Effect of Vitamin D Supplementation on Some Inflammatory Biomarkers in Type 2 Diabetes Mellitus Subjects: A Systematic Review and Meta-Analysis of Randomized Controlled Trials

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Keywords

Vitamin D · Inflammation · High-sensitivity C-reactive protein · Type 2 diabetes mellitus · Randomized controlled trials · Meta-analysis

Abstract

Background/Aims: The mechanism, by which vitamin D influences inflammatory biomarkers in type 2 diabetes mellitus (T2DM), is not very well known. Thus, a meta-analysis of randomized controlled trials was conducted to assess the effect of vitamin D supplementation on some inflammatory biomarkers in T2DM subjects. **Methods:** We searched randomized controlled trials from PubMed and the Cochrane Library in October 2017 and conducted a meta-analysis to evaluate the effectiveness of vitamin D supplementation on high-sensitivity C-reactive protein (hs-CRP), tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6). Either a fixed-effects or a random-effects model was used to calculate pooled effects. **Results:** We identified 13 studies that met our

inclusion criteria. The results indicated that the vitamin D supplementation significantly decreased the hs-CRP level by 0.45 $\mu\text{g/mL}$, whereas the vitamin D supplementation did not influence the TNF- α and IL-6. Subgroup analysis showed that vitamin D significantly lowered hs-CRP by 0.34 $\mu\text{g/mL}$ among trials with a daily vitamin D dose $\leq 4,000$ IU and by 0.31 $\mu\text{g/mL}$ among trials with time of vitamin D supplementation > 12 weeks. **Conclusions:** Vitamin D supplementation is beneficial for the reduction of hs-CRP in T2DM subjects but does not have a significant influence on TNF- α and IL-6 in T2DM subjects.

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Introduction

The prevalence of diabetes has been increasing throughout the world over recent decades and the trend is set to continue. According to the World Health Organization, there were approximately 415 million people

with diabetes in the world, which could rise to 642 million by 2040 [1]. Type 2 diabetes mellitus (T2DM) is a chronic, progressive metabolic disease characterized by persistent hyperglycemia that is accompanied by chronic inflammation [2]. A growing body of evidence has suggested that high concentrations of circulating inflammatory biomarkers, such as interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF- α) and high-sensitivity C-reactive protein (hs-CRP), might play a critical role in the development of T2DM [3–5].

It has been reported that vitamin D could play a key role in immune activation and inflammation [6, 7]. Moreover, vitamin D can prevent the destruction of pancreatic beta-cells and reduce the incidence of diabetes mellitus, possibly secondary to inhibition of proinflammatory cytokines [8–10]. Higher concentrations of proinflammatory cytokines (such as IL-6, TNF- α) and hs-CRP have been noted especially in subjects with low concentrations of vitamin D [11–13]. Vitamin D deficiency is now regarded as a potential risk factor for T2DM. A 5-year follow-up study of the Asian population has found that the incidence of type 2 diabetes in the vitamin D deficiency group was much higher than that in the vitamin D insufficiency group and vitamin D sufficient group [14]. We hypothesized that vitamin D might be involved in the pathogenesis of systemic inflammation that contributed to the development of T2DM. Some studies highlight the potential role of adipokines (IL-6, TNF- α , leptin and adiponectin) as a major link between vitamin D status and T2DM [15]. Moreover, some studies suggest that the supplementation with vitamin D could decrease proinflammatory markers concentrations [16]. However, the mechanism by which vitamin D influences inflammatory biomarkers in T2DM is not very well known. Therefore, we conducted a meta-analysis of randomized controlled trials (RCTs) to examine whether or not the supplementation of vitamin D exhibits anti-inflammatory benefits in T2DM subjects.

Methods

Literature Search

In the period from June 2017 to October 2017, we conducted a systematic search of publications written in the English language using: PubMed (Medline), Scopus, Web of Knowledge, the Cochrane Library and Embase data-bases. Through this process, we identified publications describing the effect of vitamin D supplementation on plasma or serum concentrations of pro-inflammatory cytokines and hs-CRP in T2DM subjects. The extraction was restricted to RCTs. There was no limitation based on the date of study publications. The following index terms were used: “vitamin D” OR “vitamin D₂” OR “vitamin D₃” OR “cholecalciferol”

OR “ergocalciferol” OR “calcitriol” OR “alfacalcidol” OR “paricalcitol” OR “doxercalciferol” OR “vitamin D analogues” OR “25-hydroxyvitamin D₂” OR “25-hydroxyvitamin D₃[25(OH)D₃]” OR “hydroxycholecalciferol” OR “dihydrotachysterol” AND “type 2 diabetes” OR “prediabetes” AND “supplementation” AND “adipokine” OR “cytokine” OR “inflammation” OR “high-sensitivity C-reactive protein” OR “tumor necrosis factor” OR “interleukin”. Reference lists and related records were manually reviewed.

Study Selection

The criteria for inclusion were as follows: intervention studies (RCTs, double-blind RCTs [DB-RCTs]), English-language articles, studies conducted in male and female T2DM subjects with vitamin D supplementation. Vitamin D could have been administered as a cholecalciferol (vitamin D₃) or an ergocalciferol (vitamin D₂), as well as an active form of vitamin D (1 α -hydroxyvitamin D and 1,25-dihydroxyvitamin D [1,25(OH)₂D]). All routes of administration (oral and intramuscular) were considered. The studies that combined vitamin D supplementation with calcium supplementation or additional dietary recommendations (including healthy eating, energy restriction diet, and physical activity) were also enabled. Finally, we analyzed the common inflammatory markers that most studies have measured in clinical practice.

The exclusion criteria comprised the following: studies performed in specific groups of patients (e.g., subjects with vitamin D abnormalities, that is, hyperparathyroidism; subjects suffering from hepatic disease or kidney disease; subjects with pregnancy), conference publications, and articles available only in the abstract form.

Data Extraction and Quality Assessment

The data extraction was performed independently by 2 investigators based upon the exclusion and inclusion criteria (Y.Y. and M.Z.). Discrepancies were resolved by discussion. The following data were extracted from eligible articles: the first author's name, publication year, country of origin, design details, number of participants, analyzed groups, participants with supplemental vitamin D, level of 25(OH) D₃, level of fasting blood glucose (FBG), homeostasis model assessment-insulin resistant (HOMA-IR), and IL-6, TNF- α , hs-CRP level. Publications were assessed according to the titles, abstracts, and full texts in subsequent stages. Doubts were resolved by reviewers by consensus. Each selected publication was studied critically. The Jadad scale was used to assess the study quality. The full score was 5, and a high-quality study was defined by a threshold of ≥ 3 points [17].

Data Synthesis and Analysis

The changes in inflammatory levels in both intervention and control groups were calculated as the mean difference between the end and the baseline. If SDs of these changes were not provided directly, then standard errors, median and quartiles were converted to SDs [18]. The homogeneity across studies was tested using Cochran's Q test and quantified by the I^2 statistic, which represented the percentage of heterogeneity that could be attributed to the variation across studies [19]. In the presence of significant heterogeneity ($p < 0.1$, $I^2 > 25\%$), a random-effects model was used to calculate the pooled effect size; otherwise, a fixed-effects model was applied [20]. The subgroup analyses were conducted to explore the possible sources of heterogeneity. The sensitivity analysis was recalculated to investigate its influence on the overall effect size. Funnel plots were applied to assess the publication bias of this meta-analysis

Fig. 1. Flowchart of literature search and study selection.

[21]. The results of the meta-analysis were visualized using a forest plot which illustrated the results of the individual studies and the summary effect. All of the data were analyzed by REVMAN 5.3 version. p value <0.05 was considered significant unless otherwise specified.

Results

Search Results

A total of 139 articles were found in our initial search and 88 were excluded by screening titles. After full-text review of using the Jadad scale, 6 articles with the threshold of <3 points were excluded. In addition, 32 articles were excluded in this meta-analysis if they: (1) subjects without control group; (2) subjects not involved T2DM; (3) subjects without vitamin D supplementation; (4) no associated inflammatory biomarkers; (5) no available data. Finally, a total of 13 RCTs were selected for the analysis. A flow diagram showing the selection process was presented in Figure 1.

Study Characteristic

The characteristics of the included trials were presented in Table 1. Thirteen trials [22–34] were published from 2012 to 2016, in which 7 were conducted in Iran [23, 24,

28, 29, 31, 33, 34], one was conducted in Singapore [30], Australia [27], America [32], Israel [22], UAE [26], and Finland [25]. The sample size varied from 20 [32] to 118 [28], with a total of 875 participants. Twelve trials were double blind [22–33] and one trial was single blind [34]. All of the trials had a parallel design and participants received capsules, pills, tablets, or a specially fortified yogurt drink. The duration of intervention lasted 8 [28] to 52 weeks [22] with a median of 12 weeks. The vitamin D dose was ranging from 20 IU/day [33] to 50,000 IU/week [28]. The vitamin D supplementation was used once a week in 2 studies [23, 28]. The vitamin D supplementation combined with other supplements for intervention in 4 studies [24, 27–28, 34] (Table 1).

Effect of Vitamin D Supplementation on 25(OH) D₃, FBG and HOMA-IR

The baseline concentration of 25(OH) D₃ suggested that vitamin D deficiency or insufficiency were common in the analyzed population. Compared with the baseline, vitamin D supplementation could increase the concentrations of 25(OH) D₃ in almost each of the intervention groups at the end of the trials achieving the mean levels between 17.6 ng/mL [22] and 95 ng/mL [27] (Table 2). There was no significant difference in FBG in the vitamin

Table 1. Characteristics of randomized controlled trials included in this meta-analysis

Study	Year	Country	Study design	Subjects, <i>n</i> ^a	Analyzed groups ^b	Age, years	Gender, women, %	BMI, kg/m ²		Vitamin D intervention		Combined with other supplementation	Jadad Scores
								baseline	intervention	dosage	duration, weeks		
Breslavsky et al. [22]	2013	Israel	DB-RCT	47	D group C group	66.80±9.20 65.80±9.70	85 92	27.90±4.30 29.60±4.90	27.50±3.30 29.20±5.80	1,000 IU/day	52	N/A	4
Farrokhan et al. [23]	2016	Iran	DB-RCT	60	D group C group	60.50±8.60 63.00±10.70	50 50	30.70±5.10 29.70±4.30	30.80±5.10 29.80±4.50	25,000 IU/week	26	N/A	5
Asemi et al. [24]	2016	Iran	DB-RCT	66	D group C group	65.90±11.40 65.00±11.10	45 48	29.70±5.20 29.60±4.30	29.90±5.60 29.80±4.40	200 IU/day	12	Vitamin K and calcium	4
Tuomainen et al. [25]	2015	Finland	DB-RCT	68	D group D group C group	65.70 (7.00) ^c	N/A	29.40±2.70	0.34±0.55 ^d 0.30±0.58 ^d 0.25±0.86 ^d	1,600 IU/day 3,200 IU/day	20	N/A	5
Sadiya et al. [26]	2015	UAE	DB-RCT	82	D group-1 ^f D group-2 ^f C group-1 ^f C group-2 ^f	49.00±8.00	80	37.90±6.10	N/A	6,000 IU/day 3,000 IU/day	12	N/A N/A	5
Gagnon et al. [27]	2014	Australia	DB-RCT	80	D group C group	53.80±11.90 55.30±11.10	71 67	31.10±5.70 31.90±6.20	N/A N/A	2,000 IU/day	26	Calcium	5
Tabesh et al. [28]	2014	Iran	DB-RCT	118	D group D group C group	50.20±6.60 49.80±6.10 51.00±6.10	48 50 53	N/A N/A N/A	N/A N/A N/A	50,000 IU/week 50,000 IU/week	8	Placebo Calcium	4
Ghavamzadeh et al. [29]	2014	Iran	DB-RCT	51	D group C group	52.26±2.09 49.28±2.00	58 60	28.90±0.86 27.90±0.93	29.20±0.77 28.10±0.99	400 IU/day	14	N/A	4
Dalan et al. [30]	2016	Singapore	DB-RCT	64	D group C group	52.20±8.20 54.80±10.80	58 39	27.30±5.80 28.90±6.00	27.70 28.50	2,000 IU/day or 4,000 IU/day	16	N/A	4
Jafari et al. [31]	2016	Iran	DB-RCT	59	D group C group	57.80±5.50 56.80±5.70	N/A N/A	28.00±0.82 29.30±0.72	27.84±0.80 29.56±0.76	2,000 IU/day	12	N/A	4
Al-Sofiani et al. [32]	2015	USA	DB-RCT	20	D group C group	48.00 (21.00–75.00) ^c	N/A N/A	28.80 (26.70–30.88) ^e 33.35 (27.34–35.56) ^e	28.33 (27.24–31.58) ^e 33.13 (27.42–36.44) ^e	5,000 IU/day	12	N/A	4
Akbarzadeh et al. [33]	2013	Iran	DB-RCT	70	D group C group	53.80±8.90 52.40±7.80	N/A N/A	28.30±4.40 27.00±3.40	N/A N/A	20 IU/day	12	N/A	3
Neyestani et al. [34]	2012	Iran	RCT	90	D group D group C group	51.50±5.40 49.90±6.20 50.80±6.70	N/A N/A N/A	29.20±4.40 29.10±5.50 29.90±4.70	N/A N/A N/A	500 IU 500 IU	12	Calcium	3

The data are expressed as the mean ± SD unless otherwise indicated.

* The statistically significant changes ($p < 0.05$); ^a number of subjects who completed the study; ^b D group: group receiving vitamin D supplementation; C group: group receiving matching placebo; ^c median and interquartile range (or range); ^d changes (value at the end of study minus value at baseline); ^e median (25th, 75th percentile); ^f this study was divided into 2 phases of vitamin D intervention (D group-1 and D group-2). N/A, not available; BMI, body mass index; RCT, randomized controlled trial; DB-RCT, double-blind randomized controlled trials.

Table 2. Mean changes in 25(OH)D₃, FPG, HOMA-IR, plasma hs-CRP, TNF-α, and IL-6 concentrations included in this meta-analysis

Study	Analysed groups ^a	25(OH)D ₃ , ng/mL		FBG, mg/dL		HOMA-IR		hs-CRP, μg/mL		TNF-α, pg/mL		IL-6, pg/mL	
		baseline	intervention	baseline	intervention	baseline	intervention	baseline	intervention	baseline	intervention	baseline	intervention
Breslavsky et al. [22]	D group	11.80±10.90	17.60±11.50*	144.30±74.20	155.90±55.00	4.20±4.20	6.10±5.30	5.00±0.50	4.00±0.40	N/A	N/A	N/A	N/A
	C group	11.70±6.50	14.00±5.90	147.60±65.60	150.40±63.50	6.00±8.60	12.20±22.50	5.00±0.40	5.00±0.50	N/A	N/A	N/A	N/A
Farrokhi et al. [23]	D group	16.90±3.60	34.50±10.90*	120.90±73.50	105.80±57.60	4.50±3.60	3.80±3.20*	5.90±8.60	5.10±8.50*	N/A	N/A	N/A	N/A
	C group	16.60±4.60	17.00±4.50	116.60±40.90	136.20±42.60*	4.50±2.80	5.00±2.90	5.90±4.90	6.40±5.60	N/A	N/A	N/A	N/A
Asemi et al. [24]	D group	15.00±8.60	21.40±7.30*	134.90±49.70	140.70±56.20	4.40±2.40	4.00±2.40	4.00±4.00	2.70±2.30*	N/A	N/A	N/A	N/A
	C group	13.00±6.30	13.40±6.50	122.20±46.90	145.50±70.40*	5.30±3.50	6.00±3.90	5.90±5.20	6.30±5.90	N/A	N/A	N/A	N/A
Tuomainen et al. [25]	D group	22.90±4.40	11.10±6.90 ^b *	109.80 (12.60) ^c	−1.80±5.40	1.60±0.80	0.09±0.33	1.20 (1.50) ^c	−0.10 (1.40) ^c	2,240 (480) ^c	−15.80 (265.10) ^c	1.30 (0.80) ^c	0.04 (0.70) ^c
	D group		18.00±9.40 ^b *		−3.60±6.00		0.03±0.99		−0.60 (1.00) ^c		42.50 (312.70) ^c		−0.10 (0.60) ^c
	C group		1.64±6.90 ^b		−1.80±7.20		0.13±0.71		−0.01 (2.60) ^c		46.50 (387.50) ^c		−0.20 (0.60) ^c
Sadiya et al. [26] ^e	D group-1	11.40±3.70	30.90±12.00*	167.40±50.40	176.40±55.80	N/A	N/A	7.40±7.60	7.80±10.80	N/A	N/A	N/A	N/A
	D group-2		24.90±8.30*		171.00±52.20	N/A	N/A		8.00±9.50				
	C group-1	12.20±4.50	11.50±5.20	160.20±57.60	165.60±61.20	N/A	N/A	8.00±9.20	9.50±10.40	N/A	N/A	N/A	N/A
	C group-2		10.20±4.70*		174.60±66.60	N/A	N/A		10.00±13.00				
Gagnon et al. [27]	D group	18.80±5.20	95.00 (89.00–101.00) ^c	93.60±9.00	N/A	N/A	N/A	5.52±10.82	3.62±5.68	6.31±2.18	6.60±1.43	1.20 (1.20–2.10) ^d	1.30 (1.20–1.80) ^d
	C group	17.20±5.20	N/A	99.00±10.80	N/A	N/A	N/A	2.44±2.15	2.57±2.66	6.70±2.43	7.10±2.27	1.80 (1.40–2.40) ^d	1.90 (1.40–2.80) ^d
Tabesh et al. [28]	D group	11.20±5.60	35.10±14.30*	N/A	N/A	N/A	N/A	2.30±1.60	−0.48±0.40	11.50±6.30	−2.10±2.30*	7.90±6.00	−5.00±1.00*
	D group	12.20±6.60	35.40±9.60*	N/A	N/A	N/A	N/A	1.90±1.10	−0.75±0.36	13.00±8.60	−5.00±1.60*	7.50±5.10	−5.00±1.00*
	C group	18.30±6.6	19.30±7.70	N/A	N/A	N/A	N/A	2.20±1.00	−0.06±0.26	10.20±3.60	0.26±1.30	6.70±3.00	3.00±1.00
Ghavamzadeh et al. [29]	D group	8.60±1.86	18.60±2.76*	N/A	N/A	N/A	N/A	N/A	N/A	10.43±1.12	4.89±1.24*	N/A	N/A
	C group	8.90±2.13	8.40±2.90	N/A	N/A	N/A	N/A	N/A	N/A	11.91±1.03	10.26±1.46	N/A	N/A
Dalan et al. [30]	D group	18.00±7.00	14.00±9.60 ^b *	N/A	N/A	N/A	N/A	1.60 (2.10) ^c	0.20 (0.80) ^{b,c}	N/A	N/A	N/A	N/A
	C group	17.00±11.00	0.40±4.30 ^b	N/A	N/A	N/A	N/A	1.80 (3.20) ^c	0.10 (1.80) ^{b,c}	N/A	N/A	N/A	N/A
Jafari et al. [31]	D group	24.95±1.81	34.82±1.95*	168.80±4.48	166.67±5.45	3.23±0.50	2.13±0.20*	1.32±0.19	0.98±0.04	N/A	N/A	N/A	N/A
	C group	25.15±1.71	22.50±1.16	167.48±2.78	170.62±2.66*	3.58±0.42	4.72±0.44*	1.07±0.05	1.21±0.10	N/A	N/A	N/A	N/A
Al-Sofiani et al. [32]	D group	10.17 (8.90–11.60) ^d	36.50 (29.90–39.80) ^{d,*}	169.20 (147.60–198.00) ^d	160.20 (111.60–198.00) ^d	3.17 (2.56–5.41) ^d	4.13 (2.30–6.83) ^d	N/A	N/A	53.87 (51.46–62.27) ^d	51.46 (47.83–57.48) ^d	N/A	N/A
	C group	15.50 (9.50–15.90) ^d	11.80 (9.30–13.70) ^d	144.90 (122.40–176.40) ^d	135.00 (117.00–147.60) ^{d,*}	3.82 (2.48–4.94) ^d	3.72 (2.76–4.87) ^d	N/A	N/A	59.28 (55.07–70.63) ^d	52.66 (47.83–59.88) ^d	N/A	N/A
Akbarzadeh et al. [33]	D group	43.28±32.10	34.10±31.50*	N/A	N/A	N/A	N/A	1.72±2.40	1.98±2.00	N/A	N/A	5.05±3.80	2.96±1.20
	C group	38.54±29.90	30.92±22.20*	N/A	N/A	N/A	N/A	1.93±1.80	2.11±1.80	N/A	N/A	4.75±3.80	3.24±2.10

Table 2. (continued)

Study	Analysed groups ^a	25(OH)D ₃ , ng/mL		FBG, mg/dL		HOMA-IR		hs-CRP, µg/mL		TNF-α, pg/mL		IL-6, pg/mL	
		baseline	intervention	baseline	intervention	baseline	intervention	baseline	intervention	baseline	intervention	baseline	intervention
Neyestani et al. [34]	D group	17.70±11.50	31.10±11.40*	N/A	N/A	3.30±1.80	2.70±1.50*	3.30±4.50	1.90±2.10	820.40±485.40	707.40±352.60	174.80±76.40	115.80±9.70
	D group	17.80±17.50	29.80±15.80*	N/A	N/A	3.70±3.30	3.00±1.50	5.70±9.40	1.60±1.50	794.40±689.80	755.60±406.50	155.50±61.20	116.00±25.50
	C group	16.60±17.80	14.90±17.60	N/A	N/A	3.40±1.50	5.50±3.70*	3.70±5.70	2.90±4.40	616.60±234.10	797.30±329.80*	153.60±69.50	138.10±49.70

The data are expressed as the mean ± SDs unless otherwise indicated.

* The statistically significant changes ($p < 0.05$).

^a D group: group receiving vitamin D supplementation; C group: group receiving matching placebo.

^b Changes (value at the end of study minus value at baseline).

^c Median and interquartile range (or range).

^d Median (25th, 75th percentile).

^e This study was divided into 2 phases of vitamin D intervention (D group-1 and D group-2).

25(OH) D₃, 25-hydroxyvitamin D₃; FBG, fasting plasma glucose; HOMA-IR, homeostasis model assessment-insulin resistant; hs-CRP, high-sensitivity C-reactive protein; TNF-α, tumor necrosis factor-α; IL-6, interleukin-6; N/A, not available.

D intervention group between the baseline and the end of the trials (Table 2). Moreover, there were 3 trials that showed that vitamin D supplementation have a significant reduction of HOMA-IR compared with the baseline (Table 2).

Effect of Vitamin D Supplementation on hs-CRP, TNF-α, and IL-6

Changes in hs-CRP concentration after vitamin D supplementation were assessed in eleven studies. Two out of the eleven RCTs reported reduced circulating hs-CRP concentration after the vitamin D supplementation [23, 24] (Table 2). Since there was evidence of heterogeneity ($p < 0.00001$, $I^2 = 78\%$), the random-effect model was applied. The vitamin D supplementation had a significant effect on hs-CRP concentration (standard mean differences, -0.45 [95% CI -0.77 to -0.14], $p = 0.005$; Fig. 2a). This result indicated that supplemental vitamin D significantly decreased the circulating hs-CRP concentration.

In 6 publications, the effect of vitamin D supplementation on TNF-α concentration was assessed. After the intervention period, the mean TNF-α concentration decreased in 6 included studies, but an opposite trend was noted in the control group in 4 studies [25, 27, 28, 34], other 2 studies [29, 32] had similar trends in the control group. Nevertheless, only in 2 studies, the changes of TNF-α between the intervention group and the control group in the end of the intervention was statistically significant [28, 29] (Table 2). The random-effect model was used because of the evidence of heterogeneity ($p < 0.00001$, $I^2 = 91\%$). The result showed no significant effect of vitamin D supplementation on plasma TNF-α concentration (standard mean differences, -0.75 [95% CI -1.50 to 0.01], $p = 0.005$; Fig. 2b) compared with that of the control group.

The effect of vitamin D supplementation on plasma IL-6 concentration was evaluated in 5 studies. After the intervention period, the mean concentration of IL-6 decreased in subjects who received vitamin D supplementation in 3 selected studies [28, 33, 34] and unchanged in 2 studies. Moreover, only one study showed significant differences, and there was no statistical difference in other studies (Table 2). Considering the evidence of heterogeneity ($p < 0.00001$, $I^2 = 94\%$), the random-effects model was applied. The result did not show any significant effect of vitamin D supplementation on plasma concentration of IL-6 (standard mean differences, -0.48 [95% CI -1.36 to 0.41], $p = 0.29$; Fig. 2c) compared with that of the control group.

Fig. 2. Forest plot of the random-effect meta-analysis of change in plasma hs-CRP concentration (**a**), plasma TNF- α concentration (**b**), plasma IL-6 concentration (**c**) according to the vitamin D supplementation shown as pooled standard differences in the means with 95% CIs. The horizontal lines denote the 95% CIs. The square

represents the point estimate of each study. The diamond represents the overall pooled estimate of the treatment effect. hs-CRP, high-sensitivity C-reactive protein; TNF- α , tumor necrosis factor- α ; IL-6, interleukin-6.

Sensitivity Analysis

Because of the high heterogeneity in hs-CRP, TNF- α , and IL-6 status, simple sensitivity analyses were counted by examining the impact of a single trial on the pooled effect. In the sensitivity analyses, omitting the trial by Tabesh et al. [28] resulted in a reduction of 1.64 $\mu\text{g/mL}$ (95% CI -2.06

to -1.22) in hs-CRP status, there was a significant effect on hs-CRP levels in the overall effect (standard mean differences, -0.31 [95% CI -0.49 to -0.13], $p = 0.0006$). Meanwhile, the trial by Tabesh et al. [28] also resulted in a reduction of 2.12 pg/mL (95% CI -2.57 to -1.66) in IL-6 status, and there was no significant difference in the overall results

Table 3. Results of subgroup analyses according to trial or participant characteristics

Inflammatory biomarkers	Group	Number	Net change (95% CI)	<i>p</i> (heterogeneity)	<i>I</i> ² , %	<i>p</i> (overall effect)	<i>p</i> (intergroup)
hs-CRP	Total	11	−0.45 (−0.77 to −0.14)	<0.00001	78	0.005	0.48
	Vitamin D dosage		–	–	–	–	
	≤4,000 IU/day	8	−0.34 (−0.56 to −0.12)	0.14	36	0.003	
	>4,000 IU/day	3	−0.70 (−1.67 to 0.27)	<0.00001	93	0.16	0.40
	Intervention duration		–	–	–	–	
	≤12 weeks	5	−0.60 (−1.22 to 0.03)	<0.00001	88	0.06	
TNF-α	>12 weeks	6	−0.31 (−0.54 to −0.08)	0.27	22	0.008	0.23
	Total	6	−0.75 (−1.50 to 0.01)	<0.00001	91	0.05	
	Vitamin D dosage		–	–	–	–	
	≤4,000 IU/day	3	−1.32 (−2.84 to 0.21)	<0.00001	95	0.09	0.50
	>4,000 IU/day	3	−0.24 (−1.11 to 0.62)	0.0009	86	0.58	
	Intervention duration		–	–	–	–	
IL-6	≤12 weeks	3	−0.50 (−1.25 to 0.26)	0.005	81	0.20	0.10
	>12 weeks	3	−1.10 (−2.67 to 0.47)	<0.00001	96	0.17	
	Total	5	−0.48 (−1.36 to 0.41)	<0.00001	94	0.29	
	Vitamin D dosage		–	–	–	–	0.58
	≤4,000 IU/day	3	−0.18 (−0.46 to 0.10)	0.36	1	0.20	
	>4,000 IU/day	2	−0.88 (−3.31 to 1.55)	<0.00001	98	0.48	
	Intervention duration		–	–	–	–	0.10
	≤12 weeks	3	−0.91 (−2.16 to 0.33)	<0.00001	95	0.15	
	>12 weeks	2	0.15 (−0.20 to 0.51)	0.30	8	0.39	

hs-CRP, high-sensitivity C-reactive protein; TNF-α, tumor necrosis factor-alpha; IL-6, interleukin-6.

(standard mean differences, −0.06 [95% CI −0.39 to 0.27], $p = 0.71$). None of the other trials could substantially influence the overall effect. Of note, when the trial of Tabesh was removed, improved heterogeneity across the studies ($p = 0.24$, $I^2 = 22\%$; $p = 0.15$, $I^2 = 43\%$, respectively) was observed, suggesting that this trial was the main source of heterogeneity in hs-CRP and IL-6 status. In addition, we found a trial by Ghavamzadeh et al. [29] resulted in a reduction of 3.22 pg/mL (95% CI −4.07 to −2.37) in TNF-α status; the overall effect has no significant difference (standard mean differences, −0.34 [95% CI −0.85 to 0.16], $p = 0.19$). However, when the trial of Ghavamzadeh et al. [29] was removed, evidence of heterogeneity ($p = 0.0007$, $I^2 = 79\%$) was observed, suggesting that the trial was only a small part of the heterogeneity, there may be other factor was the main source of heterogeneity in TNF-α status.

Subgroup Analysis

Evidence of heterogeneity was also observed within subgroups (Table 3). Of note, there was no evidence of heterogeneity in the low dosage of vitamin D supplement and major cycle of vitamin D intervention for the hs-CRP and IL-6. In addition, vitamin D supplementation lowered hs-CRP

by 0.34 µg/mL (95% CI −0.56 to −0.12) among trials with a daily vitamin D dosage ≤4,000 IU and by 0.31 µg/mL (95% CI −0.54 to −0.08) among trials with vitamin D intervention duration >12 weeks ($p < 0.05$), but there was no statistical difference compared with that of their counterpart. Subgroup analysis showed that the dosage of vitamin D supplementation and duration of vitamin D intervention had no significant effects on TNF-α and IL-6 concentration.

Publication Bias

The funnel plot was used to show the evidence of publication bias in hs-CRP, TNF-α, and IL-6 and the effects were plotted against their standard error in the funnel plot (Fig. 3). According to funnel plot, the publication bias was observed in these selected inflammatory biomarkers.

Discussion

Our primary result showed that supplementation with vitamin D was beneficial for the reduction of circulating hs-CRP but did not have a significant influence on TNF-α and IL-6 in the T2DM subjects.

Fig. 3. Funnel plot of standard error by standard differences in means of plasma hs-CRP concentration (a), plasma TNF- α concentration (b), plasma IL-6 concentration (c), in selected randomized trials. hs-CRP, high-sensitivity C-reactive protein; TNF- α , tumor necrosis factor-alpha; IL-6, interleukin-6.

Recently, it has been shown that there is a relationship of supplementation with vitamin D and glycemic control in adults [35]. In our analysis, we have focused on the common evaluated inflammatory biomarkers that might help to describe the relationship between vitamin D and T2DM. Our findings for reducing circulating hs-CRP by vitamin D supplementation are biologically plausible. Studies have suggested that there was a cross relationship between the inflammatory signaling pathway and the insulin receptor posterior signaling pathway [36]. The low level of chronic inflammatory response induced by nuclear factor kappa B pathway (NF- κ B) and the inhibitor kinase interference of insulin signaling pathway are important pathogenesis of T2DM. Another mechanism related to vitamin D and hs-CRP may involve the NF- κ B-dependent transcriptional activation. The NF- κ B activation participates in endogenous hs-CRP induction and activated NF- κ B may enhance the effects of activator of transcription-3 (STAT3) [37]. The STAT3 signaling is

downregulated by 25(OH) D₃ [38]. Thus, vitamin D supplementation could reduce the hs-CRP in T2DM patients by NF- κ B and STAT3 signaling.

The findings of this meta-analysis did not show any statistically significant impact of vitamin D supplementation on TNF- α and IL-6. Both TNF- α and IL-6 have been considered biomarkers of systemic inflammation [39, 40]. The vitamin D supplementation on circulating biomarkers (TNF- α , IL-6) in T2DM patients were reported to be decreased in the clinical studies [28, 29]. Vitamin D₃ could inhibit NF- κ B activity by increasing I κ B expression and was also found to suppress the production of TNF- α [41]. However, data from the human studies seemed to be inconsistent. Zittermann et al. [42] supported the idea that the high concentration of 25(OH) D₃ seemed to be necessary to maintain adequate concentration of calcitriol, which could also be suppressed by TNF- α . Whereas, Beilfuss et al. [43] observed that there was no influence on TNF- α after 52 weeks of vitamin D supplementation,

which was consistent with our findings. Vitro studies showed that the vitamin D might inhibit the production of IL-6 [44–46] and the vitamin D could be directed against IL-6 to reduce the incidence of T2DM [47]. Unfortunately, within this analysis, we did not find evidence of changes in TNF- α and IL-6 concentrations in T2DM subjects that could strongly support the merit of vitamin D supplementation.

Subgroup analysis results indicated the daily vitamin D supplementation dosage and intervention time could influence the hs-CRP changes. Food and Nutrition Board, Institute of Medicine considered that the upper level for vitamin D among people who are older than 9 years of age is 4,000 IU/day. Our results have found that the hs-CRP in T2DM patients with vitamin D dosage $\leq 4,000$ IU/day reduced compared with the vitamin D $> 4,000$ IU/day. But so far, no studies have determined a more appropriate dose of vitamin D supplements on hs-CRP. Therefore, more clinical study and animal experiments will be conducted to verify the appropriate vitamin D dosage to produce measurable biological effects on hs-CRP in humans. Moreover, in the subgroup analysis, there were no significant effects on TNF- α and IL-6 status. It might be due to the fact that few RCTs of vitamin D supplementation and TNF- α and IL-6 and the data were insufficient. Therefore, more RCTs will be conducted to analyze the relationship between vitamin D supplementation and TNF- α and IL-6 in subjects with T2DM.

There were additional other limitations existing in this meta-analysis. Heterogeneity was the primary one across studies that made our findings complicated to interpret. This was not strange, taking into account the difference in study designs, the dose of supplied vitamin D, and the intervention duration of the supplementation. However, sensitivity analyses and subgroup analyses in our study did not show that the supplemental dose of vitamin D and intervention duration together were the potential factors

influencing the pooled effect. We could not exclude other possible unmeasured confounders in this meta-analysis. In addition, the median and quartiles in some trials were converted into mean and their SDs for meta-analysis. The calculation for skewed distribution virtually reduced the precision. Finally, almost all of the trials were not designed to measure selected inflammatory biomarker level as a primary outcome.

Conclusion

In conclusion, our findings indicated the significantly favorable effect of vitamin D supplementation on the circulating hs-CRP concentration but no effect on the TNF- α and IL-6 concentration. However, the results should be interpreted with caution because of the evidence of heterogeneity. RCTs with long-term follow-up should be applied for future investigations to determine the effect of supplemental vitamin D on the inflammatory biomarkers in T2DM subjects.

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Disclosure Statement

The authors declare no conflicts of interest to disclose.

Authors Contribution

All the authors contributed to study conception and design, acquisition of data, analysis and interpretation of data, drafting of manuscript and critical revision.

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