

BMJ Open A systematic review protocol examining the effect of vitamin D supplementation on endothelial function

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ABSTRACT

Introduction: Vitamin D has potential benefits for extraskeletal health. These could include an anti-inflammatory effect as well as a reduction in endothelial dysfunction. We aim to provide quality evidence for the hypothesis that supplementation with vitamin D will improve endothelial function (EF), possibly through the abrogation of systemic inflammation.

Methods and analysis: We will conduct a systematic review of all randomised controlled trials on vitamin D supplementation and EF lasting 12 weeks or more. The search will cover the period 2000–2015 and include studies that describe direct measures of EF, markers of endothelial cell (EC) activation and if concurrently reported, indicators of systemic inflammation. Study selection will follow the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines and study quality will be assessed by the Jadad score in addition to an evaluation of allocation concealment and data analysis. If sufficient data are available, a meta-analysis will be conducted. The effect sizes will be generated using Hedges' g score, for both fixed and random effect models. I² statistics and Galbraith plots will be used to assess heterogeneity and identify their potential sources. Potential publication and small sample size bias will be assessed by visual inspections of funnel plots and also Egger's test. Meta-regression analysis (if feasible) will be conducted with restricted maximum likelihood (REML) estimation method, controlling for potential confounders (demographics, study methods, location, etc). A backward elimination process will be applied in the regression modelling procedure. Subgroup analysis, conditional on number of studies retrieved and their sample size, will be stratified on participant disease category, total dose administered, degree of 25(OH)D change and type of supplement used.

Ethics and dissemination: Formal ethical approval is not required as primary data will not be collected. The results will be disseminated through a peer-reviewed publication, conference presentation and the popular press.

Trial registration number: International Prospective Register for Systematic Reviews (PROSPERO) number CRD42014013523.

INTRODUCTION

The vascular endothelium plays a pivotal role in the detection and response to

Strengths and limitations of this study

- Systematic review and meta-analysis of randomised controlled trials.
- Will offer highest level of evidence for informed decisions.
- Potential clarification that the effect of vitamin D status on endothelial dysfunction is through inflammation.
- Availability of quality studies with direct measures of endothelial function based on the same technique.
- The many inflammatory and endothelial activation biomarkers in the literature hamper collation of outcomes.
- Key words: endothelial function, vitamin D, inflammation, systematic review, randomised controlled trials.

blood-borne signals and changes in haemodynamic forces. Endothelial dysfunction is strongly linked to cardiovascular disease (CVD),¹ and can predict the occurrence of type 2 diabetes (T2DM).² A chronic low-grade inflammation is common to many metabolic disorders,³ and also underscores endothelial dysfunction.^{4 5} Vitamin D inadequacy is now a global issue and normalisation of status has a potential protective role in conditions such as obesity, CVD and T2DM.^{6–11} Vitamin D status is related to inflammation.^{12 13} The conversion of 25(OH)D to its active form 1,25(OH)2D occurs in immune system cells such as dendritic cells, macrophages, T cells and B cells.¹⁴ The outcome of 1,25(OH)2D action in these cells is a decreased production of interferon γ (INF- γ), interleukin-12 (IL-12), interleukin-6 (IL-6), and interleukin-23 (IL-23) with an enhanced production of IL-4.¹⁴ Vitamin D may also benefit endothelial function (EF).^{15 16} The endothelium can convert 25(OH)D to its active form through a specific endothelial 1 α -hydroxylase.¹⁷ Interestingly, greater enzyme activity is stimulated by inflammatory cytokines.¹⁸ Protective effects of vitamin D may then be realised through

increased nitric oxide (NO) production, decreased oxidative stress, reduced IL-6, vascular cell adhesion molecules (VCAM) and intracellular adhesion molecule (ICAM) among other effects.¹⁶ Thus the overall impact of adequate vitamin D in this context would be to decrease both systemic inflammation and endothelial dysfunction.

One of the most important actions of endothelium is the production of NO, which plays a major role in regulating the vessel diameter and, hence resistance, throughout the arterial bed.¹⁹ However, two other endothelial cell (EC) products, endothelium-derived hyperpolarising factor and prostacyclin (PGI₂), also produce vasodilation of the underlying smooth muscle cells, and overall these actions are countered by the vasoconstrictor EC factor endothelin-1 in the regulation of vascular tone.¹⁵ Endothelial dysfunction is characterised by reduced NO-dependent vascular activity which leads to dysregulation of arterial tone.²⁰ However, there is an endothelium independent pathway as well that is determined by the activity of the smooth muscle layer. Sublingual glyceryl trinitrate (GTN) can be used to uncover the influence of this pathway,²¹ as GTN decreases smooth muscle tone leading to vasodilation.²¹ A comprehensive assessment of EF usually encompasses the testing of both pathways.

EF is assessed by testing the vascular reactivity of either coronary or peripheral arteries. Initially the invasive technique of artery catheterisation was used; this assesses endothelial-dependent vasodilation. Subsequently, non-invasive techniques like flow-mediated dilation (FMD) were developed. This is the current gold standard for measuring EF in peripheral arteries. FMD uses ultrasound imaging to detect the endothelial response to shear stress, acetylcholine infusion, and salbutamol inhalation for the assessment of the endothelial-dependent pathway, or sublingual GTN for the endothelial-independent pathway. More recently, pulse contour analysis (PCA), based on a photoplethysmographic recording of the digital volume pulse, has been used to assess EF.²² The derived variables, stiffness index and reflective index (RI) reflect large artery stiffness and small artery vascular tone, respectively.²³ Studies that employ both salbutamol and GTN in conjunction with PCA have been used to report endothelial dysfunction. On the other hand, arterial applanation tonometry uses a sensitive probe applied in turn to the carotid and femoral arteries to detect characteristics of the transmitted waveform. A derived variable is the augmentation index (AIx), the ratio of the pulse pressure at the second systolic peak to the pulse pressure of the first systolic peak.²⁴ Other studies have also employed markers of EC activation, like higher plasma levels of soluble VCAM, ICAM, P-selectin and E-selectin as indicators of endothelial dysfunction.¹⁵ Systemic inflammation is usually measured by the levels of circulating inflammatory biomarkers such as high sensitivity C reactive protein (CRP), white cell count (WCC), INF- γ , IL-12, IL-6, IL-23 and IL-4.^{14 25} CRP is an interesting marker

since its effect on EC activation⁵ could underscore its high prediction of CVD.^{5 25} Moreover, strong relationships between WCC, ICAM and fibrinogen and the prediction of CVD has also been documented.^{1 26}

Collectively, there is sufficient evidence to hypothesise that adequate vitamin D status may directly attenuate endothelial dysfunction (as supported from functional measures and/or markers of EC activation), or act indirectly through the abrogation of systemic inflammation. Two limited narrative reviews on randomised controlled trials (RCTs) did not, however, uncover consistent support for an effect of vitamin D on improvements in EF or decreases in markers of EC activation.^{15 16} Clearly there is a need to expand the scope of such findings to arrive at an evidence-based conclusion. To our knowledge there is no published systematic review that addresses our question. Previous systematic reviews in related fields have examined the links between vitamin D and CRP,²⁷ and vitamin D and blood pressure,²⁸ while a narrative review reported on vitamin D, blood pressure, endothelial and renal function of postmenopausal women.²⁹ The present systematic review protocol will evaluate potential causal interrelationships between vitamin D status and EF, and determine whether systemic inflammation is a moderator of the effect. We address our objectives through a comprehensive protocol targeting all RCTs in this area, from 2000 to 2015, in order to confirm or negate this extraskeletal role for vitamin D.

METHODS AND DESIGN

Population

The systematic review will include high quality RCTs on adults aged >20 years who have been supplemented with cholecalciferol or calcitriol, and have had measures of EF, EC activation and/or systemic inflammation before and after the interventions. The study population will be restricted to healthy subjects, and overweight or obese who suffer from glucose intolerance, CVD, metabolic syndrome (MetS) or T2DM.

Study design

This systematic review will consider only randomised controlled trials of good quality.

Search strategy

The search strategy aims to find published articles only, and will include a three-stage protocol (figure 1). An initial limited search of Medline and Scopus will be undertaken; this will be followed by analysis of the text words contained in the titles and abstracts, and of the index terms used to describe each article. A second search, using all identified keywords and index terms, will then be undertaken across all included databases. In the third step, the reference lists of key articles will be searched for additional studies. Studies will be restricted to the English language and to those published from 2000 to 2015, inclusive. The databases that will be

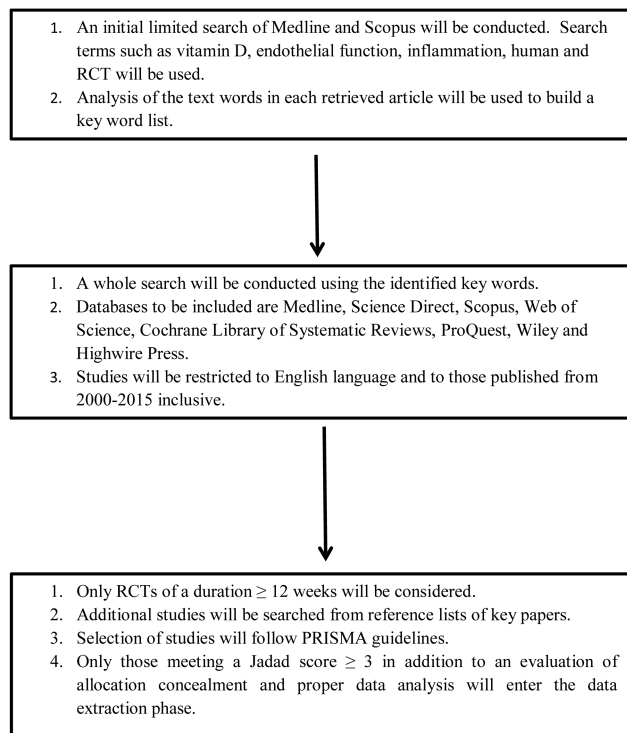


Figure 1 A schematic of the processes of the systemic review. (Randomised controlled trials (RCTs); Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)).

searched are Medline, Science Direct, Scopus, Web of Science, Cochrane Library of Systematic Reviews, ProQuest, Wiley and Highwire Press.

Study selection

Quantitative studies will be independently assessed by three reviewers and reported using the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) flow diagram.³⁰ Valid studies will then be assessed for their quality before any retrieval of information. Any disagreements that arise between the reviewers will be resolved through discussion.

Quality assessment

The three reviewers will independently check each selected article to minimise bias. All selected articles will be judged for their quality based on the Jadad score³¹ in addition to an evaluation of allocation concealment and data analysis.³²

Data extraction

Quantitative data will be extracted from papers receiving a Jadad score of 3 and over with adequate allocation concealment and proper data analysis.^{31 32} The data extracted will include all details specific to the review question and fulfils the requirements for both the narrative synthesis of outcomes and the potential meta-analysis. We will also contact corresponding authors for key information when

data are ambiguous or missing from the published study. Data extraction will be independently cross-checked.

Outcomes

The outcomes of the review will be grouped under the following headings

- ▶ EF: this will include direct measures as measured by flow mediated dilation (FMD), PCA, AIx or endothelial vasodilation/vasoconstriction following drug intervention.
- ▶ EC activation: these include circulating markers such as P-selectin, E-selectin, L-selectin, VCAM-1, ICAM-1 or von Willebrand factor.
- ▶ Systemic inflammatory molecules: these will include markers, such as nuclear factor κ -light-chain-enhancer of activated B cells (NF- κ B), pro-inflammatory cytokine IL-6, IL-12, and high-sensitivity CRP, as well as anti-inflammatory cytokines such as IL-6, IL-10, and adiponectin.

ANALYSIS

Descriptive analysis

A narrative synthesis of the outcomes of the selected studies will be presented in the final review. This will include the following:

1. Type of intervention and the control group and sample size;
2. 25(OH)D3 baseline and final measurement and other biomarkers of interest;
3. Targeted population and its characteristics; age, sex, ethnicity, disease prevalence in group, location and the distance from equator, if possible;
4. Intervention outcomes: this will include the change in 25(OH)D, measurements of EF such as FMD, AIx and PCA derived end points RI and stiffness index, systemic inflammatory biomarkers, EC activation biomarkers, time between last dose and EF measurement.

Statistical analysis

We are interested in the relationship between vitamin D supplementation and endothelial dysfunction. Endothelial dysfunction is measured in different ways among studies; therefore, we anticipate a limited ability to run a meta-analysis for this review. However, in studies which used the same end point measurements we will report pre-intervention, postintervention and overall mean change pertaining to the endothelial dysfunction outcomes of interest. The overall mean change will be calculated by subtracting the mean change in the placebo group from that in the treatment group in the studies if these had a parallel design. Standard deviation (SD) will be calculated from standard errors (SEs), or confidence interval (95% CI), or t or F value from raw data, where available, for both the placebo group and the treatment group for each study included.

Meta-analysis (where possible) will be carried out to assess the effect of vitamin D supplementation on

measures of EF and systemic/vascular inflammation. Effectiveness of vitamin D supplementation on endothelial dysfunction will be reported as standardised mean difference (SMD) for each individual study and its 95% CI. A positive SMD will denote a higher (more favourable) value in the vitamin D3 group. The effect sizes will be generated using Hedges' g score and presented using a forest plot for each study to assess the magnitude of the intervention effect on a particular outcome. The overall effect sizes will be estimated using both fixed-effects models and random-effects models. I^2 statistics and Galbraith plot will be used to assess for heterogeneity and identify the potential sources of heterogeneity. Subgroup analyses, conditional on number of studies retrieved and their sample size, will be stratified on participant disease category (eg, CVD/MetS/T2DM) or total dose administered (daily dose \times duration) (low-medium-high) or degree of 25(OH)D change (low-medium-high) or supplement used (calcitriol vs vitamin D3 alone vs calcium + vitamin D3).

Potential publication and small sample size bias will be assessed by visual inspections of funnel plots and also Egger's test. To explore the effect of main factors of interest on predicting SMD, meta-regression analysis will be conducted with restricted maximum likelihood (REML) estimation method, controlling for potential confounders (demographics, study methods, and location). Backward elimination process will be applied in the regression modelling procedure. All of the statistical analysis will be performed by using STATA V.12.0 (StataCorp, College Station, Texas, USA).³³ A p value < 0.05 will be considered statistically significant for all analyses.

CONCLUSION

This systematic review will provide evidence in support or against the hypothesis that vitamin D has a role in EF. This conclusion will stem from direct measurements of EF and/or EC activation, and indirectly through changes in biomarkers of systemic inflammation. Where sufficient data are available, we will conduct a meta-analysis to confirm the relationship between the improvement in vitamin D status and the reduction in endothelial dysfunction. Moreover, whether this occurs through a reduction in systemic inflammation will also be clarified. Overall, the review will complement the evidence base on the extraskeletal benefits of vitamin D.

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Contributors AA, MJS, JLS and JH conceived the idea, planned and designed the study protocol. AA designed the figure and wrote the first draft; YZ planned the data extraction and statistical analysis; FC provided critical insights. All authors have approved and contributed to the final written manuscript.

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