PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	THE RELATIONSHIP BETWEEN SERUM OSTEOCALCIN/ UNDERCARBOXYLATED OSTEOCALCIN AND TYPE II DIABETES: A SYSTEMATIC REVIEW/ META-ANALYSIS STUDY PROTOCOL
AUTHORS	Liu, Yihui; Liu, Xiaoying; R. Lewis, Joshua; Brock, Kaye; C.Brennan-Speranza, Tara; Teixeira-Pinto, Armando

VERSION 1 – REVIEW

REVIEWER	Setor Kunutsor University of Bristol, UK
REVIEW RETURNED	02-Jul-2018

GENERAL COMMENTS	Summary The authors plan to investigate the relationship between osteocalcin (OC) and type 2 diabetes mellitus (T2DM) using a systematic review and meta-analysis. The rationale is that there is uncertainty in the relationship between osteocalcin and metabolic outcomes and previous published reviews on the topic have been characterised by heterogeneity. The authors plan to include observational studies which have reported associations between OC and T2DM in adult humans. They will be comparing OC levels in patients with T2DM and prediabetes with their controls, evaluate the associations between OC and risk of developing T2DM, and will evaluate the heterogeneity observed in previous meta-analyses on the topic. General Comments Thank you for the opportunity to review your manuscript. The topic is indeed of relevance as there is great uncertainty regarding the association between osteocalcin and adverse metabolic outcomes such as T2DM. A number of reviews have been published on the topic and the results have not been conclusive as most of the evidence available is based on cross-sectional and case-control study designs with very few cohort studies. Kunutsor SK, Apekey TA, Laukkanen JA. Association of serum total osteocalcin with type 2 diabetes and intermediate metabolic phenotypes: systematic review and meta-analysis of observational evidence. European journal of epidemiology. 2015;30(8):599-614 Liu C, Wo J, Zhao Q, et al. Association between Serum Total Osteocalcin Level and Type 2 Diabetes Mellitus: A Systematic Review and Meta-Analysis. Hormone and metabolic research. 2015;47(11):813-9

	For instance, in the review by Kunutsor et al, circulating serum total OC levels in relation to risk of T2DM was reported in 6 cross-sectional and 3 cohort studies. There were more studies that compared levels of OC in diabetes and controls. My concern is that the issue is not about heterogeneity but rather if there are enough observational cohort studies to clarify the association between osteocalcin and T2DM? If there are not enough published studies, then this project will just be repeating what has been previously done. Since the publication of the two relevant reviews in 2015, how many observational cohort studies have been published on the topic? The English language needs considerable improvement. <u>Specific comments</u> **Title** The title should be specific. It needs to take into account the population, intervention (exposure), comparator, and outcomes. What is the primary aim of the review? Are the authors assessing OC levels in patients with T2DM compared with controls or investigating associations between OC and risk of developing T2DM? **Abstract** The abstract is missing the following: 1. Please specify the aim/objective of the review. 2. What kind of observational studies will be included? 3. This statement is very vague “A literature search was conducted in March 2017 and will be updated in early 2018 in three databases” The reviewer is unsure what this means. 4. Briefly mention methods for pooling – risk ratios and mean differences 5. “A single reviewer will perform the data extraction” How will this be verified? 6. The authors state they will be evaluating heterogeneity but fail to report how this will be done. **Strengths and limitations of this study** 1. “This review will include more eligible studies (especially of prospective studies) and increase the number of available participants” The authors have not provided any proof of this. 2. “This review will be the first study thoroughly investigating heterogeneity in the relationships between OC and T2DM with an advanced technical method of Rstudio” This is not relevant. 3. I am a bit surprised by this statement “The main limitation of the current study is that there is no qualitative assessment in this review so studies having a poor quality will not be excluded, and it may affect the study results to some extent.” The review has not yet been conducted so why report this. How do they intend to address this if it is seen as a potential limitation? **Introduction**

	1. Most of the paragraph 1 is largely irrelevant as this is nothing new. Please focus on the current topic. Provide a suitable background. 2. The authors introduce this statement “Furthermore, there are two forms of OC: the carboxylated osteocalcin (cOC) and undercarboxylated osteocalcin (ucOC)” and then go on to mention total OC. What is total OC? 3. “Several systematic reviews/meta-analyses got published in recent years, but with different conclusions and great unexplained heterogeneity among studies. They reported that OC might play a role in whole-body energy metabolism (17-20).” None of these were meta-analyses. These were all narrative reviews. How was heterogeneity quantified in these reviews? 4. “The findings of three recent systematic reviews support this hypothesis by concluding that patients with T2DM had a significantly lower OC levels compared with normal glucose controls (mean difference [95%CI] of OC (ng/ml) and p-value for each review:” Please provide their references. 5. “Therefore, this present review aims collect more evidence of TOC and ucOC in patients with T2DM and comprehensively explore possible factors that can explain the heterogeneity of the results across studies.” How do the authors intend to do this? Since the evidence is limited, have there been any published studies on the topic since the publication of the last relevant review? Have the authors conducted any scoping work to find other studies on the topic. This should be the focus of the introduction. The rationale is not very convincing and should be re-written. **Objectives** “The primary objective is to determine the associations between TOC and ucOC and the incidence of T2DM and to investigate the possible resources for heterogeneity. The secondary aim is to examine this association in patients with prediabetes and the potential remedies for heterogeneity” How does this tie in with what was reported in the abstract “We will conduct a systematic review including a meta-analysis to compare OC levels in patients with T2DM, prediabetes and standard glucose controls, and to further investigate associations between OC and risk of developing T2DM”. Please be consistent. **Eligibility criteria for studies included in the review** 1. Please be specific about the type of cohort studies 2. What about randomised controlled trials? **STATISTICAL ANALYSIS & DATA SYNTHESIS** 1. “Mean differences (MDs) with 95%CI of TOC/ucOC are produced regarding T2DM, or prediabetes and standard glucose controls.” This is not clear. 2. For risk estimates reported by per-unit or standard deviation change, quintiles, or other groupings, how do the authors intend to transform this into a consistent format to enable pooling?

	3. “We will assess publication bias of MD and risk estimates by visual inspection of the funnel plots (30).” This will only be possible if there are a minimum number of studies. This should be stated. **Risk of bias assessment** How do you intend to assess the methodological quality of included studies? This needs to be done. Please be specific. I could not take anything away from the narrative. **Discussion** 1. “Firstly, we will provide more evidence to previous investigations in analyzing OC's potential roles in T2DM by increasing the number of eligible studies and make an up-to-date analysis.” The authors are very confident of doing this but have not provided any indications that there are enough published studies to achieve this. The authors need to tone down their statements in all sections of the manuscript. 2. “The major limitation of this review is that we will only be including observational studies because there is insufficient evidence from clinical trials, which will restrict study result in specific analysis.” No indications have been provided anywhere in the introduction as to whether there are published clinical trials or not. 3. “As quality assessments are not conducted in our current study…” Why wont quality assessment be conducted. This is a requirement for every systematic review/meta-analysis. **Figure 1** The authors have indicated in the figure that 96 articles will be included in the quantitative synthesis but no breakdowns by study design and type of analysis have been provided in the figure or text.

REVIEWER	Tetsuo Nishikawa Endocrinology & Diabetes Center, Yokohama Rosai Hospital, Yokohama City, 222-0036, JAPAN
REVIEW RETURNED	10-Jul-2018

GENERAL COMMENTS	Major problems: 1. Although authors raise lack of quality assessment as a major limitation, it is still unclear why they will not quality assessment. Subgroup analysis with study type may not be sufficient in this context. Please rationalize why you do (or can) not assess study quality, otherwise quality assessment may also be needed (ref). ref: Greenland S, O’Rourke K. Meta-analysis. Modern epidemiology 3rd ed. Lippincott Williams & Wilkins, 2008:652-82 2. Page 3, Line 35: Please rephrase “updated in early 2018” and clarify the period given already July in 2018. 3. Page. 5: In introduction part, I feel insufficiency of the information about osteocalcin (OC) or undercarboxylated osteocalcin (ucOC). Today, it is well known about multifunction of OC as a bone-derived hormone in experimental research and many observational studies were reported. You should summarize these results in detail. Also you should mention about the differences between OC and ucOC.

	4. Page 5, Line 15: It is not clear why authors quote the number of diabetes/prediabetes in the U.S. while this review will not focus on US population. Please refer global epidemiology if applicable. Also, please update your information because links in the references were accessible almost a year ago. 5. Page 5, Line 15: space between 2012 and (4). There seem to be some similar errors in whole manuscript (Page 6, Line 5 etc..). Please check format and grammar again and consider Native English check if needed. 6. Page 6. Line 16: Why is this information (i.e. need for further investigation) “additional”? Please consider rephrasing. 7. Page. 7: In inclusion and exclusion criteria, why don’t you exclude the patients with rickets or osteomalacia which is severe bone disease? Moreover, why don’t you exclude the patients with severe infection such as a sepsis. How will you manage slowly progressive insulin-dependent diabetes mellitus? You should mention about denosumab and teriparatide in part of 12. Ptients with medications that affect bone metabolism. 8. Page. 8, Line. 13: Several diabetic treatments influence serum OC/ucOC level, especially insulin therapy and GLP-1 receptor analogs. You should mention about it. 9. Page. 12: In subgroup analysis and investigation of heterogeneity part, you should add subgroups based on age. Does only menopausal in women effect serum level of OC/ucOC? Is there any information about the differences of serum OC/ucOC level by age? How about the conditions of blood sampling. Does meal affect serum level of OC/ucOC? 10. Page 12, Line 31, 34: Please spell out WC, VK, and VD. 11. Page. 13, Line. 24: I feel it is a big issue that you will not conduct quality assessment. 12. Please check the order of references. Ex. 34 seems to come before 33.

VERSION 1 – AUTHOR RESPONSE

Dear BMJ-Open editorial staff,
We thank the editors and reviewers for their feedback on our manuscript. The suggestions by the reviewers are addressed as follows:

1. Although authors raise lack of quality assessment as a major limitation, it is still unclear why they will not quality assessment. Subgroup analysis with study type may not be sufficient in this context. Please rationalize why you do (or can) not assess study quality, otherwise quality assessment may also be needed (ref).

ref: Greenland S, O’Rourke K. Meta-analysis. Modern epidemiology 3rd ed. Lippincott Williams & Wilkins, 2008:652-82
The Newcastle-Ottawa scale will be used to assess risk of bias.

2. Page 3, Line 35: Please rephrase “updated in early 2018” and clarify the period given already July in 2018.
We have now replaced “updated in early 2018”, with “The latest search was performed in July 2018”.

3. Page. 5: In introduction part, I feel insufficiency of the information about osteocalcin (OC) or undercarboxylated osteocalcin (ucOC). Today, it is well known about multifunction of OC as a bone-

derived hormone in experimental research and many observational studies were reported. You should summarize these results in detail. Also you should mention about the differences between OC and ucOC.

We have now extensively revised the introduction to read as follows:

Type 2 diabetes (T2DM) results from the body becoming progressively more resistant to the effects of insulin. This is termed insulin resistance. With the influence of long-term progress, blood sugar exceeds the normal levels and patients are diagnosed with T2DM. The disease now ranks 9th in the world global health threats list (1). Currently, around 425 million people have diabetes, with 90% of these having T2DM (1). It is estimated that by 2045, this figure will have increased to 629 million people (1).

Patients with T2DM have increased levels of glucose parameters/insulin resistance indices (2). Accordingly, the methods for diagnosing diabetes are based on measuring fasting plasma glucose (FPG), haemoglobin A1c (HbA1c), fasting insulin levels (FINS) and the homeostatic model assessment-insulin resistance (HOMA-IR) (3). Patients with T2DM have increased risks of other complications such as heart attacks, strokes, diabetic retinopathy and renal disease (3). Interestingly, other diabetic complications include impaired bone remodelling and fracture risk (4,5). Although the bone mineral density (BMD) in T2DM is generally reported to be normal or slightly higher than healthy age-matched individuals, with large numbers of studies having reported increased risk of hip fractures in people with T2DM (6,7).

Osteocalcin (OC), is an osteoblast secreted protein that plays a role in the communication between the skeleton and glucose homeostasis. There are two forms of OC: uncarboxylated osteocalcin (ucOC) and carboxylated osteocalcin (cOC) (9). The cOC contributes to extracellular bone matrix while ucOC is likely the active form of OC in the circulation (11). Both cOC and ucOC are present in the circulation, and the amount of them is known as total osteocalcin (TOC) (11). TOC is considered a marker of bone turnover (12).

A potential endocrine function of OC was first suggested in 2007. Lee et al. and Ferron et al. reported OC mediated glucose homeostasis via stimulating beta-cell proliferation and adiponectin secretion in mice (13,14). The endocrine actions of OC involve increasing insulin synthesis and secretion by beta-cells and improved insulin sensitivity by promoting adiponectin secretion in adipocytes (13,14). The high-fat diet experimental study revealed that bone could become insulin resistant by inhibiting the activation of OC (15). However, reported associations between OC and T2DM in humans have yielded conflicting results. (16–19). Lerchbaum et al. reported high OC was associated with reduced risk of developing T2DM in a population-based study (OR:0.57;95%CI:[0.46,0.70]) (23). Achemlal et al. reported, in a cross-sectional study of patients with poorly controlled T2DM, serum levels of OC were significantly lower in T2DM compared with age-matched controls (24).While Bao et al. observed increased serum levels of OC were associated with improved glucose control (25). Yeap et al. found both TOC and ucOC were associated with reduced risk of developing diabetes in a cohort of community-dwelling elderly men (OR:0.60; 95%CI:[0.50,0.72] for TOC, and OR:0.55; 95%CI:[0.47,0.64] for ucOC) (26). In contrast, a case-control study with 1,635 participants by Zwakenberg et al. indicated there was no association between TOC/ucOC and the risk of T2DM (OR:0.97; 95%CI:[0.69,1.36] for TOC, and OR:0.88; 95%CI:[0.61,1.27] for ucOC) (27).

Two previously published systematic reviews/meta-analyses have reported decreased levels of serum TOC in people with T2DM compared to controls. However, these reviews only found a small number of the published studies and did not investigate ucOC (28–30). The mean differences in T2DM compared with normal glucose tolerance controls from the three reviews showed similar results (-3.31ng/ml [-4.04, -2.57] from Kunutsor et al.; -2.87 ng/ml [-3.76,-1.98] from Liu C et al. , and -2.51 ng/ml [-3.01,-2.01] from Hygum et al.) (28–30). Both of the reviews by Kunutsor et al and Liu C et al. only found a small number (n=4) of cohort studies (28,29).. Additionally, studies reporting the associations between ucOC and glucose homeostasis in T2DM have not been adequately meta-analysed (29).

Some observational studies have reported decreased OC concentrations in pre-diabetics (PD) compared to normal glucose tolerance controls, while Aoki et al. indicated an increase of OC

concentration in the early stage of diabetes (36–38). Therefore, conducting meta-analyses comparing the OC levels between PD and normal glucose controls and comparing OC levels between T2DM and PD may contribute to the investigation between OC and glucose homeostasis in patients with diabetes. Another unsolved issue in the previously published meta-analyses are the high between-study heterogeneity. Previous reviews explored different sources of heterogeneity with modest success (28,29). Starup-Linde et al. conducted subgroup analysis according to menopausal status in women, sex, and age (39).Liu C et al. attempted to explain the heterogeneity by sex and OC assay methods (29).Kunutsor et al. conducted subgroups analysis according to study design and degree of confounders of risk estimates (28).Hygum et al. performed a meta-regression analysis to investigate how much heterogeneity was explained by the haemoglobin A1c(HbA1c) levels (30).
Therefore, the present systematic review/meta-analysis will use a more comprehensive search strategy to identify more prospective studies thereby increasing statistical power. Secondly, we will search for studies reporting the association between ucOC and glucose metabolism. Thirdly, we will identify studies comparing the OC concentrations between PD and normal glucose controls, and between T2DM and PD. Lastly, by systematically exploring potential sources of heterogeneity we may explain previous conflicting findings."

4. Page 5, Line 15: It is not clear why authors quote the number of diabetes/prediabetes in the U.S. while this review will not focus on US population. Please refer global epidemiology if applicable. Also, please update your information because links in the references were accessible almost a year ago.
We have now updated the manuscript to reflect global trends.

5. Page 5, Line 15: space between 2012 and (4). There seem to be some similar errors in whole manuscript (Page 6, Line 5 etc..). Please check format and grammar again and consider Native English check if needed.
We have now checked and corrected the errors.

6. Page 6. Line 16: Why is this information (i.e. need for further investigation) "additional"? Please consider rephrasing.
As suggested, we have rewritten the introduction The reason for recruiting studies reporting the association between ucOC and T2DM is described in paragraph three.

7. Page. 7: In inclusion and exclusion criteria, why don't you exclude the patients with rickets or osteomalacia which is severe bone disease? Moreover, why don't you exclude the patients with severe infection such as a sepsis. How will you manage slowly progressive insulin-dependent diabetes mellitus? You should mention about denosumab and teriparatide in part of 12. Patients with medications that affect bone metabolism.
As advised, we have now updated the inclusion and exclusion criteria. A new statement of in part of 10 shows that patients with osteomalacia are excluded. Rickets are not mentioned as our eligible participants are adults and children are excluded from the present review. T2DM on medications will not be excluded as we will undertake sensitivity analyses to determine whether "treated" T2DM affects the overall findings. The effect of anti-glutamic medications will be analyses by subgroup analysis. In part of 13, patients with sepsis is added to the exclusion criteria. The suggested medications (denosumab and teriparatide) have been added to part 14. Patients with medications that affect bone metabolism.

8. Page. 8, Line. 13: Several diabetic treatments influence serum OC/ucOC level, especially insulin therapy and GLP-1 receptor analogs. You should mention about it.
As suggested, the anti-diabetic drugs that may affect serum OC/ucOC levels are summarized in in the item two 'Note' part, page nine. We also suggested patients with T2DM under anti-diabetic medications are not excluded but will be assessed by subgroup analysis based on the status of anti-diabetic medications status.

9. Page. 12: In subgroup analysis and investigation of heterogeneity part, you should add subgroups based on age. Does only menopausal in women effect serum level of OC/ucOC? Is there any information about the differences of serum OC/ucOC level by age? How about the conditions of blood sampling. Does meal affect serum level of OC/ucOC?
Thank you for this suggestion. Yes, serum OC is correlated with age in both men and women. We have now added subgroup analysis based on age in page 13. The conditions of blood sampling in short term and long period are captured by investigating the various glucose metabolism measurements with different responses to meals likely to be obvious in short-term vs long-term measures. After the meal, patients may have reduced levels of OC/ucOC.

10. Page 12, Line 31, 34: Please spell out WC, VK, and VD.
The above abbreviations have now been spelled out in the text.

11. Page. 13, Line. 24: I feel it is a big issue that you will not conduct quality assessment.
We will conduct a Newcastle-Ottawa quality assessment scale (NOS). Extracted data will be presented and used in the subgroup analyses to evaluate the individual study weight.

12. Please check the order of references. Ex. 34 seems to come before 33.
As suggested, all the reference order have been checked and updated accordingly.

VERSION 2 – REVIEW

REVIEWER	Setor Kunutsor University of BristoL, UK
REVIEW RETURNED	18-Aug-2018

GENERAL COMMENTS	The English language is still not up to standard. The grammar needs improvement – the authors report a lot of past tenses even though the review has not been done. The manuscript is much improved, but it appears the authors have still not addressed some of my previous comments. The title is still not appropriate. “The relationship between serum UC/UCOC and type 2 diabetes is more appropriate: a systematic review and meta-analysis study protocol” Please do not include abbreviations in the title. Abstract “Three databases (MEDLINE, EMBASE, and SCOPUS) will be searched from 1946 until July 2018 without language restrictions.” Are all three databases valid from 1946? Why not rather state that they will be searched from inception? “The risk of bias assessment would be conducted by two reviewers independently based on the Newcastle-Ottawa Scale (NOS).” The authors state they will be including intervention studies. Will the NOS also apply to these studies? Strengths and limitations of this study “The main limitation of the current study is only including observational studies (cohort, case-control and cross-sectional studies).” You stated in the abstract that you will be including

	intervention studies, so why this statement. The authors need to be consistent! Introduction “Type 2 diabetes (T2DM) results from the body becoming progressively more resistant to the effects of insulin. This is termed insulin resistance. With the influence of long-term progress, blood sugar exceeds the normal levels and patients are diagnosed with T2DM.” Largely irrelevant and not scientifically written. The first two paragraphs are too long and quite redundant. Readers don’t need all that information. You only need a statement or two on the epidemiology of T2DM! Data extraction “One author will extract data from studies that are eligible for full-text assessment. Obtained data will be examined for a second time by the same author to correct any mistakes.” This is not appropriate. Shouldn’t this be done independently by two reviewers or done by one reviewer and checked by a second reviewer? Discussion “The major limitation of this review is that we will only be including observational studies because there is insufficient evidence from clinical trials, which will restrict study result in specific analysis.” No indications have been provided anywhere in the introduction as to whether there are published clinical trials or not. This comment was raised in my previous review, but the authors do not seem to have addressed this. Moreover, the authors have indicated in the methods section that they will be including intervention studies. Have the authors done a scoping search? Have more studies been published since the previous published reviews? Are there any trials out there. The authors need to convince readers that there are more studies out there and that the intended review will be an improvement over previous ones. This has not been done at all.

REVIEWER	Tetsuo Nishikawa, MD, PhD. Endcrinology & Diabetes Center, Yokohama Rosai Hospital, Yokohama City, JAPAN
REVIEW RETURNED	25-Aug-2018

GENERAL COMMENTS	I find that the overall organization of the manuscript could be improved.

VERSION 2 – AUTHOR RESPONSE

Reviewer(s)' Comments to Author:

Reviewer: 1

Reviewer Name: Setor Kunutsor

Institution and Country: University of Bristol, UK

Please state any competing interests or state ‘None declared’: None declared

Please leave your comments for the authors below

The English language is still not up to standard. The grammar needs improvement – the authors report a lot of past tenses even though the review has not been done. The manuscript is much improved, but it appears the authors have still not addressed some of my previous comments.

We have improved the grammar in the manuscript with the help of the Editage language service.

The title is still not appropriate. “The relationship between serum UC/UCOC and type 2 diabetes is more appropriate: a systematic review and meta-analysis study protocol” Please do not include abbreviations in the title.

The title of the revised manuscript has been revised to “The relationship between serum osteocalcin/ undercarboxylated osteocalcin and type II diabetes: a systematic review and meta-analysis study protocol”

Abstract

“Three databases (MEDLINE, EMBASE, and SCOPUS) will be searched from 1946 until July 2018 without language restrictions.” Are all three databases valid from 1946? Why not rather state that they will be searched from inception?

As suggested, we have made changes to the revised manuscript. Changes show as “Three databases (MEDLINE, EMBASE, and SCOPUS) will be searched from inception until January 2019 without language restrictions.”

“The risk of bias assessment would be conducted by two reviewers independently based on the Newcastle-Ottawa Scale (NOS).” The authors state they will be including intervention studies. Will the NOS also apply to these studies?

Yes. As we only used the baseline data of some interventional studies, we believe the NOS for cross-sectional studies can be applied to those trial studies.

Strengths and limitations of this study

“The main limitation of the current study is only including observational studies (cohort, case-control and cross-sectional studies).” You stated in the abstract that you will be including intervention studies, so why this statement. The authors need to be consistent!

The reason is that we will only use the baseline data of the interventional studies before the initiation of any intervention as there are very limited interventional studies in the literature thus to analyse intervention would just be repeated the results of these studies rather than applying them to a new meta-analysis. In this case, we consider those interventional studies as cross-sectional studies. Thus, our statement implies that this present study only includes observational studies. A more precise description has been given in the revised manuscript.

Introduction

“Type 2 diabetes (T2DM) results from the body becoming progressively more resistant to the effects of insulin. This is termed insulin resistance. With the influence of long-term progress, blood sugar exceeds the normal levels and patients are diagnosed with T2DM.” Largely irrelevant and not scientifically written. The first two paragraphs are too long and quite redundant. Readers don’t need all that information. You only need a statement or two on the epidemiology of T2DM!

We have modified the “Introduction” section and made it more precise. Improved content shows as below:

“The disease burden attributed to diabetes is high. Currently, around 425 million people have diabetes, with 90% of these having T2DM.[1] It is estimated that by 2045, this figure will have increased to 629 million people.[1] Patients with T2DM present with increased levels of glucose compared to people with normal glycaemic metabolism. Additionally, patients with T2DM have increased risks of other complications such as heart attacks, strokes, diabetic retinopathy, and renal disease.[2]

Correspondingly, several organs become targets to treat, prevent or predict diabetes, such as pancreatic beta cells, muscle, liver, adipose tissue, kidney, the gastrointestinal tract, or the brain.[3] Interestingly, a recent study has identified a new potential tissue to target for the treatment diabetes: the skeleton and bone. [3] Increasing numbers of osteokines secreted by the skeleton and bone exhibit regulatory functions in glucose metabolism.[3]

Osteocalcin (OC) is an osteoblast-secreted protein that plays a role in the communication between the skeleton and glucose homeostasis. There are two forms of OC: uncarboxylated osteocalcin (ucOC) and carboxylated osteocalcin (cOC).[4] cOC contributes to the extracellular bone matrix, while ucOC is likely the active form of OC in the circulation.[5] Both cOC and ucOC are present in the circulation, and their combined levels are referred to as total osteocalcin (TOC).[5] TOC is considered a marker of bone turnover.[6]

A potential endocrine function of OC was first suggested in 2007. Lee et al. and Ferron et al. reported that OC mediated glucose homeostasis via stimulating beta-cell proliferation and adiponectin secretion in mice.[7,8] The endocrine actions of OC involve increasing insulin synthesis and secretion by beta-cells and improved insulin sensitivity by promoting adiponectin secretion from adipocytes.[7,8] The high-fat diet experimental study revealed that bone could become insulin resistant by inhibiting the activation of OC.[9] However, reported associations between OC and T2DM in humans have yielded conflicting results.[10–13] Lerchbaum et al. reported that high OC level was associated with reduced risk of developing T2DM in a population-based study (odds ratio [OR], 0.57; 95% confidence interval [CI]: 0.46, 0.70).[14] In a cross-sectional study of patients with poorly controlled T2DM, Achemlal et al. reported that serum levels of OC were significantly lower in patients with T2DM compared with age-matched controls,[15] while Bao et al. observed that increased serum levels of OC were associated with improved glucose control.[16] Yeap et al. found that both TOC and ucOC were associated with reduced risk of diabetes in a cohort of community-dwelling elderly men (OR, 0.60; 95% CI: 0.50, 0.72 for TOC, and OR, 0.55; 95% CI: 0.47, 0.64 for ucOC).[17] In contrast, a case-control study conducted by Zwakenberg et al. with 1,635 participants, indicated a lack of

association between TOC/ucOC and the risk of T2DM (OR, 0.97; 95% CI: 0.69, 1.36 for TOC, and OR, 0.88; 95% CI: 0.61, 1.27 for ucOC).[18]

Two previously published systematic reviews/meta-analyses reported decreased serum levels of TOC in people with T2DM compared to controls in 2015. However, these reviews only found a small number of published studies and did not investigate ucOC.[19–21] The mean differences in T2DM compared with normal glucose tolerance controls from the three reviews showed similar results (-3.31 ng/ml [-4.04, -2.57] from Kunutsor et al.; -2.87 ng/ml [-3.76, -1.98] from Liu C et al., and -2.51 ng/ml [-3.01, -2.01] from Hygum et al.).[19–21] Both of the reviews by Kunutsor et al. and Liu C et al. only found a small number (n=4) of cohort studies.[19,20] Additionally, studies reporting the associations between ucOC and glucose homeostasis in T2DM have not been adequately meta-analysed.[20]

An increasing number of epidemiological studies have been continuously published in the recent three years following two systematic reviews/ meta-analyses in 2015, signalling a need for up-to-date systematic review/ meta-analysis. In 2017, Takashi et al. showed that ucOC could predict insulin secretion in patients with T2DM.[22] They conducted the study in 41 Japanese patients with T2DM with a mean age of about 59 years.[22] The result showed a correlation between ucOC and homeostatic model assessment of beta-cell function ($r = 0.36$, $p = 0.011$).[22] In a cross-sectional study of 69 volunteers, OC was found to be suppressed with insulin resistance, regardless of obesity or fat mass at significantly lower levels shown in controls compared with T2DM or insulin resistant obesity.[23] However, only a few interventional studies/ clinical trials were found in our scope search in MEDLINE (Appendix). Only three clinical studies were conducted after 2015 and might be eligible for inclusion in the present review.[24–26] Ghiraldini et al. designed a clinical trial in 32 T2DM patients and 19 patients without diabetes. Baseline data indicated that OC levels were higher in systematically healthy patients than those with better-controlled T2DM while poorly controlled T2DM patients had the highest OC levels.[26]

Some observational studies have reported decreased OC concentrations in pre-diabetic patients (PD) compared to normal glucose tolerance controls, while Aoki et al. indicated an increase in OC concentration during the early stages of diabetes.[27–29] Therefore, conducting a meta-analysis comparing the OC levels between PD and normal glucose controls and comparing OC levels between T2DM and PD may contribute to our understanding of the relationship between OC and glucose homeostasis in patients with diabetes.

Another unresolved issue in the previously published meta-analyses is the high between-study heterogeneity. Previous reviews explored different sources of heterogeneity with modest success.[19,20] Starup-Linde et al. conducted subgroup analysis according to sex, age and menopausal status in women.[30] Liu C et al. attempted to explain the heterogeneity by sex and OC assay methods.[20] Kunutsor et al. conducted subgroup analyses according to study design and degree of confounders of risk estimates.[19] Hygum et al. performed a meta-regression analysis to investigate the extent to which heterogeneity was explained by haemoglobin A1c (HbA1c) levels.[21]

Therefore, the present systematic review/meta-analysis will use a more comprehensive search strategy to identify more numerous and more recent prospective studies, thereby increasing the statistical power. Secondly, we will search for studies reporting the association between ucOC and

glucose metabolism. Thirdly, we will identify studies comparing the OC concentrations between PD and normal glucose controls, and between T2DM and PD. Lastly, by systematically exploring potential sources of heterogeneity we may explain previous conflicting findings."

Data extraction

"One author will extract data from studies that are eligible for full-text assessment. Obtained data will be examined for a second time by the same author to correct any mistakes." This is not appropriate. Shouldn't this be done independently by two reviewers or done by one reviewer and checked by a second reviewer?

As suggested, we have modified the "Data extraction" section. The revised content is "Two authors will independently extract data from studies that are eligible for full-text assessment. If any discrepancy arises, a third reviewer will examine the data."

Discussion

"The major limitation of this review is that we will only be including observational studies because there is insufficient evidence from clinical trials, which will restrict study result in specific analysis." No indications have been provided anywhere in the introduction as to whether there are published clinical trials or not.

This comment was raised in my previous review, but the authors do not seem to have addressed this. Moreover, the authors have indicated in the methods section that they will be including intervention studies.

Have the authors done a scoping search? Have more studies been published since the previous published reviews? Are there any trials out there. The authors need to convince readers that there are more studies out there and that the intended review will be an improvement over previous ones. This has not been done at all.

A scope search has been applied and the results have been added in paragraph 6 of the Introduction section. Revised content shows below:

"An increasing number of epidemiological studies have been continuously published in the recent three years following two systematic reviews/ meta-analyses in 2015, signalling a need for up-to-date systematic review/ meta-analysis. In 2017, Takashi et al. showed that ucOC could predict insulin secretion in patients with T2DM.[22] They conducted the study in 41 Japanese patients with T2DM with a mean age of about 59 years.[22] The result showed a correlation between ucOC and homeostatic model assessment of beta-cell function ($r = 0.36$, $p = 0.011$).[22] In a cross-sectional study of 69 volunteers, OC was found to be suppressed with insulin resistance, regardless of obesity or fat mass at significantly lower levels shown in controls compared with T2DM or insulin resistant obesity.[23] However, only a few interventional studies/ clinical trials were found in our scope search in MEDLINE (Appendix). Only three clinical studies were conducted after 2015 and might be eligible for inclusion in the present review.[24–26] Ghiraldini et al. designed a clinical trial in 32 T2DM patients and 19 patients without diabetes. Baseline data indicated that OC levels were higher in systematically healthy patients than those with better-controlled T2DM while poorly controlled T2DM patients had the highest OC levels.[26] "

Reviewer: 2

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Please state any competing interests or state ‘None declared’: None

We have changed the statement to “None declared”

Please leave your comments for the authors below

I find that the overall organization of the manuscript could be improved.

As suggested, we have invited the language service editor to double check the manuscript organization according to the protocol guideline in BMJ-Open.