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**Enhancing Melatonin Secretion: The Methodical Consumption of Tryptophan from Whole
Cow's Milk to Regulate Sleep Quality in Individuals Aged 18-30 with Delayed Sleep-Wake
Phase Disorder**

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Abstract

Young adults in the United States are increasingly affected by DSPD (delayed sleep-wake phase disorder), a prevalent circadian rhythm disorder that delays evening sleep and morning wake times relative to the solar cycle. Although medicinal supplements have shown to produce effective results on immediate sleep induction, they lack the ability to aid in regulation and maintenance of routine sleep schedules. Alternatively, a dietary method may be able to adjust the deficits of supplements. A review of the literature on clinical nutrition and endocrinology suggests that dietary alterations through the timed consumption of tryptophan-abundant whole cow's milk may be an auxiliary option of improving sleep quality and morning alertness in individuals with DSPD. Studies on chrono-nutrition indicate that dietary components absorbed by the bloodstream can alter the circadian schedule of melatonin secretion from the pineal gland, and the timed consumption of tryptophan-abundant foods, such as whole cow's milk, can consequently spike melatonin levels before a DSPD patient's desired sleep time and promote circadian rhythm advancement. Based on the stated studies, this research proposes the MILC (Melatonin Intake through Lactalbumin (α -lac) Consumption) treatment, the consistent, timed consumption of milk. The MILC treatment may decrease a DSPD patient's morning sleepiness on the basis that disordered, high-stressed, and sleep deprived individuals are susceptible to minimal changes in hormones because their bodies naturally attempt to attain homeostatic equilibrium. The correlation between chrono-nutrition and dietary effectiveness is a novel idea, and testing is needed to quantify the optimal timings and ranges of dietary tryptophan that can produce a significant effect on the sleep quality of a DSPD patient.

Keywords: delayed sleep-wake phase disorder, chrono-nutrition, alpha-lactalbumin, tryptophan, sleep quality, circadian rhythm, melatonin, phase shift

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Introduction

Delayed sleep-wake phase disorder (DSPD) is a circadian rhythm disorder in which an individual lacks the ability to fall asleep within a socially acceptable time. The symptoms of DSPD patients are variable based on their individual routines and biological predispositions for sleep disorders. Some display signs of insomnia while others struggle with sleep latency, or the time that it takes an individual to actively initiate sleep at night. On the other hand, some patients lack total sleep efficiency timing, or the total percentage of time that individuals spend sleeping in bed. Others do not struggle with such symptoms of DSPD, but rather are delayed through circumstances such as night shifts and late responsibilities. Among all categories of patients and their symptoms, researchers have found that the greatest overarching problems are morning alertness, drowsiness, inability to focus, and decreased retention.

This sleep disorder can be traced back to the sleep-inducing hormone, melatonin; its secretion from the pineal gland is a determinant of the consistency and extent of circadian offset in sleep-deprived individuals. Those with delayed circadian schedules will encounter fluctuations in hormones, and the pineal gland's secretion of melatonin will be unable to adjust to a regular, timely pattern. The dim light melatonin onset (DLMO), or the timing of melatonin secretion patterns from the pineal gland, will shift in accordance with an individual's average delayed

sleep schedule; at minimum, the DLMO shift will be 2 hours after the socially accepted sleep onset time. This shift determines the extent of correlation between DLMO and sleep timing, and with the large delay for DSPD patients, adjustment is necessary through external, or exogenous factors such as light exposure or overall dietary intake.

Melatonin is *only* released by the pineal gland at night, but it can also be synthesized from the dietary intake of the essential amino acid, tryptophan. Tryptophan conversion can increase melatonin concentrations and induce sleep through the tryptophan–serotonin–melatonin pathway. Consumption of naturally high-tryptophan foods or drinks may advance DLMO timings and increase melatonin secretion levels up to 6 hours after intake. If timed accurately, the high-tryptophan food can be used to enhance sleep quality in DSPD patients by accelerating melatonin secretion *before* the pineal gland starts its delayed release of melatonin. Through this method, the increased melatonin levels can induce deeper sleep and enhance sleep quality in terms of morning alertness.

One specific sleep inducing, high-tryptophan drink is whole cow's milk; it has the highest tryptophan content of all milk types due to its alpha-lactalbumin protein levels. Cow's milk also contains nutrients, such as calcium and potassium, that work through alternative pathways to aid in sleep-induction. Although plant-based and other mammalian-milk types have other defining properties and nutritional contents, cow's milk is largest in terms of tryptophan; it is also one of the highest produced milk types and is widely available to the public. The later proposed MILC (Melatonin Intake through Lactalbumin (a-lac) Consumption) treatment will consist of DSPD patients intaking 1 cup of whole cow's milk at 1-2 hours before their desired sleep time, and this may improve their overall sleep health and morning functionality, even if their circadian schedule does not shift times.

Delayed Sleep-Wake Phase Disorder and Phase Shifting

Because young, delayed sleep-wake phase disorder (DSPD) patients have specific difficulties in regulating their sleep and circadian rhythms to the socially acceptable times, research suggests that the best way to improve their sleep quality would be to manipulate endogenous factors by altering melatonin levels through the intake of tryptophan.

In “Sleep Timing and Circadian Phase in Delayed Sleep Phase,” Chang et al. (2009) claimed that individuals with delayed sleep wake phase disorder have an offset sleep episode later than desired, and this can lead to difficulty in morning functionality and struggles in awakening. They added that individuals have a specialized circadian schedule based on their health, age, and lifestyle, and a delay or shift in sleep schedules based on solar cycles may lead to insufficient and non-restorative sleep when it comes to duration and quality (p. 1). Offset sleep episodes develop based on external shifts from an individual’s preset sleep schedules, suggesting that environmental stimuli may have a greater influence on sleep than genetic predispositions. Individuals with biological tendencies to have declined sleep quality can likely avoid sleep disorders if they maintain a healthy circadian schedule. Others can have circadian rhythm disorders from life circumstances and responsibilities that prevent them from maintaining a consistent sleep schedule. DSPD emerges in either case, but the extent of the disorder differs based on both an individual’s genetic inheritance and environmental influences.

Those with DSPD struggle in terms of long-term physical fatigue and mental functionality, but the symptoms can differ between geographical time zones due to countries having alterations in socially acceptable sleep timings. These differences create a wide range of sleep habits across the globe as determined by Krueger & Friedman (2009) in “Sleep Duration in the United States.” In this cross-sectional population-based study, they reasoned that the sleep

ranges and social habits of individuals in the United States indicate that the average sleep initiation time is 10 PM – 12 AM while wake time is approximately 7:00 AM. On the other hand, DSPD patients are generally unable to fall asleep before 2 AM, and if their schedules permit, they don't naturally wake up until 11:00 AM – 1:00 PM. This wide distribution of sleep times can further be attributed to individual schedules and variable intensity of the disorder.

Krueger & Friedman (2009) noted that sex is a statistically insignificant factor in determining the prevalence of sleep disorders, while age is considered key in understanding the body's ability to adjust to hormonal changes. In "A Global Quantification of "Normal" Sleep Schedules Using Smartphone Data," Walch et al. (2016) concluded that sleep timings and habits are highly dependent and quantifiable based on age because it can reflect the efficacy of the circadian clock. Individuals 55 or older had high sleep habit variability due to their need for a more regular sleep pattern because of decreased hormonal flexibility and narrower ranges of circadian phases; the younger population had a variable bedtime mainly due to higher social pressure and cues to tolerate a lack of sleep (p. 4). Both young adults and the elderly populations were found to have low levels of melatonin, making them susceptible to sleep disorders, but the greatest prevalence is in young adults due to variable ranges in sleep and wake times from unpredictable work, school, and extracurricular schedules. The sleeping disorders in the elderly can be attributed to the compounding adverse health effects that come with the natural process of aging. The age of an individual combined with their biological predisposition can amplify the effects of poor health, high BMI, diabetes, and depression. Keeping these factors regulated can allow the DSPD symptoms to be quantified to determine the extent of treatment that is necessary to adjust DSPD patients' circadian rhythms.

With age being crucial to the body's ability to adjust to stimuli, researchers have performed studies to determine the prevalence of DSPD in the young adult age range. In "Chrono-Nutrition and Diet Quality in Adolescents with Delayed Sleep-Wake Phase Disorder," Berendsen et al. (2020) studied patients with DSPD aged 13-20 due to a 7-16% prevalence of circadian disorders within that age range (p. 1). Micic et al. (2007) added to the validity of that finding in their study of "Nocturnal Melatonin Profiles in Patients with Delayed Sleep-Wake Phase Disorder and Control Sleepers." They determined that young adults between the ages of 20-30 have reduced melatonin secretion; researchers interpreted that DSPD patients fall within that age range due to the delayed hormonal secretory rates (p. 438). Both studies concluded that DSPD patients who need the greatest amount of aid in resetting their circadian rhythm are young adults, and the age controls can be used to quantify the "normal" amount of melatonin secretion and sleep that an average individual should maintain.

To understand the symptoms of sleep delay and abnormal melatonin secretion, Akerstedt & Gilberg (1986) conducted a study on the "Sleep Duration and the Power Spectral Density of the EEG," and they calculated the effects of non-restorative sleep by placing healthy subjects under restricted night sleep schedules. Non-restorative sleep is considered the amount of sleep that individuals have not sufficiently restored, and although this is a subjective measurement, it can be quantified by testing how individuals react to sleep loss. Akerstedt & Gilberg (1986) measured the subjects' daytime sleepiness through the EEG (electroencephalogram) power density analysis, a test that records brain activity and electrical signals through small sensors in the scalp. They found that individuals who were restricted to lower hours of night sleep were increasingly prone to daytime sleepiness, and they required greater recovery time to return to a "normal" level of alertness. Although this test was conducted on healthy subjects, the results can

be applied to DSPD patients who have increasingly restricted sleep schedules because of their consistent non-restorative sleep; thus, they tend to “crash” whenever they get the chance. This crash is an effort to recover sleep loss as described by Akerstedt & Gilberg (1986). Those with sleep loss need minimum power density to be recovered through restorative sleep, but excessively long sleep or napping can yield adverse effects on normal melatonin secretion times. They added that sleep deficit and less recovered percentage can be carried over in individuals, and this can reduce attention in the morning or cause sleep crashing on days off. This extended sleep crash in healthy individuals is a homeostatic attempt to recover a percentage of the prior loss. It can be assumed that DSPD patients generally have compounding sleep deficits and an inability to recover percentage. These effects can multiply because of an individual’s constraints and responsibilities that prevents normal homeostatic sleep recovery. Whenever patients “give in” to their homeostatic sleep struggles, prior loss can be partially recovered through an excessively long sleep, usually during the weekends. At times, extended sleep is necessary because it can slowly shift imbalanced melatonin rhythms to a different time, but when the individual deters back to their abnormal cycles, generally during the weekdays, then their melatonin rhythms shift back into imbalance. If the melatonin rhythms fluctuate between days, then the DSPD patient would have highly imbalanced hormone levels in their body, breeding adverse health effects.

Chang et al. (2009) studied whether there could be a solution that aids with recovery of prior loss. They investigated the exogenous, or externally rooted, causes of DSPD alongside the solar light phases to see if they could manipulate the internal, endogenous factors. In other words, researchers aimed to determine how external conditions could alter internal hormone concentrations, sleep duration, quality, latency, and initiation. They tested patients with DSPD to

determine if advancement of dim light melatonin onset (DLMO) levels can induce phase shifts, a measurement of when the body starts producing its own melatonin when the light is dim; DLMO generally occurs two hours before individuals go to bed. Chang et al. (2009) found that DSPD patients had later sleep-wake times ($p < 0.001$), but duration and efficiency of sleep remained similar. The amount of time the individuals took to go from a full state of alertness to the initial stage of sleep, or sleep latency, remained similar between the DSPD and control groups as well ($p = .5$). They found that DSPD patients were going to bed nearly 29 minutes later on weekends and waking up 50 minutes later as compared to a regular weekday ($p < 0.05$) ($p = .5$). DSPD patients and healthy individuals had similar sleep efficiencies and latencies when placed under unstressed conditions. This finding allowed them to shift the focus of their study to sleep quality, melatonin rhythms, and general alertness because these aspects are highly variable in the disordered. All three factors can be shifted through exogenous changes such as melatonin supplements, solar light cues, or bright light exposure. This modification can initiate the endogenous melatonin secreted from the pineal gland to realign and form a stable temporal relationship to obtain an optimal sleep-wake rhythm.

Although an exogenous shift in circadian phase can occur through light therapy and photic stimuli, researchers are exploring dietary methods as an alternative. In “L-Tryptophan: Basic Metabolic Functions, Behavioral Research and Therapeutic Indications,” Richard et al. (2009) discussed that the hormone melatonin correlates with sleep based on its concentration, production, and secretion from the pineal gland during the dark phase of the solar cycle. An additional way of melatonin production is through its synthesis from its essential amino acid precursor: tryptophan. Manipulating tryptophan levels could be used as a dietary alternative to supplements regarding the increase of melatonin levels and the induction of phase shifts to

improve sleep quality. Researchers emphasized that those with sleep disorders can utilize exogenous factors such as the tryptophan content in food to instigate melatonin production.

The melatonin secretion of young adults can become offset due to social times dependent on their age range, and the continuous shifting of circadian phases can lead to the formation of delayed sleep-wake phase disorder. Research suggests that manipulation of exogenous and endogenous stimuli can shift sleep quality and establish a normal temporal rhythm.

The Effects of Exogenous Factors on the Melatonin Synthesis Pathway

Because tryptophan is the sole precursor of melatonin, an increase in tryptophan intake may alter the overall melatonin profiles and concentrations to boost mood and potentially increase sleep quality through phase shifting.

As previously mentioned, Micic et al. (2015) studied the nocturnal melatonin profiles in patients with DSPD and healthy sleepers to determine how offset circadian timing can contribute to the development of delayed sleep-wake phase disorder. They determined that there was a 2–6 hour delay in the circadian rhythms of the DSPD group as compared to healthy sleepers (p. 437). However, the greatest determinant in sleep health between both types of sleepers was the initial burst of melatonin in the early part of the night and the melatonin production fluctuations throughout the night (p. 446). In “Melatonin Rhythms in Delayed Sleep Phase Syndrome,” Shibui et al. (1999) conducted a study to determine the melatonin rhythms and circadian characteristics of DSPD and healthy individuals over the course of 24 hours. Both Shibui et al. (1999) and Micic et al. (2015) observed that DSPD patients had delayed sleep onset timing and different melatonin peak timings because of their abnormal response to stimuli, but their melatonin level stages, and peaks of secretion remained relatively similar. In terms of total sleep

length, Chang et al. (2009) determined that it was statistically similar between controls and DSPD patients, while Shibui et al. (1999) dictated that DSPD patients had longer sleeps. As mentioned before, patients with DSPD have specific biological rhythms and individual sleep requirements, so differences in findings can be attributed to parameters or the extent of the disorder in an individual. However, in either case, the studies permit the assumption that the pineal gland melatonin secretion in DSPD patients is altered by time, not by functionality.

Delayed melatonin profiles can be a consequence of exogenous phase-shifting variables such as excess artificial light post-solar phase. Although some exogenous variables can aid with realigning the melatonin rhythms of DSPD patients, an excess of blue-light or artificial light at the “wrong” time can negatively affect the dim light melatonin onset (DLMO) secretion times. Micic et al. (2015) asserted that *accurately timed* exogenous phase shifting variables can alter this phenomenon by advancing the circadian clock of DSPD patients, changing the release of nightly melatonin profiles to a desired time (p. 446). Alongside bright-light therapy, dietary intake can be considered an exogenous variable that can produce a change in endogenous hormonal release timing.

Shibui et al. (1999) established a positive correlation between sleep phase markers and melatonin phase markers. Whenever melatonin levels increase during the night, the individual enters a deeper state of sleep, and the lower the melatonin levels, the closer an individual is to an alert state. An average individual has peaks in melatonin secretion from the pineal gland between 2 AM and 4 AM, gradually decreasing afterwards. However, a DSPD individual has melatonin peaks during morning sunrise hours, and if they have a morning time constraint to awaken for, their sleep cycle won't be completed. Not only does this lead to excessive lethargy and decreased alertness from the high melatonin concentrations in the morning, but it can also add to the non-

restorative sleep percentage. This cycle can be combated by advancing the melatonin onset through exogenous factors that can increase melatonin production at a desired time.

To understand the root of melatonin production, Richard et al. (2009) studied the pineal gland, or the circadian center, and they emphasized that the gland secretes melatonin in the darkness to influence neurons and the endocrine system to regulate sleep. The pineal gland solely functions during the dark phase of the solar cycle, and it is inactivated during the daytime, meaning that alternative methods need to be exercised to initiate melatonin secretion. In disordered patients, synthesis of melatonin through dietary consumption needs to occur to counteract the delayed DLMO.

Melatonin can be obtained through the intake and conversion of tryptophan, an essential amino acid that can *only* be obtained from food. Richard et al. (2009) stated that the recommended daily intake of tryptophan is 250-425 milligrams per day, and foods high in tryptophan are beneficial for sleep regulation because they can adjust melatonin levels and diurnal rhythms through conversion (p. 47). Once tryptophan enters the body through food intake, it can enter numerous pathways to create necessary building blocks and molecules in the body. One specific path, called the melatonin synthesis pathway, drives tryptophan to be converted into melatonin through a series of chemical reactions. The tryptophan in the bloodstream travels to the entrance of the brain, or the BBB (blood-brain barrier) in an attempt to enter this highly selective network of blood vessels. Only certain substances are allowed to penetrate the BBB, and tryptophan has a high affinity to enter the barrier and convert itself into 5-hydroxytryptophan (serotonin) by the tryptophan hydroxylase enzyme, a rate limiting step of serotonin synthesis. The activity of tryptophan hydroxylase can only be increased from greater tryptophan availability in the brain, meaning that a rise in tryptophan levels itself can enable the

enzyme to continue serotonin synthesis (p. 48). The 5-hydroxytryptophan then undergoes decarboxylation by removing a carboxyl group and releasing carbon dioxide to become serotonin. The neurotransmitter serotonin becomes acetylated by adding an acetyl group and methylated by adding a methyl group to produce the hormone melatonin (p. 49). In “The Bovine Protein Alpha-lactalbumin Increases the Plasma Ratio of Tryptophan to the other Large Neutral Amino Acids, and in Vulnerable Subjects Raises Brain Serotonin Activity, Reduces Cortisol Concentration, and Improves Mood Under Stress,” Markus et al. (2000) summarized this process by stating that melatonin conversion is dependent on the availability of serotonin, and serotonin is dependent on the availability of tryptophan.

Environmental, genetic, and natural factors can affect serotonin synthesis, but the greatest influence in the process occurs from the net change in tryptophan availability, which can shift its ability to enter the blood brain barrier. Markus et al. (2000) suggested that increasing tryptophan ratio and serotonin synthesis can have a beneficial side effect of boosting mood due to the neurotransmitter’s ability to reflect the effects of antidepressants (p. 1537). Serotonin can also enhance the effects of the parasympathetic nervous system by promoting digestion, metabolism, and relaxation. Stimulation of metabolism could expedite the breakdown of macromolecules into its individual components and advance the speed of further conversions.

Sleep deprivation can negatively pressure and weaken the autonomic nervous system and immune system. To counteract these effects, tryptophan can synthesize melatonin in the gut and positively increase the diversity of the colonic microbiota which endorse protection against bacterial infections, promote vitamin production, and improve the digestion of food. It can be assumed that the greatest benefit of melatonin synthesis would be for high-stressed, sleep

deprived individuals because they would be the most susceptible to treatment since their body naturally desires to move towards homeostatic equilibrium.

To determine the potential of diet-induced tryptophan as a treatment method for DSPD patients, researchers have attempted to use tryptophan to raise the serotonin and melatonin levels in the body, yielding positive changes in sleep quality through relaxation and sharpened morning alertness.

Trp:LNAA Ratio and Carbohydrate-Rich – Protein Poor Diet on DSPD Patients

Because tryptophan (Trp) is an essential amino acid, it must be derived from food and must compete with other large neutral amino acids (LNAA) to generate a high Trp:LNAA ratio, thus increasing serotonin concentrations.

Regarding the melatonin synthesized in the brain, Markus et al. (2000) established the effects of dietary proteins and carbohydrates in altering the plasma ratio of tryptophan as compared to other large neutral amino acids, also referred to as the Trp-LNAA ratio (p. 1536). The concentrations of these amino acids can determine the level of brain serotonin, stress management, depression, and mood. To understand the optimal efficiency of raising tryptophan levels through serotonin synthesis, Richard et al. (2009) studied the interaction of amino acids in the blood brain barrier (p. 48). Once ingested, nearly 95% of the tryptophan that enters the circulatory system is bound to albumin while the rest remains unbound. Unbound tryptophan has a high affinity to enter the BBB, but the bound tryptophan also gets pulled towards the BBB transporter. However, it must compete with neutral amino acids in order to be accepted by the transporter (p. 48). All competing amino acids take part in competitive inhibition to be transported across the barrier and contribute to the Trp:LNAA ratio gradient; the more

tryptophan available to compete, the greater the chance that it will enter the BBB and continue its path towards serotonin synthesis (p. 48). Due to this competition, it is optimal for tryptophan levels to be abundant, thus having a greater opportunity of entering through the transporter.

Addressing tryptophan levels, Richard et al. (2009) emphasized that one of the best ways to increase tryptophan ability in the brain can be through the increased consumption of carbohydrates and decreased consumption of proteins. These macromolecules do not immediately change the Trp:LNAA ratio, but rather contribute to the levels of amino acids circulating in the bloodstream that are available to compete. Specifically, carbohydrates decrease the LNAA concentrations and increase the tryptophan levels, while proteins increase the LNAA concentrations and decrease the tryptophan levels (p. 49). Proteins can deplete plasma tryptophan concentrations and decrease the Trp:LNAA ratio through increased peptide chain formation from protein synthesis. This process produces a net increase in the LNAA concentrations (p. 51). While proteins increase tryptophan levels, they also raise competing amino acids levels, thus negating the effects of creating a net change in the Trp:LNAA ratio. On the other hand, carbohydrates generally have a sedative property because they enable the body to absorb the tryptophan without increasing the LNAA levels. A balance of both macromolecules needs to be achieved for obtaining tryptophan (proteins) and converting tryptophan (carbohydrates) into serotonin, otherwise an excess of either molecule can exhibit adverse health effects on an individual's BMI. Evidence on tryptophan intake has shown positive health effects, but Richard et al. (2009) noticed some negatives on how tryptophan depletion can affect brain functionality. Depletion resulted in reduced neural serotonin release, decreased mood, worsened memory, and lethargy in animal subjects (p. 52). These side effects were a result of *only* tryptophan

manipulation, proving the importance of the amino acid in mental, physical, and emotional processes.

To obtain a deeper understanding of the Trp:LNAA ratio and its relationship to serotonin synthesis, Markus et al. (2000) studied how diet-induced tryptophan might be able to raise the brain serotonin levels. In accordance with Richard et al. (2009), Markus et al. (2000) acknowledged that brain serotonin synthesis positively correlates with tryptophan intake through dietary components such as carbohydrate-rich and protein-poor foods (CR-PP) (p. 1536). Markus et al. (2000) further elaborated by studying the effects of the CR-PP diet on stress-vulnerable subjects, concluding that high-stressed individuals will have decreased cortisol secretion when placed on a CR-PP diet (p. 1537). Cortisol, the primary stress hormone, is correlated with high-stress individuals such as DSPD patients who have both mental and homeostatic stress because of their sleep depletion. Tryptophan may be able to generate substantial sleep-inducing effects by synthesizing serotonin in high-stress, highly susceptible DSPD patients. Further, a CR-PP diet may be able to boost their Trp:LNAA ratio while increasing brain serotonin synthesis to boost mood and reduce stress.

According to research by Richard et al. (2009), proteins are generally known to decrease the Trp:LNAA ratio due to their tendency to increase protein synthesis and raise the levels of competing amino acids. However, Markus et al. (2000) reported that certain tryptophan-abundant foods are proteins, and they wanted to test whether the high quantity would be enough to negate the balance of amino acids and raise the Trp:LNAA ratio. If the food can overcome this block, then serotonin and melatonin synthesis would be able to proceed effectively. The researchers focused on this theory to determine how the dietary consumption of proteins with high tryptophan behaved as compared to the CR-PP diet. Intaking carbohydrates in excess is

impractical to the health of the body, and a balanced diet with proteins, in theory, can aid a DSPD patient in terms of sleep quality. Markus et al. (2000) narrowed down a particular protein with a high tryptophan concentration known as alpha-lactalbumin (a-lac). A-lac has qualities that are conducive to serotonin synthesis, and its tryptophan content may be able to overcome competitive transporter blocks, making it a potential aspect of enhancing sleep quality.

DSPD patients can be considered high-stress individuals because their bodies undergo constant homeostatic stress from sleep deprivation, making them more susceptible to treatment options, and the intake of a-lac could be used as a natural, dietary method to alter their melatonin concentrations.

Correlation Between the Trp:LNAA Ratio and the a-lac Content of Whole Cow's Milk

Because alpha-lactalbumin is a high-tryptophan protein found in large quantities in whole cow's milk, it can prove beneficial for highly susceptible, sleep-deprived individuals to increase their serotonin synthesis and decrease cortisol levels through the adjustment of the Trp:LNAA ratio.

Similar to Richard et al. (2009), Markus et al. (2000) asserted that proteins generally decrease the Trp:LNAA ratio while carbohydrates increase the Trp:LNAA ratio. However, Markus et al. (2000) highlighted one notable exception to the CR-PP rule: alpha-lactalbumin (a-lac). A-lac is a whey protein that has the highest tryptophan levels of all bovine proteins with approximately 4.8g/100g tryptophan. Researchers tested a-lac's success at raising the Trp:LNAA ratio despite it being categorized as a protein with high quantities of competing amino acids. In "Chronic Administration of Bovine Milk-Derived α -lactalbumin Improves Glucose Tolerance Via Enhancement of Adiponectin in Goto-Kakizaki Rats with Type 2 Diabetes," Yamaguchi &

Takai (2014) determined that a-lac constitutes nearly 3.5% of the total proteins in bovine milk, and one cup of whole cow's milk (237 mL) contains 284 milligrams of alpha-lactalbumin and 100 milligrams of tryptophan (p. 406). These quantities are relatively high, accounting that one cup of milk nearly contains half of the daily recommended amount of tryptophan. Yamaguchi & Takai (2014) noted that other mammalian milks contain alpha-lactalbumin, but the greatest quantity of tryptophan in terms of bovine milk would be in whole cow's milk. Not only is cow's milk highly accessible, but it also has a greater fat content that the body can utilize to create hormones that stimulate digestion and sleepiness. It is also a nutritiously dense option with essential nutrients such as calcium, potassium, vitamins, and phosphorus. DSPD patients generally lack the nutritious contents of a healthy, balanced diet because their sleep-wake timings inhibit a consistent dietary schedule. The small addition of milk into their diets could improve their overall health and push them towards easier, deeper sleep. The intake of milk can have its reservations because the majority of the world's population is lactose intolerant, and this proposition of drinking milk only applies to those who do not have this dietary restriction. However, plant-based milks, such as almond milk, could be an alternative solution due to nuts being a naturally high source of tryptophan as well. Further testing and research is needed to determine the effectiveness of other milk alternatives in lactose intolerant individuals.

To expand on the molecular components of cow's milk, Zeng et al. (2014) studied "Strategies of Functional Foods Promote Sleep in Human Being," and they found that milk intake can stimulate serotonergic activity and melatonin synthesis to induce enhanced sleep. Whole cow's milk is enriched with omega-3, polyunsaturated fats, calcium, and potassium; these molecules can affect sleep based on the pathways that they enter and the products they form in the bloodstream (p. 148). High levels of omega-3 correlate with increased conversion of

serotonin into melatonin (p. 148). Similarly, polyunsaturated fatty acids are positively associated with increased sleep efficiency and REM (rapid eye movement) sleep (p. 148). Calcium and potassium have the ability to promote sleep because of their role in the modulation of voltage-dependent channels that generate slow waves and sleep spindles (p. 152). Slow waves occur in NREM stage 3 sleep, also known as deep, delta sleep, in which individuals are difficult to awaken. The deeper the sleep, the more substantial the restorative process in DSPD patients. In context to the overarching problem, milk has the components available to work on different aspects of sleep; when consumed by a DSPD patient with sleep deprivation and excessive lethargy, the milk can enter pathways and synthesize hormones that can regulate a healthier circadian rhythm.

Although milk contains qualities that can be used to synthesize serotonin and boost mood, and Markus et al. (2000) measured the extent to which mood, digestion, and stress levels were affected by a-lac intake in vulnerable individuals. When testing subjects with various intensities of stress tolerances, the low-stress control group received casein protein, a protein high in large neutral amino acids, while the high-stress experimental group received a-lac whey protein (p. 1537). Markus et al. (2000) gathered university students aged 17-34 as their optimal test subjects because of their high vulnerability to stress, while the control group consisted of low-stress students with a mean age of 20.9 (p. 1537). The highest prevalence of DSPD patients also fall within this age parameter, and many of them are considered high stress regarding both circumstance and sleep health. Results from the researchers' study indicated that the high-stressed, high-cortisol test subjects were exceptionally prone to the effects of alpha-lactalbumin in raising their plasma Trp:LNAA ratio; there was a statistically significant 48% increase in the ratio for those who were on the a-lac diet as compared to the casein diet meaning that the high a-

lac content was successful in increasing the tryptophan concentration in the BBB (p. 1539). The experimental a-lac diet also boosted mood and reduced cortisol response in HS (high stress) individuals as compared to the LS individuals (low stress) when exposed to stressors (p. 1541). Before conducting the experiment, the HS group had greater serotonin breakdown, creating a rise in cortisol levels as a biological response to stress, and this catabolism bred depression. However, when raising tryptophan availability in the brain using a-lac, the HS group resulted in decreased depressive moods because of greater serotonin synthesis rather than breakdown (p. 1542).

Although a-lac was found to reduce stress from the tryptophan to serotonin conversion, researchers wanted to further investigate whether the serotonin effectively converted into melatonin from a-lac. Using the results of their previous study as a basis, Markus et al. (2005) conducted a double-blind, placebo controlled, follow up study on “Evening Intake of Alpha-lactalbumin Increases Plasma Tryptophan Availability and Improves Morning Alertness and Brain Measures of Attention.” The experimental group consisted of poor sleepers who had reduced alertness and cognitive deterioration, and the researchers claimed that the amplitude of these symptoms could be reduced through the regulation of tryptophan and serotonin from a-lac. When 4.8g/100g a-lac was consumed in the evening, there was a 130% rise in the plasma Trp:LNAA as compared to the placebo casein diet. Furthermore, all a-lac experimental subjects, specifically those who had the greatest initial sleep complaints, reported less sleepiness, increased alertness, and vigilance the following morning (p. 1026). This study by Markus et al. (2005) placed emphasis on poor sleepers, similar to DSPD patients, and a spike in Trp:LNAA levels resulted in increased attentiveness due to greater restoration of deep sleep through a-lac intake. The tryptophan from a-lac was successfully converted to serotonin and melatonin,

justifying that the protein successfully works on the melatonin synthesis pathway. As previously mentioned, the greater amount of melatonin circulating in an individual's system, the deeper the sleep quality, and this directly applies to DSPD patients. Even if DSPD patients are not changing the total amount of sleep that they receive due to time constraints, they can change the *quality* of their sleep to reach a greater depth for morning functionality. A notable find by Markus et al. (2005) was that an even lower intake of a-lac with minimal tryptophan levels can also show similar effects of sleep improvement in high-stressed or sleep deprived individuals because they are susceptible to minimal changes (p. 1032). Allowing DSPD patients to have greater a-lac in their diet could potentially improve their sleep because they are considered sleep deprived *and* high-stressed individuals from the high cortisol levels and sleep stress. If a minimal change can raise the melatonin levels in their system even slightly, then a diet proposal may be a feasible treatment plan.

Follow-up studies were conducted on a-lac's ability to raise the Trp:LNAA ratio as compared to other macromolecules. In "Alpha-lactalbumin Combined with a Regular Diet Increases Plasma Trp-LNAA ratio," Beulens et al. (2004) performed a study where subjects either received a carbohydrate only drink or an alpha-lactalbumin + carbohydrates drink; they found that the group that only consumed carbohydrates had a 17% decreased ratio, while the a-lac + carbohydrate group had a 16% increased Trp:LNAA ratio (p. 585). This finding justifies that the optimal diet to increase the Trp:LNAA ratio and raise melatonin levels in DSPD patients would be a balance of both carbohydrates and a-lac protein. Similarly, in "Acute Effects of Breakfasts Containing Alpha-lactalbumin, or Gelatin with or without Added Tryptophan, on Hunger, 'Satiety' Hormones and Amino Acid Profiles," Nieuwenhuizen et al. (2009) studied how the intake of a-lac protein and tryptophan separately compete in raising the Trp:LNAA ratio.

Subjects were given alpha-lactalbumin, gelatin without tryptophan, or gelatin with added tryptophan to calculate specific amino acid concentrations within the total plasma amino acid concentrations. The researchers found that the alpha-lactalbumin group had the highest Trp:LNAA ratio, and this diet also kept subjects satiated longer than the other two groups. Since the a-lac group raised the ratio better than purely tryptophan, then it can be assumed that the a-lac has more qualities in addition to tryptophan that allows it to enter the BBB efficiently.

Regarding supplements, it is possible that supplemental pills may be able to aid with sleep health, however they come with reservations and unwanted side effects such as nausea, irritability, reduced alertness, disorientation, and drowsiness. However, milk is a natural method of obtaining these amino acids and synthesizing hormones through various metabolic pathways, decreasing the potential for negative side effects; milk also contains essential vitamins and nutrients that the body can metabolize for enhanced sleep and bone growth, while the supplements simply contain one essential amino acid for specific purposes. Tryptophan can enter many pathways itself, but only one pathway is available for its conversion into melatonin. On the contrary, milk contains tryptophan + alpha-lactalbumin along with its numerous nutrients, and this combination is most favorable for sleep quality and health in vulnerable DSPD patients.

The studies mentioned above include different parameters and testing conditions, yet all reached the same conclusion that alpha-lactalbumin consumption yields the highest Trp:LNAA ratio, thus increasing sleep quality through melatonin production.

Changes in Sleep Quality Through Chrono-nutrition

Because research suggests that the effectiveness of dietary components can be associated with intake time, then milk consumed within 2 hours of a DSPD patient's desired sleep time

could yield a net improvement in sleep quality and morning functionality on the following day because of the patient's susceptibility to changes in bodily hormones.

Richard et al. (2009) briefly introduced the idea that tryptophan availability and serotonin synthesis efficiency can be impacted through the timing of consumption. The macromolecules in foods can break down and enter different synthesis pathways depending on their homeostatic necessity at that specific time. In "Chrono-Nutrition and Diet Quality in Adolescents with Delayed Sleep-Wake Phase Disorder," Berendsen et al. (2020) researched the phenomenon of timed dietary intake, or chrono-nutrition, and diet quality in adolescents with DSPD to measure the conjoined effect of diet and timed consumption to create a circadian balance (p. 1). They experimented on relatively healthy DSPD patients aged 13 to 20 years due to a 7-16% prevalence of DSPD within the age range (p. 1). In the study, the researchers noticed that sleep disordered individuals consumed their first food much later in the day when compared to controls, and they generally skipped breakfast due to abnormal wake times (p. 5). Additionally, the Eating Choices Index (ECI) score, or the measure of the healthiness of a diet, was lower for DSPD patients due to their poorer diet quality, contributing to the development of a higher-than-average BMI (p. 7). Individuals who have decreased sleep duration tend to have lower leptin levels and increased ghrelin levels, meaning that there is decreased satiation and increased hunger; researchers noted that this was associated with adverse health effects and a high BMI (p. 8). Zeng et al. (2014) added that sleep restrictions can overstimulate the hypothalamus, the brain region that is sensitive to food stimuli, to decrease leptin circulation and increase ghrelin concentrations (p. 152). In "Food selection changes under stress," Zellner et al. (2006) connected that increased cortisol and ghrelin levels cause individuals to stray from healthy, low-fat foods to high-fat foods; the fat content tends to make individuals feel emotionally secure. In application

to DSPD patients with abnormal circadian schedules and decreased sleep durations, unhealthy foods tend to stimulate their hunger hormones, making them susceptible to having a high BMI. In DSPD patients with high cortisol levels, their hypothalamus will order increased secretion of ghrelin to address poor sleep health with increased food intake. Increasing cortisol levels is a sympathetic response that causes a domino effect on decreasing digestive powers and increasing ghrelin. Since the digestive juices are not functioning at potential due to high cortisol, the food intake will have a high chance of being stored directly to fat. All these researchers similarly concluded that disruptive sleep schedules can increase ghrelin levels and contribute to adverse health effects. Shifting mealtimes to “normal” times of food intake, *combined* with milk intake, could contribute to advancing the biological clock and regulating the circulatory concentration of these hunger hormones (p. 9).

Analysis of the experiment led Berendsen et al. (2020) to conclude that the *consistent* addition of breakfast for DSPD patients could stimulate the chrono-nutritional clock to initiate digestion earlier in the day; this may positively impact the BMI, sleep health, and metabolism of a sleep disordered patient. Consistency is key in chrono-nutrition, and changing a diet or drinking milk for a single day will not exhibit immediate results. A DSPD patient should establish a routine schedule for the body to adjust to this new change, and results will only manifest with time. In contrast to these findings, Richard et al. (2009) inferred that ordinary changes in diet and eating times are unlikely to produce significantly noticeable changes in the behavior of a healthy individual (p. 50). DSPD patients are generally considered healthy apart from their sleep schedule which can nurture future health problems; thus, the statement by Richard et al. (2009) cannot necessarily be assumed for DSPD patients who are prone to negative health problems due to high stress and sleep deprivation. Researchers tend to dismiss results if

there is an absence of statistical significance, but the findings for DSPD patients cannot be easily disregarded because patients are highly susceptible to even the smallest changes in hormonal levels as stated by Markus et al. (2000) (p. 1542). In their study, the results were statistically insignificant, yet the DSPD patients reported a boost in sleep quality and an increased Trp:LNAA ratio (p. 1542). Since the subjects expressed a net improvement in their sleep quality and morning vigilance, then statistical numbers cannot always represent the subjective feelings and findings.

Regarding the previously mentioned idea of chrono-nutrition, Markus et al. (2005) found that the consumption of a-lac in the evening can be timed properly to peak at specific times and induce sleep (p. 1032). They determined that the Trp:LNAA levels reached an apex at 3 hours after the initial consumption of a-lac, and the effects settled down to a base rate 5 hours after intake; within that interval, the DSPD patients reported better sleep quality due to a deeper, REM sleep stage, and they had greater morning alertness on the following day. As stated before, supplements can have excess side effects if they are not taken at an exact time, but the same cannot necessarily be stated with milk. Milk has quantities of a-lac and tryptophan that are high in comparison to other foods, but not enough to create nausea or other side effects when taken at the wrong time. Yet, DSPD patients should aim to drink the milk approximately 1-2 hours before they plan on going to sleep because their melatonin will begin to surge during that time, allowing them to have a shorter sleep latency and maximum sleep efficiency. The following proposal of the MILC treatment mainly intends to enhance the sleep *quality* of DSPD patients, but phase advancement would be an alternative benefit. It is indeterminate whether milk is substantial enough to phase shift the circadian rhythm to a desired time, but it can allow the body to adjust accordingly.

Some individuals have schedules in which they cannot shift their schedule to a desired time because of life constraints, hence this method can mainly enable them to improve the sleep that they originally receive rather than advance their circadian schedules to an alternative time. Chrono-nutritional differences in young adults with DSPD can alter how the molecules from foods can be synthesized. In the evening, the nutrients and macromolecules obtained from milk can be utilized towards synthesizing melatonin as a preparation for sleep, working in conjunction with the night activated pineal gland.

Subjective Testing and Experimental Proposal for the MILC Treatment

Because there is subjectivity in testing for sleep quality, multiple quantifiable tests, individual ratings, and sleep EEGs are required to measure the sleep activity and depth of sleep to create an overall interpretation of sleep quality in DSPD patients.

Patients with DSPD have specific definitions of a healthy, restorative sleep because of biological predispositions, but most generally border normal-high BMIs because of their susceptibility to unhealthy lifestyles. To quantify a patient's health and diet quality, Berendsen et al. (2020) referred to the Dutch Healthy Diet (DHD) index that places exercise, food quality, and drinks on a scale (p. 3). Although this scale cannot quantify exact eating patterns in respect to time and chrono-nutrition, it still gives input on the health habits of DSPD patients. Information on chrono-nutrition and alpha-lactalbumin are scarce, but researchers are expanding on the idea that chrono-nutrition and intake of a-lac can conjunctively alter an individual's sleep quality. To verify this connection between tryptophan, sleep, and chrono-nutrition, Markus et al. (2005) tested 14 subjects who were considered "good sleepers" and 14 subjects who were considered "poor sleepers" (p. 1027). The morning after providing the subjects with evening a-lac, Markus

et al. (2005) measured the effectiveness of the protein on sleep quality by subjectively asking the participants to rate their alertness levels on the Stanford Sleepiness Scale (p. 1027). Another alertness quantification method was through physical and mental challenges in which the participants completed continuous performance tasks (CPT) to determine their agility, accuracy, and speed (p. 1027). As a last form of data quantification, the researchers performed EEGs and event-related potential (ERP) tests to analyze the eye-movement signals and deep sleep stages. These alertness and sleep tests were performed to acquire an average, quantifiable measurement of sleep quality, and in future follow-up studies, the same tests should be performed to conduct baseline comparisons.

In “Nocturnal Melatonin Profiles in Patients with Delayed Sleep-Wake Phase Disorder and Control Sleepers,” Micic et al. (2007) evaluated the rates of melatonin secretion from the dim light melatonin onset (DLMO) to acrophase, or the peak of the melatonin rhythm, based on entraining cues that can adjust the biological clock (p. 443). Exogenous light cues can delay or advance the melatonin profiles depending on the intensity of the cues. DSPD patients generally have slow, delayed melatonin secretions from the pineal gland, so Micic et al. (2007) suggested that increasing the melatonin concentration around DLMO could advance the daily circadian timing of an individual by 0.2 hours per day, resulting in slow sleep changes over time (p. 445). In the MILC treatment, one cup of whole cow’s milk could promote melatonin secretion and advance the DLMO time to shift an individual’s circadian rhythm. In, “Can tryptophan supplement intake at breakfast enhance melatonin secretion at night,” Nagashima et al. (2017) expanded on the effects of exogenous factors in relation to sleep health. They determined that bright light exposure during the early morning hours could train the body to phase shift the circadian rhythm, advancing DLMO and nocturnal melatonin secretion. Such exogenous factors

can be an additional source of phase shifting, and drinking milk alongside this treatment could generate an efficient endogenous response.

A follow up study needs to be conducted to test the efficacy of the MILC treatment, consisting of DSPD patients within the ages of 18-30 drinking approximately one cup of milk 2-3 hours before they plan on sleeping; this should be consistently timed in the evening for an extended period for results to show. Regarding test subjects, they should be considered high-stressed based on The Perceived Stress Scale (PSS) in which individuals' stress levels are categorized on subjective life situations and how overwhelmed they are with those situations. Researchers should determine the length of the study depending on parameters, but control and experimental groups should have the same diet quality based on the Dutch Healthy Diet (DHD) index. The experimental group should have a set milk intake time in the evening, preferably 2 hours before the subjects go to sleep. The lumen and amount of light exposure for the test subjects should also be kept similar because external exogenous factors can negate the effects of milk intake. To obtain results, the Trp:LNAA ratio must be measured in individuals before, during, and after the experimental manipulation of chrono-nutrition and milk consumption. As for melatonin concentrations, the DLMO profiles may be assessed through the collection of saliva to evaluate the effectiveness of the endogenous circadian rhythm and the pineal gland melatonin releases. A sleep EEG can be used to determine the sleep rhythms and stages of sleep that an individual experiences over the course of the night. To acquire a combined, quantifiable result, standardized sleep scales and deep sleep brain waves should be utilized to determine sleep quality in patients with DSPD. Certain variables such as individual biological predispositions on health need to be considered to determine a statistical range for error. Alterations to the MILC

treatment, such as adjusting the temperature of milk, can possibly affect its sleep inductive qualities and affinity to the BBB, but further research is needed on the subject.

The MILC treatment suggests that a potential solution for DSPD patients' sleep quality is the ingestion of one cup of whole cow's milk to raise melatonin levels at night and induce deeper sleep. Considering chrono-nutrition, high-stress individuals who consistently time their high-tryptophan diets can yield the greatest increase in their Trp:LNAA ratio, enabling melatonin synthesis to effectively enhance agility and morning alertness.

Discussion

Individuals who maintain a relatively healthy lifestyle and diet, even when struggling with an unbalanced sleep schedule, can counteract negative symptoms through small alterations in their lifestyles. Consider DSPD patients who are high-stressed and unhealthy in terms of both sleep and diet; these adversities inhibit internal regulation of hormonal concentrations and pave a path for serious illnesses. At the core of treating their disorder, these individuals need to obtain a balance of the sleep hormone, melatonin, to rise and fall at set times. To achieve this goal, the MILC treatment acts upon the concept of chrono-nutrition, or timed consumption, to raise the chances of molecular components entering a specific synthesizing pathway. For this theoretical treatment, DSPD patients will consistently consume 1 cup of whole cow's milk at a predetermined time based on individual needs. Over time, the individuals should sense an improvement in morning alertness and enhanced sleep quality, averting negative health effects. Synthesizing melatonin and combating an altered circadian schedule through milk can be a non-supplemental method to naturally reestablish a healthy sleep cycle. Although consumption of milk once will not establish results immediately, a consistent sleep, diet, and exercise schedule is

crucial in efficiently regulating the body. Body types can contribute to an individual's susceptibility for the MILC treatment, but further research is needed to determine and adjust specific quantities of milk and timing of consumption. This potential treatment is meant to be an alternative method to supplements, and it can *only* work if an individual does not hinder the process through unnecessary exposure to delaying exogenous factors. A DSPD patient needs to be receptive towards alternative treatment methods that target specific symptoms, and the only way to figure out the best needs for a specific individual is through trial and error.

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