PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Probiotic supplements and bone health in postmenopausal women: a meta-analysis of randomized controlled trials
AUTHORS	Yu, Jiawei; Cao, Gaoyang; Yuan, Shuohui; Luo, Cong; Yu, Jiafeng; Cai, Ming

VERSION 1 – REVIEW

REVIEWER	Klara Sjögren Centre for Bone and Arthritis Research, Institute of Medicine, University of Gothenburg, Gothenburg, Sweden.
REVIEW RETURNED	19-Aug-2020

GENERAL COMMENTS	This is to my knowledge the first SR and metaanalysis analysing the evidence of a positive effect of probiotic treatment on BMD in postmenopausal women. My main concern with this study is that the PICO is not clearly defined and one of the included studies (Lambert et al) treat with a combination of isoflavones and probiotics. The dose of isoflavones is defined but not the strain and dose of the probiotic bacteria. The sensitivity analysis showed that this study affected the results in favour of treatment. One can question if this study can be included since the dose of probiotics is unknown and combined with isoflavones that have beneficial effects on bone. If it's included the authors need to adress this in the discussion and modify their conclusion accordingly. The search dates are not specified and the authors have missed to include a large RCT that would fit the inclusion criteria; Jansson, P.A. et al, Probiotic treatment using a mix of three Lactobacillus strains for lumbar spine bone loss in postmenopausal women: a randomised, double-blind, placebo-controlled, multicentre trial, The Lancet Rheumatology, 2019. Minor comments: - The references 3-5 concerns red clover and isoflavones and not estrogen treatment. - The rodent studies concerning probiotic treatment are not correctly described and the references are wrong. -Figure legends are missing -Cannot find registration number for Review protocol

REVIEWER	Antonio Schiattarella University of Campania Luigi Vanvitelli
REVIEW RETURNED	30-Sep-2020

GENERAL COMMENTS	bmjopen-2020-041393 The Manuscript entitled “Probiotic supplements and bone health in postmenopausal women: a meta-analysis of randomized controlled trials”, deals with the relation between probiotic supplements and osteoporosis in postmenopausal women as Authors systematically searched the literature for randomized controlled trials. Authors found out that probiotic might increase lumbar and hip bone mineral density and reduce bone resorption. The paper falls within the aim of BMJ Open. The topic is interesting enough to attract the readers’ attention. The paper is well written and has important hot points. Nevertheless, authors should clarify some points and improve the introduction citing relevant and novel key articles about the topic. Authors should consider the following recommendations: - Manuscript should be further revised by a native English speaker in order to correct several typos. - Introduction: osteoporosis represents a critical complaint for menopausal women and a natural approach has been increasingly requested by patients. Authors should improve this point, at least briefly, referring to: PMID: 31466381. Methods and analysis: Authors correctly followed the recommendations of PRISMA Statement. However, you should cite this document PMID: 19621072.

REVIEWER	Ben Carter King's College London, UK
REVIEW RETURNED	19-Nov-2020

GENERAL COMMENTS	The authors have raised a very novel and useful question. However, the methods deployed are not described in adequate detail or are the results defined. The authors really need a methodologist/statistician with experience of carrying out a systematic review to look at this manuscript prior to re-submission. 1) The RoB is not adequately described - stating the Cochrane tool is not adequate 2) The outcomes are not defined or the description of how they are measured is not included, or are the comparitors 3) There is no description of the likely mechanism of action 4) Given the level of evidence of the included studies the authors should include widening the inclusion criteria to consider non-randomised trials in the evidence 5) The searches are currently 1.5 years old. This review would be out of date at the time of acceptance- this needs to be updated 6) The analysis methods are vague- they do not describe what they would do in face of heterogeneity. 7) Do not pool with a fixed effects model, there is no justification. There is very much clinical diversity that would warrant fitting a random effects model even if I-square was 0%. 8) State the subgroup analyses to be explored in the face of heterogeneity 9) The weaknesses of the evidence are not clearly stated. For example, the abstract conclusions is not appropriate as it does not reflect the evidence provided since the results section entirely misses out the study risk of bias. 10) The study level risk of bias needs to be stated throughout the review to qualify the level of evidence. From reviewing the individual studies these would not be reported with a low Risk of

	Bias. However I look forward to seeing how the authors have characterized the studies RoB.

REVIEWER	James Bentham University of Kent, UK
REVIEW RETURNED	30-Nov-2020

GENERAL COMMENTS	The authors present a well-conducted meta-analysis of RCTs studying the effects of probiotics on bone health. I have carried out a statistical review and only have two specific comments: 1. The authors don't mention any corrections of p-values for multiple testing. These corrections should be described in methods. 2. The sensitivity analyses should be described in methods, so that the plots in supplementary materials can be interpreted more easily. I also have some minor general comments: 1. The abstract states that the search was to May 2019. Is this a typo? It's stated as 2020 in inclusion and exclusion criteria. 2. The paper is well written and clear. There are just a few typos to correct, e.g,, "to identify any additional relevant studies" and several in Table 1. 3. It's stated that 218 women completed the trials, but the numbers in tables and figures add to 248. This should be clarified. 4. The authors state the limitations well, which makes the paper stronger. Perhaps it should also be mentioned that the studies were only of certain population groups (presumably mostly Japanese, Iranian and Scandinavian).

VERSION 1 – AUTHOR RESPONSE

Reviewer Comments to Author:

Reviewer: 1

Comments to the Author

1.My main concern with this study is that the PICOis not clearly defined.

A. Thanks. We modified the Inclusion criteria with the words:

(2) consideration of postmenopausal women as patients, consideration of probiotic supplement as interventions, consideration of placebo as comparison and consideration of the change of BMD and bone turnover markers as outcomes;

2.one of the included studies (Lambert et al) treat with a combination of isoflavones and probiotics. Th e dose of isoflavones is defined but not the strain and dose of the probiotic bacteria. The sensitivity an alysis showed that this study affected the results in favour of treatment.One can question if this study

can be included since the dose of probiotics is unknown and combined with isoflavones that have ben eficial effects on bone. If it's included the authors need to adress this in the discussion and modify thei r conclusion accordingly.

A. Thanks. We agree with the reviewer that the results of Lambert could probably bring in additional beneficial effects on bone. After added the data of the brilliant study of 'Jansson 2019', we find the new results indicated that probiotic supplements were associated with a significantly higher BMD in lumbar spine, however, there was no difference between probiotic supplements and BMD in hips. The results was not affected by 'Lambert et al' in the sensitivity analysis. Therefore, we modified our conclusion and added the deficiency brought by 'Lambert et al' in the Limitation Section.

3.The search dates are not specified and the authors have missed to include a large RCT that would f it the inclusion criteria; Jansson, P.A. et al, Probiotic treatment using a mix of three Lactobacillus strai ns for lumbar spine bone loss in postmenopausal women: a randomised, double-blind, placebo-controlled, multicentre trial, The Lancet Rheumatology, 2019.

A. Thanks. We update the search to November 2020. And added this brilliant work.

Minor comments:
4. The references 3-5 concerns red clover and isoflavones and not estrogen treatment.

A. Thanks. In clinic, red clover isoflavones is a very popular estrogen supplement treatment. Thanks for reminding, we have added explanation.

5.The rodent studies concerning probiotic treatment are not correctly described and the references ar e wrong.

A. Thanks. We used more accurate description as "supplementation with specific bacterial strains can increase bone density and protect against osteoporosis".
The previous references' supporting evidence was not prominent. We have cited more accurate references with prominent supporting evidence.

6.Figure legends are missing
A. Thanks. We added the figure legends at the end of the manuscript.

7.Cannot find registration number for Review protocol
A. Thanks. We are sorry that this article was not successfully registered with Cochrane

Reviewer: 2

1.Manuscript should be further revised by a native English speaker in order to correct several typos.
A. Thanks. We had a thorough language corrections with help of a native speaker.

2. Introduction: osteoporosis represents a critical complaint for menopausal women and a natural app roach has been increasingly requested by patients. Authors should improve this point, at least briefly, referring to: PMID: 31466381.

A. Thanks. This is an interesting and convincing point, we have added this point.

3.Methods and analysis: Authors correctly followed the recommendations of PRISMA Statement. How ever, you should cite this document PMID: 19621072.

A. thanks. we have cited this documents.

Reviewer: 3

Comments to the Author

1) The RoB(risk of bias) is not adequately described - stating the Cochrane tool is not adequate

A. Thanks. we modified the description of the Cochrane tool with the sentence:

The Cochrane Collaboration's tool was used for assessing risk of bias. Six domain-based evaluations (selection bias, performance bias, detection bias, attrition bias, reporting bias and other bias) were applied in the tool to assess the possible bias of randomized controlled trials. The results were displayed as low risk, unclear risk or high risk of bias.

And we added the results of quality assessment of all included studies in the Supplementary Table 3.

2) The outcomes are not defined or the description of how they are measured is not included, or are t he comparitors

A. Thanks. We modified the Inclusion criteria with the words:

BMD was measured by dual-energy X-ray absorptiometry (DXA) and BTM was measured by blood test at baseline and the end of trial;

3) There is no description of the likely mechanism of action
A. Thanks. we explained the mechanism of action with the words:

"The mechanism of action
The mechanisms of action of probiotics is as follows. Probiotics have many functional properties in humans. They function in the gastrointestinal system by modifying the microbiota composition, intestinal barrier function, and the immune system which feeds back systemic benefits to the host, including bone health. Moreover, probiotic function modifying physiological homeostasis of the intestinal flora can also benefit bone metabolism 32. Gastrointestinal inflammation and systemic inflammation are closed related to enhanced generation of potent osteoclastogenic cytokines as the main cause of bone loss 33-34. Probiotics can restore balance of the gut microbiota, preventing or moderating gut and systemic inflammation and allowing absorption of nutrients, especially in elderly people 35. Furthermore, probiotics decrease the levels of inflammatory mediators and cytokines in the gut and bone marrow 36. These changes give signals to bone cells, including osteoblasts, osteoclasts and stem cells, significantly affecting bone homeostasis. Endocrine factors (such as serotonin and incretins) secreted by intestine also remarkably affect bone cells 37. Anti-inflammatory effects are

among the underlying mechanisms by which probiotics benefit bone metabolism. There is evidence that arginine deiminase, produced by the probiotic Lactobacillus brevis CD2, has an anti-inflammatory effect 38. Supplementation of probiotics may reduce expression of pro-inflammatory and osteolytic cytokines, including TNF-α. These cytokines alter anti-osteoclastogenic cytokine expression, leading to enhanced osteoclast formation and inhibited osteoblast activity 39. Some studies found that probiotic supplementation reduces TNFα, IL-17, and RANKL expression levels in ovariectomized mice 40. These changes give signals to bone cells, such as osteoblasts, osteoclasts and stem cells, which significantly affect bone homeostasis. In this meta-anaysis, TNF-α was reported by two RCTs. One reported 19 that serum levels of TNF-were significantly lower in the probiotic-treated group; however, another study 20 showed there was no differences between probiotic and control groups. More clinical trials are needed in the future to elucidate the relationship between administration of probiotics and anti-inflammatory effects."

4) Given the level of evidence of the included studies the authors should include widening the inclusio n criteria to consider non-randomised trials in the evidence

A. Thanks. we widened the inclusion criteria to "randomized controlled trials and prospective cohort studies;" unfortunately, we did not find relevant prospective cohort studied for this meta-analysis. We discussed it in the Limitation Section.

5) The searches are currently 1.5 years old. This review would be out of date at the time of acceptanc e- this needs to be updated

A. Thanks. We update the search to November 2020. And added a large RCT that fit the inclusion criteria; Jansson, P.A. et al,

6) The analysis methods are vague- they do not describe what they would do in face of heterogeneity.

A. Thanks. we modified the analysis methods with the words:

Meta-regression was conducted to verify whether different types of probiotic supplement would introduce sources of heterogeneity. Random-effects model and subgroup analysis were used in face of heterogeneity.
Sensitivity analysis was conducted to verify the impact of each individual study on the pooled results.

7) Do not pool with a fixed effects model, there is no justification. There is very much clinical diversity t hat would warrant fitting a random effects model even if I-square was 0%.

A. Thanks. we used a random-effects model to process the data, and modified the results.

8) State the subgroup analyses to be explored in the face of heterogeneity

A. Thanks. we modified the analysis methods with the words:
Random-effects model and subgroup analysis were used in face of heterogeneity.

9) The weaknesses of the evidence are not clearly stated. For example, the abstract conclusions is n ot appropriate as it does not reflect the evidence provided since the results section entirely misses out the study risk of bias.

A. Thanks. we modified the abstract conclusions with the words：

Supplementation with probiotics can increase lumbar BMD and reduce bone resorption. More randomized controlled trials are recommended to validate the result.

Add we added the words
“The limited number of reports focusing on the association between probiotic supplement and BMD and bone turnover markers prevented us from conducting subgroup analysis and drawing conclusive summaries.”
to the Limitation section：

10) The study level risk of bias needs to be stated throughout the review to qualify the level of evidenc e. From reviewing the individual studies these would not be reported with a low Risk of Bias. However I look forward to seeing how the authors have characterized the studies RoB.

A. Thanks. We added the results of quality assessment of all included studies in the Supplementary Table 3.

Reviewer: 4

1. The authors don't mention any corrections of p-
values for multiple testing. These corrections should be described in methods.

A. Thanks. we are sorry that we don't understand the problem well. Did the reviewer indicate that P-values were adjusted for multiple testing using the false discovery rate (FDR) approach?

2. The sensitivity analyses should be described in methods, so that the plots in supplementary materi als can be interpreted more easily.

A. Thanks. we described the sensitivity analyses with the words:

Sensitivity analysis was conducted to verify the impact of each individual study on the pooled results. In the sensitivity analyses, each study was omitted to recalculate the pooled estimates.

3. The abstract states that the search was to May 2019. Is this a typo? It's stated as 2020 in inclusion and exclusion criteria.

A. Thanks. we corrected the mistake and updated the search to November 2020. And added a large RCT that fit the inclusion criteria; Jansson, P.A. et al,

2. The paper is well written and clear. There are just a few typos to correct, e.g,, "to identify any additi onal relevant studies" and several in Table 1.

A. Thanks. we corrected the mistake

3. It's stated that 218 women completed the trials, but the numbers in tables and figures add to 248. T his should be clarified.

A. Thanks. we corrected the mistake and updated the search to November 2020. And added a large RCT that fit the inclusion criteria; Jansson, P.A. et al. At last, 497 women completed the trials.

4. The authors state the limitations well, which makes the paper stronger. Perhaps it should also be m entioned that the studies were only of certain population groups (presumably mostly Japanese, Irania n and Scandinavian).

A. Thanks. we modified the Limitation section with the words:

only five randomized controlled trials with certain population groups satisfied our inclusion criteria. the limited number of reports and certain population groups focusing on the association between probiotic supplement and BMD and bone turnover markers prevented us from conducting subgroup analysis and drawing conclusive summaries. Furthermore, insufficient number of estimates inflate the impact of the results of a particular study.

VERSION 2 – REVIEW

REVIEWER	Ben Carter King's College London, UK
REVIEW RETURNED	30-Jan-2021

GENERAL COMMENTS	The review has been improved. I have a number of minor comments 1) Revise the conclusions and abstract to state that caution is needed when interpreting the effects, due to the quality and quantity of the included evidence and that the effects may be vary on presentation of new studies 2) State the study level RoB findings in the results section Discussion 3) State the number of included studies and participants in the first paragraph of the discussion as well as the number of studies with Low/Unclear/High risk of RoB 4) State the limitations of few and low quality studies and the conclusions may change on the publication of future studies

REVIEWER	James Bentham University of Kent, UK
REVIEW RETURNED	14-Jan-2021

GENERAL COMMENTS	The authors have answered my comments, and I have only one extremely minor further comment. I think this can be dealt with during the proofs stage, and I don't need to see the paper again. There is a discrepancy between the 0.27 in line 198, and 0.26 in Figure 2, which should be corrected.

VERSION 2 – AUTHOR RESPONSE

Reviewer: 4
Comments to the Author:
The authors have answered my comments, and I have only one extremely minor further comment. I think this can be dealt with during the proofs stage, and I don't need to see the paper again.
There is a discrepancy between the 0.27 in line 198, and 0.26 in Figure 2, which should be corrected.
A. thanks. We correct this mistake

Reviewer: 3
Comments to the Author:
1) Revise the conclusions and abstract to state that caution is needed when interpreting the effects, due to the quality and quantity of the included evidence and that the effects may be vary on presentation of new studies
A. Thanks. we modify the abstract with the words:
“We conclude cautiously that supplementation with probiotics could increase lumbar BMD. More randomized controlled trials are recommended to validate or update these results”
We modify the conclusion with the words:
“Our systematic review and meta-analysis showed that probiotic supplementations in postmenopausal women were associated with preserving lumbar spine BMD. The results should be interpreted with caution and more high quality RCTs are needed to validate or update these results. An appropriate supplement of probiotics could be recommended to improve bone status in postmenopausal women.”

2) State the study level RoB findings in the results section
A. Thanks. we modify the results section with the sentence:
“All the five RCTs had low risk of bias (available in Supplementary Table 2).”

Discussion
3) State the number of included studies and participants in the first paragraph of the discussion as well as the number of studies with Low/Unclear/High risk of RoB
A. Thanks. we modify the discussion section with the sentence:
“This meta-analysis included five randomized controlled trials with low risk of bias and 497 postmenopausal women.”

4) State the limitations of few and low quality studies and the conclusions may change on the publication of future studies
A. Thanks. we modify the limitation section with the sentence:
“the insufficient number of estimates inflates the impact of the results of a particular study and the conclusions may change on the publication of future studies.”