

Peer-review Comments and Author Responses

Reviewer 1

1. Abstract: I would recommend adding the main objective of this systematic review. Probably, it would fit as the last sentence of the background section.

Response: We acknowledge the reviewer's comment and added the main objective to the abstract.

Change in text: It was added to the abstract: "We aim to pave the way for future investigations, ultimately contributing to the clinical management of sleep health."

2. I would add the time interval during which the studies included in this analysis were published.

Response: We acknowledge the reviewer's comment and include the time interval in which the studies included were published. Change in text: "This mini-review follows the PRISMA guidelines for systematic reviews and examines studies published from the date of inception on databases up to September 1st, 2023, within the publication interval of 2002 to 2022."

3. "Is it possible to add a summary of the data search strategy? For example, the main inclusion criteria."

Response: We acknowledge the reviewer's comment and include the data search strategy in the abstract. Change in text: "Inclusion criteria encompassed randomized controlled trials (RCTs) and observational designs involving the ingestion of omega-3, whether through dietary sources or supplementation, and the primary outcome related to sleep quality or clinical parameters. Exclusion criteria included preclinical studies and literature reviews."

4. "I would recommend adding more information in the results section, such as the frequency/proportion of "promising results."

Response: We acknowledge the reviewer's comment and include more information about the results in the abstract. Change in text: "The review identified 19 eligible studies, consisting of 9 RCTs and 10 observational studies. The results displayed a complex relationship between omega-3 supplementation and sleep quality, with some studies suggesting positive effects, particularly in specific subpopulations, while others showed no significant impact or even negative effects on sleep. Among the RCTs, 7 showed positive and promising results in favor of omega-3 supplementation for sleep quality, while 1 RCT indicated the need for further studies, and 1 RCT suggested no benefit of omega-3 on sleep quality. In the observational studies, 7 reported positive and promising outcomes with omega-3 supplementation, while 2 indicated no benefit in improving sleep quality and 1 suggested the need for further studies. "

5. Introduction: In the introduction section, there is a link between omega-3, cardiovascular outcomes, and sleep research, while here the link is between healthy sleep and cardiovascular disease. It seems a little disconnected to me. I would try to connect the information.

Response: We understand the confusion created by poor phrasing and improved the text by clarifying the improvement of sleep patterns achieved with omega-3 can then also improve

cardiovascular outcomes. Change in text: “Improving sleep patterns minimizes the risk of cardiovascular disease (Bertisch et al., 2018)”

6. Methods: I would include the publication date of the first article included in this analysis, which is 2012 from Table 2.

Response: We acknowledge the reviewer’s comment and added information regarding the time interval during which the studies included in this analysis were published. Change in the text: “This mini-review follows the PRISMA guidelines for systematic reviews and examines studies published from the date of inception on databases up to September 1st, 2023, within the publication interval of 2002 to 2022”

7. I suggest using a standardized abbreviation for experimental clinical trials - RCT. Table 2 is great; you added the sample size (not the sample size calculation). In general, to describe the sample size calculation, we must add the power, alpha level, effect size/variance ushttps://drive.google.com/drive/folders/1LalQj9nf5WfftCsnwN9zia--nc9uX87A?usp=sharing to calculate the sample size. You correctly added the sample size, which is great.

Response: We acknowledge the reviewer’s comment and standardized the abbreviation as RCT throughout the text. Change in the text: All abbreviations referring to randomized clinical trials were changed to RCT. We corrected the term “sample size calculation” for “sample size”.

8. One minor point: I did not find a column describing the sampling method. If all studies used the convenience sampling method, maybe you can add it to the results. Not necessary in the table.

Response: We acknowledge the reviewer’s comment and indeed all studies used convenience sampling. Change in text: We added in the text: “All studies used convenience sampling methods.”

9. Table 3 - I missed the footnotes on this table and I would add a column to add the comparator group in case it exists.

Response: We acknowledged this issue and corrected the problem. Change in text: Please refer to Table 3. We added footnotes, including an explanation of the lack of comparators for the included studies (they were analyzed against non-exposure).

10. Results: When you described the interventions used in different studies, I would recommend describing them in a small table. For example, Intervention: DHA (n, %), EPA (n, %), fish intake (n, %) // Placebo: inactive compound (n, %), corn oil (n, %), and refined olive oil (n,%).

I am missing the characteristics of the observational studies.

Response: We appreciate the comment and understand the concern, however, the total dosage of supplemented omega-3 is the aspect of utmost importance, as specified in the table, and not the composition, since both DHA and EPA are considered biologically active. Equally, placebo compounds were chosen solely based on similarity, for blinding purposes, to omega-3 formulations. Change in text: Not applicable.

11. *One major point, was not clear in Table 3, designed to describe the observational studies, if any of the articles described had a control group assessment. I am also missing the risk of bias assessment for the observational studies.*

Response: We acknowledge that, and we provided a footnote rectifying the issue in Table 3 and a bias assessment for observational studies. Change in text: Refer to main document, and Table 3.

12. *Regarding the results of individual studies and syntheses: I would divide the results of individual studies and syntheses for RCT and Observational studies.*

Response: We acknowledge the comment, and reorganized our Results to better serve the purposes of this section. Change in text: Please refer to the Discussion section.

13. *Another major point, the study population of 4 studies included in this analysis (Cheruku, 2002; Jansen, 2020, Christian et al 2016, Jackson et al 2020) did not receive oral supplementation of omega-3. So, based on your inclusion criteria, I think these studies should not be included in the analysis.*

Response: We appreciate the reviewer's comment and would like to reinforce that our inclusion criteria allowed for studies with oral supplementation of omega-3 or measurement of omega-3 levels as we read in "(2) oral ingestion of omega-3 through regular diet or supplementation, or assessment of LC-PUFA plasma levels". Change in text: Not applicable.

14. *"Discussion: In the discussion, I would summarize the systematic review in the first paragraph. Then (second), I would recommend describing the studies that have shown an association between omega-3 and improving sleep patterns. In this paragraph, I will describe the limitations and potential biases in these results. As you did, for example, a heterogeneous population, age range, and outcome measurement. Later (third paragraph), I would describe the studies that have shown a worsening or lack of association between omega-3 intake and sleep patterns. Adding the potential limitation and bias to consider this data as definitive. I would add a lack of generalizability and a lack of a comparison group in observational studies as limitations. I hope these revisions help improve the clarity of your manuscript."*

Response: We acknowledge the comment, and reorganized our Discussion to better serve the purposes of this section. Change in text: Please refer to the Discussion section.

Reviewer 2

15. *Dear Authors, The article is well written, with the topic being relevant, but not new. The main issue in this article is the diversity of methodologies groups and subgroups of patients, making the article very broad and difficult to analyze to conclude. The authors conclude that more studies are needed to better understand the relationship between Omega-3 and sleep quality, but they do not provide alternatives for better results. Thank you!*

Response: Dear reviewer 2, we appreciate your comment. As you mentioned on the heterogeneity of the analyzed studies, we truly found difficulties in providing a new horizon for future researchers on the topic. We however added suggestions of what we believe future research should prioritize. Change in text: "We believe that future research on omega-3 impact

on sleep quality should be designed as RCTs focused on determining efficacious regimens and exploring population groups possibly benefited by the intervention.”

16. *Could you explain better the conflicting data mentioned in the article? What are the main challenges and inconsistencies in the medical literature on the relationship between omega-3 and sleep?*

Response: We acknowledge your doubts and improved our Discussion section to better explore such topics. Change in text: Please refer to the Discussion section.

17. *The study includes randomized controlled trials (RCTs) and observational studies. Can you discuss the advantages and limitations of each type of study in the context of investigating the relationship between omega-3 and sleep?*

Response: We acknowledge this doubt and improved our Discussion to better explore such a topic, bearing in mind the word count limit that limits extensive considerations. Change in text: Please refer to the Discussion section.

18. *The article discusses different populations studied, such as newborns, children, adults, and pregnant women. How do diverse study populations affect the interpretation of results and the generalizability of results?*

Response: We appreciate your thoughtful consideration. We improved our Discussion to discuss such topics. Change in text: Please refer to the Discussion section.

19. *According to Boone et al (2019), there are limitations to the trial because the study trusted caregiver-reported sleep; therefore, caregiver bias cannot be ruled out. In terms of assessing the risk of bias, which studies in the review are considered to be at high risk of bias, and how does this affect the overall quality of the evidence presented in the article?*

Response: We have perfected our bias risk assessment for RCTs and developed one for observational trials to satisfy this comment. Change in text: Please refer to the main document.

20. *Christian et al (2016) used the Pittsburgh Sleep Quality Index (PSQI) to assess self-reported sleep quality. The article mentions the use of objective and subjective sleep measures. Can you explain how the choice of measurement methods affects the reliability and validity of results related to omega-3 and sleep?*

Response: We appreciate your comment and have improved the Discussion section to include this aspect. Change in text: Please refer to the Discussion section.

21. *The article quotes some studies that show positive effects of omega-3 supplementation on sleep and others with different results. Can you discuss potential factors or confounders that may explain these mixed results, especially in the pediatric population?*

Response: We appreciate your comment and have improved the Discussion section to include this issue. Change in text: Please refer to the Discussion section.

22. *The paper concludes that the impact of omega-3 on sleep is inconclusive. Could you discuss the limitations of the study and suggest potential directions for future research in this area?*

Response: We appreciate your comment and have improved the Discussion section to explore suggestions for future research. Change in text: Please refer to the Discussion section.

Reviewer 3

23. Please verify previous systematic reviews and meta-analyses regarding omega-3 and sleep, PROSPERO found 29 related reviews that might be of help as these meta-analyses might offer a more specific approach to your mini review, especially the one entitled: *Omega-3 long-chain polyunsaturated fatty acid and sleep: a systematic review and meta-analysis of randomized controlled trials and longitudinal studies [CRD42020156826]*.

Response: We acknowledge the reviewer's comment and indeed the mentioned database is useful in finding further studies on this topic. However, considering the initial methodology established for this review, PROSPERO was not included for retrieval. Due to its qualitative nature, we believe the included trials fulfill the purposes of this mini-review. Change in text: Not applicable.

24. I really enjoyed reading the methodology section, especially the hard work you put into data collection. Excellent work.

Response: We are glad we conveyed properly to this reviewer our methodology. Change in text: Not applicable.

25. Figures/tables should have a title explaining what each figure or table is portraying.

Response: We acknowledge the reviewer's comment and titles have been added to each figure and/or table. Change in text: Refer to the main document.

26. Bias was only analyzed in the clinical trials, what about the observation studies which is a big chunk of the articles included in the review?

Response: We acknowledge this problem and a bias assessment is now performed for observational studies. Change in text: Please refer to the main document.

27. The discussion needs a lot of work, after reading the discussion it looks like another section of results, you are specifying the findings of each trial, this section has to be more of an analysis of what you found. The way I would approach the discussion part would be by comparing the results found in the clinical trials (as a whole group) vs the results found in the observational studies (as a whole group), including what they were analyzing and what were their results, if they were positive or negative. Finally, in the discussion, I would add at the end what are your conclusions based on the results found in both the clinical trials and observational studies. It appears that there is a benefit in certain populations, which populations? It also appears that omega-3 improves sleep quality, but in which aspect, how was it reported? Emphasize these findings, as an example (not real): From the RCTs we can see that omega-3 appears to have a benefit in improving sleep as demonstrated by 6/9 trials in which they reported an improvement in their sleep score. In summary, the discussion is NOT just writing and rephrasing the results, is

interpreting the results and how the findings could be positive or negative in which population/settings.

Response: We acknowledge the comment, and reorganized our Discussion to better serve the purposes of this section. Change in text: Please refer to the Discussion section.

28. I know English is a limitation for all of us, but if possible, consider (if possible) offering an English native to read and correct the article regarding vocabulary and grammar would boost your article's quality.

Response: We will revise and make an extra effort to correct language mistakes. Change in text: Not applicable.

29. Improve your abstract, it needs to demonstrate the findings you discovered in your review and make it attractive as it is the first thing people and reviewers read. Your abstract at the moment is not attractive and does not portray all the effort and work you did nor the results you found in your review.

Response: We acknowledge the comment, and we have improved our abstract with the contribution of this and other reviewer's comments as well. Change in text: Refer to the Abstract section.

30. Please refer to the Word document for more details.

Response: We thank the suggested modifications and will address them properly in the document. Change in text: Refer to the main document.

31. Excellent tables 2 and 3 summarize the results of the articles used as well as excellent figures, really friendly, creative, and attractive.

Response: We thank the compliment and hope reviews in this document can help us further improve the text. Change in text: Not applicable.

Reviewer 4

32. The introduction does not give a fair background of the literature, there's no explanation about sleep, stages, contributed factors, and why they think Omega 3 can have a role in sleep”

Response: We acknowledge the reviewer's comment; despite its conciseness, we sought to highlight the main mechanisms through which omega-3 improves sleep parameters - melatonin regulation and cellular membrane stability. Change in text: Refer to Introduction.

33. There's no explanation of search strategies (only tables).

Response: We better described our search strategy as suggested by the reviewer. Change in text: Please refer to the Methodology section.

34. No explanation of the risk of bias calculation (figure 2).

Response: We have added missing titles and footnotes for all figures in the article, including risk assessment, and developed a new risk assessment for observational studies. Change in text: Refer to figures.

35. *No comprehensive and coherent writing in the result section.*

Response: We acknowledge the reviewer's comment and improved the comprehension of the writing in the result section. Change in text: Please refer to the Result section.

Reviewer 5

36. *Dear authors, Congratulations on submitting this manuscript!*

Overall, the text is very good and easy to follow. I mention some specific comments in the attached Word documents for your consideration to further improve clarity.

Response: We thank the compliment. Change in text: Please refer to the Main document.

37. *“Background: Omega-3 fatty acids are known for the improvement of cardiovascular and metabolic outcomes, with increasing interest in how sleep quality could be improved with supplementation”.* I would rephrase this sentence to “Omega-3 fatty acids have well-known benefits in cardiovascular and metabolic outcomes, with increasing interest in how sleep quality may be improved with its supplementation”.

Response: We acknowledge the reviewer's comment. We perfected the text according to this and other comments. Change in text: Not applicable.

38. *Methods: 87 articles were retrieved from MEDLINE and Cochrane databases through a systematic search strategy, and 19 were included. Please refer to the comments on Figure 1 below.*

Response: We acknowledge the reviewer's comment and correct Figure 1. Change in text: Please refer to Figure 1.

39. *“Results: Different populations have been investigated for sleep clinical benefits with omega-3 supplementation, with promising results in most results, however divergent findings throughout studies persist”.*

I would rephrase this sentence to “Sleep clinical benefits with omega-3 supplementation have been investigated in different populations, with promising results in most of the studies”. If this is indeed true, I believe that the last phrase “However, divergent findings persist” is unnecessary and even contradictory.

Response: We acknowledge the reviewer's comment. We perfected the text according to this and other comments. Change in text: Not applicable.

40. *“Nonetheless, there is conflicting data on how omega-3 can benefit populations suffering from poor sleep quality or correlated disorders”.*

I would change nonetheless to HOWEVER, and would replace “populations suffering from” with “patients with poor sleep quality or correlated disorders”.

Response: We acknowledge the reviewer's comment. We perfected the text according to this and other comments. Change in text: Not applicable

41. *"In this mini-review, our objective is to comprehend the current evidence in the literature that investigates the impact of omega-3 on sleep".*

I would rephrase as follows: "The aim of this mini-review is to analyze the current evidence on the impact of omega-3 on sleep"

Response: We acknowledge the reviewer's comment. We perfected the text according to this and other comments. Change in text: Not applicable. We acknowledge the reviewer's comment.

42. *"...we aim to pave the way for future investigations..."*

I would change the way to LAY THE FOUNDATION.

Response: We acknowledge the reviewer's comment. We perfected the text according to this and other comments. Change in text: Not applicable.

43. *I would change the title to MATERIALS AND METHODS.*

Response: We acknowledge the reviewer's comment and corrected the subtitle. Change in text: Please refer to the referred section.

44. *Eligibility criteria: "(1) RCT or observational designs".*

I would change observational designs to OBSERVATIONAL STUDIES.

Response: We acknowledge the reviewer's comment and changed the term. Change in text: Please refer to the referred section.

45. *"(2) oral ingestion of omega-3 through regular diet or supplementation.."*

I would rephrase as follows: "(2) supplementation or consumption of omega-3 through regular diet".

Response: We acknowledge the reviewer's comment. However, we believe our phrasing better classifies the oral route of supplementation or diet consumption. Change in text: Not applicable.

46. *"(3) sleep quality or clinical parameters a primary outcome". I would change the primary outcome to AS PRIMARY OUTCOMES.*

Response: We acknowledge the reviewer's comment and accept it. Change in text: Refer to the referred sentence.

47. *Did you include/exclude articles in any other language other than English?*

Response: There were not any articles in other languages retrieved. Change in text: Not applicable

48. *I would write the inclusion and exclusion criteria in two separate paragraphs.*

Response: We acknowledge the reviewer's comment, however, we do not believe understanding is compromised by our paragraph format. Change in text: Not applicable.

49. *"Our population included patients with any baseline covariates"*.

If you mean that you didn't exclude patients with any underlying condition, I would rephrase as follows: "Both healthy patients or with any underlying condition were included in the mini-review".

Response: We acknowledge the reviewer's comment, and phrased this sentence. Change in text: "regardless of baseline covariates".

50. *"The intervention or exposure included ingestion of omega-3 fatty acids, while control of RCTs, placebo or other treatment. The outcome should primarily be related to sleep quality or its clinical parameters"*. *I think these sentences are redundant because they enumerate the same information explained in the inclusion criteria named before. I would rephrase inclusion criteria (2) and (3) and eventually delete these sentences.*

Response: We acknowledge the reviewer's comment, and excluded such sentences. Change in text: Refer to the main document.

51. *Information sources and Selection process: "This mini-review followed considered publications from the date of inception on databases to September 1st, 2023"*.

I find this phrase hard to understand. Do you mean you included the existing publications in the different databases available until September 1st, 2023?

I would also change the date of inception to DATE OF INCORPORATION TO DATABASES.

Response: We acknowledge the reviewer's comment partially, and changed the term to "incorporation". Change in text: Refer to the main document.

52. *Figure 1: Please change the name of the file from "Figures" to "Figure 1" to avoid confusion.*

Response: We acknowledge the reviewer's comment. Change in text: Not applicable.

53. *IDENTIFICATION: Left square. I would change registers to REGISTRIES*

Response: We acknowledge the reviewer's comment, however we followed PRISMA guidelines. Change in text: Not applicable.

54.

SCREENING: Top left square: I would change records screened to SCREENED RECORDS

Response: We acknowledge the reviewer's comment, however we followed PRISMA guidelines.

Change in text: Not applicable

55.

Top right square: I would change records excluded to EXCLUDED RECORDS.

Response: We acknowledge the reviewer's comment, however we followed PRISMA guidelines. Change in text: Not applicable

56. *Mid left square “Reports sought for retrieval”: If 66 records were screened and 40 were excluded, then 26 records were sought for retrieval, not 27. Please verify these numbers*

Response: We acknowledge the reviewer’s comment, and have corrected the numbers. Change in text: Please refer to the new Prisma Image.

57. *INCLUDED: Left square “Studies included in the review”: related to the previous comment, please verify if 26 or 27 records were sought for retrieval and then assessed for eligibility because then the number of studies included in the review changes from 19 to 18. Please verify these numbers.*

Response: We acknowledge the reviewer’s comment, and have corrected the numbers. Change in text: Please refer to the new Prisma Image.

58.

Study selection: “After analyzing the publications, only 19 met our criteria”.

Related to the feedback in Figure 1, please verify the final number of studies included, either 18 or 19.

Response: We acknowledge the reviewer’s comment, and have corrected the numbers. Change in text: Please refer to the new Prisma Image.

59. *Study characteristics: “We had no restrictions on participants’ characteristics, resulting in a varied population; including neonates, children, young adults, and pregnant women”.*

I would rephrase as follows: “There were no restrictions on participants’ characteristics, which led to a varied population, including neonates, children, young adults, and pregnant women”.

Response: We acknowledge the reviewer’s comment, however, we kept our original phrasing. Change in text: Not applicable.

60. “... the frequency in administration varied per week”. I find this last part of the phrase hard to understand. Do you mean that omega-3 was administered differently each week in the different studies?

Response: We acknowledge the reviewer’s comment, and rephrased this sentence. Change in text: “varied among studies”

61. *Results of individual studies and syntheses: “A positive association between omega 3 and most sleep parameters analyzed was observed in most studies, as shown in Tables 2 and 3”.*

Please change as shown in Tables 2 and 3 to AS SHOWN IN TABLES 2 AND 3.

Response: We acknowledged the reviewer’s comment, and corrected it. Change in text: “Tables 2 and 3”.

62. *Risk of bias in studies: “Figure 2 summarizes the risk of bias assessment”.*

Isn’t this referring to Figure 3?

Response: We acknowledge the reviewer’s comment, and corrected the numbering of the figures. Change in text: Refer to the main document.

63. *“Further studies are necessary to corroborate specific subpopulations appropriate targets of omega-3”.*

I would delete this phrase and develop this idea further in the paragraph referring to the limitations of the study at the end of the discussion section.

Response: We acknowledge the reviewer’s comment, and have perfected the Discussion section.
Change in text: Refer to the main document.

64. *“Boone (2019) also had positive results while analyzing, since they concluded toddlers born preterm did not show significant differences in sleep patterns overall with DHA and AA supplementation, but there were improvements in certain subgroups”.*

I would rephrase as follows: “Boone (2019) also shows positive results, since they concluded toddlers born preterm did not show significant differences in sleep patterns overall with DHA and AA supplementation, and there were improvements in certain subgroups”.

Response: We acknowledge the reviewer’s comment, and rephrased it. Change in text: “showed positive results”.

65. *“These subgroups involved male children and children of caregivers with depressive symptoms inside the intervention group”.*

I would replace inside with IN THE INTERVENTION GROUP.

Response: We acknowledged the reviewer’s comment, and corrected it. Change in text: “In the intervention group”.

66. *“Normality was attested using Kolmogorov Smirnov or Shapril Wils test if parametric methods were used”.*

I would rephrase as follows: “Normality was verified using...”. Additionally, please change Shapril Wils to SHAPIRO WILKS.

Response: We acknowledged the reviewer’s comment, and corrected it. Change in text: “Shapiro Wilks”.

67. *“Limitations we found in our mini-review include the heterogeneous population and outcomes measurements, jeopardizing statistical analysis of the results”.*

I would rephrase as follows: “Some of the limitations of our mini-review include the heterogeneous population and diverse outcome measurements, jeopardizing the statistical analysis of the results”.

Response: We acknowledge the reviewer’s comment. However, we kept our original phrasing.
Change in text: Not applicable.

68. *“Future research could benefit from focusing on omega-3 regimens and specific strata of the population that could take more advantage of supplementation, such as those with a low omega-3 index”.*

I would rephrase as follows: “Future research should focus on omega-3 supplementation on specific subgroups that could benefit from it, such as those with a low omega-3 index”.

Response: We acknowledge the reviewer's comment. However, we kept our original phrasing.
Change in text: Not applicable.

