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THE ROLE OF OSTEOCALCIN IN PATIENTS WITH TYPE 2 DIABETES: A SYSTEMATIC REVIEW / META-ANALYSIS STUDY PROTOCOL

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ABSTRACT

Introduction

Type 2 diabetes (T2DM) is more often associated with lifestyle factors in recent years and becoming an economic and disease burden for countries globally. Novelty findings to the preventive factors from bone for abnormal metabolic outcomes had been reported. Two mice-model studies in 2007 and 2008 indicated that osteocalcin, as a bone formation marker, may also affect glucose homeostasis and obesity measurements. Since then, many research groups developed observational studies about relationships between osteocalcin (OC) and metabolic outcomes.However, results in human studies remain controversial, with several reviews suggesting that OC may play a protective role in the development of T2DM but with a sizeable unexplained heterogeneity across studies.

Methods and analysis

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. This review will comprehensively evaluate possible explanatory factors for the heterogeneity observed in previous meta-analyses. We include observational studies which reported interested associations between OC and T2DM in adult humans. A literature search was conducted in March 2017 and will be updated in early 2018 in three databases (MEDLINE, EMBASE, and SCOPUS) without language restrictions or time limitations. Two reviewers independently screen the titles and abstracts and conduct a full-text assessment to exclude ineligible studies. Meeting with a third reviewer to address discrepancies. A single reviewer will perform the data extraction. Eligible extracted data will be pooled to meta-analyses to evaluate the interested associations and assess resources for heterogeneity if permitted. This study will report items in line with guidelines in PRISMA and MOOSE (25,26).

Registration number in PROSPERO: CRD42017073127

Keywords: osteocalcin, type 2 diabetes, prediabetes, meta-analysis

Strengths and limitations of this study

- This review will include more eligible studies (especially of prospective studies) and increase the number of available participants.
- This review will be the first study thoroughly investigating heterogeneity in the relationships between OC and T2DM with an advanced technical method of Rstudio.
- The methods of the review analysis two forms of OC (TOC and ucOC) that may specify the actual endocrine function of OC in humans.
- The design of the review considers an early stage of diabetes which indicate the relationship between OC and impaired glucose metabolism in a progressive level.
- 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.

INTRODUCTION

Type 2 diabetes (T2DM) is a preventable disease, but its prevalence has been increasing in the past four decades. This chronic disease, also known as non-insulin-dependent diabetes, results from insulin resistance and is associated with modifiable lifestyle risk factors (1, 2). People with risk factors, such as overweight or obese, inadequate physical activity and inappropriate diet, may develop T2DM via a progressive condition (1-3). There are growing cases of T2DM not only in high-income countries but also in developing countries. In the United States, about 29 million people had diabetes in 2012(4), and 86 million adults had prediabetes (5). Besides, WHO reported that diabetes cases might have a massive increase in developing countries in next decades, rising from 115 million in 2000 to 284 million in 2030 (6). Furthermore, the cost for T2DM treatments has become a severe economic burden in global. Expenditures due to the right medications on T2DM ranged from about $242 to $11,917 across countries (6).

Osteocalcin(OC), a bone turnover marker from osteoblast, has been found that may have an effect on glucose metabolism except for its natural functions in the skeleton. By nature, OC plays a role in bone remodeling, bone mineralization and calcium^{2+} homeostasis (7). In last decades, it was shown, by two mice-model studies, that OC could regulate glucose homeostasis by stimulating beta cell proliferation and adiponectin secretion (7, 8). Furthermore, there are two forms of OC: the carboxylated osteocalcin (cOC) and undercarboxylated osteocalcin (ucOC) (8, 9). Also, ucOC is the active form of OC, and the experiments focused on investigating the functions of both total osteocalcin (TOC) and ucOC in energy homeostasis (9, 10). Since then, clinical observations conducted in humans have been contributing to investigations of OC and energy metabolism in different population groups, according to their disease conditions, sex difference or regions. The results are still conflicting (11-16).

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). 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: -3.31[-4.04, -2.57], p<0.001; -2.87 [-

3.76, -1.98], p<0.001; -2.51[-3.01-2.01]), p<0.001). Another review found very similar levels of OC in T2DM and healthy controls (mean difference[95%CI] of OC (ng/ml): -0.80[-1.64,0.03], p=0.06) (21). The reviews explored different sources of heterogeneity but with modest success. Starup-Linde et al. conducted subgroup analysis according to menopausal status in women, sex, and age (21). Liu C et al. attempted to explain the heterogeneity by sex and OC assay methods (22). Kunutsor et al. conducted subgroups analysis according to study design and degree of confounders of risk estimates (23). Hygum et al. performed a meta-regression analysis to investigate how much heterogeneity was explained by the Haemoglobin A1c(HbA1c) levels (24). Additionally, as reported by Liu et al., the number of studies which investigated the association between ucOC and T2DM were limited and needed further investigation (22). 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.

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.

METHODS & ANALYSIS

We designed this study in adherence to the guideline of Preferred Reporting Items for Systematic review and Meta-analysis (PRISMA) and Meta-analysis of observational studies in epidemiology (MOOSE) (25, 26). The process of the proposed protocol shows in Figure.1, and PRISMA-checklist shows in Appendix 1.

Protocol and registration

This protocol is registered and available on PROSPERO (CRD42017073127).

Patient and public involvement statement

There is no patient or public involved in this systematic review/meta-analysis.

Eligibility criteria for studies included in the review

Inclusion and exclusion criteria

A. Participants:

Participants should be adult humans (older than 18 years old), with T2DM at baseline or developed T2DM afterward; not have any conditions that can affect bone metabolism or with medications that affect bone metabolism; could be with anti-diabetic treatments.

Exclude:

1. Children or adolescents (younger than 18 years old), pregnant or lactating women because of altered bone turnover markers levels.
2. Patients with a particular disease(s) that either affects bone metabolism or glucose metabolism:
3. Patients with type 1 diabetes and gestational diabetes as they are pathophysiological different compared with T2DM.
4. Patients with Cushing's disease or Cushing's syndrome as they have a disordered metabolism.
5. Patients with hormonal disorders. For instance, growth-hormone deficiency.
6. Patients with hyperparathyroidism or hypoparathyroidism or other diseases affect thyroid function because of increased OC levels.
7. Patients with liver dysfunction (alanine transaminase > 3 times upper limit of normal).
8. Patients with impaired kidney function as mentioned below:
   - A chronic renal disease when glomerular filtration rate of impaired renal function patients below 30ml/min • 1.73 m^2 at stage four or five, or
   - A chronic renal illness when serum creatinine over 2.07 mg/dL, or renal osteodystrophy, or kidney transplant as 21% to 50% kidney transplant recipients may develop secondary hyperparathyroidism after kidney transplantation or treated with dialysis or hemodialysis.
9. Patients with Paget's disease as they have disorder bone metabolism.
10. Patients with cancer or tumors. For example, bone cancer metastases could mediate bone turnover markers levels.
11. Patients with human immunodeficiency virus (HIV).
12. Patients with medications that affect bone metabolism:

- Antiresorptive therapy for osteoporosis and selective estrogen receptor modulators (such as bisphosphonates, alendronate, etidronate, and raloxifene).
- Estrogen replacement therapy.
- Glucocorticoids and thiazide diuretics.

13. Patients treated with surgery that directly affected hormone or thyroid function (i.e., thyroidectomy, oophorectomy and hysterectomy).

Note: We include intervention study that reported baseline data of OC and T2DM. Accordingly, we will eliminate observational studies with more than 20% of the cohort taking above non-eligible therapy.

B. Study types:

Observational studies are eligible to include, including cohort study, case-control study and cross-sectional study. Reporting eligible exposure(s) and outcome(s).

Exclude reviews, commentaries, short survey, case reports, and letters.

Exposure(s)

OC levels identified from enzyme-linked immunoassay (ELISA or EIA), Electro-chemiluminescence immunoassay (ECLIA), Immunoradiometric assay (IRMA), radioimmunoassay (RIA), hydroxylapatite binding assay (HAP). The standard unit for OC is ng/ml; thus other presented groups for OC (eg.nmol/l) will be converted to ng/ml.

Measures of OC:

- Total serum osteocalcin levels (ng/ml).
- Undercarboxylated osteocalcin levels(ng/ml).
- OC categorized as low (reference) and high groups. Tertile, quartile, or quantile are the common categories used for classing different levels of TOC or ucOC.

Outcome(s)

Measures of T2DM;

- Diabetes status categorized as type 2 diabetes disease or normal controls (reference)
- As some studies may categorize diabetes states as insulin-dependent diabetes mellitus (IDDM) and non-insulin dependent diabetes mellitus (NIDDM), NIDDM will be used and presented as T2DM.

Exclude Type 1 diabetes and gestational diabetes as they are pathophysiological different compared with T2DM.

Secondary outcome(s)

- •IGT /IFG that is the pre-diabetic state and has a higher risk of developing T2DM
- • HbA1c percentages categorized as type 2 diabetes, prediabetes and healthy controls (reference) by HbA1c rates over 6.5%, between 5.7% and 6.5% and below 5.7% respectively.
- • Fasting plasma glucose levels categorized as diabetes, prediabetes and healthy controls(reference) by FPG levels over 126mg/dl, between 100 and 126 mg/dl, and below 100 mg/dl respectively.

STUDY DESIGN

Search strategies

A comprehensive literature search within MEDLINE, EMBASE and SCOPUS databases will be conducted to source all possibly relevant studies for the present review. There is no language restriction, and non-English articles will be translated when possible and evaluated for eligibility. There is no time restriction. We might include conferences in proceeding and abstracts if necessary. Hand search will conduct reference lists of each available paper. If duplicate publications of the same study are retrieved, most relevant and up to date paper with more complete data will be included. The detailed search strategy shows in Table.1.

Table 1. Detailed search strategy in databases: Medline, Embase and Scopus.

Medline (Ovid SP)	Embase (Ovid SP)	Scopus
1. exp osteocalcin	1. exp osteocalcin	(KEY ('osteocalcin')
2. osteocalcin.mp	2. osteocalcin.mp	OR KEY ('bone AND gla AND protein')
3. bone gla protein.mp	3.bone gla protein.mp	OR KEY ('bone AND turnover AND markers'))
4. vitamin k?dependent	4.vitamin k?dependent	AND (KEY ('diabetes AND mellitus')
bone protein*.mp	bone protein*.mp	OR KEY ('hemoglobin AND a1c')
5. 1 and 2 and 3 and 4	5. 1 and 2 and 3 and 4	OR KEY ('fasting AND plasma AND glucose'))
6. exp diabetes mellitus,	6. exp non insulin	AND KEY ('human') AND (LIMIT-
Type 2/II	dependent diabetes	TO (DOCTYPE , "ar"))
7.diabetes mellitus type	mellitus	
2/II.mp	7. exp diabetes mellitus 2/II	
8. (T2D* or NIDDM or	8. (T2D* or NIDDM or “type	
“type 2” or “type II”)).tw	2” or “type II”).tw	
	9. (prediabet* or pre	
9. (non insulin$ depend$	diabet*).tw	
or nonsinulin$depend$ or	10. hyperglyc?emi*.tw	
non insulin?depend$ or	11. 6 or 7 or 8 or 9 or 10	

noninsulin?depend$).tw 10.exp Hyperglycemia 11. hyperglycemia.mp 12. hypergly?emi*.tw 13. exp Hemoglobin A/ or exp Hemoglobin A，Glycosylated 14. HbA1c.mp 15. ("HbA(1c)" or HbA1c or "HbA 1c" or ((glycosylated or glycated) adj h?emoglobin)).tw 16. 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 17. 5 and 16 18. limit 17 to humans	12. 5 and 11 13. limit 13 to (human and exclude medline journals)

Process for selecting studies

One author set up the search strategy and store search results to Endnote X7, then the search strategy and recorded search results will be checked by another investigator. Two and more independent investigators will go through the abstract screening (remove duplicate records of the same report; include eligible articles), and full-text assessment (acquire full-text of available studies, construct citation lists of eligible items). If a discrepancy arises, disagreement will be shared with investigators by email or face-to-face meetings and make a final decision.

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 mistake. All extracted data are saved in an excel sheet.

Eligible extracted items: author and publication year, study design, study base, sample size, sex and postmenopausal status in female, age, ethnicity, country, osteocalcin assay methods, obesity measurements (BMI or WC), diabetic duration, anti-diabetic medications status, VK supplementation/anti-VK drugs, VD supplementation, TOC/ucOC levels in groups, any risk estimate between TOC/ucOC and T2DM, any association between TOC/ucOC and HbA1c and/or FPG in T2DM, any association between TOC/ucOC and

prediabetes and/or IGT, any association between TOC/ucOC and standard glucose controls, any association between TOC/ucOC and HOMA-IR or HOMA-beta in T2DM.

STATISTICAL ANALYSIS & DATA SYNTHESIS

Mean differences (MDs) with 95%CI of TOC/ucOC are produced regarding T2DM, or prediabetes and standard glucose controls. Estimates of effect size will be expressed as Relative Risk (RR) with 95% confidence intervals (95% CI) in cohort study and Odds Ratio (OR) with 95% CI in case-control and cross-sectional study. OR is expressed as one increase standard deviation (SD) of OC to the risk of developing T2DM. Pearson correlation coefficient will be analyzed by investigating the relationships between TOC or ucOC and fasting insulin levels (FINS). Studies that only have medians and ranges or interquartile ranges (IQRs) will be transformed to means and standard deviations (27, 28). Furthermore, log-transformed data will be converted to raw statistics before applying to analyses (29). We will assess publication bias of MD and risk estimates by visual inspection of the funnel plots (30). We will additionally examine heterogeneity employing the I^2 statistic by study ID which quantifies inconsistency across studies to assess the impact of heterogeneity on the meta-analysis (31). I2 represents the degree of heterogeneity. I^2 thresholds of 0%-40%, 30%-60%, 50%-90% and 75%-100% indicate possibilities of low, moderate, substantial and be considerable heterogeneity (31). All meta-analyses are conducted by Rstudio (Version 1.1.419-2009-2019 Rstudio, Inc.). Metafor package will be used to produce meta-regression analyses, meta-bias analyses and assessing heterogeneities (32). Each P value below 0.05 indicates statistically significant.

Risk of bias assessment

In this review, we will assess the risk of bias by subgroup analysis based on study type. Although Newcastle Ottawa Scale (NOS) is the frequently applied tools for quality appraisal of individual study in a meta-analysis of observational studies, we found that subgroup analysis based on study design may be more feasible in this case than using NOS tools. According to previously published reviews, it is acknowledged that there are significantly more cross-sectional studies than prospective studies had been identified. Also, with considering the characteristics of NOS tools that was constructed based on the study methodology, subgroup analysis with study type may provide a similar risk of bias result as

NOS produced (34). Thereby, we believed it makes sense to apply subgroup analysis with study type to save time and optimal assessment methods.

Subgroup analysis and investigation of heterogeneity

As there is no quality assessment of individual study in this review, the risk of bias will be measured by subgroup analysis. We will use subgroup analyses to identify the single characteristic of studies concerning heterogeneity. Meta-regression will be used for continuous factors and also combine different explaining heterogeneity. Random-effects models will be used, and p-values of < 0.01 will be considered statistically significant for subgroup analyses. Pre-planned subgroup analyses to explore statistical heterogeneity will include stratification by:

- Subgroups based on study design.
- Subgroups based on sex. Additionally, a subset based on menopausal will be conducted in females.
- Subgroups based on ethnicity or race.
- Subgroups based on diabetic status (normal, prediabetes, T2DM).
- Subgroups based on anti-diabetic medication status in T2DM.
- Subgroups based on obesity measurements (BMI/WC).
- Subgroups based on OC assay methods.
- Subgroups based on VK supplementation/anti-VK drugs or VD supplementation if data available.

Publication bias & Confidence in cumulative evidence

Publication bias assessment is based on by graphical test (funnel plots) and Egger & Begg tests (30,33). The asymmetry of funnel plot suggests a higher risk of publication bias and vice versa (30). Statistically, Egger and Begg's test will be conducted respectively in Rstudio.

We will provide the confidence in results by applying the Grading of Recommendations Assessment Development and Evaluation (GRADE) tool. We also will present an evidence profile summary with GRADEpro software (http://ims.cochrane.org/gradepro). Considering items are the risk of bias with subgroup analysis with study type, consistency of results, directness of evidence and precision of the results.

DISCUSSION

The current systematic review/meta-analysis is strengthened in several ways. 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. Secondly, investigating the sources of heterogeneity explicitly by more possible factors, such as age, sex, postmenopausal status in women, study design, ethnicity or regions, OC assays and medications on T2DM. This comprehensive analysis on heterogeneity may find out the factor(s) that responses for the difference among studies. Thirdly, producing a report not only in total osteocalcin (TOC) levels but also in undercarboxylated osteocalcin (ucOC) levels. By including investigations on ucOC, it might be clearer that which form of OC plays the endocrine role in humans. Additionally, investigating the relationship in a subgroup of patients with prediabetes would give more detail about how OC influence glucose levels in a progressive T2DM status. 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. As quality assessments are not conducted in our current study, it may bias our study results because included studies with poor quality cannot be assessed. Despite disadvantages, there still be a large number of studies that could be used to pool a quantitative analysis and provide evidence according to heterogeneity problems. Our review will contribute to public health and clinical researchers for further investigations regards of the gap in the current literature.

Conflicts of interests

All authors declare that there is no conflict of interests in this study protocol.

Data statement

Technical appendix, statistical code, and dataset available from the Figshare repository, DOI: [10.6084/m9.figshare.6199364].

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Author contributions

All authors contributed to the study concept and design. YHL led the writing of the manuscript and is the primary designer of the protocol. YHL, XYL collected the data for

screening. YHL, XYL, JL, KB, TB, and AP revised protocol critically. All authors read and approve revised version and final supported versions.

Ethics and dissemination

The present study will be published in a peer-reviewed journal when completed. If appropriate, we will present novelty findings at a relevant conference.

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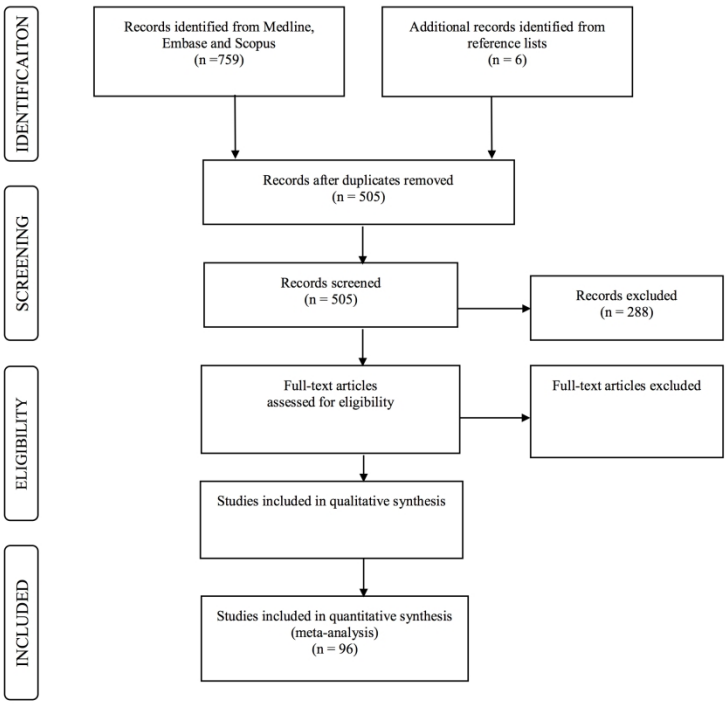

Figure 1. Process of proposed protocol (25).

The process of the proposed protocol.

209x297mm (300 x 300 DPI)

Appendix 1. PRISMA-checklist

		Reporting Item	Page Number
Identification	#1a	Identify the report as a protocol of a systematic review	Title page &Page 4
Update	#1b	If the protocol is for an update of a previous systematic review, identify as such	n/a
	#2	If registered, provide the name of the registry (such as PROSPERO) and registration number	
Contact	#3a	Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author	Title page
Contribution	#3b	Describe contributions of protocol authors and identify the guarantor of the review	Page 12
	#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
Sources	#5a	Indicate sources of financial or other support for the review	Page 12
Sponsor	#5b	Provide name for the review funder and / or sponsor	n/a
Role of sponsor or funder	#5c	Describe roles of funder(s), sponsor(s), and / or institution(s), if any, in developing the protocol	n/a
Rationale	#6	Describe the rationale for the review in the context of what is already known	Page 4
Objectives	#7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	Page 4
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	Page 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	Page 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	Page 7
Study records - data management	#11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	Page 8
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)	Page 8

Study records - 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	Page 8
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	Page 8
Outcomes and prioritization	#13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	Page 7
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	Page 10
Data synthesis	#15a	Describe criteria under which study data will be quantitatively synthesised	Page 8-9
	#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 I2, Kendall's τ)	Page 9
	#15c	Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression)	Page 10
	#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 studies)	Page 11
Confidence in cumulative evidence	#17	Describe how the strength of the body of evidence will be assessed (such as GRADE)	Page 11

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THE ASSOCIATIONS BETWEEN SERUM OC/UCOC AND PATIENTS WITH TYPE II DIABETES: A SYSTEMATIC REVIEW ／META-ANALYSIS STUDY PROTOCOL

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THE ASSOCIATIONS BETWEEN SERUM OC/UCOC AND PATIENTS WITH TYPE II DIABETES: A SYSTEMATIC REVIEW / META-ANALYSIS STUDY PROTOCOL

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ABSTRACT

Introduction

The global burden of type 2 diabetes (T2DM) is steadily increasing. Experimental studies have demonstrated a novel bone-cell secreted hormone, osteocalcin(OC), can stimulate beta-cell proliferation and improve insulin sensitivity in mice. Observational studies in humans have investigated the relationship between osteocalcin (OC) and metabolic parameters and T2DM. Importantly, few studies report on the uncarboxylated form of OC (ucOC), which is the putative active form of OC suggested to affect glucose metabolism.

Objectives

We will conduct a systematic review and meta-analysis to: 1) compare the serum OC and ucOC between T2DM and normal glucose tolerant controls (NGC); 2) to investigate the risk ratios between serum OC and ucOC and T2DM; 3) to determine the correlation coefficient between OC and ucOC and fasting insulin levels (FINS), homeostatic model assessment-insulin resistance (HOMA-IR), haemoglobin A1c (HbA1c) and fasting glucose levels (FPG); 4) to explore potential sources of between-study heterogeneity. A secondary objective is to compare the serum OC and ucOC between prediabetes (PD) and NGC, and between T2DM and PD.

Methods and analysis

This study will report items in line with the guidelines outlined in PRISMA and MOOSE (25,26). We will include observational studies (cohort, case-control and cross-sectional studies) and intervention studies with baseline data. Three databases (MEDLINE, EMBASE, and SCOPUS) will be searched from 1946 until July 2018 without language restrictions. Two reviewers will independently screen the titles and abstracts and conduct a full-text assessment to identify eligible studies. Discrepancies will be resolved by consensus with a third reviewer. The risk of bias assessment would be conducted by two reviewers independently based on the Newcastle-Ottawa Scale (NOS). Potential sources of between-study heterogeneity will be tested by meta-regression/subgroup analyses. Contour-enhanced funnel plots and Egger's test will be used to identify potential publication bias.

Registration number in PROSPERO

CRD42017073127

Keywords

osteocalcin, type 2 diabetes, prediabetes, meta-analysis

Strengths and limitations of this study

- This review will undertake a sensitive search strategy to include more eligible observational studies (cohort, case-control and cross-sectional studies) than previous meta-analyses.
- The review will assess and synthesise data on both forms of OC (TOC and ucOC), potentially being more relevant to the endocrine function in humans.
- The design of the review considers early to late stages of diabetes which will indicate whether the relationship between OC and impaired glucose metabolism is altered during progressively poorer glucose control.
- Sources of heterogeneity will be explored using meta-regression/subgroups analyses.
- The main limitation of the current study is only including observational studies (cohort, case-control and cross-sectional studies).

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. 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, large numbers of studies have reported an 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) (8). The cOC contributes to extracellular bone matrix while ucOC is likely the active form of OC in the circulation (9). Both cOC and ucOC are present in the circulation, and the amount of them is known as total osteocalcin (TOC) (9). TOC is considered a marker of bone turnover (10).

A potential endocrine function of OC was first suggested in 2007. Lee *et al*. and Ferron *et a*l. reported OC mediated glucose homeostasis via stimulating beta-cell proliferation and adiponectin secretion in mice (11,12). The endocrine actions of OC involve increasing insulin synthesis and secretion by beta-cells and improved insulin sensitivity by promoting adiponectin secretion in adipocytes (11,12). The high-fat diet experimental study revealed that bone could become insulin resistant by inhibiting the activation of OC (13). However, reported associations between OC and T2DM in humans have yielded conflicting results. (14–17). 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]) (18). 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 (19).While Bao

et al. observed increased serum levels of OC were associated with improved glucose control (20). 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) (21). In contrast, a case-control study by Zwakenberg *et al*. with 1,635 participants 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) (22).

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 (23–25). 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*.) (23–25). Both of the reviews by Kunutsor *et al* and Liu C *et al*. only found a small number (n=4) of cohort studies (23,24).. Additionally, studies reporting the associations between ucOC and glucose homeostasis in T2DM have not been adequately meta-analysed (24).

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 (26–28). 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 (23,24). Starup-Linde *et al.* conducted subgroup analysis according to sex, age and menopausal status in women, (29). Liu C *et al*. attempted to explain the heterogeneity by sex and OC assay methods (24). Kunutsor *et al.* conducted subgroup analyses according to study design and degree of confounders of risk estimates (23). Hygum *et al.* performed a meta-regression analysis to investigate the extent to which heterogeneity was explained by haemoglobin A1c(HbA1c) levels (25).

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.

OBJECTIVES

The present systematic review and meta-analysis aims to: 1) compare the serum OC and ucOC between T2DM and normal glucose tolerant controls (NGC); 2) investigate the risk ratios between serum OC and ucOC and T2DM; 3) determine the correlation coefficient between OC and ucOC and fasting insulin levels (FINS), homeostatic model assessment-insulin resistance (HOMA-IR), haemoglobin A1c (HbA1c) and fasting glucose levels (FPG); 4) explore potential sources of between-study heterogeneity. A secondary objective is to compare the serum OC and ucOC between prediabetes (PD) and NGC, and between T2DM and PD.

METHODS & ANALYSIS

We designed this systematic review and meta-analysis in adherence to the guidelines of Preferred Reporting Items for Systematic review and Meta-analysis (PRISMA) and Meta-analysis of observational studies in epidemiology (MOOSE) (30,31).The process of the proposed protocol is shown in Figure.1, and PRISMA-checklist shows in Appendix 1.

Protocol and registration

This protocol is registered and available on PROSPERO (CRD42017073127).

Patient and public involvement statement

There is no patient or public involved in this systematic review/meta-analysis.

Eligibility criteria for studies included in the review

Inclusion and exclusion criteria

Participants

Participants should be adult humans (older than 18 years old), with T2DM at baseline or developed T2DM afterward; not have any conditions that can affect bone metabolism or with medications that affect bone metabolism; could be with anti-diabetic treatments.

Exclude:

1. Children or adolescents (younger than 18 years old), pregnant or lactating women because of altered bone turnover markers levels.
2. Patients with a disease that either affects bone metabolism or glucose metabolism:
3. Patients with type 1 diabetes and/or gestational diabetes as they are pathophysiologically different from patients with T2DM.
4. Patients with Cushing's disease or Cushing's syndrome as they have a disordered metabolism.
5. Patients with hormonal disorders. For instance, growth-hormone deficiency or excess.
6. Patients with hyperparathyroidism or hypoparathyroidism or other diseases that affect thyroid function because of increased OC levels and changes in metabolism.
7. Patients with liver dysfunction (alanine transaminase > 3 times upper limit of normal).
8. Patients with impaired kidney function as described below:
   - A chronic renal disease when glomerular filtration rate of impaired renal function patients is below 30ml/min • 1.73 m^2 at stage four or five, or
   - A chronic renal illness when serum creatinine over 2.07 mg/dL, or renal osteodystrophy, or kidney transplant as 21% to 50% of kidney transplant recipients may develop secondary hyperparathyroidism after kidney transplantation or when treated with dialysis or hemodialysis.
9. Patients with Paget's disease as they have disordered bone metabolism.
10. Patients with osteomalacia as it is a severe bone disease and affects bone metabolism.
11. Patients with cancer or tumours. For example, bone cancer metastases could mediate bone turnover marker levels.
12. Patients with human immunodeficiency virus (HIV).
13. Patients with sepsis as they have disordered immune response caused by infections.
14. Patients with medications that affect bone metabolism:
    - Antiresorptive or anabolic therapy for osteoporosis and selective estrogen receptor modulators (such as bisphosphonates, alendronate, etidronate, raloxifene, denosumab and teriparatide).
    - Estrogen replacement therapy.
    - Glucocorticoids and thiazide diuretics.

15. Patients treated with surgery that directly affected hormone or thyroid function (i.e., thyroidectomy, oophorectomy and hysterectomy).

Note:

1) We include intervention studies that reported baseline data of OC and T2DM. Accordingly, we will eliminate observational studies with more than 20% of the cohort taking above non-eligible therapy.
2) We included T2DM with diabetic medications, but they will be assessed using subgroup analysis by the medication status. Anti-diabetic medications that affect OC/ucOC levels include insulin therapy, glucagon-like peptide-1 (GLP-1) receptor analogist and thiazolidinediones.

Study types

Observational studies are eligible for inclusion: cohort studies (both prospective and retrospective cohort studies), case-control study and cross-sectional study. Reporting eligible exposure(s) and outcome(s).

We will exclude reviews, commentaries, short survey, case reports, and letters.

Interventional studies (including randomised control trials) will be used if they provide eligible cross-sectional data at baseline before intervention.

Exposure(s)

OC levels are identified from enzyme-linked immunoassay (ELISA or EIA), Electro-chemiluminescence immunoassay (ECLIA), Immunoradiometric assay (IRMA), radioimmunoassay (RIA) and hydroxylapatite binding assay (HAP). The standard unit for OC is ng/ml; thus, other presented groups for OC (eg. nmol/l) will be converted to ng/ml.

Measures of OC

- Total serum osteocalcin levels (ng/ml).
- Undercarboxylated osteocalcin levels(ng/ml).
- OC categorized as low (reference) and high groups. Tertile, quartile, or quantile are the common categories used for classing different levels of TOC or ucOC.

Outcome(s)

Measures of T2DM

- Diabetes status categorized as type 2 diabetes disease or normal controls (reference)
- As some studies may categorize diabetes states as insulin-dependent diabetes mellitus (IDDM) and non-insulin dependent diabetes mellitus (NIDDM), NIDDM will be used and presented as T2DM.

Exclude Type 1 diabetes and gestational diabetes as they are pathophysiological different compared with T2DM.

Secondary outcome(s)

- IGT /IFG that is the pre-diabetic state and has a higher risk of developing T2DM
- HbA1c percentages categorized as type 2 diabetes, prediabetes and healthy controls (reference) by HbA1c rates over 6.5%, between 5.7% and 6.5% and below 5.7% respectively.
- Fasting plasma glucose levels categorized as diabetes, prediabetes and healthy controls (reference) by FPG levels over 126mg/dl, between 100 and 126 mg/dl, and below 100 mg/dl respectively.

Study design

Search strategies

A comprehensive literature search within MEDLINE, EMBASE and SCOPUS databases will be conducted to source all possible relevant studies for the present review. There is no language restriction, and non-English articles will be translated when possible and evaluated for eligibility. There is no time restriction. We may include conference proceedings and abstracts if necessary. We will further conduct reference list searches of each available paper. If duplicate publications of the same study are retrieved, the most relevant and up to date paper with more complete data will be included. The detailed search strategy shows in Table.1

Table 1. Detailed search strategy in databases: Medline, Embase and Scopus.

Medline (Ovid SP)	Embase (Ovid SP)	Scopus

1. exp osteocalcin	1. exp osteocalcin	(KEY ('osteocalcin')
2. osteocalcin.mp	2. osteocalcin.mp	OR KEY ('bone AND gla AND protein')
3. bone gla protein.mp	3.bone gla protein.mp	OR KEY ('bone AND turnover AND markers'))
4. vitamin k?dependent bone protein*.mp	4.vitamin k?dependent bone protein*.mp	AND (KEY ('diabetes AND mellitus')
5. 1 and 2 and 3 and 4	5. 1 and 2 and 3 and 4	OR KEY ('hemoglobin AND a1c')
6. exp diabetes mellitus, Type 2/II	6. exp non insulin dependent diabetes mellitus	OR KEY ('fasting AND plasma AND glucose'))
7.diabetes mellitus type 2/II.mp	7. exp diabetes mellitus 2/II	AND KEY ('human') AND (LIMIT-TO (DOCTYPE , "ar"))
8. (T2D* or NIDDM or “type 2” or “type II”)).tw	8. (T2D* or NIDDM or “type 2” or “type II”).tw	
9. (non insulin$ depend$ or nonsinulin$depend$ or non insulin?depend$ or noninsulin?depend$).tw	9. (prediabet* or pre diabet*).tw	
10.exp Hyperglycemia	10. hyperglyc?emi*.tw	
11. hyperglycemia.mp	11. 6 or 7 or 8 or 9 or 10	
12. hypergly?emi*.tw	12. 5 and 11	
13. exp Hemoglobin A/ or exp Hemoglobin A , Glycosylated	13. limit 13 to (human and exclude medline journals)	
14. HbA1c.mp		
15. (“HbA(1c)” or HbA1c or “HbA 1c” or ((glycosylated or glycated) adj h?emoglobin)).tw		
16. 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15		
17. 5 and 16		
18. limit 17 to humans		

Process for selecting studies

One author will set up the search strategy and store the search results to Endnote X7. The search strategy and recorded search results will then be checked by another investigator. Two or more independent investigators will go through the abstract screening (to remove duplicate records of the same report and to include eligible articles), and full-text assessment (to

acquire full-texts of available studies and to construct citation lists of eligible items). If a discrepancy arises, the disagreement will be shared with investigators by email or face-to-face meetings before reaching a final decision.

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. All extracted data will be saved in an excel spreadsheet.

Eligible extracted items: author and publication year, study design, study base, sample size, sex and postmenopausal status in females, age, ethnicity, country, osteocalcin assay methods, obesity measurements (body mass index or waist circumference), diabetic duration, anti-diabetic medications status, vitamin K supplementation/anti-vitamin K drugs, vitamin D supplementation, TOC/ucOC levels in groups, any risk estimate between TOC/ucOC and T2DM, any association between TOC/ucOC and HbA1c and/or FPG in T2DM, any association between TOC/ucOC and prediabetes and/or impaired glucose tolerance/impaired fasting glucose, any association between TOC/ucOC and standard glucose controls, any association between TOC/ucOC and HOMA-IR or HOMA-beta in T2DM.

Risk of bias assessment

The methodological quality will be assessed by Newcastle-Ottawa Scale (NOS). Cohort and case-control studies can be assessed by three main parts in the NOS: selection, comparability and outcome/exposure (32). The maximum score is nine points (32). The higher the score indicates a better methodological quality of the individual study (32). Cross-sectional studies can be assessed by modified NOS (33). The maximum score is ten points for the modified NOS, representing the highest quality (33). The quality assessment template can be found in supplementary materials.

Statistical analysis and data synthesis

Mean differences (MDs) with 95% CI will be calculated between T2DM and NGC, between PD and NGT, and between T2DM and PD. Estimates of effect size will be expressed as Relative Risk (RR) with 95% confidence intervals (95% CI) in cohort studies and Odds Ratio (OR) with 95% CI in case-control and cross-sectional studies. OR is expressed as one increased standard deviation (SD) of OC to the risk of developing T2DM. Papers reporting

other forms of OR will be translated to per increased SD of OC if there is logistic regression model. Pearson correlation coefficient will be analysed by investigating the relationships between TOC or ucOC and fasting insulin levels (FINS). Studies that only have medians and ranges or interquartile ranges (IQRs) will be transformed to means and standard deviations (34,35). Furthermore, log-transformed data will be converted to raw statistics before subjecting to analyses (36). We will assess publication bias of MD and risk estimates by visual inspection of the funnel plots if there are the minimum number of studies (37,38)). Egger's test will be used to assess the publication bias when there are a large number of studies (37).We will examine heterogeneity employing the I^2 statistic by study ID which quantifies inconsistency across studies to assess the impact of heterogeneity on the meta-analysis (39). I^2 represents the degree of heterogeneity. I^2 thresholds of 0%-40%, 30%-60%, 50%-90% and 75%-100% indicate possibilities of low, moderate, substantial and be considerable heterogeneity (39). All meta-analyses are conducted by Rstudio (Version 1.1.419-2009-2019 Rstudio, Inc.). Metafor package will be used to produce meta-regression analyses, meta-bias analyses and assessing heterogeneities (40). Each P value below 0.05 indicates statistically significant.

Meta-regression/subgroup analysis

Meta-regression analysis and subgroup analysis will be applied to assess the sources of heterogeneity. Meta-regression will be used for continuous factors such as age, sample size and proportion of postmenopausal in women. We will use subgroup analyses to identify potential sources of clinical, methodological or statistical heterogeneity for categorical variables. We will also generate mix-effect models to see the influence of multiple factors on the effect size. Random-effects models will be used, and p-values of < 0.01 will be considered statistically significant for subgroup analyses. Pre-planned subgroup analyses to explore statistical heterogeneity will include stratification by:

- Subgroups based on study design.
- Subgroups based on age.
- Subgroups based on sex. Additionally, a subset based on menopausal status will be conducted in females.
- Subgroups based on ethnicity or race.
- Subgroups based on diabetic status (normal, prediabetes, T2DM).
- Subgroups based on anti-diabetic medication status in T2DM.

- Subgroups based on obesity measurements (body mass index/waist circumference).
- Subgroups based on OC assay methods.
- Subgroups based on the fasting measures and spot measures.
- Subgroups based on vitamin K supplementation/anti-vitamin K drugs or vitamin D supplementation if data available.

Publication bias & Confidence in cumulative evidence

Publication bias assessment is based on graphical test (funnel plots) and Egger & Begg tests (37,38). The asymmetry of funnel plot suggests a higher risk of publication bias and vice versa (37). Statistically, Egger's and Begg's test will be conducted respectively in Rstudio.

We will provide assurance of the quality of our results by applying the Grading of Recommendations Assessment Development and Evaluation (GRADE) tool. We also will present an evidence profile summary with GRADEpro software (http://ims.cochrane.org/gradepro). The quality checklist includes the following items: risk of bias assessment, consistency of results, directness of evidence and precision of the results.

DISCUSSION

The current systematic review/meta-analysis is an update and improvement to the current literature in several ways. Firstly, we will provide more evidence compared to previous investigations in analysing the potential role/s OC plays in T2DM by increasing the number of eligible studies included in our up-to-date analysis. Secondly, we are investigating the sources of heterogeneity, explicitly by an increase in the number of factors such as age, sex, postmenopausal status in women, study design, ethnicity or regions, OC assays and medications on T2DM. This comprehensive analysis on heterogeneity may uncover the factor(s) responsible for the difference among already published studies. Thirdly, we are producing a report not only on total osteocalcin (TOC) levels but also on undercarboxylated osteocalcin (ucOC) levels. By including investigations on ucOC, it is possible we can determine the endocrine roles of both OC and ucOC in humans, if any. Additionally, investigating the relationship in a subgroup of patients with prediabetes will provide more details regarding the influence of OC (or ucOC) on glucose levels in a progressive T2DM status. The major limitation of this review is that we will only be including observational

studies as there is insufficient evidence from clinical trials, which will restrict study results in specific analyses. Despite this disadvantage, there are still a large number of studies that could be used to pool a quantitative analysis and provide evidence according to concerns with heterogeneity. Our review will contribute to public health and clinical research for further investigations regarding the gap in the current literature.

Conflicts of interests

All authors declare that there is no conflict of interests in this study protocol.

Data statement

Technical appendix, statistical code, and dataset available from the Figshare repository, DOI: [10.6084/m9.figshare.6199364].

F**unding**

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Author contributions

All authors contributed to the study concept and design. YHL led the writing of the manuscript and is the primary designer of the protocol under the guidance of AP. TBS, JL, KB and AP conceived the conceptual ideas presented in the manuscript. YHL, XYL collected the data for screening. YHL, XYL, JL, KB, TBS, and AP revised protocol critically. All authors read and approved revised version and final supported versions.

Ethics and dissemination

The present study will be published in a peer-reviewed journal when completed. If appropriate, we will present novelty findings at a relevant conference.

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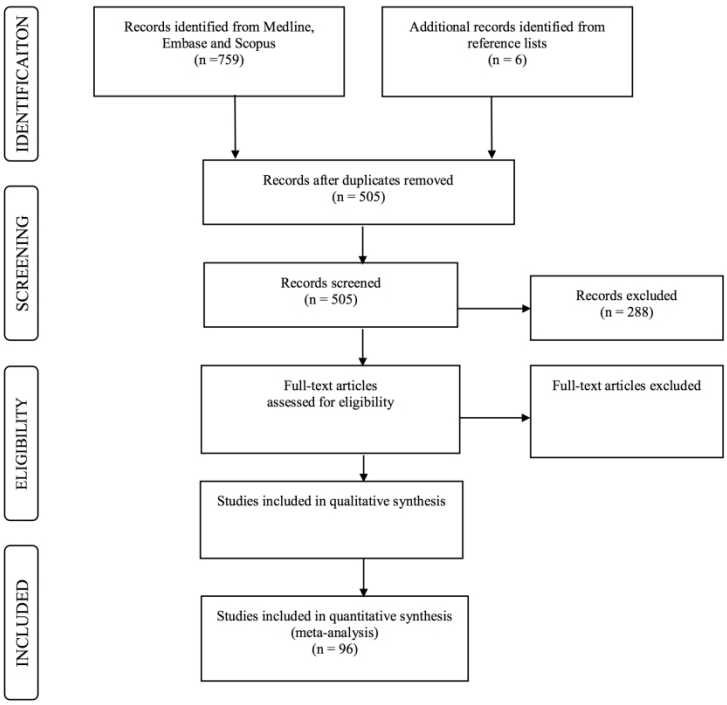

Figure 1. Process of proposed protocol (25).

The process of the proposed protocol.

209x297mm (300 x 300 DPI)

Appendix 1. Quality assessment template for cross-sectional study based on Newcastle-Ottawa-Scale (NOS)

Study number	Author	Year	Selection Bias Assessment (Maximum 5 stars)								Comparability (Maximum 2 stars)		Outcome (Maximum 3 stars)				Total score (Maximum 10 stars)
			Representativeness of the sample		Sample size		Non-respondents		Ascertainment of the exposure (risk factor)		Confounding factors are controlled		Assessment of the outcome		Statistical Test		
			selection	score	selection	score	selection	score	selection	score	selection	score	selection	score	selection	score	

Appendix 2. Quality assessment template for cohort study based on Newcastle-Ottawa-Scale (NOS)

Study number	Author	Year	Selection Bias Assessment (Maximum 4 stars)								Comparability (Maximum 2 stars)		Outcome (Maximum 3 stars)						Total score (Maximum 10 stars)
			Representativeness of the exposed cohort		Selection of the non-exposed cohort		Ascertainment of exposure		Demonstration that outcome of interest was not present at start of study		Comparability of cohorts on the basis of the design or analysis		Assessment of the outcome		Was follow-up long enough for outcomes to occur		Adequacy of follow up of cohorts		
			selection	score	selection	score	selection	score	selection	score	selection	score	selection	score	selection	score	selection	score	

Appendix 3. Quality assessment template for case-control study based on Newcastle-Ottawa-Scale (NOS)

Study number	Author	Year	Selection Bias Assessment (Maximum 4 stars)								Comparability (Maximum 2 stars)		Outcome (Maximum 3 stars)						Total score (Maximum 10 stars)
			Is the case definition adequate?		Representativeness of the cases		Selection of controls		Definition of controls		Comparability of cases and controls on the basis of the design or analysis		Assessment of the exposure		Same method of ascertainment for cases and controls		Non-response rate		
			selection	score	selection	score	selection	score	selection	score	selection	score	selection	score	selection	score	selection	score	

Appendix 1. PRISMA-checklist

		Reporting Item	Page Number
Identification	#1a	Identify the report as a protocol of a systematic review	Title page &Page 4
Update	#1b	If the protocol is for an update of a previous systematic review, identify as such	n/a
	#2	If registered, provide the name of the registry (such as PROSPERO) and registration number	
Contact	#3a	Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author	Title page
Contribution	#3b	Describe contributions of protocol authors and identify the guarantor of the review	Page 12
	#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
Sources	#5a	Indicate sources of financial or other support for the review	Page 12
Sponsor	#5b	Provide name for the review funder and / or sponsor	n/a
Role of sponsor or funder	#5c	Describe roles of funder(s), sponsor(s), and / or institution(s), if any, in developing the protocol	n/a
Rationale	#6	Describe the rationale for the review in the context of what is already known	Page 4
Objectives	#7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	Page 4
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	Page 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	Page 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	Page 7
Study records - data management	#11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	Page 8
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)	Page 8

Study records - 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	Page 8
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	Page 8
Outcomes and prioritization	#13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	Page 7
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	Page 10
Data synthesis	#15a	Describe criteria under which study data will be quantitatively synthesised	Page 8-9
	#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 I2, Kendall's τ)	Page 9
	#15c	Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression)	Page 10
	#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 studies)	Page 11
Confidence in cumulative evidence	#17	Describe how the strength of the body of evidence will be assessed (such as GRADE)	Page 11

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THE RELATIONSHIP BETWEEN SERUM OSTEOCALCIN/ UNDERCARBOXYLATED OSTEOCALCIN AND TYPE II DIABETES: A SYSTEMATIC REVIEW/ META-ANALYSIS STUDY PROTOCOL

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THE RELATIONSHIP BETWEEN SERUM OSTEOCALCIN/UNDERCARBOXYLATED OSTEOCALCIN AND TYPE II DIABETES: A SYSTEMATIC REVIEW/ META-ANALYSIS STUDY PROTOCOL

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ABSTRACT

Introduction

The global burden of type 2 diabetes (T2DM) is steadily increasing. Experimental studies have demonstrated that a novel hormone secreted by bone cells, osteocalcin (OC), can stimulate beta-cell proliferation and improve insulin sensitivity in mice. Observational studies in humans have investigated the relationship between OC and metabolic parameters and T2DM. Importantly, few studies have reported on the uncarboxylated form of OC (ucOC), which is the putative active form of OC suggested to affect glucose metabolism.

Objectives

We will conduct a systematic review and meta-analysis to: 1) compare the serum OC and ucOC between T2DM and normal glucose-tolerant controls; 2) investigate the risk ratios between serum OC and ucOC and T2DM; 3) determine the correlation coefficient between OC and ucOC and fasting insulin levels, homeostatic model assessment-insulin resistance, haemoglobin A1c, and fasting glucose levels; and 4) explore potential sources of between-study heterogeneity. The secondary objective is to compare the serum OC and ucOC between prediabetes and normal glucose-tolerant controls and between T2DM and prediabetes.

Methods and analysis

This study will report items in line with the guidelines outlined in PRISMA and MOOSE. We will include observational studies (cohort, case-control and cross-sectional studies) and intervention studies with baseline data. Three databases (MEDLINE, EMBASE, and SCOPUS) will be searched from inception until July 2018 without language restrictions. Two reviewers will independently screen the titles and abstracts and conduct a full-text assessment to identify eligible studies. Discrepancies will be resolved by consensus with a third reviewer. The risk of bias assessment will be conducted by two reviewers independently based on the Newcastle-Ottawa Scale. Potential sources of between-study heterogeneity will be tested using meta-regression/subgroup analyses. Contour-enhanced funnel plots and Egger's test will be used to identify potential publication bias.

Ethics and dissemination

Formal ethical approval is not required. We will disseminate the results to a peer-reviewed publication and conference presentation.

Registration number in PROSPERO

CRD42017073127

Keywords: osteocalcin, type 2 diabetes, prediabetes, meta-analysis

Strengths and limitations of this study

- This review will propose a sensitive search strategy to include more eligible observational studies (cohort, case-control and cross-sectional studies) than previous meta-analyses.
- The review will assess and synthesise data on both forms of OC (total OC and ucOC), potentially being more relevant to the endocrine function in humans.
- The design of the review considers the early to late stages of diabetes, which will indicate whether the relationship between OC and impaired glucose metabolism is altered during progressively poorer glucose control.
- Sources of heterogeneity will be explored using meta-regression/subgroup analyses.
- The main limitation of the current study is only including observational studies (cohort, case-control and cross-sectional studies).

INTRODUCTION

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 increased levels of glucose than people with normal glycaemic metabolism. Also, those patients have increased risks of other complications such as heart attacks, strokes, diabetic retinopathy, and renal disease.[2]

Correspondingly, several organs become the 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 treat diabetes: the skeleton and bone. Increasing numbers of osteokines secreted by skeleton and bone exhibit regulatory function 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 amount is 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 a*l. reported 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 in 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 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 1). 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-diabetics (PD) compared to normal glucose tolerance controls, while Aoki *et al*. indicated an increase in OC

concentration during the early stage of diabetes.[27–29] 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 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 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.

OBJECTIVES

The present systematic review and meta-analysis aims to: 1) compare the serum OC and ucOC between T2DM and normal glucose-tolerant controls (NGC); 2) investigate the risk ratios between serum OC and ucOC, and T2DM; 3) determine the correlation coefficient between OC and ucOC, and fasting insulin levels, homeostatic model assessment-insulin resistance (HOMA-IR), HbA1c, and fasting glucose levels (FPG); and 4) explore potential sources of between-study heterogeneity. The secondary objective is to compare the serum OC and ucOC between PD and NGC, and between T2DM and PD.

METHODS AND ANALYSIS

We designed this systematic review and meta-analysis in adherence to the guidelines of Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) and Meta-analysis of Observational Studies in Epidemiology (MOOSE).[31,32] The process of the proposed protocol is shown in Figure 1, and the PRISMA checklist shown in Appendix 2.

Protocol and registration

This protocol is registered and available on PROSPERO (CRD42017073127).

Patients and public involvement statement

There is no patient or public involved in this systematic review/meta-analysis.

Eligibility criteria for studies included in the review

Inclusion and exclusion criteria

Participants

Participants should be adult humans (older than 18 years old), with T2DM at the baseline or developed T2DM afterwards; not have any conditions that can affect bone metabolism or with medications that affect bone metabolism; and could be on anti-diabetic treatment.

Exclude:

1. Children or adolescents (younger than 18 years), and pregnant or lactating women due to altered bone turnover marker levels.
2. Patients with a disease that either affects bone metabolism or glucose metabolism.
3. Patients with type 1 diabetes and/or gestational diabetes as they are pathophysiologically different from patients with T2DM.
4. Patients with Cushing's disease or Cushing's syndrome as they have disordered metabolism.
5. Patients with hormonal disorders. For instance, growth-hormone deficiency or excess.
6. Patients with hyperparathyroidism or hypoparathyroidism or other diseases that affect thyroid function due to increased OC levels and changes in metabolism.
7. Patients with liver dysfunction (alanine transaminase level > 3 times upper limit of normal).
8. Patients with impaired kidney function as described below:

- Chronic renal disease patients with glomerular filtration rate below 30 ml/min·1.73 m^2 at stage four or five, or
- Chronic renal disease patients with serum creatinine level over 2.07 mg/dL, or renal osteodystrophy, or kidney transplant as 21–50% of kidney transplant recipients may develop secondary hyperparathyroidism after kidney transplantation or when treated with dialysis or hemodialysis.

9. Patients with Paget's disease as they have disordered bone metabolism.
10. Patients with osteomalacia as it is a severe bone disease and affects bone metabolism.
11. Patients with cancer or tumours. For example, bone cancer metastases could affect bone turnover marker levels.
12. Patients with human immunodeficiency virus infection.
13. Patients with sepsis as they have disordered immune response caused by infections.
14. Patients on medications that affect bone metabolism:
    - Antiresorptive or anabolic therapy for osteoporosis and selective oestrogen receptor modulators (such as bisphosphonates, alendronate, etidronate, raloxifene, denosumab and teriparatide).
    - Oestrogen replacement therapy.
    - Glucocorticoids and thiazide diuretics.
15. Patients treated with surgery that directly affects hormone or thyroid function (i.e., thyroidectomy, oophorectomy and hysterectomy).

Note:

1) We include intervention studies that reported baseline data of OC and T2DM. Accordingly, we will eliminate observational studies with more than 20% of the cohort taking above non-eligible therapy.
2) We included T2DM with diabetic medications, but they will be assessed using subgroup analysis by medication status. Anti-diabetic medications that affect OC/ucOC levels include insulin therapy, glucagon-like peptide-1 (GLP-1) receptor agonists, and thiazolidinediones.

Study types

Observational studies are eligible for inclusion: cohort studies (both prospective and retrospective cohort studies), case-control studies and cross-sectional studies, reporting eligible exposure(s) and outcome(s).

We will exclude reviews, commentaries, short surveys, case reports, and letters.

Interventional studies (including randomised controlled trials) will be used if they provide eligible cross-sectional data at the baseline before intervention.

Exposure(s)

OC levels are identified from enzyme-linked immunosorbent assay, electrochemiluminescence immunoassay, immunoradiometric assay, radioimmunoassay and hydroxylapatite binding assay. The standard unit for OC is ng/ml; thus, other presented groups for OC (e.g. nmol/l) will be converted to ng/ml.

Measures of OC

- Total serum OC levels (ng/ml).
- ucOC levels (ng/ml).
- OC categorised as low (reference) and high groups. Tertile, quartile, or quantile are the common categories used for classifying different levels of TOC or ucOC.

Outcome(s)

Measures of T2DM

- Diabetes status categorised as type 2 diabetes disease or normal controls (reference)
- As some studies may categorise diabetes states as insulin-dependent diabetes mellitus and non-insulin dependent diabetes mellitus (NIDDM), NIDDM will be used and presented as T2DM.

Exclude type 1 diabetes and gestational diabetes as they are pathophysiologically different compared with T2DM.

Secondary outcome(s)

- Impaired glucose tolerance/impaired fasting glucose: that is the pre-diabetic state with a higher risk of developing T2DM.

- HbA1c levels categorised as type 2 diabetes, prediabetes and healthy controls (reference) by HbA1c rates over 6.5%, between 5.7% and 6.5%, and below 5.7%, respectively.
- Fasting plasma glucose levels categorised as diabetes, prediabetes and healthy controls (reference) by FPG levels over 126 mg/dl, between 100 and 126 mg/dl, and below 100 mg/dl, respectively.

Study design

Search strategies

A comprehensive literature search within MEDLINE, EMBASE and SCOPUS databases will be conducted to source all possible relevant studies for the present review. There is no language restriction, and non-English articles will be translated when possible and evaluated for eligibility. There is no time restriction. We may include conference proceedings and abstracts if necessary. We will further conduct reference list searches of each available paper. If duplicate publications of the same study are retrieved, the most relevant and up to date paper with more complete data will be included. The detailed search strategy is shown in Table 1.

Table 1. Detailed search strategy in databases: MEDLINE, EMBASE and SCOPUS

MEDLINE (Ovid SP)	EMBASE (Ovid SP)	SCOPUS
1. exp osteocalcin	1. exp osteocalcin	(KEY ('osteocalcin')
2. osteocalcin.mp	2. osteocalcin.mp	OR KEY ('bone AND gla AND protein')
3. bone gla protein.mp	3. bone gla protein.mp	OR KEY ('bone AND turnover AND markers'))
4. vitamin k?dependent bone protein*.mp	4. vitamin k?dependent bone protein*.mp	AND (KEY ('diabetes AND mellitus')
5. 1 or 2 or 3 or 4	5. 1 and 2 and 3 and 4	OR KEY ('hemoglobin AND a1c')
6. exp diabetes mellitus, Type 2/II	6. exp non insulin dependent diabetes mellitus	OR KEY ('fasting AND plasma AND glucose'))
7. diabetes mellitus type 2/II.mp	7. exp diabetes mellitus 2/II	AND KEY ('human') AND (LIMIT-TO (DOCTYPE , "ar"))
8. (T2D* or NIDDM or “type 2” or “type II”).tw	8. (T2D* or NIDDM or “type 2” or “type II”).tw	
9. (non insulin$ depend$ or nonsinulin$depend$ or non insulin?depend$ or noninsulin?depend$).tw	9. (prediabet* or pre diabet*).tw	
10. exp Hyperglycemia	10. hyperglyc?emi*.tw	
11. hyperglycemia.mp	11. 6 or 7 or 8 or 9 or 10	
12. hypergly?emi*.tw	12. 5 and 11	
13. exp Hemoglobin A/ or exp Hemoglobin A, Glycosylated	13. limit 13 to (human and exclude medline journals)	
14. HbA1c.mp		
15. (“HbA(1c)” or HbA1c or “HbA 1c” or (glycosylated or glycated) adj h?emoglobin)).tw		
16. 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15		
17. 5 and 16		
18. limit 17 to humans		

Process for selecting studies

One author will set up the search strategy and store the search results in Endnote X7. The search strategy and recorded search results will then be checked by another investigator. Two or more independent investigators will perform the abstract screening (to remove duplicate records of the same report and to include eligible articles), and full-text assessment (to acquire full-texts of available studies and to construct citation lists of eligible items). If a discrepancy arises, the disagreement will be discussed with investigators by email or face-to-face meetings before reaching a final decision.

Data extraction

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. All extracted data will be saved in an Excel spreadsheet.

Eligible extracted items: author and publication year, study design, study base, sample size, sex and postmenopausal status in females, age, ethnicity, country, OC assay methods, obesity measurements (body mass index or waist circumference), duration of diabetes, anti-diabetic medications status, vitamin K supplementation/anti-vitamin K drugs, vitamin D supplementation, TOC/ucOC levels in groups, any risk estimate between TOC/ucOC and T2DM, any association between TOC/ucOC and HbA1c and/or FPG in T2DM, any association between TOC/ucOC and prediabetes and/or impaired glucose tolerance/impaired fasting glucose, any association between TOC/ucOC and standard glucose controls, and any association between TOC/ucOC and HOMA-IR or HOMA-beta in T2DM.

Risk of bias assessment

The methodological quality will be assessed using the Newcastle-Ottawa Scale (NOS). Cohort and case-control studies can be assessed by three main parts in the NOS: selection, comparability and outcome/exposure.[33] The maximum score is nine points.[33] A higher score indicates better methodological quality of the individual study.[33] Cross-sectional studies can be assessed using the modified NOS.[34] The maximum score is ten points for the modified NOS, representing the highest quality.[34] The quality assessment template can be found in the supplementary materials (Appendix 3).

Statistical analysis and data synthesis

Mean differences with 95% CI will be calculated between T2DM and NGC, between PD and NGT, and between T2DM and PD. Estimates of effect size will be expressed as relative risk (RR) with 95% CI for cohort studies and OR with 95% CI for case-control and cross-sectional studies. OR is expressed as one increased standard deviation (SD) of OC to the risk of developing T2DM. Papers reporting other forms of OR will be translated to per increased SD of OC if a logistic regression model is used. Pearson's correlation coefficient will be analysed by investigating the relationships between TOC or ucOC and fasting insulin levels. Studies that only have medians and ranges or interquartile ranges will be transformed to means and standard deviations.[35,36] Furthermore, log-transformed data will be converted to raw statistics before subjecting to analyses.[37] We will assess publication bias of mean differences and risk estimates by visual inspection of the funnel plots[38,39] Egger's test will be used to assess the publication bias when there is a large number of studies.[38] We will evaluate heterogeneity employing the I^2 statistic by study ID which quantifies inconsistency across studies to assess the impact of heterogeneity on the meta-analysis.[40] I^2 represents the degree of heterogeneity. I^2 thresholds of 0–40%, 30–60%, 50–90%, and 75–100% indicate possibilities of low, moderate, substantial, and considerable heterogeneity, respectively.[40] It is suggested to use Rstudio conducting meta-analysesRStudio (version 1.1.419-2009-2019; RStudio Inc.). The "metafor" package will be used to perform meta-regression analyses, meta-bias analyses and for assessing heterogeneities.[41] Each P value below 0.05 indicates statistical significance.

Meta-regression/subgroup analysis

Meta-regression analysis and subgroup analysis will be applied to assess the sources of heterogeneity. Meta-regression will be used for continuous factors such as age, sample size and proportion of postmenopausal women. We will use subgroup analyses to identify potential sources of clinical, methodological or statistical heterogeneity for categorical variables. We will also generate mix-effect models to evaluate the influence of multiple factors on the effect size. Random-effects models will be used, and p-values of < 0.01 will be considered statistically significant for subgroup analyses. Pre-planned subgroup analyses to explore statistical heterogeneity will include stratification by:

- Subgroups based on study design.
- Subgroups based on age.

- Subgroups based on sex. Additionally, a subset based on menopausal status will be assessed among females.
- Subgroups based on ethnicity or race.
- Subgroups based on diabetic status (normal, prediabetes, T2DM).
- Subgroups based on anti-diabetic medication status in T2DM.
- Subgroups based on obesity measurements (body mass index/waist circumference).
- Subgroups based on OC assay methods.
- Subgroups based on fasting measures and spot measures.
- Subgroups based on vitamin K supplementation/anti-vitamin K drugs or vitamin D supplementation if data are available.

Publication bias and confidence in cumulative evidence

Publication bias assessment is based on graphical test (funnel plots) and Egger's and Begg's tests.[38,39] The asymmetry of the funnel plot suggests a higher risk of publication bias and vice versa.[38] Statistically, Egger's and Begg's tests will be conducted using RStudio.

We will provide assurance of the quality of our results by applying the Grading of Recommendations Assessment Development and Evaluation (GRADE) tool. We will also present an evidence profile summary using GRADEpro software (http://ims.cochrane.org/gradepro). The quality checklist includes the following items: risk of bias assessment, consistency of results, directness of evidence, and precision of the results.

DISCUSSION

The current systematic review/meta-analysis constitutes an update and improvement to the current literature in several ways. Firstly, we will provide more evidence compared to previous investigations in analysing the potential role/s OC plays in T2DM by increasing the number of eligible studies included in our up-to-date analysis. Secondly, we will investigate the sources of heterogeneity, explicitly by an increase in the number of factors such as age, sex, postmenopausal status in women, study design, ethnicity or regions, OC assays, and medications on T2DM. This comprehensive analysis of heterogeneity may uncover the factor(s) responsible for the differences among already published studies. Thirdly, we will produce a report not only on TOC levels but also on ucOC levels. By including investigations on ucOC, we can determine the endocrine roles of both OC and ucOC in humans, if any.

Additionally, investigating the relationship in a subgroup of patients with prediabetes will provide more details regarding the influence of OC (or ucOC) on glucose levels in a progressive T2DM status. The major limitation of this review is that we will only be including observational studies as there is insufficient evidence from clinical trials, which will restrict study results in specific analyses. According to the search results for clinical studies, if there are any eligible interventional studies, we will include them but only use the baseline data in which case we will regard those studies as cross-sectional studies. Despite this disadvantage, there are still a large number of studies that could be used to pool a quantitative analysis and provide evidence according to concerns with heterogeneity. Our review will contribute to public health and clinical research for further investigations regarding the gap in the current literature.

Conflicts of interest

None declared.

Data statement

Technical appendix, statistical code, and dataset available from the Figshare repository, DOI: [10.6084/m9.figshare.6199364].

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Author contributions

All authors contributed to the study concept and design. YHL led the writing of the manuscript and is the primary designer of the protocol under the guidance of AP. TBS, JL, KB, and AP conceived the conceptual ideas presented in the manuscript. YHL, XYL collected the data for screening. YHL, XYL, JL, KB, TBS, and AP revised the protocol critically. All authors read and approved the revised version and final supported versions.

Ethics and dissemination

The present study will be published in a peer-reviewed journal when completed. If appropriate, we will present novelty findings at a relevant conference.

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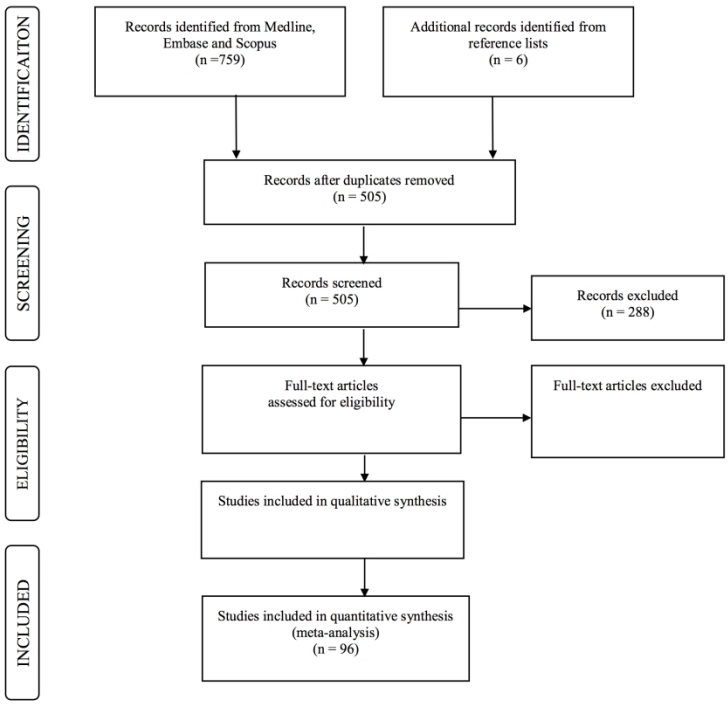

Figure 1. Process of proposed protocol (25).

The process of the proposed protocol.

209x297mm (300 x 300 DPI)

Appendix 1.

The scope search strategy to identify the trial study being published after 2015.

▼ **Search History** (19)

# ▲	Searches	Results	Type
1	exp osteocalcin/	9689	Advanced
2	osteocalcin.mp.	16198	Advanced
3	bone gla protein.mp.	631	Advanced
4	1 or 2 or 3	16354	Advanced
5	exp diabetes mellitus, Type 2/	119103	Advanced
6	diabetes mellitus type 2.mp.	119425	Advanced
7	(T2D* or NIDDM or "type 2" or "type II").tw.	229963	Advanced
8	(noninsulin$ depend$ or non insulin?depend$ or noninsulin?depend$).tw.	1409	Advanced
9	exp hyperglycemia/	33658	Advanced
10	hyperglycemia.mp.	47853	Advanced
11	hypergly?emi*.tw.	39454	Advanced
12	exp Hemoglobin A/ or exp Hemoglobin A, Glycosylated/	35398	Advanced
13	HbA1c.mp.	22933	Advanced
14	("HbA(1c)" or HbA1c or "HbA 1c" or ((glycosylated or glycated) adj h?emoglobin)).tw.	35894	Advanced
15	5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14	332918	Advanced
16	4 and 15	662	Advanced
17	limit 16 to humans	453	Advanced
18	limit 17 to yr="2015 -Current"	125	Advanced
19	limit 18 to (clinical study or randomized controlled trial)	6	Advanced

Appendix 2. PRISMA-checklist

		Reporting Item	Page Number
Identification	#1a	Identify the report as a protocol of a systematic review	Title page &Page 4
Update	#1b	If the protocol is for an update of a previous systematic review, identify as such	n/a
	#2	If registered, provide the name of the registry (such as PROSPERO) and registration number	
Contact	#3a	Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author	Title page
Contribution	#3b	Describe contributions of protocol authors and identify the guarantor of the review	Page 12
	#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
Sources	#5a	Indicate sources of financial or other support for the review	Page 12
Sponsor	#5b	Provide name for the review funder and / or sponsor	n/a
Role of sponsor or funder	#5c	Describe roles of funder(s), sponsor(s), and / or institution(s), if any, in developing the protocol	n/a
Rationale	#6	Describe the rationale for the review in the context of what is already known	Page 4
Objectives	#7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	Page 4
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	Page 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	Page 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	Page 7
Study records - data management	#11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	Page 8
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)	Page 8

Study records - 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	Page 8
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	Page 8
Outcomes and prioritization	#13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	Page 7
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	Page 10
Data synthesis	#15a	Describe criteria under which study data will be quantitatively synthesised	Page 8-9
	#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 I2, Kendall's τ)	Page 9
	#15c	Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression)	Page 10
	#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 studies)	Page 11
Confidence in cumulative evidence	#17	Describe how the strength of the body of evidence will be assessed (such as GRADE)	Page 11

Appendix 3.

Quality assessment template for cross-sectional study based on Newcastle-Ottawa-Scale (NOS)

Study number	Author	Year	Selection Bias Assessment (Maximum 5 stars)								Comparability (Maximum 2 stars)		Outcome (Maximum 3 stars)				Total score (Maximum 10 stars)
			Representativeness of the sample		Sample size		Non-respondents		Ascertainment of the exposure (risk factor)		Confounding factors are controlled		Assessment of the outcome		Statistical Test		
			selection	score	selection	score	selection	score	selection	score	selection	score	selection	score	selection	score	

Quality assessment template for cohort study based on Newcastle-Ottawa-Scale (NOS)

Study number	Author	Year	Selection Bias Assessment (Maximum 4 stars)								Comparability (Maximum 2 stars)		Outcome (Maximum 3 stars)						Total score (Maximum 10 stars)
			Representativeness of the exposed cohort		Selection of the non-exposed cohort		Ascertainment of exposure		Demonstration that outcome of interest was not present at start of study		Comparability of cohorts on the basis of the design or analysis		Assessment of the outcome		Was follow-up long enough for outcomes to occur		Adequacy of follow up of cohorts		
			selection	score	selection	score	selection	score	selection	score	selection	score	selection	score	selection	score	selection	score	

Quality assessment template for case-control study based on Newcastle-Ottawa-Scale (NOS)

Study number	Author	Year	Selection Bias Assessment (Maximum 4 stars)								Comparability (Maximum 2 stars)		Outcome (Maximum 3 stars)						Total score (Maximum 10 stars)
			Is the case definition adequate?		Representativeness of the cases		Selection of controls		Definition of controls		Comparability of cases and controls on the basis of the design or analysis		Assessment of the exposure		Same method of ascertainment for cases and controls		Non-response rate		
			selection	score	selection	score	selection	score	selection	score	selection	score	selection	score	selection	score	selection	score	