Peer-review Comments and Author Responses

Reviewer 1

1. *Comment: "Participants will receive a general orientation about sleep hygiene. Do you plan to follow up if this orientation is being adopted? If yes, how? If not, why? I assume that different behaviors (adherent vs non-adherent sleep hygiene guidance) will affect the results even if the participant is taking omega-3 supplementation. This FUP seems an important matter."*
    a. Response: We acknowledge the importance of adopting sleep hygiene and plan to implement follow-up visits.
    b. Change in text: To implement the follow-up mechanism, we will administer the Sleep Hygiene Questionnaire to participants, designed to inquire about their sleep habits and the extent to which they have integrated the provided sleep hygiene guidance into their daily routines. These surveys will be conducted in each scheduled visit to capture any changes in behavior over time. Additionally, participants may be encouraged to keep sleep journals or logs to provide more detailed insights into their sleep patterns and adherence to the guidance. These measures will not only allow us to track adherence but also provide valuable qualitative data regarding participants' experiences with implementing the sleep hygiene recommendations.

2. *Comment: "Workers will be recruited via email and participants will be notified via telephone and email all advances in the study and future scheduled visits to avoid delay or dropouts. Will be used an automatic system or a person will be in charge of these procedures? Who will have access to personal information about workers and participants? How will you guarantee the privacy and confidentiality of such personal information like these?"*
    a. Response: In this case, a person will be in charge of scheduling the visits and notifying advances of the study, this does not include results of the study, only scheduling visits or communicating any information of the study that would be considered relevant at the moment. The randomization list and allocated codes will be kept anonymous, and will be obtained by REDCap platform. To add the study would be double blinded, neither investigator, participants, data collectors, researchers administering treatments or collecting data and outcome assessors will be aware of patient allocation.
    b. Change in text: the information to this question was already answered in the protocol

3. *Comment: "For the inclusion criteria, please give examples of clinical symptoms that are compatible with night shift sleep disorder."*

a. Response: We thank the reviewer for the feedback and added the clinical symptoms of SWSD
b. Change in text: Such as insomnia and excessive sleepiness, adding an overlap of usual sleep hours with work.

4. *Comment: "In table 2 is stated when the ICF will be applied, but in the MATERIALS AND METHODS section it is not well described. The eligibility criteria will be explained and the participants who meet the criteria will be randomized. Specify in which moment the consent will be given."*
    a. Response: We thank the reviewer for the feedback and added this information
    b. Change in text: The Informed Consent Form (ICF) will be signed before the beginning of the trial.

5. *Comment: "If adverse reactions are presented, medication will be withdrawn and participants will be able to exit the study protocol if intolerance to formulations jeopardizes participation. Is the consent also expected to be withdrawn? This is not clear in the manuscript. Is this a per protocol or intention-to-treat protocol? Please clarify."*
    a. Response: we thank the reviewer for the feedback. Actually, we plan to do a combination of both. ITT + PP
    b. Change in text: Given the intervention's safety profile, we don't anticipate needing emergency unblinding. But if it becomes necessary due to a life-threatening event, we'll proceed with it. Following the per protocol principles, if such a situation arises, we'll promptly inform the principal investigator and discontinue the subject from the trial. Furthermore, we'll conduct an intention-to-treat analysis, which means participants will be analyzed based on their randomized treatment assignment, regardless of any protocol deviations.

6. *Comment: "It mentions a questionnaire will be taken the day after the second night shift of the week. Where will this questionnaire be applied? During visits? On-line? How? By whom? This is not clear."*
    a. Response: The questionnaire is referred to the Pittsburgh Sleep Quality Index (PSQI), and it would be applied during the visits. Visits would be scheduled with anticipation, so as to make sure they would take place the day after the second night shift of the week.
    b. Change in text: during the visits that would be pre scheduled

7. *Comment: "The Discussion was well written. It is mentioned a possibility of bias. How do you plan to avoid or reduce the risk of bias?"*
    a. Response: We acknowledge the bias by using a self-reporting questionnaire. We have detailed further how to address the self-reporting bias.

b. Change in text: To minimize bias in self-reported questionnaire data, we'll ensure participant blinding to treatment allocation and supplement subjective responses with objective measures (actigraphy). Rigorous quality control measures during data collection and analysis, along with transparent acknowledgment of limitations in the discussion, will further enhance the validity of our findings.

8. *Comment: "It says that Visit 1 and Visit 2 may take place in the same day, but in the case of different days, how long can take between the visits? It is not specified."*
    a. Response: We have specified the allowable duration between Visit 1 and Visit 2 in the protocol.
    b. Change in text: We specify a maximum allowable time frame of ±3 days between Visit 1 and Visit 2. This ensures clear guidelines for scheduling and maintains uniformity in participant experiences throughout the trial.

9. *Comment: "In "Administration of IP", I suggest that you to replace "IP(omega-3)" with IP dispensation or IP (omega-3 or placebo), as it gives an idea that only omega-3 will be dispensed to participants."*
    a. Response: We acknowledge the misunderstanding and have corrected the wording on the IP dispensation so that it's understood that mega-3 and placebo will be dispensed to participants.
    b. Change in text: On Table 2: IP (omega-3 or placebo).

10. *Comment: "I suggest you to add the questionnaires expected to be used for the eligibility criteria - STOP-BANG and Pittsburgh Sleep Quality Index - in the TIMELINE. As part of the procedures, they should be mentioned."*
    a. Response: We thank the reviewer for the comment. In this case, the eligibility criteria is written in the Timeline, as Eligibility criteria, that sums up all our inclusion criteria
    b. Change in text: See Manuscript.

11. *Comment: "If the Bang's Blinding is a procedure from the protocol, I suggest to add it too."*
    a. *Response: We thank the reviewer for the comment*
    b. *Change in text: The Bang´s Blinding was added in the Timeline.*

Reviewer 2

12. *Comment: "The majority of references (15 out of 23) are not recent (published more than 5 years ago). If possible, I suggest updating the references. Most journals only accept articles with more than 50% of references published in the last 5 years."*

a. Response: We acknowledge that a majority of our references are not recent. This is due to the limited availability of recent studies in our research topic. Our review is based on the existing body of research, which predominantly consists of older publications. We have ensured that our references accurately represent the available literature.
b. Change in text: None.

13. *Comment: "Dear group, I suggest to explain the main symptoms of shift work sleep disorders once is the outcome you intend to evaluate in this study ("Shift work disorder (SWD), which is characterized by insomnia and excessive sleepiness " (Int J Environ Res Public Health. 2021 Feb; 18(3): 1294.)"*
    a. Response: we thank the reviewer for the feedback and we added the main symptoms
    b. Change in text: whose main symptoms include insomnia and excessive sleepiness

14. *Comment: "Dear group, I missed physical activity as exclusion criteria. Could it interfere in the results (depending on the intensity of the exercises)? I yes, I suggest to include in the exclusion criteria list."*
    a. Response: We have addressed physical activity as an exclusion criteria.
    b. Change in text: We decided to exclude individuals engaging in high intensity physical activity, as it may adversely affect sleep outcomes regardless of omega-3 intake. To implement this, we will conduct the preference section of the PRETIE-Q questionnaire. Based on previous literature, 24 points was considered high intensity physical activity.

15. *Comment: "Dear group, the interventions were very well describe, according to CONSORT 2010 statment. I would like to do only one suggestion, including in the text that the control group will receive 2 pills daily, as it was described for the active group."*
    a. Response: We thank the reviewer for the feedback and added the number of placebo pills that the subject will receive daily
    b. Change in text: 2 daily inert pills.

Reviewer 3

16. *Comment: "Very good title for the paper, consider making a trial acronym too".*
    a. Response: We thank the reviewer for the feedback and have added a trial acronym that reflects our focus on shift-work sleep disorder and the intervention with omega-3 supplementation.

b. Change in text: Efficacy of Omega-3 in Improving Sleep Quality of Healthcare Workers with Shift-work Sleep Disorder: phase III, double-blind, placebo-controlled trial (SLEEP-O3 trial).

17. *Comment: "Very good abstract, consider adding to the methods that for more objectives measuring you will also use actigraphy, I consider it valuable as it would help to convince the quality of the study to measure sleep."*
    a. Response: We thank the reviewer for the feedback and have added actigraphy in the method section of the introduction
    b. Change in text: one of the secondary outcomes would be actigraphy as reliable sleep assessment

18. *Comment: "I would add as another keyword shift work sleep disorder or sleep disorders just in case the other is too long, as you are not only doing the research on health care personnel but health care personnel with a sleep disorder."*
    a. Response: We thank the reviewer for the feedback and have added another keyword that
    b. Change in text: omega-3 PUFA, sleep quality, health care personnel, actigraphy, shift work sleep disorder.

19. *Comment: "Very good introduction, consider adding the information regarding the mechanism of action of omega 3 to the second paragraph of the introduction, on the methods section is mention MOA of omega 3, it would be better to have it on the introduction and in more detailed."*
    a. Response: we thank the reviewer for the feedback and have added the suggestion.
    b. Change in text: In experiments with rats, omega-3 has demonstrated to protect the activation of astrocytes which is the major component of the brain-blood barrier, and fundamental for the function of the glymphatic system that is most activated during sleep and responsible for the clearance of neurotoxins generated throughout the day. This effect on astrocytes causes the avoidance of the depolarization of the aquaporins of these cells for locations that can disrupt the normal flow of the cerebrospinal fluid (CSF).

20. *Comment: "Have you consider the measurement of some biological information, such as levels of salivary cortisol or levels of melatonin, along with the actigraphy, it could provide more objective information to the trial. I mentioned it as I saw there was a study regarding the effect of night-shift work on cortisol circadian rhythm and melatonin. In case it is not feasible due to inconvenience, it might be good that you added to the limitations or as a recommendation for future studies."*

a. Response: We thank the reviewer for the feedback. We have discussed the possibility of the measure of cortisol or melatonin, but we had the limitation on the time of the day they should be measured to determine the maximum concentration, and being able because of the participants schedule to measure them in each visit at the same time.
b. Change in text: None.