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The Effect of Melatonin Upon Post-Acute Withdrawal Among Males in a Residential Treatment Program (M-Paws): A Randomized, Double-Blind, Placebo Controlled Trial

Corry D. Bondi

THE EFFECT OF MELATONIN UPON POST-ACUTE WITHDRAWAL AMONG MALES IN A RESIDENTIAL TREATMENT PROGRAM (M-PAWS): A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED TRIAL

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Corry D. Bondi, Ph.D.

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Corry D. Bondi, Ph.D.

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Vincent J. Giannetti, Ph.D. Professor of Pharmacy Administration (Committee Chair)	Khalid M. Kamal, Ph.D. Associate Professor Pharmacy Administration (Committee Member)
Paula A. Witt-Enderby, Ph.D. Marie-Clement Rodier, C.S.Sp., Endowed Chair in Scholarship Professor of Pharmacology (Committee Member)	David A. Johnson, Ph.D. Associate Professor of Pharmacology Division Head of Pharmaceutical Sciences (Committee Member)
J. Douglas Bricker, Ph.D. Dean, Mylan School of Pharmacy Professor of Pharmacology	James K. Drennen III, Ph.D. Associate Dean, Research and Graduate Programs

ABSTRACT

THE EFFECT OF MELATONIN UPON POST-ACUTE WITHDRAWAL AMONG MALES IN A RESIDENTIAL TREATMENT PROGRAM (M-PAWS): A RANDOMIZED, DOUBLE-BLIND PLACEBO-CONTROLLED TRIAL

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Thesis supervised by Vincent J. Giannetti, Ph.D.

The study goal was to assess melatonin as an adjuvant treatment along with current pharmaco- and behavioral therapy for 28 days on weekly self-reported severity of anxiety, depression, stress, and sleep complaints as well as how sleep is affecting daily life in a sample of males in recovery from chemical dependency at a single, residential treatment site, Salvation Army Harbor Light Center in Pittsburgh, PA. This study was a single-center, randomized, double-blind, placebo-controlled, parallel group trial of 28 days. Participants were randomized to melatonin (5 mg) or placebo and instructed to administer the intervention nightly at bedtime. Primary self-reported outcome measures of severity of anxiety, depression, stress, as well as sleep complaints and how sleep is affecting daily life were assessed on a weekly basis with the Generalized Anxiety Disorder Scale (GAD-7), Personal Health Questionnaire Depression Scale (PHQ-8),

Perceived Stress Scale (PSS-14), and Pittsburgh Sleep Symptom Questionnaire – Insomnia (PSSQ-1). Secondary outcome measures were to acquire participant histories, determine adherence as well as adverse events. Seventy participants (age 21 – 65, mean 40.4 ± 11 years) were enrolled with 24 completing the study in each group. Demographically, the sample consisted of those who identified as white (70%), single (74.3%), and with an education level of high school/G.E.D. or less (77.1%). Intention-to-treat analysis for all outcome measures revealed statistically significant within-groups differences over time for both groups. The study failed to demonstrate statistically between-group differences for these measures. Also, complete case analysis for each week revealed no between-group differences. Additionally, the change from Baseline and Day 28 as determined by a response of an improvement of 50% or higher in scores for each scale revealed no significant strength of association between the groups when considering worst case for the loss to follow-up. Melatonin appeared to be well tolerated with similar adverse events reported as placebo; however, there was a tendency to report more vivid dreams/nightmares as well as next day tiredness/grogginess/sleepiness. Clinical investigations into the use of melatonin as a treatment for depression, anxiety, stress, and sleep difficulties in those recovering from illicit and non-illicit drug dependency are limited and larger studies are warranted. Possible future directions include a study design that is multicenter, the inclusion of a therapy only arm, assessing various doses and timelines, assessing effects in adolescents or females, or limiting inclusion based on prescribed medications, mental health status, medical conditions, prior melatonin use, and/or a specific chemical dependency. Overall, this is the first and largest randomized, double-blind, placebo-controlled, parallel group trial assessing the

effects of melatonin upon post-acute withdrawal among males in a residential treatment program. However, the various analyses indicated insufficient evidence to suggest that melatonin and placebo were significantly different, and it may be concluded, based upon the study sample, design, and its limitations, the effect of melatonin on the assessed measures was no different than placebo. Due to the heterogeneity of the participants as evidenced by the participant histories, there exists a possibility of a Type II error that must be considered and not overlooked.

DEDICATION

Thesis is dedicated to all those who participated in this study.

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LIST OF ABBREVIATIONS

GAD-7: Generalized Anxiety Disorder Scale

MAT: Medication Assisted Treatment

PAWS: Post-Acute Withdrawal Syndrome

PHQ-8: Personal Health Questionnaire Depression Scale

PSS-14: Perceived Stress Scale

PSSQ-1: Pittsburgh Sleep Symptom Questionnaire – Insomnia

SAMHSA: Substance Abuse and Mental Health Services Administration

TEDS: Treatment Episode Data Set

U.S.: United States

USDUH: National Survey on Drug Use and Health

BACKGROUND

In the United States (U.S.), chemical dependency (i.e., substance abuse or substance use disorder) is a public health crisis that affects the behavioral and physical health of the nation. It impacts communities, families, as well as contributes to crime, homelessness, and other social problems and the etiology of chronic diseases such as heart disease and diabetes (1, 2). The economic burden is high with cost estimates of over $400 billion related to lost productivity, crime, and healthcare costs, of which, $36 billion is directly attributed to healthcare costs (3). Specifically, the costs associated with alcohol dependency are $224 billion, of which, $25 billion is attributed to healthcare costs while costs associated with illicit drug abuse is $193 billion and $11 billion, respectively (3). Thus, finding ways to counteract this crisis is of national importance.

According to Koob and Simon (2009), "drug addiction is a chronically relapsing disorder characterized by: (a) compulsion to seek and take the drug, (b) loss of control in limiting intake, and (c) emergence of a negative emotional state (e.g., dysphoria, anxiety, and irritability) when access to drug is prevented (4)." The Substance Abuse and Mental Health Services Administration (SAMHSA) conducts the National Survey on Drug Use and Health (NSDUH), the major source of information on the prevalence, patterns, and consequences of illicit and non-illicit drug use and abuse in the general U.S. civilian, noninstitutionalized population ages 12 and older (3, 5). According to the 2013 NSDUH, there were approximately 22.4 million adults (9.4% of adults) reporting using illicit drugs in 2013 in the past month and about 20.3 million adults (8.5% of adults) reported a past year substance use disorder (5). Co-morbidity of a substance use disorder along with a mental health condition is common. It is estimated that 7.7 million adults (3.2% of

adults) have a substance use disorder co-occurring with a mental health condition as defined by DSM-IV criteria (5). Importantly, individuals with mental health issues were more likely to have a substance use disorder and vice versa; at least half of those who develop an addiction have mental health conditions (6, 7).

Commonly abused drugs include opiates and narcotics (e.g., heroin & narcotic pain medications), stimulants (e.g., amphetamine and cocaine), central nervous system depressants (e.g., alcohol and benzodiazepines), hallucinogens (LSD and psilocybin), and marijuana/hashish (6). Specifically, 18 million adults (7.6% of adults) reported using marijuana or hashish followed by 4 million adults (2.5% of adults) who used prescription drugs (i.e., pain medications) for nonmedical reasons (5). Marijuana use has increased since 2007 and remains the most commonly reported first drug used (8). Use and abuse of alcohol is common with 16.2 million adults, disproportionately males, reporting being engaged in heavy drinking defined as "drinking five or more drinks on the same occasion on five or more days in the past 30 days (5, 8)."

To alleviate the financial impact on the nation, prevention and early treatment programs have been shown to be beneficial. For example, cost-benefit ratios, reported in the Institute of Medicine and National Research Council's Preventing Mental, Emotional, and Behavioral Disorders among Young People Report – 2009, range from 1:2 to 1:10 meaning every $1 of investment yields $2-$10 in savings (2). As reported in the Treatment Episode Data Set (TEDS) of admissions and discharges from substance abuse treatment facilities, Whites (60%) accounted for the most admissions followed by African-Americans (21%) and Hispanic or Latino (14%). The age range of 20-29 years (29.2%) accounted for the highest proportion of admissions while the lowest being 65

years or older (0.6%). The majority of those admitted sought treatment for alcohol abuse (41.4%) with 18.3% of those abusing alcohol with another drug. Twenty percent of admissions included heroin and other opiates, cocaine and other stimulants (17.8%), and marijuana (17.0%) (9). Effective treatments are available but too many individuals fail to get the treatment they need. The 2013 NSDUH reported that out of 22.7 million individuals 12 years or older who needed treatment for alcohol or illicit drug use, only 2.5 million received treatment at a specialty facility (5). Individuals who needed but did not receive treatment, felt a need for treatment, and made an effort face many barriers including "no health coverage/could not afford cost (37.3%)," "not ready to stop using (24.5%)," "did not know where to go for treatment (9.0%)," "had health coverage but it did not cover treatment or did cover cost (8.2%)," and "no transportation or inconvenient hours (8.0%) (5)." To address the coverage gap, the Affordable Care Act requires health plans to cover essential benefits such as treatments for substance abuse (7).

Treatment begins by first identifying the problem with the overall goals to empower the individuals to regain control of their lives as well as to improve the behavioral health; both aimed at reducing the national burden of chemical dependency (5, 10). Because treatment is not a "one size fits all approach," treatment programs aim to understand addiction, prevent relapse, and utilize various combinations of counseling, support networks, faith-based approaches, and medications (7, 11). A comprehensive approach addresses a continuum of care including the components of health promotion, prevention, treatment, and recovery. Tailoring a program to the needs and cultural background of the individual is the optimal approach for successful recovery (2). The program needs to understand the cultural context of the individual as well as implement

community-based values, traditions, and customs. Integrated treatment approaches focusing on treating mental health issues along with co-occurring substance use disorders have increased value by displaying lower costs and improved outcomes as evidenced by reduced substance use, decreased hospitalizations, improved mental health, increased housing stability, reduced arrests, and enhanced quality of life (7). Residential treatment programs apply techniques to allow individuals in recovery to recognize their behaviors and to learn how to avoid relapse (6). Talk therapy sessions directed by a therapist or counselor includes individual, group, or family in outpatient, residential, or inpatient settings (7, 11). Medication-assisted treatment (MAT) seeks to control cravings and other symptoms of withdrawal by blocking the reward pathways or induce negative feelings when the addicting drug is used (7).

Once involved in a treatment program, the individual may enter withdrawal therapy (detoxification) performed on an inpatient or outpatient basis in a supportive environment with the goal of drug cessation in a rapid and safe manner (6, 11). Depending on the drug, various approaches can be utilized such as titrating down the dose of the drug, substitution with a prescribed drug such as methadone, or combining treatment medications with behavioral therapy (11, 12). Recovery as defined by SAMHSA is "a process of change through which individuals improve their health and wellness, live self-directed lives, and strive to reach their full potential (10)." Hope is the foundation of recovery, and recovery is built upon the individual's talents, strengths, coping abilities, resources, and inherent values. During recovery, one may experience many setbacks, but the focus rests on improvements to health and wellness (10). Overall,

it is important for the individual to maintain their abstinence and to cope with life challenges without relapse (2).

Relapse after detoxification is extremely high if relapse prevention counseling is not initiated. A number of techniques to prevent relapse include seeking help immediately upon drug use, avoiding high-risk environments, maintaining one's treatment plan such as meeting with counselor, and going to support sessions (11). If relapse occurs, a new treatment program may need to be developed or the prior treatment program may need to be reinstated or adjusted (12).

Generally, individuals in recovery will transition from acute withdrawal to post-acute withdrawal. During post-acute withdrawal, recovered individuals will experience a variety of emotional and psychological symptoms (i.e., post-acute withdrawal syndrome: PAWS) including, but are not limited to, anxiety, sleep difficulties (e.g., sleep latency and duration), depression, and stress with symptoms tending to be episodic and lasting for up to two years. To alleviate PAWS, individuals are usually prescribed pharmacotherapies and/or instructed to effectively use coping techniques of practicing self-care, relaxation, and cognitive therapies (13).

Because of the symptomatology of post-acute withdrawal, melatonin therapy may be beneficial. Melatonin is widely used in a non-regulated manner to alleviate insomnia and evidence shows that melatonin contributes to the sleep/wake cycle by initiating and maintaining sleep, decreasing sleep latency, improving sleep quality, next day alertness, and quality of life (14). In addition to insomnia, evidence reported in the literature suggests that melatonin may also decrease anxiety and depressive symptoms as well as in individuals with co-morbid insomnia (15-19). Melatonin (0.75 mg, nightly, 10 days)

improved the emotional state in anxious young individuals (15). An intermediate-release formulation of melatonin, melaxen (1.5 mg, nightly, 2 weeks), decreased levels of depression and anxiety in a group of healthy volunteers (16). In alcohol dependent patients who were not consuming alcohol for at least 14 days prior, melaxen (3 to 6 mg, nightly, 3 weeks) decreased anxiety (17). Melatonin administration may be beneficial for improving sleep in individuals with co-morbid depression and sleep disturbances. Individuals with delayed sleep phase syndrome with depressive symptoms given melatonin (5 mg, 4 weeks) reported decreases in depression scores (18). In an open pilot study, patients with depression and sleep disturbances given melatonin (3 mg orally, nightly, 21 days) exhibited improved sleep quality and reduced awakening within 2 to 3 days (19)

Alcohol dependent individuals experience difficulties with sleep latency and maintenance possibly due to alterations in nocturnal melatonin levels. In a study of alcoholic individuals, circulating nocturnal melatonin levels were found to be lower during the early part of the night and had a delay in the nocturnal rise (20). In a trial of alcohol dependent patients who were not consuming alcohol for at least 14 days prior, melaxen (3 to 6 mg, nightly, 3 weeks) improved quality, latency, duration of sleep, breathing during sleep, as well as decreased daily sleepiness (17).

Research regarding nightly, orally administered melatonin has demonstrated the following: is well tolerated, has no abuse potential, does not induce rebound insomnia or withdrawal symptoms, does not affect endogenous melatonin production, does not impact psychomotor, performance, mood, or cognitive functions, does not negatively affect

hepatic and renal function (14, 21-24). These findings are important considering that it will be administered to individuals in recovery.

There is a **paucity** of literature demonstrating the effects of melatonin in individuals experiencing PAWS. As aforementioned, previous studies have shown that melatonin therapy is beneficial in alleviating anxiety, depressive symptoms, and insomnia. However, no randomized, double-blind, placebo-controlled trials have been conducted in males who are experiencing PAWS. The goal of this study is to test a therapeutic approach that incorporates the addition of melatonin to the current treatment program of males who are in a residential treatment program for chemical dependency. The purpose of the study was to assess the effect of 5 mg melatonin compared to placebo as an adjuvant treatment along with their current pharmaco- and behavioral therapy for 28 days on weekly self-reported severity of anxiety, depression, stress, and sleep complaints as well as how sleep is affecting daily life in a sample of males in recovery from chemical dependency at a single, residential treatment site.

PURPOSE, HYPOTHESIS, and OUTCOME MEASURES

Purpose

The purpose of the study was to assess the effect of 5 mg melatonin compared to placebo as an adjuvant treatment along with their current pharmaco- and behavioral therapies for 28 days on weekly self-reported severity of anxiety, depression, stress, and sleep complaints as well as how sleep is affecting daily life in a sample of males in recovery from chemical dependency at a single, residential treatment site.

Hypotheses

1) Melatonin along with current pharmacotherapy and behavioral treatment regimen will affect sleep complaints and how sleep is affecting daily life compared to placebo along with current pharmacotherapy and behavioral treatment regimen treatment regimen in males participating in a residential treatment program.

2**)** Melatonin along with current pharmacotherapy and behavioral treatment regimen will affect severity of anxiety, depression, and stress compared to placebo along with current pharmacotherapy and behavioral treatment regimen in males participating in a residential treatment program.

Primary outcome measures

1.) To determine the effect of melatonin or placebo along with current treatment regimen on the change in severity of anxiety as measured by the Generalized Anxiety Disorder Scale (GAD-7).

2.) To determine the effect of melatonin or placebo along with current treatment regimen on the change in depressive symptoms as measured by the Personal Health Questionnaire Depression Scale (PHQ-8).

3.) To determine the effect of melatonin or placebo along with current treatment regimen on the change in stress as measured by the Perceived Stress Scale (PSS-14).

4.) To determine the effect of melatonin or placebo along with current treatment regimen on the change in sleep complaints and how sleep is affecting daily life as measured by the Pittsburgh Sleep Symptom Questionnaire – Insomnia (PSSQ-1).

5.) To determine response of each individual as an improvement of 50% or higher in score for each scale (i.e., change from Baseline to Day 28).

Secondary outcome measures

1.) To acquire participant histories (social, medical, medication, preventive, mental health, chemical dependency, and melatonin use, if any)

2.) To determine adverse events experienced while taking the intervention.

3.) To determine adherence to study interventions of melatonin or placebo

INTRODUCTION

Substance use disorder (SUD)

Substance use disorder is defined as "a dependence on legal or illegal drugs or medication" and is diagnosed by a licensed mental health professional using criteria in the Statistical Manual of Mental Disorders (DSM-V), published by the American Psychiatric Association (11). According to Koob and Simon (2009), "drug addiction is a chronically relapsing disorder characterized by: (a) compulsion to seek and take the drug, (b) loss of control in limiting intake, and (c) emergence of a negative emotional state (e.g., dysphoria, anxiety, and irritability) when access to drug is prevented (4)." The following is a selected list of addiction symptoms and behaviors provided by the Mayo Clinic (for a more complete list consult reference): "feeling that you have to use the drug regularly," "having intense urges for the drug," "needing more of the drug to get same effect," "maintain a supply of the drug," "spending more money on the drug," and "focusing more and more time and energy on getting and using the drug (11)."

Exposure to risk factors greatly increases the probability of becoming dependent on drugs and these risk factors vary with the type of drug, environment, genetics, and development (11, 12). Specific factors include family history of addiction, lack of family involvement, male gender, initiating drug use at an early age, mental illness, peer pressure, and as a coping mechanism to deal with mental health issues including, but not limited to, depression and anxiety (11, 12). At least half of those who become dependent struggle with mental health issues (6). Moreover, the 2013 NSDUH estimated that 7.7 million adults (3.2% of adults) had a substance use disorder along with a mental health issue as defined by DSM-IV criteria (5).

Before becoming dependent, an individual progresses through several stages of drug use: experimental use, regular use, problem/risky use, and then addiction/dependence (6). While chemically dependent, the individual physically needs the drug and larger doses of the drug in order to function normally in their daily life and continues to use despite the deleterious effects to their physical and mental health as well as to a host of societal problems including work, family, financial, and legal. The individual also finds it difficult to cease drug use because discontinuance leads to cravings (psychological dependence) and abrupt stoppage leads to withdrawal symptoms (11).

Neurobiology

Addiction

To date, the reinforcing effects of addicting drugs have been linked to site of action - receptors and transporters, and the neurocircuitry involved - dopamine and opioid systems (4). In general, the brain is a collection of neurons that communicate through the use of chemicals called neurotransmitters (e.g., serotonin, gamma-aminobutyric acid (GABA), and dopamine). The process of addiction results in modifications in the brain thus resulting in changes in the behavior of the individual. Withdrawal from the addicting drug produces dysphoria suggesting alterations of the same neural systems involved with its reinforcing effects (4). Addicting drugs act via mimicking endogenous neurochemicals, or by overstimulating the reward system of the brain. For example, heroin and marijuana mimic endogenous neurochemicals to exert actions through receptors. Dopaminergic reward systems (movement control, emotion, motivation, and pleasure) of the brain are modified during development of addiction and stimulants, such

as amphetamine and cocaine, produce effects via increasing release of dopamine or preventing its reuptake by the neurons from the synapse resulting in an overstimulation and the subsequent euphoric effects. Over time, the neuroadaptation is to produce less dopamine or reduce the number of dopamine receptors thereby attenuating the effect of dopamine on the reward system leading to the use of more drug to prevent dysphoria – drug tolerance (12). Human imaging studies revealed decreases in dopaminergic function (25) and the mesocorticolimbic dopamine system has been an area of focus of the positive reinforcing effects of addicting drugs (26). Evidence suggests that dopamine-independent neurocircuitry located in the nucleus accumbens and amygdala have a role in reward. Research is demonstrating a role of nondopaminergic systems being involved with drug dependence. Other neurochemicals are affected during addiction; for example, changes in glutamate levels may have a role in affecting cognition. In summary, long-term use of drugs leads to neuroadaptations in neurochemical levels and brain regions controlling a multitude of functions such as executive, cognition, and behavior (12). Modifications of the reward system leads to increased intake of addicting drugs, and it is these adaptive changes that lead to addiction. Dopamine-dependent and -independent actions by dopamine, opioid peptides, serotonin, and GABA are involved with positive reinforcement while modifications in the reward system during dependence include the decreases in the aforementioned as well as recruitment of stress systems that contribute the negative motivational state during cessation (27).

Cessation

Because of the neuroadaptations that occurred in the brain during addiction, cessation of drug use is difficult (12). As drug use progresses, tolerance is achieved

where the amount of drug needed to produce the same euphoric effect increases due to changes in neurocircuitry and molecular targets (e.g., receptors). To avoid the negative effects of drug cessation, the individual needs the drug to prevent the physical illness, cravings, and dysphoria (11). Addicting drugs produce dysphoria upon cessation suggesting that the neuroadaptations that occurred during addiction may involve the same systems involved with the positive reinforcing effects. As such, the dysphoria and anxiety associated with cessation probably involve decreases in the reward system and recruitment of stress neurocircuitry (4). Collectively, during drug cessation, decreases in levels of neurochemicals and resulting transmission occur with dopamine, serotonin, GABA, and dynorphin but increase levels and transmission of glutamate and norepinephrine suggesting that neuroadaptations occur in systems that are involved with positive reinforcing effects during dependence as well as systems involved with stress and arousal (4, 27).

After drug detoxification, the individual will experience less physical symptoms but more psychological and emotional symptoms due to the remodification of the neuroadaptations where the brain chemistry is attempting to return to pre-drug state. This second stage of withdrawal is commonly known as PAWS. The symptoms include, but are not limited to, anxiety, sleep disturbances (e.g., sleep latency and duration), and depression; these symptoms may occur for up to two years post drug cessation. The symptoms of PAWS tend to be episodic; for example, symptoms may last for days and then disappear before re-expressing at a later date. Treatment modalities usually involve instruction on the use coping techniques such as practicing self-care, relaxation, and patience (13).

Melatonin (N-acetyl-5-methoxytryptamine)

Melatonin therapy may be beneficial to those experiencing PAWS. In the U.S., melatonin is readily available as a non-regulated, nutraceutical and used for treating sleep disorders. It is commonly found in doses of 0.5 mg, 1 mg, 3 mg, and 5 mg. Melatonin contributes to regulating the sleep/wake cycle, and its synthesis and release follows a diurnal rhythm. In response to darkness, melatonin is synthesized in and then secreted from the pineal gland with peak plasma levels occurring at 02:00 (2 am) (28, 29); however, exposure to light attenuates norepinephrine release from sympathetic nerve terminals resulting in reduced synthesis of melatonin (30, 31). Because of variation in daily light exposure, seasonal fluctuations exist; for example, during the summer months when the day is longer, the duration of action of melatonin will be shorter compared to winter months (32).

The actions of melatonin are through receptor independent (e.g., free radical scavenging) and dependent mechanisms (e.g., MT_1 or MT_2 melatonin receptors). Upon receptor binding, the melatonin/melatonin receptor complex elicits effects on intracellular proteins that may ultimately impact gene transcription (32-38). Melatonin receptors are ubiquitously expressed throughout the body including expression in the brain (39, 40).

Melatonin has very low toxicity with a high margin of safety. However, adverse events have been reported; for example, orally administered melatonin may cause vivid dreaming or daytime grogginess, but these events usually dissipate with continued use. Other adverse events an individual may experience include drowsiness, headache, dizziness, small changes in blood pressure, or nausea. Research regarding nightly, orally administered melatonin has demonstrated the following: is well tolerated, has no abuse

potential, does not induce rebound insomnia or withdrawal symptoms, does not affect endogenous melatonin production, does not impact psychomotor, performance, mood, or cognitive functions, does not negatively affect hepatic and renal function (14, 21-24).

Due to its lipophilicity, melatonin readily distributes to most tissues and crosses the blood-brain barrier. In humans, oral bioavailability is about 33% with serum levels peaking within one hour. Melatonin undergoes phase I metabolism in the liver by the cytochrome P450 enzyme, CYP1A2, to 6-hydroxymelatonin before phase II metabolism by sulfotransferases to 6-sulfatoxymelatonin (αMT6). Up to 85% of a given dose is excreted in the urine (41). Importantly, melatonin levels are affected by various classes of drugs (e.g., psychotropic medications) that induce, inhibit, or act as a substrate of CYP1A2 (42).

Melatonin and mood disorders

Levels of melatonin and/or αMT6

The noradrenergic system is involved in the production of melatonin as well as in the pathophysiology of depression. Individuals with depression have disruption of the nightly rhythm of melatonin whereby the onset of melatonin secretion occurs later in the night. Results of a study of 14 inpatients (7 males and 7 females) with major depression at the end of a psychotropic medication-free period (14 matched controls for age, gender, season, and hormonal treatment), published by scientists at the Universite de Liege in Belgium, revealed a significant delay in the nightly melatonin peak in depressive patients suggesting a phase-shifting of melatonin production; however, there were no differences in mean level or peak of melatonin. In the depressive group, urinary levels of αMT6 were higher in the morning compared to night time levels while the urinary levels of the

control group displayed the characteristic lowering from night to morning (43). The degree of depression as well as living in a different hemisphere may impact the secretion of melatonin. In a study of 32 psychotropic medication-free patients (9 males and 23 females) with major depression (32 matched controls for age and gender) and another 15 drug-free outpatients (5 males and 10 females) with major depression (matched controls for age, gender, body mass index, and season) from São Paulo, Brazil, the following was documented, urinary levels of αMT6 during the 24 hour and 6 hour periods were similar between depressed and control groups suggesting that alterations in nightly melatonin production may occur only in more severe depression and in the northern hemisphere (44).

Melatonin as a treatment

In anxious young individuals, melatonin (0.75 mg, nightly, 10 days) improved their emotional state (15). Studies investigating the effects of melatonin using an intermediate-release dosage form on anxiety and depression demonstrated an improvement in levels of depression and anxiety. For example, in a group of healthy volunteers, melaxen (melatonin IR, 1.5 mg, 2 weeks) decreased levels of depression and anxiety (16).

Melatonin and sleep disorders – insomnia

Levels of melatonin and/or αMT6

Individuals suffering from insomnia experience late onset of sleep or early morning awakening which may be a result of a shift in the circadian rhythm by either delaying or advancing it, respectively. Individuals with insomnia have alterations in the nightly secretion pattern of melatonin. One of the first studies to investigate the possible

alteration in secretion pattern in individuals experiencing difficulties maintaining sleep (i.e., experiencing frequent awakenings) was performed by a group at University of Gottingen in Germany. Results of the study revealed that plasma levels of melatonin increased earlier in the evening and were lower in the middle of the night compared to controls (peak value of 82.5 ± 26.5 pg/ml versus 116.8 ± 13.5 pg/ml, respectively). Moreover, the levels of melatonin were the most severely reduced in individuals who experienced difficulties for more than five years (peak value 72.1 ± 25.0 pg/ml) (45).

Melatonin as a treatment

Because of its role in circadian systems, melatonin supplementation has been shown to be beneficial for the treatment of sleep dysfunctions. A meta-analysis investigating the effects of exogenously administered melatonin on sleep conducted by Brzezinski et al. (2005) determined, that even though the pooled data were heterogeneous, melatonin reduced sleep onset latency by 4 to 7.5 minutes, increased sleep efficiency (the ratio of total sleep time to time in bed) by 2.2%, and increased total sleep duration by 12.8 minutes (46). Results from a prospective 6 to 12 month open-label study of 244 community dwelling adults (aged 20 to 80 years) with insomnia (112 completed study 6 months and the other 96 completed 12 months) demonstrated that prolonged-release melatonin (2 mg, nightly, 6 to 12 month followed by a 2 week withdrawal) significantly increased the number of nights with sleep quality reported as "good" or "very good" compared to before treatment (22). In the European Union (E.U.), Circadin (prolonged-release 2 mg melatonin) was approved in 2007 for short-term treatment of primary insomnia in individuals aged 55 and older. Clinical trials have

demonstrated improvements in onset of sleep latency, sleep quality, next day alertness, and quality of life (21).

However, melatonin has also been shown to improve sleep latency in individuals without sleep dysfunctions. In a group of young healthy volunteers without sleep disorders, melatonin (0.3 or 1 mg, nightly) reduced onset of sleep latency as well as latency to stage 2 sleep. Additionally, neither dose altered sleep architecture, the pattern of sleep as it changes between sleep stages (14).

Melatonin and comorbidities

Melatonin as a treatment

Individuals experiencing major depressive disorder frequently experience sleep difficulties. Antidepressant medications are generally ineffective in combating sleep issues, and the addition of benzodiazepines to the medication regimen is not without concerns. Because of its role in circadian systems and its low abuse potential, melatonin supplementation may be beneficial for improving sleep in individuals with comorbidities such as depression. Results of those completing a double-blind, placebo-controlled trial beginning with 24 outpatients 22 - 65 years of age (19 completed study) demonstrated that the 10 individuals with major depressive disorder given slow release melatonin (5 mg up to 10 mg) along with fluoxetine for four weeks reported significant improvement of scores on the Pittsburgh Sleep Quality Index (PSQI) compared to the nine taking placebo and fluoxetine. However, no differences were detected in the rate of improvement in depressive symptoms compared to those given fluoxetine plus placebo. Of importance, the slow-release melatonin did not increase the onset of fluoxetine (47). In an open pilot study, elderly individuals with signs of depression and sleep disturbances

as well as those only experiencing sleep disturbances were given melatonin (3 mg, nightly, 21 days) and within 2 to 3 days improvements in sleep quality and reduced awakenings were demonstrated in those with or without depression (19). The largest RCT at time of publication involved 33 individuals with major depressive disorder where 15 were given slow release melatonin (6 mg, nightly, 4 weeks). Findings demonstrated significant improvement of subjective but not objective measures of sleep as well as for mood; however, results were not specific to melatonin (i.e., placebo effect) (24). In a randomized, double-blind, crossover, placebo-controlled study testing if exogenous melatonin can reduce depressive symptoms in individuals with delayed sleep phase syndrome (DSPS), individuals with DSPS and depressive symptoms (n = 8) and DSPS without depressive symptoms (n = 12) were enrolled. Interventions were given for four weeks with one week washout in between. Melatonin decreased depression scores as assessed by the Epidemiologic Studies Depression Scale (CES-D) and Hamilton Depression Scale-17 in both study groups compared to placebo. Assessment of αMT6 revealed those with DSPS and depressive symptoms had alterations in melatonin rhythms compared to those without depressive symptoms (18).

SYSTEMATIC LITERATURE REVIEW

Even though melatonin and its analogues have been used in studies of anxiety, depression, and insomnia, the purpose of this study was to investigate the effect of melatonin upon PAWS among individuals in recovery from chemical dependency. Therefore, the specific objectives of this systematic review were: 1.) to identify studies that assessed melatonin levels in the blood and/or αMT6 levels in the urine of chemically dependent individuals or those who have undergone withdrawal, 2.) to identify studies that used melatonin or its analogs as a treatment in chemically dependent individuals or those who have undergone withdrawal, and 3.) the use of melatonin to facilitate the withdrawal of benzodiazepine administration and/or its effect on health conditions during benzodiazepine withdrawal.

The literature search was conducted in Pubmed on June 7, 2016 with no time restriction (Figure 1). The search was set to return only studies conducted in humans. After the search was performed, the results were further filtered manually by the author to exclude meta-analyses, reviews, systematic reviews, studies without an abstract available, and published study protocols. Abstracts written in English language were included regardless of original language of respective article. Articles were included if they met criteria as stated in the aforementioned objectives. The search strategy included the following terms: ("Melatonin"[tiab] OR "Melatonin"[OT] OR "Melatonin"[mesh] OR "Ramelteon"[tiab] OR "Ramelteon"[OT] OR "6-sulfatoxymelatonin"[tiab] OR "6-sulfatoxymelatonin"[OT]) AND ("Chemical Dependency"[tiab] OR "Substance abuse"[tiab] OR "Substance use disorder"[tiab] OR "Addiction"[tiab] OR "Alcoholism"[tiab] OR "Illicit drug use"[tiab] OR "Drug dependence"[tiab] OR

“Substance dependence”[tiab] OR “Alcohol dependent”[tiab] OR “substance withdrawal”[tiab] OR “post acute withdrawal”[tiab] OR “Chemical Dependency”[OT] OR “Substance abuse”[OT] OR “Substance use disorder”[OT] OR “Addiction”[OT] OR “Alcoholism”[OT] OR “Illicit drug use”[OT] OR “Drug dependence”[OT] OR “Substance dependence”[OT] OR “Alcohol dependent”[OT] OR “substance withdrawal”[OT] OR “post acute withdrawal”[OT] OR “Substance-Related Disorders”[Mesh] OR “Behavior, Addictive”[Mesh] OR “Street Drugs”[Mesh]).

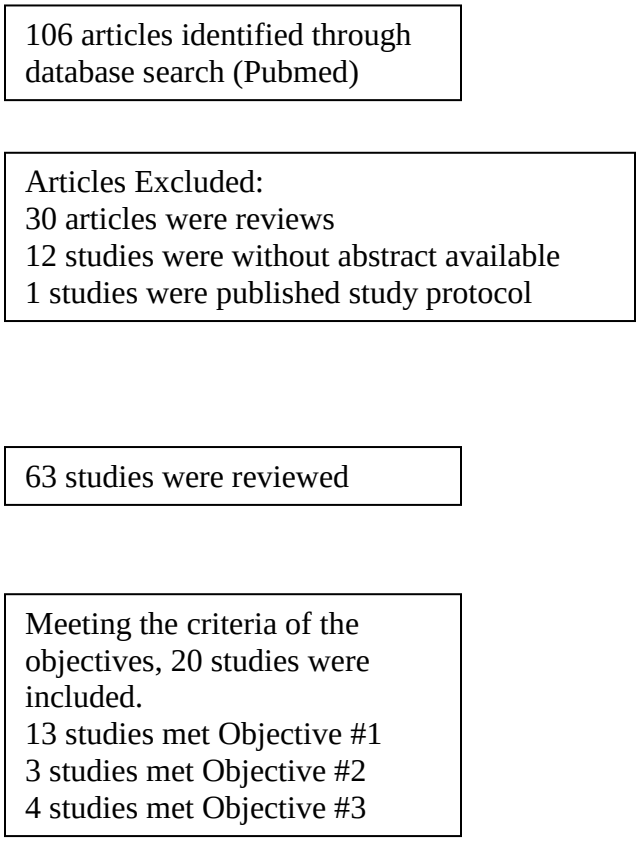

Figure 1. Schematic representation of methodology used and selection criteria.

Melatonin and chemical dependency

Objective #1: Levels of melatonin and/or αMT6

Substance use disorder

Compared to alcohol use disorder (9 articles), the literature review revealed fewer articles (4 articles) investigating plasma levels of melatonin and/or its metabolite in individuals with substance use disorder demonstrating a necessity for more research. Veit and colleagues investigated circadian hormone profiles in 13 cases of politoxicomania compared to 10 persons in a good state of health. Cases were divided up into three groups: Group 1: complete abstinence, Group 2: no hard drug intake, and Group 3 acute relapse after a prolonged period of abstinence. Melatonin levels were higher in the group of "abstinents" compared to acute relapsive cases (48).

As part of a larger study, an exploratory study of 21 adolescents, 14 males and seven females, (mean age of 16.3 ± 1.35 yr, range 14 – 19 yr) who experience sleep complaints or daytime sleepiness and have completed substance abuse treatment eight weeks prior were enrolled. Participants kept sleep diaries, wore an actiwatch for seven days, completed behavioral and psychological measures, and spent one night for dim light melatonin onset assessment where salivary samples were collected every 30 minutes beginning at 19:30 to 03:30. Participants demonstrated disordered sleep and older adolescents showed later dim light onset. Considering that adolescents commonly have a delayed phase, a substantial number of participants had early dim light onsets. Overall, the authors highlight the following: the participants have a wide range of dim light onset with delays associated with the older adolescents, the onsets were associated with longer sleep onset latency, and finally, onsets and shorter phase angles between sleep offset and

dim light onset were significantly related to higher severity of substance abuse issues (49).

A study sought to investigate 24 hour levels of urine αMT6 in 11 opiate-dependent individuals during opiate withdrawal while undergoing in-patient methadone detoxification. Levels were assessed during methadone stabilization and on Days 6 and 12 of withdrawal treatment. Compared to stabilization (i.e., baseline), urine levels were significantly higher on Day 6 but not on Day 12. There existed a correlation between withdrawal symptom score severity and urine levels during stabilization and Day 6 (50).

In 13 females with heroin addiction (mean age of 31.7 ± 2.4 yr) compared to 17 healthy females (mean age of 30.0 ± 1.7 yr), levels of plasma melatonin were significantly lower at baseline and six months after heroin withdrawal suggesting melatonin levels did not fully recover. In those with heroin addiction, three and six months after drug withdrawal, melatonin levels significantly increased compared to baseline, but there existed no significant difference between three and six months (51).

Alcohol use disorder

In healthy men, acute alcohol exposure may not influence nocturnal melatonin secretion in contrast to chronic exposure in those with dependency (52). Alcohol dependent individuals experience difficulties with latency and maintenance of sleep that may be attributed to phase-shifting and decreased levels of nocturnal melatonin secretion.

In a study of 10 male chronic alcoholic individuals before and after two weeks of abstinence, urine melatonin levels as collected in two fractions (08:00 – 20:00) and (20:00 – 08:00) revealed that 24 hour levels were higher in individuals during alcohol intake compared to sex and age-matched controls. Moreover, higher day fraction levels

were detected in these individuals as well as after alcohol withdrawal. Overall, the ratio of night fraction over day fraction approximated "1" during intake and became greater than "1" after withdrawal like the controls (53).

As an addition to a larger, worldwide, multinational control study sampling depressed and abstinent alcohol dependent men and women from Sweden and California, urinary melatonin levels were found to be similar between the two control groups as well as between the depressed and alcoholic groups. However, the levels of the depressed and alcoholic groups were significantly lower than the control groups (54).

The 24 hour, day- and nighttime melatonin levels were assessed in 10 alcohol dependent men during active drinking and two weeks after withdrawal. Results revealed increased daytime levels of urine melatonin and the inversion of the ratio between night- and daytime levels during active drinking that normalized upon withdrawal (55).

A study of 24 hour plasma levels of melatonin in eight chronic alcohol dependent males in a detoxification program and eight healthy controls, levels were assessed on the first day of alcohol withdrawal and after 14 days. The mean 24 hour levels were higher during acute withdrawal compared to after 14 days of abstinence and those of the healthy controls. Significance could not be determined due to larger inter-individual differences. Based on cosinor analysis, there existed a loss of circadian periodicity in the acute phase but periodicity was significantly restored after 14 days (56).

Research regarding ten chronically alcohol dependent individuals (mean 11.7 year alcohol use with range 2 to 30 year, mean 219 g alcohol daily with range from 60 g to 360 g, and mean age 47.3 years with range 33 to 64 years), nighttime levels of blood

melatonin were disrupted where more than 50% had low secretion (<30 pg/mL) during the four days of alcohol withdrawal (57).

Another study investigated melatonin levels in alcohol dependent individuals with or without delirium tremens during and 1 month after withdrawal. Individuals with delirium tremens had disrupted serum levels during withdrawal that normalized after withdrawal; however, those without delirium tremens had normal rhythm during and after withdrawal (58).

In the absence of major pre-existing or concomitant psychiatric disorders, 11 alcohol dependent individuals, who underwent withdrawal 14 days prior, had sampling of blood melatonin performed every 30 minutes beginning at 22:00 to 06:30. Results revealed that levels were lower during the early part of the night as well as a delay in the onset of the plateau or peak value compared to controls with the delay correlating with the prolonged sleep latency as determined by polysomnography (20).

A study investigated the inversion of melatonin circadian rhythm in seven alcohol dependent individuals during acute withdrawal (along with benzodiazepines) and 15 days later (without psychotropic medications). Results demonstrated that in over half the individuals the levels of urine αMT6 showed that the inversion of melatonin rhythm persisted during acute withdrawal and continued to persist at 15 days in three individuals (59).

Research investigating dim light melatonin onset in 52 abstinent alcohol dependent individuals compared to 19 age- and sex matched healthy controls showed a slower rate of rise of levels of salivary melatonin and decreased maximal peak in

abstinent individuals. Specifically focusing on when melatonin levels were increasing, there existed a significant delay of 18 minutes in abstinent individuals (60).

Objective #2: Melatonin as a treatment

In the U.S., melatonin is readily available as a non-regulated, nutraceutical commonly used by individuals experiencing sleep disorders such as insomnia. Because of the high abuse potential of hypnotic medications (e.g., benzodiazepines) or issues with tolerability and daytime sedation associated with use of sedating psychotropic medications, melatonin may be a safer and more appealing treatment option. Moreover, literature shows melatonin may have anxiolytic and antidepressive actions (15, 16, 18, 24). However, research is limited as to the effects of melatonin on anxiety, depression, and insomnia in individuals in recovery from chemical dependency.

Eryshev and collaborators investigated the effects of melaxen in 45 alcohol dependent individuals with sleep and mild mood disorders. Initiated 14 days after alcohol withdrawal, melaxen (melatonin IR, 3 to 6 mg, nightly, 3 weeks) decreased the mood disorders especially anxiety as assessed with the Hamilton Anxiety Rating Scale (HAM-A). In addition to studying the effects of melaxen on anxiety and depression, self-reported sleep quality and sleepiness were also assessed with a sleep quality questionnaire and the Epworth sleepiness scale, respectively. Melaxen improved sleep onset and duration and quality of sleep as well as decreased daily sleepiness (17).

Case series studies have been performed to assess the effects of ramelteon or agomelatine, structural analogs of melatonin that act as MT_1 and MT_2 melatonin receptor agonists with agomelatine also acting as a 5-hydroxytryptamine$_{2c}$ antagonist, in alcohol dependent individuals with insomnia in the absence of other psychiatric disorders except

nicotine dependence. Both studies were limited by a small sample size and an absence of a placebo group. Ramelteon (8 mg, nightly, 4 weeks) was taken by four females and one male, ages 32 - 53 years with alcohol dependency. Abstinence was initiated in the past 2 - 13 weeks. Remelteon improved insomnia scores on the Insomnia Severity Index (ISI), reduced onset of sleep latency by about 0.5 hour, and increased total sleep time by more than 1 hour (61).

Agomelatine (25 mg to 50 mg, nightly, 6 weeks) was investigated in abstinent eight males and one female, mean age of 47.2 ± 11.2 years, with mean alcohol dependency of 20 ± 8.3 years. Several individuals were weaned off their sleep-promoting substances; however disulfram treatment was maintained. After six weeks of treatment, global sleep quality scores obtained from the PSQI significantly decreased (62).

Objective #3: Melatonin use in benzodiazepine withdrawal

Following an extensive literature review, four articles revealed mixed results for use of melatonin to facilitate benzodiazepine withdrawal. In an RCT of 34 older individuals (9 men, 25 women, mean 68 ± 13 yr) who were undergoing benzodiazepine treatment for six months, melatonin (2 mg controlled release: Circadin) or placebo was administered nightly for six weeks. Individuals were directed to reduce their dosage of medications by 50% during week 2, 75% during weeks 3 and 4, and then to discontinue completely. By the end of the study, 14 of 18 (77%) individuals in the melatonin group discontinued medications compared to 4 of 16 (25%) in the placebo group. Sleep quality scores were significantly increased in the melatonin group compared to placebo group indicating an improvement in sleep quality. When melatonin was administered to

individuals in the placebo group, six more discontinued their benzodiazepine usage. At 6 month follow-up, 19 (79%) of those who discontinued usage of benzodiazepines and who continued using melatonin remained abstinent and had a significantly improved sleep quality compared to baseline scores (63).

A randomized, double-blind, placebo-controlled, cross-over control study of 80 individuals enrolled at a community methadone maintenance clinic was performed to investigate the effect of melatonin in reducing sleep difficulties during benzodiazepine withdrawal. Melatonin (5 mg/day) or placebo was administered for 6 weeks with a 1 week washout before cross-over for another 6 weeks. Results revealed that the discontinuation rate of those 61 individuals who completed the six weeks of treatment was similar. In those that continued using benzodiazepines, sleep quality as measured by the PSQI improved in those who took melatonin first compared to those who took placebo first; however, no difference was found between groups in those who ceased usage (64).

A randomized, double-blind, placebo-controlled, cross-over study was conducted investigating the effect of melatonin on behavioral disorders and sleep in 22 individuals (7 men, 15 women over 65 years of age) and the facilitation of hypnotic medication cessation in 14 of those individuals with melatonin (5 mg/day) or placebo being administered for two months. Sleep disorders were measured with the Northside Hospital Sleep Medicine Institute (NHSMI) and behavioral disorders with Yesavage Geriatric Depression Scale (GDS) and Goldberg Anxiety Scale (GAS). Melatonin improved sleep quality scores compared to baseline and placebo and improved the scores

of behavioral disorders assessed. During melatonin treatment, hypnotic medication cessation occurred in nine out of 14 individuals (65).

An RCT investigated the efficacy of melatonin as an adjuvant in sedative withdrawal in 92 men and women, over 55 years of age presenting with primary insomnia and long-term sedative usage. Melatonin (controlled release, 2 mg) or placebo were administered during the 1 month withdrawal along with psychosocial support. After 1 month, the reduction of sedative use was similar between groups. At the 6 month follow-up, similar numbers of individuals remained abstinent (n = 14 melatonin and n = 20 placebo), but the doses of those still using were significantly higher in the melatonin group compared to placebo. These findings suggest that melatonin provided no benefit compared to placebo when attempting to withdraw from sedative use (66).

Summary of literature review

As stated previously, melatonin levels begin to rise during the hours of darkness. In individual with depression, the onset of nightly melatonin secretion occurs later which may be related to severity of depression. The evidence suggests, however limited, that nightly administration of melatonin provides a benefit in individuals experiencing mood disorders such as depression and anxiety. Investigations into melatonin secretion in those suffering from insomnia reveal alterations in the nightly secretion of melatonin. Of interest, one of the first studies to investigate the possible alteration in secretion pattern in individuals experiencing difficulties maintaining sleep (i.e., experiencing frequent awakenings) found plasma levels of melatonin increased earlier in the evening and were lower in the middle of the night compared to controls. Moreover, levels of melatonin were the most severely reduced in individuals who experienced sleep difficulties for more

than five years. A meta-analysis investigating the effects of exogenously administered melatonin on sleep determined that melatonin reduced sleep onset latency by 4 to 7.5 minutes, increased sleep efficiency (the ratio of total sleep time to time in bed) by 2.2%, and increased total sleep duration by 12.8 minutes. When focusing on those individuals experiencing major depressive disorder with sleep difficulties, research also suggests that melatonin may be beneficial for improving sleep in individuals with comorbidities such as depression. Collectively, these findings provide evidence of the effectiveness of melatonin administration in those dealing with depression, anxiety, insomnia, or with comorbidities of insomnia and depression. Even though the research was conducted in those individuals not in recovery from illicit or non-illicit drug use, these findings provide a rationale for investigating its effects in individuals in recovery from illicit drug and alcohol use.

Individuals in recovery have a high prevalence of mental health symptoms along with substance abuse history. Also, sleep complaints and how their difficulties with sleep impact their daily life are common and a cause of concern. After drug detoxification, the individual will experience psychological and emotional symptoms (i.e., PAWS). The symptoms include, but are not limited to, anxiety, sleep disturbances, and depression; these symptoms may occur for up to two years post drug cessation and tend to be episodic. These individuals also have multiple prescription medication use including psychotropic medications such as antidepressants that are generally ineffective in treating sleep issues. Because of the high abuse potential of hypnotic medications (e.g., benzodiazepines) or issues with tolerability and daytime sedation associated with use of sedating psychotropic medications (e.g., trazadone and quetiapine), melatonin may be a

safer and more appealing treatment option. Thus, melatonin administration may be beneficial to those experiencing PAWS. In the U.S., melatonin is readily available as a non-regulated, nutraceutical and commonly used for treating sleep disorders because melatonin contributes to regulating the sleep/wake cycle. Melatonin has very low toxicity with a high margin of safety and research regarding its use has demonstrated the following: it is well tolerated; has no abuse potential; does not induce rebound insomnia or withdrawal symptoms; does not affect endogenous melatonin production; does not impact psychomotor, performance, mood, or cognitive functions; and does not negatively affect hepatic and renal function. These are important concerns when considering administering melatonin to individuals in recovery.

The purpose of this study was to investigate the effect of melatonin upon post-acute withdrawal among males in recovery from chemical dependency. Therefore, three specific objectives were formulated before conducting the systematic review: 1.) to identify studies that assessed melatonin levels in the blood and/or αMT6 levels in the urine of chemically dependent individuals or those who have undergone withdrawal, 2.) to identify studies that used melatonin or its analogs as a treatment in chemically dependent individuals or those who have undergone withdrawal, and 3.) the use of melatonin to facilitate the withdrawal of benzodiazepine administration and/or its effect on health conditions during benzodiazepine withdrawal. The systematic literature review uncovered 20 articles relating to the three objectives. The majority of articles explored melatonin levels in the blood and/or αMT6 levels in the urine of individuals during or after withdrawal from alcohol (9 articles) or other substance use (4 articles). The consensus of literature provides evidence that in abstinent alcohol dependent individuals,

nightly melatonin secretion tends to be phase-shifted (i.e., delayed) with decreased nocturnal levels suggesting that temporal disruption and magnitude of melatonin secretion may explain why alcohol dependent individuals experience difficulties with latency and maintenance of sleep.

Only three and four articles met the criteria for Objective #2 and #3, respectively. Based on the review, there seems to be a paucity of studies investigating the use of melatonin as a treatment in this population. Even though the case series studies involving melatonin analogues revealed improvements in sleep in abstinent alcohol dependent individuals with insomnia in the absence of other psychiatric disorders, these studies were limited by a small sample size and an absence of a placebo group. Regarding Objective #3, the literature revealed mixed results for use of melatonin to facilitate benzodiazepine withdrawal. Overall, research is limited as to the effects of melatonin on anxiety, depression, and insomnia in individuals in recovery from chemical dependency.

METHODS

Drugs

Melatonin [73-31-4] (#5250)

Trial design

Full board approval was obtained from the Institutional Review Board at Duquesne University and then registered as a clinical trial on Clinicaltrial.gov (Identifier: NCT02431728, Received: April 28, 2015) prior to the study implementation. The trial was a single-center, randomized, double-blind, placebo-controlled, parallel-group trial conducted in males 18 years of age and older who are in a residential treatment program for chemical dependency at the Salvation Army Harbor Light Center (865 West North Avenue, Pittsburgh, Pennsylvania, 15233) in the U.S. Convenience sampling was used to recruit individuals from July 2015 to December 2015. A total sample of 70 participants were enrolled and block randomized with an allocation ratio of 1:1 for the interventions, 5 mg melatonin and placebo. Financial compensation of $5.00 U.S. was initiated at Day 7 and continued at each follow-up (Day 14, Day 21 & Day 28). Intention-to-treat and complete case analyses were performed; however, no interim analysis was performed to assess efficacy. Participants completed study materials in a designated room at the center.

Briefly, this study involved the completion of four validated surveys assessing self-reported severity of anxiety (Generalized Anxiety Disorder Scale; GAD-7), depression (Personal Health Questionnaire Depression Scale; PHQ-8), stress (Perceived Stress Scale; PSS-14), and sleep complaints and how is sleep affecting daily life (Pittsburgh Sleep Symptom Questionnaire – Insomnia; PSSQ-1) at five time points

(Baseline, Day 7, Day 14, Day 21, and Day 28). At enrollment, an individual expressing interest was provided with an informed consent form by the investigator (CDB) and was instructed to read the consent form prior to the investigator (CDB) verbally reviewing the contents of the form. At this time, questions were addressed, if any. It was paramount that informed consent was obtained through the willingness of the individual and not through a perception of coercion. The investigator stressed that participation was voluntary, would not involve any foreseeable financial costs, and lack of participation would not affect their treatment or status at the center. Furthermore, it was stressed that the participant can withdraw at any time; however, the data collected will be used.

Upon providing informed consent, a business-sized card labeled with the study identification number was provided. If needed, this card will be presented at the weekly follow-ups in order to obtain his opaque manilla envelope, labeled with his unique study identification number, containing the four self-report surveys and financial compensation. After informed consent, the participant completed the health history form containing questions addressing social, medical, medication, preventive, mental health, chemical dependency, and melatonin histories. The participant proceeded to complete the four self-report surveys, GAD-7, PHQ-8, PSS-14, and PSSQ-1. If the participant had requested help, the investigator (CDB) read the survey question/s, and then provided an interpretation of what the question/s was/were asking, if needed. Upon completion, all forms were enclosed in the manilla envelope by the participant, and then placed into a secured container by the investigator (CDB). The study intervention (a capsule card containing either 5 mg melatonin and Avicel® or the placebo containing only Avicel®) was provided along with specific instructions to administer one capsule by mouth at

bedtime (center "lights out" at 24:00) for 28 days. If a dose was missed, the capsule was to remain sealed in the card. The investigator (CDB) stressed that it was imperative to maintain adherence to current pharmacotherapies as prescribed. To address any adverse events relating to the interventions, the participant was encouraged to report any adverse events to the investigator (CDB). The participant was also encouraged at the weekly follow-ups to report if any new symptoms were experienced during the past week other than those experienced at time of study entrance. All adverse events were documented. At the conclusion of each weekly follow-up, willingness to remain in the study was verbally assessed. Those participants who completed the 28-day study were prompted to answer the question, "Do you believe you were taking melatonin – Yes or No?" To protect the anonymity and confidentiality of the participants, all paperwork were de-identified but contained the unique identification number. All data containing materials were secured in the office of the investigator (CDB) at Duquesne University. Only the principle investigator (VJG) and the co-investigators (CDB & PWE) had access to the data. Dr. Adam Gordon, the physician on record at the center, was aware of the study.

Participants

Eligible participants were males 18 years of age and older who had a recent history of chemical dependency and also have co-occurring mental health diagnosis, medical conditions, legal system involvement, and/or a history of homelessness. Study inclusion criteria were residence at the Harbor Light Center, willingness to participate in the 28-day study, willingness to provide social, medical, medication, preventive, mental health, chemical dependency, and melatonin histories, willingness to complete self-assessments of severity of anxiety, depression, stress, and sleep complaints and how sleep

is affecting daily life, willingness to administer daily at bedtime the intervention, and the ability to read and speak English. Participants were excluded if currently self-administering melatonin or had an adverse history with melatonin supplementation.

Recruitment

Recruitment was conducted via study flyers displayed at the center, counselors notifying residents, residents notifying residents, investigator (CDB) notifying residents twice a week before one of their therapy sessions, and investigator (CDB) having discussions with interested resident/s.

Settings and locations

The study was conducted at the Salvation Army Harbor Light Center (865 West North Avenue, Pittsburgh, Pennsylvania, 15233). Pittsburgh is a major city located in Allegheny County with a population of 300,000 and 1,230,000, respectively (67). From 2005 to 2010, the metropolitan statistical area (MSA), counties of Allegheny, Armstrong, Beaver, Butler, Fayette, and Washington, consists of 2.1 million persons aged 12 or older with 1.9 million being adults aged 18 or older. According to the NSDUH Report: Substance Use and Mental Disorders in the Pittsburgh MSA, an estimated annual mean of 281,000 persons aged 12 or older (13.4%) used an illicit drug and about 182,000 persons aged 12 or older (8.7%) had a substance use disorder in the past year. Also, an estimated 116,000 adults aged 18 or older (6.1%) experienced a major depressive episode in the past year. These rates were similar to rates of the State and nation (68).

The Harbor Light Center is a Pennsylvania Department of Drug & Alcohol Program (DDAP) licensed, medically-monitored long-term (3-month), and residential substance abuse rehabilitation program for men. It is a 40-bed, residential treatment

program for men 18 years of age and older who have a recent history of chemical dependency along with co-occurring mental health diagnosis, medical conditions, legal system involvement, and/or a history of homelessness. Requirements for program entry are residency in Allegheny County and possessing a valid Pennsylvania state identification. Residents follow a daily schedule that includes therapy sessions and three meals. The program is aimed to individuals with varying degrees of dependency, and the facility is a place where men are cared for physically, mentally, and spiritually in order to allow each man to realize his worth, value, and personhood. Services provided include group therapy that incorporates relapse prevention, gratitude, life skills, psychiatric medication evaluation, and rational emotive and mental health therapies. Additionally, individual therapy, assignment to an Allegheny County D&A case manager to assist with housing and other community-based resources, and meetings with Office of Vocational Rehabilitation counselor to aid with educational resources and work are offered. MAT is not provided.

Interventions

Participants were randomly assigned to receive capsules containing either 5 mg melatonin plus Avicel® filler or the placebo containing only Avicel®. Avicel® is a microcrystalline cellulose powder commonly used by the pharmaceutical industry. All study capsules were compounded by Jeffreys Drug Store (1 North Central Avenue, Ste. #1, Canonsburg, PA, 15317). The capsules were packaged in non-child resistant, foil-backed cards delivering a 30-day supply (Washington Medical Equipment). A number from 1 to 30 appeared next to each foil-backed capsule corresponding to the day in the study. Even though the study was for 28 days, two extra capsules were provided in case

of quality, loss, or sanitary issues were encountered. Capsules were clear, matched for size, and the formulation within appeared as a white, microcrystalline powder. Melatonin and Avicel® are tasteless. Capsule content was independently confirmed by the laboratory of Kevin J. Tidgewell, Ph.D. (Assistant Professor of Medicinal Chemistry, Graduate School of Pharmaceutical Sciences, Duquesne University, Pittsburgh, PA) using high performance liquid chromatography (See Appendix).

Outcomes

The primary outcome measures were to determine the effect of melatonin or placebo along with current treatment regimen on the change in severity of: 1.) anxiety as measured by the GAD-7, 2.) depression as measured by the PHQ-8, 3.) stress as measured by the PSS-14, and 4.) sleep complaints and how sleep is affecting daily life as measured by the PSSQ-1. Surveys were completed at five different time points (Baseline, Day 7, Day 14, Day 21, and Day 28). Also, the change from Baseline and Day 28 was determined by a response of an improvement of 50% or higher in the survey scores for each scale. Secondary outcome measures were to acquire participant histories (social, medical, medication, preventive, mental health, chemical dependency, and melatonin use), determine any adverse events through self-report, and determine adherence to study interventions.

Instruments

Evaluation methods utilized structured, self-reported surveys: GAD-7, PHQ-8, PSS-14, and PSSQ-1. An investigator generated form was used to collect social, medical, medication, preventive, mental health, chemical dependency, and melatonin histories. Importantly, each designated time frame as indicated on the surveys was used

to assess baseline measures; then, each time frame was changed to "over the last seven days" for the other follow-up times. For the PSSQ-1, mean scores of each subscale were determined, and the scale was not used to assign a diagnosis of insomnia disorder.

The GAD-7 measures self-reported severity of anxiety. It is a 7-item scale assessing severity as measured by a symptom checklist over the last two weeks. It employs a 4-point scale with the response options of "Not at all" (0 pts), "Several days" (1 pt), "More than half the days" (2 pts), and "Nearly every day" (3 pts). Severity is based on the sum total where 15 - 21 is considered "severe anxiety (69)."

The PHQ-8 measures self-reported degree of depression. It is an 8-item scale assessing degree as measured by a symptom checklist over the last two weeks. It employs a 4-point scale with the response options of "Not at all" (0 pts), "Several days" (1 pt), "More than half the days" (2 pts), and "Nearly every day" (3 pts). The higher the sum total the greater the degree of depression; for example, a score of 20 or more is considered severe major depression (70).

The PSS-14 measures self-reported degree of stress. It is a 14-item scale assessing degree as measured by a checklist of the individual's thoughts and feelings during the past month. It employs a 5-point scale with the response options of "Never" (0 pts), "Almost Never" (1 pt), "Sometimes" (2 pts), "Fairly Often" (3 pts), and "Very Often" (4 pts). Because some questions are positively stated, scores are obtained by reversing the scoring on items 4, 5, 6, 7, 9, 10, and 13, for example a score of "4" becomes a score of "0". The scores are summed with higher score indicating more perceived stress (71).

The PSSQ-1 measures self-reported severity of sleep complaints and how sleep affects daily life. It is a 13-item scale that assesses severity during the past month with two subscales: Sleep complaints (Questions 1-5) and how sleep is affecting daily life (Questions 6-13). Sleep complaints are assessed by a 6-point scale with the response options of "Never" (0 pts), "Do not know" (1 pt), "Rarely" (2 pts), "Sometimes" (3 pts), "Frequently" (4 pts), and "Always" (5 pts) and asks "How long has the symptom lasted." To assess for how sleep is affecting daily life, it employs a five point scale with the response options "Not at all" (0 pts), "A little bit" (1 pt), "Moderately" (2 pts), "Quite a bit" (3 pts), and "Extremely" (4 pts) (72). Mean scores for each subscale were determined.

Sample size

The study involved repeated measures (Baseline, Day 7, Day 14, Day 21, Day 28), within-between (Intervention by Time) research design. The independent variables were intervention (melatonin and placebo) and time (Baseline, Day 7, Day 14, Day 21, and Day 28). The dependent variable was mean self-reported score. G*power 3.1.9 was used to perform a power analysis for ANOVA: Repeated measures, within-between interaction. Statistical power (1 - β_{error} probability) of 0.80 with a small effect size of 0.15 was selected. The Type 1 error (α) probability was 0.05. Based on the analysis (total sample size = 56) and adjusted to account for predicted loss to follow-up of <20%, a total of 70 individuals were enrolled.

Randomization

To ensure equal treatment allocation of 1:1, block randomization with a block size of four and a scheme of AABB, BBAA, ABAB, BABA, ABBA, and BAAB (sequence repeated) was used to randomize all 70 participants (73).

Allocation concealment mechanism and implementation

The consent form, health history form, surveys, financial compensation envelope, capsule card labeled with a study ID number, and study identification card (business sized card with ID number) were contained in, according to allocation sequence, sequentially numbered (1-70), opaque manilla envelopes individually secured by a metal clasp. Allocation concealment (e.g., preparing envelopes) was performed by a student volunteer from the Mylan School of Pharmacy at Duquesne University. Implementation procedures were performed by investigator (CDB). After receiving informed consent, the manilla envelope was opened revealing the study identification card, and then the study identification number was placed on the front of the envelope.

Blinding/Masking

Study interventions (i.e., capsule cards) were provided by the manufacturer to the principal investigator (VJG) in two boxes labeled "A" and "B." Sealed envelopes containing the key were provided by the pharmacy. One sealed envelope was maintained in a secured location in the office of the principle investigator (VJG) and another sealed envelope was provided to the center director (SL). A sealed envelope was provided to the center director for safety purposes (e.g., hospitalization of participant). According to allocation sequence, capsule cards were then labeled on the back in the lower right-hand corner with the study identification number. Another set of sealed envelopes were

generated enclosing the key for this scheme. These envelopes were also provided to and maintained by the principle investigator (VJG) and the center director (SL). The overall randomization key, linking box letter to study identification number, was maintained as an electronic file on the computer of the investigator (CDB). The investigators and participants were blind to intervention allocation. Participants were also unaware of the exact intervention received. Upon completion of the last participant completing the last survey day, the study was unmasked to the investigators (VJG, CDB, & PWE) by comparing the randomization key to the contents of both unsealed envelopes.

Statistical methods

No interim analysis was performed to assess efficacy. Data obtained from the health histories were tallied and reported as percentages, as warranted. To determine if significant differences exist in the data obtained from the health histories between the two groups, Fisher's exact, Chi-square, or unpaired *t*-tests (two-tailed) were performed. The study involved a repeated measures (Baseline, Day 7, Day 14, Day 21, Day 28), within-between (Intervention by Time) research design. Intention-to-treat analysis, in which all participants were analyzed in the group to which they were assigned, and complete case analysis, in which all participants who completed the 28 day study, were performed. To analyze the data obtained from those fulfilling the intention-to-treat and complete case criteria, a two-way ANOVA (Intervention by Time, two-tailed) followed by a Tukey's multiple comparison test was performed.

The change from Baseline to Day 28 of each participant was dichotomized as either a response or no response. Response was defined as improvement of 50% or greater in the survey scores (GAD-7, PHQ-8, PSS-14, and PSSQ-1) from Baseline to Day

28. For those lost to follow-up, the participants were assumed to have no response (i.e., assumed worst case). The proportion of response/no response were compared across groups with contingency table analysis by performing a Fisher's exact test (two-tailed, CI 95%). Strength of association were reported as relative risk (95% CI). GraphPad Prism 6 software (GraphPad Software Inc., La Jolla, CA) was used to perform the statistical analyses. Significance level was $\alpha = 0.05$ (CI 95%) for all tests. Significance, if any, was defined as $p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$, and $p \leq 0.0001$ as indicated by (*), (**), (***), and (****), respectively.

RESULTS

Participant recruitment, allocation, follow-up, and analysis

From July 2015 to December 2015, 70 potential participants were randomized to either the experimental group (Melatonin, n = 35) or the comparison group (Placebo, n = 35). It was revealed at Day 21 that one participant was not taking the intervention (i.e., placebo), and the data was excluded except for baseline. Intention-to-treat and complete case analyses were performed.

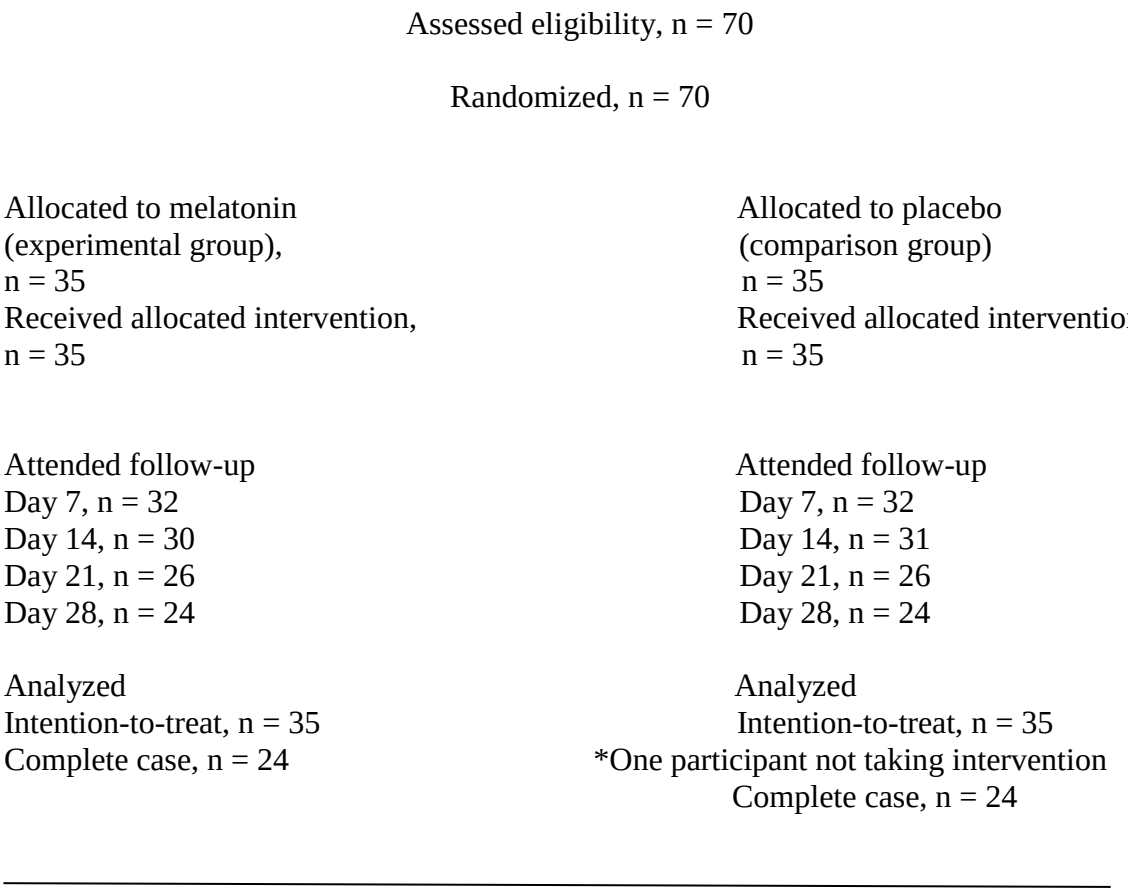

Figure 2. Flow diagram for participant recruitment, allocation, follow-up, and analysis.

Reasons for loss to follow-up

Table 1 presents the reasons and percent of loss to follow-up. The study retained 68.6% of the participants (a loss to follow-up of 31.4%), and was similar between the two groups. The most prevalent reason was non-illicit or illicit drug relapse. These participants were detected at the center to be under the influence of alcohol (n = 2), benzodiazepines (n = 2), heroin (n = 3), or opiates (n = 3). Because of administrative rule violations, four participants had to leave the center. Two participants withdrew due to adverse events of tiredness (melatonin) and diarrhea (placebo). One individual withdrew immediately after completing surveys at baseline (placebo). One death by possible drug overdose was recorded. This participant was allowed to leave the center for the weekend and was found deceased on a city street. Statistical analysis revealed no significant differences between the two groups for loss to follow-up.

Table 1

Loss to Follow-up

Variable	Total	Melatonin	Placebo
n	70	35	35
Loss to Follow-up, n (%)			
Baseline	0 (0.0)	0 (0.0)	0 (0.0)
D7	6 (8.6)	3 (8.6)	3 (8.6)
D14	9 (12.9)	5 (14.2)	4 (11.4)
D21	18 (25.7)	9 (25.7)	9 (25.7)
D28	22 (31.4)	11 (31.4)	11 (31.4)
Reasons, n (%, % yes)	22 (31.4)	11 (15.7)	11 (15.7)
Adverse Event	2 (2.9, 9.1)	1 (2.9, 9.1)	1 (2.9, 9.1)
Adherence w/ Intervention	1 (1.4, 4.5)	0 (0.0, 0.0)	1 (2.9, 9.1)
Administrative	4 (5.7, 18.2)	1 (2.9, 9.1)	3 (8.6, 27.3)
Death (drug overdose)	1 (1.4, 4.5)	1 (2.9, 9.1)	0 (0.0, 0.0)
Relapse	10 (14.3, 45.5)	5 (14.3, 45.5)	5 (14.3, 45.5)
Withdrew	2 (2.9, 9.1)	1 (2.9, 9.1)	1 (2.9, 9.1)
Work	1 (1.4, 4.5)	1 (2.9, 9.1)	0 (0.0, 0.0)
Unknown	1 (1.4, 4.5)	1 (2.9, 9.1)	0 (0.0, 0.0)

% yes is defined as the number of those lost to follow-up for a specific reason divided by the total number lost to follow-up multiplied by 100.

Baseline self-reported social and current medication histories

Table 2 presents the self-reported social and current medication histories at baseline. The sample had a mean age of 40.4 ± 11 years (range of 21 – 65 and median 39), and consisted mostly of participants who identified as white (70%), single (74.3%), and with an education level of high school/G.E.D. or less (77.1%). Age was not reported by two participants in the Melatonin group and one participant in the Placebo group. Sixty-five participant (92.9%) reported currently taking prescribed medications (i.e., those medications expected to be taken during the 28-day study). The mean number of medications prescribed was 3.5 ± 2.2 (range of 0 - 11, median 3, and mode 3). There were 75 different prescribed pharmacotherapies including nutraceuticals for a total of 242 with most medications being used for treating conditions of the central nervous and cardiovascular systems. If all nutraceuticals (e.g., vitamins, minerals, and supplements), except for folic acid (vitamin B9) because of its use in treating anemia as well as folate deficiency associated with alcoholism and liver disease, were excluded, there remained 70 different pharmacotherapies accounting for 231 total medications (Data not shown). One participant reported antivirals for HIV/AIDS. Statistical analyses revealed no significant differences between the two groups for age, race, marital status, education, medication history, or mean number of medications prescribed.

Table 2

Self-reported Social and Current Medication Histories at Baseline

Variable	Total	Melatonin	Placebo
n	70	35	35
Age, years			
*Mean, SD**	40.4 ± 11.0	39.5 ± 11.9	41. 3 ± 10.3
Range	21 - 65	21 - 65	25 - 63
Median	39	37	41
Race, n (%)			
White	49 (70.0)	25 (71.4)	24 (68.6)
Black/African American	17 (24.3)	8 (22.9)	9 (25.7)
Other	4 (5.7)	2 (3.2)	2 (3.2)
Marital Status, n (%)			
Single	52 (74.3)	26 (74.3)	29 (74.3)
Divorced	14 (20)	5 (14.3)	9 (25.7)
Other	4 (5.7)	4 (11.4)	0 (0.0)
Education, n (%)			
High School/G.E.D. or less	54 (77.1)	30 (85.7)	24 (68.6)
Technical School	3 (4.3)	1 (2.9)	2 (5.7)
College (attended or completed)	13 (18.6)	4 (11.4)	9 (25.7)
Medication History, n (%)			
Yes	65 (92.9)	32 (91.4)	33 (94.3)
Medications			
Mean, SD	3.5 ± 2.2	3.7 ± 2.3	3.2 ± 2.2
Range	0 - 11	0 - 9	0 - 11
Median	3	4	3
Mode, n	3 (15)	5 (7)	3 (10)
Different Medications, #	75	53	58
Medications, #	242	129	113
Antiviral (HIV/AIDS), # (%)	2 (0.8)	0 (0.0)	2 (1.8)
*Cardiovascular, # (%)**	43 (17.8)	29 (22.5)	14 (12.4)
Central Nervous, # (%)	157 (64.8)	82 (63.6)	75 (66.4)
Endocrine, # (%)	9 (3.7)	2 (1.5)	7 (6.2)
Gastrointestinal, # (%)	9 (3.7)	3 (2.3)	6 (5.3)
*Nutraceuticals, # (%)**	11 (4.5)	8 (6.2)	3 (2.7)
Respiratory, # (%)	1 (0.4)	0 (0.0)	1 (0.9)
*Other, # (%)**	10 (4.1)	5 (3.9)	5 (4.4)

*Age was not reported by two participants in the Melatonin group and by one participant in the Placebo group.

*Nutraceuticals included vitamins, minerals, and supplements; however, folic acid (vitamin B9) was included in "Cardiovascular." NSAIDS were included in "Other;" however, aspirin was included in "Cardiovascular."

Baseline self-reported medical history

Table 3 presents the self-reported medical histories identifying the total number of conditions reported for each system as well as the most reported condition for each system at baseline. Sixty–two participants (88.6%) reported a medical condition diagnosed by a doctor with a mean of 3.0 ± 3.1 (range 0 – 18, median 2, and mode 1). In aggregate, the participants tended to present with histories of high blood pressure, heartburn/acid reflux, hepatitis, arthritis, and/or neuromuscular symptoms related to disc herniation. One participant reported a history of HIV/AIDS. However, the category of "Neurological/Psychiatric Events" did not address mental health conditions such as anxiety, bipolar, depression, and schizophrenia; these were reserved for the mental health history questionnaire (See Table 5). Statistical analyses revealed no significant differences between the two groups for the mean number of diagnosed medical conditions as well as the number of participants reporting conditions for each system.

Table 3

Self-reported Medical History at Baseline

Variable	Total	Melatonin	Placebo
n	70	35	35
Medical History, n (%)			
Yes	62 (88.6)	32 (91.4)	30 (85.7)
Medical Conditions			
Mean, SD	3.0 ± 3.1	3.6 ± 3.5	2.5 ± 2.4
Range	0 - 18	0 - 18	0 - 11
Median	2	3	2
Mode, n	1 (17)	1 (8)	2 (9)
Cardiovascular, #	35	24	11
Yes, n (%)	23 (32.9)	15 (42.9)	8 (22.9)
High Blood Pressure, n (%, % yes)	20 (28.6, 87.0)	13 (37.1, 86.7)	7 (20.0, 87.5)
High Cholesterol, n (%, % yes)	7 (10.0, 30.4)	6 (17.1, 40.0)	1 (2.9, 12.5)
Endocrine, #	9	3	6
Yes, n (%)	9 (12.9)	3 (8.6)	6 (17.1)
Diabetes, n (%, % yes)	7 (10.0, 77.8)	2 (5.7, 66.7)	5 (14.3, 83.3)
Gastrointestinal, #	25	14	11
Yes, n (%)	23 (32.9)	13 (37.1)	10 (28.6)
Heartburn/Acid Reflux, n (%, % yes)	18 (25.7, 78.3)	10 (71.4, 76.9)	8 (22.9, 80.0)
Genitourinary/Renal, #	10	8	2
Yes, n (%)	7 (10.0)	6 (17.1)	1 (2.9)
Kidney Stones, n (%, % yes)	4 (5.7, 57.1)	4 (11.4, 66.7)	0 (0.0, 0.0)
Hepatic/Gall Bladder, #	22	11	11
Yes, n (%)	18 (25.7)	9 (25.7)	9 (25.7)
Hepatitis, n (%, % yes)	17 (24.3, 94.4)	9 (25.7, 81.8)	8 (22.9, 88.9)
Musculoskeletal/Spinal, #	48	29	19
Yes, n (%)	31 (44.3)	17 (48.6)	14 (40.0)
Arthritis, n (%, % yes)	10 (14.3, 32.3)	7 (20.0, 41.2)	3 (8.6, 21.4)
Cervical or Lumbar Disc, n (%, % yes)	12 (17.1, 38.7)	6 (17.1, 35.3)	6 (17.1, 42.9)
Neck/Back Pain, n (%, % yes)	16 (22.9, 51.6)	9 (25.7, 52.9)	7 (20.0, 50.0)
Neurological/Psychiatric, #	33	21	12
Yes, n (%)	19 (27.1)	11 (31.4)	8 (22.9)
Migraines/Headaches, n (%, % yes)	7 (10.0, 36.8)	5 (14.3, 45.5)	2 (5.7, 25.0)
Numbness/Tingling, n (%, % yes)	7 (10.0, 36.8)	5 (14.3, 45.5)	2 (5.7, 25.0)
Respiratory/Ears/Nose/Throat, #	25	12	13
Yes, n (%)	20 (28.6)	10 (28.6)	10 (28.6)
Asthma, n (%, % yes)	5 (7.1, 25.0)	2 (5.7, 20.0)	3 (8.6, 30.0)
Sleep Apnea, n (%, % yes)	6 (8.6, 30.0)	2 (5.7, 20.0)	4 (11.4, 40.0)
Other, #	6	3	3
Yes, n (%)	6 (8.6)	3 (8.6)	3 (8.6)
HIV/AIDS, n (%, % yes)	1 (1.4, 16.7)	1 (2.9, 33.3)	0 (0.0, 0.0)

% yes is defined as the number of those reporting a specific condition divided the total number reporting a condition multiplied by 100.

Baseline self-reported use of nicotine delivery products, consumption of caffeinated beverages, and preferred type of exercise

Table 4 presents the self-reported use of nicotine delivery products, consumption of caffeinated beverages, or preferred type of exercise at baseline. Sixty-three participants (90%) reported use of nicotine delivery products with cigarettes being the preferred mode. The vast majority (94.3%) also consumed caffeinated beverages with coffee being preferred. The participants were physically active with about three quarters (72.9%) of them choosing some form of exercise with lifting weights being preferred. Statistical analyses revealed no significant differences between the two groups for the use of nicotine delivery products, consumption of caffeinated beverages, or preferred type of exercise

Table 4

Self-reported Nicotine, Caffeinated Beverages, and Exercise Histories at Baseline

Variable	Total	Melatonin	Placebo
n	70	35	35
Nicotine, n (%, % yes)			
Yes, n (%)	63 (90.0)	32 (91.4)	31 (88.6)
Chewing tobacco	4 (5.7, 6.3)	2 (5.7, 6.3)	2 (5.7, 6.5)
Cigarettes	56 (80.0, 88.9)	31 (88.6, 96.9)	25 (71.4, 80.6)
Snuff	12 (17.1, 19.0)	5 (14.3, 15.6)	7 (20.0, 22.6)
Vapor	0 (0.0, 0.0)	0 (0.0, 0.0)	0 (0.0, 0.0)
Caffeinated Beverages, n (%, % yes)			
Yes, n (%)	66 (94.3)	33 (94.3)	33 (94.3)
Coffee	55 (78.6, 83.3)	26 (74.3, 78.8)	29 (82.9, 87.9)
Soda	35 (50.0, 53.0)	20 (57.1, 60.6)	15 (42.9, 45.5)
Tea	21 (30.0, 31.8)	10 (28.6, 30.3)	11 (31.4, 33.3)
Exercise, n (%, % yes)			
Yes, n (%)	51 (72.9)	24 (68.6)	27 (77.1)
Weights	36 (51.4, 70.6)	17 (48.6, 70.8)	19 (54.3, 70.4)
Aerobics/Pilates/Yoga	10 (14.3, 19.6)	2 (5.7, 8.3)	8 (11.4, 29.6)
Running	9 (12.9, 17.6)	2 (5.7, 8.3)	7 (20.0, 25.9)
Sports	6 (8.6, 11.8)	2 (5.7, 8.3)	4 (11.4, 14.8)
Other	16 (22.3, 31.4)	7 (20.0, 29.2)	9 (25.7, 33.3)

% yes is defined as the number of those reporting a specific use divided by the total number reporting a use multiplied by 100.

Self-reported mental health conditions diagnosed by a medical professional

Table 5 presents the self-reported mental health histories at baseline. Ninety-one percent (91.4%) reported a history of mental health conditions with diagnosis obtained mostly in a hospital setting (71.9% of those reporting a diagnosis). On average, 1.9 ± 0.9 conditions were reported; specifically, 49 participants reported comorbidity of at least two conditions accounting for 76.6% of those reporting a mental health history. Most were diagnosed with depression followed by anxiety while 62.5% of those reporting a mental health history were comorbid for depression and anxiety with or without other mental health conditions. Statistical analyses revealed no significant differences between the two groups for reported mental health history, mean number of conditions, location of diagnosis as well as the number of participants diagnosed or comorbid.

Table 5

Self-reported Mental Health History at Baseline

Variable	Total	Melatonin	Placebo
n	70	35	35
Mental Health History, n (%)			
Yes	64 (91.4)	32 (91.4)	32 (91.4)
Conditions			
Mean, SD	1.9 ± 0.9	1.9 ± 0.9	1.8 ± 0.9
Median	2	2	2
Mode, (n)	2 (32)	2 (16)	2 (16)
Diagnosis, n (%, % yes)			
Anxiety	45 (64.2, 70.3)	26 (74.3, 81.3)	19 (54.3, 59.4)
Bipolar	24 (34.3, 37.5)	11 (31.4, 34.3)	13 (37.1, 40.6)
Depression	56 (80.0, 87.5)	27 (77.1, 84.4)	29 (82.9, 90.6)
Schizophrenia	5 (7.1, 7.8)	2 (5.7, 6.3)	3 (8.6, 9.4)
Comorbid, n (%, % yes)			
Total (≥2 conditions)	49 (70.0, 76.6)	25 (71.4, 78.1)	24 (68.6, 75.0)
Depression & Anxiety	40 (57.1, 62.5)	22 (62.9, 68.8)	18 (51.4, 56.3)
Location, n (%, % yes)			
Hospital	46 (65.7, 71.9)	22 (62.8, 68.8)	24 (68.6, 75.0)
PCP/Clinic/Rehab Center	12 (17.1, 18.8)	6 (17.1, 18.8)	6 (17.1, 18.8)
Other	6 (8.6, 9.4)	4 (11.4, 12.5)	2 (5.7, 6.3)

% yes is defined as the number of those reporting a specific diagnosis/location divided by the total number reporting a diagnosis/location multiplied by 100.

Self-reported illicit and non-illicit drug history at baseline

Table 6 presents the self-reported illicit and non-illicit drug histories at baseline. On average, 2.5 ± 1.8 drugs (range of 1 -10, median 2, and mode 2). Alcohol use (60.0%) was the most reported followed by heroin (51.4%) and crack cocaine (38.6); however, 15.7% reported only using alcohol and 10.0% reported only using heroin. One of the most reported multiple drug use was alcohol and cocaine or crack cocaine (27.1%). Another popular usage was heroin and opiate drugs being reported by 20.0% of the participants, and 17.1% reported using alcohol and heroin or opiate drugs. Statistical analyses revealed no significant differences between the two groups for the mean number of illicit and non-illicit drug used as well as for each illicit and non-illicit drug used.

Table 6

Self-reported Illicit and Non-illicit Drug History

Variable	Total	Melatonin	Placebo
n	70	35	35
Mean, SD	2.5 ± 1.8	2.4 ± 1.8	2.6 ± 1.7
Range	1 - 10	1 - 10	1 - 10
Median	2	2	2
Mode, (n)	2 (29)	2 (15)	2 (14)
Illicit & Non-illicit Drugs, n (%)			
Alcohol	42 (60.0)	21 (60.0)	21 (60.0)
Alcohol only	11 (15.7)	7 (20.0)	4 (11.4)
Alcohol & Cocaine or Crack Cocaine	19 (27.1)	9 (25.7)	10 (28.6)
Alcohol & Heroin or Opiate Drugs	12 (17.1)	8 (22.9)	4 (11.4)
Amphetamine	6 (8.6)	3 (8.6)	3 (8.6)
Benzodiazepines	4 (5.7)	2 (5.7)	2 (5.7)
Cocaine	16 (22.9)	5 (14.3)	11 (31.4)
Crack Cocaine	27 (38.6)	12 (34.3)	15 (42.9)
Cocaine & Crack Cocaine	10 (14.3)	5 (14.3)	5 (14.3)
Hash	2 (2.9)	1 (2.9)	1 (2.9)
Heroin	36 (51.4)	20 (57.1)	16 (45.7)
Heroin only	7 (10.0)	4 (11.4)	3 (8.6)
Heroin & Opiate Drugs	14 (20.0)	7 (20.0)	7 (20.0)
LSD	3 (4.3)	1 (2.9)	2 (5.7)
Marijuana	20 (28.6)	10 (28.6)	10 (28.6)
Marijuana & Alcohol	14 (20.0)	7 (20.0)	7 (20.0)
Marijuana & Cocaine or Crack Cocaine	14 (20.0)	6 (17.1)	8 (22.9)
MDMA	1 (1.4)	1 (2.9)	0 (0.0)
Opiate Drugs	17 (24.2)	8 (22.9)	9 (25.7)
PCP	2 (2.9)	1 (2.9)	1 (2.9)

Self-reported melatonin history at baseline

Table 7 presents the self-reported melatonin history at baseline. Thirty participants (42.9%) reported a history of melatonin use with 100.0% indicating usage for sleep. Most participants obtained the melatonin from a clinician (53.3%) most likely in a hospital, rehabilitation center, or provided to them (46.7%). Only 40.0% could recall the dose taken with between 3 to 10 mg being the most popular dose range. Seventy percent of participants who reported a history believed melatonin had an effect. Most of the participants (70.0%) reported a prior usage of greater than one month before study enrollment. Statistical analyses revealed no significant differences between the two groups for prior history, reason for use, motivation, place acquired, time of last use, recall dose taken, or believed it helped.

Table 7

Self-reported Melatonin History at Baseline

Variable	Total	Melatonin	Placebo
n	70	35	35
Prior History, n (%)			
Yes	30 (42.9)	17 (48.6)	13 (37.1)
Reason, n (% yes)			
Sleep	30 (100.0)	17 (100.0)	13 (100.0)
Motivation, n (% yes)			
Clinician	16 (53.3)	11 (64.7)	5 (38.5)
Self	11 (36.7)	6 (35.3)	5 (38.5)
Both	3 (10.0)	0 (0.0)	3 (23.1)
Acquired, n (% yes)			
Hospital/Rehab Center/Provided	14 (46.7)	8 (47.1)	6 (46.2)
Pharmacy	5 (16.7)	1 (5.9)	4 (30.8)
Retail	8 (26.7)	5 (29.4)	3 (23.1)
Other	1 (3.3)	1 (5.9)	0 (0.0)
Unknown/No Response	3 (10.0)	2 (11.8)	1 (7.7)
Last Use, n (% yes)			
< 1 week	1 (3.3)	1 (5.9)	0 (0.0)
≤ 1 month	7 (23.3)	5 (29.4)	2 (15.4)
> 1 month to ≤ 1 year	10 (33.3)	6 (35.3)	4 (30.8)
> 1 year	11 (36.7)	5 (29.4)	6 (46.2)
Unknown/No Response	1 (3.3)	0 (0.0)	1 (7.7)
Recall Dose, n (% yes)			
Yes, n (%)	12 (40.0)	9 (52.9)	3 (23.1)
1 mg	0 (0.0)	0 (0.0)	0 (0.0)
3 - 10 mg	10 (83.3)	8 (88.9)	2 (66.7)
>10 mg	2 (16.7)	1 (11.1)	1 (33.3)
Believe It Helped?, n (% yes)			
Yes	21 (70.0)	11 (64.7)	10 (76.9)
No	5 (16.7)	4 (23.5)	1 (5.9)
Unknown/No Response	4 (13.3)	2 (11.8)	2 (15.4)

Self-reported belief intervention taken was melatonin

Table 8 presents the self-reported belief of those who completed the 28-day study that the intervention taken was melatonin. Twenty-one participants (43.8%) reported a history of melatonin use. Seventeen participants (81.0%) reported that the prior use helped. Interestingly, thirty-four participants (70.8%) believed that the intervention they were taking was melatonin while fourteen participants (29.2%) believed it was placebo. Fisher's exact test revealed there was a significant difference in identified taken intervention between the groups, $p = 0.0084$. Twenty-two participants (45.8%) correctly identified the intervention they were provided while 26 participants (54.2%) were incorrect. Moreover, 12 participants (25.0%) who incorrectly identified the provided intervention reported a prior history of melatonin. Statistical analyses revealed no significant differences between the two groups for prior history, prior use helped, and belief intervention taken was melatonin.

Table 8

Self-reported Belief Intervention taken was Melatonin

Variable	Total	Melatonin	Placebo
n (completed 28 days)	48	24	24
Prior History, n (%)			
Yes	21 (43.8)	11 (45.8)	10 (41.7)
No	27 (56.3)	13 (54.2)	14 (58.3)
Prior Use Helped, n (%)			
Yes	17 (81.0)	8 (72.7)	9 (90.0)
No	2 (9.5)	2 (18.2)	0 (0.0)
Unknown/No Response	2 (9.5)	1 (9.1)	1 (10.0)
Belief Intervention was Melatonin, n (%)			
Yes	34 (70.8)	16 (66.7)	18 (75.0)
No	14 (29.2)	8 (33.3)	6 (25.0)
Identified Taken Intervention, n (%) **			
Correct	22 (45.8)	16 (66.7)	6 (25.0)
Yes Prior History	9 (18.8)	7 (29.2)	2 (8.3)
No Prior History	13 (27.1)	9 (37.5)	4 (16.7)
Incorrect	26 (54.2)	8 (33.3)	18 (75.0)
Yes Prior History	12 (25.0)	4 (16.7)	8 (33.3)
No Prior History	14 (29.2)	4 (16.7)	10 (41.7)

Asterisks indicate significance, $p < 0.05$, $p < 0.01$, $p < 0.001$, and $p < 0.0001$ as indicated by *, **, ***, and ****, respectively.

Self-reported adherence to interventions at 28 days (capsule count)

Table 9 presents the adherence to interventions of those who completed the 28-day study. Forty-five capsule cards were returned to the investigator (CDB). On average, 27.2 ± 1.4 capsules were taken by the participants. Specifically, forty-three participants took the intervention as directed (i.e., 1 capsule per day); however, two participants took more than directed. After identification, these participants were provided an additional capsule card and again verbally given specific instructions to administer one capsule by mouth at bedtime for the remainder of the study. Two participants did not return the capsule card but verbally informed the investigator (CDB) that all capsules had been taken. Statistical analyses revealed no significant differences between the two groups for mean number of capsules taken or adherence as directed.

Table 9

Adherence to Interventions at 28 days (Capsule Count)

Variable	Total	Melatonin	Placebo
n (completed 28 days)	48	24	24
Returned Cards, n	45	22	23
Capsules Count			
Mean, SD	27.2 ± 1.4	27.5 ± 1.3	26.9 ± 1.5
Adherence, n (%)			
As Directed	43 (89.6)	20 (83.3)	23 (95.8)
> Directed	2 (4.2)	2 (8.3)	0 (0.0)
Verbal Confirmation	2 (4.2)	1 (4.2)	1 (4.2)
Unknown	1 (2.1)	1 (4.2)	0 (0.0)

Self-reported adverse events

Table 10 presents the self-reported adverse events experienced during the 28-day study. To address any adverse events relating to the interventions, the participant was encouraged to report any adverse events to the investigator (CDB) or at the weekly assessments to identify any new symptoms experienced other than those at time of study entrance. Twenty participants (28.6%) reported experiencing an adverse event. The most reported adverse events were "fatigue/groggy/tired/sleepy," "headache," "nightmares/vivid dreams," and "sleeplessness/wakefulness." From July to the end of January, the investigator observed a number of individuals at the center experiencing symptoms of the common cold; thus, the symptoms of the common cold are most likely not attributable to the intervention. Statistical analyses revealed no significant differences between the two groups for number of participants reporting an adverse event.

Table 10

Self-reported Adverse Events

Variable	Total	Melatonin	Placebo
n	70	35	35
Reported, n (%)	20 (28.6)	13 (37.1)	7 (20.0)
Adverse Events, #	26	18	8
Common Cold	2	2	0
Diarrhea	2	0	2
Fainted	1	1	0
Fatigue/Groggy/Tired/Sleepy	6	4	2
Headache	4	2	2
Nausea	1	1	0
Nightmares/Vivid Dreams	4	4	0
Sleeplessness/Wakefulness	5	3	2
Sleep Latency	1	1	0

Intention-to-treat: mean self-reported GAD-7 scores

Figure 3 presents the mean self-reported GAD-7 scores of the melatonin and placebo groups at baseline and each weekly assessment. Values are mean ± SEM, GAD-7 scores (0 – 21). Higher scores indicate more severity of anxiety. The two-way ANOVA was conducted to investigate score differences in intervention and time among participants. The results revealed no significant interaction between factors (Intervention by Time), $F(4, 283) = 0.6362$, $p = 0.6370$, $\omega^2 = 0.7571$. No significant main effect for intervention, $F(1, 283) = 0.5794$, $p = 0.4472$, $\omega^2 = 0.1724$ was determined. However, the two-way ANOVA results revealed a significant main effect for time, $F(4, 283) = 12.51$, $p < 0.0001$, $\omega^2 = 14.88$. Results of the Tukey post hoc test revealed no significant between group differences but revealed significant within group differences especially for Baseline to Day 28 for both groups (See Table 11).

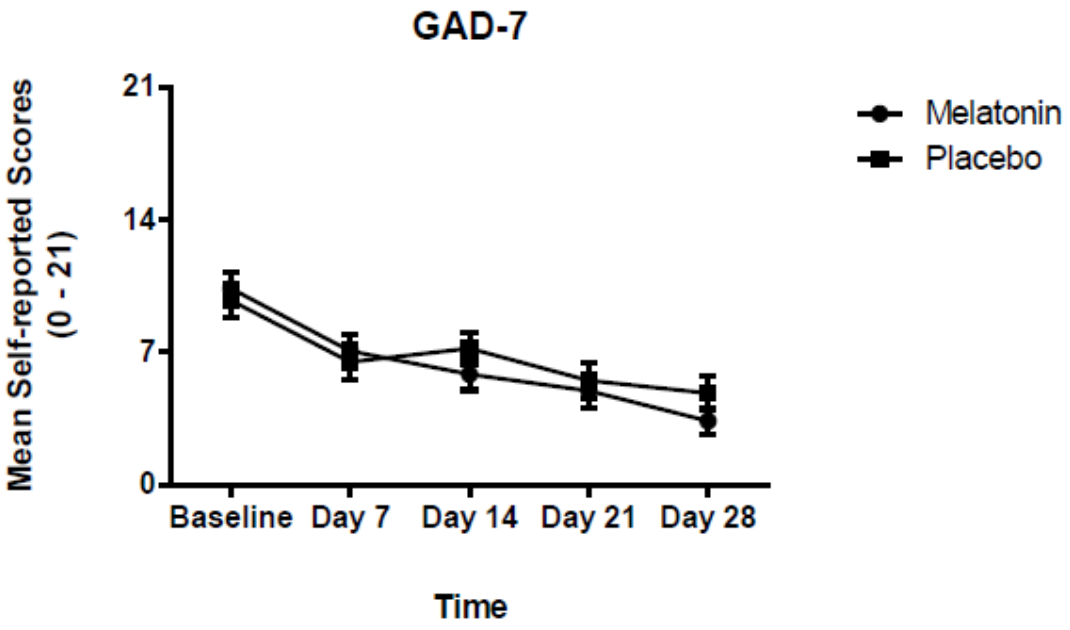

Figure 3. The mean self-reported GAD-7 scores of the melatonin and placebo groups at baseline and each weekly assessment. Values are mean ± SEM, GAD-7 scores (0 – 21). Higher scores indicate more severity of anxiety. The two-way ANOVA was conducted to investigate score differences in intervention and time among participants. The results revealed no significant interaction between factors (Intervention by Time) and no significant main effect for intervention. However, the results revealed a significant main effect for time. The Tukey post hoc test revealed no significant between group differences but revealed significant within group differences especially for Baseline to Day 28 for both groups. Melatonin (n = 35) and Placebo (n = 35).

Intention-to-treat: mean self-reported PHQ-8 scores

Figure 4 presents the mean self-reported PHQ-8 scores of the melatonin and placebo groups at baseline and each weekly assessment. Values are mean ± SEM, PHQ-8 scores (0 – 24). Higher scores indicate more degree of depression. The two-way ANOVA was conducted to investigate score differences in intervention and time among participants. The results revealed no significant interaction between factors (Intervention by Time), $F(4, 283) = 0.8085$, $p = 0.5206$, $\omega^2 = 0.9557$. No significant main effect for intervention, $F(1, 283) = 0.2558$, $p = 0.6134$, $\omega^2 = 0.0756$ was determined. However, the two-way ANOVA results revealed a significant main effect for time, $F(4, 283) = 12.97$, $p < 0.0001$, $\omega^2 = 15.34$. Results of the Tukey post hoc test revealed no significant between group differences but revealed significant within group differences especially for Baseline to Day 28 for both groups (See Table 11).

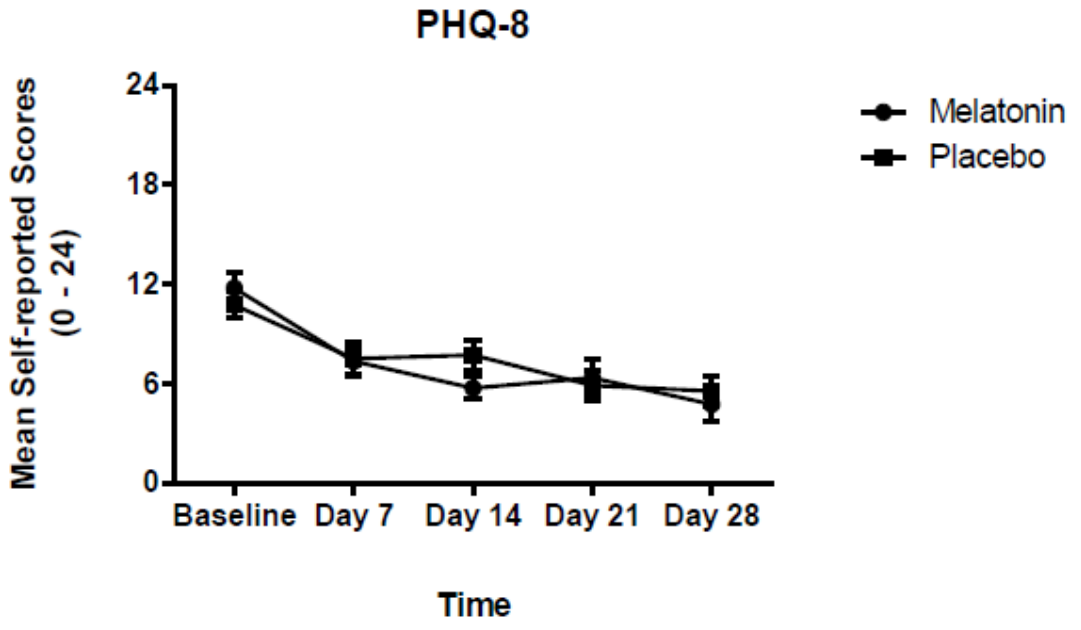

Figure 4. The mean self-reported PHQ-8 scores of the melatonin and placebo groups at baseline and each weekly assessment. Values are mean ± SEM, PHQ-8 scores (0 – 24). Higher scores indicate more degree of depression. The two-way ANOVA was conducted to investigate score differences in intervention and time among participants. The results revealed no significant interaction between factors (Intervention by Time) and no significant main effect for intervention. However, the results revealed a significant main effect for time. Results of the Tukey post hoc test revealed no significant between group differences but revealed significant within group differences especially for Baseline to Day 28 for both groups. Melatonin (n = 35) and Placebo (n = 35).

Intention-to-treat: mean self-reported PSS-14 scores

Figure 5 presents the mean self-reported PSS-14 scores of the melatonin and placebo groups at baseline and each weekly assessment. Values are mean ± SEM, PSS-14 scores (0 – 56). Higher scores indicate more degree of stress. The two-way ANOVA was conducted to investigate score differences in intervention and time among participants. The results revealed no significant interaction between factors (Intervention by Time), $F(4, 283) = 0.3180$, $p = 0.8658$, $\omega^2 = 0.3666$. No significant main effect for intervention, $F(1, 283) = 0.5188$, $p = 0.4719$, $\omega^2 = 0.1495$ was determined. However, the two-way ANOVA results revealed a significant main effect for time, $F(4, 283) = 15.54$, $p < 0.0001$, $\omega^2 = 17.92$. Results of the Tukey post hoc test revealed no significant between group differences but revealed significant within group differences especially for Baseline to Day 28 for both groups (See Table 11).

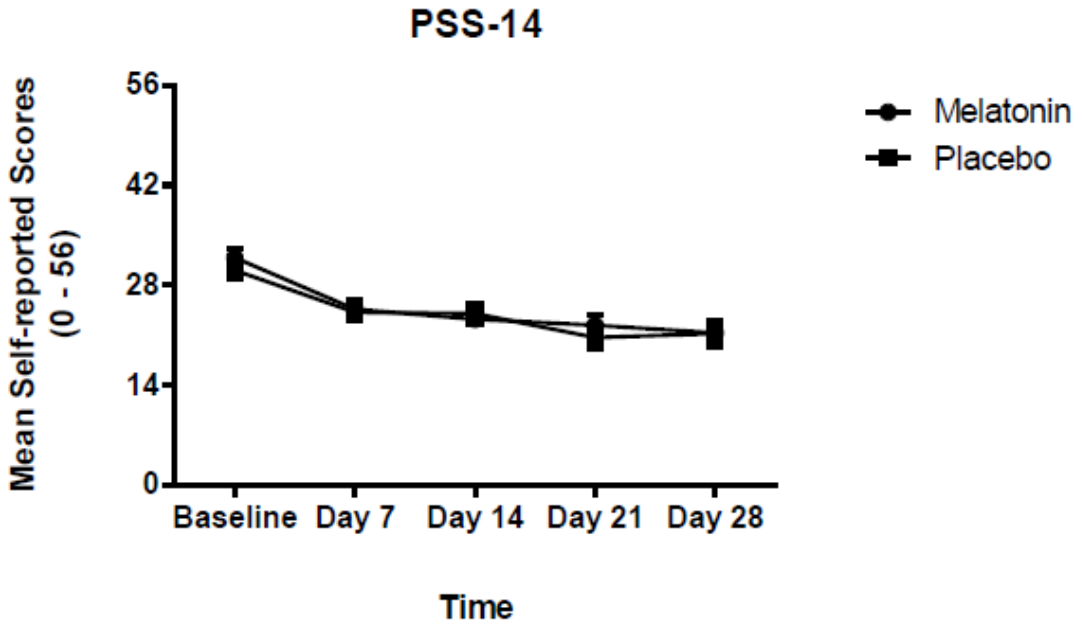

Figure 5. The mean self-reported PSS-14 scores of the melatonin and placebo groups at baseline and each weekly assessment. Values are mean ± SEM, PSS-14 scores (0 – 56). Higher scores indicate more degree of stress. The two-way ANOVA was conducted to investigate score differences in intervention and time among participants. The results revealed no significant interaction between factors (Intervention by Time) and no significant main effect for intervention. However, the results revealed a significant main effect for time. Results of the Tukey post hoc test revealed no significant between group differences but revealed significant within group differences especially for Baseline to Day 28 for both groups. Melatonin (n = 35) and Placebo (n = 35).

Intention-to-treat: mean self-reported PSSQ-1 sleep complaints scores

Figure 6 presents the mean self-reported PSSQ-1 sleep complaints scores of the melatonin and placebo groups at baseline and each weekly assessment. Values are mean ± SEM, PSSQ-1 scores (0 – 25). Higher scores indicate more frequency of sleep complaints. The two-way ANOVA was conducted to investigate score differences in intervention and time among participants. The results revealed no significant interaction between factors (Intervention by Time), $F (4, 283) = 0.3877$, $p = 0.8174$, $\omega^2 = 0.5059$. No significant main effect for intervention, $F (1, 283) = 0.5397$, $p = 0.4632$, $\omega^2 = 0.1761$ was determined. However, the two-way ANOVA results revealed a significant main effect for time, $F (4, 283) = 5.359$, $p < 0.0004$, $\omega^2 = 6.993$. Results of the Tukey post hoc test revealed no significant between group differences but revealed a significant within group difference for Baseline to Day 28 for only the melatonin group (See Table 11).

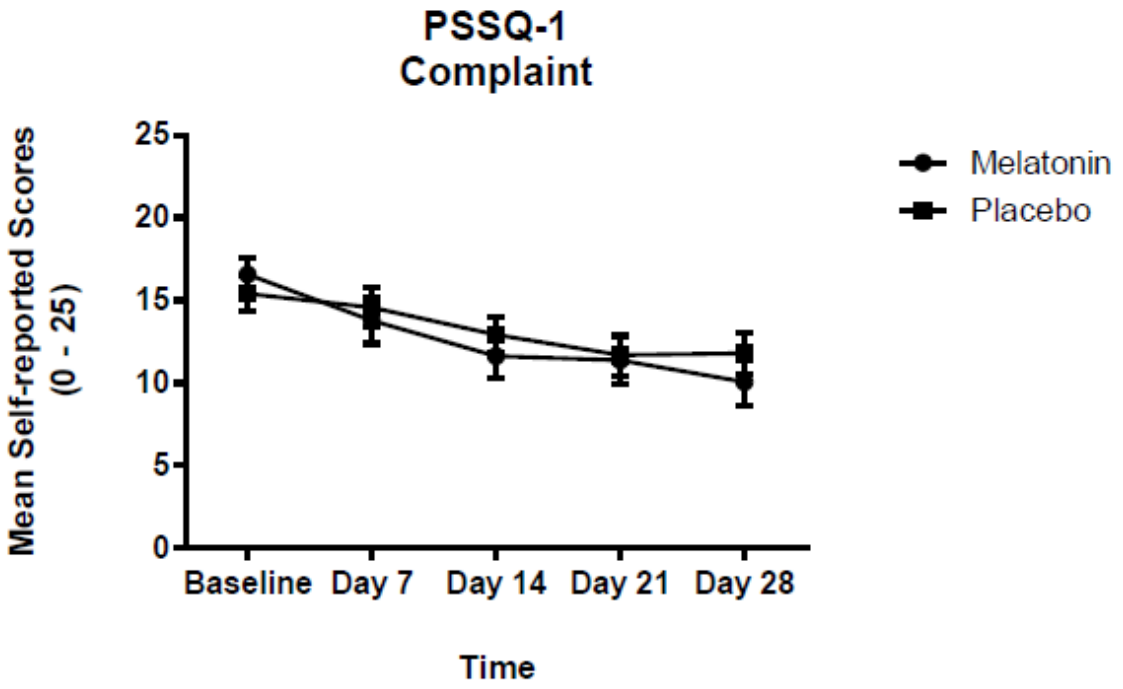

Figure 6. The mean self-reported PSSQ-1 sleep complaint scores of the melatonin and placebo groups at baseline and each weekly assessment. Values are mean ± SEM, PSSQ-1 scores (0 – 25). Higher scores indicate more frequency of sleep complaints. The two-way ANOVA was conducted to investigate score differences in intervention and time among participants. The results revealed no significant interaction between factors (Intervention by Time) and no significant main effect for intervention. However, the results revealed a significant main effect for time. Results of the Tukey post hoc test revealed no significant between group differences but revealed a significant within group difference for Baseline to Day 28 for only the melatonin group. Melatonin (n = 35) and Placebo (n = 35).

Intention-to-treat: mean self-reported PSSQ-1 sleep affecting daily life scores

Figure 7 presents the mean self-reported PSSQ-1 sleep affecting daily life scores of the melatonin and placebo groups at baseline and each weekly assessment. Values are mean ± SEM, PSSQ-1 scores (0 – 32). Higher scores indicate more effect on daily life. The two-way ANOVA was conducted to investigate score differences in intervention and time among participants. The results revealed no significant interaction between factors (Intervention by Time), $F(4, 283) = 0.3946$, $p = 0.8124$, $\omega^2 = 0.4941$. No significant main effect for intervention, $F(1, 283) = 0.02420$, $p = 0.8765$, $\omega^2 = 0.007574$ was determined. However, the two-way ANOVA results revealed a significant main effect for time, $F(4, 283) = 8.713$, $p < 0.0001$, $\omega^2 = 10.91$. Results of the Tukey post hoc test revealed no significant between group differences but revealed a significant within group difference for Baseline to Day 28 for only the melatonin group (See Table 11).

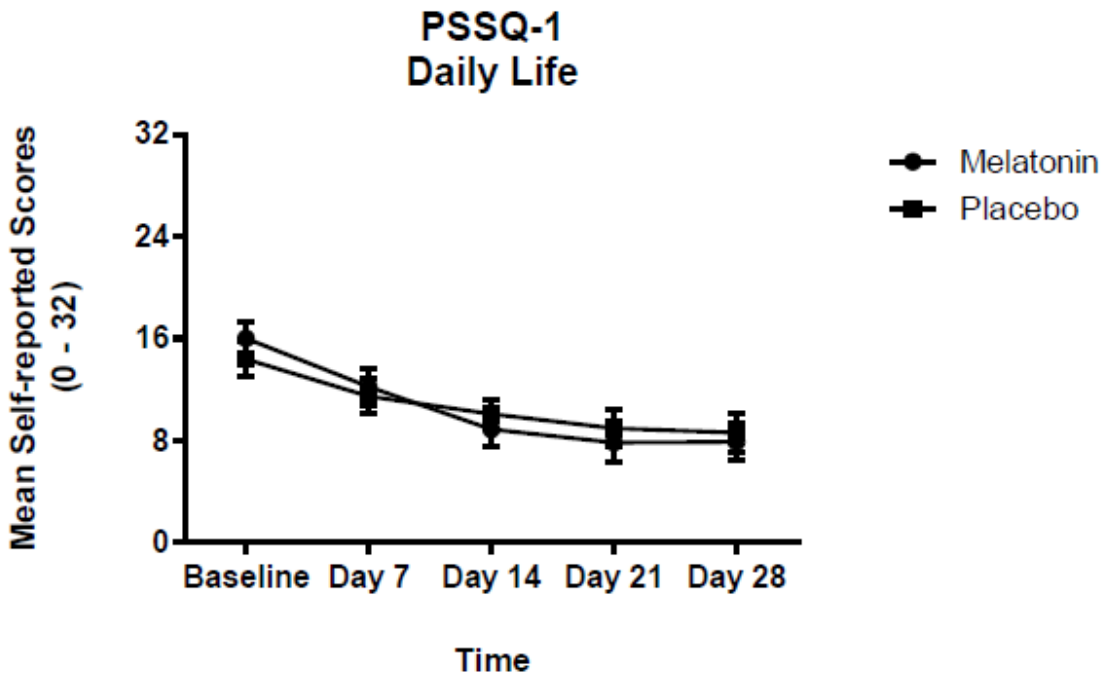

Figure 7. The mean self-reported PSSQ-1 sleep affecting daily life scores of the melatonin and placebo groups at baseline and each weekly assessment. Values are mean ± SEM, PSSQ-1 scores (0 – 32). Higher scores indicate more effect on daily life. The two-way ANOVA was conducted to investigate score differences in intervention and time among participants. The results revealed no significant interaction between factors (Intervention by Time) and no significant main effect for intervention. However, the results revealed a significant main effect for time. Results of the Tukey post hoc test revealed no significant between group differences but revealed a significant within group difference for Baseline to Day 28 for only the melatonin group. Melatonin (n = 35) and Placebo (n = 35).

Complete case: mean self-reported GAD-7 scores

Figure 8 presents the mean self-reported GAD-7 scores of the melatonin and placebo groups at baseline and each weekly assessment. Values are mean ± SEM, GAD-7 scores (0 – 21). Higher scores indicate more severity of anxiety. The two-way ANOVA was conducted to investigate score differences in intervention and time among participants. The results revealed no significant interaction between factors (Intervention by Time), $F(4, 230) = 0.3903$, $p = 0.8155$, $\omega^2 = 0.5176$. No significant main effect for intervention, $F(1, 230) = 2.394$, $p = 0.1231$, $\omega^2 = 0.7939$ was determined. However, the two-way ANOVA results revealed a significant main effect for time, $F(4, 230) = 16.91$, $p < 0.0001$, $\omega^2 = 22.43$. Results of the Tukey post hoc test revealed no significant between group differences but revealed significant within group differences especially for Baseline to Day 28 for both groups (See Table 11).

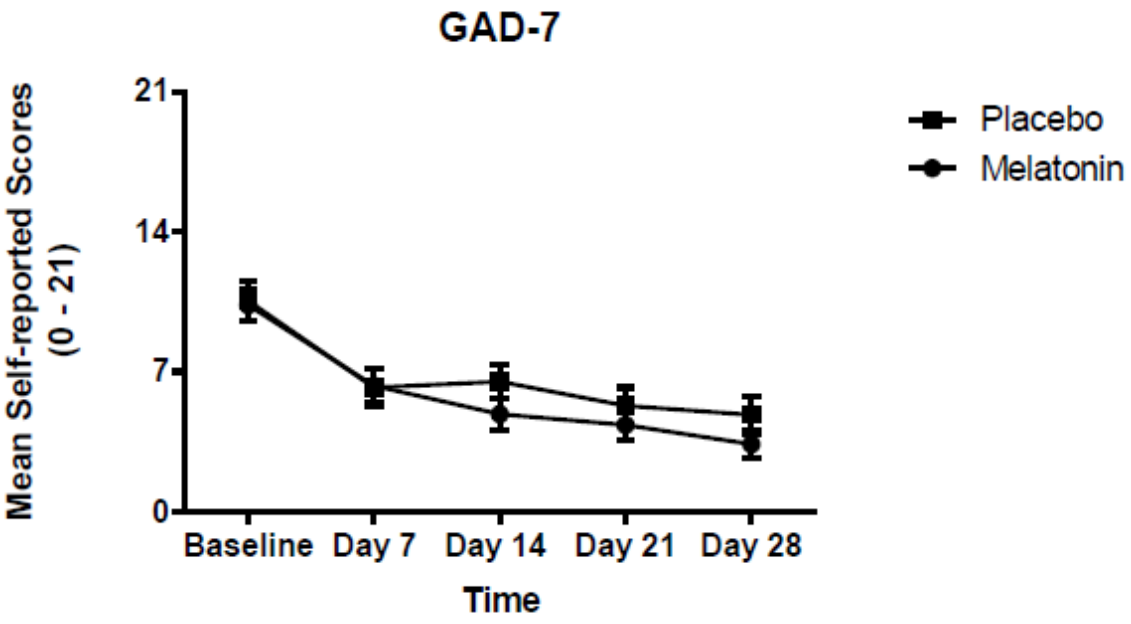

Figure 8. The mean self-reported GAD-7 scores of the melatonin and placebo groups at baseline and each weekly assessment. Values are mean ± SEM, GAD-7 scores (0 – 21). Higher scores indicate more severity of anxiety. The two-way ANOVA was conducted to investigate score differences in intervention and time among participants. The results revealed no significant interaction between factors (Intervention by Time) and no significant main effect for intervention. However, the results revealed a significant main effect for time. The Tukey post hoc test revealed no significant between group differences but revealed significant within group differences especially for Baseline to Day 28 for both groups. Melatonin (n = 24) and Placebo (n = 24).

Complete case: mean self-reported PHQ-8 scores

Figure 9 presents the mean self-reported PHQ-8 scores of the melatonin and placebo groups at baseline and each weekly assessment. Values are mean ± SEM, PHQ-8 scores (0 – 24). Higher scores indicate more degree of depression. The two-way ANOVA was conducted to investigate score differences in intervention and time among participants. The results revealed no significant interaction between factors (Intervention by Time), $F(4, 230) = 0.3410$, $p = 0.8501$, $\omega^2 = 0.4588$. No significant main effect for intervention, $F(1, 230) = 1.311$, $p = 0.2534$, $\omega^2 = 0.4409$ was determined. However, the two-way ANOVA results revealed a significant main effect for time, $F(4, 230) = 16.16$, $p < 0.0001$, $\omega^2 = 21.74$. Results of the Tukey post hoc test revealed no significant between group differences but revealed significant within group differences especially for Baseline to Day 28 for both groups (See Table 11).

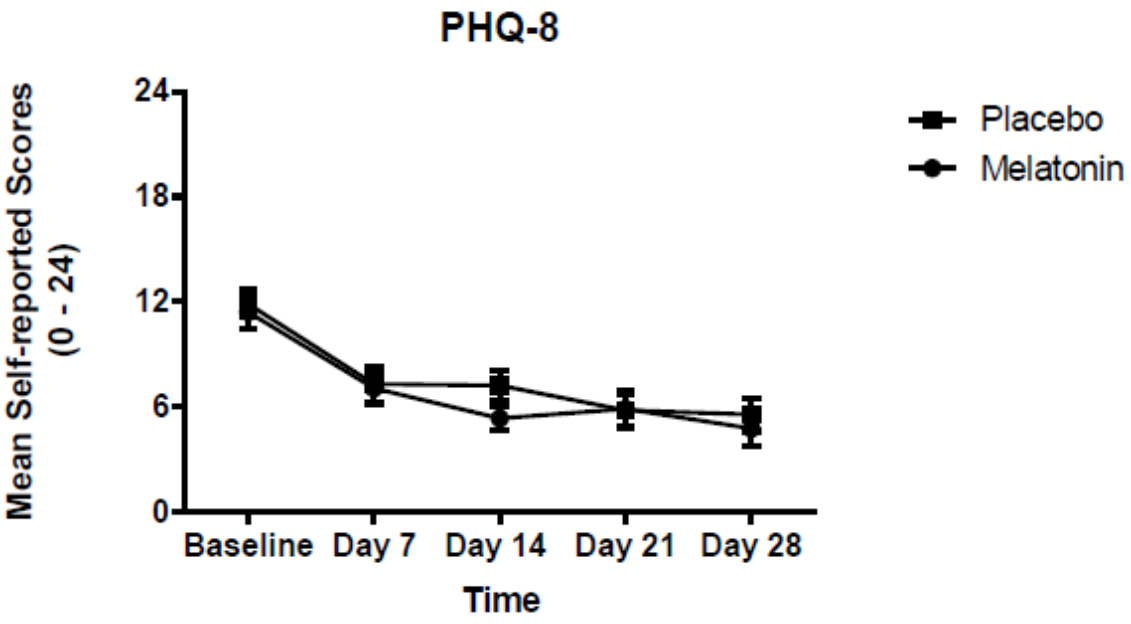

Figure 9. The mean self-reported PHQ-8 scores of the melatonin and placebo groups at baseline and each weekly assessment. Values are mean ± SEM, PHQ-8 scores (0 – 24). Higher scores indicate more degree of depression. The two-way ANOVA was conducted to investigate score differences in intervention and time among participants. The results revealed no significant interaction between factors (Intervention by Time) and no significant main effect for intervention. However, the results revealed a significant main effect for time. Results of the Tukey post hoc test revealed no significant between group differences but revealed significant within group differences especially for Baseline to Day 28 for both groups. Melatonin (n = 24) and Placebo (n = 24).

Complete case: mean self-reported PSS-14 scores

Figure 10 presents the mean self-reported PSS-14 scores of the melatonin and placebo groups at baseline and each weekly assessment. Values are mean ± SEM, PSS-14 scores (0 – 56). Higher scores indicate more degree of stress. The two-way ANOVA was conducted to investigate score differences in intervention and time among participants. The results revealed no significant interaction between factors (Intervention by Time), $F(4, 230) = 0.2534$, $p = 0.9074$, $\omega^2 = 0.3395$. No significant main effect for intervention, $F(1, 230) = 0.6704$, $p = 0.4137$, $\omega^2 = 0.2246$ was determined. However, the two-way ANOVA results revealed a significant main effect for time, $F(4, 283) = 16.71$, $p < 0.0001$, $\omega^2 = 22.39$. Results of the Tukey post hoc test revealed no significant between group differences but revealed significant within group differences especially for Baseline to Day 28 for both groups (See Table 11).

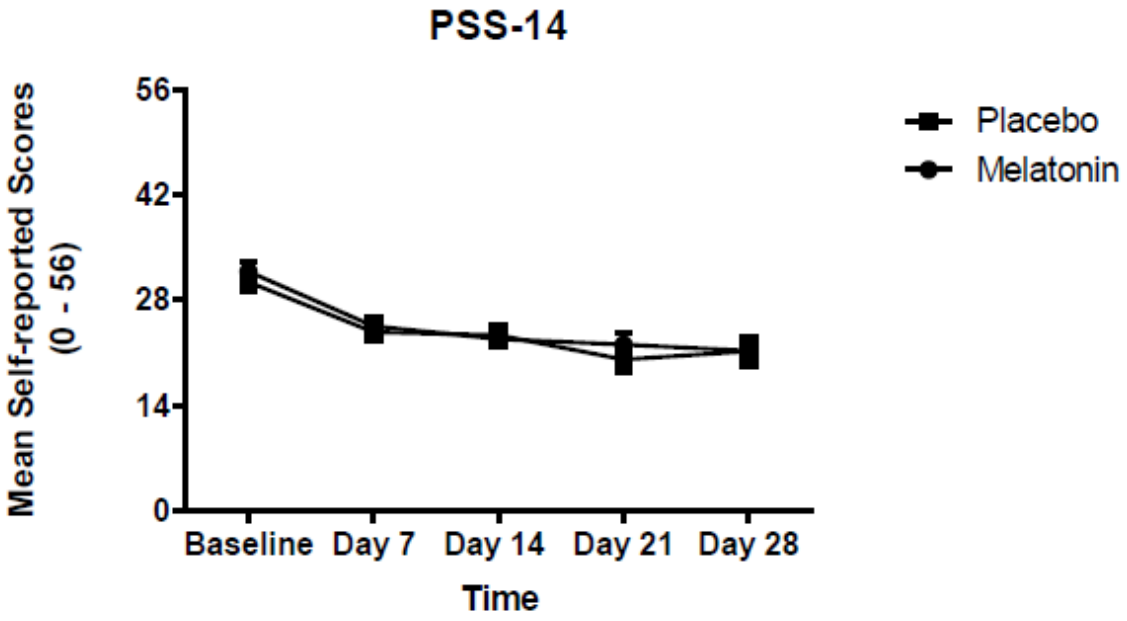

Figure 10. The mean self-reported PSS-14 scores of the melatonin and placebo groups at baseline and each weekly assessment. Values are mean ± SEM, PSS-14 scores (0 – 56). Higher scores indicate more degree of stress. The two-way ANOVA was conducted to investigate score differences in intervention and time among participants. The results revealed no significant interaction between factors (Intervention by Time) and no significant main effect for intervention. However, the results revealed a significant main effect for time. Results of the Tukey post hoc test revealed no significant between group differences but revealed significant within group differences especially for Baseline to Day 28 for both groups. Melatonin (n = 24) and Placebo (n = 24).

Complete case: mean self-reported PSSQ-1 sleep complaints scores

Figure 11 presents the mean self-reported PSSQ-1 sleep complaints scores of the melatonin and placebo groups at baseline and each weekly assessment. Values are mean ± SEM, PSSQ-1 scores (0 – 25). Higher scores indicate more frequency of sleep complaints. The two-way ANOVA was conducted to investigate score differences in intervention and time among participants. The results revealed no significant interaction between factors (Intervention by Time), $F(4, 230) = 0.2352$, $p = 0.9183$, $\omega^2 = 0.3624$. A significant main effect for intervention, $F(1, 230) = 5.817$, $p = 0.0167$, $\omega^2 = 2.241$ was determined. Also, the two-way ANOVA results revealed a significant main effect for time, $F(4, 230) = 5.703$, $p = 0.0002$, $\omega^2 = 8.788$. Results of the Tukey post hoc test revealed no significant between group differences and no significant within group differences especially for Baseline to Day 28 for both groups (See Table 11).

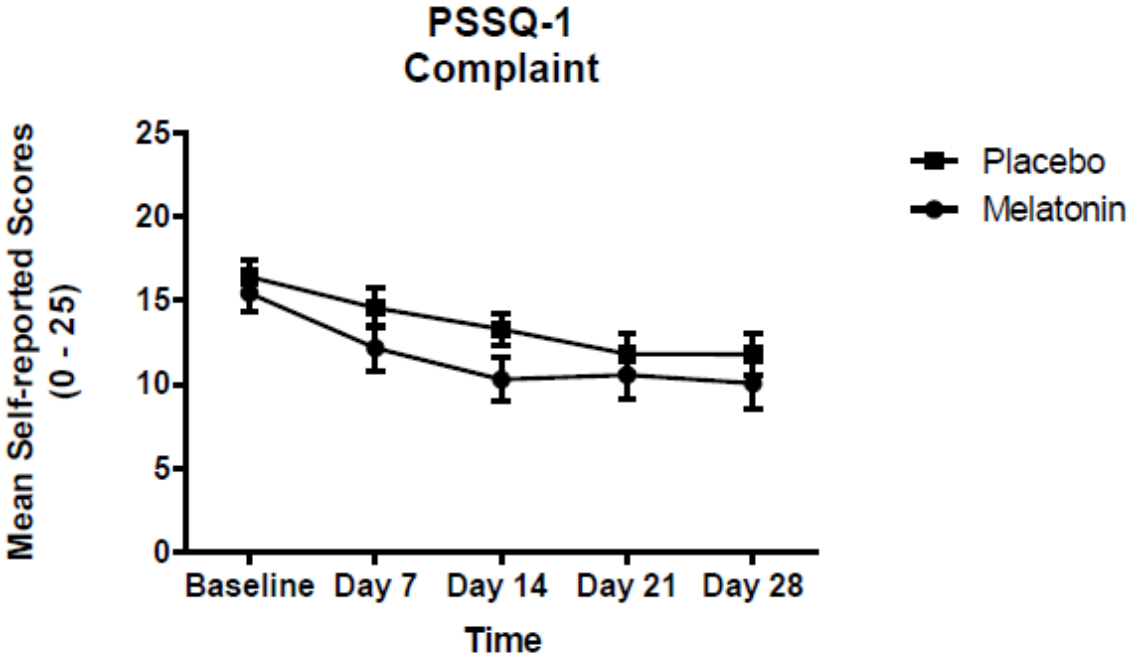

Figure 11. The mean self-reported PSSQ-1 sleep complaint scores of the melatonin and placebo groups at baseline and each weekly assessment. Values are mean ± SEM, PSSQ-1 scores (0 – 25). Higher scores indicate more frequency of sleep complaints. The two-way ANOVA was conducted to investigate score differences in intervention and time among participants. The results revealed no significant interaction between factors (Intervention by Time) but a significant main effect for intervention. Also, the results revealed a significant main effect for time. Results of the Tukey post hoc test revealed no significant between group differences and no significant within group differences for Baseline to Day 28 for the groups. Melatonin (n = 24) and Placebo (n = 24).

Complete case: mean self-reported PSSQ-1 sleep affecting daily life scores

Figure 12 presents the mean self-reported PSSQ-1 sleep affecting daily life scores of the melatonin and placebo groups at baseline and each weekly assessment. Values are mean ± SEM, PSSQ-1 scores (0 – 32). Higher scores indicate more effect on daily life. The two-way ANOVA was conducted to investigate score differences in intervention and time among participants. The results revealed no significant interaction between factors (Intervention by Time), $F(4, 230) = 0.3359$, $p = 0.8536$, $\omega^2 = 0.4712$. No significant main effect for intervention, $F(1, 230) = 1.891$, $p = 0.1704$, $\omega^2 = 0.6631$ was determined. However, the two-way ANOVA results revealed a significant main effect for time, $F(4, 230) = 12.99$, $p < 0.0001$, $\omega^2 = 18.22$. Results of the Tukey post hoc test revealed no significant between group differences but revealed significant within group differences especially for Baseline to Day 28 for both groups (See Table 11).

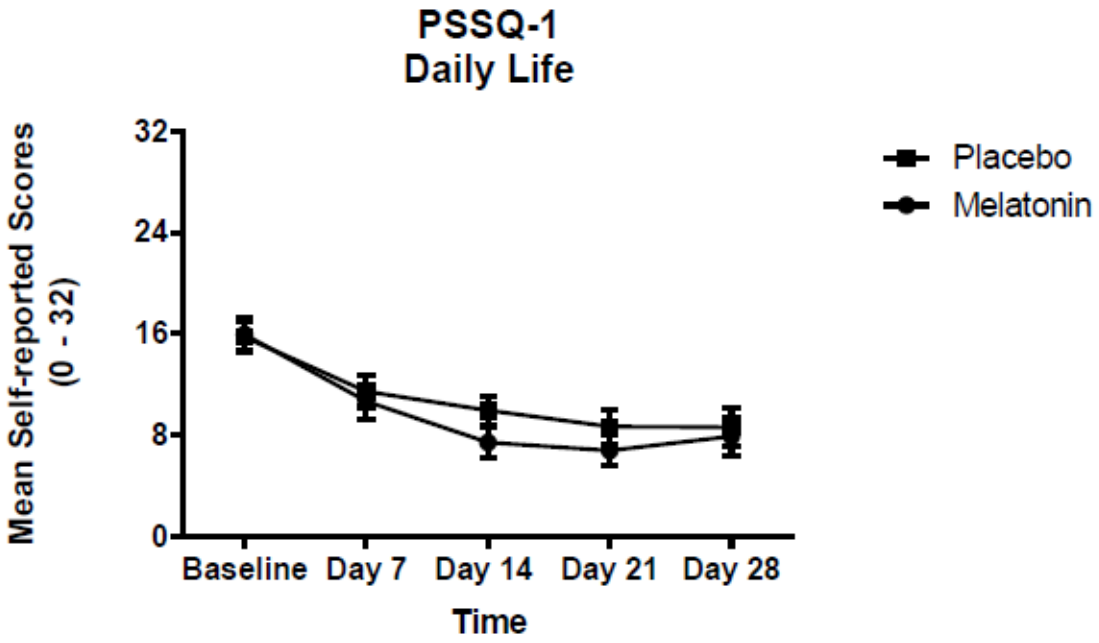

Figure 12. The mean self-reported PSSQ-1 sleep affecting daily life scores of the melatonin and placebo groups at baseline and each weekly assessment. Values are mean ± SEM, PSSQ-1 scores (0 – 32). Higher scores indicate more effect on daily life. The two-way ANOVA was conducted to investigate score differences in intervention and time among participants. The results revealed no significant interaction between factors (Intervention by Time) and no significant main effect for intervention. However, the results revealed a significant main effect for time. Results of the Tukey post hoc test revealed no significant between group differences but revealed significant within group differences especially for Baseline to Day 28 for both groups. Melatonin (n = 24) and Placebo (n = 24).

Mean difference and percent change from baseline to day 28

Table 11 presents the mean difference and percent change from Baseline to Day 28 of each outcome measure of the interventions. Values are mean difference (95% confidence interval) with significance indicated by asterisks.

Table 11

Mean Difference and Percent Change from Baseline to Day 28

Outcome Measure	Melatonin	Placebo
GAD-7		
ITT		
Baseline ± SEM	10.37143 ± 0.835238	9.742857 ± 0.938748
Day 28 ± SEM	3.375 ± 0.711735	4.833333 ± 0.926006
Mean Difference	6.996 (2.935, 11.06)****	4.910 (0.8483, 8.971)**
% Change	67.46	50.39
CC		
Baseline ± SEM	10.33333 ± 0.795432	10.54167 ± 0.970342
Day 28 ± SEM	3.375 ± 0.711735	4.833333 ± 0.926006
Mean Difference	6.958 (3.111, 10.81)****	5.708 (1.861, 9.556)***
% Change	67.33	54.15
PHQ-8		
ITT		
Baseline ± SEM	11.77143 ± 0.914942	10.74286 ± 0.833483
Day 28 ± SEM	4.75 ± 0.984021	5.541667 ± 0.947879
Mean Difference	7.021 (2.844, 11.20)****	5.201 (1.023, 9.379)**
% Change	59.65	48.42
CC		
Baseline ± SEM	11.41667 ± 0.948247	11.875 ± 0.788603
Day 28 ± SEM	4.75 ± 0.984021	5.541667 ± 0.947879
Mean Difference	6.667 (2.559, 10.77)****	6.333 (2.225, 10.44)****
% Change	58.39	53.33
PSS-14		
ITT		
Baseline ± SEM	31.85714 ± 1.207221	30.05714 ± 1.252857
Day 28 ± SEM	21.29167 ± 1.594237	21.20833 ± 1.91578
Mean Difference	10.57 (4.172, 16.96)****	8.849 (2.455, 15.24)***
% Change	33.17	29.44
CC		
Baseline ± SEM	31.79167 ± 1.200392	30.41667 ± 1.27656
Day 28 ± SEM	21.29167 ± 1.594237	21.20833 ± 1.91578
Mean Difference	10.50 (4.101, 16.90)****	9.208 (2.810, 15.61)***
% Change	33.03	30.27
PSSQ-1		
Sleep Complaints		
ITT		
Baseline ± SEM	16.57143 ± 1.031671	15.4 ± 1.118372
Day 28 ± SEM	10.08333 ± 1.490206	11.79167 ± 1.220268
Mean Difference	6.488 (0.7428, 12.23)*	3.608 (-2.137, 9.354)
% Change	39.15	23.43

CC		
Baseline ± SEM	15.45833 ± 1.066694	16.41667 ± 0.99429
Day 28 ± SEM	10.08333 ± 1.490206	11.79167 ± 1.220268
Mean Difference	5.375 (-0.1545, 10.90)	4.625 (-0.9045, 10.15)
% Change	34.77	28.17
Quality of life		
ITT		
Baseline ± SEM	16.00 ± 1.32589	14.4 ± 1.394587
Day 28 ± SEM	7.916667 ± 1.510731	8.625 ± 1.466797
Mean Difference	8.083 (1.735, 14.43)**	5.775 (-0.5736, 12.12)
% Change	50.52	40.10
CC		
Baseline ± SEM	15.95833 ± 1.288727	15.75 ± 1.234343
Day 28 ± SEM	7.916667 ± 1.510731	8.625 ± 1.466797
Mean Difference	8.042 (2.110, 13.97)***	7.125 (1.194, 13.06)**
% Change	50.39	45.24

Mean difference (Baseline - Day 28).

Percent change [(Baseline - Day 28) / Baseline] * 100

Values in parentheses are 95% confidence interval.

ITT = Intention-to-treat; CC = Complete case

Asterisks indicate significance, $p < 0.05$, $p < 0.01$, $p < 0.001$, and $p < 0.0001$ as indicated by *, **, ***, and ****, respectively.

Contingency table analysis and strength of association

Table 12 presents the results of the Fisher's exact test (two-tailed, CI 95%) and the strength of association reported as relative risk (95% CI) of the change following the intervention. No significant differences were detected. All confidence intervals of the strength of association included the null value of 1, thus concluding there exists insufficient evidence to suggest that the interventions were significantly different.

Table 12

Contingency Table Analysis and Strength of Association

Outcome Measure	P value	Relative Risk (95% CI)
GAD-7	1.0000	1.063 (0.6466 to 1.746)
PHQ-8	0.6279	1.231 (0.7014 to 2.160)
PSS-14	0.5401	0.6250 (0.2260 to 1.724)
PSSQ-1		
Complaints	0.5613	1.500 (0.5973 to 3.767)
Daily life	0.7972	1.200 (0.5980 to 2.408)

DISCUSSION

Based upon the results of the systematic review, three identified studies investigated the use of melatonin in those recovering from alcohol dependency. All three studies investigated the effect of melatonin on sleep measures while only one study included assessments of its antidepressant and anxiolytic effects. Therefore, clinical investigations into the use of melatonin as a treatment for depression, anxiety, stress, and sleep difficulties in those recovering from illicit and non-illicit drug dependency is limited and more studies are warranted. This is the first and largest randomized, double-blind, placebo-controlled trial assessing the effects of melatonin upon post-acute withdrawal among males in a residential treatment program. The purpose of the study was to assess the effect of 5 mg melatonin compared to placebo as an adjuvant treatment along with their current pharmaco- and behavioral therapies for 28 days on weekly self-reported severity of anxiety, depression, stress, and sleep complaints as well as how sleep is affecting daily life in a sample of males in recovery from chemical dependency at a single, residential treatment site.

Even though the results for all outcome measures revealed statistically significant within-groups differences over time for both groups, post hoc analyses revealed the study lacked sufficient evidence to demonstrate statistically significant between-group differences for these measures. Additionally, contingency table analysis as well as the relative degree of association between response for participants who are taking melatonin compared to those taking placebo (i.e., relative risk) revealed no significant strength of association between the groups (i.e., confidence interval included null value of 1) when considering worst case for the loss to follow-up. Overall, the various analyses indicated

there exists insufficient evidence to suggest that melatonin and placebo were significantly different, and it may be concluded, based upon the study sample, design, and its limitations, the effect of melatonin on the assessed measures was no different than placebo.

The mean age of the sample was 40.4 ± 11 years (range 21 – 65 and median 39) and consisted mostly of those who identified as white (70%), single (74.3%), and with an education level of high school/G.E.D. or less (77.1%). Black/African-American enrollment was at 24.3%. The sample was similar to the racial demographics reported in the Treatment Episode Data Set (TEDS) of nationwide admissions into substance abuse treatment facilities where it was reported that Whites and African-Americans account for 60% and 21%, respectively (9). The participants have a variety of medical issues with 88.6% reporting a medical condition and also present with multiple medication use with an average of 3.5 ± 2.2 (range 0 – 11 and median 3) medications. Interestingly, a total of 75 different medications were prescribed including antiviral (HIV/AIDS), cardiovascular, central nervous, endocrine, gastrointestinal, respiratory, nutraceuticals (i.e., vitamins, minerals, and nutritional supplements), and other medications with 157 total medications being prescribed for treating conditions of the central nervous system. Collectively, the histories suggest that these individuals experience a tremendous burden of neurological disorders such as cervical or lumbar disc radiculopathies and mood disorders. As detailed, 91.4% reported a history of diagnosed mental health conditions. Of those reported, depression followed by anxiety were the most reported with 62.5% reporting co-occurring depression and anxiety with or without other mental health conditions. The prevalence of mental health issues is substantially higher in the study sample compared to

the U.S. general population. This difference may be due to the lack of psychiatric services for the population at the center resulting in over diagnosis by non-psychiatric physicians. As stated in 2013 NSDUH report, 3.2% of adults had both a substance use disorder and any mental illness and 1% of adults had both a substance use disorder and a serious mental illness (5). Of interest, 24.3% of participants reported a history of hepatitis. However, based upon the expected medication usage for the 28 days, no medications specific to hepatitis treatment were reported. Unfortunately, the study did not investigate if these participants had undergone treatment in the past or were expecting to be treated in the future.

To add to their future health and medical burden, 90% of the participants reported use of nicotine delivery products with cigarettes being the most favored. Caffeinated beverage consumption was high with coffee being preferred. Of note, the center had a coffee maker, tea packets, and soda dispensing machine available for resident use. It may be surmised that the residents are substituting or maintaining use of more socially acceptable stimulants (nicotine and caffeine) while trying to remain abstinent from much harder drugs. Regarding fitness activities, about three quarters of the participants did some form of exercise during the week with weight training being the most preferred. One reason for such a high use is the availability of an onsite recreation room that housed weight training equipment.

Regarding chemical dependency, the participants reported a history of multiple illicit and non-illicit drug use. The most frequently reported number of drugs used was two with a history of alcohol use being the most prevalent (60%); specifically 15.7% reported abuse of alcohol only while 44.3% abused alcohol and another drug; these

findings vary from the TEDS report that stated 41.4% of admissions sought treatment for alcohol abuse (23.1% alchohol only) and 18.3% of those for abusing alcohol with another drug. Reported stimulant abuse was higher than the TEDS reported findings of 17.8% for cocaine and other stimulants. In line with the TEDS report of 20% of those seeking treatment sought treatment for heroin and other opiates, 20% of the sample reported a history of abuse of heroin and opiate drugs.

History of prior melatonin use was surveyed to investigate if more participants were willing to participate that may have had a positive experience of use, in contrast to those not willing to participate who may have had an adverse experience. As reported, almost half of the sample (42.9%) indicated prior use with 100% using it for treating their sleep difficulties, and 70.0% believed it helped. Overall, about 30.0% out of all the participants had a favorable opinion of melatonin at study entrance. Even though it is readily available on store shelves, over half of the participants (53.3%) received melatonin from a clinician mostly in a hospital or rehabilitation center at reported doses of 3 to 10 mg. Interestingly, although there is a body of evidence that suggests that melatonin is efficacious for sleep, there is a paucity of evidence-based literature of its efficacy in this population.

Even though it is difficult to specifically ascertain issues with study masking or efficacy of the intervention, the belief of the participant completing 28 days regarding intervention allocation was assessed by asking “Do you believe you were taking melatonin?” The rationale for the inquiry was because 30.0% of the participants had a positive prior melatonin experience and may have remained cognizant of its effects. Surprisingly, thirty-four participants (70.8%) believed the assigned intervention taken

was melatonin. To provide more evidence of their belief, most of the returned capsule cards were missing the two extra provided capsules suggesting the participants kept the capsules. Because of the high percentage of participants believing the assigned intervention taken was melatonin, twenty-two participants (45.8%) correctly identified the intervention while 26 participants (54.2%) were incorrect. Interestingly, 12 participants (25.0%) who incorrectly identified the provided intervention reported a prior history of melatonin use. It would seem that a prior history of use would suggest the ability to recall similarities between the previous effect and the current effect; however this appeared to not be the case.

Although adherence, as determined by capsule counts, was very high, questions about the true adherence remain. For example, one individual was assessed to Day 21 but left the center, and the capsule card located in the room was untouched. Potentially, this capsule card could have been returned to the investigator devoid of capsules by disposing of the capsules before entering the designated room for Day 28 assessment. Also, the nightly administration of the capsules was the responsibility of the participant and no direct observation of capsule administration by staff or investigators was conducted.

To assess for adverse events, the participants were prompted to report any new symptoms during the past week; however, because of the nature of the participants, they may have underreported events out of the unfounded fear of being withdrawn from the study or being accustomed to not revealing too much information (i.e., incriminate oneself) suggesting a potential reporting bias. Overall, the adverse events were similar between the interventions except for a tendency of melatonin to induce more

nightmares/vivid dreams and next day fatigue/grogginess which are commonly associated with melatonin use. One reason for the increase in next day effects may be attributed to a lack of established bedtime by the study or the center. Therefore, the experimental intervention may have been taken later in the night; thus, the 5 mg dose may have resulted in a carryover effect to late morning. Importantly, melatonin levels are influenced by various classes of drugs (e.g., psychotropic medications) that induce, inhibit, or act as a substrate of CYP1A2 (42). Thus, there exists the possibility that the prescribed medications may have affected the metabolism of melatonin. Even though majority of participants had prior history of melatonin use, they expressed a keen interest and concern about the potential adverse events associated with melatonin, and its potential impact in their recovery.

Both groups resulted in a rapid improvement in measured outcomes from Baseline to Day 28 with the sharpest improvement occurring from Baseline to Day 7. This improvement may be attributed to the individual being in a stable, supportive environment, having access to necessary resources, as well as being provided pharmaco- and behavioral therapies. Because the study incorporated weekly measures in an effort to allow for a detection of an effect of melatonin on a weekly basis instead of at the final endpoint of 28 days, the impact of the effect of melatonin compared to placebo on measured outcomes at each weekly assessment was further investigated by conducting two-way ANOVA (Intervention by Outcome) with the complete cases. In aggregate, no between-group differences were detected (Data not shown). To further support the results of the intention-to-treat analyses as well as to assure that the sample of those completing the study were similar in outcomes (i.e., results not affected by loss to follow-

up), complete case analyses with two-way ANOVA (Intervention by Time) for all outcomes were also performed. Overall, both the intention-to-treat and complete case analyses generated similar results (i.e., no between group differences for all outcomes). Additionally, the proportion of those having a response for each group revealed no significant strength of association between the groups (i.e., confidence interval included null value of 1) considering worst case for the loss to follow-up.

Limitations and generalizability

Because the study was conducted at the Salvation Army Harbor Light Center in Pittsburgh, PA, it was not a multicenter study but a single center study, and the center has a unique approach to treatment where men are cared for physically, mentally, and spiritually in order to allow each man to realize his worth, value, and personhood. Also, the study was age, gender, and geographically restricted to males over the age of 18 who are residents of Allegheny County with a valid Pennsylvania identification; thus investigations into the effects in adolescents, females, or those residing outside of Allegheny County were not possible. Because sampling and enrollment occurred from July 2015 to December 2015, there existed the potential that time of year may have influenced endogenous melatonin duration of action because research has shown that duration of action of endogenous melatonin is affected by the season due to variation in light exposure (32). Thus, future studies may want to consider limiting enrollment to one season. Most of the participants in the study were enrolled within a week to two weeks upon entrance into the center. Thus, there exists the possibility that some of the participants may have been still experiencing the acute phase of withdrawal instead of being in post-acute withdrawal. Unfortunately, the study did not include assessment of

levels of melatonin thus limiting the knowledge of levels of melatonin in the participants at inclusion and upon completion of study. If levels of melatonin were assessed, for example at each follow-up, then a correlation between the levels of melatonin to outcome could be performed. Additionally, the data obtained from assessing levels of melatonin could be used to provide insight into why participants responded or not. Also, the levels would provide additional data for intervention adherence along with the capsule counts. The inclusion and exclusion criteria in the study was broad and did not limit eligibility for type of chemical dependency, mental health status, prescribed medication (e.g., antidepressant, anxiolytic, and/or sedating psychotropic drugs), prior melatonin use, and/or medical conditions. Once accepted into the center, the residents follow a daily schedule that includes group and individual therapy sessions, meals, and activities. However, it must be emphasized that individuals at the center are heterogeneous and present with a complex history of chemical dependency along with potentially co-occurring mental health and medical conditions, limited formal education, societal issues, the episodic nature of symptoms of PAWS, and multiple medication use that adds to the difficulty of studying the effect of melatonin on the measured outcomes. Even though the outcomes measured were conducted with readily available, brief, and valid instruments, a few individuals had requested help during survey completion; thus, more individuals may have had needed help but were self-conscious as not to inquire for help. As aforementioned, the instruments used are valid and reliable for measuring self-reported psychiatric symptoms, the literature reporting the validity and reliability in use with a population in recovery is limited thus adding difficulty of generalizability to this population (74, 75). Any one of these could potentially impact the outcomes (i.e.,

improvement) thereby reducing between-group differences or the potential therapeutic effect of melatonin leading to a possible Type II error, failure to reject the null hypothesis (i.e., accepting the null hypothesis) that is false. Group algorithms and subsequent subgroup analyses were not conducted because of the lack of significant between-group differences as well as the limited sample number thereby these analyses would have been underpowered.

In retrospect, the study would have been aided by the inclusion of a therapy alone arm to assess the effects of therapy alone without the adjuvant addition of the interventions. The milieu of a total therapeutic environment may have accounted for the decrease observed in the measured outcomes independent of the effect of treatment or placebo. While reading the consent form, the participants were informed of the likelihood of being assigned to either the melatonin or placebo group potentially biasing the perception of intervention assignment and the outcomes. Because of the high percentage of belief the intervention taken was melatonin, the participants may have had or developed through further research during participation, ideas of the efficacy and value of melatonin as a treatment. Moreover, spillover effect may have occurred because participants had the potential to freely discuss the effects of the interventions among their fellow residents potentially biasing their perception of efficacy. From observation, the participants were actively aware of the pharmacology and adverse events associated with their prescribed pharmacotherapies. Because of the overall residential and rehabilitative environment of the center as well as the various types of support provided by it, there exists the possibility that participants may be motivated to demonstrate improvement in symptoms over time to maintain residency at the center. This would bias the results by

falsely demonstrating an improvement over time as well as masking any true effect of the experimental intervention, if one exists.

It is difficult to assess the impact of the psychotropic medications on the measured outcomes because the study lacked questions addressing medication indication (e.g., sleep versus mood disorders) and the length of time the individual has been taking the medication. Although the participant histories form asked for current medication and its dosage regimen, it was difficult for a few individuals to recall the list of prescribed medications as well as the dosage regimen. Considering medications such as antidepressants may take weeks before therapeutic effect is observed, there is uncertainty as to duration of therapy and their adherence. The improvement in outcome measures may be result of the efficacy of psychotropic medications thereby reducing between-group differences and/or potentially attenuated or masked the effect of melatonin, if any. Another possibility is that the efficacy is similar between melatonin and psychotropic medications, and the generated data did not suggest a potentiating effect with concomitant use.

The outcomes were measured weekly (Monday or Thursday) and was based on day of enrollment. Analysis revealed no between-group differences comparing those surveyed on Monday or Thursday (Data not shown). Importantly, each designated time frame indicated on the surveys was changed to "over the last seven days" for the follow-up times. This change was implemented to alleviate the influence of recall bias. Because the individuals were assessed with subjective, self-reported measures, there is the potential for recall bias. For example, the individuals may be more acutely aware of how they were feeling on those days closer to the assessment than earlier days. Moreover,

their physical and mental state may have influenced their ability to recall, thus biasing the subjective measures. Self-reported measures, in part, rely on the subjective experiences of the participant and as such bring into question the reliability of such measures. No objective measures were conducted to complement the self-reported measures especially using physiological measures; specifically for example, assessment of sleep with polysomnography, which would have been not feasible at the center, or wrist actigraphy. Thus, no correlations between subjective and objective measures were able to be performed to determine accuracy of the self-reported measures.

Because the participant histories as well as the self-reported outcome measures may not have been completed accurately, the collected data is susceptible to recall bias as well as the mood or willingness/openness of the participant to provide accurate information, and as such, questions remain as to the reliability and validity of the data. However, no significant between-group differences were detected for all outcome measures suggesting that any bias with regards to accuracy were similar. Also, inter-individual variability in the outcome measures may affect the detection of between-group differences. Although underpowered, no between-group differences were detected after conducting additional analyses, excluding those participants with either mild anxiety or depression (Data not shown). A deeper investigation into how the high belief that the intervention taken was melatonin may have influenced the results is warranted.

As aforementioned, previous literature investigating the use of melatonin is limited and suffers from either lack of placebo control, small sample size, or sample consisting of only abstinent alcohol dependent individuals. Because of the limited data, the power analysis was calculated with a small effect size in order to enroll a larger

number of individuals to allow for adequate power to capture a small melatonin effect (> 0.15). Conversely, if larger effect sizes were selected, the number of individuals needed to be enrolled would have been less. Even though there were no significant between-group differences, the mean differences from Baseline to Day 28 of the melatonin group were larger for all outcomes compared to placebo suggesting a potential very small effect of melatonin that may be detectable with a larger "n."

The study lacked a run-in period to help address if exclusions needed to be made; for example, participants not adhering to prescribed medications, potential to disregard study protocol, or at a high risk for loss to follow-up. Even though the power analysis included an adjustment for a predicted loss to follow-up of <20%, the loss to follow-up was 31.4% suggesting the study may be slightly underpowered. Based upon the returned probability values, none of the between-group comparisons closely trended toward statistical significance. Coincidentally, both groups had the same loss to follow-up. The rate of loss to follow-up was consistent throughout the weeks with the highest occurring between Day 14 and Day 21 for both groups. Loss to follow-up due to drug relapse was the highest at 45.5% suggesting the increased difficulty in maintaining abstinent even being in a supportive environment and provided needed resources. Those that relapsed were detected to be under the influence of alcohol, benzodiazepines, heroin, or opiates. Two participants relapsed on benzodiazepines. Strikingly, only four participants self-reported benzodiazepine history. The reason may be attributed to the patient history not directing addressing benzodiazepine use but indirectly through the term "Other." Of those who relapsed, a higher prevalence of heroin, opiates, and marijuana usage, but lower usage of alcohol was reported compared to the sample. Also, mental health

disorders such as anxiety, bipolar as well as being comorbid for ≥2 were more prevalent. Another factor contributing to the loss to follow-up was administrative rule/policy violations possibly due to the center having a close to zero tolerance approach to violations. Melatonin and placebo appeared to be well tolerated and each group had only one individual withdrawing due to an adverse event. The individual lost to follow-up due to adherence with intervention is the same individual who was determined to not be taking the study intervention after discovery of the full capsule card upon leaving the center shortly after completing Day 21. Only the Baseline data was included in the analyses. Unfortunately, one individual who was out on a weekend pass given by the center was discovered deceased on a street in Pittsburgh. The event leading to cause of death was still under investigation at time of study conclusion, but it was suggested that it was a possible drug overdose. Because of the ambiguity, loss to follow-up was recorded as "Death" but not "Relapse." It is unlikely that melatonin may have influenced the potential drug relapse leading to death or death, in general. Relapse rates were the same between the interventions, and melatonin has a high margin of safety. Block randomization with a block size of four was utilized to ensure equal treatment allocation, but a larger block size could have been used to ensure greater unpredictability as to treatment allocation.

Positively, the study met the recruitment and eligibility goal of 70 individuals in a time frame of six months. Unfortunately, a motivating factor may have been the weekly financial compensation. These individuals enrolled at the center tended to be of lower socioeconomic status and without current earning power (i.e., without a source of

income). Therefore, enrollment in the study provided access to funds that allowed them to purchase items such as cigarettes, an unintended consequence.

Future directions

Possible future directions include a study design that is multicenter, the inclusion of a therapy only arm, assessing various doses and timelines, assessing effects in adolescents or females, assessing levels of melatonin at inclusion and study completion, assessing nocturnal levels of melatonin, or limiting inclusion based on prescribed medications, mental health status, medical conditions, prior melatonin use, and/or a specific chemical dependency. Additionally, more clearly defined medication histories could be considered when enrolling participants. Future studies may incorporate a protocol that ensures participants are actively taking the intervention nightly as well as consider establishing a specified bedtime range. If the effect of melatonin is indeed very small, future studies should enroll a larger sample. Also, it is recommended that a future power analysis include adjusting for at least a loss to follow-up greater than 30%. Because of the loss to follow-up was the highest between Day 14 and Day 21 for both groups, a study design considering a run-in may want to include a run-in length of at least two weeks.

Conclusions

Based upon the review of the literature, this is the first and largest randomized, double-blind, placebo-controlled trial assessing the effects of melatonin upon post-acute withdrawal among males in a residential treatment program. The purpose of the study was to assess melatonin as an adjuvant treatment along with their current pharmaco- and behavioral therapy for 28 days on weekly self-reported severity of anxiety, depression,

stress, and sleep complaints as well as how sleep is affecting daily life in a sample of individuals in recovery from chemical dependency at a single, residential treatment site. In summary, the various analyses indicated there exists insufficient evidence to suggest that the melatonin and placebo were significantly different, and it may be concluded, based upon the study sample, design (e.g., inclusion and exclusion criteria) and limitations, the effect of melatonin on the assessed self-reported outcome measures was no different than taking placebo. However, due to the heterogeneity of the participants as evidenced by the participant histories or by chance alone, there exists a possibility of a Type II error that must be considered and not overlooked.

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APPENDIX

Capsule content was independently confirmed by the laboratory of Kevin J. Tidgewell, Ph.D. (Assistant Professor of Medicinal Chemistry, Graduate School of Pharmaceutical Sciences, Duquesne University, Pittsburgh, PA) using high performance liquid chromatography. The laboratory was blinded/masked as to intervention group of capsules provided. Results of analysis is below:

Cap	Area (210)	Height (210)	Area (222)	Height (222)
1	139.9546	71.647	144.1421	78.336
1	139.5083	74.768	145.025	81.618
1	139.2811	75.171	144.4363	82.393
Avg. 1	139.5813333	73.862	144.5344667	80.78233333
25	89.4659	48.61	96.1752	53.47
25	89.1073	50.113	95.9579	55.13
25	90.3399	50.553	96.9673	55.613
Avg. 25	89.6377	49.75866667	96.3668	54.73766667
27	0	0	0	0
27	0	0	0	0
27	0	0	0	0
Avg. 27	0	0	0	0
41	99.1313	55.18	109.4784	60.998
41	98.5686	56.892	109.1269	62.9
41	98.7854	57.271	109.4738	63.352
Avg. 41	98.82843333	56.44766667	109.3597	62.41666667
42	0	0	0	0
42	0	0	0	0
42	0	0	0	0
Avg. 42	0	0	0	0
60	0	0	0	0
60	0	0	0	0
60	0	0	0	0
Avg. 60	0	0	0	0

	210 nm		222 nm			
	Area	Height	Area	Height	**Average/ pill**	**St. Dev./pill**
Cap 1 [c]	67.158 15	64.341 4	63.882 76	64.037 77	**64.85501 86**	**1.547191 5**
Cap 25 [c]	43.057 57	43.371 03	42.789 89	43.471 86	**43.17258 967**	**0.310177 82**
Cap 41 [c]	47.492 61	49.190 59	48.479 55	49.535 51	**48.67456 459**	**0.902322 42**
Average	**52.569 44**	**52.301 01**	**51.717 4**	**52.348 38**		
St. Dev.	**12.827 32**	**10.825 67**	**10.912 84**	**10.567 56**		
Average (all)	**52.234 06**					
St. Dev. (all)	**9.6586 65**					
Average of Averages (J7 - N7)	**52.234 06**					
St. Dev. (J7-N7)	**0.3637 63**					

Results of capsule content in milligrams for melatonin group

41.6 comes from ug/mL melatonin/pill

$$\frac{\textbf{41.6ug/ml}}{\textbf{5mg mel}} = \frac{\textbf{average/ tab}}{\textbf{X}}$$

7.800481 mg of melatonin: Capsule 1
5.192308 mg of melatonin: Capsule 25
5.853365 mg of melatonin: Capsule 41