PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Selenium and bone health: a protocol for a systematic review and meta-analysis
AUTHORS	Wang, Ning; Xie, Dongxing; Wu, Jing; Wu, Ziying; he, hongyi; Yang, Zidan; Yang, Tuo; Wang, Yilun

VERSION 1 – REVIEW

REVIEWER	Jan J. Stepan Charles University Faculty of Medicine 1 Institute of Rheumatology Prague Czech Republic
REVIEW RETURNED	24-Jan-2020

GENERAL COMMENTS	This manuscript is presented as a study protocol aimed to perform a systematic review and meta-analysis on the associations between selenium and bone mineral density and risk of fracture. The article is clearly written, is concise, clear and well organized. Major comment There is a major flaw in the study that would prevent a sound interpretation of the data. According to the Methods, … no sex, ethnicity, economic status, geographical limitations, or education restrictions will be applied. The meta-analysis should be conclusive as to whether changes in bone status associated with selenium excess and deficiency are from selenium, independent of other important risk factors. Effects of selenoproteins in antioxidant defense is strongly modified by intakes of other antioxidants (vitamin E, b-carotene). Also, associations between selenium, BMD and fractures is strongly modified by various factors, such as nutritional status, menopausal status, smoking status. calcium and vitamin D supply. Minor comments 1. Title. The outcomes of the study will include only BMD and the prevalence or incidence of osteoporosis and osteoporotic fractures, rather than bone health. 2. Page 6, line 38. What is the meaning of this sentence; does it refer to the idiopathic, involutional or secondary osteoporosis? 3. Page 8, line 33. The participants in the included studies must be … with data of BMD or diagnosis of osteoporosis or osteoporotic fractures. What criteria (except for BMD) will be used to establish osteoporosis? 4.. Page 8, line 50. BMD values measured with Lunar and Hologic machines differ substantially. Therefore, T-score instead of BMD values should be used. 6. Page 16. Reference 3 should be properly cited.

REVIEWER	Aleksandra Buha Department of Toxicology University of Belgrade-Faculty of Pharmacy Serbia
REVIEW RETURNED	13-Feb-2020

GENERAL COMMENTS	Please specify if there will be any restrictions regarding age of participants.

REVIEWER	Erin Gaffney-Stomberg DoD, USA
REVIEW RETURNED	18-Feb-2020

GENERAL COMMENTS	A well-conducted systemic review and meta-analysis would be a welcomed addition to the literature. The study design and analysis seem appropriate, although a statistical review by a statistician with experience in meta-analyses would be helpful. Overall, the protocol manuscript is well-written, and I have only minor suggested edits: 1. Clarify in the abstract and methods that the existing literature search will be restricted to human studies. 2. Introduction, p6, line 8: "...the ability of the human body..." 3. Introduction, p6, line 35: "...proxy measure that accounts..." 4. Introduction, p6, line 45: "nutritional support" typically refers to total parenteral nutrition, or feeding through alternative means such as a feeding tube. Suggest re-wording to "adequate nutrition" or "optimal nutrition" 5. Eligibility criteria, p8, line 33-34: "...studies must have provided information on dietary intake or serum levels of selenium, and BMD measures or diagnosis..." 6. Discussion, p13, lines 4-6: " Osteoporosis is becoming..." 7. Discussion, p13, line 20: "...might have been too small to achieve..."

REVIEWER	robberecht harry University of Antwerp Department of Pharmaceutical Sciences Belgium.
REVIEW RETURNED	20-Feb-2020

GENERAL COMMENTS	1) small remarks: abbreviated title and keywords: use small letter fi selenium and bone health, …..the same for reference 11 in the title. 2) I am not that strong in statistics, sorry for that 3) the limitations of the study are not that highlighted 4) I should not use the reference 15 and 20. In 15 there other antioxidants and all measured effects are modified by smoking status 5) what is the most interesting to readers and the scientific community is the discussion (conclusions are not included !). I should omit the two mentioned references for cited reasons. The whole page 13 is fattening of the article. Most of the things are already said. Due to the small amount of publications no definite conclusions can be drawn, i agree on that, but some reasons for found contradictory results ought to be mentioned.

REVIEWER	Dr Sonia Gran University of Nottingham, UK
REVIEW RETURNED	31-Mar-2020

GENERAL COMMENTS	This is a very well written protocol. I just noticed that prospective citations e.g. using google scholar, and looking at grey literature were not included. Could the authors consider using these to make their search strategy more comprehensive?

VERSION 1 – AUTHOR RESPONSE

Reviewer #1:

Major comment: There is a major flaw in the study that would prevent a sound interpretation of the data. According to the Methods, … no sex, ethnicity, economic status, geographical limitations, or education restrictions will be applied. The meta-analysis should be conclusive as to whether changes in bone status associated with selenium excess and deficiency are from selenium, independent of other important risk factors. Effects of selenoproteins in antioxidant defense is strongly modified by intakes of other antioxidants (vitamin E, b-carotene). Also, associations between selenium, BMD and fractures is strongly modified by various factors, such as nutritional status, menopausal status, smoking status. calcium and vitamin D supply
Response: Thanks for the suggestion. First, to minimize the heterogeneity between included studies, subgroup analyses based on different sex, ethnicity, economic status, geographical limitations, or education restrictions will be conducted where feasible. Second, to adjust the effect of various factors, such as nutritional status, menopausal status, smoking status. calcium and vitamin D supply on the association between selenium and bone health, we will extract “adjusted” effect sizes from each study. Then, both unadjusted risk estimates and adjusted estimates will be pooled in our meta-analysis. The above two methods are both effective approach that has been commonly adopted in some high-quality meta-analyses(1. Yang Y, Li W, Zhu H, et al. Prognosis of unrecognised myocardial infarction determined by electrocardiography or cardiac magnetic resonance imaging: systematic review and meta-analysis. BMJ. 2020;369:m1184; 2. Willems RPJ, van Dijk K, Ket JCF, et al. Evaluation of the Association Between Gastric Acid Suppression and Risk of Intestinal Colonization With Multidrug-Resistant Microorganisms: A Systematic Review and Meta-analysis. JAMA Intern Med. 2020;180(4):561-571.).
Action: “Then, both the adjusted and unadjusted effect sizes (mean difference [MD], odds ratio [OR], relative risk [RR], hazard ratio [HR] or beta coefficient [β]) will be either extracted directly or calculated based on the information in the original studies whenever possible” (Page 10, lines 20, in the clean copy of the revised manuscript)
“Unadjusted risk estimates and adjusted estimates will be pooled in the meta-analysis.” (Page 11, lines 17, in the clean copy of the revised manuscript)
“Finally, to minimize the heterogeneity between included studies, subgroup analyses based on different age, sex, ethnicity, economic status, geographical limitations, or education restrictions will be conducted where feasible.” (Page 12, lines 4, in the clean copy of the revised manuscript)

Comment 1: Title. The outcomes of the study will include only BMD and the prevalence or incidence of osteoporosis and osteoporotic fractures, rather than bone health.
Response: There are some previous high-quality studies that use bone health to represent BMD and fracture risk (1. Hampton T. Experts urge early investment in bone health. JAMA. 2004;291(7):811-

812; 2. Bundred NJ. Optimising bone health in survivors of breast cancer. Lancet Oncol. 2007;8(2):89-91.). So it is appropriate to use bone health to generalize BMD, osteoporosis and osteoporotic fractures in our article. We have explained it in the manuscript, and we will further satisfy the reviewers in the next revision if there are any other comments.
Action: “Bone health is mainly reflected in diseases caused by bone mass loss, such as osteoporosis and fragility fracture.” (Page 6, lines 5, in the clean copy of the revised manuscript)

Comment 2: Page 6, line 38. What is the meaning of this sentence; does it refer to the idiopathic, involutional or secondary osteoporosis?
Response: Thanks for the suggestion. In order to avoid ambiguity, we replaced “aetiology” with “risk factors” in this sentence.
Action: “However, osteoporosis can occur without a known underlying cause, and its risk factors have not been fully elucidated.” (Page 6, lines 15, in the clean copy of the revised manuscript)

Comment 3: Page 8, line 33. The participants in the included studies must be … with data of BMD or diagnosis of osteoporosis or osteoporotic fractures. What criteria (except for BMD) will be used to establish osteoporosis?
Response: Thanks for the suggestion. The diagnostic criteria of osteoporosis shall be based on the each included study, such as the presence of a fragility fracture or using BMD criteria. We will also add the description of the osteoporosis diagnostic criteria of each included study in our review.
Action: “The diagnostic criteria of osteoporosis shall be based on the included articles, such as the presence of a fragility fracture or using BMD criteria.” (Page 9, lines 3, in the clean copy of the revised manuscript)

Comment 4: Page 8, line 50. BMD values measured with Lunar and Hologic machines differ substantially. Therefore, T-score instead of BMD values should be used.
Response: Thanks for the suggestion. We will give priority to extracting T-score for analysis. When T-score is not available, we will try to analyze BMD measured with Lunar and Hologic machines separately.
Action: “There will be no restriction on site and measuring method, but we will give priority to extracting T-score for analysis rather than to extracting BMD values. When T-score is not available, we will try to analyze BMD measured with Lunar and Hologic machines separately.” (Page 9, lines 6, in the clean copy of the revised manuscript)

Comment 5: Page 16. Reference 3 should be properly cited.
Response: We agree with the Reviewer’s comment and have revised the manuscript accordingly.
Action: “3. NIH Consensus Development Panel on Osteoporosis Prevention, Diagnosis, and Therapy. Osteoporosis prevention, diagnosis, and therapy. JAMA 2001;285:785-95.” (Page 17, lines 6, in the clean copy of the revised manuscript)

Reviewer #2:

Comment 1: Please specify if there will be any restrictions regarding age of participants.
Response: Thanks for your suggestion. We have revised the manuscript and there will be no restriction on age of participants. Furthermore, we will conduct subgroup analyses based on age where feasible.
Action: “No age, sex, ethnicity, economic status, geographical limitations, or education restrictions will be applied.” (Page 8, lines 16, in the clean copy of the revised manuscript)
“Finally, to minimize the heterogeneity between included studies, subgroup analyses based on different age, sex, ethnicity, economic status, geographical limitations, or education restrictions will be conducted where feasible.” (Page 12, lines 4, in the clean copy of the revised manuscript)

Reviewer #3:

Comment 1: Clarify in the abstract and methods that the existing literature search will be restricted to human studies.
Response: Thanks for the suggestion. In the revised manuscript we clarified that we will only include human studies.
Action: “Both interventional and observational studies in humans will be included.” (Page 3, lines 15, in the clean copy of the revised manuscript)
“The meta-analysis will include both interventional and observational studies in humans, inclusive of case-control studies, randomized controlled trials, cross-sectional studies and cohort studies which focus on the associations between dietary intake or serum levels of selenium and BMD, osteoporosis, or osteoporotic fractures.” (Page 8, lines 8, in the clean copy of the revised manuscript)

Comment 2: Introduction, p6, line 8: "...the ability of the human body..."
Response: We agree with the Reviewer’s comment and have revised the manuscript accordingly.
Action: “Bone health affects the ability of the human body to stay active throughout life, and the degradation of bone health can cause considerable morbidity and mortality.” (Page 2, lines 6, in the clean copy of the revised manuscript)

Comment 3: Introduction, p6, line 35: "...proxy measure that accounts..."
Response: We agree with the Reviewer’s comment and have revised the manuscript accordingly.
Action: “Osteoporosis is practically diagnosed by the presence of a fragility fracture or low bone mineral density (BMD) which is a commonly used proxy measure that accounts for approximately 70% of bone strength” (Page 6, lines 13, in the clean copy of the revised manuscript)

Comment 4: Introduction, p6, line 45: "nutritional support" typically refers to total parenteral nutrition, or feeding through alternative means such as a feeding tube. Suggest re-wording to "adequate nutrition" or "optimal nutrition"
Response: We agree with the Reviewer’s comment and have revised the manuscript accordingly.
Action: “Nutrition has a significant influence on bone health and adequate nutrition is among the crucial cornerstones of the prevention of osteoporosis.” (Page 6, lines 19, in the clean copy of the revised manuscript)

Comment 5: Eligibility criteria, p8, line 33-34: "...studies must have provided information on dietary intake or serum levels of selenium, and BMD measures or diagnosis..."
Response: We agree with the Reviewer’s comment and have revised the manuscript accordingly.
Action: “The participants in the included studies must have provided information on dietary intake or serum levels of selenium, and BMD measures or diagnosis of osteoporosis or osteoporotic fractures.” (Page 8, lines 14, in the clean copy of the revised manuscript)

Comment 6: Discussion, p13, lines 4-6: " Osteoporosis is becoming..."
Response: We agree with the Reviewer’s comment and have deleted this sentence.

Comment 7: Discussion, p13, line 20: "...might have been too small to achieve..."
Response: We agree with the Reviewer’s comment and have revised the manuscript accordingly.
Action: “The sample size of previous studies might have been too small to achieve sufficient statistical power, which could explain, at least in part, why the statistical difference in some instances was not reached, despite the obvious trends” (Page 13, lines 17, in the clean copy of the revised manuscript)

Reviewer #4:

Comment 1: small remarks: abbreviated title and keywords: use small letter fi selenium and bone health, …..the same for reference 11 in the title.
Response: Thanks for the suggestion. We have revised the manuscript accordingly.
Action: “Abbreviated title: Selenium and bone health” (Page 1, lines 15, in the clean copy of the revised manuscript)
“Keywords: selenium, osteoporosis, serum, dietary, meta-analysis” (Page 1, lines 17, in the clean copy of the revised manuscript)
“13. Beukhof CM, Medici M, van den Beld AW, et al. Selenium status is positively associated with bone mineral density in healthy aging European men. PLoS One 2016;11:e0152748.” (Page 17, lines 23, in the clean copy of the revised manuscript)

Comment 2: the limitations of the study are not that highlighted
Response: Thanks for the suggestion. We have revised the limitation section in the revised manuscript.
Action: “Both prospective and retrospective studies will be included in this meta-analysis, which may also incur bias and impact the final results .” (Page 5, lines 14, in the clean copy of the revised manuscript)

Comment 3: I should not use the reference 15 and 20. In 15 there other antioxidants and all measured effects are modified by smoking status
Response: Thanks for the suggestion. First, the data that have not been adjusted by smoking status will be calculated based on the data given in reference 15 and 20. Second, subgroup analyses based on smoking status could be conducted through these two articles.

Comment 4: what is the most interesting to readers and the scientific community is the discussion (conclusions are not included !). I should omit the two mentioned references for cited reasons. The whole page 13 is fattening of the article. Most of the things are already said. Due to the small amount of publications no definite conclusions can be drawn, i agree on that, but some reasons for found contradictory results ought to be mentioned.
Response: Thanks for the suggestion. We have cut down the unnecessary sentences and revised the discussion section in the manuscript.
Action: “The health of the skeletal system is important for elderly people.1 An in-depth understanding of the relationship between selenium and bone health is useful for designing early life interventions. To our best knowledge, there are at least 10 studies that have investigated the associations between dietary or serum selenium concentrations and BMD, osteoporosis, or osteoporotic fractures. Of them, two studies suggested that serum and dietary selenium were likely to positively correlate with BMD,13 14 and three studies showed that dietary selenium was negatively associated with osteoporotic fractures.17 18 24 On the contrary, one study found no association between dietary selenium and BMD,25 and four studies reported that neither serum nor dietary selenium was related to osteoporosis.15 16 26 27 Contradictory results of these studies might be related to the differences in study design and sample characteristics. So it's still controversial whether the contents of selenium can directly influence BMD and affect the pathogenesis of osteoporosis. The sample size of previous studies might have been too small to achieve sufficient statistical power, which could explain, at least in part, why the statistical difference in some instances was not reached, despite the obvious trends. Therefore, the aim of this systematic review and meta-analysis is to summarize the available evidence to investigate the associations between selenium and bone health.” (Page 13, lines 5, in the clean copy of the revised manuscript)

Reviewer #5:

Comment 1: I just noticed that prospective citations e.g. using google scholar, and looking at grey literature were not included. Could the authors consider using these to make their search strategy

more comprehensive?
Response: Thanks for the suggestion. We will search grey literature for potential eligible studies, e.g., ClinicalTrials.gov and google scholar.
Action: “And Grey literature will also be searched included study registries (e.g., ClinicalTrials.gov and google scholar).” (Page 9, lines 15, in the clean copy of the revised manuscript)

VERSION 2 – REVIEW

REVIEWER	Jan J. Stepan Charles University, Faculty of Medicine 1, Institute of Rheumatology, Prague, Czech Republic
REVIEW RETURNED	22-Jul-2020

GENERAL COMMENTS	1. The hypothesis to be tested is not supported by analysis of mode of action of selenium on bone quality. 2. The associations between selenium and bone mineral density and risk of fracture cannot be assessed independent of pathophysiology of decrease in BMD and increase in risk of fracture. In the protocol, different types of osteoporosis (such as bone loss due to sex hormone deficiency, glucocorticoid induced osteoporosis, involutional osteoporosis, osteoporosis associated with vitamin deficiency, etc.) are not taken into account. 3. In the Methods, assessment of bone mineral density is poorly specified.

REVIEWER	robberecht harry University of Antwerp, Belgium. Department of Pharmaceutical Sciences
REVIEW RETURNED	12-Aug-2020

GENERAL COMMENTS	the article has been enhanced in quality, however it is still not conclusive enough and limitations are not all enumerated.

VERSION 2 – AUTHOR RESPONSE

Reviewer #1:

Comment 1 The hypothesis to be tested is not supported by analysis of mode of action of selenium on bone quality
Response: Thanks for your suggestion, and we have given more details regarding our hypothesis. We want to test the hypothesis that selenium is associate with bone mineral density or osteoporosis by including relevant studies in this field. In order to test the hypothesis more reliably, we will try to minimize the effect of confounder by extracting “adjusted” effect sizes from each relevant study.
Action: “Thus, we aimed to systematically examine the existing literature in this field to test the hypothesis that dietary or serum selenium concentrations are associate with BMD, osteoporosis, or osteoporotic fractures.” (Page 7, lines 14, in the clean copy of the revised manuscript)

Comment 2 The associations between selenium and bone mineral density and risk of fracture cannot be assessed independent of pathophysiology of decrease in BMD and increase in risk of fracture. In the protocol, different types of osteoporosis (such as bone loss due to sex hormone deficiency, glucocorticoid induced osteoporosis, involutional osteoporosis, osteoporosis associated with vitamin deficiency, etc.) are not taken into account.

Response: Thanks for your suggestion. We will catalog and categorize the primary and secondary osteoporosis in included studies. And we will try to conduct subgroup analyses based on different types of osteoporosis, or exclude secondary osteoporosis if relevant studies are not enough for subgroup analysis.
Action: “Osteoporosis is classified as “primary” and “secondary”, and secondary osteoporosis is due to certain clinical disorders independent of age and estrogen deficiency. We will catalog and categorize the different types of primary and secondary osteoporosis according included studies.” (Page 9, lines 6 in the clean copy of the revised manuscript)
“Finally, to minimize the heterogeneity between included studies, subgroup analyses based on different age, sex, ethnicity, economic status, geographical limitations, education restrictions, or causes of osteoporosis will be conducted where feasible.” (Page 12, lines 8 in the clean copy of the revised manuscript)

Comment 3 In the Methods, assessment of bone mineral density is poorly specified.
Response: Thanks for your suggestion, and we have given more details regarding the assessment of bone mineral density.
Action: “The diagnostic criteria of osteoporosis shall be based on the WHO criteria. T-score between 0 and -1 is considered normal and T-score ≤-2.5 is considered osteoporosis. BMD corresponds to osteopoenia if T-score ranges between -1 and -2.5.” (Page 9, lines 3, in the clean copy of the revised manuscript)

Reviewer #4:

Comment 1 The article has been enhanced in quality, however it is still not conclusive enough and limitations are not all enumerated.
Response: Thanks for your suggestion and we have revised the manuscript. In addition, our subsequent review and meta-analysis will make a sufficiently detailed discussion on the conclusion when we complete our analysis.
Action: “We wish to investigate the associations between selenium and the different types of primary and secondary osteoporosis, however, such subgroup analysis is difficult or even impossible given the small number of studies will probably be included.” (Page 5, lines 16, in the clean copy of the revised manuscript)
“Selenoproteins expressed in human fetal osteoblasts would appear to protect the bone against oxidative stress, which may contribute to the development of osteoporosis by inhibiting osteoblastic differentiation of bone marrow stromal cells. The trace element selenium, as a critical constituent of selenoproteins, is much more likely to be an essential role in the associations between selenium and bone mineral density.” (Page 13, lines 11, in the clean copy of the revised manuscript)

VERSION 3 – REVIEW

REVIEWER	Jan Stepan Charles University Faculty of Medicine 1, Institute of Rheumatology, Prague, Czech Republic.
REVIEW RETURNED	25-Sep-2020

GENERAL COMMENTS	The limitations pointed out by reviewer have been adequately incorporated into the manuscript.