OPTIMIZING EXO-MEL ADMINISTRATION TO ENHANCE SLEEP PROMOTION: A RANDOMIZED DOUBLE-BLIND TRIAL

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Original Research Protocol: “OPTIMIZING EXO-MEL ADMINISTRATION TO ENHANCE SLEEP PROMOTION: A RANDOMIZED DOUBLE-BLIND TRIAL”

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The study is coordinated by the Sleep Laboratory (Sonnolab) – Institute of Physiology, University of Pisa.
Prof. Ugo Faraguna is the scientific lead.

Collaborators at the University of Pisa include: Prof. Paola d'Ascanio, Dr. Simone Bruno, Dr. Francy Jurany Cruz-Sanabria, Dr. Maria Paola Tramonti Fantozzi, and Dr. Francesco Daddoveri. External collaborator: Dr. Marco Di Galante.

The scientific lead will train and authorize researchers to collect or analyze pseudonymized data.
Thesis students and/or interns from the University of Pisa working under the supervision of the above-mentioned faculty member will also be trained and authorized at a later time, as will any additional collaborators.

Title of the Study

OPTIMIZING EXO-MEL ADMINISTRATION TO ENHANCE SLEEP PROMOTION: A RANDOMIZED DOUBLE-BLIND TRIAL

Background and Rationale

Several epidemiological studies show that sleep disorders are present in about one-third of the adult population. Difficulties in falling asleep and frequent nighttime awakenings (symptoms related to insomnia) are the most common. Consistently, among the sleep disorders recognized by the International Classification of Sleep Disorders (ICSD), insomnia is the most frequent and is associated with a wide range of conditions such as cardiovascular and metabolic diseases, impaired immune function, cognitive dysfunction, and psychiatric disorders.

Approximately 60% of adults treated for insomnia-related symptoms are prescribed sedative and/or hypnotic drugs. In the past decade, the prevalence of sleep disorders and insomnia diagnoses has increased significantly. Along with this rise, there has been

an even greater increase in the volume of hypnotic prescriptions (benzodiazepines (BZ), non-benzodiazepines (Z-drugs and ramelteon)). Due to the side effects and risk profiles associated with these medications, efforts have been made to reduce the prescription of hypnotics by healthcare providers.

Although a decrease in hypnotic prescriptions was observed between 2008 and 2015, insomnia symptoms remain a major public health issue, highlighting the need to optimize current treatment options.

Available substances for insomnia treatment include BZs and benzodiazepine receptor agonists (BZRAs), antidepressants, antipsychotics, antihistamines, herbal products, and melatonin. Among these, melatonin stands out as a promising option, with several studies reporting its efficacy in improving sleep and its favorable safety profile, even at high doses. Its chronobiotic action is widely recognized, given its ability to synchronize circadian rhythms. However, its role as a sleep-promoting agent remains debated due to inconsistent findings. Many potential sources of heterogeneity in experimental design—such as dose and timing of administration—may explain these contradictory results. A systematic investigation of these parameters is essential to understand melatonin's role in sleep promotion and insomnia treatment.

Several meta-analyses have assessed melatonin’s efficacy in treating primary sleep disorders. These studies have shown that it reduces sleep onset latency (SOL), increases sleep efficiency (SE), and extends total sleep time (TST) across various clinical conditions, including insomnia, schizophrenia, Alzheimer’s disease, and in healthy volunteers.

Similarly, melatonin has been found to be more effective than placebo in reducing SOL in primary insomnia and in Delayed Sleep-Wake Phase Disorder (DSWPD), and in regulating sleep-wake patterns in blind individuals. However, other meta-analyses indicate that SOL reduction is less significant in primary insomnia compared to DSWPD, suggesting that melatonin’s effectiveness may vary by diagnosis—being more effective as a chronobiotic than as a sleep-promoting agent.

None of these studies reached definitive conclusions about optimal treatment parameters. Only one meta-analysis investigated dose-response effects, showing that longer treatments (7 to 126 days) and higher doses (0.5–5 mg) are associated with greater effects on SOL and TST. That study was a step forward in identifying effective dosing for melatonin in clinical contexts, but the findings need replication in less heterogeneous samples (e.g., adult vs. pediatric populations). Moreover, the influence of important parameters such as timing of administration—which may affect effectiveness—was not evaluated.

For instance, it is known that the sleep-promoting action of exogenous melatonin is strongly influenced by the phase of endogenous melatonin secretion. Yet, the authors did not analyze how the time of day when melatonin is administered might have affected its impact on sleep parameters.

Finally, a recent meta-analysis conducted by our research group suggests that administering 4 mg of melatonin three hours before bedtime maximizes its effectiveness. This is not the treatment schedule currently suggested by manufacturers (i.e., 1–2 mg close to bedtime). If confirmed by clinical studies, these findings could lead to a reevaluation of melatonin's role as a treatment for sleep disorders.

Study Objectives

Primary Objective
To assess the effect of melatonin administered at a dose of 4 mg three hours before the desired bedtime compared to 2 mg administered at bedtime.
The primary outcome of the study is sleep onset latency.

Secondary Objectives
Additional outcomes considered indicators of good sleep quality will include:

- Total sleep duration
- Sleep efficiency
- Sleep continuity
- Sleep micro- and macroarchitecture

Study Population

Participants will be healthy volunteers recruited from among the staff and associates of the sleep laboratory (Sonnolab) of the Institute of Physiology, and their acquaintances, after providing written informed consent to participate in the study.

For the sample size calculation, we assume a difference (effect size) between the group receiving 2 mg of melatonin at bedtime and the group receiving 4 mg of melatonin three hours before bedtime equal to 0.54 (Standardized Mean Difference) with a within-group standard deviation of 20.41 minutes. Given these parameters, setting the alpha error at 0.05 and the power at 90%, a sample of 12 participants is needed to detect the indicated effect size as statistically significant. Accounting for a 20% dropout rate, the expected sample size for this study is at least 16 participants.

Inclusion Criteria

- Both genders (50-50 distribution)
- Age between 18 and 45 years
- Fluent in the Italian language
- Review and signing of the informed consent form and the privacy policy

Exclusion Criteria

- Diagnosis of sleep disorder or circadian rhythm disorder
- Diagnosis of neurological, psychiatric, or internal medical conditions that may interfere with sleep
- Ongoing treatment with medications that may interfere with sleep, including hypnotics or melatonin
- Travel to a country with at least a two-hour time zone difference in the two months preceding or during the study
- Shift work
- Confirmed pregnancy
- Breastfeeding

Study Design

All participants who agree to take part in the experiment will be asked to complete a screening questionnaire to verify inclusion and exclusion criteria, as well as to provide general demographic and lifestyle information. Participants will also wear a wrist actigraph for one week to confirm their reported sleep habits through objective data (see detailed list in the appendix).

Each participant who meets the eligibility criteria will receive, in randomized order, the following melatonin administration conditions:

- 4 mg three hours before the desired bedtime
- 4 mg at bedtime
- 2 mg three hours before the desired bedtime

- 2 mg at bedtime

This will follow a Latin square crossover design. Each administration will be separated by at least one week to ensure a complete washout of the supplement. In total, each participant will receive four doses of melatonin (12 mg total) over approximately one and a half months.

Sleep will be monitored using an EEG Holter device during the night following treatment administration. After the device is set up in the sleep lab at the Institute of Physiology, participants will be allowed to return home and sleep in their usual environment. Although EEG Holter is a non-invasive method, it may cause some discomfort to participants who have never worn it before. Therefore, the first night of recording will be considered as a habituation night, and no active treatment will be administered.

During each experimental condition, participants will also be given a paper questionnaire including a sleep diary and some questions related to the treatment (e.g., time of intake, any adverse reactions, and whether they believe they received 2 mg or 4 mg of melatonin).

The 2 mg dose is the one recommended by the American Academy of Sleep Medicine (AASM) to promote sleep onset and is therefore considered the reference value against which we aim to test the dosage that emerged as most effective from our studies (4 mg).

Data Collection and Statistical Analysis

The following personal data will be collected for each participant:

1. Personal information necessary for informed consent. These will be stored at the University of Pisa for five years after the study concludes, in a locked cabinet at the administrative office with restricted access, available only to the scientific lead and authorized personnel. Access may be granted for audits or ethical inspections.

2. OPTIONAL contact information, the refusal of which does not lead to exclusion from the study. Participants will be asked for their email address and phone number for potential follow-up or invitations to future studies.

3. Sensitive personal data, including health-related and biometric information.

4. General information collected via questionnaires (habits, opinions, etc.).

Data listed in points (2) and (3) will be collected using questionnaires and/or non-invasive or minimally invasive techniques. Questionnaires may be administered in paper or online format, depending on what is agreed with the participant. Experiments will be conducted under the supervision of the scientific lead and/or authorized personnel.

Data will be processed as follows: the scientific lead and/or authorized researchers will store paper-based data in a locked cabinet with restricted access. The re-identification key will be held by the scientific lead and destroyed once all data analyses serving the research objectives are completed. Once anonymized (i.e., once the re-identification key is destroyed), the data may be reused for scientific or statistical research.

If physiological parameter analysis reveals unsought but clinically relevant findings that may indicate a latent pathology, the participant will be promptly informed and provided with the recorded material so they may consult a physician of their choice.

APPENDIX: Evaluation Tools

Below are the questionnaires and non-invasive techniques used in the study.
The selected questionnaires are based on authoritative literature references or were designed specifically to address the research questions. Additional or substitute questionnaires/tasks may be included if similar in nature and scope. Any methodological changes will be reported to the Ethics Committee for review.

QUESTIONNAIRES

Reduced Morningness-Eveningness Questionnaire (rMEQ)
The rMEQ is a self-administered questionnaire with 5 multiple-choice questions about daily habits (e.g., preferred time for physical activity, morning alertness, appetite, fatigue).
Scores are translated into a final score identifying the participant's chronotype.
Scores range from 4 to 26. Chronotype categories:

- <11: Evening type
- 11-17: Intermediate type
- >17: Morning type

Epworth Sleepiness Scale (ESS)
The ESS is a self-administered questionnaire to measure general daytime sleepiness.
It includes 8 items rated from 0 to 3:

- 0 = No chance of dozing
- 1 = Slight chance
- 2 = Moderate chance
- 3 = High chance
  Total scores:
- 0–6: Normal sleepiness
- 7–8: Average sleepiness
- 9–24: Excessive sleepiness

Pittsburgh Sleep Quality Index (PSQI)
The PSQI evaluates sleep quality and disturbances over one month, including:

- Subjective sleep quality
- Sleep latency
- Sleep duration
- Habitual sleep efficiency
- Sleep disturbances
- Use of sleep medication
- Daytime dysfunction

Global score:

- 0 = Best
- 21 = Worst
- ≤5 = Good sleep quality
- 5 = Poor sleep quality

OTHER TECHNIQUES

Below are the techniques that will be used in the study, based on currently available device models. These may be supplemented or replaced with updated or equivalent versions.

Electroencephalography (EEG)

EEG measures cortical activation using electrodes placed on the scalp.
The signal includes five types of brain waves, each with different frequencies and amplitudes, which reflect the participant's cognitive and arousal state.
EEG remains the gold standard for analyzing and classifying sleep stages.

Actigraphy

For actigraphy, a certified actigraphic device (Fitbit Inspire 2 or equivalent) will be used. Raw actigraphy data will be exported and analyzed using dedicated software, certified by the Italian Ministry of Health and registered under sleepActa srl, a University of Pisa spin-off company directed by Prof. Ugo Faraguna.

The devices will record rest-activity patterns over several days and estimate key sleep parameters:

- Sleep Latency (SL)
- Total Sleep Time (TST)
- Wake After Sleep Onset (WASO)
- Sleep Efficiency (SE = TST / time in bed)

Actigraphy is a validated method for determining circadian rhythm patterns and is the preferred tool for assessing and diagnosing circadian rhythm sleep disorders. DEVICE REGISTRATION DETAILS: Registration ID (BD/RDM): 1763747. Class: 1. National Classification of Medical Devices (CND): Z12030682. Data Collected by the Device;

- Full Name
- Date of Birth
- Recording Start Date
- Recording End Date
- Total Recording Duration
- Start and End Time of Recording
- Mean WASO (Wake After Sleep Onset)
- Mean Total Sleep Time (TST)

- Mean Sleep Efficiency (SE%)
- Overall Sleep Assessment

Legend:

- WASO (Wake After Sleep Onset): Total minutes of wakefulness between first sleep onset and final awakening
- TST (Total Sleep Time): Total duration of sleep during the recording period
- SE% (Sleep Efficiency): Percentage of minutes spent asleep relative to total time in bed

In addition to accelerometer sensors, the smartbands used in the study will also include photoplethysmographic sensors capable of detecting pulsations in the peripheral vascular bed, allowing for heart rate monitoring.

Bibliography

[1] G. C. Hisler, D. Muranovic, and Z. Krizan, "Changes in sleep difficulties among the U.S. population from 2013 to 2017: results from the National Health Interview Survey," *Sleep Health*, vol. 5, no. 6, pp. 615–620, Dec. 2019, doi: 10.1016/j.sleh.2019.08.008.

[2] Chokroverty S., *Sleep Disorders Medicine*. New York, NY: Springer New York, 2017. doi: 10.1007/978-1-4939-6578-6.

[3] M. J. Sateia, "International Classification of Sleep Disorders-Third Edition," *Chest*, vol. 146, no. 5, pp. 1387–1394, Nov. 2014, doi: 10.1378/chest.14-0970.

[4] M. Partinen and E. Kronholm, "Epidemiology: Principles and Application in Sleep Medicine," in *Sleep Disorders Medicine*, New York, NY: Springer New York, 2017, pp. 485–521. doi: 10.1007/978-1-4939-6578-6_28.

[5] R. J. Ozminkowski, S. Wang, and J. K. Walsh, "The Direct and Indirect Costs of Untreated Insomnia in Adults in the United States," *Sleep*, vol. 30, no. 3, pp. 263–273, Mar. 2007, doi: 10.1093/sleep/30.3.263.

[6] M. E. Moloney, T. R. Konrad, and C. R. Zimmer, "The Medicalization of Sleeplessness: A Public Health Concern," *Am J Public Health*, vol. 101, no. 8, pp. 1429–1433, Aug. 2011, doi: 10.2105/AJPH.2010.300014.

[7] S. Dujardin, A. Pijpers, and D. Pevernagie, "Prescription Drugs Used in Insomnia," *Sleep Med Clin*, vol. 13, no. 2, pp. 169–182, Jun. 2018, doi: 10.1016/j.jsmc.2018.03.001.

[8] D. F. Kripke, R. D. Langer, and L. E. Kline, “Hypnotics’ association with mortality or cancer: a matched cohort study,” *BMJ Open*, vol. 2, no. 1, p. e000850, Feb. 2012, doi: 10.1136/bmjopen-2012-000850.

[9] A. S. Halme, S.-G. Beland, M. Preville, and C. Tannenbaum, “Uncovering the source of new benzodiazepine prescriptions in community-dwelling older adults’,” *Int J Geriatr Psychiatry*, vol. 28, no. 3, pp. 248–255, Mar. 2013, doi: 10.1002/gps.3818.

[10] Nicholas J.G. Pimlott, Janet E. Hux, Lynn M. Wilson, Meldon Kahan, Cindy Li, and Walter W. Rosser, “Educating physicians to reduce benzodiazepine use by elderly patients: a randomized controlled trial,” *CMAJ*, Apr. 2003.

[11] M. E. Moloney, G. Ciciurkaite, and R. L. Brown, “The medicalization of sleeplessness: Results of U.S. office visit outcomes, 2008–2015,” *SSM Popul Health*, vol. 8, p. 100388, Aug. 2019, doi: 10.1016/j.ssmph.2019.100388.

[12] C. M. Morin *et al.*, “Insomnia disorder,” *Nat Rev Dis Primers*, vol. 1, no. 1, p. 15026, Sep. 2015, doi: 10.1038/nrdp.2015.26.

[13] D. Riemann *et al.*, “European guideline for the diagnosis and treatment of insomnia,” *J Sleep Res*, vol. 26, no. 6, pp. 675–700, Dec. 2017, doi: 10.1111/jsr.12594.

[14] F. Cruz-Sanabria *et al.*, “Melatonin as a Chronobiotic with Sleep-promoting Properties,” *Curr Neuropharmacol*, vol. 20, Feb. 2022, doi: 10.2174/1570159X20666220217152617.

[15] N. Buscemi *et al.*, “The efficacy and safety of exogenous melatonin for primary sleep disorders a meta-analysis,” *J Gen Intern Med*, vol. 20, no. 12, pp. 1151–1158, Dec. 2005, doi: 10.1111/j.1525-1497.2005.0243.x.

[16] Z. Menczel Schrire *et al.*, “Safety of higher doses of melatonin in adults: A systematic review and meta-analysis,” *J Pineal Res*, vol. 72, no. 2, Mar. 2022, doi: 10.1111/jpi.12782.

[17] J. Arendt and D. J. Skene, “Melatonin as a chronobiotic,” *Sleep Med Rev*, vol. 9, no. 1, pp. 25–39, Feb. 2005, doi: 10.1016/j.smrv.2004.05.002.

[18] I. v. Zhdanova, “Melatonin as a hypnotic: Pro,” *Sleep Med Rev*, vol. 9, no. 1, pp. 51–65, Feb. 2005, doi: 10.1016/j.smrv.2004.04.003.

[19] C. J. van den Heuvel, S. A. Ferguson, M. Mila Macchi, and D. Dawson, “Melatonin as a hypnotic: Con,” *Sleep Med Rev*, vol. 9, no. 1, pp. 71–80, Feb. 2005, doi: 10.1016/j.smrv.2004.07.001.

[20] A. Brzezinski *et al.*, “Effects of exogenous melatonin on sleep: a meta-analysis,” *Sleep Med Rev*, vol. 9, no. 1, pp. 41–50, Feb. 2005, doi: 10.1016/j.smrv.2004.06.004.

[21] E. Ferracioli-Oda, A. Qawasmi, and M. H. Bloch, “Meta-Analysis: Melatonin for the Treatment of Primary Sleep Disorders,” *PLoS One*, vol. 8, no. 5, p. e63773, May 2013, doi: 10.1371/journal.pone.0063773.

[22] F. Auld, E. L. Maschauer, I. Morrison, D. J. Skene, and R. L. Riha, “Evidence for the efficacy of melatonin in the treatment of primary adult sleep disorders,” *Sleep Med Rev*, vol. 34, pp. 10–22, Aug. 2017, doi: 10.1016/j.smrv.2016.06.005.

[23] J. K. Wyatt, D.-J. Dijk, A. R.-D. Cecco, J. M. Ronda, and C. A. Czeisler, “Sleep-Facilitating Effect of Exogenous Melatonin in Healthy Young Men and Women Is Circadian-Phase Dependent,” *Sleep*, vol. 29, no. 5, pp. 609–618, May 2006, doi: 10.1093/sleep/29.5.609.

[24] M. J. Sateia, D. J. Buysse, A. D. Krystal, D. N. Neubauer, and J. L. Heald, “Clinical Practice Guideline for the Pharmacologic Treatment of Chronic Insomnia in Adults: An American Academy of Sleep Medicine Clinical Practice Guideline,” *Journal of Clinical Sleep Medicine*, vol. 13, no. 02, pp. 307–349, Feb. 2017, doi: 10.5664/jcsm.6470.

[25] Cruz-Sanabria F, Bruno S, Crippa A, Frumento P, Scarselli M, Carmassi C, Faraguna U. Optimizing the use of melatonin as a sleep-promoting drug: a systematic review of randomized controlled trials and dose-response meta-analysis. *Under preparation*

[26] K. Kanagarajan *et al.*, “Morningness-Eveningness questionnaire in bipolar disorder,” *Psychiatry Res*, vol. 262, pp. 102–107, Apr. 2018, doi: 10.1016/j.psychres.2018.02.004.

[27] L. Tonetti and V. Natale, “Discrimination between extreme chronotypes using the full and reduced version of the Morningness-Eveningness Questionnaire,” *Chronobiol Int*, vol. 36, no. 2, pp. 181–187, Feb. 2019, doi: 10.1080/07420528.2018.1525392.

[28] M. W. Johns, “A New Method for Measuring Daytime Sleepiness: The Epworth Sleepiness Scale,” *Sleep*, vol. 14, no. 6, pp. 540–545, Nov. 1991, doi: 10.1093/sleep/14.6.540.

[29] D. J. Buysse, C. F. Reynolds, T. H. Monk, S. R. Berman, and D. J. Kupfer, “The Pittsburgh sleep quality index: A new instrument for psychiatric practice and research,” *Psychiatry Res*, vol. 28, no. 2, pp. 193–213, May 1989, doi: 10.1016/0165-1781(89)90047-4.

[30] Kryger, M. H., Roth, T., & Dement, W. C. (2010). Principles and Practice of Sleep Medicine E-Book. Elsevier Health Sciences.