THE EFFECTS OF MICROBIAL ECOSYSTEM THERAPEUTIC-2 ON SLEEP DISTURBANCES IN INDVIDUALS WITH MAJOR DEPRESSIVE DISORDER

By

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A thesis submitted to the Centre for Neuroscience Studies

in conformity with the requirements for

the degree of Master of Science

Queen's University

Kingston, Ontario, Canada

October 2023

ABSTRACT

Objective: The primary objective of this thesis is to evaluate the effects of microbial ecosystem therapeutic-2 (MET-2) in comparison to a placebo on sleep disturbances in individuals with major depressive disorder (MDD) during a 6-week treatment period, with follow-up assessments at 12- and 24-weeks post-treatment. The secondary objectives are to assess changes in mood at the same intervals and to assess any correlations between sleep and mood.

Methods: Data from this thesis were derived from the METDA study, a phase 2 double-blind placebo-controlled trial that recruited individuals with MDD in the Kingston and Toronto areas. Participants were randomly assigned to receive either MET-2 or a placebo for 6 weeks. Longitudinal effects of MET-2 were explored in a follow-up sub-study at 12- and 24-weeks post-treatment. The Pittsburgh Sleep Quality Index (PSQI) and the Montgomery Åsberg Depression Rating Scale (MADRS) evaluated changes in sleep and mood, respectively, at each time point.

Results: Among the 27 participants included in the analysis of this thesis, a two-way repeated measures ANOVA showed significant improvements in PSQI scores within the MET-2 group (n= 13) compared to the placebo group (n= 14) from baseline to week 6 (p= 0.044) and baseline to week 18 (p= 0.011), after adjusting for sex and age. Although MADRS scores did not display inter-group differences, intra-group trends revealed notable mood improvements in MET-2 recipients from baseline to week 6 (p<0.001) and baseline to week 12 (p= 0.003).

Conclusion: This study is the first to examine the potential efficacy of MET-2 in mitigating sleep disturbances in individuals with MDD, suggesting the potential benefits in treating sleep-related symptoms in MDD. The results of this study contribute to a growing body of research on gut repopulation as a treatment method for a variety of psychiatric illnesses, offering new insights and directions for future investigations.

ACKNOWLEDGMENTS

I would like to take this opportunity to thank the individuals who have made significant contributions towards the successful completion of my dissertation.

First and foremost, I would like to thank my supervisor, Dr. Roumen Milev, whose guidance and support were instrumental in shaping the direction of my master's experience. His expertise in the field of neuropsychiatry has been essential, and I am truly grateful for his mentorship throughout this process.

Next, I wish to extend my thanks to my thesis committee, Dr. Alina Marin and Dr. Ruzica Jokic, for assisting and supporting me in the completion of my dissertation. I also wish to thank the administrative teams at Providence Care Hospital and Centre for Neuroscience Studies for helping coordinate my master's and for always providing support when needed.

I am extremely grateful to my lab mates Arthi Chinna Meyyappan, Cassandra Sgarbossa, Emily Kasurak and Evan Forth for their constant encouragement, insightful advice, and their ability to keep me grounded when life gets overwhelming. I have learned a great deal from them and am so proud to have been a part of such a supportive research community.

Finally, I would like to thank my family and friends who have consistently supported me throughout my academic journey. Their encouragement, love, and support have been truly invaluable, and I would not be where I am or accomplished what I have without them.

Sincerely,

Hayley Bromley

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LIST OF ABBREVIATIONS

AE	Adverse Event
ANOVA	Analyses of Variance
ANCOVA	Analyses of Covariance
ANS	Autonomic Nervous System
BBB	Blood Brain Barrier
BDI	Beck Depression Inventory
BPD1	Bipolar I Disorder
CBT	Cognitive Behavioral Therapy
CBT-I	Cognitive Behavioral Therapy for Insomnia
CNS	Central Nervous System
CRF	Corticotrophin-Releasing Factor
CRF	Case Report Form
DSM	Diagnostic and Statistical Manual of Mental Disorders
ENS	Enteric Nervous System
FMT	Fecal Microbiota Transplant
GALT	Gut-Associated Lymphoid Tissue
GBA	Gut-Brain Axis
GCP	Good Clinical Practice
GF-mice	Germ-Free Mice
HPA-axis	Hypothalamic-Pituitary-Adrenal axis
ICD-10	International Classification of Diseases
IFN	Interferon

IL	Interleukin
ITT	Intention to treat
LOCF	Last Observation Carried Forward
MADRS	Montgomery Åsberg Depression Rating Scale
MAOI	Monoamine Oxidase Inhibitors
MET-2	Microbial Ecosystem Therapeutic-2
MDD	Major Depressive Disorder
MDE	Major Depressive Episode
NREM	Non-Rapid Eye-Movement
PHQ-9	Patient Health Questionnaire-9
PSQI	Pittsburgh Sleep Quality Index
RCT	Randomized Controlled Trial
REB	Research Ethics Board
REM	Rapid Eye-Movement
SCFA	Short Chain Fatty Acid
SCN	Suprachiasmatic Nucleus
SNRI	Serotonin and Norepinephrine Reuptake Inhibitor
SSRI	Selective Serotonin Reuptake Inhibitor
SWS	Slow Wave Sleep
TCA	Tricyclic Antidepressant
TNF	Tumour Necrosis Factor
WHO	World Health Organization

CHAPTER 1: Purpose of Thesis

1.1 General Overview

Major depressive disorder (MDD) is a prevalent and debilitating mental health disorder that significantly contributes to the global burden of disease (World Health Organization, 2012). MDD affects millions of individuals of all ages worldwide, with a significantly increased risk of suicide for these individuals being 20 times higher compared to the general population (Lépine & Briley, 2011). MDD manifests through persistent feelings of sadness and/or loss of interest or pleasure in one's surroundings. It presents a range of symptoms that cause significant distress or impairment, including changes in appetite and even thoughts of suicide. MDD often follows a severe and chronic course, with affected individuals experiencing multiple recurrent episodes throughout their lives. Depression leads to impairments in academic, occupational, and interpersonal functioning, as well as high rates of medical and psychiatric comorbidities (Thaipisuttikul et al., 2014). The global economic cost of major depressive disorder is substantial; according to some estimates it may be responsible for up to $1 trillion each year alongside anxiety disorders (The Lancet Global, 2020).

Sleep disturbances that result in decreased sleep quality are one of the most common and debilitating symptoms in individuals with MDD, playing a crucial role in both the manifestation and progression of the disorder (Nutt et al., 2008). Among the various symptoms present in MDD, disturbances in sleep stand out particularly due to their substantial impact on an individual’s well-being and overall functioning. Sleep disturbances often manifest in the form of sleep disorders, such as insomnia and hypersomnia’s, of which are highly comorbid with MDD (Li et al., 2021). While not every individual with depression will meet the diagnostic criteria for

insomnias or hypersomnia's, many exhibit symptoms consistent with these sleep disorders. Specifically, about three quarters of individuals with depression have difficulties initiating or maintaining sleep, including early morning awakenings. In addition, hypersomnia, or excessive sleepiness, is also commonly observed, often resulting in prolonged sleep durations or significant daytime sleepiness (Nutt et al., 2008). Although sleep disorders have a close relationship with MDD, any sleep disturbances may contribute to the overall burden of the disorder and are closely intertwined with other depressive symptoms, exacerbating the negative effects on mood, cognition, and daily functioning (Murphy & Peterson, 2015). The bidirectional relationship between sleep disturbances and depression adds complexity to the clinical understanding of these conditions. Sleep disturbances can function as both a prodromal symptom and a result of depression (Murphy & Peterson, 2015). In certain instances, sleep disturbances precede the onset of depressive episodes, potentially indicating early indicators or risk factors for developing MDD (Murphy & Peterson, 2015). Further, ongoing, and unresolved sleep problems can perpetuate and exacerbate depressive symptoms, contributing to a chronic and recurring trajectory of the disorder. On the other hand, depressive symptoms can disrupt normal sleep (Murphy & Peterson, 2015).

Standard treatments for MDD primarily involve pharmacological interventions, with selective serotonin reuptake inhibitors (SSRIs) being the first line treatment and most widely prescribed method targeting symptomatic remission (Karrouri et al., 2021). However, while SSRIs are effective for mood stabilization, they present a variable impact on sleep disturbances. Early in treatment, these medications can potentially exacerbate sleep issues, leaving residual sleep symptoms even after mood stabilization (Nutt et al., 2008). This often necessitates the introduction of drugs such as benzodiazepines to counteract these effects, however, these types

of drugs come with many limitations, such as potential dependence and withdrawal (Nutt et al., 2008). Specific antidepressants, including those with activating effects like SSRIs, can further disrupt sleep in the short term (Wichniak et al., 2017). Conversely, those with sedative qualities might offer rapid sleep enhancing effects but present long-term risks of oversedation (Wichniak et al., 2017). While cognitive behavioral therapy for insomnia (CBT-I) is the gold standard for insomnia, its efficacy in treating the comorbidity of sleep disruptions and MDD remains an uncertainty (Feng et al., 2020). Moreover, even when pharmacological approaches are combined with psychotherapies like CBT-I, approximately one third of patients will still not respond to treatment (Murphy & Peterson, 2015). Indeed, sleep-related symptoms in MDD frequently remain unaddressed post-treatment, conferring greater risks of depressive relapse and recurrence (Nutt et al., 2008).

Recently, there has been increasing recognition of the gut-brain axis (GBA), a bidirectional communication system between the gastrointestinal microbiota and the brain. The gut microbiota, which varies significantly among individuals, is hypothesized to impact brain function, sleep, and mood and anxiety symptoms through GBA signaling (Carabotti, 2015; Wang et al., 2022). Promising studies, both in preclinical and clinical models, have shown that gut repopulation techniques such as fecal microbiota transplant (FMT) can alleviate psychiatric symptoms by reintroducing healthy bacteria to the gut (Chinna Meyyappan et al., 2020). As a result, many studies have suggested that the GBA may provide the basis for a hypothesis that explains sleep disturbances in depression (Li et al., 2018). Gut repopulation techniques such as FMT therefore have the potential to be a novel treatment for targeting both depression and its associated sleep disturbances.

The purpose of this thesis is to evaluate the efficacy of a novel microbe therapy, microbial ecosystem therapeutic-2 (MET-2), against a placebo, in addressing sleep disturbances in MDD. Unlike FMT, MET-2 offers a safer alternative due to its lyophilized, lab grown, encapsulated formulation. The data used and presented in this thesis are sourced from the METDA study, a randomized controlled trial and subsequent follow-up, conducted at Providence Care Hospital in Kingston, Ontario and at the Centre for Addiction and Mental Health in Toronto, Ontario. This research was funded by NuBiyota LLC. Participants diagnosed with MDD were recruited from the Kingston and Toronto communities and were administered either encapsulated MET-2 or a placebo for a duration of 6 weeks. Throughout the treatment period and at 12- and 24-week post-treatment, sleep disturbances and depressive symptoms were evaluated using the Pittsburgh Sleep Quality Index (PSQI) and Montgomery Asberg Depression Rating Scale (MADRS), respectively.

1.2 Objectives

1.2.1 Primary Objective

The primary objective of this thesis was to evaluate alterations in sleep disturbances among individuals with MDD following MET-2 administration. Assessments were made from baseline to the end of the 6-week treatment and continued at 12- and 24-weeks post-treatment. Comparisons were drawn using the PSQI, against a placebo group.

1.2.2 Secondary Objectives

The secondary objectives of this thesis were to assess mood changes using the MADRS from baseline through the end of treatment and during the 12- and 24-week follow-up periods, as well as to examine potential correlations between sleep disturbances and mood alterations.

CHAPTER 2: Literature Review

2.1 Major Depressive Disorder

Major Depressive Disorder (MDD) is a global health concern with a substantial prevalence and an increasing burden. According to the World Health Organization (WHO), depression affects more than 300 million individuals worldwide, making it the leading cause of disability (World Health Organization, 2023). Furthermore, the lifetime prevalence of MDD is approximately 16%, indicating a significant risk of developing this condition at any point in one's life (Kessler et al., 2005).

MDD is characterized by persistent feelings of sadness, loss of interest or pleasure in daily activities, and a wide range of cognitive, emotional, and physical symptoms. This disorder affects individuals across diverse demographic backgrounds and has profound implications for both personal well-being and societal functioning (Kennedy, 2008). In comparison to the general population, patients with depression endure substantial functional impairments in many domains (Hammer-Helmich et al., 2018). It has been shown that the functional disparities seen in depression are as devastating, if not more, as those found in chronic medical diseases, such as diabetes mellitus and cardiovascular disease (Hammer-Helmich et al., 2018)

The economic burden of depression is also extensive, with substantial costs to workplace productivity. Individuals with depression lose, on average, six hours of productive work a week, and depression accounts for nearly 20% of all workdays lost (Stewart et al., 2003). Moreover, the likelihood of unemployment is seven times higher for individuals with depression, underscoring the significant impact of depression on productivity and the overall output of an economy (Lerner et al., 2010; Stewart et al., 2003).

2.1.1 Clinical Presentation

The clinical manifestations of MDD exhibit a diverse range of symptoms and considerable variability in terms of severity and duration among patients (Kennedy, 2008). While ongoing research explores the potential use of biomarkers for diagnosing depression, currently, there are not widely accepted or utilized biomarkers in clinical practice. Consequently, the most effective diagnostic approach for depression involves a comprehensive clinical assessment conducted by a trained healthcare professional, such as a psychiatrist or psychologist (García-Gutiérrez et al., 2020). This assessment typically employs a combination of methods, including evaluation of the individual's symptoms, medical history, and psychosocial factors. The Diagnostic and Statistical Manual of Mental Disorders (DSM-5) provides the primary guidelines for diagnosing depression. Additionally, self-report questionaries or rating scales, such as the Beck Depression Inventory (BDI) or the Patient Health Questionnaire-9 (PHQ-9), may be used as a supplementary tool to assess the severity and presence of depressive symptoms ((UK), 2010; Beck, 1961).

To confirm a major depressive episode (MDE), the DSM-5 criteria require that an individual must exhibit five or more symptoms during the same two-week period. At least one of the symptoms should be either (1) depressed mood or (2) loss of interest or pleasure, known as anhedonia, persisting for the specified duration ((UK), 2010). The other symptoms may be any of the following: (3) Significant (more than 5 percent in a month) unintentional weight loss/gain or decrease/increase in appetite; (4) sleep disturbance (insomnia or hypersomnia); (5) psychomotor changes (agitation or retardation) severe enough to be observable by others; (6) tiredness, fatigue, or low energy, or decrease efficiency with which routine tasks are completed; (7) a sense of worthlessness or excessive, inappropriate, or delusional guilt; (8) impaired ability

to think, concentrate, or make decisions; (9) recurrent thoughts of death (Chand & Arif, 2023). Additionally, the confirmation of an MDE requires the absence of any previous manic or hypomanic episodes in the patient's history. The wide range of possible symptom combinations contributes to the significant intraclass heterogeneity within MDD (Kennedy, 2008).

2.1.2 Comorbidity

Extensive research based on epidemiological surveys has provided substantial evidence highlighting the close association between depression and other mental disorders, particularly anxiety disorders and substance use disorders (Steffen et al., 2020). Approximately 50-60% of individuals who have experienced depression during their lifetime also report having at least one anxiety disorder. A large-scale survey in the United States found that 14% of individuals with MDD in the past year also had an alcohol use disorder, and 4.6% had a drug use disorder. Among those with a lifetime history of MDD, 40% had an alcohol use disorder, and 17% had a drug use disorder (Steffen et al., 2020).

Furthermore, the comorbidity of depression and sleep disorders has been well-documented, highlighting a bidirectional relationship between the two (Staner, 2010). About three quarters of patients with depression exhibit insomnia symptoms, and hypersomnia is seen in approximately 40% of adults and 10% of elderly patients with depression. Notably, 41% of participants with depression had symptoms severe enough to qualify for an insomnia diagnosis as per the DSM-IV (Nutt et al., 2008). The presence of psychiatric comorbidity alongside depression has been associated with increased MDD severity, including a higher risk of suicidality, slower recovery, elevated risk of chronicity, recurrence, treatment resistance and more frequent use of medical services compared to individuals with "pure" depression (Steffen

et al., 2020). Given the wide-range comorbidities and the profound implications for personal well-being and societal health, it is crucial to prioritize the management of this disorder.

2.1.3 Pathophysiology

The complexity of MDD stems from its multifaceted clinical and etiological features. While a comprehensive understanding of its pathophysiology is crucial for optimal treatment, the precise mechanisms of depression remain partially understood, explaining why many interventions fail to achieve desired results in many individuals (Jesulola et al., 2018). Despite these challenges, there are currently a few recognized mechanisms that are hypothesized to contribute to MDD pathophysiology.

At the neurobiological level, MDD is associated with dysregulation in several neurotransmitter systems, including serotonin, norepinephrine, and dopamine. The monoamine hypothesis of depression proposes that imbalances in these neurotransmitters play a crucial role in the pathophysiology of MDD (Jesulola et al., 2018). For instance, serotonin, often referred to as the “feel-good” neurotransmitter, is instrumental in mood regulation. Meanwhile, dopamine modulates reward and motivation functions, and norepinephrine affects the functioning of the prefrontal cortex, which regulates behavior (Jesulola et al., 2018). The importance of monoaminergic systems in depression is clear, as they are instrumental to the various behavioral symptoms associated with the disorder, including low mood, reduced motivation, fatigue, and psychomotor agitation (Jesulola et al., 2018). Modern antidepressants, grounded in the monoamine hypothesis, focus on restoring synaptic monoamine neurotransmitter balance to alleviate depressive symptoms. A significant limitation of the monoamine hypothesis is its inability to account for the delayed response of antidepressants. While the hypothesis would

suggest immediate effects, these drugs typically require several weeks to demonstrate therapeutic benefits (Racagni & Popoli, 2008). Furthermore, monoamine-targeting antidepressants don't benefit all patients as up to 30% of those with MDD remain unresponsive following treatment, and roughly a third of responders continue to experience residual symptoms post-treatment (Al, 2012; Boku et al., 2018).

In addition to monoamine dysfunction, growing evidence suggests that disturbances in other neurobiological systems may contribute to the pathophysiology of MDD. The hypothalamic-pituitary-adrenal (HPA) axis, which regulates the body's stress response, is often dysregulated in individuals with MDD (Varghese & Brown, 2001). Research on the HPA axis have shown that those with depression often have increased secretion of corticotrophin-releasing factor (CRF) and cortisol, weakened glucocorticoid feedback, and compromised corticosteroid receptor signaling (Jesulola et al., 2018). Furthermore, patients with depression who fail to restore proper functioning of their HPA axis after treatment tend to experience poorer clinical outcomes and prognosis (Mickey et al., 2018; Owashi et al., 2008). Despite this understanding, it should be noted that previous studies exploring HPA axis-regulating treatments, such as glucocorticoid receptor antagonists, have shown limited success in alleviating depression symptoms (Aubry, 2013; Stetler & Miller, 2011).

2.1.4 Current Interventions

Every year, approximately 7% of American adults experience MDD, yet only half seek professional help. Among those who do seek care, only 20% receive adequate treatment (Wichniak et al., 2017). Treatments for MDD primarily comprises pharmacotherapy, psychotherapy, and lifestyle changes, often beginning in primary care (Karrouri et al., 2021).

Despite drug therapy being the most prescribed, around 20% of patients do not adhere, mostly due to side effects or insufficient relief (Gartlehner et al., 2008; Xing et al., 2011). Most patients don't achieve full remission with their initial treatment. This has prompted research on combining medication and psychotherapy, revealing better outcomes for both short- and long-term depression management compared to single-modality treatments for some patients (Dunlop, 2016). The combination of pharmacotherapy with psychotherapy, such as CBT, has been shown to be more effective than either one alone (Cuijpers et al., 2014). However, psychotherapy often isn't covered by insurance, posing a financial challenge for patients.

2.1.4.1 Pharmacotherapy

Antidepressant medications play a central role in MDD treatment. Among the various antidepressants available, second-generation antidepressants, including SSRIs like fluoxetine and sertraline, are the most prescribed for their efficacy and limited side effects (Mojtabai & Olfson, 2014). These drugs modulate serotonin levels by blocking the reuptake of serotonin, therefore increasing the amount of serotonin remaining in the synaptic cleft and thus improving mood (Chu & Wadhwa, 2023). Despite side effects like sleep disturbances, sexual dysfunction and weight changes, SSRIs remain the first-line treatment for MDD due to their overall effectiveness (Karrouri et al., 2021).

In cases where SSRIs are not effective or well-tolerated, other classes of antidepressants, such as serotonin-norepinephrine reuptake inhibitors (SNRIs), tricyclic antidepressants (TCAs), and monoamine oxidase inhibitors (MAOIs), are considered (Taylor, 2008). The choice is often based on individual reactions and side effects. The process of finding the most suitable antidepressant often relies on a trial-and-error approach.

SNRIs, which include drugs like desvenlafaxine and duloxetine, block serotonin and norepinephrine reuptake, prolonging their effects (Fanelli et al., 2021). Although SNRIs are similar in effectiveness to SSRIs, they may be associated with more side effects such as increased blood pressure or gastrointestinal symptoms (Santarsieri & Schwartz, 2015).

MAOIs exhibit their effects by inhibiting the monoamine oxidase enzyme, which is responsible for breaking down various neurotransmitters in the brain, leading to increased levels in the brain. Due to severe potential side effects, including hepatotoxicity and hypertensive crises, the use of MAOIs has decreased in frequency over time (López-Muñoz et al., 2007).

TCAs, including amitriptyline and imipramine, are comparably effective to the other classes of antidepressants. TCAs inhibit the reuptake of norepinephrine and serotonin by binding to their transporters, primarily affecting these neurotransmitter levels. This broader receptor interaction increases the likelihood of TCAs inducing undesirable side effects, and therefore TCAs should be prescribed only after first-line antidepressants have failed (Karrouri et al., 2021; Tatsumi et al., 1997).

2.1.4.2 Cognitive Behavioral Therapy

Among the various psychotherapeutic approaches, CBT is one of the most extensively studied and evidence-based interventions for depression (Cuijpers et al., 2014). CBT focuses on identifying and modifying negative thought patterns and behaviors that contribute to depressive symptoms. By providing adaptive coping strategies and positive thinking, CBT aims to improve emotional regulation and well-being. CBT is recommended both as an initial treatment and as an augmentation strategy to antidepressants, such as SSRIs. Evidence supports its use in both scenarios (Nakagawa et al., 2017). Several studies have shown that CBT can be as effective as

antidepressant medications, particularly for mild to moderate depression. The combination of CBT with pharmacotherapy has also been shown to lead to better treatment outcomes, especially for those with more severe depression (Cuijpers et al., 2014).

2.2 Sleep

Sleep is integral to human well-being, supporting various physiological and cognitive functions like memory consolidation, immune regulation, and emotional processing (Diekelmann & Born, 2010). The National Sleep Foundation suggests 7-9 hours of sleep per night for adults aged 18-64, but individual needs can vary (Hirshkowitz et al., 2015). Though sleep duration is important, it's not the sole determinant of restfulness. Here, the multifaceted concept of 'sleep quality' comes into play, encompassing both objective and subjective dimensions of sleep. While the primary emphasis of this thesis revolves around subjective sleep quality, delving into the objective scope remains relevant to a more holistic understanding of sleep.

2.2.1 The Topography of Normal Sleep

Sleep architecture refers to the overall structure and organization of the different sleep stages that occur during a typical night's sleep. A balanced proportion of different sleep stages, cycling throughout the night, is indicative of healthy sleep (Walker, 2009). Understanding sleep architecture also provides insights into overall sleep quality.

2.2.1.1 Stages of Sleep

Normal sleep architecture is comprised of non-rapid-eye-movement (NREM) sleep and rapid eye-movement (REM) sleep (Walker, 2009). During NREM, the brain and body undergo

restorative processes. In contrast, REM sleep, which is associated with vivid dreaming, demonstrates increased brain activity (Rasch & Born, 2013; Walker, 2009). Polysomnography, the gold standard tool for studying objective sleep, monitors brain activity, eye movements, muscle activity, and heart rhythm, helping to identify the primary sleep stages: wakefulness, NREM Stage 1, NREM Stage 2, NREM Stage 3, and REM (Feriante & Araujo, 2023). Distinct EEG patterns are correlated with each stage of sleep, involving beta, alpha, theta, and delta waves (Feriante & Araujo, 2023).

2.2.1.2 The Sleep Cycle

Sleep begins with NREM stage 1, acting as a transitional phase from wakefulness to deep sleep, followed by REM sleep, alternating cyclically throughout the night (Colten & Altevogt, 2006). On average, most of a night's sleep is spent in NREM, constituting approximately 75-80% of sleep, while REM contributes 20-25% (Colten & Altevogt, 2006). In healthy young individuals, the first cycle lasts around 70 to 100 minutes, extending to 90 to 120 minutes in subsequent cycles (Colten & Altevogt, 2006). NREM stage 2 gradually becomes the predominant NREM phase, with stage 3, referred to as slow-wave sleep (SWS), sometimes fading entirely. As the night advances, sleep shifts from a dominance of SWS to REM sleep, with most experiencing four to five REM periods at about 90-minute intervals (Thase, 2006). These REM periods intensify and lengthen, especially towards the night's end (Colten & Altevogt, 2006). Termed "paradoxical sleep", REM sleep shows rapid eye movements with EEG activity resembling wakefulness (Siegel, 2005). Around 80% of vivid dreams are recalled from REM sleep, with muscle paralysis preventing physical enactment of dreams (Dement &

Kleitman, 1957). Although the precise function of NREM and REM alterations isn't fully clear, abnormal sleep stage patterns are associated with sleep disorders (Colten & Altevogt, 2006).

2.2.1.3 Sleep Regulation

The regulation of sleep involves two processes: circadian rhythms and homeostatic mechanisms (Thase, 2006). Circadian rhythms, governed by internal "clocks" like the suprachiasmatic nucleus (SCN), regulate our sleep-wake cycles and hormone releases. Disruptions in these rhythms have been associated with disorders like MDD, with clock genes dysregulation being associated with insomnia and depression (Thase, 2006).

Homeostatic regulation, on the other hand, ensures we sleep mostly at night, aligning with melatonin and growth hormone releases. Extended wakefulness increases the need for sleep, with chemicals like adenosine playing a potential role. These rhythms are further influenced by various neurotransmitters, ensuring a balance between sleep and arousal (Thase, 2006).

2.2.2 Other Measures of Sleep

Sleep quality, as a subjective measure, includes various dimension of sleep that offer insights into the restfulness and satisfaction of an individual's experience (Buysse et al., 1989). One of the most clinically validated instruments to assess this subjective sleep quality is the Pittsburgh Sleep Quality Index (PSQI). The PSQI is designed to capture several components of sleep over a one-month interval, encompassing components such as subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medications, and daytime dysfunction (Buysse et al., 1989). With its high reliability and validity,

the PSQI has become a gold standard in both clinical and research settings to measure the overall sleep quality of individuals (Larche et al., 2021).

Sleep quality encompasses a broad spectrum of elements, including sleep latency, the duration of time taken to transition from wakefulness to sleep. Prolonged sleep latency can often be indicative of issues initiating sleep, which can be due to anxiety, stress, or an overly active mind (Nelson et al., 2022). Another element is sleep efficiency, a metric that compares the total time spent asleep to the overall time spent in bed. A high sleep efficiency suggests that most of the time in bed is spent asleep, whereas a lower efficiency can indicate frequent awakenings (Nelson et al., 2022).

Although detailed sleep stages are more accurately assessed with objective tools, such as polysomnography, individuals can report perceived durations to their sleep cycle, like frequent nocturnal awakenings or a feeling of never reaching deep sleep. In essence, objective measures detail the architecture and physiology of sleep, but it is the subjective experiences, as captured by tools like the PSQI, that elucidate how a person feels during their waking hours and how effectively they function in daily life.

2.2.3 The Relationship Between Sleep and Depression

Sleep disturbances are intrinsically linked with MDD. Over 90% of individuals with depression report sleep disruptions, ranging from insomnia, experienced by roughly 75% of MDD patients, to hypersomnia (Franzen & Buysse, 2008), making sleep-related complaints almost synonymous with depression (Nutt et al., 2008). These disturbances manifest as increased sleep onset latency, decreased sleep efficiency, early morning wakening's and alterations in sleep duration, less in insomnia and more in hypersomnia (Hutka et al., 2021; Nutt et al., 2008).

Polysomnographic recordings also indicate profound changes in sleep architecture among patients with depression, with marked disturbances in REM sleep patterns and a decrease in SWS (Hutka et al., 2021; Wichniak et al., 2017). Specifically, shortened REM latency and increased REM duration are most characteristic of depression (Wang et al., 2015). Disturbances in REM sleep, linked to emotional processing, may contribute to the emotional dysregulation seen in depression (Wang et al., 2015). Additionally, a shift in sleep stages is highlighted, with a notable reduction in SWS (Armitage, 2007; Borbély et al., 1984). Several studies indicate this SWS reduction in MDD patients, with varying results across age groups (Bovy et al., 2022).

The consequences of sleep disturbances in depression are profound. For instance, a study revealed that 97% of individuals with depression reported sleep difficulties, with 59% of them experiencing significant impairment in their quality of life (Nutt et al., 2008). Not only do sleep disturbances intensify depressive symptoms, they also contribute to an increased likelihood of suicidal ideation and reduced treatment effectiveness, whether through medication or psychotherapy (Asarnow & Manber, 2019; Franzen & Buysse, 2008; Manber et al., 2008; Mason & Harvey, 2014). Sleep disturbance in depression is associated with higher attrition rates, leading many to stop treatment prematurely (Christensen et al., 2016). Moreover, higher baseline insomnia is correlated to a decreased likelihood of MDD remission, regardless of whether insomnia symptoms improve or not (Mason et al., 2020). Studies have found that addressing sleep disturbances could markedly improve depressive symptoms in patients (Franzen & Buysse, 2008).

Notably, the interplay between sleep and depression is bidirectional, in that symptoms of insomnia contribute to depression and symptoms of depression disrupt sleep (Mason & Harvey, 2014). Recent evidence suggests that sleep disturbances may act as potential precursors to

depression, challenging the traditional view of them merely being by-products of depressive illness (Baglioni et al., 2011; Fang et al., 2019). Their presence can predict depression onset, with studies showing that those with insomnia, for example, face a 2-4 times higher risk of developing MDD (Breslau et al., 1996). Furthermore, persistent insomnia remains the most frequent residual symptom in patients with MDD and is considered a key predictor of relapse (Fang et al., 2019). The occurrence of sleep disturbances either before or concurrently with other depressive symptoms highlights their role as diagnostic entities capable of triggering the onset of depression (Fang et al., 2019).

2.2.3.1 Neurophysiological Mechanisms

At the neurophysiological level, there is clear evidence of a mechanistic link between sleep and depression (Li et al., 2022). Neurotransmitter imbalances, notably within the monoamine system, have been identified as key players in both disturbed sleep and depression. These neurotransmitters, in addition to their involvement in mood regulation as per the monoamine theory of depression, are also important for maintaining sleep-wake cycles, particularly the initiation and maintenance of REM sleep. Altered neurotransmitter release can also interrupt the regular alternation of REM and NREM sleep (Li et al., 2022).

Additionally, the HPA axis, which governs our body's stress responses, is implicated in both sleep and mood disorders. Dysregulation in the HPA axis results in altered cortisol secretion. Elevated cortisol levels can not only disrupt sleep architecture but also contribute to MDD pathophysiology. Cortisol serves an important role in the stress response system, as well as in a classic circadian pattern given its release from the adrenal gland (Asarnow, 2020).

2.2.4 Current Interventions for Disturbed Sleep in MDD

Addressing sleep disturbances in the context of depression is crucial, given the potential for improved symptom resolution and remission. Both pharmacological and non-pharmacological approaches have demonstrated effectiveness in addressing the co-occurring conditions.

2.2.4.1 Pharmacotherapy

Antidepressant medications serve as primary treatments for MDD and often target associated sleep disturbances. They directly impact sleep neurophysiology, with some improving sleep-related issues, and others exacerbating them (Hutka et al., 2021; Sharpley & Cowen, 1995).

Nearly all SSRIs, SNRIs, and MAOIs are associated with REM sleep suppression, marked by increased REM latency, reduced REM sleep duration, and diminished REM density (Wichniak et al., 2017). Some of these drugs are known to further deteriorate sleep quality as a side effect (Fang et al., 2019; Wang et al., 2015). By elevating the brain's levels of monoamines, such as serotonin, antidepressants can affect REM sleep (Gillin et al., 1994). The suppression of REM is often most pronounced during the early stages of treatment but may gradually diminish over time (Wilson & Argyropoulos, 2005). However, even after mood improvement, some of these medications might still cause residual sleep symptoms (Nutt et al., 2008). Notably, MAOIs may even lead to a complete absence of REM sleep for extended periods (Wilson & Argyropoulos, 2005). Another drug, Mirtazapine, categorized as an atypical antidepressant, also suppresses REM sleep (Winokur et al., 2003). A rebound in REM sleep, sometimes surpassing baseline levels, is observed in many individuals after discontinuing these medications (Wang et al., 2015). Interestingly, escitalopram, an SSRI, appears not to suppress REM sleep (Holshoe,

2009). While depression is characterized by increased REM sleep and many antidepressants suppress REM, this doesn't directly translate to therapeutic efficacy. The influence of antidepressants on sleep goes beyond merely REM modulation, therefore addressing one aspect of sleep architecture does not assure overall sleep or mood improvement.

While many antidepressants can disrupt sleep, others, notably sedative types like nefazodone, mirtazapine, and certain TCAs, can decrease sleep latency, enhance sleep efficiency, and increase time in SWS without significant REM interference (Wichniak et al., 2017). Notably, nefazodone and mirtazapine exert antagonist effects on certain serotonin receptors and minimally inhibit monoamine transporters, targeting insomnia in patients with depression (Thase, 2006). In some instances, just a single dose of these medications, especially mirtazapine, can enhance sleep quality (Schmid et al., 2006). However, the sedative properties can be problematic in long-term use due to oversedation, potentially requiring dose adjustments that could impact treatment efficacy (Wichniak et al., 2017).

Agomelatine, a unique antidepressant that targets melatonergic receptors, has been reported to reduce sleep latency and enhance sleep continuity by increasing SWS and daytime alertness without suppressing REM sleep (Wichniak et al., 2017) However, its antidepressant mechanism and relative effectiveness are still topics of research and debate (Leung, 2021).

Other medications, such as the psychostimulant modafinil, offers potential against fatigue and sleepiness associated with depression. However, its efficacy remains debated across studies (Fava et al., 2007). Benzodiazepines, common sedative-hypnotics, are used to treat sleep disorders like insomnia by amplifying the action of GABA, the brain's primary inhibitory neurotransmitter (Pagel & Parnes, 2001). The newer non-benzodiazepines, or z-drugs, offer improved sleep with fewer risks of dependency and insomnia rebound, likely due to their distinct

mechanisms of action (Schifano et al., 2019). Although not typically effective for symptoms of depression due to their depressive action on the central nervous system (CNS), combination studies using antidepressants with these drugs show promising results. For instance, combining fluoxetine with eszopiclone resulted in better sleep and depression outcomes (Krystal et al., 2007). However, both benzodiazepines and z-drugs come with potential concerns such as dependence and cognitive disturbances (Brett & Murnion, 2015). Therefore, non-pharmacological interventions or safer alternatives should serve as primary treatments, with hypnotic drug use closely monitored for potential side effects and risks.

Sedative antidepressants, like mirtazapine, can serve as effective treatments for patients with co-existing depression and sleep disturbances (Dolder et al., 2012). Such medications can also reduce benzodiazepine reliance, mitigating the risk of sleep aid addiction in patients with depression (Hashimoto et al., 2016). Nevertheless, concerns about drug resistance, potential side effects, and discontinuation symptoms should always be considered (Fang et al., 2019).

2.2.4.2 Cognitive Behavioral Therapy for Insomnia

Cognitive behavioral therapy for insomnia (CBT-I) has become a frontline intervention in managing both depression and coexisting insomnia, with numerous studies demonstrating its effectiveness. For instance, a study involving adults with coexistent depression and insomnia found that participants receiving CBT-I experienced pronounced improvements in both insomnia severity and depressive symptoms when compared to a control group (Manber et al., 2008). A recent meta-analysis further supported these findings, showing marked improvements in sleep metrics, including sleep onset latency, total sleep time, and wake after sleep onset, after undergoing CBT-I. Additionally, depressive symptoms also saw noteworthy reductions (Wu et

al., 2015). Another study spanning three years confirmed the benefits of addressing insomnia rather than just targeting depression, thus advocating for the integration of CBT in treating individuals with both conditions (Blom et al., 2017).

However, while CBT-I primarily targets insomnia symptoms, it may not directly address the underlying depressive symptoms. Although improved sleep can potentially elevate mood, the broader emotional and physiological aspects of MDD may not be fully addressed (Baglioni et al., 2011). Implementing CBT-I requires consistent participation and adherence, a challenge for those with depression facing motivation issues, lethargy, or therapy engagement challenges. For example, those exhibiting pronounced depressive symptoms might be more inclined to abort group CBT-I sessions (Ong et al., 2008). There's also evidence suggesting that those with childhood-onset depression and insomnia may not benefit from CBT-I compared to those who manifested these conditions later in life (Edinger et al., 2017).

Addressing sleep disturbances in MDD requires a significant understanding of both the subjective and objective aspects of sleep, as these disturbances not only signal alterations in brain neurotransmitter functions, but also exert profound impacts on quality of life. While sedative antidepressants and CBT-I present promising avenues for those struggling with depression and sleep disturbances, barriers like financial constraints and sustained therapy engagement remain. The persistent nature of sleep disturbances in depression, persisting even post-treatments, underscores the need for enhanced therapeutic strategies. These are vital to optimize patient well-being and reduce the likelihood of recurrent depressive episodes.

2.3 The Gut Microbiome

2.3.1 The Microbiota Gut-Brain Axis

The human body is home to trillions of microorganisms, collectively termed the microbiome, pivotal in maintaining overall health (Kho & Lal, 2018). Recent years have witnessed a revolutionary surge in our understanding of microbiota biology, revealing their significant role to the pathophysiology of brain disorders (Wang et al., 2022). Often considered our body's secondary genome, approximately 3.8×10^{13} microorganisms inhabit the gastrointestinal (GI) tract, with the colon owning the densest microbial concentration (Kho & Lal, 2018; Sender et al., 2016). Colonization of the gut begins soon after birth, influenced by factors such as birthing method and breastfeeding. As we age, the microbial composition stabilizes, though its specific makeup varies widely due to influences like genetics, diet, metabolism, age, geographic location, antibiotic use, and stress (Martin et al., 2016). Generally, the gut microbiota consists of bacteria predominantly from three major phyla: *Firmicutes,* *Bacteroidetes,* and *Actinobacteria* (Tap et al., 2009). This diverse microbiota ecosystem aids in the proper functioning and development of the immune system, CNS circuitry, and GI operations (Yong et al., 2020).

A rapidly growing area of study is the bidirectional communication between the gut microbiome and the brain, termed the microbiota-gut-brain axis (GBA). This communication network includes the central nervous system (CNS), the autonomic nervous system (ANS), the enteric nervous system (ENS), the immune system and the HPA axis (Carabotti, 2015; Yong et al., 2020). Understanding this intricate communication system showcases its role in maintaining vital systems like GI and immune homeostasis, while also influencing higher cognitive functions (Carabotti, 2015). Any disruptions in this system, whether resulting from diseases or stress, can

lead to imbalances in the gut, mood swings, cognitive dysfunctions, and altered inflammatory levels. The gut microbiota and the brain communicate bidirectionally most notably through neural, immune, and humoral pathways (Yong et al., 2020).

2.3.1.1 Neural and Humoral Routes

A significant link between the gut microbiota and our nervous system is thought to initiate within the ENS. Termed "the second brain," the ENS exhibits neuronal complexity comparable to the brain, autonomously governing gut-related functions, and the immune system (Breit et al., 2018). Gut metabolites, such as short-chain fatty acids (SCFAs), including butyrate, propionate, and acetate, have a profound effect on the ENS. Generated during the fermentation of non-digestible carbohydrates by gut microbiota, these SCFAs, though indigestible, critically influence gut functions. Notably, butyrate and propionate affect serotonin synthesis in enterochromaffin cells (Reigstad et al., 2015). Studies using germ-free (GF) mice highlight the essential role of gut microbiota in maintaining neuron excitability (McVey Neufeld et al., 2013).

The gut microbiota and the brain communicate bidirectionally, employing neural and humoral pathways (Appleton, 2018). A primary conduit for this communication is the tenth cranial nerve, the vagus nerve. This nerve relays information between the GI tract and the CNS, significantly influencing the activities of the ENS, particularly in areas like intestinal permeability and gut inflammation (Powley, 2000; Yong et al., 2020). Additionally, microbial metabolites interact with the brain through the systemic (humoral) circulation, while the brain sends chemical messengers, such as cytokines and glucocorticoids, to influence gut physiology (Luan et al., 2019).

2.3.1.2 Immune, Endocrine, and Neurotransmitter Systems

The gut, specifically the gut-associated lymphoid tissue (GALT), contains approximately 70% of the body's immune system, making it the body's largest immune organ (Vighi et al., 2008). Specific immune cells in the gut respond to foreign antigens as they penetrate the intestinal mucosal barrier, triggering the production of inflammatory cytokines such as interleukins (ILs), tumour necrosis factor (TNF)- α and interferon (IFN)-γ (Sherwin et al., 2016). These cytokines can then influence neural pathways in the brainstem, hypothalamus, and limbic system or directly penetrate the blood-brain barrier (BBB) through the humoral pathway (Yong et al., 2020).

Additionally, the HPA axis, a major stress-response system, plays an integral role. When activated by increased systemic pro-inflammatory cytokines or other stressors, it triggers a series of hormonal releases that ultimately affect areas such as the gut by modulating inflammation (Carabotti, 2015; Tsigos & Chrousos, 2002). Specifically, HPA axis stimulation can influence the release of corticotropin-releasing factor (CRF), which then stimulates the secretion of adrenocorticotropic hormone (ACTH) from the pituitary gland (Carabotti, 2015). ACTH stimulates adrenal gland release of cortisol which inhibits CRF release, creating a negative feedback loop. This stress hormone, cortisol, is known as the body's major stress hormone that influences multiple organs, such as the brain. Cortisol, a primary glucocorticoid produced as a result of HPA axis activation, plays a crucial role in regulating inflammatory responses throughout the body, including the gut (Tsigos & Chrousos, 2002).

Simultaneously, the gut microbiota produces brain neurotransmitters critical to brain function. This includes GABA, glutamate, and monoamines like serotonin, while also modulating their precursor availability (Oleskin et al., 2016). These neurotransmitters are

involved in modulating ENS signalling, ultimately influencing the GBA (Karl et al., 2018). For instance, microbes that ferment carbohydrates produce butyrate, a SCFA, which then triggers the synthesis of serotonin from intestinal cells (Reigstad et al., 2015). The complex interplay of neural, immune, and hormonal pathways facilitates the brain's influence over various intestinal activities, underscoring the significance of the GBA (Carabotti, 2015).

2.3.2 GBA Dysregulation and its Implications in MDD

Following the discovery of the GBA, research has underscored the importance of a healthy microbiota for normal brain function. A balanced state of the gut, or eubiosis, is characterized by a healthy ratio of potentially beneficial bacteria to potentially harmful bacteria, primarily represented by the phyla *Firmicutes* and *Bacteroidetes*. This balance is integral for physiological homeostasis (Salami, 2021; Zhang et al., 2015). Dysbiosis, the offset of this balance, can be triggered by various factors such as dietary shifts, lifestyle changes, excessive stress, and antibiotic consumption. In this altered state, the gut may see a decline in intestinal gut microbiota diversity or a rise in pathogenic bacterial populations (Salami, 2021). This imbalance can elevate acetylcholine and cortisol levels, compromising the intestinal barrier, a condition termed "leaky gut syndrome" (Clapp et al., 2017). The weakened barrier permits bacterial toxins to penetrate through, causing inflammation in the GI tract. This inflammation, in turn, prompts the release of cytokine and neurotransmitters, adding stress to the microbiome (Clapp et al., 2017; Yong et al., 2020). These cascading effects, paired with the increased permeability of the BBB, can influence brain function, potentially leading to depression (Raffaele et al., 2021). Indeed, high levels of pro-inflammatory cytokines have been identified in individuals with depression (Howren et al., 2009).

Typically, the immune system and the HPA axis can mount an adaptive response. However, chronic stress hinders this response. Sustained inflammation, induced by elevated pro-inflammatory cytokines, can cause persistent activation of the HPA axis. This chronic inflammation impairs the immune system's ability to recognize and respond to cortisol's inhibitory signals, disrupting the negative feedback inhibition of the HPA axis (Schiepers et al., 2005).

Moreover, chronic stress and gut inflammation can also alter the distribution and abundance of the bacteria in the gut. Such shifts in composition can exacerbate gut inflammation and escalate intestinal permeability and cytokine production (Yong et al., 2020). These changes can also influence neuroactive metabolite production, altering host neurotransmitter circuitry, a key component in depression pathophysiology. A compromised neurotransmitter system in the ENS might impact gut motor function, which is crucial for sustaining microbial diversity in the gut (Salami, 2021).

Thus, chronic stress triggers a vicious cycle leading to an impaired GBA, marked by inflammation, HPA axis overactivity, and gut imbalances (Raffaele et al., 2021). Significantly, those with depression react more intensely to stress, emphasizing the GBA's role in these conditions (Mendonça-De-Souza et al., 2007).

2.3.2.1 Altered Gut Microbiota Composition in Depression

There are evident alterations in gut microbiome patterns among those with depression. Many studies have highlighted these changes, a notable one being a systematic review that aimed to distinguish the differences in microbiota composition in individuals with depression compared to their healthy counterparts (Barandouzi et al., 2020). Key findings included noted discrepancies

in both alpha-diversity (the diversity of organisms within individual samples) and beta-diversity (the diversity of organisms between different samples) of the microbiota among the two groups. Phylum level analysis showed inconsistencies in the abundance of *Firmicutes*, *Bacteroidetes,* and *Proteobacteria* in the depressed cohort. Conversely, *Actinobacteria* and *Fusobacteria* phyla were markedly abundant in depressed individuals. Both family- and genus-level data revealed more consistent findings of either lower or higher abundances of certain bacteria between groups (Barandouzi et al., 2020). These findings encourage further exploration to steer findings into novel clinical strategies that could enhance outcomes in depression.

2.3.3 The Bidirectionality of Disturbed Sleep and Gut Microbiota

The intricate bidirectional relationship between sleep disturbances and gut microbiota is becoming increasingly evident (Wang et al., 2022). Disruptions in the GBA have been associated with sleep disorders such as insomnia, sleep apnea, and circadian rhythm disorders (Wang et al., 2022). Notably, both sleep and gut microbiota showcase circadian rhythms, emphasizing a synchronized relationship (Li et al., 2018; Voigt et al., 2014). The bidirectional communication between sleep and the gut microbiota is influenced by various mechanisms, including the vagus nerve, HPA-axis, homeostatic and inflammatory regulation processes, and brain function modulation influence this bidirectional communication, much like the mechanisms pertaining to the GBA and depression. Neurotransmitters and microbial metabolites, including tryptophan, melatonin, serotonin, GABA, and SCFAs further mediate this relationship (Osadchiy et al., 2019). The intertwined nature of these factors underscores the importance of understanding their interactions, with potential implications for holistic health interventions.

2.3.3.1 Mechanisms Linking Sleep Regulation and the GBA

Serotonergic System

Serotonin plays a crucial role in regulating sleep architecture. A decline in serotonin concentrations in the brain have been associated with changes in NREM sleep (Nakamaru-Ogiso et al., 2012). Similarly, antibiotic-induced gut microbiome depletion diminishes intestinal serotonin and alters NREM patterns, suggesting that serotonin may bridge gut microbiome interactions with brain sleep regulation (Ogawa et al., 2020).

Microbial Metabolites

Alterations in gut SCFA-producing microbiota have been correlated with disrupted sleep patterns (Maki et al., 2020). Butyrate, a specific SCFA, when introduced externally, has been shown to enhance NREM sleep in rats, suggesting a possible bidirectional link between gut microbiota and sleep via microbial metabolites (Szentirmai et al., 2019)

The Vagus Nerve

The role of the vagus nerve in linking gut microbiota and sleep has been highlighted in mice studies where severing the vagus nerve negated the inflammation resulting from sleep deprivation (Zhang et al., 2021).

The Immune System

A reciprocal relationship exists between sleep and the immune response. Sleep deprivation intensifies inflammatory reactions and susceptibility to infections, whereas inflammation can disturb sleep patterns (Sen et al., 2021). Vagotomised mice lack of

inflammatory response post sleep deprivation suggests gut microbiota mediates this inflammation (Zhang et al., 2021). Further, chronic inflammation from leaky gut syndrome can impact circadian rhythm and induce sleep disturbances (Irwin, 2023)

The Blood Brain Barrier

The BBB's permeability is susceptible to the influences of the gut microbiota. For instance, FMT from conventional mice to GF mice mitigates their increased BBB permeability (Braniste et al., 2014). Similarly, antibiotics, which deplete gut microbiota, can also compromise the BBB permeability (Sun et al., 2021). Rodent studies have shown sleep is vital for BBB integrity, whereby sleep restriction led to a widespread breakdown of the BBB, suggesting that sleep disturbances might impact the BBB via interactions with the gut microbiota (He et al., 2014).

2.3.3.2 Altered Gut Microbiota Compositions in Sleep Disturbances

Both the gut microbiome and the host's circadian rhythm and sleep cycles influence each other. Notably, variations within the gut microbiome have been shown to influence the expression of the host's circadian genes (Li et al., 2018). As such, maintaining a balanced gut microbiota is important for optimal circadian rhythm regulation (Parkar et al., 2019). However, the literature presents somewhat varied findings concerning the exact relationship between sleep disruptions and microbiota composition. A key consideration is that many studies assess gut microbiota at single time points, often overlooking the fact that gut microbiota undergo rhythmic fluctuations throughout the day. While various studies in the literature have explored the potential influence of microbial composition on different aspects of both objective and subjective

sleep disruptions, the complex relationship between the two remains an ongoing field of study. In the context of this thesis, with a particular emphasis on microbial therapeutic interventions and the use of the PSQI scale, we will focus on studies measuring sleep quality, an important aspect of sleep with significant clinical implications.

Sleep Quality Studies

Recent studies have underscored the importance of sleep quality in relation to gut microbiota. A positive correlation has been observed between self-reported sleep quality, as measured by the PSQI, and microbial diversity. Specifically, participants reporting better sleep quality demonstrated a richer microbial diversity in the gut, as measured by the Shannon index (Grosicki et al., 2020). This observation aligns with findings that connect gut microbiota diversity with objective measures of sleep quality, such as actigraphy-based assessments of sleep duration and efficiency (Smith et al., 2019). In contrast, two earlier studies did not identify significant impacts on microbiome diversity following short-term sleep restriction (Benedict et al., 2016; Zhang et al., 2017). However, a more extended study proposed that minor, short-term alterations in sleep might not exert notable effects on gut microbiota diversity (Smith et al., 2019). Moreover, a positive association was noted between sleep quality and the *Firmicutes/Bacteroidetes* (F/B) ratio, with the presence of higher relative abundance of butyrate-producing genera *Blautia* and *Ruminococcus,* both from the *Firmicutes* phylum, and a decreased presence of *Prevotella,* from the *Bacteroidetes* phylum (Grosicki et al., 2020). Collectively, these results strengthen an expanding body of research, which suggests a connection between the gut microbiome and its by-products with sleep physiology.

The *Actinobacteria* phylum showed a negative correlation with the number of awakenings during sleep, indicating that *Actinobacteria* richness contributes to enhanced sleep quality (Smith et al., 2019). Studies in mice illustrated a reduction in the proportion of *Actinobacteria* in the gut microbiome after sleep disruption (Poroyko et al., 2016). However, in humans, one study identified that specific members of the *Actinobacteria* phylum increased in response to sleep restrictions (Benedict et al., 2016). Another recent study revealed that high sleep quality individuals exhibited a gut microbiome marked by an abundance of bacteria from the *Verrucomicrobia* and *Lentisphaerae* phyla (Anderson et al., 2017; Morwani-Mangnani et al., 2022). Yet, another study did not find any significant relationships between sleep measures and the richness or diversity within these phyla, demonstrating the inconsistencies across trials (Smith et al., 2019).

Given the established connection between gut microbiota and sleep quality, it's vital to examine this relationship within the framework of MDD, especially considering the prominent link between sleep disturbances and depressive disorders. As sleep disturbances remain one of the hallmark features of depression, it becomes crucial to explore innovative interventions targeting this domain.

2.3.5 Microbiota-Targeted Interventions for Sleep Disturbances in Depression

Given the clearer understanding of the relationship between sleep, depression, and the gut microbiome, microbiota-targeted interventions are being heavily explored for their therapeutic potential to alter the gut microbiota and mitigate dysbiosis, as seen in psychiatric pathology. The motivation for this exploration stems from two primary factors: the search for therapeutic

alternatives that sidestep the complications associated with traditional sleep and depression treatments, and the promising regulatory potential of these interventions on the GBA. In this regard, microbiota-targeted interventions, including probiotics, prebiotics, synbiotics, postbiotics, and FMT, have emerged as potential strategies for managing psychiatric illnesses such as MDD.

2.3.5.1 Probiotics

Probiotics are live microorganisms, primarily beneficial bacterial strains like *Lactobacillus* and *Bifidobacterium,* that confer health benefits when consumed sufficiently. Available in capsules, powders, and fermented foods, they modulate the gut microbiota to promote a balance of beneficial bacterial over harmful bacteria. The effects and mechanisms of action of probiotics are strain-specific, and their efficacy can vary depending on the probiotic strain and the host's individual characteristics (Zmora et al., 2018).

Recent research underscores the potential of probiotics in sleep enhancement. Specific strains like *Lactobacillus fermentum* PS150TM have been noted for improving sleep in mice with caffeine-induced sleep disturbances and increasing NREM sleep duration (Lin et al., 2021; Lin et al., 2019). *Lactobacillus brevis* ProGA28 was shown to mitigate stress-related sleep issues (Lai et al., 2022).

In clinical trials, diverse *Lactobacillus* and *Bifidobacterium* strains have yielded promising results in enhancing both sleep quality and mood (Dickerson et al., 2018; Marotta et al., 2019). Specifically, a study in 2021 found positive results from probiotic supplementation on both mood and sleep. A mixture of *Lactobacillus reuteri* NK33 and *Bifidobacterium adolescentis* NK98 were found to improve depression and sleep disturbances significantly compared to their

placebo counterparts (Lee et al., 2021). In patients with MDD, supplementation with *Lactobacillus fermentum* NS9 significantly improved sleep quality and reduced sleep latency (Liu et al., 2016). Another study found that a multispecies probiotic improved multiple sleep parameters, including sleep efficiency and sleep duration, in patients with depression (Huang et al., 2016). These findings highlight the potential of probiotics as a novel and promising approach to manage sleep disturbances in depression.

2.3.5.2 Prebiotics

Prebiotics are indigestible substances that, when metabolized by microorganisms, foster beneficial effects on the host by modulating the composition and function of these microorganisms (Pandey et al., 2015). Research on rats has indicated that dietary prebiotics can promote NREM sleep and affect certain gut microbiota-produced metabolites (Fei et al., 2021). One significant finding revealed that prebiotic-supplemented rats had higher levels of *Parabacteroides distasonis* and *Ruminiclostridium 5*, which were related to adjustments in body temperature rhythms after a light/dark shift (Thompson et al., 2021). Another study found that a prebiotic diet increased both NREM and REM sleep durations in rats, also highlighting *Parabacteroides distasonis* as a potential contributor to these sleep enhancements (Bowers et al., 2022).

In human studies, early-life prebiotic supplementation in infants led to delayed nap onset and sustained daytime alertness, possibly due to microbiota changes (Colombo et al., 2021). Additionally, another study including individuals with depression observed enhanced sleep quality and REM sleep duration following the administration of a prebiotic galactooligosaccharide supplement (Schmidt et al., 2015). However, participants given the

prebiotic inulin in a cross-over study did not experience any mood or sleep quality alterations compared to placebo counterparts (Smith, 2005). Collectively, these findings from preclinical and clinical studies, although promising, require further investigation to establish a clearer understanding of their potential for sleep disturbances in depression.

2.3.5.3 Synbiotics

Synbiotics are combinations of probiotics and prebiotics designed to work synergistically (Pandey et al., 2015). Military field training often leads to sleep deprivation, with soldiers experiencing sleep loss and sleepiness. In this context, one study found that symbiotic ice cream improved soldiers' resistance to sleepiness during field training (Valle et al., 2021). In the context of depression, symbiotic treatment led to significant reductions in depressive symptoms, although no measures of sleep were assessed (Ghorbani et al., 2018). Additional research is needed to evaluate the therapeutic potential of synbiotics in managing sleep disturbances in depression.

2.3.5.4 Postbiotics

Postbiotics are the metabolic by-products or components of probiotics that offer health benefits to the host (Salminen et al., 2021). One study demonstrated that using heat-inactivated *Lactobacillus gasseri* CP2305 notably enhanced sleep quality in chronically stressed students (Nishida et al., 2017). Unlike strategies that utilize live microorganisms, postbiotics are perceived as safer due to their absence of biological activity (Salminen et al., 2021). However, the evidence regarding postbiotics' impact on sleep and depression remains limited.

2.3.5.5 FMT

FMT is a therapeutic approach that involves transferring a complete microbial community into the intestinal tract of a recipient from a donor to treat various diseases (Smits et al., 2013). While its primary clinical application has been for patients suffering from persistent Clostridium difficile infections, its therapeutic potential is being explored for a broader range of conditions (Smits et al., 2013). These include inflammatory bowel diseases, irritable bowel syndrome, anorexia nervosa, cancer, and even neuropsychiatric issues such as depression (Kang et al., 2022). Although limited, some studies have also proposed the potential of FMT in treating insomnia and other sleep disorders (Kang et al., 2022). A recent systematic review highlighted the promising impact of FMT on psychiatric disorders specifically. All studies cited within the review reported reduced symptoms of depression and anxiety post healthy microbiota transplantation, across both preclinical and clinical contexts. A specific study in the review even showcased improved sleep metrics (Chinna Meyyappan et al., 2020). Furthermore, research indicates FMT might alleviate sleep disruptions in irritable bowel syndrome patients, while also correlating enhanced depressive scores with post-FMT microbiome diversity changes (Kurokawa et al., 2018).

Despite its potential, several challenges limit FMT's therapeutic promise. The primary concerns relate to safety, the procedure's invasiveness, and its implications. The invasive nature of FMT could deter patients with MDD, impacting treatment adherence and leading to premature discontinuation. Moreover, potential complications and side effects, especially in immunocompromised individuals, remain a significant concern. Identifying ideal donor-recipient matches also presents a challenge, considering the vast diversity of individual gut microbiomes

(Bibbò et al., 2020). Lastly, optimal timing, frequency, and the long-term effects of FMT procedures remain unknown.

While the current literature investigating gut-repopulation techniques for psychiatric illness like depression is promising, challenges such as individual variability, safety concerns, and standardization are of relevance. Another significant limitation is the lack of comprehensive studies, underscoring an urgent need for deeper investigation using larger-scale clinical trials. In this context, this thesis introduces MET-2, a novel microbe therapy containing defined microbial communities in capsule form. The aim is to ascertain MET-2's potential as a novel solution for addressing both disturbed sleep and depression.

CHAPTER 3: METHODOLOGY

3.1 Introduction to the METDA Study

This thesis uses data from the METDA study: a phase 2, double-blind, placebo-controlled trial evaluating the safety and efficacy of MET-2 in patients with major depression. The METDA study was conducted at two sites: Providence Care Hospital in Kingston, Ontario and the Centre for Addition and Mental Health in Toronto, Ontario.

The METDA study was designed to assess the efficacy of MET-2 against a placebo in mitigating depressive symptoms across a 6-week treatment duration. The primary objective was to assess mood using the Montgomery Asberg Depression Rating Scale (MADRS) at 5 different time points throughout the study. Secondary objectives included analyzing changes in mood, anxiety, sleep disturbance, early stress, gastrointestinal symptoms, and the tolerability of MET-2. These evaluations employed a range of clinical scales, including the HAM-A, GAD-7, CGI, SHAPS, QIDS-SR16, and the PSQI. Side effects and gastrointestinal symptoms were assessed using the TSES and GSRS scales, respectively.

A component of the METDA study was a follow-up sub study, which was designed to monitor the longitudinal effects of MET-2 at both 12- and 24-weeks post-treatment discontinuation. Therefore, this thesis also uses data from this sub study.

This thesis primarily investigates the impact of MET-2 on sleep disturbances in individuals with depression, with mood symptoms assessed as a secondary measure. The methods discussed in this chapter are based on the METDA study protocol. A specific set of METDA study data, encompassing PSQI and MADRS scores along with demographic information, was extracted for the analyses presented.

**This protocol has been detailed in a published manuscript (Chinna Meyyappan et al., 2021).*

3.2 Participants

A total of 29 eligible adult participants between the ages of 18 and 45 currently in a major depressive episode (MDE) were recruited from the Kingston and Toronto areas. All potential study participants were initially pre-screened over the phone by one of the site coordinators to deem eligibility based off a few pre-screening questions. If eligible, individuals were then invited to the study site for an in-depth screening appointment. At the initial screening visit, individuals were given ample time to read over the consent form and to have any questions answered by the site coordinator. Consent was then obtained from the participant by a site coordinator and overseen by the principal investigator. Following informed consent, participants were screened to ensure they fit the inclusion and exclusion criteria. Participants were then evaluated for a diagnosis of MDD based on the Mini-International Neuropsychiatric Interview (MINI) and a score of 15 or higher on the MADRS. All participants had to be in a current MDE and could not be taking any antidepressant medication currently. See **Table 1** and **Table 2** for full study criteria.

Table 1: Inclusion Criteria

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1. *Between 18 and 45 years of age (inclusive) and able to provide informed consent.*
2. *Not pregnant. Individuals who are capable of becoming pregnant must agree to use appropriate birth control measures unless otherwise exempted by investigator and sponsor for exceptional circumstances.*
3. *Willing to participate in follow up as part of the study.*
4. *Diagnosis of MDE as determined by the MINI*
5. *Current depressive episode with a MADRS score of ≥15.*
6. *Able to understand and comply with the requirements of the study.*
7. *Able to provide stool, urine, and blood samples.*
8. *Those who do not choose to use antidepressants for moderate-severe depression.*

Table 2: Exclusion Criteria

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1. *History of chronic diarrhea*
2. *Need for regular use of agents that affect GI motility (narcotics such as codeine or morphine, agents such as loperamide or metoclopramide)*
3. *Colostomy*
4. *Elective surgery that will require preoperative antibiotics planned within 6 months of enrolment.*
5. *History of bariatric surgery.*
6. *Pregnant, breastfeeding, or planning to get pregnant in the next 6 months.*
7. *Any condition for which, in the opinion of the investigator, the patient should be excluded from the study.*
8. *Current use of any antidepressant/antianxiety drug (eligible to participate after a 4-week washout period)*
9. *More than three depressive episodes throughout lifetime*
10. *Having failed two or more anti-depressant treatments in the past or a antidepressant treatment during current depressive episode*
11. *Use of any antibiotic drug in the past 4 weeks (may be eligible to participate after a 1-month washout period)*
12. *History of alcohol or substance use disorder in the past 12 months based on the MINI 7.0 Section I: Alcohol use disorder and Section J: Substance use disorder.*
13. *Daily use of probiotic product in the past 2 weeks (may be eligible to participate after a 2-week washout period)*
14. *Use of any type of laxative in the last 2 weeks.*
15. *Consumption of products fortified in probiotics (may be eligible to participate after a 2-week washout period)*
16. *High suicidal risk, as measured by MADRS item 10 score equal to 4 or higher.*
17. *Current psychotic symptoms*
18. *History of epilepsy or uncontrolled seizures*
19. *Immunodeficiency (immuno-compromised and immuno-suppressed patients, e.g., acquired immune deficiency syndrome [AIDS], lymphoma, patients undergoing long-term corticosteroid treatment, chemotherapy, and allograft patients)*
20. *Unstable medical conditions or serious diseases/conditions (e.g., cancer, cardiovascular, renal, lung, diabetes, psychiatric illness, bleeding disorders, etc.)*
21. *The use of natural health products (e.g., St. John's Wort, passionflower, etc.) that affect depression.*
22. *History of Electroconvulsive therapy*

3.3 Recruitment

Participants were recruited through a combination of methods, including targeted Facebook ads, public transit advertisements, and posters distributed around Kingston and Toronto University campuses. Additionally, announcements were made on the Department of Psychiatry Queen's website and the Queen's Centre for Neuroscience website.

3.4 Investigational Product

Microbial ecosystem therapeutic (MET) is an innovative treatment method that aims to repopulate the gut with healthy bacteria. MET was developed as an alternative to fecal microbiota transplantation (FMT). MET is a biological compound that consists of live microbes that naturally reside in the gut of a healthy individual. While stool contains a diverse and complex community of microbes, including bacteria, fungi, and viruses, MET focuses on using defined mixtures of isolated strains of intestinal bacteria for treatment.

Specifically, MET-2 is a refined version of the original MET-1 mixture. MET-2 is derived from healthy donor stool and was developed to alleviate symptoms of early depression and restore normal gut flora. It consists of 40 lyophilized bacteria strains, carefully selected from a healthy 25-year-old donor for their safety profile and purified for use.

To enhance patient compliance and convenience, MET-2 is administered orally in capsule form. This method of administration allows for easier intake over consecutive days compared to rectal administration, as seen with FMT. Furthermore, the use of capsules ensures the controlled delivery of fecal colonies, which increases safety compared to using raw fecal material in rectal suspension.

Each MET-2 capsule contains 0.5 g of product, with a concentration of 3.2 X 10^5-10^{11} colony forming units (CFU) per capsule. An initial loading dose of 5 g (equivalent to 10 capsules) is typically administered to deliver a therapeutic dose of MET-2 ranging from 10^8 to 10^{12} CFU. MET-2 capsules were produced in adherence to Good Manufacturing Practice guidelines at the University of Guelph and were transported securely under anaerobic conditions at room temperature (Chinna Meyyappan et al., 2021).

3.5 Study Design

3.5.1 METDA Randomized Controlled Trial

The METDA study was an 8-week, phase 2, double-blind, randomized placebo-controlled, clinical trial used to measure the effects of MET-2 on symptoms of depression using pre- and post- treatment scores. A total of 15 participants were randomly allocated to receive MET-2 treatment and a total of 14 participants were randomly allocated to receive placebo alternative. All participants consumed either MET-2 or placebo alternative daily for 6 weeks. The study consisted of 6 visits total: a screening visit, a baseline visit (week 0), 3 treatment-period visits (week 2, 4, and 6), and a 2-week follow-up visit (week 8) as outlined in **Table 3**.

3.5.2 METDA Longitudinal Follow-Up

In addition to the METDA study RCT, a longitudinal follow-up sub study was conducted to assess long-term effects of MET-2 on depressive symptoms. Participants who completed at least 5 weeks of either MET-2 or placebo treatment were invited to participate. Consent to participate was obtained during the last visit of the METDA RCT: the week 8 visit. The sub study consisted of two additional visits, occurring 12- and 24-weeks post-treatment discontinuation (week 18 and week 30), as outlined in **Table 3**.

Table 3: Summary of Study Visits

Procedures	Visit	1	2	3	4	5	6	7	8
	Week	Screening[a]	0 (BL)	2	4	6	8 (FU)	18 (FU)	30 (FU)
	Study Day	-10 to -1	0[b]	14[b]	28[b]	42[b]	68[b]	126[b]	210[b]
Study Criteria and ICF Review and Signing		X[c]							
MINI Diagnostic Interview		X							
Demographics		X[c]							
Medical and Antidepressant History		X[c]							
Randomization to Treatment or Placebo group			X[c]						
MADRS		X[c]	X	X	X	X	X	X	X
HAM-A			X	X	X	X	X	X	X
YMRS		X[c]	X	X	X	X	X	X	X
GAD-7			X	X	X	X	X	X	X
CGI			X	X	X	X			
SHAPS			X	X	X	X			
QIDS-SR16			X	X	X	X			
PSQI			X	X	X	X		X	X
TSES			X	X	X	X			
GSRS			X	X	X	X			
CECA						X			
AE Monitoring			X	X	X	X	X		
Treatment Administration[e]			X[c]		X[c]				

AE= adverse event; BL = baseline; CECA = Childhood Experience of Care and Abuse Questionnaire; CGI = Clinical Global Impressions Scale; FU = follow up; GAD-7 = Generalized Anxiety Disorder Scale; GSRS = Gastrointestinal Symptom Rating Scale; HAM-A = Hamilton Anxiety Rating Scale; MADRS = Montgomery-Asberg Depression Rating Scale; MINI = Mini-International Neuropsychiatric Interview; PSQI = Pittsburgh Sleep Quality Index; QIDS-SR16 = Quick Inventory of Depressive Symptomatology; SAE = serious adverse event; SHAPS = Snaith-Hamilton Pleasure Scale; TSES = Toronto Side Effects Scale.

[a] Time between screening to baseline can be up to 10 days.

[b] Note that all windows will reset to the previous visit (i.e. +/- 4 days from last treatment visit). All participants will be provided with four extra day's worth of investigational product to account for this window.

[c] In-hospital component of visits.

[d] The serum pregnancy test is only to be performed to confirm pregnancy if a urine pregnancy test is positive.

[e] Note that although subjects take the product daily, they only pick-up the product from research personnel at two timepoints.

3.6 Treatment of Patients

3.6.1 Identity and Administration of Study Drug

MET-2 and placebo were both provided in 0.5g capsules. The placebo, filled with cellulose, mirrored the appearance of MET-2 capsules to maintain blinding. Participants were given a 4-week supply of MET-2 or placebo capsules at the baseline visit and a subsequent 2-week supply at the week 4 visit. Distinct vials differentiated loading/booster doses from the daily maintenance doses.

Eligible participants began the regimen with a loading dose of 5g (10 capsules) of MET-2 on days 1-2. The maintenance dose, set at 1.5g (3 capsules), was introduced on days 3-14. Days 15-16 involved another booster dose, followed by the continuation of the maintenance dose on days 17-28. At week 4, participants were analyzed for treatment response. Responders, identified by more than a 50% decrease in MADRS scores from baseline, continued with the maintenance dose until day 42. Non-responders received a second booster dose on days 29-30, followed by the continuation of the maintenance dose until day 42 (see **Table 4**). After the initial dose on day 1, a 30-minute observation period was mandated to ensure no immediate adverse reactions.

Table 4: MET-2 Dosing Schedule

Dosing	**Treatment Period**		
	Baseline to Week 2	**Weeks 2 to 4**	**Weeks 4 to 6**
Loading/Booster Dose (5.0 g/day MET-2[a])	Days 1 and 2 only	Days 15 and 16 only	NR: Days 29 and 30 only[b]
Maintenance Dose (1.5 g/day MET-2)	Days 3-14	Days 17-28	R:Days 29-42 NR: Days 31-42

NR = non-responders; R = responders

[a] The present study involves 6 weeks of regular maintenance dose with a 2-day loading dose/booster at baseline and Week 2, during which period patients will not take the maintenance dose.

[b] Only non-responders will receive the booster dose at this point (Week 4).

3.7 Study Visits

3.7.1 METDA RCT

Due to the COVID-19 pandemic, participants were only required to attend 4 out of the 6 RCT visits in person: Screening, Baseline, Week 4, and Week 8. Week 2 and Week 6 visits were conducted over the phone. Please see **Table 3** for a more detailed description of the study visits.

Screening Visit (Day -10 to -1)

During screening, the following data was collected: demographic information and medical/antidepressant history, MINI diagnostic interview and MADRS scores, vital signs (including blood pressure, pulse, temperature, and weight), and blood samples to ensure the participant was in good health to participate. Participants who met the eligibility criteria (see **Table 1** and **Table 2**) proceeded to the baseline visit.

Baseline Visit (Week 0)

The baseline visit included participant randomization into either the treatment or placebo group. To ensure participant safety, pregnancy tests (specific to women participants), a physical exam performed by the study doctor, and vital signs were taken at the beginning of the visit. Mood (MADRS) and sleep (PSQI) assessments were then administered prior to the first dose of treatment administration at the clinic in front of the site coordinator.

Treatment Period Visits (Weeks 2, 4, and 6)

During each treatment visit, participants underwent mood (MADRS) and sleep (PSQI) assessments. Specifically, at the week 4 in-person visit, vitals and blood samples were collected

to ensure participant safety. Additionally, the second supply of treatment was dispensed at the week 4 visit.

Follow Up Visit (Week 8)

This 2-week follow-up visit was a part of the METDA RCT, however, for the purpose of this thesis, no data from this visit was used in the analysis.

3.7.2 METDA Longitudinal Follow-Up

At the final METDA RCT visit, participants were consented to the longitudinal follow-up had they wished to participate. Both follow-up visits were conducted over the phone. Please see **Table 3** for a more detailed description of the study visits.

12- and 24- Week Longitudinal Follow-Up Visits (Week 18 and 30)

The first follow up visit occurred 12-weeks post treatment discontinuation, at week 18, whereas the second follow up visit occurred 24-weeks post treatment discontinuation, at week 30. During these visits, both PSQI and MADRS scores were collected.

3.7 Treatment Compliance

To monitor treatment adherence, unused clinical trial material was recorded, and participants' mood charts were reviewed, which were personal logs used to document the following: mood, hours of sleep, adverse events, etc. In the event of non-compliance, patients received retraining after the initial occurrence. Had non-compliance continued, they were subsequently withdrawn from the study.

3.8 Discontinuation Criteria

At any point during the treatment or study, if the participant, principal investigator, or research personnel determined that it was not in the best interest of the participant to continue, the participant may have been discontinued from treatment or withdrawn from the study. The following are examples of potential reasons for treatment discontinuation:

- *Withdrawal of consent*
- *Non-compliance with study procedures*
- *Adverse event that in the opinion of the investigator would be in the best interest of the patient to discontinue study treatment.*
- *Protocol violation requiring discontinuation of study treatment*
- *Lost to follow-up*
- *Request by NuBiyota LLC for early termination of study*
- *Newly pregnant patient*

If a participant had to withdraw because of an adverse event (AE), the investigator monitored and provided necessary treatment until the issue was resolved or stabilized. All participants had the right to withdraw at any time and for any reason, without facing any repercussions and the investigator made efforts to understand the reasons behind any withdrawals.

3.9 Clinical Outcome Measures

3.9.1 Sleep

Sleep disturbances were evaluated at all treatment visits, excluding the week 8 visit as previously mentioned. The primary outcome measure of this thesis was changes in sleep disturbances, assessed biweekly using the PSQI, from baseline to the end of the RCT and at the end of each follow-up visit. The PSQI is a 19-item tool assessing various aspects of sleep. Each item has a score ranging from 0 to 3, with higher scores indicating poorer sleep quality. The total

score ranges from 0 to 21, with a higher score indicating worse sleep quality. Total scores were reported in this thesis and used in the analysis.

3.9.2 Mood

Depression symptoms were the primary outcome of the METDA RCT and follow-up and were therefore assessed at all treatment visits. The secondary outcome measure of this thesis included changes in depressive symptoms, evaluated biweekly using the MADRS from baseline to the end of the RCT and at the end of each follow-up visit. The MADRS is a 10-item diagnostic questionnaire used to measure the severity of depressive episodes in patients with mood disorders. Each item on the MADRS is scored out of 6, where 0 indicates the absence of symptoms and 6 indicates symptoms of maximum severity. The overall score thus ranges from 0 (no depression) to 60 (severe depression).

3.9.3 Correlation

Another secondary outcome measure of this thesis involved gauging correlations between sleep disturbances and depressive symptoms. This correlation assessment was carried out using both the PSQI and MADRS scores from baseline to the end of the RCT. It's worth noting that only one analysis was carried out using the RCT data sample, and not the smaller follow-up sample sizes. This is because the RCT dataset had the largest sample size, providing a more robust and representative data set that is statistically appropriate for correlation analyses. A larger sample size ensures greater reliability in detecting meaningful differences between sleep disturbances and mood.

3.10 Statistical Analysis

All statistical tests in this thesis were conducted using the IBM Statistical Package for the Social Sciences (SPSS) version 29.0.1.0 with a significance level set at 0.05. At baseline, PSQI and MADRS scores between groups were compared using independent samples *t* tests, while demographic characteristics of participants were described using means with standard deviations.

Adhering to the intention-to-treat (ITT) principle, all participants data were included in the RCT analysis, excluding those without a post-baseline visit. For participants who withdrew early from the study, their last observation was carried forward to week 6 using the last observation carried forward (LOCF) method. This approach was solely used for the baseline to week 6 analysis, as it was not suitable for the long-term follow-up visits.

Considering the varied participation in follow-up visits, clinical data was separated and analyzed in three batches: baseline to week 6 (RCT), baseline to week 18 (12-week follow-up), and baseline to week 30 (24-week follow up).

For both PSQI and MADRS scores, changes from baseline to each endpoint were assessed using a two-way repeated measures ANOVA to observe differences both between and within the experimental and placebo groups. Pairwise comparisons facilitated the evaluation of within-group differences. When required, potential confounders were controlled for in the statistical models using analyses of covariance (ANCOVA). If sphericity was compromised, Greenhouse-Geisser corrections were used. To contrast the changes in overall scores from baseline to each endpoint between groups, independent samples *t* tests were used.

Correlations between sleep and depression scores from baseline to week 6 were established through a bivariate correlation analysis using the Pearson correlation coefficient. For visual representation, graphs were created using GraphPad Prism10.

3.11 Data Management

Data from the clinical scales were recorded on case report forms (CRF) and source documents, and then stored at Providence Care Hospital and Centre for Addiction and Mental Health. The data was then transferred to REDCap, a secure online database software. Only the study team had access to the database, ensuring data confidentiality. To protect privacy, participant data was analyzed using randomized assigned study numbers. Participants' study files were securely kept in a designated locked room, accessible only to the study coordinator and investigators, for a period of 25 years in accordance with the guidelines set by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use.

3.12 Data Monitoring

Monitoring visits were conducted by NuBiyota LLC representatives in accordance with the ICH Guidelines for Good Clinical Practices (GCP). Information collected during the study was treated as highly confidential, and the anonymity of each participant was always protected. The Investigator granted permission to NuBiyota LLC (or its designated representatives) and relevant regulatory authorities to perform on-site monitoring and auditing of study documentation as necessary. Participants were identified solely by study numbers and were not disclosed in any publications or reports.

The data collected for the study was securely stored in a locked area under the responsibility of the study doctor for a period of 25 years. Only the study team, representatives of the Research Ethics Board (REB), and representatives of Health Canada had access to the data. In some cases, the study data may have been transferred to the aforementioned individuals or groups for processing purposes related to the study, product registration, and scientific purposes, while ensuring compliance with applicable laws and regulations.

3.13 Adverse Events

During the safety reporting period, careful monitoring was conducted for the development of adverse events (AEs) in each patient. A questionnaire was used to collect information on the frequencies and severity of AEs from the time of consent until the completion of the study, as well into the follow-up sub study. This data was collected for use in the METDA RCT and Follow-Up but will not be presented in this thesis. AEs were assessed and documented at all in-hospital visits and phone calls, and they were categorized based on their frequency, severity, and causality.

Site coordinators engaged in discussions with the patients during each visit to inquire about the occurrence of AEs, and this information was recorded in the site's source documents. Details of the AEs, such as their duration (start and stop dates and times), severity, outcome, treatment, and their relationship to the study drug or other causes, were documented in the patient CRF.

Adverse events were collected by:

1. *Any symptoms or events self-reported spontaneously by the patient*
2. *Any significant changes in blood work*
3. *The patient was asked open-ended questions to answer about their health (e.g., "how are you feeling since we saw you last in clinic?")*
4. *Any clinically relevant abnormalities noted by the investigator during follow up interviews.*

All AEs were recorded in an AE Log for each patient by the Research Study Coordinator during study follow-up encounters. Any instances that led to AEs or untoward medical occurrences at any dose were reported to the Research Ethics Board (REB).

3.13.1 Serious Adverse Events

During the entirety of the study, no serious adverse events (SAE) were reported. If a SAE occurred, it would have been promptly reported to the REB, and immediate medical attention would have been provided. A SAE refers to any undesirable medical incident that:

- *Results in death*
- *Is life-threatening.*
- *Requires inpatient hospitalization or prolongation of existing hospitalization.*
- *Results in persistent or significant disability/incapacity*
- *The AE results in a congenital anomaly/birth defect*
- *Is an important medical event. The investigator should apply medical and scientific judgement to assess whether an AE should be classified as a SAE due to medical significance, even if no other seriousness criteria is met.*

3.13.2 Requirement to Report

AEs that met the criteria of being serious, unexpected, and potentially or probably related to the study drug would have been reported promptly to the REB and Health Canada in compliance with C.05.014 of the Canadian Food and Drug Regulations. The Investigator bears the responsibility of reporting serious, unexpected, and possibly or probably related AEs to the REB, while NuBiyota LLC is responsible for reporting such AEs to Health Canada.

3.13.3 Reporting Procedures

If the investigator or designated representative had become aware of a participant experiencing a SAE, they would have been responsible for reporting it to the Medical Monitor within 24 hours, regardless of the expectedness or relationship to the investigational drug. This reporting would have been done by completing a Serious Adverse Event Form, which includes:

- *Participant's study number*
- *Participant's gender*
- *Date of first dose of investigational drug(s)*

- *Date of last dose of investigational drug(s), if applicable*
- *AE term*
- *Time and date of occurrence of the event*
- *A brief description of the event, outcome to date, and any actions taken.*
- *The seriousness criteria that were met*
- *Concomitant medication at onset of the event*
- *Relevant medical history information*
- *Relevant laboratory test findings*
- *Investigator's opinion of the relationship to investigational drug(s) ("Is there a reasonable possibility that the investigational drug caused the SAE? Yes or No?")*

3.14 Approvals and Registration

This trial was conducted following the protocol, adhering to GCP, and in accordance with relevant local regulatory requirements and laws. Ethics approval was granted from the Queen's University Health Sciences and Affiliated Teaching Hospitals Research Ethics Board (protocol no. 6025187) on **March 28, 2019** (See **Appendix A**). A no objection letter from Health Canada was collected for the use of MET-2, in compliance with Part C, Division 5 of the Food and Drug Regulations. This clinical trial was registered on Clinicaltrials.gov on October 26, 2020 (NCT04602715) and was funded by NuBiyota LLC.

Had the investigator made alterations to the study protocol, including modifications to eligibility criteria, informed consent procedures, outcomes, or analyses, these changes were relayed to the sponsor, the REB, Health Canada, and the trial registry. When necessary, all participants were notified and re-consented.

CHAPTER 4: RESULTS

4.1 Study Population Demographics

The study population consisted of 29 participants total, with 15 participants randomized to the experimental group and 14 to the placebo group. Participants were enrolled between March 2021 and December 2022. Data was analyzed on an intention-to-treat (ITT) basis. With regards to the RCT only, any missing clinical data values were inputted using the last observation carried forward (LOCF) method. In contrast, follow-up data was analyzed separately without using the LOCF method due to its distinct phase and different set of participants. Using the LOCF method for the follow-up analysis could introduce bias, as it may not accurately reflect the natural progression or changes occurred so long after the RCT phase.

Of the initial cohort in the RCT, 27 participants were included in the analysis after excluding 2 for lacking a post-baseline visit. In terms of the follow-up visits, 15 participants attended the 12-week follow-up and 11 attended the 24-week follow-up, with all follow-up participants being included in their respective analysis. The decline in participant attendance primarily resulted from participants not proceeding from the RCT. Therefore, to maintain the integrity and accuracy of the results, the RCT and both sets of follow-up data were analyzed independently.

Further details on enrollment and study flow are provided in **Figure 1**, which presents information on pre-screens, screenings, screen-fails, enrollments, study completions, and withdrawals related to the RCT. It also depicts how many participants from the initial cohort completed the 12-week and 24-week follow-ups. A significant number of potential participants were screened out during both the pre-screening and screening stages, primarily because they did

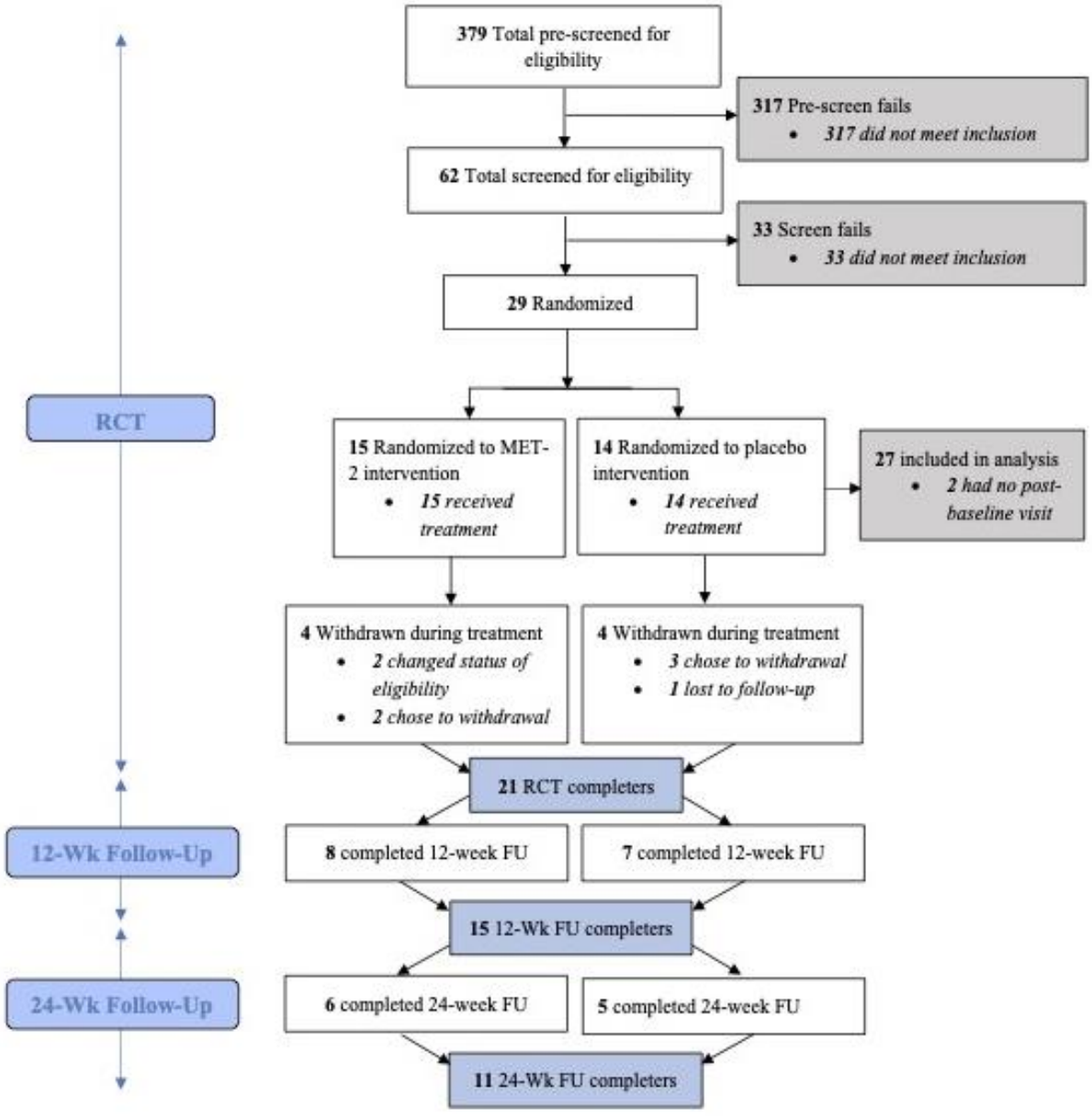
not meet the inclusion criteria. A common reason for exclusion was ongoing use of antidepressant medication.

During the treatment phase, 8 participants were withdrawn due to issues like non-compliance and changes in eligibility. Two of these participants had no post-baseline observation, and were, therefore, excluded from the subsequent analyses. Thus, 27 participants (13 in the experimental group and 14 in the placebo group) were retained for statistical analysis, with each having at least one post-baseline observation.

Of the 27 participants analyzed, 22 were females with a mean age (±SD) of 27.95 ± 8.21, and 5 were males with a mean age of 26.6 ± 4.39. The overall mean age of the cohort was 27.70 ± 7.59. Participant ages ranged from 18 to 42, fitting the study's age inclusion criteria of 18-45, with individuals from 11 distinct ethnicities. All participants were in a current major depressive episode at baseline, as confirmed by the MINI psychiatric interview and MADRS. A total of 24 participants had a diagnosis of MDD, while 3 were diagnosed with Bipolar 1 Disorder (BPD1).

The average PSQI score for the cohort was 8.56 ± 3.77. The MET-2 cohort averaged 9.76 ± 3.61, while the placebo cohort averaged 7.43 ± 3.67. The average baseline MADRS score for the entire cohort was 24.44 ± 4.27. When separated by treatment groups, the MET-2 cohort had an average score of 24.23 ± 5.05, and the placebo cohort scored 24.64 ± 3.59 on average. Mann-Whitney U tests indicated no significant differences between the experimental and placebo groups regarding baseline PSQI (U= 129; p= 0.068) and MADRS scores (U= 89.5, p= 0.943). Baseline characteristics and demographic information can be found in **Table 5**.

Figure 1: Flow Diagram of MET-2 RCT and Follow-Up Visits



RCT= randomized controlled trial; Wk= week; FU= follow-up

Table 5: Baseline Sample Characteristics and Demographic Information

Baseline characteristic	MET-2 (n=13)		Placebo (n=14)		Full Sample (n=27)	
	n	%/SD	*n*	%/SD	*n*	%/SD
Mean age (years)	28.3	8.0	27.1	7.4	27.7	7.6
Sex						
Female	11	84.6%	11	78.6%	22	81.5%
Male	2	15.4%	3	21.4%	5	18%
Ethnicity						
White	7	53.8%	8	57.1%	15	55.6%
Black	1	7.7%	2	14.3%	3	11.1%
South Asian	0	0%	2	14.3%	2	7.4%
Chinese	0	0%	1	7.1%	1	3.7%
Southeast Asian	1	7.7%	0	0%	1	3.7%
West Asian	1	7.7%	0	0%	1	3.7%
Tibetan	1	7.7%	0	0%	1	3.7%
Persian	1	7.7%	0	0%	1	3.7%
Arab	0	0%	1	7.1%	1	3.7%
Latin American/Hispanic	1	7.7%	0	0%	1	3.7%
Mean baseline PSQI score	9.77	3.61	7.43	3.67	8.56	3.77
Mean baseline MADRS score	24.23	5.05	24.64	3.59	24.44	4.27

SD: standard deviation; *PSQI:* Pittsburgh Sleep Quality Index; *MADRS*: Montgomery-Åsberg Depression Rating Scale

4.2 Clinical Measures

4.2.1 RCT

4.2.1.1 PSQI

Several domains of sleep were measured using the PSQI at baseline, week 2, week 4, and week 6. A two-way repeated measures ANOVA revealed a significant main effect of time on PSQI scores for both the experimental and placebo groups, F (2.355, 58.877) = 7.065, p= 0.001. However, the interaction effect, which would show if the changes over time differed between the groups, was not statistically significant, F (2.355, 58.877) = 2.767, p= 0.062, after Greenhouse-Geisser correction (**Figure 2A**). When accounting for demographic covariates, specifically sex and age, a significant difference emerged between the groups over time, p= 0.044) (**Figure 2B**).

Further analysis using pairwise comparisons showed significant reductions in the experimental group's mean PSQI scores from baseline (9.77 ± 3.61) to week 4 (7.77 ± 2.98, p= 0.004) and week 6 (6.62 ± 3.15, p<0.001). In contrast, the placebo group experienced a smaller, non-significant decrease in mean PSQI scores from baseline (7.43 ± 3.67) to week 4 (6.86 ± 3.55, p= 0.353) and week 6 (6.50 ± 3.13, p= 0.234) (**Figure 2A**).

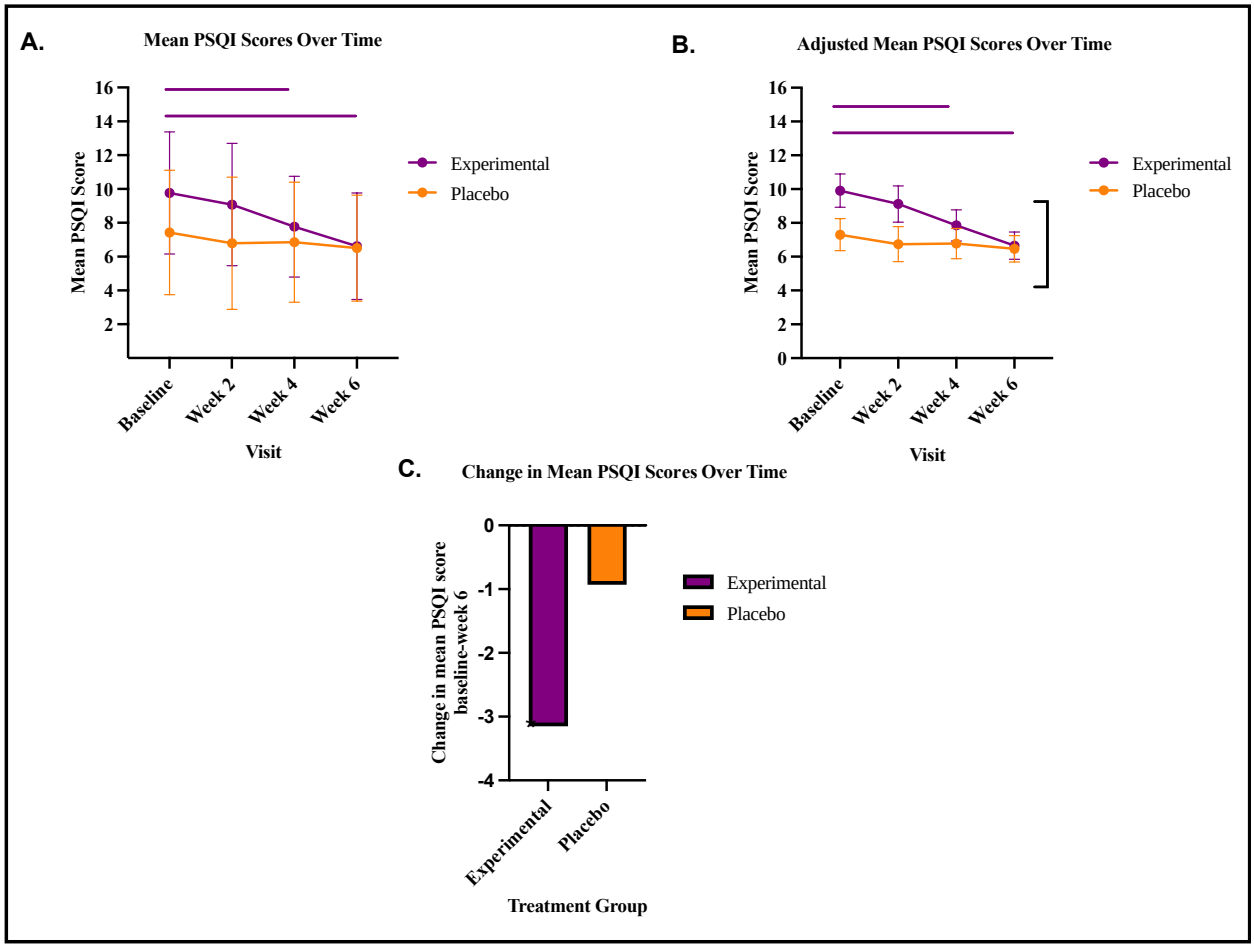
Additionally, an independent samples t-test comparing the overall change in PSQI scores from baseline to week 6 was conducted, revealing a notably greater reduction in PSQI scores for the treatment group (-3.15 ± 2.54) compared to the placebo group (-0.93 ± 3.10) from baseline to week 6. This between-group difference was statistically significant, t (25) = 2.029, p= 0.027 (one-sided) (**Table 6** and **Figure 2C**).

Table 6: Clinical RCT Statistical Analysis Results

	MET 2 (n=13)					Placebo (n=14)					
Measure	**Baseline Mean (SD)**	**Week 2 Mean (SD**	**Week 4 Mean (SD)**	**Week 6 Mean (SD)**	**Change from baseline**	**Baseline Mean (SD)**	**Week 2 Mean (SD**	**Week 4 Mean (SD)**	**Week 6 Mean (SD)**	**Change from baseline**	***p***
Sleep Quality											
PSQI*	9.77 (3.61)	9.08 (3.62)	7.77 (2.98)	6.62 (3.15)	-3.15	7.43 (3.67)	6.79 (3.91)	6.86 (3.55)	6.50 (3.13)	-0.93	**0.027[a]**
Global Depression Symptomology											
MADRS	24.23 (5.05)	15.84 (5.16)	12.69 (8.15)	12.77 (7.22)	-11. 46	24.64 (3.59	19.64 (6.59)	17.21 (5.35)	16.57 (7.94)	-8.07	0.131[a]

SD: standard deviation; *PSQI*: Pittsburgh Sleep Quality Index; *MADRS*: Montgomery-Åsberg Depression Rating Scale

Figure 2A-C: Trajectories and Change in Mean PSQI Scores from Baseline to Week 6



A. *Mean trajectory PSQI scores from baseline to week-2, week-4 and post-intervention (week 6)* ***B.*** *Mean trajectory PSQI scores from baseline to week-2, week-4 and post-intervention (week 6) after accounting for demographic covariates; sex= 1.19 and age= 27,7037* ***C.*** *Overall change in PSQI scores from baseline to post-intervention (week 6).*

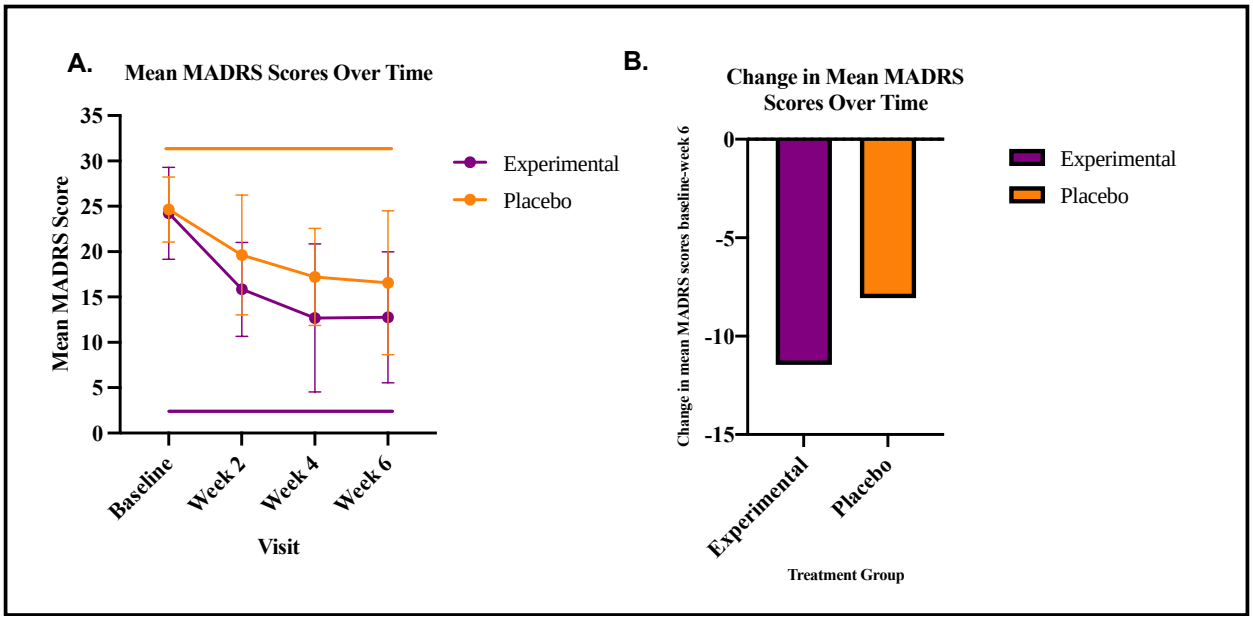
4.2.1.2 MADRS

A two-way repeated measures ANOVA indicated that MADRS scores improved significantly over time in both groups, F (3,75) = 27.089, p< 0.001, sphericity assumed.

Pairwise comparisons showed that participants MADRS scores in the experimental group significantly improved from baseline (24.23±5.05) to week 6 (12.77±7.22, p<0.001), while participants in the placebo group also significantly improved from baseline (24.64±3.59) to week 6 (16.57±7.94, p<0.001). Although the experimental group exhibited lower mean scores compared to the placebo group over time, analyses showed no statistically significant group differences over time, F (3, 75) = 1.119, p= 0.347) (**Figure 3A**)**.** Further analyses controlling for sex and age at baseline still did not yield any significant group differences over time F (3, 69) = 1.361, p= 0.262).

An independent samples t-test was again conducted to compare mean MADRS changes over time between the 2 groups, however, there was no statistically significant difference found, t (25) = 1.146, p= 0.131, although a notably greater reduction in MADRS scores for the treatment group compared to the placebo group was evident (**Table 6** and **Figure 3B**).

Figure 3A-B: Trajectories and Change in Mean MADRS Scores from Baseline to Week 6



A. *Mean trajectory MADRS scores from baseline to week-2, week-4, and post-intervention (week 6)* ***B.*** *Overall change in MADRS scores from baseline to post-intervention (week 6).*

4.2.2 12-Week Follow-Up

4.2.2.1 PSQI

Among the 27 participants included in the RCT analysis, 15 participants (8 experimental and 7 placebo) completed the first follow-up visit, which was conducted 12-weeks post-treatment discontinuation. A one-way repeated measures ANOVA did not reveal a significant main effect of time on PSQI scores for both groups, F (2.321, 30.167) = 2.642, p= 0.080, or a significant interaction between time and treatment group, F (2.321, 30.167) = 2.642, p= 0.065). When accounting for demographic covariates, specifically sex and age, a significant difference emerged between the groups over time, p= 0.011 (**Figure 4B**).

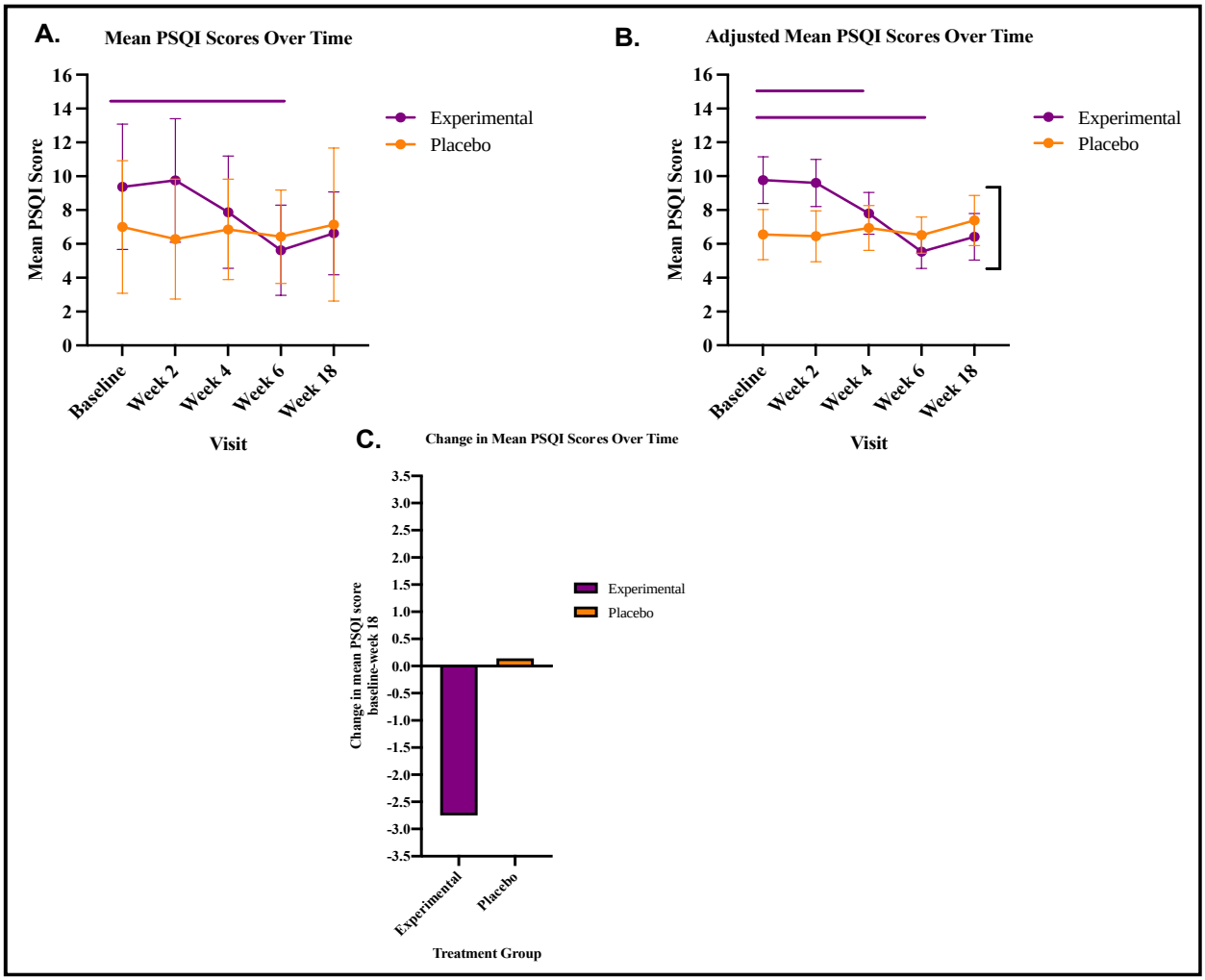
Pairwise comparisons revealed significant improvements in PSQI scores in the experimental group from baseline (9.38 ± 3.70) to week 6 (5.63 ± 2.67, p= 0.006), and a noticeable yet non-significant improvement to week 18 (6.63 ± 2.45, p= 0.111). The period between week 6 and week 18 reflected a modest increase in scores, but this too lacked statistical significance (p= 0.383). In contrast, the placebo group, although showing a slight reduction in PSQI scores from baseline (7.00 ± 3.92) to week 6 (6.43 ± 2.76), was not significant (p= 0.645). The placebo group also showed worsening PSQI scores from baseline to week 18 (7.14 ± 4.53, p= 0.935), and week 6 to week 18 (p= 0.383) (**Figure 4A**).

Additionally, an independent samples t-test comparing the overall change in mean PSQI scores from baseline to week 18 was conducted, showing a greater mean reduction in PSQI scores in the experimental group compared to the placebo group, although this difference was not statistically significant, t (13) = 1.230, p= 0.120 (**Table 7** and **Figure 4C**).

Table 7: Clinical 12-Week Follow-up Statistical Analysis Results

	MET 2 (n=13)						Placebo (n=14)						
Measure	**Baseline Mean (SD)**	**Week 2 Mean (SD**	**Week 4 Mean (SD)**	**Week 6 Mean (SD)**	**Week 18 Mean (SD)**	**Change from baseline**	**Baseline Mean (SD)**	**Week 2 Mean (SD**	**Week 4 Mean (SD)**	**Week 6 Mean (SD)**	**Week 18 Mean (SD)**	**Change from baseline**	***p***
Sleep Quality													
PSQI	9.38 (3.70)	9.75 (3.65)	7.88 (3.31)	5.63 (2.67)	6.63 (2.45)	-2.75	7.00 (3.92)	6.29 (3.55)	6.86 (2.97)	6.43 (2.76)	7.14 (4.52)	-0.14	0.120
Global Depression Symptomology													
MADRS	25.63 (4.17)	17.88 (4.22)	13.75 (9.50)	11.75 (6.76)	14.50 (8.77)	-11.13	25.00 (3.56)	19.43 (7.30)	15.29 (3.86)	13.29 (8.04)	16.29 (10.52)	-8.71	0.299

Figure 4A-C: Trajectories and Change in Mean PSQI Scores from Baseline to Week 18



A. *Mean trajectory PSQI scores from baseline to week-2, week-4, post-intervention (week 6), and 12-week follow-up (week 18)* ***B.*** *Mean trajectory PSQI scores from baseline to week-2, week-4, post-intervention (week 6), and 12-week follow-up (week 18) after accounting for demographic covariates; sex= 1.19 and age= 27,7037* ***C.*** *Overall change in PSQI scores from baseline to 12-week follow up (week 18).*

4.2.2.2 MADRS

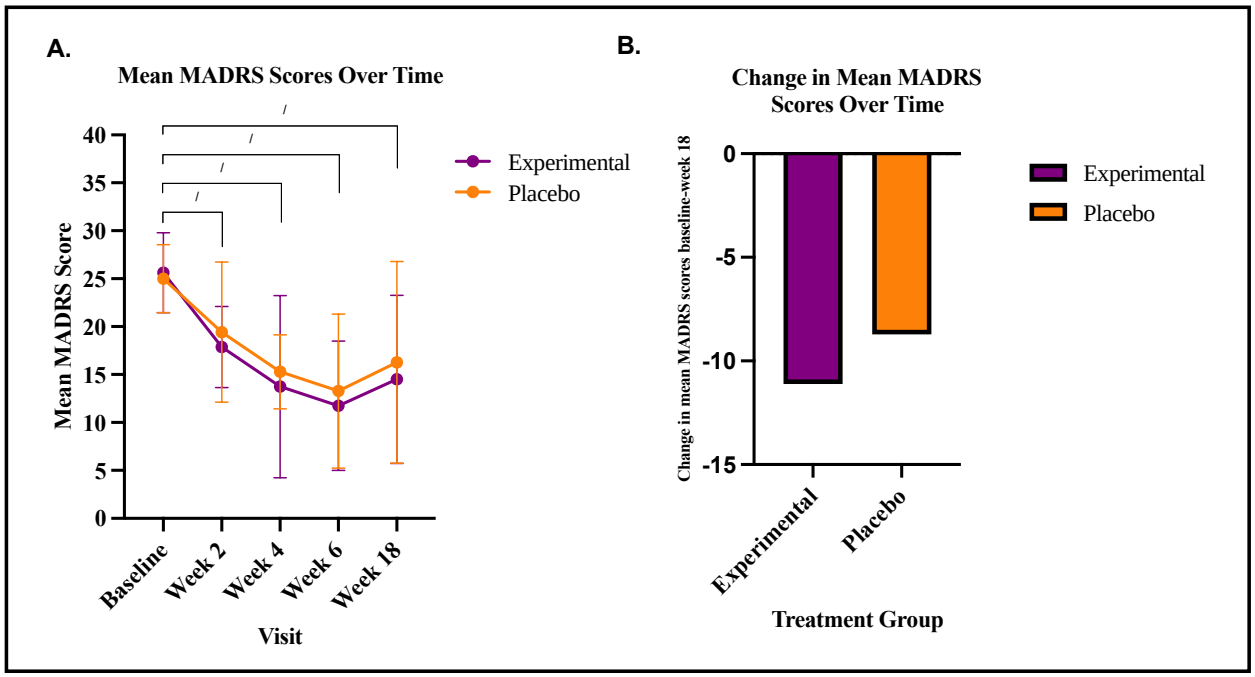
A two-way repeated measures ANOVA found a significant main effect of time on MADRS scores for both the experimental and placebo groups, F (4, 52) = 12.454, p<0.001.

Pairwise comparisons revealed significant reductions in MADRS scores in the experimental group from baseline to week 2 (p= 0.004), week 4 (p<0.001), week 6 (p<0.001), and week 18 (p= 0.003). Similarly, significant reductions in MADRS scores were also seen in the placebo group from baseline to week 2 (p= 0.036), week 4 (p= 0.001), week 6 (p<0.001), and week 18 (p= 0.019). Both groups MADRS scores slightly worsened from week 6 (mean= 12.47 ± 7.15) to week 18 (15.33 ± 9.31) (**Figure 5A**).

No significant interaction between time and treatment group was found during analysis, F (4, 52) = 0.125, p= 0.973. Further analyses controlling for sex and age at baseline also did not find any statistically significant differences between experimental and placebo groups, F (4, 44) = 0.448, p= 0.773.

Additionally, an independent samples t-test comparing the overall change in mean MADRS scores from baseline to week 18 was conducted, showing a greater mean reduction in MADRS scores in the experimental group compared to the placebo group, although this difference was not statistically significant, t (13) = 0.539, p= 0.299 (**Table 7** and **Figure 5B**).

Figure 5A-B: Trajectories and Change in Mean MADRS Scores from Baseline to Week 18



A. *Mean trajectory MADRS scores from baseline to week-2, week-4, post-intervention (week 6), and 12-week follow-up (week 18).* ***B.*** *Overall change in MADRS scores from baseline to 12-week follow up (week 18).*

4.2.3 24-Week Follow-Up

4.2.3.1 PSQI

Of the 27 participants included in the RCT analysis, 11 participants (6 MET-2, 5 placebo) completed the second follow-up visit, at 24-weeks post-treatment discontinuation.

Results from a two-way repeated measures ANOVA found that there was no significant main effect of time on the PSQI measure, F (2.114, 19.029) = 1.908, p= 0.174. Additionally, there was no significant time*treatment group interaction, F (2.114, 19.029) = 1.302, p= 0.296. Further analyses controlling for sex and age at baseline again did not demonstrate any statistically significant differences between groups, F (1.824, 12.765) = 1.904, p= 0.190.

However, post hoc tests revealed that participants in the experimental group significantly improved from baseline (9.67 ± 4.23) to week 6 (6.00 ± 3.03, p= 0.037), from week 2 (9.83 ± 4.22) to week 6 (6.00 ± 3.03, p= 0.001) and week 30 (6.33 ± 3.93, p= 0.002). Scores worsened from week 6 to week 18, but did improve from week 18 to week 30, although showing no significance. In contrast, the placebo group showed very minimal non-significant improvements from baseline (6.40 ± 4.38) to week 6 (5.80 ± 3.03), week 18 (5.80 ± 4.09) and week 30 (5.80 ± 3.11). Mean PSQI scores in the placebo group remained the same from week 6 to week 30 (**Figure 6A**).

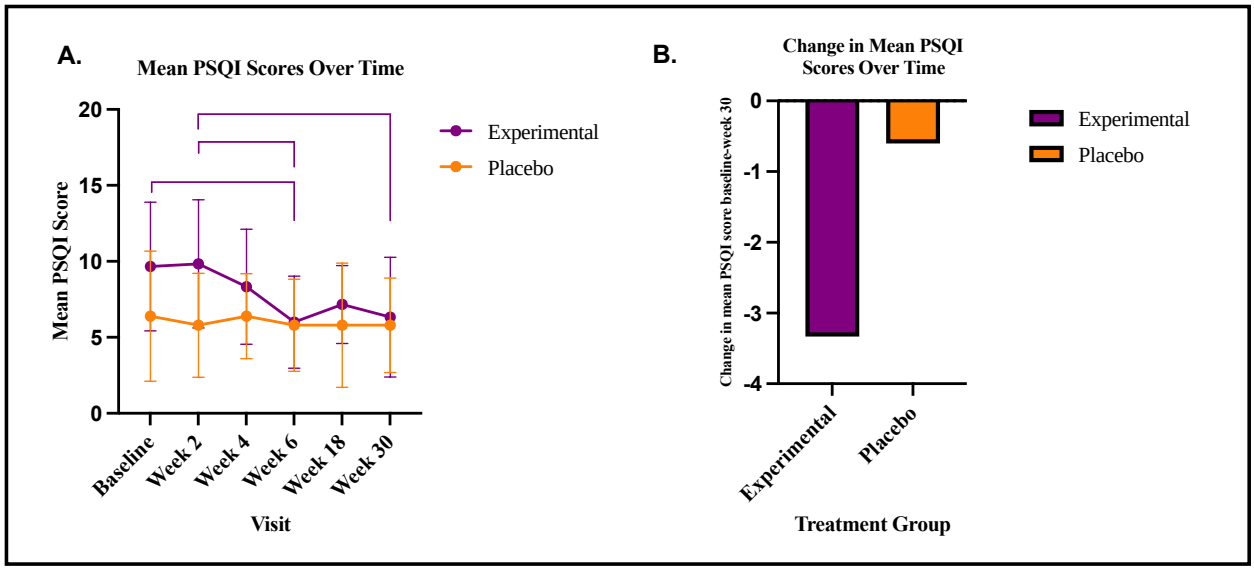
Independent samples t-tests were carried out to compare the overall change in mean PSQI scores from baseline to week 30. This difference was not statistically significant, although there was a greater mean reduction in PSQI scores in the experimental group compared to the placebo group over time, t (9) = 1.089, p= 0.152 (**Table 8** and **Figure 6B**).

Table 8: Clinical 24-Week Follow-Up Statistical Analysis Results

	MET 2 (n=13)							Placebo (n=14)							
Measure	**Baseline Mean (SD)**	**Week 2 Mean (SD**	**Week 4 Mean (SD)**	**Week 6 Mean (SD)**	**Week 18 Mean (SD)**	**Week 30 Mean (SD)**	**Change from baseline**	**Baseline Mean (SD)**	**Week 2 Mean (SD**	**Week 4 Mean (SD)**	**Week 6 Mean (SD)**	**Week 18 Mean (SD)**	**Week 30 Mean (SD)**	**Change from baseline**	***p***
Sleep Quality															
PSQI	9.67 (4.23)	9.83 (4.22)	8.33 (3.78)	6.00 (3.03)	7.17 (2.56)	6.33 (3.93)	-3.33	6.40 (4.38)	5.80 (3.42)	6.40 (2.79)	5.80 (3.03)	5.80 (4.09)	5.80 (3.11)	-0.60	0.152
Global Depression Symptomology															
MADRS	25.67 (4.84)	17.83 (4.31)	16.50 (9.05)	13.67 (6.02)	15.50 (9.52)	12.17 (11.36)	-13.50	25.00 (2.55)	18.80 (8.11)	14.80 (3.35)	10.80 (4.97)	12.40 (8.23)	11.20 (8.56)	-13.80	0.479

SD: standard deviation; *PSQI*: Pittsburgh Sleep Quality Index; *MADRS*: Montgomery-Åsberg Depression Rating Scale

Figure 6A-B: Trajectories and Change in Mean PSQI Scores from Baseline to Week 30



A. *Mean trajectory PSQI scores from baseline to week-2, week-4, post-intervention (week 6), 12-week follow-up (week 18), and 24-week follow-up (week 30).* ***B.*** *Overall change in PSQI scores from baseline to 24-week follow-up (week 30).*

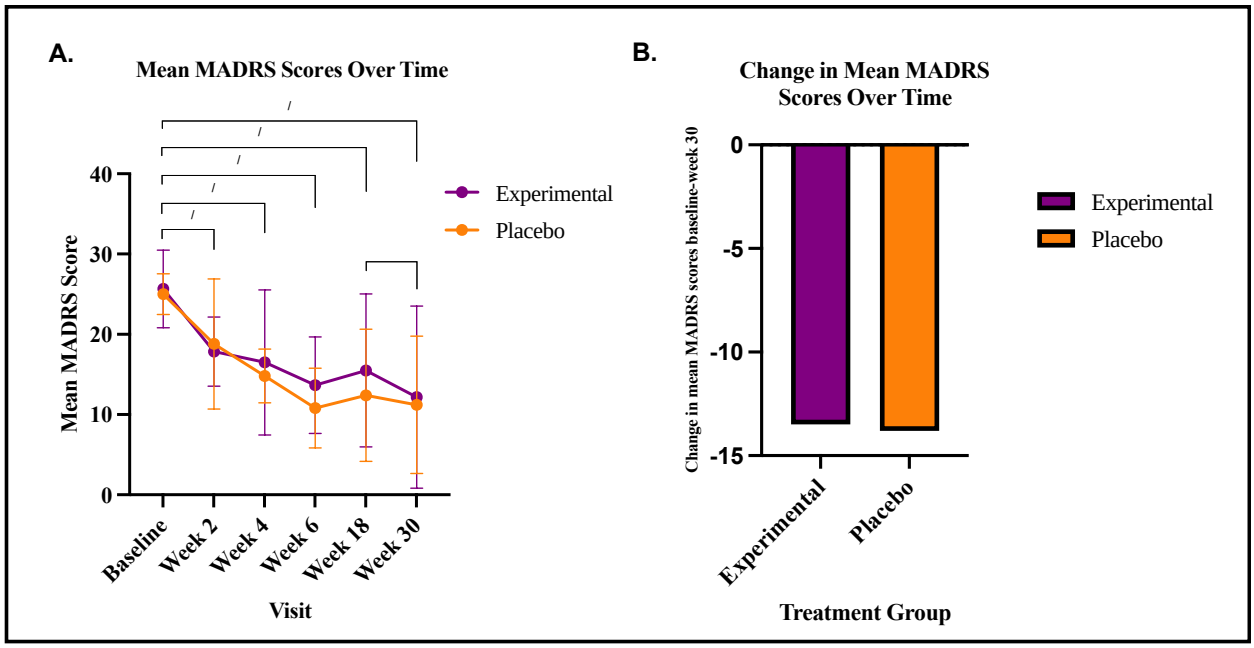
4.2.3.2 MADRS

There was a significant main effect of time on MADRS scores, F (5,45) = 9.845, p< 0.001. Post hoc tests confirmed that those in the experimental group significantly improved from baseline (25.67 ± 3.15) to week 6 (13.67 ± 6.02, p= 0.001), to week 18 (15.50 ± 9.52, p= 0.016), and to week 30 (12.17 ± 11.36, p= 0.006). However, participants did not improve from week 6 (13.67 ± 6.02) to week 18 (15.50 ± 9.52) but did improve significantly from week 18 (15.50 ± 9.52) to week 30 (12.17 ± 11.36, p= 0.037). Participants in the placebo group also significantly improved from baseline (25.00 ± 2.56) to week 6 (10.80 ± 4.97, p<0.001), to week 12 (12.40 ± 8.23, p= 0.008) and to week 24 (11.20 ± 8.56, p= 0.008). Participants in the placebo group showed the same trends as the experimental group from week 6 to week 18, and week 18 to week 30, with all trends not being significant (**Figure 7A**).

However, the interaction between time and treatment group was not significant. Under the sphericity assumed condition, F (5,45) = 0.218, p= 0.953. Further analyses controlling for sex and age at baseline, measured using demographic data at screening, still did not yield any statistically significant differences between experimental and placebo groups, F (5,35) = 0.774, p= 0.575.

Independent samples t-tests were carried out to compare the overall change in mean MADRS scores from baseline to week 30. This difference was not statistically significant, as both groups demonstrated very similar reduction in PSQI scores over time, t (9) = -0.54, p= 0.479 (**Table 8** and **Figure 7B**).

Figure 7A-B: Trajectories and Change in Mean MADRS Scores from Baseline to Week 30



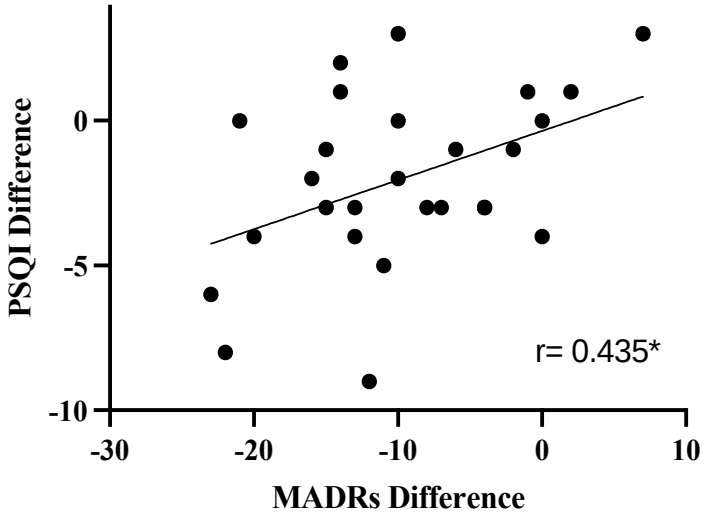
A. *Mean trajectory MADRS scores from baseline to week-2, week-4, post-intervention (week 6), 12-week follow-up (week 18), and 24-week follow-up (week 30).* ***B.*** *Overall change in MADRS scores from baseline to 24-week follow-up (week 30).*

4.3 Correlations Findings

The Pearson correlation analysis revealed a significant moderate correlation between the changes in PSQI and MADRS scores from baseline to week 6, r (25) = 0.435, *p*= 0.023. At baseline, the relationship between scores was positive but not significant, r (25) = 0.345, *p*= 0.078. By week 6, a significant moderate correlation emerged, r (25) = 0.465, *p*= 0.015 in PSQI scores

Supporting this, non-parametric tests at week 6 showed significant relationship using both Kendall's tau_b, τ (25) = 0.313, *p*= .028 and Spearman's rho, ρ (25) = 0.437, *p*= 0.023.

Figure 8: Correlation Between Changes in PSQI and MADRS from Baseline to Week 6



PSQI= Pittsburgh Sleep Quality Index; MADRS= Montgomery Åsberg Depression Rating Scale; r= Pearson's correlation coefficient; *= represents significance

CHAPTER 5: DISCUSSION

5.1 Summary of Main Findings

The aim of this thesis was to evaluate the efficacy of a novel microbe therapy, MET-2, against a placebo in managing sleep and mood disturbances among patients with MDD. The clinical data for this evaluation was sourced from the phase 2 RCT and the succeeding follow-up MET-2 study. Our primary investigation focused on changes in sleep disturbances, while our secondary outcomes investigated the effects of MET-2 on mood. Results indicate that MET-2 could have a potential advantage in improving sleep disturbances, with some indications of its possible influence on mood regulation, compared to a placebo.

5.2 Effects of MET-2 on Sleep

5.2.1 RCT Data and Baseline Observations

Clinical findings on our primary outcome measure, the PSQI, did not yield any statistically significant differences between groups. However, deeper analysis offers an alternative perspective. Independent samples t-tests revealed a statistically significant decline in PSQI scores for the MET-2 group compared to the placebo over the treatment period. In addition, pairwise comparisons further emphasized MET-2's efficacy by demonstrating significant improvements from baseline to both week-4 and week-6, trends that were absent in the placebo group. Finally, when sex and age were factored in as covariates, a statistically significant result was in fact yielded between the two groups, demonstrating MET-2's superiority in treating sleep disturbances against a placebo. This suggests that sex and age may be important covariates in determining sleep outcomes in depression.

A notable observation is the higher initial baseline PSQI score in the MET-2 group relative to the placebo group. Given that both groups had baseline scores exceeding 5 – a threshold indicating significant sleep disturbances as per guidelines provided by authors such as (Chiu & Hsu, 2016) – it is possible to postulate that MET-2 may offer pronounced benefits to those with more severe baseline sleep disturbances. Conversely, MET-2's impact could be diminished or harder to discern in those with mild to moderate sleep disturbances.

Drawing parallels with previous research, our findings are in line with the outcomes of the MET-2 phase 1 clinical trial, an open-label pilot study, which documented significant reductions in PSQI scores across an 8-week treatment span (Chinna Meyyappan et al., 2022). Similarly, a recent double-blind placebo-controlled trial that used a probiotic mixture and a placebo alternative on healthy subjects for 6 weeks (Marotta et al., 2019). Analogous to our study, the baseline PSQI values differed between the groups and although direct group comparisons didn't reveal significant differences, post-hoc analysis did unveil significant improvements from the onset to week 6 in the probiotic cohort (Marotta et al., 2019). This evidence presents a persuasive argument for the use of microbial therapies, like MET-2, which seems to be especially beneficial for individuals suffering severe sleep disturbances, in a depressed population.

5.2.2 Long-Term Effects and Follow-up Observations

At the 12-week follow-up, PSQI scores continued to suggest better sleep metrics for the MET-2 group compared to the placebo group. Although these group differences did not reach statistical significance, they became significant when accounting for sex and age from baseline to week 18, further emphasizing the potential significance of these demographic factors in sleep

outcomes. A noticeable pattern emerged where both groups saw a slight worsening of PSQI scores from week 6 to week 18. This could be attributed to the cessation of MET-2 administration post-treatment, indicating either a transient effect of MET-2 or an adaptive response of the gut microbiota over time. In other words, either the effects of MET-2 are short-lived, or the gut adjusts to MET-2 over time, which may lessen its initial benefits. This observation raises questions about MET-2's duration of action and its lasting impacts.

By the 24-week follow-up, there remained no notable group differences over time, even after controlling for covariates. However, significant intra-group differences were seen in the MET-2 group over time, particularly from baseline to week 30. In contrast, the placebo group did not display any significant changes within the group. Interestingly, a slight improvement in PSQI scores from week 18 to week 30 was observed in the treatment group. Intriguingly, the mean PSQI score at week 30 closely aligned with the week 6 score in the MET-2 group. As previously discussed, the temporary increase seen in PSQI scores post-treatment to week-12 might suggest MET-2's effects are not permanent. It's possible that upon discontinuing therapy, the gut microbiota may revert to its original composition, reverting the therapeutic benefits. Yet, the gradual improvement in PSQI scores for the MET-2 group from week 18 to week 30 complicates this possible explanation. These results might indicate that while MET-2's initial effects diminish over time, it may still trigger longer-term changes either in the gut or potentially in the CNS that subtly influence sleep patterns. The near identical mean PSQI scores at end of treatment and the 24-week follow-up offer a unique perspective. One possibility is that MET-2's efficacy plateaus after a certain period, beyond which no additional benefits can be obtained. Alternatively, MET-2 may initiate various adaptive changes in the body that eventually lead to improved sleep quality after a longer duration, despite the initial relapse.

5.3 Effects of MET-2 on Mood

5.3.1 RCT Data

Our secondary outcome measure, the MADRS, found no significant differences between groups. Although there was a notably greater reduction in MADRS scores for the MET-2 group compared to the placebo group, both groups were found to significantly improve over time.

These findings corroborate existing literature, which identified significant amelioration of depressive symptoms post-microbe therapy, as measured by the MADRS. This was observed in an open-label study assessing the impacts of probiotics on depression (Wallace & Milev, 2021) and was consistent with our MET-2 pilot study that also reported significant reductions in MADRS scores (Chinna Meyyappan et al., 2022). However, the absence of a significant distinction between MET-2 and placebo groups argues that MET-2 may not be conclusively superior in treating depressive symptoms compared to a placebo. This might also suggest that while MET-2 demonstrates promise in improving sleep, its direct influence on depressive symptoms remains unclear. Other intervening variables, such as potential placebo effects or the limited sample size, might contribute to these observations. Yet, the notable improvements in the MET-2 group could have clinical relevance, warranting further investigation.

5.3.2 Long-Term Effects and Follow-up Observations

From the end of treatment to the 12-week follow-up, a mild deterioration in MADRS scores was evident in both groups. This trend may be the case of a gradual waning of the treatment's effects over time, a pattern that was with the PSQI during the same time point. These findings parallel a recent RCT examining the effects of probiotics on depressed individuals. Their findings revealed improvements across both treatment and placebo groups within a 4-week

RCT, although a sharper decline in Hamilton depression rating scale (HAM-D) scores was noted in the probiotic group compared to their placebo counterparts (Schaub et al., 2022). Yet, at their 4-week follow-up, modest improvements persisted in the probiotic group in contrast to our 12-week follow-up, which showed a decline in scores (Schaub et al., 2022). The HAM-D, like the MADRS, is a measure designed to assess depressive symptomology. While this similarity allows comparisons between our studies to be relevant, it's also worth noting that the differences in these measures could account for the discrepancies seen between studies. In addition, the dissimilarities observed could be due to the much shorter follow-up period of just 4 weeks, hinting at potential attrition as time advances, or their much larger sample size, which provides greater statistical power and potentially more meaningful results found.

By the 24-week mark, both groups mirrored earlier trends seen by PSQI scores: following a regression in MADRS scores from the end of treatment to week 18, modest improvements were visible from week 18 to week 30. Once again, no significant intergroup variations were observed. However, a significant improvement in MADRS scores was recorded from week 18 to week 30 exclusively for the MET-2 group. In parallel with the PSQI findings, it's plausible that MET-2's effects plateau after a specific period, to which no further enhancements can be observed. Alternatively, MET-2 might trigger adaptive bodily responses that, over an extended period, eventually result in better mood outcomes, despite the initial regressions. On the other hand, these observations may just be the result of a limited sample size. Given the novelty of this research area, few studies provide follow-up data as comprehensive as ours, limiting the available literature for comparative analysis.

5.4 Correlation Findings between Mood and Sleep

Previous studies have demonstrated a strong relationship between sleep and mood (Lastella et al., 2014). This was also evident in the analysis of our study. In this thesis, we conducted a correlation analysis to assess the strength and direction of the relationship between sleep quality and mood over the 6-week treatment. Our findings revealed that improvements in sleep, as measured by the PSQI, were moderately positively correlated with enhancements in mood, as measured by the MADRS, and the correlation was significant. This implies that as sleep quality improved, there was a concurrent increase in mood improvement. The observed correlation in our study underscores the multifaceted potential of MET-2. As MET-2 seems to positively impact sleep quality, it could indirectly also be influential in mood regulation. This offers an avenue for further exploration: Could MET-2 be employed as a holistic therapy, addressing both sleep and mood dimensions simultaneously? Depression and sleep disturbances share an interconnected relationship, with each potentially exacerbating the other. It's notable that when individuals experience sleep issues, the severity of their depressive symptoms often intensifies, underlining the interconnectedness of these two conditions. Additionally, persistent sleep disturbances can elevate the risk of developing depression in the first place or lead to a higher risk of depressive relapse (Nutt et al., 2008). Given this connection, the therapeutic landscape requires a comprehensive solution. A novel treatment, such as MET-2, that can target and potentially alleviate both depression and its co-occurring sleep disturbances has the potential to transform clinical practice. Such an approach would not only offer more holistic relief to patient but also reduce the duration and intensity of treatment, leading to faster recovery and improved quality of life.

5.5 Evaluation of MET-2 in Clinical Contexts

5.5.1 Comparisons with Existing Microbial Therapy

While no studies have directly compared the efficacy of MET-2 against a placebo in sleep and mood, there are a handful of clinical studies that have investigated the use of microbial therapy on mood or sleep in various indications. The literature reviewed in this thesis suggests that techniques for repopulating the gut can influence psychiatric symptoms. Notably, FMT has gathered attention for its potential to enhance mood and sleep in both preclinical and clinical domains (Chinna Meyyappan et al., 2020).

However, while FMT might offer symptom relief, it brings with it certain limitations. Some significant challenges include its invasive nature and its limited duration of benefits, often lasting just 3-6 months, leading to concerns about its long-term clinical feasibility (He et al., 2017). Furthermore, FMT introduces variability as it depends on individual donor microbial profiles, potentially leading to inconsistent therapeutic outcomes. There is also the risk of potential pathogen transmission, even with rigorous donor screening (Park & Seo, 2021).

In contrast, our research highlights the many promising attributes of MET-2. We observed rapid therapeutic benefits from MET-2, findings that align well with our initial pilot study (Chinna Meyyappan et al., 2022). The primary MET-2 study, while outside the scope of this thesis, documents its safety and tolerability over extended periods, demonstrating MET-2's potential for long-term application. MET-2's standardized nature means it can promise more consistent outcomes compare to FMT, without any risk of pathogen transmission. Additionally, as a non-invasive therapy, MET-2 might be more patient-friendly, making it easier to administer and potentially more acceptable to a broader patient demographic. While there is extensive research on FMT compared to MET-2, when considering the balance between efficacy, safety,

and patient acceptability, MET-2 seems to offer a more viable alternative or supplement to existing microbial therapies like FMT.

5.5.2 MET-2's Therapeutic Advantages

A notable aspect of MET-2 is its rapid onset of therapeutic response. Data from our RCT and prior pilot demonstrate that most patients experience symptomatic relief within the first two weeks of MET-2 therapy (Chinna Meyyappan et al., 2022). This is significant when compared to standard antidepressants, which often take longer to display full therapeutic effects. This potentially expedited relief from depressive and disturbed sleep symptoms could be transformative for many patients, especially those in acute distress, thereby reducing the overall burden of disease and improving quality of life for these individuals in the short-term. Given its distinct mode of action targeting the gut-brain axis, MET-2 might circumvent common drawbacks associated with tradition antidepressant therapies such as side effects, drug interactions, and varied patient response rates. The intrinsic difference in MET-2's mode of intervention may also make it less stigmatizing than traditional chemical interventions. Nonetheless, how society perceives such treatments remains an area for exploration.

5.5.3 Potential Challenges and Considerations

While MET-2's efficacy and tolerability highlight its potential as a therapeutic for improving sleep in MDD, certain factors may impede its widespread clinical acceptance. The safety profile of MET-2, alike other microbial therapies such as FMT, have not been fully elucidated, and societal perceptions or possible stigmas attached to its use are yet to be clarified, factors which could lead to poor treatment adherence.

Despite its promise, MET-2's therapeutic longevity, especially post-treatment, requires further investigation and more rigorous testing before advocating its use in clinical contexts. It's important to determine whether MET-2 could complement or even replace current antidepressant treatment in certain patient demographics. Such shifts in treatment paradigms will heavily depend on further empirical evidence, alongside assessments of production scalability, cost, and patient access. Given that the research surrounding this treatment is still in its infancy, its challenging to definitively ascertain the viability of MET-2 in treating psychiatric conditions such as MDD and comorbid sleep disturbances, emphasizing the need for further diverse and large-scale investigations.

5.6 METDA Study Limitations

5.6.1 Sample Size and Generalizability

The main limitation of the MET-2 study, alike others in the field of microbe therapy, was its small sample size. This not only limits the study's statistical power but also raises the potential for type II errors, potentially overlooking MET-2's true effects. While we identified statistically significant differences between groups in certain measures, no significance was found in our secondary objective measure, the MADRS, or in much of the follow-up data. Such small cohorts might fail to detect subtle differences between treatment groups. In essence, the risk of type II errors means there's a possibility we failed to detect a difference that truly exists, potentially underestimating MET-2's true therapeutic impact.

This limitation also has repercussions for the generalizability of our findings. Consequently, while our findings are relevant to our specific cohort, they may not readily apply to broader populations, especially males, given their limited representation in our sample. The reduced

cohort size also increases vulnerability to random variations, with outliers potentially skewing the results and thus potential to find significant differences between groups. Moreover, with smaller sample studies, there's often a heightened margin of error, suggesting the effects of MET-2 identified could vary in different settings or populations. Such limitations underscore the importance of replication in larger cohorts to affirm MET-2's therapeutic potential.

5.6.2 External Influences

Further, it is plausible that other factors not accounted for in our study, such as changes in diet or physical activity may have affected both the short and long-term outcomes of our results. Notably, changes in diet stand out as a significant confounder, given the close relationship between dietary intake and the gut microbiota composition. While MET-2 introduces beneficial microbes, factors such as dietary changes and medication use can rapidly disrupt this balance. Although we documented medication use throughout the study, assessing dietary habits may have enhanced our analysis.

Moreover, the episodic nature of depression and its symptoms, including sleep disturbances, makes it challenging to distinguish between genuine therapeutic effects and the natural fluctuations of the disorder. This variability underscores the need for continued long-term, follow-up studies with larger cohorts to truly determine the short-term and sustained benefits of MET-2.

5.6.3 Subjective vs. Objective Measures

While subjective sleep measures didn't highlight significant differences at specific follow-up timepoints, specifically the 12-week follow-up without adjusting for covariates, or the

24-week follow-up, incorporating objective evaluations would be of value. As sleep disturbances in depression are multidimensional, addressing not just subjective disturbances, but also objective measures such as polysomnography, might offer additional insight into the potential benefits of MET-2.

5.7 Future Research

Exploring the use of native microbe therapies, such as MET-2, as potential treatments for MDD and comorbid sleep disturbances in upcoming research holds considerable promise that warrants further investigation. Future studies should focus on the expansion of sample size and participant diversity. The observed gender imbalance and constrained sample size in our research demonstrates the need for more inclusive and extensive participation recruitment in future endeavors, which will enhance generalizability and reduce the susceptibility to type II errors.

Our findings also examine the potential influence of external factors like diet or physical activity, in addition to concurrent medication use, on treatment outcomes. This highlights a potential avenue for future studies: the incorporation of more rigorous controls or intricate participant-monitoring systems, by using daily logs to capture these variables.

While our research did incorporate longitudinal follow-up studies at 12- and 24-weeks post-treatment, a limitation to many clinical studies, there was a lack of statistical significance yielded between the 2 groups, which is most likely due from the low sample size. Although statistical significance was low, there are promising trends in the data that warrant further investigation. Given the cyclical nature of depression, it’s plausible that extending observational periods could offer deeper insights into MET-2’s long-term efficacy.

Additionally, future studies should focus on comparing the efficacy of MET-2 against established depressive treatments such as SSRIs. Such studies should ideally set the new intervention against standard treatments, moving beyond solely placebo comparisons. Furthermore, there is also value in exploring MET-2 as an adjunct or complementary treatment to existing therapeutic approaches.

However, it is essential that future research endeavors to understand the mechanisms by which MET-2 could have sustained effects on sleep disturbances in depression. Larger, longer-term studies, ideally incorporating multiple time points for assessment, are needed to validate our findings, and shed light on the potential factors affecting the long-term outcomes.

5.8 Conclusion

The MET-2 RCT and its subsequent follow-up study were the first to examine the potential benefits of MET-2, a novel microbe therapy, against a placebo in addressing sleep disturbances in MDD. Results from this thesis indicate that MET-2 is highly efficacious in treating sleep quality disturbances compared to a placebo across 6 weeks of treatment, and at a 12-week follow-up adjusted for age and sex. Such findings are in line with emerging research that identifies the pivotal role gut microbiota might play in influencing brain function and mood disorders via the GBA. These insights substantiate the theory that interventions altering the gut microbiota, like MET-2, could have a direct impact on depressive symptoms and associated sleep disturbances in MDD.

However, while there was evident improvement in mood symptoms across the 6-week treatment and subsequent follow-up durations, no significant differences were discovered between the MET-2 and placebo cohorts. These outcomes may be attributed to factors such as a

limited participant pool, high interpatient variability, and the unpredictable trajectory of depression.

Our research contributes to the growing body of research that suggests manipulating gut microbiota could be instrumental in managing sleep disturbances in MDD. While our findings require further investigation, they raise interesting questions about the potential of therapies such as MET-2 to provide a novel approach in treating sleep disturbances in depression, and possibly other related mental health disorders. While our study provides valuable longitudinal insights on MET-2, it alone is insufficient to conclusively determine its efficacy, emphasizing the need for further diverse and large-scale investigations to truly understand MET-2's therapeutic potential.

REFERENCES

(UK), N. C. C. f. M. H. (2010). Depression in Adults with a Chronic Physical Health Problem: Treatment and Management. In *NICE Clinical Guidelines, No. 91* (Vol. Appendix 12). British Psychological Society (UK). https://www.ncbi.nlm.nih.gov/books/NBK82926/

Al, H. (2012). Treatment-resistant depression: therapeutic trends, challenges, and future directions. *Patient Preference and Adherence*, 369. https://doi.org/10.2147/ppa.s29716

Anderson, J. R., Carroll, I., Azcarate-Peril, M. A., Rochette, A. D., Heinberg, L. J., Peat, C., Steffen, K., Manderino, L. M., Mitchell, J., & Gunstad, J. (2017). A preliminary examination of gut microbiota, sleep, and cognitive flexibility in healthy older adults. *Sleep medicine*, *38*, 104-107. https://doi.org/10.1016/j.sleep.2017.07.018

Appleton, J. (2018). The Gut-Brain Axis: Influence of Microbiota on Mood and Mental Health. *Integrative medicine (Encinitas, Calif.)*, *17*(4), 28-32.

Armitage, R. (2007). Sleep and circadian rhythms in mood disorders. *Acta Psychiatrica Scandinavica*, *115*(s433), 104-115. https://doi.org/10.1111/j.1600-0447.2007.00968.x

Asarnow, L. D. (2020). Depression and sleep: what has the treatment research revealed and could the HPA axis be a potential mechanism? *Current opinion in psychology*, *34*, 112-116. https://doi.org/10.1016/j.copsyc.2019.12.002

Asarnow, L. D., & Manber, R. (2019). Cognitive Behavioral Therapy for Insomnia in Depression. *Sleep Medicine Clinics*, *14*(2), 177-184. https://doi.org/10.1016/j.jsmc.2019.01.009

Aubry, J.-M. (2013). CRF system and mood disorders. *Journal of chemical neuroanatomy.*, *54*, 20-24. https://doi.org/10.1016/j.jchemneu.2013.09.003

Baglioni, C., Battagliese, G., Feige, B., Spiegelhalder, K., Nissen, C., Voderholzer, U., Lombardo, C., & Riemann, D. (2011). Insomnia as a predictor of depression: A meta-analytic evaluation of longitudinal epidemiological studies. *Journal of affective disorders*, *135*(1), 10-19. https://doi.org/10.1016/j.jad.2011.01.011

Barandouzi, Z. A., Starkweather, A. R., Henderson, W. A., Gyamfi, A., & Cong, X. S. (2020). Altered Composition of Gut Microbiota in Depression: A Systematic Review. *Frontiers in psychiatry.*, *11*. https://doi.org/10.3389/fpsyt.2020.00541

Beck, A. T. (1961). An Inventory for Measuring Depression. *Archives Of General Psychiatry*, *4*(6), 561. https://doi.org/10.1001/archpsyc.1961.01710120031004

Benedict, C., Vogel, H., Jonas, W., Woting, A., Blaut, M., Schürmann, A., & Cedernaes, J. (2016). Gut microbiota and glucometabolic alterations in response to recurrent partial sleep deprivation in normal-weight young individuals. *Molecular metabolism (Germany)*, *5*(12), 1175-1186. https://doi.org/10.1016/j.molmet.2016.10.003

Bibbò, S., Settanni, C. R., Porcari, S., Bocchino, E., Ianiro, G., Cammarota, G., & Gasbarrini, A. (2020). Fecal Microbiota Transplantation: Screening and Selection to Choose the Optimal Donor. *Journal of Clinical Medicine*, *9*(6), 1757. https://doi.org/10.3390/jcm9061757

Blom, K., Jernelöv, S., Rück, C., Lindefors, N., & Kaldo, V. (2017). Three-Year Follow-Up Comparing Cognitive Behavioral Therapy for Depression to Cognitive Behavioral Therapy for Insomnia, for Patients With Both Diagnoses. *Sleep.*, *40*(8). https://doi.org/10.1093/sleep/zsx108

Boku, S., Nakagawa, S., Toda, H., & Hishimoto, A. (2018). Neural basis of major depressive disorder: Beyond monoamine hypothesis. *Psychiatry and Clinical Neurosciences*, *72*(1), 3-12. https://doi.org/10.1111/pcn.12604

Borbély, A. A., Tobler, I., Loepfe, M., Kupfer, D. J., Ulrich, R. F., Grochocinski, V., Doman, J., & Matthews, G. (1984). All-night spectral analysis of the sleep EEG in untreated depressives and normal controls. *Psychiatry research.*, *12*(1), 27-33. https://doi.org/10.1016/0165-1781(84)90135-5

Bovy, L., Weber, F. D., Tendolkar, I., Fernández, G., Czisch, M., Steiger, A., Zeising, M., & Dresler, M. (2022). Non-REM sleep in major depressive disorder. *NeuroImage : clinical.*, *36*, 103275. https://doi.org/10.1016/j.nicl.2022.103275

Bowers, S. J., Summa, K. C., Thompson, R. S., González, A., Vargas, F., Olker, C., Jiang, P., Lowry, C. A., Dorrestein, P. C., Knight, R., Wright, K. P., Fleshner, M., Turek, F. W., & Vitaterna, M. H. (2022). A Prebiotic Diet Alters the Fecal Microbiome and Improves Sleep in Response to Sleep Disruption in Rats. *Frontiers in neuroscience.*, *16*. https://doi.org/10.3389/fnins.2022.889211

Braniste, V., Al-Asmakh, M., Kowal, C., Anuar, F., Abbaspour, A., Tóth, M., Korecka, A., Bakocevic, N., Ng, L. G., Guan, N. L., Kundu, P., Gulyás, B., Halldin, C., Hultenby, K., Nilsson, H., Hebert, H., Volpe, B. T., Diamond, B., & Pettersson, S. (2014). The gut microbiota influences blood-brain barrier permeability in mice. *Science translational medicine*, *6*(263), 263ra158-263ra158. https://doi.org/10.1126/scitranslmed.3009759

Breit, S., Kupferberg, A., Rogler, G., & Hasler, G. (2018). Vagus Nerve as Modulator of the Brain-Gut Axis in Psychiatric and Inflammatory Disorders. *Frontiers in Psychiatry*, *9*, 44-44. https://doi.org/10.3389/fpsyt.2018.00044

Breslau, N., Roth, T., Rosenthal, L., & Andreski, P. (1996). Sleep disturbance and psychiatric disorders: A longitudinal epidemiological study of young Adults. *Biological psychiatry (1969)*, *39*(6), 411-418. https://doi.org/10.1016/0006-3223(95)00188-3

Brett, J., & Murnion, B. (2015). Management of benzodiazepine misuse and dependence. *Australian Prescriber*, *38*(5), 152-155. https://doi.org/10.18773/austprescr.2015.055

Buysse, D. J., Reynolds, C. F., Monk, T. H., Berman, S. R., & Kupfer, D. J. (1989). The Pittsburgh sleep quality index: A new instrument for psychiatric practice and research. *Psychiatry research*, *28*(2), 193-213. https://doi.org/10.1016/0165-1781(89)90047-4

Carabotti, M. (2015). The gut-brain axis: interactions between enteric microbiota, central and enteric nervous systems. *Annals of Gastroenterology*, *28*(2), 203-209.

Chand, S., & Arif, H. (2023). Depression. In *StatPearls [Internet]*. StatsPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK430847/

Chinna Meyyappan, A., Forth, E., & Milev, R. (2022). Microbial Ecosystem Therapeutic-2 Intervention in People With Major Depressive Disorder and Generalized Anxiety Disorder: Phase 1, Open-Label Study. *Interactive Journal of Medical Research*, *11*(1), e32234. https://doi.org/10.2196/32234

Chinna Meyyappan, A., Forth, E., Wallace, C. J. K., & Milev, R. (2020). Effect of fecal microbiota transplant on symptoms of psychiatric disorders: a systematic review. *BMC psychiatry*, *20*(1). https://doi.org/10.1186/s12888-020-02654-5

Chinna Meyyappan, A., Sgarbossa, C., Vazquez, G., Bond, D. J., Müller, D. J., & Milev, R. (2021). The Safety and Efficacy of Microbial Ecosystem Therapeutic-2 in People With Major Depression: Protocol for a Phase 2, Double-Blind, Placebo-Controlled Study. *JMIR Research Protocols*, *10*(9), e31439. https://doi.org/10.2196/31439

Chiu, N.-Y., & Hsu, W.-Y. (2016). Sleep Disturbances in Methadone Maintenance Treatment (MMT) Patients. *Neuropathology of Drug Addictions and Substance Misuse*, 608-615. https://doi.org/10.1016/B978-0-12-800634-4.00062-7

Christensen, H. P., Batterham, P. J. P., Gosling, J. A. B., Ritterband, L. M. P., Griffiths, K. M. P., Thorndike, F. P. P., Glozier, N. P., O'Dea, B. P., Hickie, I. B. P., & Mackinnon, A. J. P. (2016). Effectiveness of an online insomnia program (SHUTi) for prevention of depressive episodes (the GoodNight Study): a randomised controlled trial. *The Lancet. Psychiatry*, *3*(4), 333-341. https://doi.org/10.1016/S2215-0366(15)00536-2

Chu, A., & Wadhwa, R. (2023). Selective Serotonin Reuptake Inhibitors. In *StatPearls [Internet]*. StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK554406/

Clapp, M., Aurora, N., Herrera, L., Bhatia, M., Wilen, E., & Wakefield, S. (2017). Gut Microbiota's Effect on Mental Health: The Gut-Brain Axis. *Clinics and Practice*, *7*(4), 987. https://doi.org/10.4081/cp.2017.987

Colombo, J., Carlson, S. E., Algarín, C., Reyes, S., Chichlowski, M., Harris, C. L., Wampler, J. L., Peirano, P., & Berseth, C. L. (2021). Developmental effects on sleep–wake patterns in infants receiving a cow's milk-based infant formula with an added prebiotic blend: a Randomized Controlled Trial. *Pediatric research*, *89*(5), 1222-1231. https://doi.org/10.1038/s41390-020-1044-x

Colten, H. R., & Altevogt, B. M. (2006). *Sleep Disorders and Sleep Deprivation: An Unmet Public Health Problem*.

Cuijpers, P., Karyotaki, E., Weitz, E., Andersson, G., Hollon, S. D., & van Straten, A. (2014). The effects of psychotherapies for major depression in adults on remission, recovery and improvement: A meta-analysis. *Journal of affective disorders.*, *159*, 118-126. https://doi.org/10.1016/j.jad.2014.02.026

Dement, W., & Kleitman, N. (1957). The relation of eye movements during sleep to dream activity: An objective method for the study of dreaming. *Journal of experimental psychology*, *53*(5), 339-346. https://doi.org/10.1037/h0048189

Dickerson, F., Adamos, M., Katsafanas, E., Khushalani, S., Origoni, A., Savage, C., Schweinfurth, L., Stallings, C., Sweeney, K., Goga, J., & Yolken, R. H. (2018). Adjunctive probiotic microorganisms to prevent rehospitalization in patients with acute mania: A randomized controlled trial. *Bipolar disorders.*, *20*(7), 614-621. https://doi.org/10.1111/bdi.12652

Diekelmann, S., & Born, J. (2010). The memory function of sleep. *Nature Reviews Neuroscience*, *11*(2), 114-126. https://doi.org/10.1038/nrn2762

Dolder, C., Nelson, M., & Iler, C. (2012). The effects of mirtazapine on sleep in patients with major depressive disorder. *Annals of Clinical Psychiatry*, *24*(3), 215-224.

Dunlop, B. W. (2016). Evidence-Based Applications of Combination Psychotherapy and Pharmacotherapy for Depression. *FOCUS*, *14*(2), 156-173. https://doi.org/10.1176/appi.focus.20150042

Edinger, J. D., Manber, R., Buysse, D. J., Krystal, A. D., Thase, M. E., Gehrman, P., Fairholme, C. P., Luther, J., & Wisniewski, S. (2017). Are Patients with Childhood Onset of Insomnia and Depression More Difficult to Treat Than Are Those with Adult Onsets of These Disorders? A Report from the TRIAD Study. *Journal of clinical sleep medicine*, *13*(02), 205-213. https://doi.org/10.5664/jcsm.6448

Fanelli, D., Weller, G., & Liu, H. (2021). New Serotonin-Norepinephrine Reuptake Inhibitors and Their Anesthetic and Analgesic Considerations. *Neurology International*, *13*(4), 497-509. https://doi.org/10.3390/neurolint13040049

Fang, H., Tu, S., Sheng, J., & Shao, A. (2019). Depression in sleep disturbance: A review on a bidirectional relationship, mechanisms and treatment. *Journal of Cellular and Molecular Medicine*, *23*(4), 2324-2332. https://doi.org/10.1111/jcmm.14170

Fava, M., Thase, M. E., DeBattista, C., Doghramji, K., Arora, S., & Hughes, R. J. (2007). Modafinil Augmentation of Selective Serotonin Reuptake Inhibitor Therapy in MDD Partial Responders with Persistent Fatigue and Sleepiness. *Annals of Clinical Psychiatry*, *19*(3), 153-159. https://doi.org/10.1080/10401230701464858

Fei, N., Choo-Kang, C., Reutrakul, S., Crowley, S. J., Rae, D., Bedu-Addo, K., Plange-Rhule, J., Forrester, T. E., Lambert, E. V., Bovet, P., Riesen, W., Korte, W., Luke, A., Layden, B. T., Gilbert, J. A., & Dugas, L. R. (2021). Gut microbiota alterations in response to sleep length among African-origin adults. *PloS one*, *16*(9), e0255323. https://doi.org/10.1371/journal.pone.0255323

Feng, G., Han, M., Li, X., Geng, L., & Miao, Y. (2020). The Clinical Effectiveness of Cognitive Behavioral Therapy for Patients with Insomnia and Depression: A Systematic Review and Meta-Analysis. *Evidence-Based Complementary and Alternative Medicine*, *2020*, 1-14. https://doi.org/10.1155/2020/8071821

Feriante, J., & Araujo, J. (2023). Physiology, REM Sleep. In *StatPearls [Internet]*. StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK531454/

Franzen, P. L., & Buysse, D. J. (2008). Sleep disturbances and depression: risk relationships for subsequent depression and therapeutic implications. *Dialogues in Clinical Neuroscience*, *10*(4), 473-481. https://doi.org/10.31887/dcns.2008.10.4/plfranzen

García-Gutiérrez, M. S., Navarrete, F., Sala, F., Gasparyan, A., Austrich-Olivares, A., & Manzanares, J. (2020). Biomarkers in Psychiatry: Concept, Definition, Types and Relevance to the Clinical Reality [Review]. *Frontiers in Psychiatry*, *11*. https://doi.org/10.3389/fpsyt.2020.00432

Gartlehner, G., Thieda, P., Hansen, R. A., Gaynes, B. N., Deveaugh-Geiss, A., Krebs, E. E., & Lohr, K. N. (2008). Comparative risk for harms of second-generation antidepressants : a systematic review and meta-analysis. *Drug safety.*, *31*(10), 851-865. https://doi.org/10.2165/00002018-200831100-00004

Ghorbani, Z., Nazari, S., Etesam, F., Nourimajd, S., Ahmadpanah, M., & Razeghi Jahromi, S. (2018). The Effect of Synbiotic as an Adjuvant Therapy to Fluoxetine in Moderate Depression: A Randomized Multicenter Trial. *Archives of Neuroscience*, *5*(2). https://doi.org/10.5812/archneurosci.60507

Gillin, J. C., Jernajczyk, W., Valladares-Neto, D. D. C., Golshan, S., Lardon, M., & Stahl, S. M. (1994). Inhibition of REM sleep by ipsapirone, A 5HT1A agonist, in normal volunteers. *Psychopharmacology*, *116*(4), 433-436. https://doi.org/10.1007/bf02247474

Grosicki, G. J., Riemann, B. L., Flatt, A. A., Valentino, T., & Lustgarten, M. S. (2020). Self-reported sleep quality is associated with gut microbiome composition in young, healthy individuals: a pilot study. *Sleep medicine*, *73*, 76-81. https://doi.org/10.1016/j.sleep.2020.04.013

Hammer-Helmich, L., Haro, J. M., Jönsson, B., Tanguy Melac, A., Di Nicola, S., Chollet, J., Milea, D., Rive, B., & Saragoussi, D. (2018). Functional impairment in patients with

major depressive disorder: the 2-year PERFORM study. *Neuropsychiatric Disease and Treatment, Volume 14*, 239-249. https://doi.org/10.2147/ndt.s146098

Hashimoto, T., Shiina, A., Hasegawa, T., Kimura, H., Oda, Y., Niitsu, T., Ishikawa, M., Tachibana, M., Muneoka, K., Matsuki, S., Nakazato, M., & Iyo, M. (2016). Effect of mirtazapine versus selective serotonin reuptake inhibitors on benzodiazepine use in patients with major depressive disorder: a pragmatic, multicenter, open-label, randomized, active-controlled, 24-week trial. *Annals of General Psychiatry*, *15*(1). https://doi.org/10.1186/s12991-016-0115-1

He, J., Hsuchou, H., He, Y., Kastin, A. J., Wang, Y., & Pan, W. (2014). Sleep Restriction Impairs Blood–Brain Barrier Function. *The Journal of Neuroscience*, *34*(44), 14697-14706. https://doi.org/10.1523/jneurosci.2111-14.2014

He, Z., Li, P., Zhu, J., Cui, B., Xu, L., Xiang, J., Zhang, T., Long, C., Huang, G., Ji, G., Nie, Y., Wu, K., Fan, D., & Zhang, F. (2017). Multiple fresh fecal microbiota transplants induces and maintains clinical remission in Crohn's disease complicated with inflammatory mass. *Scientific reports*, *7*(1). https://doi.org/10.1038/s41598-017-04984-z

Hirshkowitz, M., Whiton, K., Albert, S. M., Alessi, C., Bruni, O., DonCarlos, L., Hazen, N., Herman, J., Katz, E. S., Kheirandish-Gozal, L., Neubauer, D. N., O'Donnell, A. E., Ohayon, M., Peever, J., Rawding, R., Sachdeva, R. C., Setters, B., Vitiello, M. V., Ware, J. C., & Adams Hillard, P. J. (2015). National Sleep Foundation's sleep time duration recommendations: methodology and results summary. *Sleep health.*, *1*(1), 40-43. https://doi.org/10.1016/j.sleh.2014.12.010

Holshoe, J. M. (2009). Antidepressants and Sleep: A Review. *Perspectives in Psychiatric Care*, *45*(3), 191-197. https://doi.org/10.1111/j.1744-6163.2009.00221.x

Howren, M. B., Lamkin, D. M., & Suls, J. (2009). Associations of depression with C-reactive protein, IL-1, and IL-6: a meta-analysis. *Psychosomatic Medicine*, *71*(2), 171-186. https://doi.org/10.1097/PSY.0b013e3181907c1b

Huang, R., Wang, K., & Hu, J. (2016). Effect of Probiotics on Depression: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Nutrients*, *8*(8), 483. https://doi.org/10.3390/nu8080483

Hutka, P., Krivosova, M., Muchova, Z., Tonhajzerova, I., Hamrakova, A., Mlyncekova, Z., Mokry, J., & Ondrejka, I. (2021). Association of Sleep Architecture and Physiology with Depressive Disorder and Antidepressants Treatment. *International journal of molecular sciences*, *22*(3), 1333. https://doi.org/10.3390/ijms22031333

Irwin, M. R. (2023). Sleep disruption induces activation of inflammation and heightens risk for infectious disease: Role of impairments in thermoregulation and elevated ambient temperature. *Temperature*, *10*(2), 198-234. https://doi.org/10.1080/23328940.2022.2109932

Jesulola, E., Micalos, P., & Baguley, I. (2018). Understanding the pathophysiology of depression: From monoamines to the neurogenesis hypothesis model - are we there yet? . *Behavioural Brain Research*, *341*, 79–90. https://doi.org/10.1016/j.bbr.2017.12.025

https://www.sciencedirect.com/science/article/abs/pii/S0166432817318521?via%3Dihub

Kang, Y., Kang, X., & Cai, Y. (2022). The gut microbiome as a target for adjuvant therapy in insomnia disorder. *Clinics and research in hepatology and gastroenterology.*, *46*(1), 101834. https://doi.org/10.1016/j.clinre.2021.101834

Karl, J. P., Hatch, A. M., Arcidiacono, S. M., Pearce, S. C., Pantoja-Feliciano, I. G., Doherty, L. A., & Soares, J. W. (2018). Effects of Psychological, Environmental and Physical

Stressors on the Gut Microbiota. *Frontiers in microbiology., 9*. https://doi.org/10.3389/fmicb.2018.02013
Karrouri, R., Hammani, Z., Benjelloun, R., & Otheman, Y. (2021). Major depressive disorder: Validated treatments and future challenges. *World J Clin Cases*, *9*(31), 9350-9367. https://doi.org/10.12998/wjcc.v9.i31.9350
Kennedy, S. H. (2008). Core symptoms of major depressive disorder: relevance to diagnosis and treatment. *Dialogues in Clinical Neuroscience*, *10*(3), 271-277. https://doi.org/10.31887/dcns.2008.10.3/shkennedy
Kessler, R., Berglund, P., Demler, O., Jin, R., Merikangas, K., & Walters, E. (2005). Lifetime Prevalence and Age-of-Onset Distributions
of DSM-IV Disorders in the National Comorbidity
Survey Replication. *Archives Of General Psychiatry*, *62*.
Kho, Z. Y., & Lal, S. K. (2018). The Human Gut Microbiome – A Potential Controller of Wellness and Disease. *Frontiers in microbiology., 9*. https://doi.org/10.3389/fmicb.2018.01835
Krystal, A., Fava, M., Rubens, R., Wessel, T., Caron, J., Wilson, P., Roth, T., & McCall, W. V. (2007). Evaluation of eszopiclone discontinuation after cotherapy with fluoxetine for insomnia with coexisting depression. *Journal of clinical sleep medicine*, *3*(1), 48.
Kurokawa, S., Kishimoto, T., Mizuno, S., Masaoka, T., Naganuma, M., Liang, K.-C., Kitazawa, M., Nakashima, M., Shindo, C., Suda, W., Hattori, M., Kanai, T., & Mimura, M. (2018). The effect of fecal microbiota transplantation on psychiatric symptoms among patients with irritable bowel syndrome, functional diarrhea and functional constipation: An open-label observational study. *Journal of affective disorders*, *235*, 506-512. https://doi.org/10.1016/j.jad.2018.04.038
Lai, C.-T., Chen, C.-Y., She, S.-C., Chen, W.-J., Kuo, T. B. J., Lin, H.-C., & Yang, C. C. H. (2022). Production of Lactobacillus brevis ProGA28 attenuates stress-related sleep disturbance and modulates the autonomic nervous system and the motor response in anxiety/depression behavioral tests in Wistar–Kyoto rats. *Life sciences.*, *288*, 120165. https://doi.org/10.1016/j.lfs.2021.120165
Larche, C. L., Plante, I., Roy, M., Ingelmo, P. M., Ferland, C. E., & Pillar, G. (2021). The Pittsburgh Sleep Quality Index: Reliability, Factor Structure, and Related Clinical Factors among Children, Adolescents, and Young Adults with Chronic Pain. *Sleep disorders.*, *2021*, 1-8. https://doi.org/10.1155/2021/5546484
Lastella, M., Lovell, G. P., & Sargent, C. (2014). Athletes' precompetitive sleep behaviour and its relationship with subsequent precompetitive mood and performance. *European journal of sport science : EJSS.*, *14*(sup1), S123-S130. https://doi.org/10.1080/17461391.2012.660505
Lee, H. J., Hong, J. K., Kim, J.-K., Kim, D.-H., Jang, S. W., Han, S.-W., & Yoon, I.-Y. (2021). Effects of Probiotic NVP-1704 on Mental Health and Sleep in Healthy Adults: An 8-Week Randomized, Double-Blind, Placebo-Controlled Trial. *Nutrients*, *13*(8), 2660. https://doi.org/10.3390/nu13082660
Lépine, J. P., & Briley, M. (2011). The increasing burden of depression. *Neuropsychiatr Dis Treat*, *7*(Suppl 1), 3-7. https://doi.org/10.2147/ndt.S19617
Lerner, D., Adler, D. A., Rogers, W. H., Chang, G., Lapitsky, L., McLaughlin, T., & Reed, J. (2010). Work Performance of Employees with Depression: The Impact of Work

Stressors. *American Journal of Health Promotion*, *24*(3), 205-213. https://doi.org/10.4278/ajhp.090313-quan-103

Leung, S. M. (2021). Drug use evaluation: A two-year retrospective review of the effectiveness and tolerability of agomelatine versus mirtazapine in patients with depressive disorder. *Brain and Behavior*, *11*(8). https://doi.org/10.1002/brb3.2311

Li, M., Cui, J., Xu, B., Wei, Y., Fu, C., Lv, X., Xiong, L., & Qin, D. (2021). Sleep Disturbances and Depression Are Co-morbid Conditions: Insights From Animal Models, Especially Non-human Primate Model. *Front Psychiatry*, *12*, 827541. https://doi.org/10.3389/fpsyt.2021.827541

Li, M., Cui, J., Xu, B., Wei, Y., Fu, C., Lv, X., Xiong, L., & Qin, D. (2022). Sleep Disturbances and Depression Are Co-morbid Conditions: Insights From Animal Models, Especially Non-human Primate Model. *Frontiers in psychiatry.*, *12*. https://doi.org/10.3389/fpsyt.2021.827541

Li, Y., Hao, Y., Fan, F., & Zhang, B. (2018). The Role of Microbiome in Insomnia, Circadian Disturbance and Depression. *Front Psychiatry*, *9*, 669. https://doi.org/10.3389/fpsyt.2018.00669

Lin, A., Shih, C.-T., Chu, H.-F., Chen, C.-W., Cheng, Y.-T., Wu, C.-C., Yang, C. C. H., & Tsai, Y.-C. (2021). Lactobacillus fermentum PS150 promotes non-rapid eye movement sleep in the first night effect of mice. *Scientific reports*, *11*(1). https://doi.org/10.1038/s41598-021-95659-3

Lin, A., Shih, C.-T., Huang, C.-L., Wu, C.-C., Lin, C.-T., & Tsai, Y.-C. (2019). Hypnotic Effects of Lactobacillus fermentum PS150TM on Pentobarbital-Induced Sleep in Mice. *Nutrients*, *11*(10), 2409. https://doi.org/10.3390/nu11102409

Liu, W.-H., Chuang, H.-L., Huang, Y.-T., Wu, C.-C., Chou, G.-T., Wang, S., & Tsai, Y.-C. (2016). Alteration of behavior and monoamine levels attributable to Lactobacillus plantarum PS128 in germ-free mice. *Behavioural brain research.*, *298*, 202-209. https://doi.org/10.1016/j.bbr.2015.10.046

López-Muñoz, F., Álamo, C., Juckel, G., & Assion, H.-J. (2007). Half a Century of Antidepressant Drugs. *Journal of clinical psychopharmacology.*, *27*(6), 555-559. https://doi.org/10.1097/jcp.0b013e3181bb617

Luan, H., Wang, X., & Cai, Z. (2019). Mass spectrometry-based metabolomics: Targeting the crosstalk between gut microbiota and brain in neurodegenerative disorders. *Mass Spectrometry Reviews*, *38*(1), 22-33. https://doi.org/10.1002/mas.21553

Maki, K. A., Burke, L. A., Calik, M. W., Watanabe-Chailland, M., Sweeney, D., Romick-Rosendale, L. E., Green, S. J., & Fink, A. M. (2020). Sleep fragmentation increases blood pressure and is associated with alterations in the gut microbiome and fecal metabolome in rats. *Physiological genomics*, *52*(7), 280-292. https://doi.org/10.1152/physiolgenomics.00039.2020 (Metabolites and Physiology)

Manber, R., Edinger, J. D., Gress, J. L., San Pedro-Salcedo, M. G., Kuo, T. F., & Kalista, T. (2008). Cognitive behavioral therapy for insomnia enhances depression outcome in patients with comorbid major depressive disorder and insomnia. *Sleep (New York, N.Y.)*, *31*(4), 489-495. https://doi.org/10.1093/sleep/31.4.489

Marotta, A., Sarno, E., Del Casale, A., Pane, M., Mogna, L., Amoruso, A., Felis, G. E., & Fiorio, M. (2019). Effects of Probiotics on Cognitive Reactivity, Mood, and Sleep Quality. *Frontiers in psychiatry.*, *10*. https://doi.org/10.3389/fpsyt.2019.00164

Martin, R., Makino, H., Yavuz, A. C., Ben-Amor, K., Roelofs, M., Ishikawa, E., Kubota, H., Swinkels, S., Sakai, T., Oishi, K., Kushiro, A., & Knol, J. (2016). Early-Life events, including mode of delivery and type of feeding, siblings and gender, shape the developing gut microbiota. *PloS one*, *11*(6), e0158498-e0158498. https://doi.org/10.1371/journal.pone.0158498

Mason, B. L., Davidov, A., Minhajuddin, A., & Trivedi, M. H. (2020). Focusing on insomnia symptoms to better understand depression: A STARD report. *Journal of affective disorders*, *260*, 183-186. https://doi.org/10.1016/j.jad.2019.08.094

Mason, E. C., & Harvey, A. G. (2014). Insomnia before and after treatment for anxiety and depression. *Journal of affective disorders.*, *168*, 415-421. https://doi.org/10.1016/j.jad.2014.07.020

McVey Neufeld, K. A., Mao, Y. K., Bienenstock, J., Foster, J. A., & Kunze, W. A. (2013). The microbiome is essential for normal gut intrinsic primary afferent neuron excitability in the mouse. *Neurogastroenterology & Motility*, *25*(2), 183-e188. https://doi.org/10.1111/nmo.12049

Mendonça-De-Souza, A. C. F., Souza, G. G. L., Vieira, A., Fischer, N. L., Souza, W. F., Rumjanek, V. M., Figueira, I., Mendlowicz, M. V., & Volchan, E. (2007). Negative affect as a predisposing factor for cortisol release after an acute stress—the impact of unpleasant priming. *Stress*, *10*(4), 362-367. https://doi.org/10.1080/10253890701379999

Mickey, B. J., Ginsburg, Y., Sitzmann, A. F., Grayhack, C., Sen, S., Kirschbaum, C., Maixner, D. F., & Abelson, J. L. (2018). Cortisol trajectory, melancholia, and response to electroconvulsive therapy. *Journal of psychiatric research*, *103*, 46-53. https://doi.org/10.1016/j.jpsychires.2018.05.007

Mojtabai, R., & Olfson, M. (2014). National Trends in Long-Term Use of Antidepressant Medications. *The journal of clinical psychiatry.*, *75*(2), 169-177. https://doi.org/10.4088/jcp.13m08443

Morwani-Mangnani, J., Giannos, P., Belzer, C., Beekman, M., Slagboom, E., & Prokopidis, K. (2022). Gut microbiome changes due to sleep disruption in older and younger individuals : a case for sarcopenia? *Sleep (New York, N.Y.)*, *45*(12), 1-13. https://doi.org/10.1093/sleep/zsac239

Murphy, M. J., & Peterson, M. J. (2015). Sleep Disturbances in Depression. *Sleep Med Clin*, *10*(1), 17-23. https://doi.org/10.1016/j.jsmc.2014.11.009

Nakagawa, A., Mitsuda, D., Sado, M., Abe, T., Fujisawa, D., Kikuchi, T., Iwashita, S., Mimura, M., & Ono, Y. (2017). Effectiveness of Supplementary Cognitive-Behavioral Therapy for Pharmacotherapy-Resistant Depression. *The Journal of Clinical Psychiatry*, *78*(8), 1126-1135. https://doi.org/10.4088/jcp.15m10511

Nakamaru-Ogiso, E., Miyamoto, H., Hamada, K., Tsukada, K., & Takai, K. (2012). Novel biochemical manipulation of brain serotonin reveals a role of serotonin in the circadian rhythm of sleep-wake cycles. *European journal of neuroscience : EJN.*, *35*(11), 1762-1770. https://doi.org/10.1111/j.1460-9568.2012.08077.x

Nelson, K. L., Davis, J. E., & Corbett, C. F. (2022). Sleep quality: An evolutionary concept analysis. *Nursing forum.*, *57*(1), 144-151. https://doi.org/10.1111/nuf.12659

Nishida, K., Sawada, D., Kawai, T., Kuwano, Y., Fujiwara, S., & Rokutan, K. (2017). Para-psychobiotic Lactobacillus gasseri <scp>CP</scp> 2305 ameliorates stress-related symptoms and sleep quality. *Journal of Applied Microbiology*, *123*(6), 1561-1570. https://doi.org/10.1111/jam.13594

Nutt, D., Wilson, S., & Paterson, L. (2008). Sleep disorders as core symptoms of depression. *Dialogues Clin Neurosci*, *10*(3), 329-336. https://doi.org/10.31887/DCNS.2008.10.3/dnutt

Ogawa, Y., Miyoshi, C., Obana, N., Yajima, K., Hotta-Hirashima, N., Ikkyu, A., Kanno, S., Soga, T., Fukuda, S., & Yanagisawa, M. (2020). Gut microbiota depletion by chronic antibiotic treatment alters the sleep/wake architecture and sleep EEG power spectra in mice. *Scientific reports*, *10*(1). https://doi.org/10.1038/s41598-020-76562-9

Oleskin, A. V., El'-Registan, G. I., & Shenderov, B. A. (2016). Role of neuromediators in the functioning of the human microbiota: "Business talks" among microorganisms and the microbiota-host dialogue. *Microbiology*, *85*(1), 1-22. https://doi.org/10.1134/s0026261716010082

Ong, J. C., Kuo, T. F., & Manber, R. (2008). Who is at risk for dropout from group cognitive-behavior therapy for insomnia? *Journal of Psychosomatic Research*, *64*(4), 419-425. https://doi.org/10.1016/j.jpsychores.2007.10.009

Osadchiy, V., Martin, C. R., & Mayer, E. A. (2019). The Gut–Brain Axis and the Microbiome: Mechanisms and Clinical Implications. *Clinical Gastroenterology and Hepatology*, *17*(2), 322-332. https://doi.org/10.1016/j.cgh.2018.10.002

Owashi, T., Otsubo, T., Oshima, A., Nakagome, K., Higuchi, T., & Kamijima, K. (2008). Longitudinal neuroendocrine changes assessed by dexamethasone/CRH and growth hormone releasing hormone tests in psychotic depression. *Psychoneuroendocrinology.*, *33*(2), 152-161. https://doi.org/10.1016/j.psyneuen.2007.10.011

Pagel, J. F., & Parnes, B. L. (2001). Medications for the Treatment of Sleep Disorders. *The Primary Care Companion For CNS Disorders*, *3*(3), 118-125. https://doi.org/10.4088/pcc.v03n0303

Pandey, K. R., Naik, S. R., & Vakil, B. V. (2015). Probiotics, prebiotics and synbiotics- a review. *Journal of Food Science and Technology*, *52*(12), 7577-7587. https://doi.org/10.1007/s13197-015-1921-1

Park, S.-Y., & Seo, G. S. (2021). Fecal Microbiota Transplantation: Is It Safe? *Clinical Endoscopy*, *54*(2), 157-160. https://doi.org/10.5946/ce.2021.072

Parkar, S., Kalsbeek, A., & Cheeseman, J. (2019). Potential Role for the Gut Microbiota in Modulating Host Circadian Rhythms and Metabolic Health. *Microorganisms*, *7*(2), 41. https://doi.org/10.3390/microorganisms7020041

Poroyko, V. A., Carreras, A., Khalyfa, A., Khalyfa, A. A., Leone, V., Peris, E., Almendros, I., Gileles-Hillel, A., Qiao, Z., Hubert, N., Farré, R., Chang, E. B., & Gozal, D. (2016). Chronic Sleep Disruption Alters Gut Microbiota, Induces Systemic and Adipose Tissue Inflammation and Insulin Resistance in Mice. *Scientific reports*, *6*(1), 35405. https://doi.org/10.1038/srep35405

Powley, T. L. (2000). Vagal input to the enteric nervous system. *Gut*, *47*(90004), 30iv-32. https://doi.org/10.1136/gut.47.suppl_4.iv30

Racagni, G., & Popoli, M. (2008). Cellular and molecular mechanisms in the long-term action of antidepressants. *Dialogues in Clinical Neuroscience*, *10*(4), 385-400. https://doi.org/10.31887/dcns.2008.10.4/gracagni

Raffaele, A., Vatta, F., Votto, M., Licari, A., Ruffoli, M., Brunero, M., Marseglia, G., & Riccipetitoni, G. (2021). Eosinophilic colitis in children: a new and elusive enemy? *Pediatric surgery international*, *37*(4), 485-490. https://doi.org/https://dx.doi.org/10.1007/s00383-020-04832-8

Rasch, B., & Born, J. (2013). About Sleep's Role in Memory. *Physiological reviews.*, *93*(2), 681-766. https://doi.org/10.1152/physrev.00032.2012

Reigstad, C. S., Salmonson, C. E., Iii, J. F. R., Szurszewski, J. H., Linden, D. R., Sonnenburg, J. L., Farrugia, G., & Kashyap, P. C. (2015). Gut microbes promote colonic serotonin production through an effect of short-chain fatty acids on enterochromaffin cells. *The FASEB Journal*, *29*(4), 1395-1403. https://doi.org/10.1096/fj.14-259598

Salami, M. (2021). Interplay of Good Bacteria and Central Nervous System: Cognitive Aspects and Mechanistic Considerations. *Frontiers in neuroscience*, *15*, 613120-613120. https://doi.org/10.3389/fnins.2021.613120

Salminen, S., Collado, M. C., Endo, A., Hill, C., Lebeer, S., Quigley, E. M. M., Sanders, M. E., Shamir, R., Swann, J. R., Szajewska, H., & Vinderola, G. (2021). The International Scientific Association of Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of postbiotics. *Nature Reviews Gastroenterology & Hepatology*, *18*(9), 649-667. https://doi.org/10.1038/s41575-021-00440-6

Santarsieri, D., & Schwartz, T. (2015). Antidepressant efficacy and side-effect burden: a quick guide for clinicians. *Drugs in Context*, *4*, 1-12. https://doi.org/10.7573/dic.212290

Schaub, A.-C., Schneider, E., Vazquez-Castellanos, J. F., Schweinfurth, N., Kettelhack, C., Doll, J. P. K., Yamanbaeva, G., Mählmann, L., Brand, S., Beglinger, C., Borgwardt, S., Raes, J., Schmidt, A., & Lang, U. E. (2022). Clinical, gut microbial and neural effects of a probiotic add-on therapy in depressed patients: a randomized controlled trial. *Translational psychiatry*, *12*(1). https://doi.org/10.1038/s41398-022-01977-z

Schiepers, O. J. G., Wichers, M. C., & Maes, M. (2005). Cytokines and major depression. *Progress in neuro-psychopharmacology & biological psychiatry*, *29*(2), 201-217. https://doi.org/10.1016/j.pnpbp.2004.11.003

Schifano, F., Chiappini, S., Corkery, J. M., & Guirguis, A. (2019). An Insight into Z-Drug Abuse and Dependence: An Examination of Reports to the European Medicines Agency Database of Suspected Adverse Drug Reactions. *The international journal of neuropsychopharmacology.*, *22*(4), 270-277. https://doi.org/10.1093/ijnp/pyz007

Schmid, D. A., Wichniak, A., Uhr, M., Ising, M., Brunner, H., Held, K., Weikel, J. C., Sonntag, A., & Steiger, A. (2006). Changes of Sleep Architecture, Spectral Composition of Sleep EEG, the Nocturnal Secretion of Cortisol, ACTH, GH, Prolactin, Melatonin, Ghrelin, and Leptin, and the DEX-CRH Test in Depressed Patients during Treatment with Mirtazapine. *Neuropsychopharmacology*, *31*(4), 832-844. https://doi.org/10.1038/sj.npp.1300923

Schmidt, K., Cowen, P. J., Harmer, C. J., Tzortzis, G., Errington, S., & Burnet, P. W. J. (2015). Prebiotic intake reduces the waking cortisol response and alters emotional bias in healthy volunteers. *Psychopharmacology*, *232*(10), 1793-1801. https://doi.org/10.1007/s00213-014-3810-0

Sen, P., Molinero-Perez, A., O'Riordan, K. J., McCafferty, C. P., O'Halloran, K. D., & Cryan, J. F. (2021). Microbiota and sleep: awakening the gut feeling. *Trends in molecular medicine*, *27*(10), 935-945. https://doi.org/10.1016/j.molmed.2021.07.004

Sender, R., Fuchs, S., & Milo, R. (2016). Revised Estimates for the Number of Human and Bacteria Cells in the Body. *PLOS Biology*, *14*(8), e1002533. https://doi.org/10.1371/journal.pbio.1002533

Sharpley, A. L., & Cowen, P. J. (1995). Effect of pharmacologic treatments on the sleep of depressed patients. *Biological psychiatry.*, *37*(2), 85-98. https://doi.org/10.1016/0006-3223(94)00135-p

Sherwin, E., Sandhu, K. V., Dinan, T. G., & Cryan, J. F. (2016). May the Force Be With You: The Light and Dark Sides of the Microbiota–Gut–Brain Axis in Neuropsychiatry. *CNS Drugs*, *30*(11), 1019-1041. https://doi.org/10.1007/s40263-016-0370-3

Siegel, J. M. (2005). Chapter 10 - REM Sleep. In W. B. Saunders (Ed.), *Principles and Practice of Sleep Medicine (Fourth Edition)* (pp. 120-135). https://doi.org/10.1016/B0-72-160797-7/50017-3.

Smith, A. P. (2005). The concept of well-being: relevance to nutrition research. *British Journal of Nutrition*, *93*(S1), S1-S5. https://doi.org/10.1079/bjn20041351

Smith, R. P., Easson, C., Lyle, S. M., Kapoor, R., Donnelly, C. P., Davidson, E. J., Parikh, E., Lopez, J. V., & Tartar, J. L. (2019). Gut microbiome diversity is associated with sleep physiology in humans. *PloS one*, *14*(10), e0222394. https://doi.org/10.1371/journal.pone.0222394

Smits, L. P., Bouter, K. E. C., de Vos, W. M., Borody, T. J., & Nieuwdorp, M. (2013). Therapeutic Potential of Fecal Microbiota Transplantation. *Gastroenterology (New York, N.Y. 1943)*, *145*(5), 946-953. https://doi.org/10.1053/j.gastro.2013.08.058

Staner, L. (2010). Comorbidity of insomnia and depression. *Sleep Med Rev*, *14*(1), 35-46. https://doi.org/10.1016/j.smrv.2009.09.003

Steffen, A., Nübel, J., Jacobi, F., Bätzing, J., & Holstiege, J. (2020). Mental and somatic comorbidity of depression: a comprehensive cross-sectional analysis of 202 diagnosis groups using German nationwide ambulatory claims data. *BMC psychiatry*, *20*(1). https://doi.org/10.1186/s12888-020-02546-8

Stetler, C., & Miller, G. E. (2011). Depression and Hypothalamic-Pituitary-Adrenal Activation: A Quantitative Summary of Four Decades of Research. *Psychosomatic Medicine*, *73*(2), 114-126. https://doi.org/10.1097/PSY.0b013e31820ad12b

Stewart, W. F., Ricci, J. A., Chee, E., Hahn, S. R., & Morganstein, D. (2003). Cost of Lost Productive Work Time Among US Workers With Depression. *JAMA*, *289*(23), 3135. https://doi.org/10.1001/jama.289.23.3135

Sun, N., Hu, H., Wang, F., Li, L., Zhu, W., Shen, Y., Xiu, J., & Xu, Q. (2021). Antibiotic-induced microbiome depletion in adult mice disrupts blood-brain barrier and facilitates brain infiltration of monocytes after bone-marrow transplantation. *Brain, behavior, and immunity.*, *92*, 102-114. https://doi.org/10.1016/j.bbi.2020.11.032

Szentirmai, É., Millican, N. S., Massie, A. R., & Kapás, L. (2019). Butyrate, a metabolite of intestinal bacteria, enhances sleep. *Scientific reports*, *9*(1), 7035-7035. https://doi.org/10.1038/s41598-019-43502-1

Tap, J., Mondot, S., Levenez, F., Pelletier, E., Caron, C., Furet, J.-P., Ugarte, E., Muñoz-Tamayo, R., Paslier, D. L. E., Nalin, R., Dore, J., & Leclerc, M. (2009). Towards the human intestinal microbiota phylogenetic core. *Environmental Microbiology*, *11*(10), 2574-2584. https://doi.org/10.1111/j.1462-2920.2009.01982.x

Tatsumi, M., Groshan, K., Blakely, R. D., & Richelson, E. (1997). Pharmacological profile of antidepressants and related compounds at human monoamine transporters. *European journal of pharmacology.*, *340*(2-3), 249-258. https://doi.org/10.1016/s0014-2999(97)01393-9

Taylor, D. (2008). Antidepressant drugs and cardiovascular pathology: a clinical overview of effectiveness and safety. *Acta Psychiatrica Scandinavica*, *118*(6), 434-442. https://doi.org/10.1111/j.1600-0447.2008.01260.x

Thaipisuttikul, P., Ittasakul, P., Waleeprakhon, P., Wisajun, P., & Jullagate, S. (2014). Psychiatric comorbidities in patients with major depressive disorder. *Neuropsychiatr Dis Treat*, *10*, 2097-2103. https://doi.org/10.2147/ndt.S72026

Thase, M. E. (2006). Depression and sleep: pathophysiology and treatment. *Dialogues in Clinical Neuroscience*, *8*(2), 217-226. https://doi.org/10.31887/dcns.2006.8.2/mthase

The Lancet Global, H. (2020). Mental health matters. *The Lancet Global Health*, *8*(11), e1352. https://doi.org/10.1016/s2214-109x(20)30432-0

Thompson, R. S., Gaffney, M., Hopkins, S., Kelley, T., Gonzalez, A., Bowers, S. J., Vitaterna, M. H., Turek, F. W., Foxx, C. L., Lowry, C. A., Vargas, F., Dorrestein, P. C., Wright, K. P., Knight, R., & Fleshner, M. (2021). Ruminiclostridium 5, Parabacteroides distasonis, and bile acid profile are modulated by prebiotic diet and associate with facilitated sleep/clock realignment after chronic disruption of rhythms. *Brain, behavior, and immunity.*, *97*, 150-166. https://doi.org/10.1016/j.bbi.2021.07.006

Tsigos, C., & Chrousos, G. P. (2002). Hypothalamic–pituitary–adrenal axis, neuroendocrine factors and stress. *Journal of Psychosomatic Research*, *53*(4), 865-871. https://doi.org/10.1016/s0022-3999(02)00429-4

Valle, M. C. P. R., Vieira, I. A., Fino, L. C., Gallina, D. A., Esteves, A. M., Da Cunha, D. T., Cabral, L., Benatti, F. B., Marostica Junior, M. R., Batista, Â. G., Santos, R., Pastore, G. M., Sartoratto, A., Sivieri, K., Tizioto, P. C., Coutinho, L. L., & Antunes, A. E. C. (2021). Immune status, well-being and gut microbiota in military supplemented with synbiotic ice cream and submitted to field training: a randomised clinical trial. *British Journal of Nutrition*, *126*(12), 1794-1808. https://doi.org/10.1017/s0007114521000568

Varghese, F. P., & Brown, E. S. (2001). The Hypothalamic-Pituitary-Adrenal Axis in Major Depressive Disorder. *The Primary Care Companion For CNS Disorders*, *3*(4), 151-155. https://doi.org/10.4088/pcc.v03n0401

Vighi, G., Marcucci, F., Sensi, L., Di Cara, G., & Frati, F. (2008). Allergy and the gastrointestinal system. *Clinical and experimental immunology*, *153*(s1), 3-6. https://doi.org/10.1111/j.1365-2249.2008.03713.x

Voigt, R. M., Forsyth, C. B., Green, S. J., Mutlu, E., Engen, P., Vitaterna, M. H., Turek, F. W., & Keshavarzian, A. (2014). Circadian Disorganization Alters Intestinal Microbiota. *PloS one*, *9*(5), e97500. https://doi.org/10.1371/journal.pone.0097500

Walker, M. P. (2009). The Role of Sleep in Cognition and Emotion. *Annals of the New York Academy of Sciences*, *1156*(1), 168-197. https://doi.org/10.1111/j.1749-6632.2009.04416.x

Wallace, C. J. K., & Milev, R. V. (2021). The Efficacy, Safety, and Tolerability of Probiotics on Depression: Clinical Results From an Open-Label Pilot Study. *Frontiers in psychiatry.*, *12*. https://doi.org/10.3389/fpsyt.2021.618279

Wang, Y.-Q., Li, R., Zhang, M.-Q., Zhang, Z., Qu, W.-M., & Huang, Z.-L. (2015). The Neurobiological Mechanisms and Treatments of REM Sleep Disturbances in Depression. *Current Neuropharmacology*, *13*(4), 543-553. https://doi.org/10.2174/1570159x13666150310002540

Wang, Z., Wang, Z., Lu, T., Chen, W., Yan, W., Yuan, K., Shi, L., Liu, X., Zhou, X., Shi, J., Vitiello, M. V., Han, Y., & Lu, L. (2022). The microbiota-gut-brain axis in sleep disorders. *Sleep Med Rev*, *65*, 101691. https://doi.org/10.1016/j.smrv.2022.101691

Wichniak, A., Wierzbicka, A., Walęcka, M., & Jernajczyk, W. (2017). Effects of Antidepressants on Sleep. *Current Psychiatry Reports*, *19*(9). https://doi.org/10.1007/s11920-017-0816-4

Wilson, S., & Argyropoulos, S. (2005). Antidepressants and Sleep. *Drugs*, *65*(7), 927-947. https://doi.org/10.2165/00003495-200565070-00003

Winokur, A., DeMartinis, N. A., McNally, D. P., Gary, E. M., Cormier, J. L., & Gary, K. A. (2003). Comparative Effects of Mirtazapine and Fluoxetine on Sleep Physiology Measures in Patients With Major Depression and Insomnia. *The journal of clinical psychiatry.*, *64*(10), 1224-1229. https://doi.org/10.4088/jcp.v64n1013

World Health Organization. (2012). *Depression* World Health Organization Retrieved June 8th from https://www.who.int/health-topics/depression#tab=tab_1

World Health Organization. (2023). Depression. https://www.who.int/news-room/fact-sheets/detail/depression

Wu, J. Q., Appleman, E. R., Salazar, R. D., & Ong, J. C. (2015). Cognitive Behavioral Therapy for Insomnia Comorbid With Psychiatric and Medical Conditions. *JAMA Internal Medicine*, *175*(9), 1461. https://doi.org/10.1001/jamainternmed.2015.3006

Xing, S., DiPaula, B. A., Lee, H. Y., & Cooke, C. E. (2011). Failure to Fill Electronically Prescribed Antidepressant Medications. *The primary care companion for CNS disorders.* https://doi.org/10.4088/pcc.10m00998blu

Yong, S. J., Tong, T., Chew, J., & Lim, W. L. (2020). Antidepressive Mechanisms of Probiotics and Their Therapeutic Potential. *Frontiers in neuroscience*, *13*, 1361-1361. https://doi.org/10.3389/fnins.2019.01361

Zhang, S. L., Bai, L., Goel, N., Bailey, A., Jang, C. J., Bushman, F. D., Meerlo, P., Dinges, D. F., & Sehgal, A. (2017). Human and rat gut microbiome composition is maintained following sleep restriction. *Proceedings of the National Academy of Sciences*, *114*(8), E1564-E1571. https://doi.org/10.1073/pnas.1620673114

Zhang, Y., Xie, B., Chen, X., Zhang, J., & Yuan, S. (2021). A key role of gut microbiota-vagus nerve/spleen axis in sleep deprivation-mediated aggravation of systemic inflammation after LPS administration. *Life sciences.*, *265*, 118736. https://doi.org/10.1016/j.lfs.2020.118736

Zhang, Y.-J., Li, S., Gan, R.-Y., Zhou, T., Xu, D.-P., & Li, H.-B. (2015). Impacts of Gut Bacteria on Human Health and Diseases. *International journal of molecular sciences*, *16*(12), 7493-7519. https://doi.org/10.3390/ijms16047493

Zmora, N., Zilberman-Schapira, G., Suez, J., Mor, U., Dori-Bachash, M., Bashiardes, S., Kotler, E., Zur, M., Regev-Lehavi, D., Brik, R. B.-Z., Federici, S., Cohen, Y., Linevsky, R., Rothschild, D., Moor, A. E., Ben-Moshe, S., Harmelin, A., Itzkovitz, S., Maharshak, N., . . . Elinav, E. (2018). Personalized Gut Mucosal Colonization Resistance to Empiric Probiotics Is Associated with Unique Host and Microbiome Features. *Cell*, *174*(6), 1388-1405.e1321. https://doi.org/10.1016/j.cell.2018.08.041

APPENDIX A HSREB APPROVAL

QUEEN'S UNIVERSITY HEALTH SCIENCES & AFFILIATED TEACHING HOSPITALS RESEARCH ETHICS BOARD (HSREB)

HSREB Initial Ethics Clearance

March 28, 2019

Dr. R V Milev
Department of Psychiatry
Queen's University

TRAQ #: 6025187
Department Code: PSIY-634-18
Study Title: "The Safety, Efficacy, and Tolerability of Microbial Ecosystem Therapeutic-2 in People with Major Depression and/or Generalized Anxiety Disorder"
Co-Investigator: A. Chinna Meyyappan
Review Type: Full Board
Date of Full Board Meeting: January 21, 2019
Date Ethics Clearance Issued: March 28, 2019
Ethics Clearance Expiry Date: March 28, 2020

Dear Dr. Milev:

The Queen's University Health Sciences & Affiliated Teaching Hospitals Research Ethics Board (HSREB) has reviewed the application and granted ethics clearance for the documents listed below. Ethics clearance is granted until the expiration date noted above.

- Protocol: METDA-Protocol v.2019MAR11
- Consent: METDA-ICF v.2019MAR11
- Survey/Questionnaires: First Contact Email Script v.2019FEB18
- METDA Prescreening Form v2 2019FEB18
- Medical History form v.2018OCT23
- Demographics Form Questionnaire v.2018OCT23
- Recruitment Materials: 2METDA Advertisement v.2019JAN07
- Recruitment Materials: METDA Advertisement v.2019JAN07
- METDA Adverse Event Log v.2019JAN07
- METDA SAE Report Form v.2019JAN07
- CECA Interview – adult v.2018OCT26
- CECA Rating Form v.2018OCT24
- CECA Vignette Form v.2018OCT24
- Black Dog Mood Chart v.2018OCT23

Documents Acknowledged:

- METDA Budget v.2019JAN07
- METDA - Investigator's Brochure v.2019JAN07
- MINI 6.0.0. Questionnaire v.2010OCT10
- GAD-7 Questionnaire v.2014JUL07
- MADRS Questionnaire v.2018OCT23
- Pittsburgh Sleep Quality Index Questionnaire v. 1989
- QIDS-SR16 Questionnaire v.2018OCT23
- TSES Toronto Side Effects Scale v.2011NOV21
- SHAPS Questionnaire v.2013OCT08
- CGI – Severity of Illness & Global Improvement Scale v.2018OCT23
- MET2 - CDiff-Safety Report v.2019MAR11
- CVs (R. Milev, A. Chinna Meyyappan, E. Hawken, C. Wallace, A. Forsyth)
- CORE/CCHRP Certificates (R. Milev, E. Hawken, A. Forsyth, C. Wallace, A. Chinna Meyyappan)
- Clinical Trial Agreement - NuBiyota_QU v.2019FEB10
- METDA Health Canada No Objection Letter v.2019JAN28
- METDA SAE Guidelines v.2019JAN07
- Milev – Local Biohazard Risk Assessment v2 2018OCT28
- Milev – Emergency Procedures v.2018OCT28
- Dr. Vazquez Protocol Peer Review v.2018OCT24
- C of A Final Drug Product – NB-BMR – 010 (Capsules) v.2018OCT23

Amendments: No deviation from, or changes to the protocol should be initiated without prior written clearance or an appropriate amendment from the HSREB, except when necessary to eliminate immediate hazard(s) to study participants or when the change(s) involves only administrative or logistical aspects of the trial.

Renewals: Prior to the expiration of your ethics clearance you will be reminded to submit your renewal report through TRAQ. Any lapses in ethical clearance will be documented on the renewal form.

Completion/Termination: The HSREB must be notified of the completion or termination of this study through the completion of a study closure report in TRAQ.

Reporting of Serious Adverse Events: Any unexpected serious adverse events occurring locally must be reported within 2 working days or earlier if required by the study sponsor. All other serious adverse events must be reported within 15 days after becoming aware of the information.

Reporting of Complaints: Any complaints made by participants or persons acting on behalf of participants must be reported to the Research Ethics Board within 7 days of becoming aware of the complaint.

Note: All documents supplied to participants must have the contact information for the Research Ethics Board.

Investigators please note that if your trial is registered by the sponsor, you must take responsibility to ensure that the registration information is accurate and complete.

Yours sincerely,

Albert F. Clark

Albert F. Clark, PhD
Chair, Queen's University Health Sciences and Affiliated Teaching Hospitals Ethics Board

The HSREB operates in compliance with, and is constituted in accordance with, he requirements of the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans (TCPS 2); the international Conference on Harmonisation Good Clinical Practice Consolidated Guideline (ICH GCP); Part C, Division 5 of the Food and Drug Regulations; Part 4 of the Natural Health Product Regulations; Part 3 of the Medical Devices Regulations, and the provisions of the Ontario Personal Health Information Protection Act (PHIPA 2004) and its applicable regulations. The HSREB is qualified through the CTO REB Qualification Program and is registered with the U.S. Department of Health and Human Services (DHHS) Office for Human Research Protection (OHRP). Federalwide Assurance Number: FWA#: 00004184, IRB#: 00001173

HSREB members involved in the research project do not participate in the review, discussion or decision.