Response to Reviewers

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Editor

World Journal of Orthopedics

RE: WJO-44584, entitled "Linkage of Microbiota and Osteoporosis: A mini literature review."

Dear Reviewers and Editors:

We wanted to thank you for your constructive feedback towards "Linkage of Microbiota and Osteoporosis: A mini literature review." We greatly appreciate your comments and suggestions. We have carried out the revisions that the reviewers suggested to manuscript WJO-44584. We hope that our revision has improved the paper to a level of your satisfaction. Below are the changes and answers to the reviewers' questions and concerns.

Reviewer #1:

Comment 1: The Authors reviewed the Literature to prove there is a strong correlation between gut microbe dysregulation and decreased bone density. But the organization of whole article was too loose and a little disordered. The first section could be deleted and readjusted into 3 sections:

Response 1: Thank you for your comment. This article addresses an interesting topic, but we can see how it may not have been the most concise or cohesive narrative. We found your input beneficial to focus more on the gut microbiota: how its dysregulation and disruption can lead to pro-inflammatory processes promote bone resorption and impair calcium transport, and what can be done to prevent or correct this.

Action 1: In order to make the manuscript more direct, concise, and cohesive, we changed the following:

We followed the suggestion to delete the first section discussing the demographics and cost of osteoporosis. We then kept the definitions of the two types of osteoporosis to explain to readers that this correlation between gut dysbiosis and osteoporosis was correlated with inflammation and disease pathology instead of decrease in estrogen. We then structured our paper to fit the three given suggestions. This made the paper more succinct and directed towards the gut microbiota and its inflammatory effects.

Comment #2: Readjust into 3 sections: 1. Gut microbe Dysregulation and decreased bone density:

Response 2: Narrowing our focus to three sections would give readers a clear picture of what is the known correlation, potential mechanisms, and potential therapies. In this first section, we elaborated on osteoporosis, a normal gut microbiota and correlations between dysregulation and its effects on bone density.

Action #2: In Gut Microbe Dysregulation and Decreased bone density the following changes were made:

We outlined how the development of the gut microbiota was symbiotic and took many years. We discussed the normal number of bacteria and composition of major phyla. We then discussed the

dysregulation patterns associated with decreased bone volume, indicating what phyla were most associated with inflammation and decreased bone volume.

Comment 3: Readjust into three sections: The possible mechanism of gut dysbiosis lead to osteoporosis.

Response 3: After identifying correlation between gut dysbiosis and decreased bone density, this section would make most sense to discuss the potential mechanisms that relate dysbiosis toward bone resorption and osteoporosis.

Action 3: in Possible Mechanism of gut dysbiosis lead to osteoporosis, the following were addressed:

After noting correlations, we used the reviewer's advice to identify possible mechanisms of dysbiosis that may lead to osteoporosis. We noted the increase in diversity or sensitivity to certain phyla and bacteria may elicit an immune pro-inflammatory response. We discussed how this inflammatory response may activate CD4+ T cells, pro-inflammatory cytokines that may cause systemic damage, but at the site of the bones: activate osteoclasts, which begin bone resorption and lead to decreased bone volume. Furthermore, we discussed how inflammatory response in the gut may lead to decreased calcium absorption. With decreased calcium absorption, the bone lost to reabsorption cannot readily be replaced therefore leading to decreased bone volume, without adequate replacement and osteoporosis.

Comment 4: Readjust into three sections: Normalize gut microbiota can treat or prevent occurrence of osteoporosis. Because this topic is very new the literature needs to be recited in detail.

Response 4: This section had the least literature since the topic is so new. Most of what was contained in the literature was cited in murine models or retrospective studies. We consolidated most of the significant research for mediating gut microbiota to prevent, decrease the rate, or reverse osteoporosis exacerbated by gut dysregulation.

Action 4: In "Normalize gut microbiota can treat or prevent occurrence of osteoporosis the following changes were made:

We discussed many of the most promising prosed therapies to correct gut microbiota dysbiosis according to the literature. We discussed probiotics as a supplementation to supplement the microbiota with bacteria that would be immunoprotective and promote a normal gut flora such as *Bacilis subtilis* and *Lactobacillis reuteri*. These bacterial species are correlated to increased osteoblast activity and promotion of bone growth. Next, we discussed prebiotics as a potential therapy. Prebiotics are high fiber carbohydrates that promote the growth of Bacteroidetes. They promote production of short chain fatty acids, decreased pH, reduced inflammation, and normal gut microbiota. Next, we discussed Antibiotics, both as a potential cause of dysbiosis (selecting for harmful pro-inflammatory bacteria i.e. *Clostridium difficile* and as a potential therapy: clearing out dysregulated GM. Once gut microbiota has been cleared out it can be repopulated. A fecal matter transplant is a cheap and effective way to promote a population already with a diversified population. Next, we discussed exercise as a potential therapy to promote a normal gut microbiota.

Reviewer #2:

Comment 1: Therapies to normalize gut microbiota in patients with osteoporosis or prevent occurrence of osteoporosis to be investigated include: high fiber prebiotic diets to promote growth of

normal gut bacteria and short chain fatty acid production. Probiotics to encourage growth of normal gut microbes, and antibiotic treatment followed by fecal matter transplant. The following needs to be addressed in this manuscript. (i) Authors could describe the potential therapy of gut microbiota and metabolic functions by showing it in figures or in a table for easy comprehension.

Response 1:

We wanted to thank this reviewer for the insightful response of how to make this article more comprehensible and clinically relevant by displaying discussed potential therapies in a table or a figure. This would indeed help to draw attention to potential means of preventing or treating the dysbiosis contributing to osteoporosis. We recognize however that the literature in this field is limited since this is such a new topic of study so we itemized the research that had sufficient literature substance in table format.

Action: 1 Authors could describe the potential therapy of gut microbiota and metabolic functions by showing it in the figures or tables for easy comprehension, is addressed below:

We then tried to take this advice to discuss some of the therapies to prevent or minimize damage of osteoporosis by targeting the gut microbiota. We were able to make one table discussing potential therapies to correct gut dysbiosis. Broad spectrum antibiotics were discussed as a potential therapy to clear a dysregulated gut microbiota. This followed by a fecal matter transplant could facilitate a normalization of the gut microbiota and reduced inflammatory response. Next, we discussed pre-biotics and their ability to promote growth of normal gut microbiota which has increased Bacteroidetes, decreased biodiversity and is associated with decreased inflammatory response. We also discussed the potential therapy of probiotics, which include supplementation of immunoprotective flora such as lactobacilli, found in many dairy products. These probiotics are associated with decreased inflammation and IL-10 which is not associated with cytokine storm and decreased bone density.

Comment #2: (ii) Authors could describe the effect (positive/negative) of antibiotic treatment on osteoporosis therapy.

Response 2: We agree this is a very interesting and potentially useful topic that would merit great interest for future research. If more clinically relevant research had been completed, then this would surely merit a table highlighting it as a known therapy. However, insufficient literature exists to implicate antibiotic use in osteoporosis other than as a correlation and a proposed mechanism, which is why we highlighted it as we did.

Action 2:

Although we briefly discussed the effects of positive/negative antibiotic therapy in our review, there was insufficient clinical data to make this a separate table. Therefore we did address positive/negative antibiotic treatment in the body of our third subsection and gave antibiotic therapy followed by fecal matter transplant as a point in our table, but we did not have significant clinical data to make antibiotic treatment of osteoporosis its own entity.

We did add two sources to constitute what clinically relevant information was known in this field:

26. **Francino M P.** "Antibiotics and the Human Gut Microbiome: Dysbioses and Accumulation of Resistances." *Frontiers in Microbiology* 6 (2016). doi:10.3389/fmicb.2015.01543.
27. **Brandt LJ**. "American Journal of Gastroenterology Lecture: Intestinal Microbiota and the Role of Fecal Microbiota Transplant (FMT) in Treatment of C. Difficile Infection." *The American Journal of Gastroenterology* 108, no. 2 (2013): 177-85. doi:10.1038/ajg.2012.450.

Comment 3: (iii) Authors could describe the evidences or studies of microbiota application in the clinical studies and show it in tables.

Response 3: We appreciate and recognize the importance of clinical studies to demonstrate microbiota mediation to treat osteoporosis. However, since this topic is still such a new and minimally studied venture, most of the research is retrospective or murine in nature. The clinically relevant research we found was included in the body of the manuscript, and we encouraged future research in clinically relevant trials to demonstrate the effectivity of microbiota manipulation in osteoporosis prevention/management.

Action 3: The changes made in response to "Describe the evidences or studies of microbiota application in the clinical studies and show it in tables" are provided below:

Furthermore, since this is such a new topic of study, there was insufficient literature to discuss the clinical studies of this content in a table form. We used this feedback to note the importance of future research to be done to make these associations and why this field may be so beneficial to understanding the nature of the relationship of the gut flora, inflammation, and osteoporosis.

Therefore, we used this reviewer's feedback associated with therapies with sufficient literature to best strengthen our paper.

Again, we wanted to thank the reviewers for their insightful feedback and in making interesting topic of gut microbiota and osteoporosis a more cohesive narrative.

Sincerely,

Authors