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Selenium and bone health: a protocol for a systematic review and meta-analysis

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Title: Selenium and bone health: a protocol for a systematic review and meta-analysis

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ABSTRACT

Introduction Bone health affects the ability of human body to stay active and the degradation of bone health can cause considerable morbidity and mortality. The factors related to bone health play an important role in preventing osteoporosis and its adverse consequences. However, the aetiology of osteoporosis has not been fully elucidated. The deficiency of the trace element selenium may be one of the risk factors for the development of osteoporosis. Previous studies have investigated the effects of selenium on osteoporosis; however, the results are inconclusive. Therefore, the present study aimed to systematically examine the existing literature on the associations between dietary or serum selenium and bone mineral density (BMD), osteoporosis, or osteoporotic fractures, and to quantify such associations through meta-analysis.

Methods and analysis PubMed, Embase and Cochrane Library will be searched by applying the specified search strategy to identify relevant studies up to October 2019. The outcomes will include BMD and the prevalence or incidence of osteoporosis and osteoporotic fractures. For the dietary or serum selenium and BMD, osteoporosis, or osteoporotic fractures pooled analyses, estimates will be expressed as the mean difference, and the pooled odds ratio, relative risk, hazard ratio or beta coefficient and corresponding 95% confidence intervals. The heterogeneity of the studies and the publication bias will be investigated accordingly. To assess the quality and the risk of bias of the included studies, the Newcastle-Ottawa Scale or the Cochrane risk of bias assessment tool will be used where appropriate.

Ethics and dissemination Since no private and confidential patient data will be contained in the reporting, approval from an ethics committee is not required. The results will be published in a peer-reviewed journal. The study raises no ethical issues.

PROSPERO registration number CRD42019147188

Strengths and limitations of the study

- As the first meta-analysis that evaluated the associations between dietary selenium intake or serum selenium concentrations and BMD, osteoporosis, or osteoporotic fractures, the findings of this study will deepen the existing knowledge base on the pathogenesis of osteoporosis, and promote the development of effective preventive or treatment strategies.
- Two investigators will perform the study selection, data extraction and quality assessments independently, and disagreements will be resolved by discussions.
- Both the quality and the risk of bias will be properly assessed for the included studies by applying the Newcastle-Ottawa Quality Scale or the Cochrane risk of bias assessment tool where appropriate.
- The differences in study design and sample characteristics may lead to a high level of heterogeneity.

INTRODUCTION

Bone health affects the ability of human body to stay active throughout life, and the degradation of bone health can cause considerable morbidity and mortality.[1 2] The various factors related to bone health at different ages play an important role in preventing osteoporosis and its adverse consequences.[3] Osteoporosis, which is characterized by low bone mass and microarchitectural deterioration of bone tissue, is a systemic skeletal disease that can result in increased bone fragility and increased fracture risk as a consequence.[3 4] With an ageing population, the socioeconomic and medical impact of osteoporosis will increase rapidly. It is estimated that by 2020, there will be over 200 million people worldwide affected by osteoporosis, and the expenses related to osteoporosis will rise to about $25.3 billion by 2025.[5] Osteoporosis is practically diagnosed by the presence of a fragility fracture or low bone mineral density (BMD) which is a commonly used proxy measure what accounts for approximately 70% of bone strength.[3] However, osteoporosis can occur without a known underlying cause, and its aetiology has not been fully elucidated.

Nutrition has a significant influence on bone health and nutritional support is among the crucial cornerstones of the prevention of osteoporosis.[6] The trace element selenium, as a critical constituent of about 25 selenoenzymes, plays an essential role in a variety of physiological processes and it has been confirmed that the beneficial effects of selenium are related to human health.[7 8] Some studies collectively suggested that the preservation of selenoproteins in bone was essential to normal skeletal

development.[9] Animal studies have shown that selenium-deprivation can retard growth and change bone metabolism.[10] Such effects are associated with reduced BMD, impaired bone microarchitecture and increased bone resorption. Previous clinical studies have investigated the effects of selenium on bone health, however, the results are inconclusive. Some suggested that serum and dietary selenium were positively correlated with BMD,[11 12] while other showed that neither serum nor dietary selenium was associated with osteoporosis.[13 14] Meanwhile, some case-control studies revealed that the dietary intake of selenium was associated with reduced risk of osteoporotic hip fracture.[15 16]

Meta-analysis is an effective tool for revealing trends that may not be apparent, and it also helps in establishing clinical policies and guidelines. Thus, we aimed to systematically examine the existing literature on the associations between dietary or serum selenium concentrations and BMD, osteoporosis, or osteoporotic fractures, and to quantify such associations through meta-analysis where feasible.

METHODS

Study design

The aim of this meta-analysis is to investigate the associations between dietary intake or serum levels of selenium and BMD, osteoporosis, or osteoporotic fractures. This protocol was developed according to the Preferred Reporting Items for Systematic Review and Meta-Analyses Protocols (PRISMA-P) 2015 Statement,[17] and has been

registered in the international prospective register of systematic reviews PROSPERO network (registration number: CRD42019147188).

Eligibility criteria

Types of studies

The meta-analysis will include both interventional and observational studies, 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.

Types of participants

The participants in the included studies must be with information on dietary intake or serum levels of selenium, and with data of BMD or diagnosis of osteoporosis or osteoporotic fractures. No sex, ethnicity, economic status, geographical limitations, or education restrictions will be applied.

Types of exposure/intervention

Two main forms of exposure will be included, i.e., dietary intake selenium and serum levels of selenium.

Types of outcome measures

Primary outcomes will include BMD values and the prevalence or incidence of

osteoporosis. The secondary outcome will be the prevalence or incidence of osteoporotic fractures. There will be no restriction on site and measuring method.

Information sources

Systematic literature searches will be undertaken across PubMed, Embase and Cochrane Library by applying the specified search strategy to identify relevant studies up to October 2019 on each platform or database. In addition, the references of the retrieved literature will be manually searched, and the relevant studies and systematic reviews will be scanned for additional eligible studies.

Search strategy

Keyword terms or medical subject heading terms (MESH) will be used to search for eligible studies in the databases mentioned above. The electronic search strategy is presented in **Table 1**. All the search terms will be adapted for different syntax rules of the databases.

Study selection

The search results from the three electronic databases will be sent to Endnote. After removing duplicates, two investigators will be responsible for screening the titles and abstracts of all the retrieved literature to identify eligible studies. In case that the eligibility of a study cannot be determined, the full text will be reviewed for inclusion according to prespecified inclusion and exclusion criteria. There will be no restriction

on the publication date and language. The study selection process will be summarized based on the PRISMA flow diagram. Disagreements between the two investigators will be resolved by discussing with a third investigator.

Data extraction

Two investigators will be engaged to independently extract the following data in a standardized collection form: publication information (author, year of publishing); study information (country of origin, study setting, data sources, study period); demographic information (sample size, age, and sex distribution); exposure information (dietary or serum selenium concentrations); outcome information (BMD, osteoporosis, osteoporotic fractures). Then, the 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. If the relevant data is not reported in an included study, the missing information will be obtained by contacting the authors directly as far as possible. The two investigators responsible for reviewing the literature will resolve any disagreements through discussions, and a third independent investigator may be involved into the discussion if required.

Assessment of risk of bias

The Cochrane risk of bias assessment tool will be used to assess the quality of RCTs,[18] while the Newcastle-Ottawa Quality Scale (NOS) will be used to assess the

quality of observational studies.[19] Two investigators will be engaged to conduct the risk of bias assessment independently, and disagreements will be resolved through discussions.

Data analysis

Firstly, the characteristics of included studies will be summarized using baseline tables and narrative texts. The proposed study will calculate the MD, the pooled OR, RR, HR or β and the corresponding 95% CIs. Then, Cochrane's Q test and the I^2 statistics will be used to assess the heterogeneity of the included studies, where *p* value > 0.05 of the Q statistics and I^2 value < 50% indicate statistical homogeneity. If the included studies are found to be statistically homogeneous, the fixed effects models will be used to pool the data during meta-analysis; otherwise, the random effects models will be used instead. Statistical analyses will be conducted in Review Manager-5.3 (Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014) and Stata-11.0 (StataCorp LP, College Station, TX) statistical software. Statistical significance is considered at *p* value < 0.05.

Assessment of publication bias

If the number of included studies is greater than 10, a funnel plot will be constructed and the Egger test will be conducted to observe the publication bias. The existence of publication bias is confirmed when the funnel plot shows an asymmetric pattern. Then, the bias, if any, will be explained through discussions after assessment.

Patient and public involvement

As our systematic review will be implemented based on published studies, no patients and members of the public will be directly involved. All the data to be used in this study already exists in the published literature and/or the aforementioned sources.

Ethics and dissemination

As this meta-analysis will be performed based on published studies, and no private and confidential patient data will be contained in the reporting, approval from an ethics committee is not required. The results will be disseminated through publication in a peer- reviewed journal. The study raises no ethical issues.

DISCUSSION

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,[11 12] and three studies showed that dietary selenium was negatively associated with osteoporotic fractures.[15 16 20] On the contrary, one study found no association between dietary selenium and BMD,[21] and four studies reported that neither serum nor dietary selenium was related to osteoporosis.[13 14 22 23]

The health of the skeletal system is important for elderly people.[1] Nowadays, osteoporosis is becoming increasingly more prevalent with population aging around the world, presenting a severe public health problem in the future.[5] Selenium is an essential element for bones, and plays an important role in skeletal development.[10] An in-depth understanding of the relationship between selenium and bone health is useful for designing early life interventions. The sample size of previous studies might have not always been big enough 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. The results of this study will deepen the existing knowledge base on the pathogenesis of osteoporosis, and promote the development of preventive or treatment strategies in this field.

COMPETING INTERESTS

The authors declare that they have no conflict of interest.

PATIENT CONSENT FOR PUBLICATION

Not required.

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Data curation: Jing Wu, Ziying Wu, Hongyi He, Zidan Yang

Methodology: Jing Wu, Ziying Wu, Hongyi He, Zidan Yang

Writing – original draft: Ning Wang and Dongxing Xie

Writing – review & editing: Yilun Wang and Tuo Yang

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Table 1 Electronic search strategy in Pubmed

Number	Search terms
1	selenium[mesh] or "selenious acid"[mesh] or selen*[tiab] or selepen[tiab] or organoselen*[tiab] or natriumselen*[tiab] or methylseleninic[tiab] or methylselenium[tiab] or selenomethionin*[tiab] or selenit*[tiab]
2	bone demineralization[mesh] or bone density[mesh] or osteopenia[mesh] or osteoporosis[mesh] or bone densitometry[mesh] or osteoporo*[tiab] or bone densit*[tiab] or bone loss[tiab] or osteomalacia[tiab] or osteodystrophy[tiab] or bone demineralization[tiab] or osteopenia[tiab] or bone mass[tiab] or bone mineral content*[tiab] or bone defect*[tiab] or bone strength[tiab] or BMC[tiab] or "fracture"[mesh] or fracture* [tiab]
3	1 AND 2

Reporting checklist for protocol of a systematic review.

Based on the PRISMA-P guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

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		Reporting Item	Page Number
Title			
Identification	#1a	Identify the report as a protocol of a systematic review	P1
Update	#1b	If the protocol is for an update of a previous systematic review, identify as such	n/a

Registration			
	#2	If registered, provide the name of the registry (such as PROSPERO) and registration number	P4, P7-8
Authors			
Contact	#3a	Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author	P1-2
Contribution	#3b	Describe contributions of protocol authors and identify the guarantor of the review	P14-15
Amendments			
	#4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments	n/a
Support			
Sources	#5a	Indicate sources of financial or other support for the review	P14
Sponsor	#5b	Provide name for the review funder and / or sponsor	P14
Role of sponsor or funder	#5c	Describe roles of funder(s), sponsor(s), and / or institution(s), if any, in developing the protocol	P14
Introduction			
Rationale	#6	Describe the rationale for the review in the context of what is	P6-7

		already known	
Objectives	#7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	P8-9
Methods			
Eligibility criteria	#8	Specify the study characteristics (such as PICO, study design, setting, time frame) and report characteristics (such as years considered, language, publication status) to be used as criteria for eligibility for the review	P8-9
Information sources	#9	Describe all intended information sources (such as electronic databases, contact with study authors, trial registers or other grey literature sources) with planned dates of coverage	P9
Search strategy	#10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	P18
Study records - data management	#11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	P10-11
Study records - selection process	#11b	State the process that will be used for selecting studies (such as two independent reviewers) through each phase of the review (that is, screening, eligibility and inclusion in meta-analysis)	P9-10
Study records - data collection	#11c	Describe planned method of extracting data from reports (such as piloting forms, done independently, in duplicate), any	P10

process		processes for obtaining and confirming data from investigators	
Data items	#12	List and define all variables for which data will be sought (such as PICO items, funding sources), any pre-planned data assumptions and simplifications	P10
Outcomes and prioritization	#13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	P8
Risk of bias in individual studies	#14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	P10-11
Data synthesis	#15a	Describe criteria under which study data will be quantitatively synthesised	P10-11
Data synthesis	#15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data and methods of combining data from studies, including any planned exploration of consistency (such as I2, Kendall's τ)	P11
Data synthesis	#15c	Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression)	n/a
Data synthesis	#15d	If quantitative synthesis is not appropriate, describe the type of summary planned	n/a
Meta-bias(es)	#16	Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting within	P11

		studies)	
Confidence in cumulative evidence	#17	Describe how the strength of the body of evidence will be assessed (such as GRADE)	n/a

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Secondary Subject Heading:	Public health
Keywords:	Bone diseases < ORTHOPAEDIC & TRAUMA SURGERY, PUBLIC HEALTH, Orthopaedic & trauma surgery < SURGERY

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ABSTRACT

Introduction Bone health affects the ability of human body to stay active and the degradation of bone health can cause considerable morbidity and mortality. The factors related to bone health play an important role in preventing osteoporosis and its adverse consequences. However, the risk factors of osteoporosis has not been fully elucidated. The deficiency of the trace element selenium may be one of the risk factors for the development of osteoporosis. Previous studies have investigated the effects of selenium on osteoporosis; however, the results are inconclusive. Therefore, the present study aimed to systematically examine the existing literature on the associations between dietary or serum selenium and bone mineral density (BMD), osteoporosis, or osteoporotic fractures, and to quantify such associations through meta-analysis.

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PROSPERO registration number CRD42019147188

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- Two investigators will perform the study selection, data extraction and quality assessments independently, and disagreements will be resolved by discussions.
- Both the quality and the risk of bias will be properly assessed for the included studies by applying the Newcastle-Ottawa Quality Scale or the Cochrane risk of bias assessment tool where appropriate.
- The differences in study design and sample characteristics may lead to a high level of heterogeneity.
- Both prospective and retrospective studies will be included in this meta-analysis, which may also incur bias and impact the final results .

INTRODUCTION

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.[1 2] The various factors related to bone health at different ages play an important role in preventing osteoporosis and its adverse consequences.[3] Bone health is mainly reflected in diseases caused by bone mass loss, such as osteoporosis and fragility fracture.[4 5] Osteoporosis, which is characterized by low bone mass and microarchitectural deterioration of bone tissue, is a systemic skeletal disease that can result in increased bone fragility and increased fracture risk as a consequence.[3 6] With an ageing population, the socioeconomic and medical impact of osteoporosis will increase rapidly. It is estimated that by 2020, there will be over 200 million people worldwide affected by osteoporosis, and the expenses related to osteoporosis will rise to about $25.3 billion by 2025.[7] 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.[3] However, osteoporosis can occur without a known underlying cause, and its risk factors have not been fully elucidated.

Nutrition has a significant influence on bone health and adequate nutrition is among the crucial cornerstones of the prevention of osteoporosis.[8] The trace element selenium, as a critical constituent of about 25 selenoenzymes, plays an essential role in a variety of physiological processes and it has been confirmed that the beneficial

effects of selenium are related to human health.[9 10] Some studies collectively suggested that the preservation of selenoproteins in bone was essential to normal skeletal development.[11] Animal studies have shown that selenium-deprivation can retard growth and change bone metabolism.[12] Such effects are associated with reduced BMD, impaired bone microarchitecture and increased bone resorption. Previous clinical studies have investigated the effects of selenium on bone health, however, the results are inconclusive. Some suggested that serum and dietary selenium were positively correlated with BMD,[13 14] while other showed that neither serum nor dietary selenium was associated with osteoporosis.[15 16] Meanwhile, some case-control studies revealed that the dietary intake of selenium was associated with reduced risk of osteoporotic hip fracture.[17 18]

Meta-analysis is an effective tool for revealing trends that may not be apparent, and it also helps in establishing clinical policies and guidelines. Thus, we aimed to systematically examine the existing literature on the associations between dietary or serum selenium concentrations and BMD, osteoporosis, or osteoporotic fractures, and to quantify such associations through meta-analysis where feasible.

METHODS

Study design

The aim of this meta-analysis is to investigate the associations between dietary intake or serum levels of selenium and BMD, osteoporosis, or osteoporotic fractures. This

protocol was developed according to the Preferred Reporting Items for Systematic Review and Meta-Analyses Protocols (PRISMA-P) 2015 Statement,[19] and has been registered in the international prospective register of systematic reviews PROSPERO network (registration number: CRD42019147188).

Eligibility criteria

Types of studies

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.

Types of participants

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. No age, sex, ethnicity, economic status, geographical limitations, or education restrictions will be applied.

Types of exposure/intervention

Two main forms of exposure will be included, i.e., dietary intake selenium and serum levels of selenium.

Types of outcome measures

Primary outcomes will include T-score , BMD values and the prevalence or incidence of osteoporosis. The diagnostic criteria of osteoporosis shall be based on the included articles, such as the presence of a fragility fracture or using BMD criteria. The secondary outcome will be the prevalence or incidence of osteoporotic fractures. 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.

Information sources

Systematic literature searches will be undertaken across PubMed, Embase and Cochrane Library by applying the specified search strategy to identify relevant studies up to October 2019 on each platform or database. In addition, the references of the retrieved literature will be manually searched. And Grey literature will also be searched included study registries (e.g., ClinicalTrials.gov and google scholar). The relevant studies and systematic reviews will be scanned for additional eligible studies.

Search strategy

Keyword terms or medical subject heading terms (MESH) will be used to search for eligible studies in the databases mentioned above. The electronic search strategy is presented in **Table 1**. All the search terms will be adapted for different syntax rules of

the databases.

Study selection

The search results from the three electronic databases will be sent to Endnote. After removing duplicates, two investigators will be responsible for screening the titles and abstracts of all the retrieved literature to identify eligible studies. In case that the eligibility of a study cannot be determined, the full text will be reviewed for inclusion according to prespecified inclusion and exclusion criteria. There will be no restriction on the publication date and language. The study selection process will be summarized based on the PRISMA flow diagram. Disagreements between the two investigators will be resolved by discussing with a third investigator.

Data extraction

Two investigators will be engaged to independently extract the following data in a standardized collection form: publication information (author, year of publishing); study information (country of origin, study setting, data sources, study period); demographic information (sample size, age, and sex distribution); exposure information (dietary or serum selenium concentrations); outcome information (T-score , BMD values, the prevalence or incidence of osteoporosis or osteoporotic fractures). 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. If the relevant data is not reported in an included study, the missing information will be obtained by contacting the authors directly as far as possible. The two investigators responsible for reviewing the literature will resolve any disagreements through discussions, and a third independent investigator may be involved into the discussion if required.

Assessment of risk of bias

The Cochrane risk of bias assessment tool will be used to assess the quality of RCTs,[20] while the Newcastle-Ottawa Quality Scale (NOS) will be used to assess the quality of observational studies.[21] Two investigators will be engaged to conduct the risk of bias assessment independently, and disagreements will be resolved through discussions.

Data analysis

Firstly, the characteristics of included studies will be summarized using baseline tables and narrative texts. The proposed study will calculate the MD, the pooled OR, RR, HR or β and the corresponding 95% CIs. Unadjusted risk estimates and adjusted estimates will be pooled in the meta-analysis. Then, Cochrane's Q test and the I^2 statistics will be used to assess the heterogeneity of the included studies, where *p* value > 0.05 of the Q statistics and I^2 value < 50% indicate statistical homogeneity.[22] [23] If the included studies are found to be statistically homogeneous, the fixed effects models will be used to pool the data during meta-analysis; otherwise, the random

effects models will be used instead. Statistical analyses will be conducted in Review Manager-5.3 (Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014) and Stata-11.0 (StataCorp LP, College Station, TX) statistical software. Statistical significance is considered at p value < 0.05. 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.

Assessment of publication bias

If the number of included studies is greater than 10, a funnel plot will be constructed and the Egger test will be conducted to observe the publication bias. The existence of publication bias is confirmed when the funnel plot shows an asymmetric pattern. Then, the bias, if any, will be explained through discussions after assessment.

Patient and public involvement

As our systematic review will be implemented based on published studies, no patients and members of the public will be directly involved. All the data to be used in this study already exists in the published literature and/or the aforementioned sources.

Ethics and dissemination

As this meta-analysis will be performed based on published studies, and no private and confidential patient data will be contained in the reporting, approval from an

ethics committee is not required. The results will be disseminated through publication in a peer- reviewed journal. The study raises no ethical issues.

DISCUSSION

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. The results of this study will deepen

the existing knowledge base on the pathogenesis of osteoporosis, and promote the development of preventive or treatment strategies in this field.

COMPETING INTERESTS

The authors declare that they have no conflict of interest.

PATIENT CONSENT FOR PUBLICATION

Not required.

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AUTHOR'S CONTRIBUTIONS

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Data curation: Jing Wu, Ziying Wu, Hongyi He, Zidan Yang

Methodology: Jing Wu, Ziying Wu, Hongyi He, Zidan Yang

1 Writing – original draft: Ning Wang and Dongxing Xie

2 Writing – review & editing: Yilun Wang and Tuo Yang

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Table 1 Electronic search strategy in Pubmed

Number	Search terms
1	selenium[mesh] or "selenious acid"[mesh] or selen*[tiab] or selepen[tiab] or organoselen*[tiab] or natriumselen*[tiab] or methylseleninic[tiab] or methylselenium[tiab] or selenomethionin*[tiab] or selenit*[tiab]
2	bone demineralization[mesh] or bone density[mesh] or osteopenia[mesh] or osteoporosis[mesh] or bone densitometry[mesh] or osteoporo*[tiab] or bone densit*[tiab] or bone loss[tiab] or osteomalacia[tiab] or osteodystrophy[tiab] or bone demineralization[tiab] or osteopenia[tiab] or bone mass[tiab] or bone mineral content*[tiab] or bone defect*[tiab] or bone strength[tiab] or BMC[tiab] or "fracture"[mesh] or fracture* [tiab]
3	1 AND 2

Reporting checklist for protocol of a systematic review.

Based on the PRISMA-P guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

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In your methods section, say that you used the PRISMA-Preporting guidelines, and cite them as:

Moher D, Shamseer L, Clarke M, Ghersi D, Liberati A, Petticrew M, Shekelle P, Stewart LA. Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P) 2015 statement. Syst Rev. 2015;4(1):1.

		Reporting Item	Page Number
Title			
Identification	#1a	Identify the report as a protocol of a systematic review	P1
Update	#1b	If the protocol is for an update of a previous systematic review, identify as such	n/a

Registration			
	#2	If registered, provide the name of the registry (such as PROSPERO) and registration number	P4, P7-8
Authors			
Contact	#3a	Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author	P1-2
Contribution	#3b	Describe contributions of protocol authors and identify the guarantor of the review	P14-15
Amendments			
	#4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments	n/a
Support			
Sources	#5a	Indicate sources of financial or other support for the review	P14
Sponsor	#5b	Provide name for the review funder and / or sponsor	P14
Role of sponsor or funder	#5c	Describe roles of funder(s), sponsor(s), and / or institution(s), if any, in developing the protocol	P14
Introduction			
Rationale	#6	Describe the rationale for the review in the context of what is	P6-7

		already known	
Objectives	#7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	P8-9
Methods			
Eligibility criteria	#8	Specify the study characteristics (such as PICO, study design, setting, time frame) and report characteristics (such as years considered, language, publication status) to be used as criteria for eligibility for the review	P8-9
Information sources	#9	Describe all intended information sources (such as electronic databases, contact with study authors, trial registers or other grey literature sources) with planned dates of coverage	P9
Search strategy	#10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	P18
Study records - data management	#11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	P10-11
Study records - selection process	#11b	State the process that will be used for selecting studies (such as two independent reviewers) through each phase of the review (that is, screening, eligibility and inclusion in meta-analysis)	P9-10
Study records - data collection	#11c	Describe planned method of extracting data from reports (such as piloting forms, done independently, in duplicate), any	P10

process		processes for obtaining and confirming data from investigators	
Data items	#12	List and define all variables for which data will be sought (such as PICO items, funding sources), any pre-planned data assumptions and simplifications	P10
Outcomes and prioritization	#13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	P8
Risk of bias in individual studies	#14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	P10-11
Data synthesis	#15a	Describe criteria under which study data will be quantitatively synthesised	P10-11
Data synthesis	#15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data and methods of combining data from studies, including any planned exploration of consistency (such as I2, Kendall's τ)	P11
Data synthesis	#15c	Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression)	n/a
Data synthesis	#15d	If quantitative synthesis is not appropriate, describe the type of summary planned	n/a
Meta-bias(es)	#16	Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting within	P11

		studies)	
Confidence in cumulative evidence	#17	Describe how the strength of the body of evidence will be assessed (such as GRADE)	n/a

BMJ Open

Selenium and bone health: a protocol for a systematic review and meta-analysis

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Primary Subject Heading:	Nutrition and metabolism
Secondary Subject Heading:	Public health
Keywords:	Bone diseases < ORTHOPAEDIC & TRAUMA SURGERY, PUBLIC HEALTH, Orthopaedic & trauma surgery < SURGERY

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Title: Selenium and bone health: a protocol for a systematic review and meta-analysis

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ABSTRACT

Introduction Bone health affects the ability of human body to stay active and the degradation of bone health can cause considerable morbidity and mortality. The factors related to bone health play an important role in preventing osteoporosis and its adverse consequences. However, the risk factors of osteoporosis has not been fully elucidated. The deficiency of the trace element selenium may be one of the risk factors for the development of osteoporosis. Previous studies have investigated the effects of selenium on osteoporosis; however, the results are inconclusive. Therefore, the present study aimed to systematically examine the existing literature on the associations between dietary or serum selenium and bone mineral density (BMD), osteoporosis, or osteoporotic fractures, and to quantify such associations through meta-analysis.

Methods and analysis PubMed, Embase and Cochrane Library will be searched by applying the specified search strategy to identify relevant studies up to October 2019. Both interventional and observational studies in humans will be included. The outcomes will include BMD and the prevalence or incidence of osteoporosis and osteoporotic fractures. For the dietary or serum selenium and BMD, osteoporosis, or osteoporotic fractures pooled analyses, estimates will be expressed as the mean difference, and the pooled odds ratio, relative risk, hazard ratio or beta coefficient and corresponding 95% confidence intervals. The heterogeneity of the studies and the publication bias will be investigated accordingly. To assess the quality and the risk of bias of the included studies, the Newcastle-Ottawa Scale or the Cochrane risk of bias

assessment tool will be used where appropriate.

Ethics and dissemination Since no private and confidential patient data will be contained in the reporting, approval from an ethics committee is not required. The results will be published in a peer-reviewed journal. The study raises no ethical issues.

PROSPERO registration number CRD42019147188

Strengths and limitations of the study

- As the first meta-analysis that evaluated the associations between dietary selenium intake or serum selenium concentrations and BMD, osteoporosis, or osteoporotic fractures, the findings of this study will deepen the existing knowledge base on the pathogenesis of osteoporosis, and promote the development of effective preventive or treatment strategies.
- Two investigators will perform the study selection, data extraction and quality assessments independently, and disagreements will be resolved by discussions.
- Both the quality and the risk of bias will be properly assessed for the included studies by applying the Newcastle-Ottawa Quality Scale or the Cochrane risk of bias assessment tool where appropriate.
- Both prospective and retrospective studies will be included in this meta-analysis, which may also incur bias and impact the final results. And the differences in study design and sample characteristics may lead to a high level of heterogeneity.
- 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.

INTRODUCTION

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.[1 2] The various factors related to bone health at different ages play an important role in preventing osteoporosis and its adverse consequences.[3] Bone health is mainly reflected in diseases caused by bone mass loss, such as osteoporosis and fragility fracture.[4 5] Osteoporosis, which is characterized by low bone mass and microarchitectural deterioration of bone tissue, is a systemic skeletal disease that can result in increased bone fragility and increased fracture risk as a consequence.[3 6] With an ageing population, the socioeconomic and medical impact of osteoporosis will increase rapidly. It is estimated that by 2020, there will be over 200 million people worldwide affected by osteoporosis, and the expenses related to osteoporosis will rise to about $25.3 billion by 2025.[7] 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.[3] However, osteoporosis can occur without a known underlying cause, and its risk factors have not been fully elucidated.

Nutrition has a significant influence on bone health and adequate nutrition is among the crucial cornerstones of the prevention of osteoporosis.[8] The trace element selenium, as a critical constituent of about 25 selenoenzymes, plays an essential role in a variety of physiological processes and it has been confirmed that the beneficial

effects of selenium are related to human health.[9 10] Some studies collectively suggested that the preservation of selenoproteins in bone was essential to normal skeletal development.[11] Animal studies have shown that selenium-deprivation can retard growth and change bone metabolism.[12] Such effects are associated with reduced BMD, impaired bone microarchitecture and increased bone resorption. Previous clinical studies have investigated the effects of selenium on bone health, however, the results are inconclusive. Some suggested that serum and dietary selenium were positively correlated with BMD,[13 14] while other showed that neither serum nor dietary selenium was associated with osteoporosis.[15 16] Meanwhile, some case-control studies revealed that the dietary intake of selenium was associated with reduced risk of osteoporotic hip fracture.[17 18]

Meta-analysis is an effective tool for revealing trends that may not be apparent, and it also helps in establishing clinical policies and guidelines. 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.

METHODS

Study design

The aim of this meta-analysis is to investigate the associations between dietary intake or serum levels of selenium and BMD, osteoporosis, or osteoporotic fractures. This

protocol was developed according to the Preferred Reporting Items for Systematic Review and Meta-Analyses Protocols (PRISMA-P) 2015 Statement,[19] and has been registered in the international prospective register of systematic reviews PROSPERO network (registration number: CRD42019147188).

Eligibility criteria

Types of studies

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.

Types of participants

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. No age, sex, ethnicity, economic status, geographical limitations, or education restrictions will be applied.

Types of exposure/intervention

Two main forms of exposure will be included, i.e., dietary intake selenium and serum levels of selenium.

Types of outcome measures

Primary outcomes will include T-score , BMD values and the prevalence or incidence of osteoporosis. The diagnostic criteria of osteoporosis shall be based on the WHO criteria.[20] 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. Osteoporosis is classified as “primary” and “secondary”, and secondary osteoporosis is due to certain clinical disorders independent of age and estrogen deficiency.[21] We will catalog and categorize the different types of primary and secondary osteoporosis according included studies. The secondary outcome will be the prevalence or incidence of osteoporotic fractures. 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.

Information sources

Systematic literature searches will be undertaken across PubMed, Embase and Cochrane Library by applying the specified search strategy to identify relevant studies up to October 2019 on each platform or database. In addition, the references of the retrieved literature will be manually searched. And Grey literature will also be searched included study registries (e.g., ClinicalTrials.gov and google scholar). The relevant studies and systematic reviews will be scanned for additional eligible studies.

Search strategy

Keyword terms or medical subject heading terms (MESH) will be used to search for eligible studies in the databases mentioned above. The electronic search strategy is presented in **Table 1**. All the search terms will be adapted for different syntax rules of the databases.

Study selection

The search results from the three electronic databases will be sent to Endnote. After removing duplicates, two investigators will be responsible for screening the titles and abstracts of all the retrieved literature to identify eligible studies. In case that the eligibility of a study cannot be determined, the full text will be reviewed for inclusion according to prespecified inclusion and exclusion criteria. There will be no restriction on the publication date and language. The study selection process will be summarized based on the PRISMA flow diagram. Disagreements between the two investigators will be resolved by discussing with a third investigator.

Data extraction

Two investigators will be engaged to independently extract the following data in a standardized collection form: publication information (author, year of publishing); study information (country of origin, study setting, data sources, study period); demographic information (sample size, age, and sex distribution); exposure information (dietary or serum selenium concentrations); outcome information (T-

score , BMD values, the prevalence or incidence of osteoporosis or osteoporotic fractures). 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. If the relevant data is not reported in an included study, the missing information will be obtained by contacting the authors directly as far as possible. The two investigators responsible for reviewing the literature will resolve any disagreements through discussions, and a third independent investigator may be involved into the discussion if required.

Assessment of risk of bias

The Cochrane risk of bias assessment tool will be used to assess the quality of RCTs,[22] while the Newcastle-Ottawa Quality Scale (NOS) will be used to assess the quality of observational studies.[23] Two investigators will be engaged to conduct the risk of bias assessment independently, and disagreements will be resolved through discussions.

Data analysis

Firstly, the characteristics of included studies will be summarized using baseline tables and narrative texts. The proposed study will calculate the MD, the pooled OR, RR, HR or β and the corresponding 95% CIs. Unadjusted risk estimates and adjusted estimates will be pooled in the meta-analysis. Then, Cochrane's Q test and the I^2

statistics will be used to assess the heterogeneity of the included studies, where *p* value > 0.05 of the Q statistics and I^2 value < 50% indicate statistical homogeneity.[24] [25] If the included studies are found to be statistically homogeneous, the fixed effects models will be used to pool the data during meta-analysis; otherwise, the random effects models will be used instead. Statistical analyses will be conducted in Review Manager-5.3 (Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014) and Stata-11.0 (StataCorp LP, College Station, TX) statistical software. Statistical significance is considered at *p* value < 0.05. 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.

Assessment of publication bias

If the number of included studies is greater than 10, a funnel plot will be constructed and the Egger test will be conducted to observe the publication bias. The existence of publication bias is confirmed when the funnel plot shows an asymmetric pattern. Then, the bias, if any, will be explained through discussions after assessment.

Patient and public involvement

As our systematic review will be implemented based on published studies, no patients and members of the public will be directly involved. All the data to be used in this study already exists in the published literature and/or the aforementioned sources.

Ethics and dissemination

As this meta-analysis will be performed based on published studies, and no private and confidential patient data will be contained in the reporting, approval from an ethics committee is not required. The results will be disseminated through publication in a peer- reviewed journal. The study raises no ethical issues.

DISCUSSION

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. 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.[26 27] 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. 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 28] On the contrary, one study found no association between dietary selenium and BMD,[29] and four studies reported that

neither serum nor dietary selenium was related to osteoporosis.[15 16 30 31] 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. The results of this study will deepen the existing knowledge base on the pathogenesis of osteoporosis, and promote the development of preventive or treatment strategies in this field.

COMPETING INTERESTS

The authors declare that they have no conflict of interest.

PATIENT CONSENT FOR PUBLICATION

Not required.

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AUTHOR'S CONTRIBUTIONS

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1 Writing – original draft: Ning Wang and Dongxing Xie

2 Writing – review & editing: Yilun Wang and Tuo Yang

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Table 1 Electronic search strategy in Pubmed

Number	Search terms
1	selenium[mesh] or "selenious acid"[mesh] or selen*[tiab] or selepen[tiab] or organoselen*[tiab] or natriumselen*[tiab] or methylseleninic[tiab] or methylselenium[tiab] or selenomethionin*[tiab] or selenit*[tiab]
2	bone demineralization[mesh] or bone density[mesh] or osteopenia[mesh] or osteoporosis[mesh] or bone densitometry[mesh] or osteoporo*[tiab] or bone densit*[tiab] or bone loss[tiab] or osteomalacia[tiab] or osteodystrophy[tiab] or bone demineralization[tiab] or osteopenia[tiab] or bone mass[tiab] or bone mineral content*[tiab] or bone defect*[tiab] or bone strength[tiab] or BMC[tiab] or "fracture"[mesh] or fracture* [tiab]
3	1 AND 2

Reporting checklist for protocol of a systematic review.

Based on the PRISMA-P guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

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In your methods section, say that you used the PRISMA-Preporting guidelines, and cite them as:

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		Reporting Item	Page Number
Title			
Identification	#1a	Identify the report as a protocol of a systematic review	P1
Update	#1b	If the protocol is for an update of a previous systematic review, identify as such	n/a

Registration			
	#2	If registered, provide the name of the registry (such as PROSPERO) and registration number	P4, P7-8
Authors			
Contact	#3a	Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author	P1-2
Contribution	#3b	Describe contributions of protocol authors and identify the guarantor of the review	P14-15
Amendments			
	#4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments	n/a
Support			
Sources	#5a	Indicate sources of financial or other support for the review	P14
Sponsor	#5b	Provide name for the review funder and / or sponsor	P14
Role of sponsor or funder	#5c	Describe roles of funder(s), sponsor(s), and / or institution(s), if any, in developing the protocol	P14
Introduction			
Rationale	#6	Describe the rationale for the review in the context of what is	P6-7

		already known	
Objectives	#7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	P8-9
Methods			
Eligibility criteria	#8	Specify the study characteristics (such as PICO, study design, setting, time frame) and report characteristics (such as years considered, language, publication status) to be used as criteria for eligibility for the review	P8-9
Information sources	#9	Describe all intended information sources (such as electronic databases, contact with study authors, trial registers or other grey literature sources) with planned dates of coverage	P9
Search strategy	#10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	P18
Study records - data management	#11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	P10-11
Study records - selection process	#11b	State the process that will be used for selecting studies (such as two independent reviewers) through each phase of the review (that is, screening, eligibility and inclusion in meta-analysis)	P9-10
Study records - data collection	#11c	Describe planned method of extracting data from reports (such as piloting forms, done independently, in duplicate), any	P10

process		processes for obtaining and confirming data from investigators	
Data items	#12	List and define all variables for which data will be sought (such as PICO items, funding sources), any pre-planned data assumptions and simplifications	P10
Outcomes and prioritization	#13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	P8
Risk of bias in individual studies	#14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	P10-11
Data synthesis	#15a	Describe criteria under which study data will be quantitatively synthesised	P10-11
Data synthesis	#15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data and methods of combining data from studies, including any planned exploration of consistency (such as I2, Kendall's τ)	P11
Data synthesis	#15c	Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression)	n/a
Data synthesis	#15d	If quantitative synthesis is not appropriate, describe the type of summary planned	n/a
Meta-bias(es)	#16	Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting within	P11

		studies)	
Confidence in cumulative evidence	#17	Describe how the strength of the body of evidence will be assessed (such as GRADE)	n/a