) and human organism examination (level of cortisol, serum cytokines, and well-being of the subject) at the baseline of research and after the treatment. Insomnia, stress, depression, anxiety, and GIT status may be assessed using standard questionnaires. It was proposed to investigate mild insomnia with mild symptoms of IBS, depression, and anxiety using a mixture of multi-strain composition as a probiotic for at least four weeks. Issues with the stability of positive gut microbiota changes after the treatment are still open.

The following conclusions may be made according to the results review:

Diet, lifestyle, and life's place significantly impact GM composition as well as on insomnia.

According to the results review, my opinion is that prebiotics and probiotics administration may restore GM alteration (or shift of microbial composition) that appeared after chronic neurologic, psychiatric, or somatic disturbances and improve sleep. However, there are more data about GM intervention should be accumulated and analyzed for correlation at baseline, after 4-week probiotics intervention and in follow up related to GM, metabolome, human condition.

Strains selection for probiotics mixture should be done considering the ability of microbial strains to produce sleep inductors such as GABA melatonin and degrade starch and OS to SCFA (particularly butyrate).

The exact mechanism of action of strain or mixture of strain on the gut, brain, and host is unknown, but probiotics administration has a good safety profile and may be used as non-pharmacological therapy.

Exploration of microbiota, metabolome in the gut, gut mucus, and epithelium (by zonulin and fecal calprotectin) together with salivatory cortisol (for acute stress and insomnia) and hair cortisol (for chronic stress and insomnia) and serum cytokines allow supposing mechanism of action (if improvement be reached)

Considering that the current use of probiotics is at the start point of research, it makes sense to enroll healthy subjects (subclinical insomnia) or those with mild stage of the disorder.

Insomnia subjects with mild forms of comorbidities like IBS, obesity, depression, anxiety, stress, and dementia may be also enrolled (due to the mechanism of action to insomnia and comorbidity may be the same - through BGMA).

The prevalence of strains in GM and their impact on gut health/human health and sleep should be further investigated for beneficial strains composition and their association with metabolites to define the most optimal probiotics mixture for treatment/alleviation of each separate disorder (or disorder combinations) and to define markers (in gut microbiome, or host serum) which may help with a better treatment choice.

As usual, a double-blinded placebo-controlled design should be used for the probiotic studies on this stage of insomnia research.

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