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# Associations between socioeconomic factors and proinflammatory cytokines in children, adolescents and young adulthood: A systematic review protocol



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| Primary Subject Heading: | Epidemiology |
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# Associations between socioeconomic factors and proinflammatory cytokines in children, adolescents and young adulthood: A systematic review protocol

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**Keywords**: inflammatory markers, cytokines, socioeconomic factors, systematic review, meta-analysis

**Abstract**

*Introduction:* There is now substantial evidence of a social gradient in bone health. Social stressors, related to socioeconomic status, are suggested to produce an inflammatory response marked by increased levels of proinflammatory cytokines. Here we focus on the particular role in the years before the achievement of peak bone mass, encompassing childhood, adolescence and young adulthood. An examination of such associations will help explain how social factors such as occupation, level of education and income may affect later-life bone disorders. This paper presents the protocol for a systematic review of existing literature regarding associations between socioeconomic factors and proinflammatory cytokines in those aged 6 to 24 years.

*Methods and analysis:* We will conduct a systematic search of PubMed, OVID, and CINAHL databases to identify articles that examine associations between socioeconomic factors and levels of proinflammatory cytokines, known to influence bone health, during childhood, adolescence or young adulthood. The findings of this review have implications for the equitable development of peak bone mass regardless of socioeconomic factors. Two independent reviewers will determine the eligibility of studies according to pre-determined criteria, and studies will be assessed for methodological quality using a published scoring system. Should statistical heterogeneity be non-significant, we will conduct a meta-analysis; however, if heterogeneity prevent numerical syntheses, we will undertake a best-evidence analysis to determine whether socioeconomic differences exist in the levels of proinflammatory cytokines from childhood through to young adulthood.

*Ethics and dissemination:* This study will be a systematic review of published data, and thus ethics approval is not required. In addition to peer-reviewed publication, these findings will be presented at professional conferences in national and international arenas.

*Strengths and limitations of this study:*

- This systematic review will provide a comprehensive assessment of the existing literature regarding associations between socioeconomic factors and levels of proinflammatory cytokines known to influence bone, in ages from childhood (6 years) to young adulthood (24 years).
- Study selection and data extraction will be performed by one author and confirmed by a second, and assessment of methodology will be conducted independently by two authors.
- The findings will have implications for research into the possible role of inflammation as a mediator between socioeconomic factors and bone health in later life.
- Possible limitations of this review include heterogeneity due to variation in the (i) measurement of socioeconomic factors, (ii) study populations, particularly with respect to age ranges, and (iii) methods used to measure proinflammatory cytokine levels.

## Introduction

A social gradient in the majority of chronic, non-communicable diseases has been well-documented. In addition, there is now considerable evidence of a social gradient in bone health; whereby worsening levels of social disadvantage is associated with lower bone mineral density (BMD) and increased fracture risk, independent of age and other clinical risk factors, such as body-mass index, dietary factors, smoking and alcohol consumption[1-5]. However, the potential causal mechanism(s) underpinning the social gradient in bone health are not well understood.

Recently, we conceptualised a role for inflammation in the relationship between social disadvantage and low BMD and the associated risk of fracture in adults[6]. We postulated an epigenetic process across the life course, whereby social stressors resulted in heightened oxidative stress and increased inflammatory reactivity, with subsequent effects on phenotypic expression of disease risk. One indication of this process would be an association between social disadvantage and heightened levels of inflammation during the years of infancy, childhood and early adulthood, that is, before the attainment of peak bone mass. Such an association would have a particularly marked effect on BMD for the remainder of life and hence on the risk for osteoporosis and fracture in later life. Across the lifespan, proinflammatory biomarkers known to be associated with bone accrual are the cytokines interleukin-1 beta (IL-1ß), interleukin-6 (IL-6), interleukin-8 (IL-8), C-reactive protein (CRP) and tumor necrosis factor alpha (TNFα).

This systematic review protocol proposes to collate and synthesise the available evidence regarding whether social stressors during childhood, adolescence and early adult life may increase the levels of proinflammatory cytokines. The need for this review is imperative, as there appears some contradictory and complex associations, which will impede progress toward understanding early life precursors for poorer bone health in later life. For instance, one study of Canadian schoolchildren found that the effects of IL-6 levels vary with the trajectory of socioeconomic status (SES), measured as parent-reported housing data during childhood, and that a number of interactions between social and health factors affected levels of IL-6[7]. Another study, of Canadian adolescents, found that SES as measured by family

income moderated the effect of "family chaos" on some proinflammatory biomarkers (IL-1A; IL-6; IL-8; and a composite measure of all cytokines measured), but not all (CRP; IL-10)[8].

Hence there appears to be a *prima facie* rationale for systematically examining past work on associations between levels of proinflammatory cytokines and socioeconomic factors in the life course from childhood up to and including young adulthood. Here, we present the protocol for a systematic review of this literature, which adheres to the Preferred Reporting Items for Systematic reviews and Meta-Analyses Protocol (PRISMA-P) guidelines[9].

## Objectives

This systematic review will:

1. identify published studies examining the associations between socioeconomic factors and the levels of proinflammatory cytokines known to influence bone health, in ages from childhood (6 years) up to and including young adulthood (24 years);
2. evaluate the methodological quality of all eligible studies according to a previously employed scoring system[10 11];
3. analyse the combined level of evidence of all studies, and conduct a subgroup analysis to examine the findings from studies deemed to be of high methodological quality (determined by quality assessment score above the median) to determine if any bias is observed.

## Methods

### Eligibility criteria

The criteria for inclusion in this review will be: full-text articles published in English that are epidemiological cohort, case-control and/or cross-sectional studies, and which examine associations between socioeconomic factors measured at the individual or area level, and proinflammatory cytokines. A study will be eligible if it examines any, or all, of the proinflammatory cytokines commonly known to be associated with bone accrual: IL-1ß, IL-6, IL-8, CRP and TNFα.

Grey literature, opinions pieces, commentaries, unpublished theses, and conference presentations will be excluded. Furthermore, given that the purpose of this review is to ascertain whether differences exist in the levels of proinflammatory cytokines across socioeconomic factors, randomised controlled trials (RCT) will be excluded unless baseline data, or data from the control arm of RCTs, pertain to proinflammatory cytokines levels prior to intervention. In that instance, data from the control arm would be equivalent to a cohort study and would thus be included.

**Socioeconomic factors**

For this review the prime variables of interest are socioeconomic factors: these may be measured at the individual level, including, but not limited to income, education, occupation, employment status, type of residence, and marital status. Socioeconomic factors may also be measured at the household or area level, and/or include composite measures of socioeconomic parameters: these composite measures may be based on country- or region-specific administrative boundaries including government or statistical areas, or census collection districts, amongst others.

**Proinflammatory cytokine measurement**

The cytokines to be included are IL-1ß, IL-6, IL-8, CRP and TNFα, which are known to have effects on bone health[6] . Included studies may have measured a different range of biomarkers. It is also important to account for which of the two differing general methods for measuring cytokine levels that a study has used: Some studies measure circulating cytokine levels *in vivo* while others measure cytokine levels *in vitro* which involves the stimulation of white blood cells by one of a range of agents [7]. As these methods will give differing results for the same participants, in the case of a meta-analysis being performed, this review will follow the methods of Steptoe et al. whereby studies measuring circulating levels and stimulated levels were investigated separately[12].

**Age**

Eligible studies will be restricted to those examining participants who are children, adolescents or in young adulthood, which, according to Medical Subject Headings (MeSH) encompasses those aged from 6 years up to and including 24 years of age (MeSH categories 'child', 'adolescent' and 'young adult'). The rationale for this age range, is to examine the particular associations between cytokine levels and socioeconomic factors before the achievement of peak bone mass. Peak bone mass is defined as the achievement of the highest possible level of stable bone density[18]: estimation of the age which peak bone mass occurs depends on the skeletal site and within individuals by a range of hereditable and environmental factors, however, total bone mass is considered to peak between the early to late 20s[13].

**Information sources and search strategy**

We will perform a computer-generated search strategy using databases for medical, health and social sciences (PubMed, OVID and CINAHL) to identify relevant literature, with no limits set on the date of publication. Standard medical subject headings (MeSH) and keywords will be applied to capture the broadest range of publications to compare against the predetermined inclusion criteria. The full search string to be applied will be: "(Cytokines OR interleukin OR C-reactive protein) AND (socioeconomic factors OR socioeconomic status OR poverty OR social class OR income OR education OR residence OR occupation OR marital status) AND (child OR adolescent OR young adult OR youth)". Relevant truncation will be used as appropriate to each database. Duplicate articles will be identified and removed using the relevant functionality of the reference management application Endnote. Reference lists of relevant studies that fulfill the eligibility criteria will be independently hand-searched. Study selection and data extraction will be performed by one author (NJF) and confirmed by a second (SLB-O), where another opinion is necessary to address any eligibility- or data-related disagreement, this will be independently provided by a third author (GD).

**Assessment of methodological quality of included articles**

The methodological quality of included studies will be independently investigated by two reviewers (NJF and DG) using the assessment and scoring system of Lievense and colleagues[10 11], as previously employed for other systematic reviews in the musculoskeletal field[14-16]. That scoring system evaluates the methodological quality of included studies in the

following way. For each design-specific criteria that a study meets (Figure 1), it will receive a score of 1, and otherwise 0. Scores will be presented as a percentage of the maximum possible score for each particular study design, whereby cohort studies are determined to be the optimum design due to their inherent qualities, followed by case-control and cross-sectional study designs.

In the case that any discrepancies in scores cannot be reconciled by the scorers, a third reviewer will make a final judgement at a single consensus meeting (GD). To assess relative methodological quality, studies will be categorised as high quality if the percentage score is above the median of all scores.

**Item Criterion applicable to Cohort (C), Case-Control (CC) or Cross Sectional (CS) study designs**

*Study population*

1 Selection at uniform point C/CC/CS

2 Cases and controls drawn from the same population CC

3 Participation rate >80% for cases/cohort C/CC

4 Participation rate >80% for controls CC

*Assessment of risk factor*

5 Exposure assessment blinded C/CC/CS

6 Exposure measured identically for cases and controls CC

7 Exposure assessed according to validated measures C/CC/CS

*Assessment of outcome*

8 Outcome assessed identically in studied population C/CC/CS

9 Outcome reproducibly C/CC/CS

10 Outcome assessed according to validated measures C/CC/CS

*Study design*

11 Prospective design used C/CC

12 Follow-up time >12 months C

13 Withdrawals <20% C

*Analysis and data presentation*

14 Appropriate analysis techniques used C/CC/CS

15 Adjusted for at least age, and sex C/CC/CS

**Figure 1:** Criteria list for the assessment of methodological quality, modified from Lievense et al. [10 11]

**Presenting and reporting results and data synthesis**

Details of the protocol for this systematic review have been registered with PROSPERO, the International Prospective Register of Systematic Reviews (CRD42016045271), and can be accessed at <https://www.crd.york.ac.uk/PROSPERO/display_record.asp?ID=CRD42016045271>. The results of this review will be presented according to the framework of the PRISMA-P reporting guidelines[9]. The process of study selection and reasons for the exclusion of any studies will be outlined in a QUORUM diagram, including observational studies[17]. The following key information will be extracted from papers and included in the review: author(s); year of study; sample size; study design; country from where the sample was drawn (and region, state or district if available); population description, including age ranges; description of socioeconomic factors, including the measurement and tool; and the specific proinflammatory cytokines and any other markers assessed and the methods used to measure them. A description of the modelling methods used by each study including the factors accounted for in each model, specifically anti-inflammatory biomarkers, the statistical results and a summary of the findings will also be provided.

We will conduct a meta-analysis, controlling for heterogeneity, if statistically appropriate. Should statistical heterogeneity preclude a numerical synthesis, we will conduct a best-evidence synthesis to assess the level of evidence from 'strong' to 'no evidence', based on the published methods of Lievense et al[10 11] (Table 1), and as previously published in the musculoskeletal field[14-16].

**Table 1: Criteria for ascertainment of evidence level for best-evidence synthesis, adapted from Lievense et al. [11]**

| Level of Evidence | Criteria for inclusion in best-evidence synthesis |
|---|---|
| Strong evidence | Generally consistent findings in:<br>Multiple high-quality cohort studies |
| Moderate evidence | Generally consistent findings in:<br>One high-quality cohort study and >2 high quality case-control studies<br>>3 high quality case-control studies |
| Limited evidence | Generally consistent findings in:<br>Single cohort study<br>One or two case-control studies or<br>Multiple cross-sectional studies |
| Conflicting evidence | Inconsistent findings in <75 % of the studies |
| No evidence | No studies could be found |

## Ethics and dissemination

As this review will be using published data, it does not require ethical clearance. We will adhere to standard ethical and governance standards regarding data management, and the presentation and discussion of our findings. Findings of this systematic review will be disseminated in a peer-reviewed scientific journal, and will be presented and discussed at relevant national and international conferences and meetings.

## Conclusion

To the best of our knowledge, this is the first systematic review that proposes to investigate the associations between socioeconomic factors and levels of proinflammatory cytokines in children, adolescents and young adults. Given the role of proinflammatory cytokines on bone, this information may contribute the evidence-base regarding how social factors during early life may influence the development of musculoskeletal disorders such as osteoporosis later in life.

## Authors' contributions

NJF, GD and SLB-O conceptualised the research question for this protocol; all authors edited and revised the research question. All authors contributed to the development of the e-search strategy, and NJF and SLB-O confirmed the e-search strategy. NJF and SLB-O developed the methodological processes; all authors edited, revised and approved the methodological processes. SLB-O and NJF drafted the manuscript, and all authors edited and contributed to the writing of this paper. SLB-O is the guarantor of the review. All authors read and approved the final version, and guarantee the protocol.

## Funding

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## Competing interests

None of the authors have any relevant conflicts of interest related to the work under consideration for publication. SLB-O has received speaker fees from Amgen. GD has received speaker fees from Amgen, Sanofi, and Lilly Australia.

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**PRISMA-P (Preferred Reporting Items for Systematic review and Meta-Analysis Protocols) 2015 checklist: recommended items to address in a systematic review protocol***

| Section and topic | Item No | Checklist item | Man. page no. |
|---|---|---|---|
| ADMINISTRATIVE INFORMATION | | | |
| Title: | | | |
| Identification | 1a | Identify the report as a protocol of a systematic review | p. 2 (Abstract), p. 4. |
| Update | 1b | If the protocol is for an update of a previous systematic review, identify as such | NA |
| Registration | 2 | If registered, provide the name of the registry (such as PROSPERO) and registration number | PROSPERO ID: CRD42016045271 |
| Authors: | | | |
| Contact | 3a | Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author | p. 1(title page) |
| Contributions | 3b | Describe contributions of protocol authors and identify the guarantor of the review | pp. 11. |
| 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 | NA |
| Support: | | | |
| Sources | 5a | Indicate sources of financial or other support for the review | p. 11. |
| Sponsor | 5b | Provide name for the review funder and/or sponsor | NA |
| Role of sponsor or funder | 5c | Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol | NA |
| **INTRODUCTION** | | | |
| Rationale | 6 | Describe the rationale for the review in the context of what is already known | pp. 4-5 |
| Objectives | 7 | Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO) | p. 5. |
| **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 | pp. 5-6. |
| 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 | p. 7. |
| 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 | p. 7. |
| Study records: | | | |
| Data management | 11a | Describe the mechanism(s) that will be used to manage records and data throughout the review | p. 9. |
| 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) | p. 7-8. |
| Data collection process | 11c | Describe planned method of extracting data from reports (such as piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators | p. 9. |
| 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 | p. 6-7. |
| Outcomes and prioritization | 13 | List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale | p. 6. |
| 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 | p. 7-8 |
| Data synthesis | 15a | Describe criteria under which study data will be quantitatively synthesised | p. 9. |
| | 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 $I^2$, Kendall's $\tau$) | p. 9. |
| | 15c | Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression) | p. 9. |
| | 15d | If quantitative synthesis is not appropriate, describe the type of summary planned | p. 9. |
| Meta-bias(es) | 16 | Specify any planned assessment of meta-bias(es) (such as publication bias across studies, | NA |

| | | | |
|---|---|---|---|
| | | selective reporting within studies) | |
| Confidence in cumulative evidence | 17 | Describe how the strength of the body of evidence will be assessed (such as GRADE) | p. 9-10 |

*** It is strongly recommended that this checklist be read in conjunction with the PRISMA-P Explanation and Elaboration (cite when available) for important clarification on the items. Amendments to a review protocol should be tracked and dated. The copyright for PRISMA-P (including checklist) is held by the PRISMA-P Group and is distributed under a Creative Commons Attribution Licence 4.0.**

*From: Shamseer L, Moher D, Clarke M, Ghersi D, Liberati A, Petticrew M, Shekelle P, Stewart L, PRISMA-P Group. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. BMJ. 2015 Jan 2;349(jan02 1):g7647.*

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- Possible limitations of this review include heterogeneity due to variation in the (i) measurement of socioeconomic factors, (ii) study populations, particularly with respect to age ranges, and the impact of heritable factors and race/ethnicity on associations, and (iii) methods used to measure proinflammatory cytokine levels.

## Introduction

A social gradient in the majority of chronic, non-communicable diseases has been well-documented. In addition, there is now considerable evidence of a social gradient in bone health; whereby worsening levels of social disadvantage is associated with lower bone mineral density (BMD) and increased fracture risk, independent of age and other clinical risk factors, such as body-mass index, dietary factors, smoking and alcohol consumption.[1-5] However, the potential causal mechanism(s) underpinning the social gradient in bone health are not well understood.

Recently, we conceptualised a role for inflammation in the relationship between social disadvantage and low BMD and the associated risk of fracture in adults.[6] We postulated an epigenetic process across the life course, whereby social stressors resulted in heightened oxidative stress and increased inflammatory reactivity, with subsequent effects on phenotypic expression of disease risk. One indication of this process would be an association between social disadvantage and heightened levels of inflammation during the years of infancy, childhood and early adulthood, that is, before the attainment of peak bone mass. Such an association would have a particularly marked effect on BMD for the remainder of life and hence on the risk for osteoporosis and fracture in later life. Across the lifespan, proinflammatory biomarkers known to be associated with bone accrual are the cytokines interleukin-1 beta (IL-1ß), interleukin-6 (IL-6), interleukin-8 (IL-8), C-reactive protein (CRP) and tumor necrosis factor alpha (TNFα).

This systematic review protocol proposes to collate and synthesise the available evidence regarding whether social stressors during childhood, adolescence and early adult life may increase the levels of proinflammatory cytokines. The need for this review is imperative, as there appears some contradictory and complex associations, which will impede progress toward understanding early life precursors for poorer bone health in later life. For instance, one study of Canadian schoolchildren found that the effects of IL-6 levels vary with the trajectory of socioeconomic status (SES), measured as parent-reported housing data during childhood, and that a number of interactions between social and health factors affected levels of IL-6.[7] Another study, of Canadian adolescents, found that SES as measured by family

income moderated the effect of "family chaos" on some proinflammatory biomarkers (IL-1A; IL-6; IL-8; and a composite measure of all cytokines measured), but not all (CRP; IL-10).[8]

Hence there appears to be a *prima facie* rationale for systematically examining past work on associations between levels of proinflammatory cytokines and socioeconomic factors in the life course from childhood up to and including young adulthood. Here, we present the protocol for a systematic review of this literature, which adheres to the Preferred Reporting Items for Systematic reviews and Meta-Analyses Protocol (PRISMA-P) guidelines.[9]

## Objectives

This systematic review will:

1. identify published studies examining the associations between socioeconomic factors and the levels of proinflammatory cytokines known to influence bone health, in ages from childhood (6 years) up to and including young adulthood (30 years);
2. evaluate the methodological quality of all eligible studies according to a previously employed scoring system;[10, 11]
3. analyse the combined level of evidence of all studies, and conduct a subgroup analysis to examine the findings from studies deemed to be of high methodological quality (determined by quality assessment score above the median) to determine if any bias is observed.

## Methods

### Eligibility criteria

The criteria for inclusion in this review will be: full-text articles published in English that are epidemiological cohort, case-control and/or cross-sectional studies, and which examine associations between socioeconomic factors measured at the individual or area level, and proinflammatory cytokines. A study will be eligible if it examines any, or all, of the proinflammatory cytokines commonly known to be associated with bone accrual: IL-1ß, IL-6, IL-8, CRP and TNFα.

Grey literature, opinions pieces, commentaries, unpublished theses, and conference presentations will be excluded. Furthermore, given that the purpose of this review is to ascertain whether differences exist in the levels of proinflammatory cytokines across socioeconomic factors, randomised controlled trials (RCT) will be excluded unless baseline data, or data from the control arm of RCTs, pertain to proinflammatory cytokines levels prior to intervention. In that instance, data from the control arm would be equivalent to a cohort study and would thus be included.

**Socioeconomic factors**

For this review the prime variables of interest are socioeconomic factors: these may be measured at the individual level, including, but not limited to income, education, occupation, employment status, type of residence, and marital status. Socioeconomic factors may also be measured at the household or area level, and/or include composite measures of socioeconomic parameters: these composite measures may be based on country- or region-specific administrative boundaries including government or statistical areas, or census collection districts, amongst others.

**Proinflammatory cytokine measurement**

The cytokines to be included are IL-1ß, IL-6, IL-8, CRP and TNFα, which are known to have effects on bone health.[6, 12] Included studies may have measured a different range of biomarkers. It is also important to account for which of the two differing general methods for measuring cytokine levels that a study has used: Some studies measure circulating cytokine levels *in vivo* while others measure cytokine levels *in vitro* which involves the stimulation of white blood cells by one of a range of agents.[7] As these methods will give differing results for the same participants, in the case of a meta-analysis being performed, this review will follow the methods of Steptoe et al. whereby studies measuring circulating levels and stimulated levels were investigated separately.[13]

**Age**

Eligible studies will be restricted to those examining participants who are children, adolescents or in young adults, which, according to Medical Subject Headings (MeSH) encompasses those aged from 6 years up to and including 30 years of age (MeSH categories 'child', 'adolescent' and 'young adult'). The rationale for this age range, is to examine the particular associations between cytokine levels and socioeconomic factors before the achievement of peak bone mass. Peak bone mass is defined as the achievement of the highest possible level of stable bone density:[14] estimation of the age which peak bone mass occurs depends on the skeletal site and within individuals by a range of hereditable and environmental factors, however, total bone mass is considered to peak between the early to late 20s.[14]

**Information sources and search strategy**

We will perform a computer-generated search strategy using databases for medical, health and social sciences (PubMed, OVID and CINAHL) to identify relevant literature, with no limits set on the date of publication. Standard medical subject headings (MeSH) and keywords will be applied to capture the broadest range of publications to compare against the predetermined inclusion criteria. The full search string to be applied will be: "(Cytokines OR interleukin OR C-reactive protein) AND (socioeconomic factors OR socioeconomic status OR poverty OR social class OR income OR education OR residence OR occupation OR marital status) AND (child OR adolescent OR young adult OR youth)". Relevant truncation will be used as appropriate to each database. Duplicate articles will be identified and removed using the relevant functionality of the reference management application Endnote. Reference lists of relevant studies that fulfill the eligibility criteria will be independently hand-searched. Study selection and data extraction will be performed by one author (NJF) and confirmed by a second (SLB-O), where another opinion is necessary to address any eligibility- or data-related disagreement, this will be independently provided by a third author (GD).

**Assessment of methodological quality of included articles**

The methodological quality of included studies will be independently investigated by two reviewers (NJF and DG) using the assessment and scoring system of Lievense and colleagues,[10, 11] as previously employed for other systematic reviews in the musculoskeletal field.[15-17] That scoring system evaluates the methodological quality of

included studies in the following way. For each design-specific criteria that a study meets (Figure 1), it will receive a score of 1, and otherwise 0. Scores will be presented as a percentage of the maximum possible score for each particular study design, whereby cohort studies are determined to be the optimum design due to their inherent qualities, followed by case-control and cross-sectional study designs.

In the case that any discrepancies in scores cannot be reconciled by the scorers, a third reviewer will make a final judgement at a single consensus meeting (GD). To assess relative methodological quality, studies will be categorised as high quality if the percentage score is above the median of all scores.

**Presenting and reporting results and data synthesis**

Details of the protocol for this systematic review have been registered with PROSPERO, the International Prospective Register of Systematic Reviews (CRD42016045271). The results of this review will be presented according to the framework of the PRISMA-P reporting guidelines.[9] The process of study selection and reasons for the exclusion of any studies will be outlined in a QUORUM (Quality of Reporting of Meta-Analyses) diagram, including observational studies.[18] The following key information will be extracted from papers and included in the review: author(s); year of study; sample size; study design; country from where the sample was drawn (and region, state or district if available); population description, including age ranges; description of socioeconomic factors, including the measurement and tool; and the specific proinflammatory cytokines and any other markers assessed and the methods used to measure them. A description of the modelling methods used by each study including the factors accounted for in each model, specifically anti-inflammatory biomarkers, the statistical results, and a summary of the findings will also be provided.

We will conduct a meta-analysis, controlling for heterogeneity, if statistically appropriate. Should statistical heterogeneity preclude a numerical synthesis, we will conduct a best-evidence synthesis to assess the level of evidence from ‘strong’ to ‘no evidence’, based on the published methods of Lievense et al[10, 11] (Table 1), and as previously published in the musculoskeletal field.[15-17]

**Table 1: Criteria for ascertainment of evidence level for best-evidence synthesis, adapted from Lievense et al.[10, 11]**

| Level of Evidence | Criteria for inclusion in best-evidence synthesis |
|---|---|
| Strong evidence | Generally consistent findings in:<br>Multiple high-quality cohort studies |
| Moderate evidence | Generally consistent findings in:<br>One high-quality cohort study and >2 high quality case-control studies<br>>3 high quality case-control studies |
| Limited evidence | Generally consistent findings in:<br>Single cohort study<br>One or two case-control studies or<br>Multiple cross-sectional studies |
| Conflicting evidence | Inconsistent findings in <75 % of the studies |
| No evidence | No studies could be found |

## Ethics and dissemination

As this review will be using published data, it does not require ethical clearance. We will adhere to standard ethical and governance standards regarding data management, and the presentation and discussion of our findings. Findings of this systematic review will be disseminated in a peer-reviewed scientific journal, and will be presented and discussed at relevant national and international conferences and meetings.

## Conclusion

To the best of our knowledge, this is the first systematic review that proposes to investigate the associations between socioeconomic factors and levels of proinflammatory cytokines in children, adolescents and young adults. Given the role of proinflammatory cytokines on bone, this information may contribute the evidence-base regarding how social factors during early life may influence the development of musculoskeletal disorders such as osteoporosis later in life.

**Figure 1:** Criteria list for the assessment of methodological quality, modified from Lievense et al.[10, 11]

## Authors' contributions

Nick J Fredman, Gustavo Duque and Sharon L Brennan-Olsen conceptualised the research question for this protocol; Nick J Fredman, Gustavo Duque, Rachel L Duckham, Darci Green and Sharon L Brennan-Olsen edited and revised the research question. Nick J Fredman, Gustavo Duque, Rachel L Duckham, Darci Green and Sharon L Brennan-Olsen contributed to the development of the e-search strategy, and Nick J Fredman and Sharon L Brennan-Olsen confirmed the e-search strategy. Nick J Fredman and Sharon L Brennan-Olsen developed the methodological processes; Nick J Fredman, Gustavo Duque, Rachel L Duckham, Darci Green and Sharon L Brennan-Olsen edited, revised and approved the methodological processes. Sharon L Brennan-Olsen and Nick J Fredman drafted the manuscript, and Nick J Fredman, Gustavo Duque, Rachel L Duckham, Darci Green and Sharon L Brennan-Olsen edited and contributed to the writing of this paper. Sharon L Brennan-Olsen is the guarantor of the review. Nick J Fredman, Gustavo Duque, Rachel L Duckham, Darci Green and Sharon L Brennan-Olsen read and approved the final version, and guarantee the protocol.

## Funding

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## Competing interests

None of the authors have any relevant conflicts of interest related to the work under consideration for publication. SLB-O has received speaker fees from Amgen. GD has received speaker fees from Amgen, Sanofi, and Lilly Australia.

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| Item Criterion applicable to Cohort (C), Case-Control (CC) or Cross Sectional (CS) study designs |
|---|
| *Study population* |
| 1 Selection at uniform point C/CC/CS |
| 2 Cases and controls drawn from the same population CC |
| 3 Participation rate >80% for cases/cohort C/CC |
| 4 Participation rate >80% for controls CC |
| *Assessment of risk factor* |
| 5 Exposure assessment blinded C/CC/CS |
| 6 Exposure measured identically for cases and controls CC |
| 7 Exposure assessed according to validated measures C/CC/CS |
| *Assessment of outcome* |
| 8 Outcome assessed identically in studied population C/CC/CS |
| 9 Outcome reproducibly C/CC/CS |
| 10 Outcome assessed according to validated measures C/CC/CS |
| *Study design* |
| 11 Prospective design used C/CC |
| 12 Follow-up time >12 months C |
| 13 Withdrawals <20% C |
| *Analysis and data presentation* |
| 14 Appropriate analysis techniques used C/CC/CS |
| 15 Adjusted for at least age, and sex C/CC/CS |

Figure 1: Criteria list for the assessment of methodological quality, modified from Lievense et al.[10, 11]

84x89mm (300 x 300 DPI)

**PRISMA-P (Preferred Reporting Items for Systematic review and Meta-Analysis Protocols) 2015 checklist: recommended items to address in a systematic review protocol***

| Section and topic | Item No | Checklist item | Man. page no. |
|---|---|---|---|
| ADMINISTRATIVE INFORMATION | | | |
| Title: | | | |
| Identification | 1a | Identify the report as a protocol of a systematic review | p. 2 (Abstract), p. 4. |
| Update | 1b | If the protocol is for an update of a previous systematic review, identify as such | NA |
| Registration | 2 | If registered, provide the name of the registry (such as PROSPERO) and registration number | PROSPERO ID: CRD42016045271 |
| Authors: | | | |
| Contact | 3a | Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author | p. 1(title page) |
| Contributions | 3b | Describe contributions of protocol authors and identify the guarantor of the review | pp. 11. |
| 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 | NA |
| Support: | | | |
| Sources | 5a | Indicate sources of financial or other support for the review | p. 11. |
| Sponsor | 5b | Provide name for the review funder and/or sponsor | NA |
| Role of sponsor or funder | 5c | Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol | NA |
| **INTRODUCTION** | | | |
| Rationale | 6 | Describe the rationale for the review in the context of what is already known | pp. 4-5 |
| Objectives | 7 | Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO) | p. 5. |
| **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 | pp. 5-6. |
| 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 | p. 7. |
| 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 | p. 7. |
| Study records: | | | |
| Data management | 11a | Describe the mechanism(s) that will be used to manage records and data throughout the review | p. 9. |
| 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) | p. 7-8. |
| Data collection process | 11c | Describe planned method of extracting data from reports (such as piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators | p. 9. |
| 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 | p. 6-7. |
| Outcomes and prioritization | 13 | List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale | p. 6. |
| 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 | p. 7-8 |
| Data synthesis | 15a | Describe criteria under which study data will be quantitatively synthesised | p. 9. |
| | 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 $I^2$, Kendall's $\tau$) | p. 9. |
| | 15c | Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression) | p. 9. |
| | 15d | If quantitative synthesis is not appropriate, describe the type of summary planned | p. 9. |
| Meta-bias(es) | 16 | Specify any planned assessment of meta-bias(es) (such as publication bias across studies, | NA |

| | | | |
|---|---|---|---|
| | | selective reporting within studies) | |
| Confidence in cumulative evidence | 17 | Describe how the strength of the body of evidence will be assessed (such as GRADE) | p. 9-10 |

*** It is strongly recommended that this checklist be read in conjunction with the PRISMA-P Explanation and Elaboration (cite when available) for important clarification on the items. Amendments to a review protocol should be tracked and dated. The copyright for PRISMA-P (including checklist) is held by the PRISMA-P Group and is distributed under a Creative Commons Attribution Licence 4.0.**

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