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Oxidative stress bridges the gut microbiota and the occurrence of frailty syndrome

Running title: Oxidative stress, gut microbiota and Frailty.

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Answer to reviewers:

Reviewer #1:

Scientific Quality: Grade B (Very good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Minor revision

Specific Comments to Authors: Authors in the article entitled "Oxidative stress builds a bridge between gut microbiota and the occurrence of frail syndrome" evaluated associations between gut microbiota, oxidative stress and frailty syndrome. Presented review in interesting and clear way shows this important and novel topic. Growing research interest in associations between gut microbiota and host health makes this article in line with works by others. Frailty syndrome is an important clinical process common in internal medicine and geriatric departments. This review evaluated current knowledge on actions of gut microbiota together with synthesis of gut microbiota-related metabolites and their role in oxidative stress pathophysiology and development of frailty syndrome. The originality of presented article is closely associated with justifying these correlations and showing changes in gut microbiota as a promising new potential marker of frailty syndrome. This article is well-written and interesting for the readers. Despite general positive reception there are also limitations of presented review that I would like to address below. I hope that authors will be able to clarify them in the rebuttal.

1. Authors at first wrote that *C. difficile* abundance is negatively correlated with frailty. While later on in the review there is a statement that "Moreover, the development of frailty is found to be closely related to an increase in the abundance of *Clostridium*[14]." This discrepancy should be closely justified and clarified since it is not possible that it can increase and decrease at the same time. Additionally, currently *Clostridium difficile* has been replaced by the new nomenclature and is called *Clostridioides difficile* and this term should be used in scientific literature.

Response: We thank the reviewer for the advice. We have checked carefully with these two references. And we found that reference 11 listed different species of *Clostridioides* in detail. While reference 14 only listed a general *Clostridioides* and did not distinct different species. So, we decide only keep reference 11 in the revision of the manuscript.

2. On the Page 8-9 authors wrote that "These studies demonstrate that frailty is always accompanied by an increase in oxidative stress markers and a decrease in antioxidant enzymes". It is preferable not to use terms like always in scientific literature, or use it when scientific knowledge on one topic is certain. Are there any scientific papers that show

opposite associations? In which frailty syndrome was not associated with increase in markers of oxidative stress? I would suggest alleviate this statement.

Response: We thank the reviewer for the suggestion. We have revised the statement in the manuscript. Line 228.

3. Similarly to point 2. Authors in presented review on page 11 wrote that: "[39]. In this TMAO-induced frailty model, the abundance of Firmicutes was increased, and the abundance of Bacteroidetes was significantly decreased in the gut. The ratio of Firmicutes to Bacteroidetes (F/B) was significantly decreased." When abundance of Firmicutes increase and Bacteroidetes decrease their ratio cannot decrease. Please clarify what is the case in this situation, since prevalence of Bacteroidetes is beneficial and normal, while increase in F/B ratio is most commonly associated with dysbiosis and metabolic diseases.

Response: We first apologized for confusing the word "decrease" and "increase" in F/B ratio. It has been corrected now.

4. Authors in presented review evaluated effects of TMA, TMAO, SCFA and hydrogen sulfide as microbiota-dependent metabolites. Authors provided a variety of results showing their role in oxidative stress what is appreciated. I would only suggest expanding this description to metabolites of tryptophan. Since indole-3-propionic acid was proven to have strong anti-inflammatory potential and acts as a free radicals scavenger with the potential similar to melatonin. Recently a review of beneficial roles of IPA has been published summarizing its positive impact on host cells and functions.

Response: We thank that the reviewer's suggestion rich the evidence of gut microbiota-dependent metabolites and frailty and oxidative stress. We have added this reference.

5. Authors should closely check the whole manuscript the overall reception is postive, however; there are some editorial mistakes like "Eggerthella Lenta"/"Eggerthella lenta" etc.

Response: We apologize for the spelling mistakes in the manuscript. We have checked the grammar and words carefully. And we had the manuscript language edited by a professional company now.

Reviewer #2:

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Major revision

Specific Comments to Authors: The topic addressed by this manuscript is quite interesting and relevant, however, some questions emerged while reading it.

Firstly, in point 1. the name of the bacteria is written in lower case, so it must be corrected with the capital letter.

Response: Thank you and it has been corrected now.

In point 4, the authors use two similar terms, vegetables and legumes, so I think only one should be included. I also think that there should be a differentiation in the text and add a concluding topic instead of being together with the whole text.

Response: Thank you for the suggestion. We have added a reference about the Mediterranean diet. In this reference, the authors listed the component of Mediterranean diet in details. We found that the vegetables and legumes are listed separately. So we cite them also separately. We added a subtitle to distinguish diet and TCMs. Different diets and TCMs are distinguished by segments.

Finally, the authors present a very good image but do not fit well into the article. I would like to see the figure better explored and related to the text.

Response: Thank you for the suggestion to the figure. We have added key words of different part of the manuscript in the figure, and showed the relationships between these parts. To make it more related to the text, we have added indexes for different parts of the figure in the corresponding paragraphs.

Reviewer #3:

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Major revision

Specific Comments to Authors:

Major 1. the relationship between aging and frailty syndrome (in the text) or frail syndrome (in the title) needs to be clarified in part 1. What is the definition of frailty syndrome or frail syndrome? Aging is not equal to frailty syndrome, right? Besides aging, which are the most important influencing factors of frailty syndrome.

Response: We added the investigation centenarians in the manuscript. These data showed that frailty is not an inevitable result of aging. The incidence of frailty increases with age, indicating that age is an influencing factor, but it is not the only one. Inflammation and oxidative stress may cause frailty. Our review suggests that gut microbiota and its metabolites can regulate inflammation and oxidative stress, thus affecting frailty.

2. in the part 1, the title is gut microbiota and frailty, actually, the content is to discuss the relation of gut microbiota and aging. How to explain?

Response: As mentioned in introduction, frailty is correlated with age. And frailty was often studied in aging animal models or in elderly people at first. So in this paragraph, we first described the gut microbiota change with age. Then we transit from chronological age to physiological age, proposed frailty. What follows is all about frailty, not aging. Furthermore, the studies about centenarians mentioned in the introduction also suggested that frailty differed from aging.

3. in the part 2, OS and frailty are concentrated. So, both gut microbiota and OS are related with frailty. Your point in the title is that OS is a bridge for gut microbiota and the frailty. How to strengthen your point, not let people confused with maybe the gut microbiota is a bridge of OS and frailty.

Response: Gut microbiota are organisms, so they are unlikely to have a direct molecular-molecular effect on our body. They can only produce effects by an

intermedia biological macromolecule with the corresponding targets of the human body. The literature cited in our review showed that the gut microbiota and their metabolites are closely related to oxidative stress and can regulate the level of oxidative stress. Therefore, we proposed that the oxidative stress related factors are these biological macromolecules that play a direct role, connecting the gut microbiota and their metabolites with the body, affecting the frailty. We added this discussion to the review to clarify our views. Of course, there are interactions between these factors. Under pathological conditions, changes in the level of oxidative stress may also affect the composition of gut microbiota. However, considering the direct action of oxidative stress factors on our body, in this review, we focus on how oxidative stress bridges the gut microbiota and frailty.

Besides, in line 340, this paragraph with intervention strategies using traditional Chinese medicine, because as you mentioned that “Chinese traditional medicine have been found to improve frailty by regulating the gut microbiota and oxidative stress”. You need to demonstrate that the medicine can improve the syndrome by influencing the gut microbiota, and the mediator or bridge is OS, not only to show us that traditional medicine in China can regulate both OS and gut microbiota, in my opinion.

Response: As an intervention means, traditional Chinese medicine can act on both gut microbiota and oxidative stress, both of which can be a target for frailty treatment. As we showed in the figure1, TCMs can act on either the bridge (OS), or the end of the bridge (GM), to improve frailty. And we also discussed that oxidative stress moleculars had a direct action on the macromoleculas of our organisms, while gut microbiota are also organisms, should produce an action by a moleculus, such as oxidative stress. Thus, we clarify that OS bridges GM and frailty.

Minor 1. line number should be added.

Response: The line numbers have been added.

2. line 52. ROS was first mentioned, it needs to have a full name: reactive oxygen species.

Response: The full name has been added.

3. ling 78-89, as to the relationship between frailty syndrome and aging, it needs a point and supported evidence.

Response: We have added evidence that the incidence of frailty increases with age, indicating that age is an influencing factor.

4. Fifty referenced papers are not too many for a review manuscript. Line 249, about the relationship between gut microbiota and NAFLD, there are some published reviews, such as “Intestinal dysbiosis in nonalcoholic fatty liver disease (NAFLD): focusing on the gut-liver axis (doi: 10.1080/10408398.2021.1966738.)”. Line 341, Chinese traditional medicine has the capacity of antioxidation. Such as the papers: doi: 10.3389/fcimb.2021.798052 and doi: 10.1039/d1fo01422f. Besides there was a paper published related to chlorogenic acid, for example, doi: 10.3389/fmicb.2021.784211. eCollection 2021. ...

Response: We thank the reviewer supply these studies for us and enrich the review. We have added these references into the manuscript. And the total number of cited references increased to 73.

Reviewer #4:

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Major revision

Specific Comments to Authors:

1) The authors reporting some different study regarding the antioxidant role of traditional Chinese medicine and they suggested that it could help to improve frailty. The cited studies are focused on Chinese population and so the same effects on the Western population are not taken for granted. Please discuss this point

Response: We thank the suggestion from reviewers. In our opinion, most of the available literatures are limited to the data obtained from Asian populations to date. However, considering that most modern pharmacological researches are done with standard experimental animals, the anti-frailty effect of these traditional Chinese medicines may be potentially extended to Western populations based on these basic research data. However, it still needs to be more widely investigated involving more population. We have added this discussion in the manuscript.

2) The author reported that the Mediterean Diet (MD) changes the composition of the gut microbiota and alleviate frailty, it is right but also a diet with Khorasan wheat (Baldi S et al World J Gastroenterol. 2022 May 14;28(18):1965-1980. doi: 10.3748/wjg.v28.i18.1965.) or Butyrate-integration (Emm G et al. Circ Res. 2021 Jan 22;128(2):278-280. doi: 10.1161/CIRCRESAHA.120.317789.) have the same positive and relevant effects, please discuss e cited these important papers

Response: We thank the reviewer supply these studies about diet on frailty for us. We have added these references into the manuscript.

3) The authors cited *Cistanche deserticola* and *Eumcommia ulmoides*, please reported the name in italicus style and add some informations regardi these microorganisms

Response: The font has been changed into italics style. We also added the information with two references about these two TCMs.

4) Regarding the SCFA the authors have missed the role in pathological conditions such as gastrointestinal disorders (1: Baldi S et al Nutrients. 2021 Feb 26;13(3):742. doi: 10.3390/nu13030742 2: Niccolai E et al. World J Gastroenterol. 2019 Sep 28;25(36):5543-5558. doi: 10.3748/wjg.v25.i36.5543.) Please add and discuss these studies

Response: We thank the reviewer supply these studies about SCFA for us. We have added these references into the manuscript.

5) I suggest a language revision of the manuscript

Response: Thank you and we have had the manuscript language edited by a professional company now.