Special Article

Vitamin D supplementation for improvement of chronic low-grade inflammation in patients with type 2 diabetes: a systematic review and meta-analysis of randomized controlled trials

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Background: *Vitamin D has been proposed to have anti-inflammatory properties; however, the effect of vitamin D supplementation on inflammation in type 2 diabetes has not been established.* ***Objective:*** *The aim of this systematic review and meta-analysis was to examine the effect of vitamin D supplementation on inflammatory markers in patients with type 2 diabetes and to identify relevant gaps in knowledge.* ***Data sources:*** *MEDLINE, CINAHL, Embase, and EBM Reviews were searched systematically from inception to January 25, 2017.* ***Study selection:*** *Randomized controlled trials (RCTs) investigating the effects of vitamin D supplementation (any form, route, and duration, and with any cosupplementation) compared with placebo or usual care on inflammatory markers in patients with type 2 diabetes were selected.* ***Data extraction:*** *Study and sample characteristics and aggregate outcome data were extracted, risk of bias was determined, and quality of evidence was assessed using the Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) approach.* ***Results:*** *Twenty-eight RCTs were included, 20 of which had data available for pooling. In meta-analyses of 20 RCTs ($n = 1270$ participants), vitamin D–supplemented groups had lower levels of C-reactive protein (standardized mean difference [SMD] -0.23; 95%CI, -0.37 to -0.09; $P = 0.002$) and tumor necrosis factor α (SMD -0.49; 95%CI, -0.84 to -0.15; $P = 0.005$), a lower erythrocyte sedimentation rate (SMD -0.47; 95%CI, -0.89 to -0.05; $P = 0.03$), and higher levels of leptin (SMD 0.42; 95%CI, 0.04–0.81; $P = 0.03$) compared with control groups. No differences were observed for adiponectin, interleukin 6, or E-selectin (all $P > 0.05$). In meta-regression and subgroup analyses, age, sex, body mass index, duration of diabetes, baseline vitamin D status, and dose and duration of supplementation did not alter the results.* ***Conclusions:*** *This meta-analysis provides level 1 evidence that vitamin D supplementation may reduce chronic low-grade inflammation in patients with type 2 diabetes.* ***Systematic Review Registration:*** *PROSPERO CRD42016047755. Available at: https://www.crd.york.ac.uk/prospero/display_record.php?RecordID=47755 (9/15/2016).*

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INTRODUCTION

Type 2 diabetes is a major cause of morbidity and mortality, and its prevalence has nearly doubled since 1980, largely reflecting the rise in associated risk factors, primarily obesity and physical inactivity.[1] Chronic low-grade inflammation is common in most chronic diseases, including obesity, type 2 diabetes, and cardiovascular disease.[2] While reducing obesity is effective in delaying the onset and progression of type 2 diabetes, weight loss strategies on a population scale are difficult to achieve and maintain over the long term.[2] Therefore, the identification of additional, easily modifiable risk factors is urgently needed to slow the increasing incidence of type 2 diabetes.

Increasing evidence suggests that vitamin D may be involved in the pathophysiology of type 2 diabetes via its effects on glucose metabolism, insulin signaling, and inflammation.[2] The anti-inflammatory properties of vitamin D are thought to be a primary mechanism by which vitamin D affects glycemic control and risk of type 2 diabetes.[3] This is supported by studies in human cells and animal models showing that 1,25-dihydroxyvitamin D (1,25[OH]D) improves insulin sensitivity by inhibiting cytokine-induced apoptosis of beta cells.[4,5] Cross-sectional studies in type 2 diabetes reported inverse associations between circulating 25-hydroxyvitamin D (25[OH]D) concentrations and levels of inflammatory markers, including C-reactive protein (CRP),[6] tumor necrosis factor α (TNF-α),[7] and interleukin (IL) 6,[8] yet results are conflicting.[7,9] Intervention studies have also reported inconsistent results. Some showed that vitamin D supplementation improved inflammatory marker profiles in patients with type 2 diabetes,[10,11] while others did not.[12,13]

Despite evidence suggesting that the link between vitamin D and type 2 diabetes may be mediated by inflammation, the effect of vitamin D supplementation on inflammation in type 2 diabetes has not been summarized quantitatively. To date, meta-analyses of vitamin D supplementation in patients with type 2 diabetes have not examined inflammation, but instead have focused on glycemic endpoints, with conflicting results.[14–16] Meta-analyses of vitamin D supplementation in other populations, such as patients with chronic heart failure,[17] obese or overweight adults,[18] and mixed participant samples (healthy, overweight/obese, or with various chronic diseases)[19,20] also showed varying results: some showed improved inflammatory profiles following vitamin D supplementation,[17,19] while others did not.[18,20]

There is a lack of consensus regarding the effects of vitamin D supplementation on inflammation, particularly in type 2 diabetes. This systematic review and meta-analysis of all existing randomized controlled trials (RCTs) examining the effect of vitamin D supplementation on inflammatory markers in patients with type 2 diabetes aims to address this knowledge gap.

METHODS

This systematic review conforms to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) standards (see Appendix S1 in the Supporting Information online)[21] and is part of a wider evidence synthesis of the effects of vitamin D on inflammation in multiple diseases. The methods for this work were specified a priori in a published protocol.[22] A protocol for this meta-analysis is registered on PROSPERO (no. CRD42016047755).

Data sources and literature searches

Studies were identified by systematically searching electronic databases using the relevant search terms and prespecified criteria outlined in the protocol.[22] Literature was searched up to January 25, 2017, and was limited to studies in humans, with no limits on language or publication date. The search was conducted using the following electronic databases: MEDLINE via Ovid; Medline In-Process and Other Nonindexed Citations via Ovid; CINAHL (Cumulative Index to Nursing and Allied Health Literature); Embase via Ovid; and Evidence-Based Medicine Reviews via Ovid.[22]

Reference lists of relevant studies or systematic reviews were searched manually for additional studies. Conference abstracts and protocols were excluded but were used to search for relevant full-text articles. Where required data were not reported, corresponding authors were contacted and asked to provide de-identified aggregate outcome measures for the purpose of meta-analysis. To identify gray literature, a manual online search was performed along with searches of the US National Library of Medicine (ClinicalTrials.gov) and the Australian New Zealand Clinical Trials (anzctr.org.au) registries.

Study selection

Selection criteria using the PICOS (Population, Intervention, Comparison, Outcomes, Study design) framework established a priori were used to determine eligibility of articles (Table 1).[22] Briefly, eligibility criteria were as follows: (1) RCTs or systematic reviews of RCTs; (2) male or female participants of any age, ethnicity, socioeconomic status, or pregnancy status, with controlled or uncontrolled type 2 diabetes for any

Table 1 **PICOS criteria for inclusion and exclusion of studies**

	Inclusion criteria	Exclusion criteria
Participants	Males and female patients with type 2 diabetes mellitus (controlled or uncontrolled) on any treatment regimen for any duration; of any age, ethnicity, socioeconomic status, geographic area, or pregnancy status; with any comorbidity	Individuals with prediabetes, type 1 diabetes, or gestational diabetes, or any studies in participants without type 2 diabetes
Intervention	Any type of vitamin D supplementation (D_2, D_3, calcitriol, analogues) administered in any form (oral, intravenous, or intramuscular) alone or combined with other intervention(s), of any dosage, and for any duration	Studies without vitamin D supplementation
Comparison	Placebo or usual care; any other nonpharmacological interventions or pharmacological interventions	Studies with no control/comparator group
Outcomes	Inflammatory biomarkers, including but not limited to the following: all interleukins, TNF-α, TGF-β1, CRP, MCP-1, IFN-γ, NF-κB, MIF, fibrinogen, leptin, resistin, visfatin, adiponectin, omentin	Studies with no inflammatory marker outcomes measured
Study type	RCTs in humans; systematic reviews of RCTs	
Language	No limit	
Year of publication	No limit	

Abbreviations: CRP, C-reactive protein; IFN-γ, interferon gamma; MCP-1, monocyte chemoattractant protein 1; MIF, macrophage migration inhibitory factor; NF-κB, nuclear factor κB; RCTs, randomized controlled trials; TGF-β1, transforming growth factor β1, TNF-α, tumor necrosis factor α.

duration, with or without comorbidities, on any treatment regimen; (3) vitamin D supplementation in any form (including active metabolites and analogues), dose, or duration, administered orally, intravenously, or intramuscularly or added to food, alone or combined with calcium or other pharmacological or nonpharmacological interventions; (4) vitamin D compared with placebo, usual care, or any pharmacological or nonpharmacological interventions; (5) measurement of inflammatory markers (including but not limited to CRP, TNF-α, IL-6, and adipokines such as leptin and adiponectin) as outcomes (Table 1).

The literature search process is shown in Figure 1. Titles and abstracts were screened, and those meeting or suspected to meet eligibility criteria were retrieved for full-text review. To avoid missing articles in which inflammatory markers were not the primary endpoints, the full texts of all studies of vitamin D supplementation in type 2 diabetes were scanned for inflammatory markers. Full-text articles were assessed for eligibility by 2 independent reviewers (A.M. and N.N.), and disagreements were resolved by consensus or by consulting a third reviewer (B.dC.).

Data extraction and quality assessment

Data were extracted by 2 independent reviewers (A.M. and N.N.), both of whom cross-checked all extracted data and computed data entries for meta-analysis. Information extracted included the following: author of study; year of study publication; study design and setting; country of study; primary endpoint; inclusion/exclusion criteria; number of participants (male/female); age, ethnicity, smoking status, baseline body mass index (BMI), baseline 25(OH)D level, diabetes duration, and comorbidities of participants; type of intervention; dose, administration route, frequency, and duration of vitamin D supplementation and control; inflammatory markers assessed and methods of assessment; and mean or median follow-up value with SDs, SEs, 95%CIs, or interquartile ranges for all inflammatory markers.

Risk of bias was assessed at the study level by 2 independent reviewers (A.M. and N.N.) using a critical appraisal template (see Appendix S2 in the Supporting Information online) with prespecified criteria.[22] Aspects of study quality were investigated using a descriptive component approach as described previously[22]; these included randomization and allocation methods; blinding of participants, investigators, and outcome assessors; conflicts of interest of authors; presence of prespecified selection criteria; outcome assessment and reporting; and statistical issues, including powering and data analysis. Using this approach, each study was assigned a risk-of-bias rating (see Appendix S2 in the Supporting Information online). Disagreement was resolved by consensus.

Quality of the evidence was assessed at the outcome level using the Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) approach.[23] The quality of each outcome was graded by 2 independent reviewers (A.M. and N.N.) as high, moderate, low, or very low, on the basis of risk of bias, inconsistency (heterogeneity), indirectness (heterogeneous

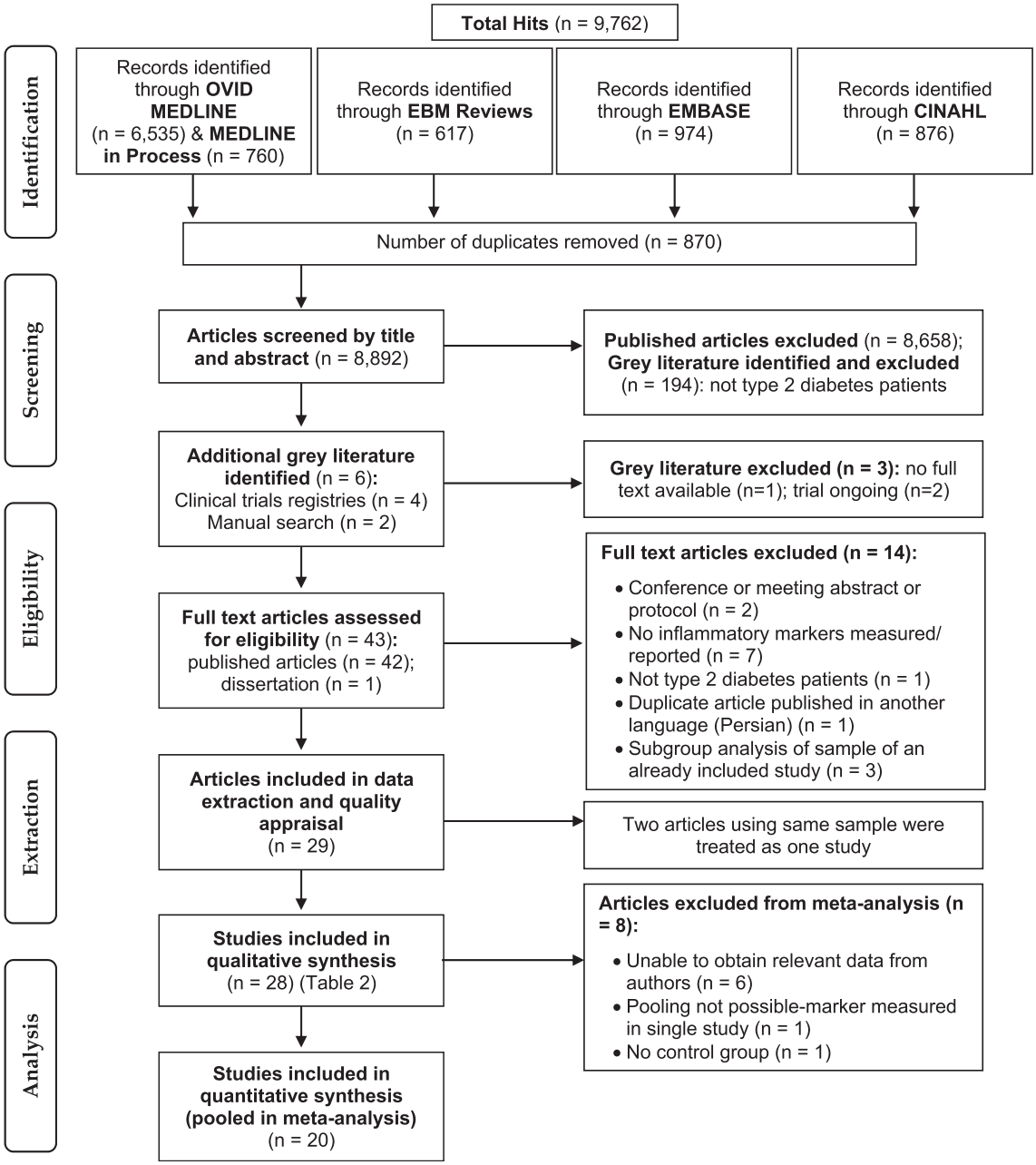


Figure 1 **Flow diagram of the literature search process.**

participants, outcomes, or interventions), and imprecision (upper or lower limit of 95%CI > 0.5). Interpretation of the grading scores is presented in Table S1 in the Supporting Information online.[23]

Data synthesis and analysis

Aggregated effect measures (means and SDs) at the end of the supplementation period were extracted and pooled for meta-analysis where appropriate. Where SE was reported, it was converted to SD using the following formula: $\mathrm{SD} = \mathrm{SE} \times (\sqrt{n})$. For studies reporting more than 1 time point for follow-up, data from the longest period were used in the primary analysis. Other time points were used in subgroup analysis by duration, where applicable. Because studies used different methods and assays and reported substantially different concentrations of inflammatory markers, data were analyzed in line with Cochrane guidelines[24] using DerSimonian and Laird random-effects models along with Cohen's *d* to calculate the standardized mean difference (SMD) ± the pooled SD for differences between the intervention and placebo groups at follow-up. For clinical reference, the weighted mean differences

(WMDs) have also been reported; however, all meta-analyses were performed using SMDs. Results are presented in forest plots.

Statistical heterogeneity was assessed using the I^2 test, where I^2 values greater than 50% indicated moderate to high heterogeneity. Sensitivity analyses were performed in which studies with high risk of bias were excluded to determine the effect of those studies on the results. Descriptive analyses were conducted for studies deemed clinically heterogeneous or for studies that presented insufficient data for pooling.

Where the number of studies was sufficient, subgroup and meta-regression analyses were performed to adjust for predetermined factors presumed to cause variations in outcomes, including age, sex, BMI and vitamin D status at baseline, duration of diabetes, and dose and duration of the intervention. Publication bias was assessed by visual inspection of funnel plots and by the Egger et al.[25] and Begg and Mazumdar[26] statistical tests. Meta-analyses and subgroup analyses were performed using Review Manager version 5.3, and meta-regression and publication bias were analyzed using the Comprehensive Meta-Analysis software version 3. Statistical significance for all analyses was set at $P < 0.05$.

RESULTS

The literature search and screening process is shown in Figure 1. The database search and the screening of bibliographies and gray literature yielded 9762 articles, of which 870 were duplicates (Figure 1). After titles and abstracts of the remaining 8892 articles were screened, 40 articles remained eligible for full-text assessment. Three additional eligible articles were identified by manual search and by search of the clinical trials registries for gray literature. In total, 43 articles were screened in full text, of which 29 articles with 28 unique samples (n = 1780) met the inclusion criteria for qualitative synthesis (2 articles by Shab-Bidar et al.[11,27] used the same sample and were treated as 1 study).

Study characteristics

Descriptive data of the studies included in the qualitative synthesis are summarized in Table 2[10–13,27—51] and detailed in Table S2 in the Supporting Information online. Of the 28 included studies, 12 were conducted in Iran and all were published in English and were of parallel design. Sample sizes ranged from 15 to 118 participants. The mean age of participants ranged from 39 to 69 years, and the mean BMI ranged from 24.9 to 37.8 kg/m^2. The mean duration of diabetes, reported in 17 studies, ranged from 3.7 to 12.7 years. The baseline 25(OH)D level was reported in 26 studies, and ranged from 21.8 to 102.1 nmol/L. Most studies (n = 20) had vitamin D–deficient participants (25[OH]D < 50 nmol/L) at baseline. For studies that included both male and female participants (n = 26), the mean proportion of females was 48.6% (range, 24.1%–86.7%). Eleven studies included participants with diabetic complications such as heart, kidney, or fatty liver disease (Table 2).

All studies reported using cholecalciferol supplementation, apart from 1 that did not specify[46] and 4 that used active forms of vitamin D (calcitriol, n = 2[28,49]; paricalcitol, n = 1[48]; alfacalcidol, n = 1[40]). Cholecalciferol doses ranged from 200 IU to 6000 IU daily (n = 16), or from 50 000 to 60 000 IU weekly or biweekly (n = 4), to a single bolus dose of 300 000 IU (n = 3) (Table 2). Oral supplementation was used in most studies, although intramuscular injections were used in 2 studies[37,46] and vitamin D–fortified yogurt in 3 studies[10,11,27,36]; 1 study did not specify the form of supplementation.[41] Intervention duration ranged from 8 weeks to 12 months, being 12 weeks (n = 11) or 24 weeks (n = 9) in most studies. Cosupplementation with calcium was used in 4 studies,[10,11,27,38,44] and 1 study used both calcium and vitamin K.[29] Most studies used a placebo control group (n = 24), although 2 compared vitamin D with usual care,[38,40] 3 compared vitamin D with calcium supplementation,[10,11,27,44] and 1 compared 2 different doses of vitamin D supplementation (ie, no control group)[41] (Table 2).

Eight studies reported inflammatory markers as primary outcomes.[10,28,31,35,42,46,47,51] C-reactive protein was the most commonly reported inflammatory marker, measured in 20 of the 28 studies. Other commonly measured markers included TNF-α (n = 8), IL-6 (n = 7), total adiponectin (n = 7), leptin (n = 5), IL-10 (n = 2), E-selectin (n = 2), erythrocyte sedimentation rate (ESR) (n = 2), and osteoprotegerin (n = 2) (Table 2).

Risk-of-bias assessment

Results of the risk-of-bias assessment are shown in Table S3 in the Supporting Information online. Of the 28 studies, 1 did not perform[40] and 2 did not report[38,41] blinding of both the participants and the investigators, and 2 reported blinding of the participants only.[11,27,49] Four studies did not report dropout rates,[28,38,41,49] and 1 did not report baseline characteristics of participants.[12] Selective reporting was evident in 7 studies (Table S3 in the Supporting Information online). Overall, most studies were rated as having low (n = 13) or moderate (n = 5) risk of bias, while 7 had high risk of bias and 3 had unclear risk of bias due to insufficient

Table 2 **Characteristics of studies included in the systematic review of the effects of vitamin D supplementation on inflammation in patients with type 2 diabetes**

Reference(s), country	Design[a]; setting	No. and characteristics of participants	Mean or median age, BMI, and 25(OH)D level of participants at baseline[b]	Intervention and control arms	Frequency/duration of supplementation	Markers of inflammation (primary)[c]	Pooled
Akbarzadeh et al. (2013),[28] Iran	RCT; university outpatient clinic	70, with no comorbidities	53.1 y; 27.7 kg/m^2; 102.1 nmol/L	I: 0.5 μg oral calcitriol P: placebo	Daily for 12 wk	CRP, IL-6, IL-18 (primary)[c]	Yes
Al-Sofiani et al. (2015),[12] Saudi Arabia	RCT; university hospital primary care clinic	20, with VitD deficiency	54.9 y; 27.7 kg/m^2 (median); 31.9 nmol/L (median)	I: 5000 IU oral VitD_3 P: placebo	Daily for 12 wk	TNF-α, IL-6, OPG, leptin, adiponectin	No[d]
Asemi et al. (2016),[29] Iran	RCT; university cardiology clinic	66, overweight with CHD	65.5 y; 29.7 kg/m^2; 34.9 nmol/L	I: 200 IU oral VitD_3 + 90 μg vitamin K + 500 mg Ca P: placebo (cellulose)	Daily for 12 wk	CRP	Yes
Barchetta et al. (2016),[30] Italy	RCT; university diabetes outpatient clinic	55, with fatty liver/suspected NAFLD	58.6 y; 30.1 kg/m^2; 44.1 nmol/L	I: 2000 IU oral VitD_3 P: placebo	Daily for 24 wk	CRP, adiponectin	Yes
Baziar et al. (2014),[31] Iran	RCT; university research center diabetes clinic	81, with VitD deficiency/insufficiency	51.5 y; 27.3 kg/m^2; 37.3 nmol/L	I: 50 000 IU oral VitD_3 P: placebo (paraffin)	Weekly for 8 wk	Adiponectin (primary)[c]	Yes
Breslavsky et al. (2013),[32] Israel	RCT; hypertension outpatient clinic	32, with hypertension/dyslipidemia	66.3 y; 28.8 kg/m^2; 29.4 nmol/L	I: 1000 IU oral VitD_3 P: placebo (microcrystalline cellulose)	Daily for 12 mo	CRP, leptin, adiponectin	Yes
Dalan et al. (2016),[33] Singapore	RCT; tertiary hospital diabetes clinic	64, with VitD insufficiency	53.5 y; 28.1 kg/m^2; 43.8 nmol/L (median)	I: 4000 IU or 2000 IU oral VitD_3, based on 25(OH)D level P: placebo	Daily for 16 wk	CRP, E-selectin	Yes
Farrokhian et al. (2017),[34] Iran	RCT; university cardiology clinic	60, overweight; with CAD and VitD deficiency	61.8 y; 30.2 kg/m^2; 41.8 nmol/L	I: 50 000 IU oral VitD_3 P: placebo	Biweekly for 6 mo	CRP	Yes
Flores et al. (2010),[51] Mexico	RCT; research unit of social security institute	99, overweight/obese postmenopausal women	56.8 y; 30.7 kg/m^2; 54.6 nmol/L	I: 4000 IU oral VitD_3 P: placebo	Daily for 6 mo	CRP (primary)[c]	No[d]
Ghavamzadeh et al. (2014),[35] Iran	RCT; university diabetes clinic	51, with no comorbidities	50.8 y; 28.4 kg/m^2; 21.8 nmol/L	I: 400 IU oral VitD_3 P: placebo (purified coconut/palm oil)	Daily for 14 wk	TNF-α, leptin (primary)[c]	Yes
Jafari et al. (2016),[36] Iran	RCT; research center registry	59, postmenopausal women	57.3 y; 28.7 kg/m^2; 62.5 nmol/L	I: 2000 IU VitD_3 in fortified yogurt P: placebo (plain yogurt)	Daily for 12 weeks	CRP, omentin	Yes
Jehle et al. (2014),[37] Switzerland	RCT; hospital ambulatory care facilities	55, with no comorbidities	65.3 y; 28.5 kg/m^2; 32.1 nmol/L	I: 300 000 IU ± 150 000 IU intramuscular VitD_3 P: placebo (NaCl)	Single bolus, with or without an additional half bolus, follow-up at 6 mo	CRP, FGF-23	Yes
Kampmann et al. (2014),[13] Denmark	RCT; primary and secondary care diabetes clinics	15, with VitD deficiency	59.3 y; 33.9 kg/m^2; 32.9 nmol/L	I: 11 200 IU, then 5600 IU oral VitD_3 P: placebo	Daily for 12 wk	CRP, TNF-α, IL-6, IL-10	No[d]
Kota et al. (2011),[38] India	RT; tertiary hospital department	30, with pulmonary TB and VitD deficiency	39.3 y; BMI NR; 29.8 nmol/L	I: 60 000 IU oral VitD_3/wk + 1000 mg Ca/d P: usual care	Weekly for 12 wk	ESR	Yes
Maggi et al. (2014),[39] Italy	RCT; outpatient diabetic foot unit	30, with diabetic foot complications	69.0 y; 29.0 kg/m^2; 30.8 nmol/L	I: 300 000 IU oral VitD_3 P: placebo	Single bolus, follow-up at 24 wk	TNF-α, OPG, leptin, adiponectin	Yes
Munisamy et al. (2016),[40] Malaysia	Open RT; university outpatient clinic	60, with diabetic nephropathy	56.9 y; 28.6 kg/m^2; 55.9 nmol/L	I: 0.25 μg oral alfacalcidol P: usual care	Daily for 6 mo	CRP	Yes

(continued)

Table 2 **Continued**

Reference(s), country	Design[a]; setting	No. and characteristics of participants	Mean or median age, BMI, and 25(OH)D level of participants at baseline[b]	Intervention and control arms	Frequency/duration of supplementation	Markers of inflammation (primary)[c]	Pooled
Neyestani et al. (2012),[10] Iran	RCT; local diabetes society registry	90, with no comorbidities	51.2 y; 29.6 kg/m^2; 43.0 nmol/L	I: 1000 IU $VitD_3$ + 300 mg Ca in fortified yogurt Co: 1000 IU $VitD_3$ + 500 mg Ca in fortified yogurt P: 300 mg Ca in plain yogurt	Daily for 12 wk	CRP, TNF-α, IL-1β, IL-6, IFN-γ, RBP-4, LCN-2, OPN, adiponectin (primary)[c]	Yes
Patel et al. (2010),[41] USA	RT; setting NR	24, with VitD deficiency	57.5 y; 32.4 kg/m^2; 41.4 nmol/L	I_1: 400 IU $VitD_3$ (route NR) I_2: 1200 IU $VitD_3$ (route NR)	Daily for 4 mo	Adiponectin	No[e]
Rad et al. (2015),[42] Iran	RCT; setting NR	58, with no comorbidities	50.1 y; 28.3 kg/m^2; 37.7 nmol/L	I: 4000 IU oral $VitD_3$ P: placebo	Daily for 2 mo	TBP-2 (primary)[c]	No[f]
Razzaghi et al. (2016),[43] Iran	RCT; diabetic foot unit	60, with diabetic foot ulcers	59.1 y; 26.1 kg/m^2; 44.2 nmol/L	I: 50 000 IU oral $VitD_3$ P: placebo	Biweekly for 12 wk	CRP, ESR	Yes
Ryu et al. (2014),[44] Korea	RCT; university hospital	62, with VitD deficiency	55.6 y; 24.9 kg/m^2; 28.7 nmol/L	I: 2000 IU oral $VitD_3$ + 200 mg Ca P: 200 mg Ca	Daily for 24 wk	CRP	Yes
Sadiya et al. (2015),[45] UAE	RCT; tertiary diabetes facility	82, obese with VitD deficiency	48.5 y; 37.8 kg/m^2; 29.5 nmol/L	I: 6000 IU, then 3000 IU oral $VitD_3$ P: placebo (starch)	Daily for 6 mo	CRP	Yes
Shab-Bidar et al. (2011, 2012),[11,27] Iran	RCT; local diabetes society registries	100, overweight or obese	52.5 y; 29.3 kg/m^2; 38.3 nmol/L	I: 1000 IU $VitD_3$ + 340 mg Ca in fortified yogurt P: 340 mg Ca	Daily for 12 wk	CRP, TNF-α, IL-6, IL-10, E-selectin, endothelin-1, IL-2	Yes
Shaseb et al. (2016),[46] Iran	RCT; university diabetes clinic	95, with ischemic heart disease	55.0 y; 27.2 kg/m^2; 76.5 nmol/L	I: 300 000 IU intramuscular VitD (form of VitD NR) P: placebo	Single bolus, follow-up at 8 wk	CRP (primary)[c]	Yes
Tabesh et al. (2014),[47] Iran	RCT; research center	118, with VitD insufficiency	50.6 y; BMI NR; 36.9 nmol/L	I_1: 50 000 IU oral $VitD_3$/wk I_2: 1000 mg Ca/d Co: 50 000 IU $VitD_3$/wk + 1000 mg Ca/d P: placebo	Weekly for 8 wk	CRP, TNF-α, IL-6, leptin, adiponectin (primary)[c]	No[d]
Thethi et al. (2015),[48] USA	Multicenter RCT; setting NR	46, with CKD	64.0 y (median); BMI NR; 25(OH)D NR	I: 1.0 μg oral paricalcitol P: placebo	Daily for 12 wk	CRP, TNF-α, IL-6, MCP-1	No[d]
Tiryaki et al. (2016),[49] Turkey	RCT; setting NR	98, with CKD	51.0 y; 27.8 kg/m^2; 25(OH)D NR	I: 0.25 μg oral calcitriol P: placebo	Daily for 6 mo	CRP	Yes
Yiu et al. (2013),[50] China	RCT; outpatient clinic	100, with VitD insufficiency	65.4 y; 25.5 kg/m^2; 53.7 nmol/L	I: 5000 IU oral VitD3 P: placebo (microcrystalline cellulose)	Daily for 12 wk	CRP	No[d]

Abbreviations: BMI, body mass index; Ca, calcium; CAD, coronary artery disease; CHD, coronary heart disease; CKD, chronic kidney disease; Co, cosupplemented group; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; FGF-23, fibroblast growth factor 23; I, intervention group; IFN-γ, interferon gamma; IL, interleukin; IU, international units; LCN-2, lipocalin-2; MCP-1; monocyte chemoattractant protein 1; NaCl, sodium chloride; NAFLD, nonalcoholic fatty liver disease; NR, not reported; OPG, osteoprotegerin; OPN, osteopontin; P, placebo or control group; RBP-4, retinol-binding protein 4; TB, tuberculosis; TBP-2, thioredoxin binding protein 2; TNF-α, tumor necrosis factor α; RCT, randomized controlled trial; RT, randomized trial; UAE, United Arab Emirates; VitD, vitamin D; 25(OH)D, 25-hydroxyvitamin D.

[a]All studies had a parallel design.

[b]Data reported as means unless otherwise specified.

[c]Denotes that inflammation markers were reported as the primary outcome in the trial.

[d]All or some relevant outcome data could not be obtained from authors of the studies cited.

[e]No control group.

[f]Pooling not possible; marker measured in a single study.

information (see Table S3 in the Supporting Information online).

Meta-analysis and sensitivity analysis

Of the 28 studies, 1 was excluded from meta-analysis because it did not have a control group,[41] and 6 were excluded because necessary outcome data were not available.[12,13,47,48,50,51] Finally, 1 study could not be pooled because it reported on a single inflammatory marker not measured in any other study (thioredoxin-binding protein 2)[42] (Figure 1). Overall, 20 studies with a total of 1270 participants were included in the meta-analysis. Fifteen studies were pooled for CRP (n = 978), 5 for adiponectin (n = 252), 3 for leptin (n = 107) and TNF-α (n = 135), and 2 for E-selectin ($n = 164$), IL-6 ($n = 130$), and ESR ($n = 90$). Forest plots of the results are shown in Figures 2, 3, and 4 and Figures S1A, S1B, and S1C in the Supporting Information online.

Using a random-effects model, pooling of 15 studies showed a significant difference in CRP levels between the vitamin D and control groups (SMD −0.23, 95%CI, −0.38 to −0.09, $P = 0.002$; $P_{het} = 0.2$, $I^2 = 23\%$; WMD −0.65 mg/L, 95%CI, −1.20 to −0.11) (Figure 2) [10,28–30,32–34,36,37,40,43–46,49]. Similar results were observed when studies that did not use cholecalciferol supplementation were excluded[28,40,46,49] (SMD −0.32, 95%CI, −0.50 to −0.13, $P = 0.0008$; $P_{het} = 0.2$, $I^2 = 29\%$; WMD −1.23 mg/L, 95%CI, −2.16 to −0.30). Further exclusion of studies that cosupplemented vitamin D with calcium[10,29,44] did not significantly alter the results (SMD −0.30, 95%CI, −0.50 to −0.09, $P = 0.006$; $P_{het} = 0.3$, $I^2 = 22\%$; WMD −1.35 mg/L, 95%CI, −2.77 to 0.08). The effect of vitamin D supplementation on CRP levels remained significant in a sensitivity analysis that only included studies deemed as having low risk of bias (n = 10) (SMD −0.33, 95%CI, −0.52 to −0.14, $P = 0.0006$; $P_{het} = 0.2$, $I^2 = 32\%$; WMD −1.44 mg/L, 95%CI, −2.52 to −0.36).

Levels of TNF-α were lower after vitamin D supplementation vs after placebo, as shown by a pooled SMD of −0.49 (95%CI, −0.84 to −0.15), P=0.005; $P_{het} = 0.4$, $I^2 = 0\%$ (WMD −3.38 ng/L, 95%CI, −6.79 to 0.03) (Figure 3)[10,35,39]. All studies reporting data for TNF-α used cholecalciferol supplementation, and exclusion of 1 study that used cosupplementation with calcium[10] did not alter the results (SMD −0.69, 95%CI, −1.16 to −0.22, $P = 0.004$; $P_{het} = 0.6$, $I^2 = 0\%$; WMD −3.35 ng/L, 95%CI, −6.79 to 0.08). However, the effect on TNF-α was attenuated in sensitivity analysis when 1 study judged as having a high risk of bias[35] was excluded (SMD −0.33, 95%CI, −0.76 to 0.10, $P = 0.1$; $P_{het} = 0.6$, $I^2 = 0\%$. WMD −1.85 ng/L, 95%CI, −4.57 to 0.87).

Leptin levels were higher after vitamin D supplementation than after placebo, with a pooled SMD of 0.42 (95%CI, 0.04 to 0.81), $P = 0.03$; $P_{het} = 0.5$, $I^2 = 0\%$ (WMD 7.20 µg/L, 95%CI, 0.92 to 13.47) (Figure 4A). [32,35,39] All studies reporting data for leptin had supplemented cholecalciferol alone. Results were no longer significant in sensitivity analysis after 1 study with high risk of bias was excluded[35] (SMD 0.49, 95%CI, −0.33 to 1.31, $P = 0.2$; $P_{het} = 0.1$, $I^2 = 56\%$; WMD 5.75 µg/L, 95%CI, −2.88 to 14.38).

For adiponectin levels, the pooled SMD was 0.18 (95%CI, −0.07 to 0.43; $P = 0.2$; $P_{het} = 0.5$, $I^2 = 0\%$), and the WMD was 0.89 µg/L (95%CI, −2.17 to 3.95)

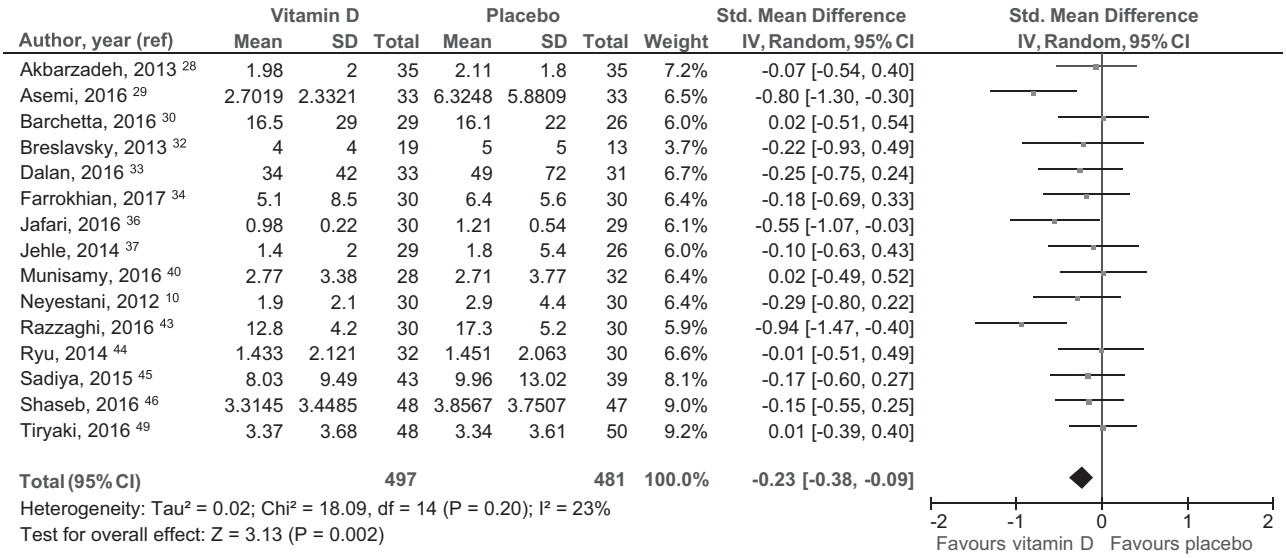


Figure 2 **Forest plot showing results of a meta-analysis of the effects of vitamin D supplementation on C-reactive protein**. Data reported as standardized mean differences with 95%CIs.

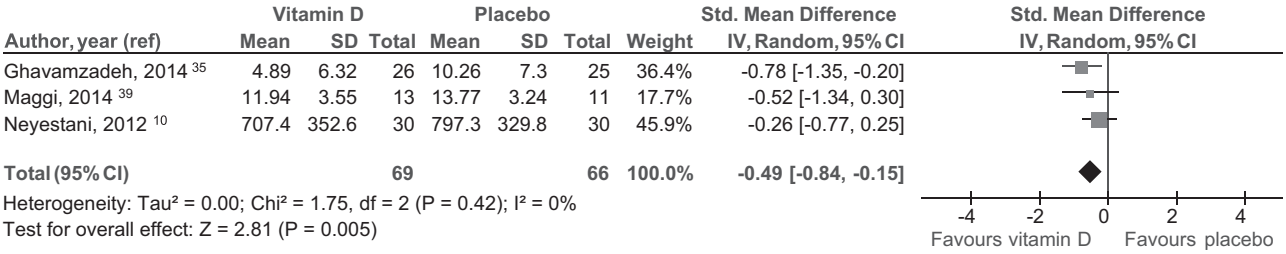
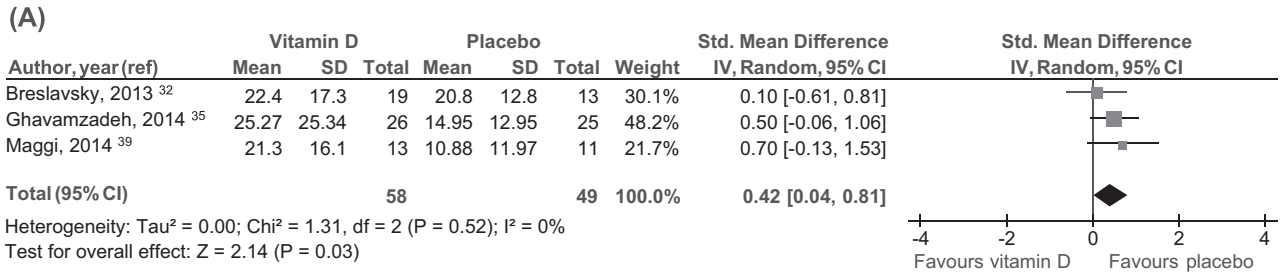


Figure 3 **Forest plot showing results of a meta-analysis of the effects of vitamin D supplementation on tumor necrosis factor α.** Data reported as standardized mean differences with 95%CIs.



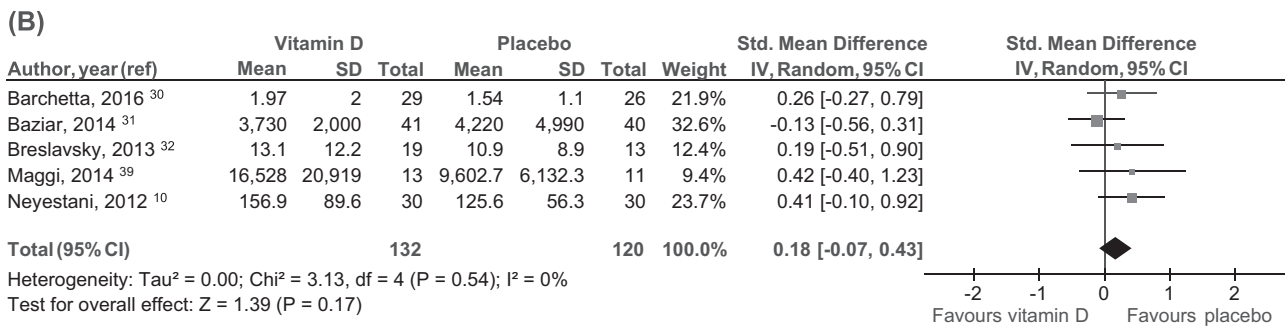


Figure 4 **Forest plot showing results of a meta-analysis of the effects of vitamin D supplementation on the adipokines leptin (A) and adiponectin (B).** Data reported as standardized mean differences with 95%CIs.

(Figure 4B[10,30–32,39]), indicating no effect of vitamin D supplementation. All studies reporting data for adiponectin levels used cholecalciferol supplementation, and results were unchanged when 1 study that cosupplemented vitamin D with calcium[10] was excluded ($P = 0.5$). None of the studies that assessed adiponectin had a high risk of bias.

Vitamin D supplementation did not change IL-6 levels (SMD −0.37, 95%CI, −0.82 to 0.07, $P = 0.1$; $P_{het} = 0.2$, $I^2 = 38\%$; WMD −9.35 ng/L, 95%CI, −30.60 to 11.89) (see Figure S1A in the Supporting Information online), and there was no effect for E-selectin, but significant heterogeneity was observed (SMD 0.03, 95%CI, −0.88 to 0.94, $P = 0.9$; $P_{het} = 0.004$, $I^2 = 88\%$; WMD 2.26 ng/ml, 95%CI, −8.82 to 13.34) (see Figure S1B in the Supporting Information online). The ESR was lower in vitamin D supplementation groups than in placebo groups (SMD −0.47, 95%CI, −0.89 to −0.05, $P = 0.03$; $P_{het} = 0.6$, $I^2 = 0\%$; WMD −8.14 mm/h, 95%CI, −17.39 to 1.12) (see Figure S1C in the Supporting Information online). For IL-6, E-selectin, and ESR, further exploratory analysis to account for heterogeneity, risk of bias, or other moderating variables was not possible because only 2 studies were pooled for each marker.

Subgroup analysis and meta-regression

Prespecified study and sample characteristics thought to be clinically relevant to the outcomes were assessed in subgroup analyses and by meta-regression, but only for CRP and adiponectin, as there were not enough studies ($n \leq 3$) to evaluate the remaining markers. Studies were stratified by age (< 60 years, ≥ 60 years), sex (> 50% female, ≤ 50% female), baseline BMI (< 30 kg/m^2, ≥ 30 kg/m^2), baseline vitamin D status (deficient,

25(OH)D < 50 nmol/L; nondeficient, 25(OH)D ≥ 50 nmol/L), dosage regimen (≤2000 IU/d, > 2000 IU/d; and daily vs bolus dose), and duration of vitamin D supplementation (≤12 weeks, > 12 weeks). For both CRP and adiponectin, there were no significant differences between any of the subgroups analyzed (all $P > 0.05$) (data not shown).

Meta-regression analyses using the study and sample characteristics described above showed no influence of age, sex, BMI, diabetes duration, baseline vitamin D status, or the dose or duration of supplementation on either CRP or adiponectin (all $P > 0.05$) (data not shown).

Publication bias and GRADE assessment

Based on visual inspection of funnel plots (see Figure S2 in the Supporting Information online) and on the tests of Egger et al.[25] and Begg and Mazumdar[26] (see Table S4 in the Supporting Information online), there was no evidence of publication bias for CRP, TNF-α, leptin, or adiponectin. Levels of IL-6, ESR, and E-selectin were not assessed for publication bias because of the small number of studies (all n = 2).

The quality of the evidence for each outcome, evaluated using the GRADE approach,[23] is presented in Table S5 in the Supporting Information online. For CRP and adiponectin, the quality of evidence was high, since most studies had a low risk of bias with low statistical and clinical heterogeneity and narrow CIs. For leptin, the evidence was deemed to be of moderate quality, owing to imprecision (wide 95%CIs). The evidence for TNF-α, IL-6, E-selectin, and ESR was deemed to be of low quality, owing to both imprecision and indirectness as well as to low numbers of studies and potential reporting bias (see Table S5 in the Supporting Information online).

Descriptive analysis

Five studies measuring CRP, TNF-α, and IL-6 were excluded because of unavailable data. Of these, 1 reported reduced CRP, TNF-α, and IL-6 following 1000 IU of cholecalciferol daily for 12 weeks,[11] and another reported reduced TNF-α and IL-6, but not CRP, after 50 000 IU of cholecalciferol weekly for 8 weeks.[47] The remaining 3 studies found no effect on IL-6 or TNF-α[12,13,48] or on CRP[13,48,50] after 5000 IU of cholecalciferol or 1 μg of paricalcitol daily for 12 weeks (Table 2). Of the 2 excluded studies that measured leptin and adiponectin, 1 found no effect of 5000 IU of cholecalciferol supplementation daily for 12 weeks,[12] while the other found reduced leptin levels after 50 000 IU of cholecalciferol weekly for 8 weeks[47] (Table 2).

For IL-10 and osteoprotegerin, 2 studies reported that cholecalciferol supplementation of 1000 IU daily for 3 months or a single bolus of 300 000 IU (with levels measured 6 months later) resulted in increased IL-10 and osteoprotegerin, respectively[11,39]; however, no effect was found in 2 studies of 5000 IU daily for 3 months[12,13] (Table 2). For markers reported in single studies, 1000 to 2000 IU of cholecalciferol daily for 3 months decreased IL-1β, retinol-binding protein 4, fibrinogen, and endothelin-1 and increased omentin levels,[10,27,36] and a bolus of 300 000 IU increased fibroblast growth factor 23 after 6 months.[37] No differences were observed in single studies reporting on IL-2, IL-18, thioredoxin-binding protein 2, interferon gamma, monocyte chemoattractant protein 1, lipocalin 2, or osteopontin[10,11,28,42,48] (Table 2).

DISCUSSION

This is the first systematic review and meta-analysis of RCTs investigating the effects of vitamin D supplementation on inflammatory markers in type 2 diabetes. Beneficial effects of vitamin D supplementation on CRP, TNF-α, leptin, and ESR were observed, with most studies found to have low heterogeneity and low to moderate risk of bias. Results for CRP remained significant in sensitivity analysis; however, differences in TNF-α and leptin were attenuated after studies with high risk of bias were excluded. Subgroup and meta-regression analyses for CRP and adiponectin showed that results were not influenced by age, sex, diabetes duration, or baseline BMI or vitamin D status. Dose and duration of supplementation also did not influence the results in meta-regression; however, the number of studies may have been too small to detect influences from these parameters.

Comparison with previous studies

The biological plausibility of these findings is supported by experimental and epidemiological studies. Results of this meta-analysis showed that CRP, TNF-α, and ESR were lower in vitamin D–supplemented groups than in control groups. Involvement of vitamin D in the functioning of these cytokines is supported by the presence of the nuclear vitamin D receptor in nearly all immune cells, including monocytes, macrophages, and activated T and B lymphocytes.[52] Cell culture studies showed that vitamin D promotes monocyte differentiation to macrophages and diminishes the ability of macrophages to release inflammatory cytokines and chemokines.[53] Vitamin D also suppresses the proliferation and stimulatory abilities of T cells and monocytes from healthy participants and patients with type 2 diabetes, thereby

downregulating proinflammatory cytokines such as CRP, TNF-α, IL-1, IL-6, and IL-8 while upregulating anti-inflammatory cytokines such as IL-10.[53] Moreover, absence of the vitamin D receptor has been shown to enhance the activity of nuclear factor κB, a transcription factor that plays a key role in inflammation and immunoregulation, whereas vitamin D treatment arrested nuclear factor κB translocation and weakened nuclear factor κB activity.[54] Cell culture studies also suggest that vitamin D may produce anti-inflammatory effects by targeting cellular stress response and signaling pathways.[55] For instance, vitamin D stimulates the redox-sensitive transcription factor nuclear factor erythroid-derived 2–related factor 2, which in turn induces a network of cytoprotective genes, termed vitagenes.[55,56] These vitagenes play a key role in cellular defense mechanisms such as redox homeostasis and detoxification.[56] They also regulate a number of proteins, including heat-shock proteins, which have been shown to promote cytoprotection in several conditions and processes such as inflammation, cancer, aging, and neurodegenerative disorders.[56]

Data from animal studies have shown that intraperitoneal injection of vitamin D_3 attenuated diabetic periodontitis by reducing serum TNF-α levels in diabetic mice,[57] while administration of 1,25(OH)D to nonobese, diabetes-prone mice modulated chemokine and cytokine expression and prevented or delayed the onset of diabetes.[4] Experimental and animal models therefore support the finding that vitamin D may have important anti-inflammatory effects.

With regard to observational studies, some researchers have found inverse associations between 25(OH)D and inflammatory markers such as CRP,[6] TNF-α,[7] and IL-6[8] in patients with type 2 diabetes, while others have not.[7,9] Interventions have also shown inconsistent findings, as evident from the RCTs in this review; some have found that vitamin D supplementation reduced CRP,[10,29] TNF-α,[27,47] and IL-6[27,47] and increased IL-10 and osteoprotegerin,[11,39] while others found no effect.[12,13,48] Discrepancies between study results may be attributable to different dosage regimens of vitamin D and different comorbidities in participants as well as to insufficient power to detect differences in inflammatory markers. It is possible that differences in IL-6 were not detected in this meta-analysis because of the small number of included studies, since data for pooling were available for only 2 of the 7 studies that measured IL-6 (Table 2). Importantly, 2 good-quality RCTs[11,47] that were excluded from meta-analysis (because requested data was not available) both reported that 1000 IU daily and 50 000 IU weekly of cholecalciferol supplementation for 2 to 3 months reduced IL-6 concentrations in patients with type 2 diabetes. Inclusion of these studies may have altered the effects for IL-6, and results should therefore be interpreted with caution. Similarly, ESR and E-selectin were measured in only 2 studies, highlighting the need for further studies before the effects of vitamin D supplementation on these markers can be ascertained.

In the present meta-analysis, leptin levels at follow-up were higher in the vitamin D group than in the placebo group. Direct regulation of adipokine gene expression by vitamin D is supported by the presence of the vitamin D receptor in adipose tissue and preadipocytes.[58] Moreover, data from in vivo and ex vivo animal models have shown that 1,25(OH)D directly stimulates leptin production by adipose tissue in a vitamin D receptor–dependent manner.[58] However, the opposite effect was shown in human adipose tissue: vitamin D treatment in vitro inhibited leptin secretion.[59] Discrepancies in the relationship between vitamin D and leptin are also seen in observational studies and RCTs. A systematic review of 14 cross-sectional studies in humans (none of which included patients with type 2 diabetes) reported both positive and negative associations between vitamin D and leptin in the included studies.[60] Similarly, RCTs identified in the present review reported both higher[35,39] and lower[47] leptin levels following vitamin D supplementation. It is possible that these inconsistent results reflect a U-shaped, rather than dose–linear, response of leptin to vitamin D in humans.[61] It should be noted that results in this meta-analysis may have been nullified by the addition of 2 excluded studies, 1 that reported decreased leptin following vitamin D supplementation[47] and another that found no effect.[12] Indeed, results were no longer significant after a study with high risk of bias[35] was excluded and should therefore be interpreted with caution. Future research elucidating the molecular interactions between vitamin D and leptin is needed to accurately define the role of each of these molecules in mitigating inflammation in chronic diseases such as type 2 diabetes.

Lastly, no differences in adiponectin levels were found between vitamin D and placebo groups. This conflicts with findings from experimental studies in which 1,25(OH)D increased adiponectin levels by downregulating the TNF-α gene, known to regulate adiponectin synthesis.[62] Moreover, vitamin D is thought to increase adiponectin by downregulating the adipose tissue renin–angiotensin system, since higher angiotensin levels lead to the production of dysfunctional adipocytes and decreased adiponectin production.[31] Observational studies have also found positive associations between serum 25(OH)D and circulating adiponectin levels in patients with type 2 diabetes[63] or metabolic syndrome.[64] In contrast, with the exception of 1 trial,[10] all RCTs

included in this review reported that vitamin D supplementation had no effect on adiponectin levels. Residual confounding may explain why the associations seen in observational studies are not consistent with results from RCTs. Moreover, the lack of findings in this meta-analysis could be due to the measurement of total adiponectin instead of high-molecular-weight adiponectin in the included RCTs. High-molecular-weight adiponectin is the active form and could therefore be a more sensitive measure for assessing adiponectin levels in vivo,[20] and it is more strongly associated with diabetes than total adiponectin.[65] Since the included RCTs measured only total adiponectin, results should be interpreted in light of this potential limitation. Nevertheless, these findings suggest that total adiponectin levels may not be affected by vitamin D supplementation, and therefore further studies are needed to establish the effects of vitamin D supplementation on high-molecular-weight adiponectin in type 2 diabetes.

Comparison with previous meta-analyses

Findings from this meta-analysis in type 2 diabetes are consistent with those of some, but not all, previous systematic reviews and meta-analyses in different population groups. Vitamin D supplementation reduced CRP and TNF-α in a meta-analysis of patients with chronic heart failure (7 RCTs)[17] and reduced CRP in another meta-analysis of mixed population groups (healthy, overweight/obese, and with different diseases; 10 RCTs).[19] In contrast, vitamin D had no effect on CRP, TNF-α, or IL-6 in a meta-analysis of overweight and obese adults (13 RCTs),[18] or on adipokines, including leptin and adiponectin, in another meta-analysis of mixed population groups (9 RCTs).[20] Systematic reviews of RCTs (without meta-analyses) also found that vitamin D supplementation had no effect on inflammation in healthy individuals.[66,67] Discrepancies between existing meta-analyses as well as disagreement between previous findings and the findings of the present review could be partly attributable to the inclusion of healthy populations in previous reviews, as it is suggested that vitamin D has more pronounced effects when the immune system is stimulated, such as in the presence of inflammatory or chronic diseases, including type 2 diabetes.[68] Randomized controlled trials and systematic reviews of RCTs have consistently shown that vitamin D improves inflammation in those with existing inflammatory diseases such as systemic lupus erythematosus,[69] inflammatory bowel disease,[70] and chronic obstructive pulmonary disease.[71] The present study extends current knowledge by showing that vitamin D supplementation also improves inflammatory profiles in type 2 diabetes, another disease characterized by systemic inflammation.

Strengths and weaknesses

This meta-analysis has several strengths. All studies included had a randomized controlled design, which is the gold standard for establishing causality. Rigorous international gold-standard methodology was applied, and international reporting standards were followed; moreover, the protocol was published a priori to ensure transparency. The search strategy was comprehensive and included non–English language publications and gray literature. The results report data for several inflammatory marker endpoints, providing a comprehensive overview of the effects of vitamin D on the inflammatory milieu that underlies type 2 diabetes.

Some limitations should be noted. First, as for any meta-analysis, the strength of the evidence depends on the number and quality of the included studies. Although most studies had a low to moderate risk of bias, results for TNF-α and leptin were no longer significant after excluding studies with a high risk of bias and thus should be interpreted with caution. Nevertheless, randomization, blinding, and the use of a control group were considered the most important aspects in the meta-analysis, and only 3 studies did not satisfy these criteria[38,40,49] (see Table S3 in the Supporting Information online). Second, the inclusion of several inflammatory marker endpoints resulted in a small number of studies for some markers, including IL-6, ESR, and E-selectin, for which exploratory analyses and meta-regression could not be performed. Third, meta-regression may not have detected the influence of relevant clinical factors because of the small number of studies, and it was not possible to adjust for all potential effect modifiers such as insulin or statin use or comorbidity status. Fourth, publication bias for some markers cannot be ruled out when there were few studies or when it was not possible to obtain all necessary data from authors. Finally, most studies were conducted in Iran, which limits the generalizability of these findings to other ethnic groups.

This review also highlights important weaknesses in the literature. Most studies had small samples, with 100 or fewer participants reported for all but 1 study ($n = 118$).[47] Although individual studies had mostly low to moderate risk of bias, the quality of evidence across studies was low for several markers (see Table S5 in the Supporting Information online). Most studies did not report ethnicity, which has been linked to vitamin D receptor polymorphisms that affect the metabolism and biological function of vitamin D, particularly in type 2 diabetes.[8] Smoking status and diabetes duration, both

factors that may influence inflammatory status in patients with type 2 diabetes, were also not reported in several studies. Finally, none of the studies reported long-term outcomes, hence inferences about whether improved inflammation following vitamin D supplementation translates to decreased morbidity or mortality in type 2 diabetes cannot be made.

Clinical implications

The finding of a beneficial effect of vitamin D supplementation on inflammation has potentially important implications in the context of diabetes. First, it is widely accepted that a systemic low-grade inflammatory state not only coexists but also precedes the development of diabetes.[72] Second, if vitamin D supplementation can improve inflammatory marker levels, as shown here, there may be important benefits for patients with type 2 diabetes, given that elevated cytokines promote insulin resistance, dyslipidemia, and atherosclerosis, while dysregulated adipokines can affect energy homeostasis, lipid and glucose metabolism, angiogenesis, and vascular remodeling.[20] Although reducing obesity through lifestyle modification is the front-line treatment for preventing progression of type 2 diabetes, weight loss strategies are often hindered by low participant adherence and poor sustainability.[2] This meta-analysis suggests that vitamin D supplementation may be a beneficial adjunct therapy to reduce subclinical inflammation in patients with type 2 diabetes, potentially preventing or delaying disease progression. However, large-scale RCTs investigating the effects of vitamin D supplementation on inflammatory markers, with assessment of clinical endpoints and long-term outcomes, are needed to establish whether reduced inflammation translates into improved health outcomes for patients with type 2 diabetes.

CONCLUSION

In summary, this meta-analysis provides level 1 evidence of the beneficial effect of vitamin D supplementation on inflammatory markers in type 2 diabetes. Larger and longer-term clinical trials are needed to establish whether improvements in inflammation following vitamin D supplementation would result in clinically meaningful health outcomes for these patients.

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Declaration of interest. The authors have no relevant interests to declare.

Supporting Information

The following Supporting Information is available through the online version of this article at the publisher's website.

Appendix S1 **PRISMA 2009 Checklist**

Appendix S2 **Template for critical appraisal of randomized controlled trials**

Table S1 **Grading the quality of the evidence (adapted from Atkins et al.[S1])**

Table S2 **Study and sample characteristics of studies included in systematic review of the effects of vitamin D supplementation on inflammation markers in type 2 diabetes patients**

Table S3 **Risk-of-bias assessment of studies included in a systematic review of the effects of vitamin D supplementation on inflammation markers in type 2 diabetes patients**

Table S4 **Assessment of publication bias calculated from Egger's and Begg's tests**

Table S5 **GRADE assessment of the effect of vitamin D supplementation on inflammatory markers meta-analyses**

Figure S1 **Forest plot of meta-analysis of effects of vitamin D supplementation on interleukin-6 (A), E-selectin (B), and erythrocyte sedimentation rate (C) in type 2 diabetes patients.**

Figure S2 **Funnel plots of all endpoints with more than 2 studies for identification of publication bias.**

REFERENCES

1. World Health Organization. Global report on diabetes. http://www.who.int/diabetes/global-report/en/. Published 2016. Accessed February, 2017.
2. Pittas A, Lau J, Hu F, et al. The role of vitamin D and calcium in type 2 diabetes: a systematic review and meta-analysis. J Clin Endocrinol Metab. 2007;92:2017–2029.
3. Mitri J, Pittas AG. Vitamin D and diabetes. Endocrinol Metab Clin North Am. 2014;43:205–232.
4. Gysemans CA, Cardozo AK, Callewaert H, et al. 1,25-Dihydroxyvitamin D_3 modulates expression of chemokines and cytokines in pancreatic islets: implications for prevention of diabetes in nonobese diabetic mice. Endocrinology. 2005;146:1956–1964.
5. Riachy R, Vandewalle B, Moerman E, et al. 1,25-dihydroxyvitamin D_3 protects human pancreatic islets against cytokine-induced apoptosis via down-regulation of the fas receptor. Apoptosis. 2006;11:151–159.
6. Kuloglu O, Gur M, Seker T, et al. Serum 25-hydroxyvitamin D level is associated with aortic distensibility and left ventricle hypertrophy in newly diagnosed type 2 diabetes mellitus. Diab Vasc Dis Res. 2013;10:546–549.
7. Haidari FP, Zakerkish MD, Karandish MP, et al. Association between serum vitamin D level and glycemic and inflammatory markers in non-obese patients with type 2 diabetes. Iran J Med Sci. 2016;41:367–373.
8. Mackawy AM, Badawi ME. Association of vitamin D and vitamin D receptor gene polymorphisms with chronic inflammation, insulin resistance and metabolic syndrome components in type 2 diabetic Egyptian patients. Meta Gene. 2014;2:540–556.
9. Šebeková K, Stürmer M, Fazeli G, et al. Is vitamin D deficiency related to accumulation of advanced glycation end products, markers of inflammation, and oxidative stress in diabetic subjects? Biomed Res Int. 2015;2015:958097. doi:10.1155/2015/958097
10. Neyestani TR, Nikooyeh B, Alavi-Majd H, et al. Improvement of vitamin D status via daily intake of fortified yogurt drink either with or without extra calcium ameliorates systemic inflammatory biomarkers, including adipokines, in the subjects with type 2 diabetes. J Clin Endocrinol Metab. 2012;97:2005–2011.
11. Shab-Bidar S, Neyestani TR, Djazayery A, et al. Improvement of vitamin D status resulted in amelioration of biomarkers of systemic inflammation in the subjects with type 2 diabetes. Diabetes Metab Res Rev. 2012;28:424–430.
12. Al-Sofiani ME, Jammah A, Racz M, et al. Effect of vitamin D supplementation on glucose control and inflammatory response in type II diabetes: a double blind, randomized clinical trial. Int J Endocrinol Metab. 2015;13:e22604. doi:10.5812/ijem.22604
13. Kampmann U, Mosekilde L, Juhl C, et al. Effects of 12 weeks high dose vitamin D_3 treatment on insulin sensitivity, beta cell function, and metabolic markers in patients with type 2 diabetes and vitamin D insufficiency—a double-blind, randomized, placebo-controlled trial. Metabolism. 2014;63:1115–1124.
14. George PS, Pearson ER, Witham MD. Effect of vitamin D supplementation on glycaemic control and insulin resistance: a systematic review and meta-analysis. Diabet Med. 2012;29:e142–e150.
15. Krul-Poel YHM, ter Wee MM, Lips P, et al. The effect of vitamin D supplementation on glycaemic control in patients with type 2 diabetes mellitus: a systematic review and meta-analysis. Eur J Endocrinol. 2017;176:R1–R14.
16. Nigil Haroon N, Anton A, John J, et al. Effect of vitamin D supplementation on glycemic control in patients with type 2 diabetes: a systematic review of interventional studies. J Diabetes Metab Disord. 2015;14:3. doi:10.1186/s40200-015-0130-9
17. Jiang WL, Gu HB, Zhang YF, et al. Vitamin D supplementation in the treatment of chronic heart failure: a meta-analysis of randomized controlled trials. Clin Cardiol. 2016;39:56–61.
18. Jamka M, Wozniewicz M, Walkowiak J, et al. The effect of vitamin D supplementation on selected inflammatory biomarkers in obese and overweight subjects: a systematic review with meta-analysis. Eur J Nutr. 2016;55:2163–2176.
19. Chen N, Wan Z, Han SF, et al. Effect of vitamin D supplementation on the level of circulating high-sensitivity C-reactive protein: a meta-analysis of randomized controlled trials. Nutrients. 2014;6:2206–2216.
20. Dinca M, Serban M-C, Sahebkar A, et al. Does vitamin D supplementation alter plasma adipokines concentrations? A systematic review and meta-analysis of randomized controlled trials. Pharmacol Res. 2016;107:360–371.
21. Liberati A, Altman DG, Tetzlaff J, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. PLoS Med. 2009;6. doi.org/10.1371/journal.pmed.1000100
22. Mousa A, Misso M, Teede H, et al. Effect of vitamin D supplementation on inflammation: protocol for a systematic review. BMJ Open. 2016;6:e010804. doi:10.1136/bmjopen-2015-010804.
23. Atkins D, Best D, Briss PA, et al. Grading quality of evidence and strength of recommendations. BMJ. 2004;328:1490. doi:10.1136/bmj.328.7454.1490
24. Higgins JT, Green S, eds. *Cochrane Handbook for Systematic Reviews of Interventions*. Version 5.1.0 [updated March 2011]. London, UK: the Cochrane Collaboration; 2011.
25. Egger M, Davey Smith G, Schneider M, et al. Bias in meta-analysis detected by a simple, graphical test. BMJ. 1997;315:629–634.
26. Begg CB, Mazumdar M. Operating characteristics of a rank correlation test for publication bias. Biometrics. 1994;50:1088–1101.
27. Shab-Bidar S, Neyestani TR, Djazayery A, et al. Regular consumption of vitamin D-fortified yogurt drink (Doogh) improved endothelial biomarkers in subjects with type 2 diabetes: a randomized double-blind clinical trial. BMC Med. 2011;9:125. doi:10.1186/1741-7015-9-125
28. Akbarzadeh M, Eftekhari MH, Dabbaghmanesh MH, et al. Serum IL-18 and hsCRP correlate with insulin resistance without effect of calcitriol treatment on type 2 diabetes. Iran J Immunol. 2013;10:167–176.
29. Asemi Z, Raygan F, Bahmani F, et al. The effects of vitamin D, K and calcium co-supplementation on carotid intima-media thickness and metabolic status in overweight type 2 diabetic patients with CHD. Br J Nutr. 2016;116:286–293.
30. Barchetta I, Del Ben M, Angelico F, et al. No effects of oral vitamin D supplementation on non-alcoholic fatty liver disease in patients with type 2 diabetes: a randomized, double-blind, placebo-controlled trial. BMC Med. 2016;14:92. doi:10.1186/s12916-016-0638-y
31. Baziar N, Jafarian K, Shadman Z, et al. Effect of therapeutic dose of vitamin D on serum adiponectin and glycemia in vitamin D-insufficient or deficient type 2 diabetic patients. Iran Red Crescent Med J. 2014;16:e21458. doi:10.5812/ircmj.21458
32. Breslavsky A, Frand J, Matas Z, et al. Effect of high doses of vitamin D on arterial properties, adiponectin, leptin and glucose homeostasis in type 2 diabetic patients. Clin Nutr. 2013;32:970–975.
33. Dalan R, Liew H, Assam PN, et al. A randomised controlled trial evaluating the impact of targeted vitamin D supplementation on endothelial function in type 2 diabetes mellitus: the DIMENSION trial. Diab Vasc Dis Res. 2016;13:192–200.
34. Farrokhian A, Raygan F, Bahmani F, et al. Long-term vitamin D supplementation affects metabolic status in vitamin D–deficient type 2 diabetic patients with coronary artery disease. J Nutr. 2017;147:384–389.
35. Ghavamzadeh S, Mobasseri M, Mahdavi R. The effect of vitamin D supplementation on adiposity, blood glycated hemoglobin, serum leptin and tumor necrosis factor-α in type 2 diabetic patients. Int J Prev Med. 2014;5:1091–1098.
36. Jafari T, Faghihimani E, Feizi A, et al. Effects of vitamin D-fortified low fat yogurt on glycemic status, anthropometric indexes, inflammation, and bone turnover in diabetic postmenopausal women: a randomised controlled clinical trial. Clin Nutr. 2016;35:67–76.
37. Jehle S, Lardi A, Felix B, et al. Effect of large doses of parenteral vitamin D on glycaemic control and calcium/phosphate metabolism in patients with stable type 2 diabetes mellitus: a randomised, placebo-controlled, prospective pilot study. Swiss Med Wkly. 2014;144:w13942. doi:10.4414/smw.2014.13942
38. Kota SK, Jammula S, Kota SK, et al. Effect of vitamin D supplementation in type 2 diabetes patients with pulmonary tuberculosis. Diab Metab Syndr. 2011;5:85–89.
39. Maggi S, Siviero P, Brocco E, et al. Vitamin D deficiency, serum leptin and osteoprotegerin levels in older diabetic patients: an input to new research avenues. Acta Diabetol. 2014;51:461–469.
40. Munisamy S, Daud KM, Mokhtar SS, et al. Effects of 1α-calcidol (alfacalcidol) on microvascular endothelial function, arterial stiffness, and blood pressure in type II diabetic nephropathy patients. Microcirculation. 2016;23:53–61.
41. Patel P, Poretsky L, Liao E. Lack of effect of subtherapeutic vitamin D treatment on glycemic and lipid parameters in type 2 diabetes: a pilot prospective randomized trial. J Diabetes. 2010;2:36–40.
42. Rad EY, Attar MJH, Koohdani F, et al. Effect of vitamin D supplementation on thioredoxin binding protein 2 gene expression in patients with diabetes type 2. Curr Top Nutraceutical Res. 2015;13:55–60.
43. Razzaghi R, Pourbagheri H, Momen-Heravi M, et al. The effects of vitamin D supplementation on wound healing and metabolic status in patients with diabetic foot ulcer: a randomized, double-blind, placebo-controlled trial. J Diabet Complications. 2016;31:766–772.
44. Ryu OH, Chung W, Lee S, et al. The effect of high-dose vitamin D supplementation on insulin resistance and arterial stiffness in patients with type 2 diabetes. Korean J Intern Med. 2014;29:620–629.

45. Sadiya A, Ahmed SM, Carlsson M, et al. Vitamin D supplementation in obese type 2 diabetes subjects in Ajman, UAE: a randomized controlled double-blinded clinical trial. Eur J Clin Nutr. 2015;69:707–711.
46. Shaseb E, Tohidi M, Abbasinazari M, et al. The effect of a single dose of vitamin D on glycemic status and C-reactive protein levels in type 2 diabetic patients with ischemic heart disease: a randomized clinical trial. Acta Diabetol. 2016;53:575–582.
47. Tabesh M, Azadbakht L, Faghihimani E, et al. Calcium–vitamin D cosupplementation influences circulating inflammatory biomarkers and adipocytokines in vitamin D–insufficient diabetics: a randomized controlled clinical trial. J Clin Endocrinol Metab. 2014;99:E2485–E2493.
48. Thethi TK, Bajwa MA, Ghanim H, et al. Effect of paricalcitol on endothelial function and inflammation in type 2 diabetes and chronic kidney disease. J Diabet Complications. 2015;29:433–437.
49. Tiryaki O, Usalan C, Sayiner ZA. Vitamin D receptor activation with calcitriol for reducing urinary angiotensinogen in patients with type 2 diabetic chronic kidney disease. Ren Fail. 2016;38:222–227.
50. Yiu YF, Yiu KH, Siu CW, et al. Randomized controlled trial of vitamin D supplement on endothelial function in patients with type 2 diabetes. Atherosclerosis. 2013;227:140–146.
51. Flores M, Barquera S, Macias N, et al. Vitamin D supplementation reduces C-reactive protein and insulin resistance in women with type 2 diabetes mellitus [abstract]. FASEB J. 2010; 24(1 suppl):342.1. fasebj.org/doi/10.1096/fasebj.24.1_supplement.342.1
52. Holick MF. Vitamin D status: measurement, interpretation, and clinical application. Ann Epidemiol. 2009;19:73–78.
53. Guillot X, Semerano L, Saidenberg-Kermanac'h N, et al. Vitamin D and inflammation. Joint Bone Spine. 2010;77:552–557.
54. Harant H, Andrew PJ, Reddy GS, et al. 1α,25-dihydroxyvitamin D_3 and a variety of its natural metabolites transcriptionally repress nuclear-factor-κB-mediated interleukin-8 gene expression. Eur J Biochem. 1997;250:63–71.
55. Berridge MJ. Vitamin D cell signalling in health and disease. Biochem Biophys Res Commun. 2015;460:53–71.
56. Calabrese V, Cornelius C, Mancuso C, et al. Redox homeostasis and cellular stress response in aging and neurodegeneration. In: Uppu RM, Murthy SN, Pryor WA, eds. Free Radicals and Antioxidant Protocols. Totowa, NJ: Humana Press; 2010:285–308.
57. Li H, Xie H, Fu M, et al. 25-hydroxyvitamin D3 ameliorates periodontitis by modulating the expression of inflammation-associated factors in diabetic mice. Steroids. 2013;78:115–120.
58. Kong J, Chen Y, Zhu G, et al. 1, 25-Dihydroxyvitamin D_3 upregulates leptin expression in mouse adipose tissue. J Endocrinol. 2013;216:265–271.
59. Menendez C, Lage M, Peino R, et al. Retinoic acid and vitamin D_3 powerfully inhibit *in vitro* leptin secretion by human adipose tissue. J Endocrinol. 2001;170:425–431.
60. Hajimohammadi M, Shab-Bidar S, Neyestani TR. Vitamin D and serum leptin: a systematic review and meta-analysis of observational studies and randomized controlled trials. Eur J Clin Nutr. 2017;71:1144–1153.
61. Maetani M, Maskarinec G, Franke AA, et al. Association of leptin, 25-hydroxyvitamin D, and parathyroid hormone in women. Nutr Cancer. 2009;61:225–231.
62. Vilarrasa N, Vendrell J, Maravall J, et al. Is plasma 25(OH)D related to adipokines, inflammatory cytokines and insulin resistance in both a healthy and morbidly obese population?. Endocrinology. 2010;38:235–242.
63. Al-Daghri NM, Al-Attas OS, Alokail MS, et al. Hypovitaminosis D associations with adverse metabolic parameters are accentuated in patients with type 2 diabetes mellitus: a body mass index-independent role of adiponectin? J Endocrinol Invest 2013;36:1–6.
64. Nimitphong H, Chanprasertyothin S, Jongjaroenprasert W, et al. The association between vitamin D status and circulating adiponectin independent of adiposity in subjects with abnormal glucose tolerance. Endocrine. 2009;36:205–210.
65. Husemoen LLN, Skaaby T, Martinussen T, et al. Investigating the causal effect of vitamin D on serum adiponectin using a mendelian randomization approach. Eur J Clin Nutr. 2014;68:189–195.
66. Agbalalah T, Hughes SF, Freeborn EJ, et al. Impact of vitamin D supplementation on endothelial and inflammatory markers in adults: a systematic review. J Steroid Biochem Mol Biol. 2017;17:30015–30018.
67. Zuk A, Fitzpatrick T, Rosella LC. Effect of vitamin D_3 supplementation on inflammatory markers and glycemic measures among overweight or obese adults: a systematic review of randomized controlled trials. PLoS One. 2016;11:e0154215. doi:10.1371/journal.pone.0154215
68. Jorde R, Sneve M, Torjesen PA, et al. No effect of supplementation with cholecalciferol on cytokines and markers of inflammation in overweight and obese subjects. Cytokine. 2010;50:175–180.
69. Abou-Raya A, Abou-Raya S, Helmii M. The effect of vitamin D supplementation on inflammatory and hemostatic markers and disease activity in patients with systemic lupus erythematosus: a randomized placebo-controlled trial. J Rheumatol. 2013;40:265–272.
70. Nicholson I, Dalzell AM, El-Matary W. Vitamin D as a therapy for colitis: a systematic review. J Crohns Colitis. 2012;6:405–411.
71. Rezk NASA, Aly NYA, Hewidy AAH. Effect of vitamin D replacement in chronic obstructive pulmonary disease patients with vitamin D deficiency. Egypt J Chest Dis Tuberc. 2015;64:353–357.
72. Flores M. A role of vitamin D in low-intensity chronic inflammation and insulin resistance in type 2 diabetes mellitus? Nutr Res Rev. 2005;18:175–182.