

Peer-review Comments and Author Responses

Reviewer 1

1. *Congratulations for a very well written article. Please review the citation on the main manuscript of Appendix 1 and 2, and table S2.*

Dear Reviewer. We appreciate the time you have dedicated to read and review our manuscript, and we are thankful for your supporting comments. We have reviewed the citations and made appropriate corrections. In the updated manuscript you can find these corrections highlighted.

Reviewer 2

2. *Dear authors, as I was reading your mini review I thought that the discussion was very well written and explained, but the other sections were a bit confusing. I thought you repeated in different parts on the article how you did the risk of bias assessment.*

Dear Reviewer. We appreciate the time you have dedicated to read and review our manuscript and for your feedback as it helps see our readers' perspective. Based on your comments we found that the Risk of Bias (RoB) assessment was repeated in two sections (methods and results). Therefore, we deleted the RoB assessment from the results section, and left the complete description only in the Methods section.

3. *It was not clear for me the population you choose, and if they were healthy adults or not, and what was the mechanism of action, or where could you find spermidine.*

The mini review included adult participants of any age. We added this clarification in the methods section, in the eligibility criteria. However, the studies found only included older adults between 60-96 years old, which was described in the results section. There was no restriction on the health status of the population.

The natural source of spermidine is diet, since spermidine can be derived from “unprocessed plant-derived foods including the durian fruit, shitake mushrooms, fresh green pepper, wheat germ, amaranth grain, cauliflower and broccoli, just to mention a few, but also products resulting from fermentation processes that involve polyamine-generating bacteria and fungi, e.g. soybean products such as natto or many types of mature cheese” (Madeo et al. 2018). However, the review assessed the effect of spermidine supplementation in any form, which has been clarified in the methods section. As for mechanism of action, spermidine has been linked to multiple mechanisms such as anti-inflammatory effects, DNA and RNA stabilization, enzymatic modulation, increased autophagy and many more processes that in one way or another have an impact in age-related diseases. However, in this specific mini review, our goal is to assess if there is a positive correlation between the use of spermidine supplementation and improved cognitive functions, hence, we improved our description of spermidine’s main mechanism of action through the induction of autophagy for its anti-aging effect in the introduction section.

However, we do not include a more detailed pathophysiological mechanism of action since we think it may distract our readers.

4. *The outcome measurements were different in all the studies as you mention in Outcome measurement, how can you relate all of them to spermidine?*

Although all outcome measurements were different, all three studies are assessing cognitive function in patients that received a spermidine supplementation compared to placebo/ regular diet/ non-placebo comparators as per the eligibility criteria in the review. Since the outcome measurements were different, we did not conduct a meta-analysis but a narrative description of the results of each study. Additionally, since all the included studies were randomized controlled trials, we can assume that the effect is only a result of the intervention, as confounders have been controlled by randomization and blinding. The risk of bias that may limit these conclusions are described and were assessed for each study.

5. *Have you looked for articles that assess spermidine intake but in the diet? Is there a minimum level of spermidine suggested?*

Dietary intake of spermidine can vary widely depending on geographical and cultural influences (Soda et al., 2018). We have collectively reviewed a number of articles addressing spermidine intake in diet and there is no consensus on the minimum or maximum levels of spermidine intake or recommendations. As described above, our review was focused on spermidine supplementation only (in any form) to assess its effects on cognitive function, and not on dietary spermidine intake. The authors of the studies did not control for dietary spermidine intake in the included patients.

6. *I shared a file where you can find my comments. Some of them were because in that paragraph the information was not clear. Congratulations to all the authors! I know all the hard work you put in this mini review.*

We hope we were able to address all your concerns, in the edited version of the mini review you can find highlighted the adjustments we made based on your recommendations. And again, thank you for your time and feedback.

Reviewer 3

7. *You used good search strategies to cover a variety of keywords that are relevant.*

We appreciate the time you have dedicated to read and review our manuscript. Thank you very much for your supporting comment on the search strategy. Although the recommendation states revisions required, we did not find the suggestions or required revisions.

Reviewer 4

8. *I want to congratulate Zarama V. et al. for presenting this interesting mini review. Importantly, as pointed out by the authors, few studies have addressed this research question, so it is a novel and relevant work choice. In general, the manuscript is well structured, and the review process and methodology are comprehensive. However, there are some comments that I believe might improve the quality of the work submitted, especially regarding repeated information.*

Dear reviewer. We appreciate the time you have dedicated to read and review our manuscript. We would like to thank you for your feedback and supporting comments. We have made adjustments based on your suggestions, which are highlighted in the updated manuscript.

Reviewer 5

9. *Great work on presenting the data you've gathered. Risk of bias was well accessed, and this is one of the major concerns when we consider reviews.*

Dear Reviewer. We appreciate the time you have dedicated to read and review our manuscript. We thank you for your feedback and supporting comments!

Reviewer 6

10. *Dear Authors. I hope this message finds you well. I wanted to let you know that we have reviewed your paper, and overall, it looks excellent. Your research is valuable and contributes significantly to the field. We appreciate your hard work and dedication in producing such a comprehensive study. However, there are a few minor language and clarity improvements that we would like you to address before we can proceed with publication. These revisions will help ensure that your paper is as clear and concise as possible for our readers. I have already uploaded the document with the suggested changes to our website for your convenience. Please take the time to review these suggestions and make the necessary adjustments. If you have any questions or need further clarification on any of the proposed changes, do not hesitate to reach out to us. We are here to assist you and ensure that your paper is of the highest quality.*

Dear Reviewer. We appreciate the time you have dedicated to read and review our manuscript. We would like to thank you for your feedback and supporting comments. We would like to thank you for your feedback. We have made the necessary adjustments based on the suggestions you provided. It is crucial for us to make our manuscript as clear and understandable for our readers. In the updated manuscript you can see all adjustments highlighted.