



# Vitamin D improves levels of hormonal, oxidative stress and inflammatory parameters in polycystic ovary syndrome: a meta-analysis study

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**Background:** Hyperandrogenism (HA), inflammation, and oxidative damage play key roles in the pathophysiology of polycystic ovary syndrome (PCOS). Whether vitamin D adjuvant therapy improves hormonal, inflammation, and oxidative damage in PCOS patients has aroused widespread interest, but the results are controversial. To evaluate the effect of vitamin D supplementation on hormonal, oxidative stress, and inflammatory parameters in patients with PCOS.

**Methods:** A literature search was conducted in Medline, the Cochrane Library, EMBASE, and Web of Science for studies related to PCOS and vitamin D supplementation. All reports of randomized controlled trials (RCTs) published before December 2019 were identified. The fixed-effects model or random-effects model was used to calculate pooled estimates of standardized differences in means (SMD) with 95% confidence intervals (CI).

**Results:** A total of 956 identified studies were retrieved, and eighteen RCTs involving 1,060 participants were ultimately included in the current meta-analysis. The pooled results demonstrated that vitamin D supplementation in patients with PCOS resulted in a significant improvement in serum total testosterone (TT), high sensitivity C-reactive protein (hs-CRP), total antioxidant capacity (TAC), and malondialdehyde (MDA). No significant effect on free testosterone (FT), dehydroepiandrosterone sulfate (DHEA-S), sex hormone-binding globulin (SHBG), free androgen index (FAI), nitric oxide (NO), and total glutathione (GSH) levels was found. Subgroup analysis showed that vitamin D supplementation reduced hs-CRP and MDA irrespective of the treatment course, type of vitamin D intervention, supplementation frequency, and dosage. Twelve weeks of vitamin D supplementation improved TT and TAC while low-dose vitamin D supplementation ( $\leq 1,000$  IU/day) improved TT and DHEA-S. Vitamin D co-supplementation reduced TT, FT, and DHEA-S, while a daily supplementation regime improved TT, DHEA-S, and TAC in patients with PCOS.

**Conclusions:** The current meta-analysis demonstrates that vitamin D supplementation in patients with PCOS resulted in an improvement in the levels of TT, hs-CRP, TAC, and MDA, but did not affect FT, DHEA-S, SHBG, FAI, NO, and GSH levels. Vitamin D co-supplementation, low-dose vitamin D supplementation ( $\leq 1,000$  IU/day), and daily supplementation frequency appeared to be more conducive to improving hormones, inflammation, and oxidative stress in PCOS patients.

**Keywords:** Vitamin D; polycystic ovary syndrome (PCOS); hormonal; oxidative stress; inflammation; meta-analysis

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## Introduction

Polycystic ovary syndrome (PCOS) is the most common reproductive endocrine disease in women, with a rate of 5–20% (1). It is characterized by hyperandrogenism (HA), polycystic ovary, ovulation dysfunction, and metabolic disorders. It can also increase the risk of mood disorders, cardiovascular and cerebrovascular diseases, type 2 diabetes, venous thromboembolism, pregnancy diseases, endometrial cancer, and other diseases (1-3). The pathogenesis of PCOS has not been fully elucidated, but current studies have shown that HA, inflammation, and oxidative damage jointly play a vicious cycle in the pathophysiology of the disease (4-8).

Vitamin D deficiency (VDD), which can aggravate HA and insulin resistance, is very common in patients with PCOS, the prevalence rate being as high as 67–85% (9-11). Numerous animal studies have shown that vitamin D can reduce inflammatory reactions and regulate the physiological balance between oxidation and antioxidants (12-15). However, based on clinical randomized controlled trials (RCTs) (16-33), the effects of vitamin D supplementation on hormone levels, inflammation, and oxidative stress in patients with PCOS are still controversial. The current systematic review and meta-analysis aimed to explore the efficacy of vitamin D supplementation at improving hormone metabolism, inflammation, and oxidative stress levels in patients with PCOS. These studies will play an indispensable role in developing new or adjuvant therapies to treat PCOS in patients. We present the following article in accordance with the PRISMA reporting checklist (available at <http://dx.doi.org/10.21037/apm-20-2201>).

## Methods

### Protocol and registration

This meta-analysis was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines (34). This study was registered with PROSPERO (Registration No. 42019129803).

### Search strategy

Two authors (Junfeng Zhao and Baixue Li) independently searched databases including Medline, the Cochrane Library, EMBASE, and Web of Science until December 2019. The search was conducted with keywords “polycystic ovary syndrome” or “PCOS” with any of the following keywords: “vitamin D”, “ergocalciferols”, “cholecalciferol”,

“25-hydroxyvitamin D2”, “hydroxycholecalciferols”, “calcifediol”, “calcitriol”, “24,25-dihydroxyvitamin D3” or “dihydrotachysterol”. Review articles and their cited studies were searched manually. There were no restrictions on language, publishing years, and publishing types.

### Selection criteria

All RCTs, double-blinded, single-blinded, or open-label trials and parallel or cross-over trials with follow-up time and sample size were included in this meta-analysis. Studies with PCOS women over 18 years old who were administered vitamin D in any dose and dosage form compared to placebo, positive drug, or blank control were included. The outcome indicators analyzed were levels or changes of serum hormones, oxidative stress, and inflammatory markers before and after vitamin D or placebo treatment.

Studies that were not RCTs were excluded. Animal studies, *in vitro* studies, and observational studies were excluded. Studies not assessing primary data, letters to the editor, conference papers, articles only available in abstract form, duplicate publications, and secondary research papers were all excluded.

### Data extraction

Two researchers (Junfeng Zhao and Baixue Li) independently reviewed and screened the literature according to the selection criteria and collected information using a pre-designed data extraction table. Any disagreements were resolved through a cross check, discussion, or consultation with the third researcher (Qi Zhang). Where original data was not reported, other information provided in the literature (such as CIS, P value, and interquartile spacing) was used to calculate the missing data according to the formula provided in the Cochrane manual (35). Where original data was not directly provided in the text or table, it was extracted from the figure. When data was incomplete, the relevant author was contacted, and/or a search was performed on the Clinical Register ([clinicaltrials.gov](http://clinicaltrials.gov)). The following contents were extracted from the selected literature: author, year of publication, study design, sample size, intervention vitamin D dose, course of treatment, average age, body mass index (BMI), serum 25 hydroxyvitamin D (25(OH)D).

Before and after the intervention, outcome indicators including total testosterone (TT), free testosterone (FT),

**Table 1** Risk of bias assessment in the included studies

Study	Other bias	Selective in reporting	Complete outcome data	Blinding of outcome assessment	Blinding of participants and personnel	Allocation concealment	Random sequence generation
Bonakdaran (16) 2012	U	U	L	L	U	U	U
Dravecka (17) 2016	U	U	L	L	H	U	L
Fatemi (18) 2017	L	L	L	L	L	U	L
Foroozanfard (19) 2015	L	L	L	L	L	L	L
Garg (20) 2015	U	U	L	L	L	U	L
Irani (21) 2015	U	U	H	L	L	U	U
Jafari-Sfidvajani (22) 2018	L	L	L	L	L	U	L
Jamilian (23) 2017	L	L	L	L	L	L	L
Jamilian (24) 2018	L	L	L	L	L	L	L
Javed (25) 2019	U	L	L	L	U	U	L
Maktabi (26) 2017	U	U	L	L	L	L	L
Maktabi (27) 2018	U	U	L	L	L	L	L
Nasri (28) 2018	U	L	L	L	L	U	U
Ostadmohammadi (29) 2019	L	L	L	L	L	L	L
Rahimi-Ardabili (30) 2013	L	L	L	L	L	L	L
Raja-Khan (31) 2014	L	L	L	L	L	L	L
Razavi (32) 2016	U	L	L	L	U	L	L
Trummer (33) 2018	U	L	H	L	L	L	L

H, high risk; L, low risk; U, unclear risk.

dehydroepiandrosterone sulfate (DHEA-S), sex hormone-binding globulin (SHBG), free androgen index (FAI), high sensitivity C-reactive protein (hs-CRP), nitric oxide (NO), total antioxidant capacity (TAC), malondialdehyde (MDA) and total glutathione (GSH)

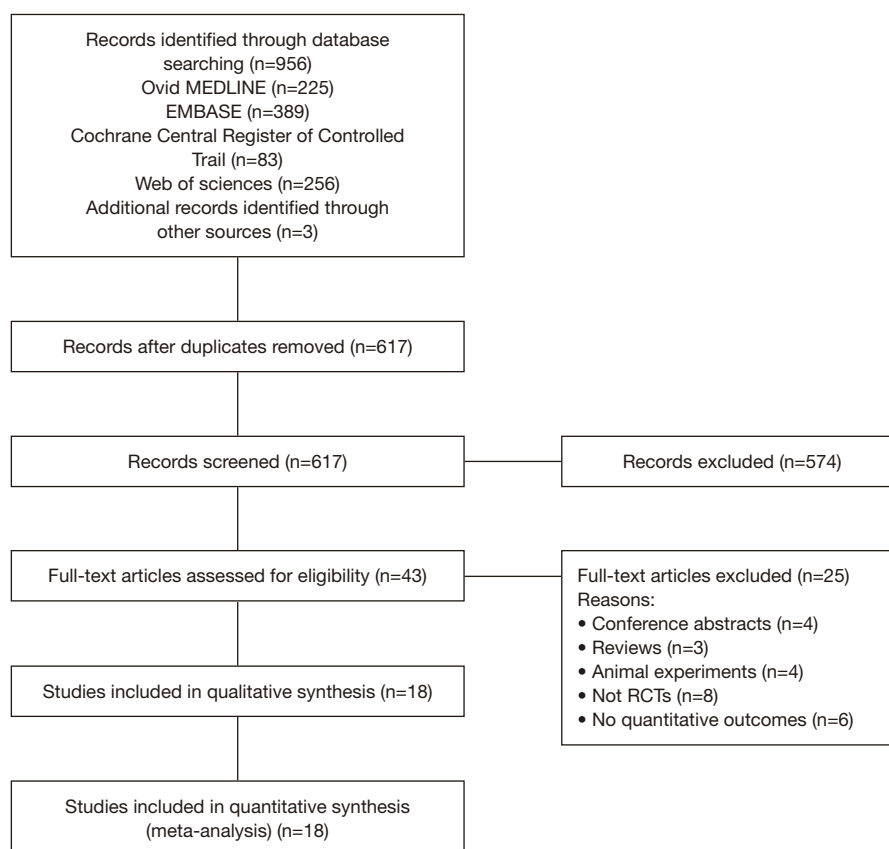
### Quality assessment

The quality of the included studies was reviewed according to the Cochrane system evaluation guide manual (35,36). The evaluation contents included random sequence generation, allocation concealment, blinding of outcome assessment, blinding of participants and personnel, incomplete outcome data, selective outcome reporting, and other bias. An answer of “yes” indicated a low risk of bias, an answer of “no” indicated a high risk of bias, and an answer of “unclear” indicated an uncertain risk of bias. The evaluation was conducted independently

by two investigators (Junfeng Zhao and Baixue Li), and any disagreements were resolved through discussion or consultation with a third investigator (Qi Zhang). The results of quality assessment are shown in *Table 1*.

### Statistical analysis

Data was analyzed using Revman 5.3 and Stata 12.0 software and the standardized mean difference (SMD) and 95% confidence interval (CI) of all outcome indicators were calculated. Q test and  $I^2$  test were used to analyze the heterogeneity. In studies with large heterogeneity ( $P < 0.10$  or  $I^2 > 50\%$ ), sensitivity analysis was used to determine the source of heterogeneity and evaluate the stability and reliability of the meta-analysis results. Subgroup analysis was performed examining Vitamin D dosage ( $\leq 1,000$  or  $> 1,000$  IU/day), intervention duration (8, 12 or 24 weeks), intervention type (vitamin D alone or with a co-supplement)



**Figure 1** Flow diagram of study selection.

and supplementation manner (daily intake or weekly intake). Funnel plot (Revman 5.3), Egger's and Begg's tests (Stata 12.0 software) were used to determine publication bias (heterogeneity considered present when  $P < 0.05$ ). Any publication bias was adjusted using the trim-and-fill method (37) (Stata 12.0 software), with  $P < 0.05$  interpreted as statistically different.

## Results

### *Search results and study characteristics*

The search flow chart is shown in *Figure 1*. A total of 956 literatures were retrieved and duplicates removed. After literatures were screened through reading titles and abstracts, 43 were selected for full-text reading. Of these, 25 were excluded and eighteen studies were finally included (16-33). *Table 2* summarizes the basic characteristics of the included studies. A total of 1,060 subjects were included in the eighteen studies, of which 573 were randomly assigned to

the vitamin D group, and the rest were in the control groups. Seventeen control group were placebo-controlled and one was a blank control group. Three studies combined vitamin D with calcium (19,27,32), one study combined vitamin D with vitamin E (18), one study combined vitamin D with polyunsaturated fatty acids (PUFA) (24), one study combined vitamin D with evening primrose oil (EPO) (28), and one study combined vitamin D with probiotics (29). In all other studies, patients were supplemented with vitamin D alone (16,19,21-23,25,26,30,31,33). In the study where metformin was used, it was administered to both the control and study group once a day for 20 weeks (20). The sample size across the studies ranged from 23 to 119, and the course of treatment ranged from 8 to 24 weeks. The average vitamin D supplement dose ranged from 20 to 12,000 IU/day.

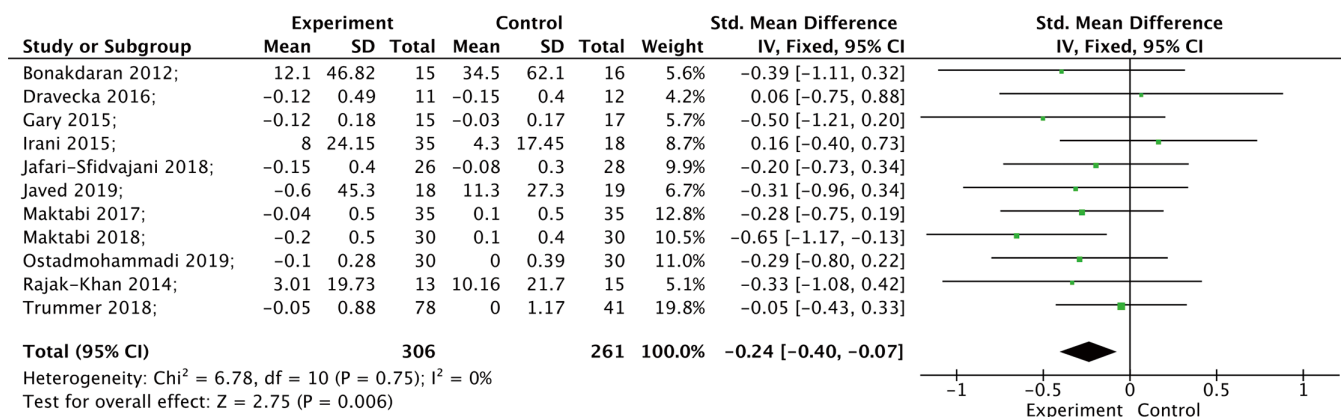
### *Results of quality assessment*

As shown in *Table 1*, Among the 18 studies (16-33), 8

Table 2 Characteristics of included studies

Study	No. of trials (n), VG/CG	Intervention (dose)	Control	Duration (weeks)	Age (year), mean ± SD, VG/CG	BMI (kg/m <sup>2</sup> ), mean ± SD VG/CG	Vitamin D (ng/mL), mean ± SD		Outcome indicators
							Baseline VG/CG	Final VG/CG	
Bonakdaran (16) 2012	15/16	Vitamin D (0.5 µg/day)	Placebo	12	24.7±3.3/25.2±7.9	24.8±5.3/25.3±5.1	11.4±8.2/19.9±16.5	20.1±16.2***/19.0±15.3	TT, DHEA-S
Dravecka (17) 2016	11/12	Vitamin D (1 µg/day) + Metformin	Metformin	24	29.2±5.4/27.6±4.9	33.1±7.1/29.8±5.4	22.0±8.4/24.3±6.8	23.2±8.2/23.5±6.5	TT, FT, DHEA-S, SHBG, FAI
Fatemi (18) 2017	44/46	Vitamin D (50,000 IU/2 week) + Vitamin E	Placebo	8	28.1±4.2/28.1±3.7	26.5±3.0/26.1±3.6	7.4±8.5/9.5±11.9	26±9.4/9.8±12.6	TAC, MDA
Foroozanfard (19) 2015	26/26/26	Vitamin D (50,000 IU/week) Vitamin D + Calcium (50,000 IU/week)	Placebo	8	NA	NA	NA	NA	hs-CRP, NO, TAC, GSH, MDA
Garg (20) 2015	15/17	Vitamin D (4,000 IU/day) + Metformin	Placebo+ Metformin	24	22.0±4.6/22.8±4.5	26.8±4.5/26.7±6.1	7.7±6.0/6.8±2.4	31.5±13.8***/6.7±2.3	TT, DHEA-S
Irani (21) 2015	35/18	Vitamin D (50,000 IU/week)	Placebo	8	30.5±1.0/29.6±1.7	30.0±1.0/28.0±1.6	NA	NA	TT, FT, DHEA-S, SHBG
Jafari-Sfidvajani (22) 2018	26/28	Vitamin D (50,000 IU/week)	Placebo	12	28.4±6.2/27.8±5.7	31.1±4.9/31.6±4.9	15.8±4.8/14.9±5.5	43.3±12.6***/25.3±10.1***	TT, DHEA-S, SHBG, FAI
Jamilian (23) 2017	30/30/30	Low-dose Vitamin D (1,000 IU/day) High-dose Vitamin D Placebo (4,000 IU/day)	Placebo	12	26.0±5.0/28.0±5.0/25.0±5.0	33.0±5.0/31.0±6.0/30.0±6.0	12.6±3.4/12.6±2.7/12.9±2.4	18.5±4.9***/24.6±3.3***/13.1±2.5	hs-CRP, NO, TAC, GSH, MDA
Jamilian (24) 2018	30/30	Vitamin D (50,000 IU/2 week) + PUFA	Placebo	12	26.8±4.4/25.1±3.7	27.1±7.0/27.4±3.9	12.1±3.1/12.4±2.7	21.9±6.3***/12.7±3.2	hs-CRP, NO, TAC, GSH, MDA
Javed (25) 2019	18/30	Vitamin D (3,200 IU/day)	Placebo	12	NA	35.4±10.6/33.8±7.2	25.6±11.4/30.9±11.1	90.4±19.5***/47.6±20.5***	TT, SHBG, FAI, hs-CRP
Maktabi (26) 2017	35/35	Vitamin D (25,000 IU/week)	Placebo	12	NA	NA	12.8±4.5/14.5±5.1	27.5±9.8***/14.4±5.2	TT, FT, DHEA-S, SHBG, FAI, hs-CRP, NO, TAC, GSH, MDA
Maktabi (27) 2018	30/30	Vitamin D (400 IU/day) + Calcium, magnesium and zinc Placebo	Placebo	12	23.8±5.7/24.8±4.8	24.0±3.7/25.5±4.9	10.1±4.9/10.8±4.6	18.0±10.3***/10.9±4.5	TT, SHBG, FAI, hs-CRP, NO, TAC, GSH, MDA
Nasri (28) 2018	30/30	Vitamin D (1,000 IU/day) + EPO Placebo	Placebo	12	26.4±8.1/25.4±4.7	26.4±4.7/26.9±4.9	15.9±2.9/14.6±2.1	26.7±7.8***/14.1±1.9	TAC, GSH, MDA
Ostadmohammadi (29) 2019	30/30	Vitamin D (25,000 IU/week) + Probiotics Placebo	Placebo	12	24.4±4.7/25.4±5.1	24.3±4.2/25.1±4.9	11.1±4.1/12.9±3.2	24.4±5.6***/13.3±2.7	TT, SHBG, hs-CRP, NO, TAC, GSH, MDA
Rahimi-Ardabili (30) 2013	24/26	Vitamin D (50,000 IU/20 day) Placebo	Placebo	12	26.8±4.7/27±3.7	29.1±4.62/28.3±3.5	6.9±2.80/7.3±2.9	23.4±6.14***/8.6±4.0	hs-CRP
Raja-Khan (31) 2014	13/15	Vitamin D (12,000 IU/day) Placebo	Placebo	12	28.2±5.2/28.7±5.6	37.2±4.5/35.0±9.8	19.9±9.4/22.2±6.8	67.3±28.6***/22.4±7.0	TT, FT, hs-CRP
Razavi (32) 2016	27/27	Vitamin D (400 IU/day) + Calcium Placebo	Placebo	8	NA	NA	14.4±2.9/14.6±5.5	19.7±3.1***/14.5±6.0	FT, DHEA-S, hs-CRP, NO, TAC, GSH, MDA
Trummer (33) 2018	78/41	Vitamin D (20,000 IU/week) Placebo	Placebo	24	25.4±4.6/27.2±5.5	20.2±7.8/19.9±7.3	15.6±5.3/15.6±5.6	28.7±6.4***/18.2±9.4*	TT, FT

BMI, body mass index; VG, vitamin D supplementation group; CG, control group; TT, total testosterone; DHEA-S, dehydroepiandrosterone sulfate; FT, free testosterone; SHBG, sex hormone binding globulin; FAI, free androgen index; TAC, total antioxidant capacity; MDA, malondialdehyde; hs-CRP, high sensitivity C-reactive protein; NO, nitric oxide; GSH, glutathione; NA, not provided; PUFA, omega-3 polyunsaturated fatty acids; EPO, evening primrose oil. \*P<0.05; \*\*P<0.01; \*\*\*P<0.001.



**Figure 2** Effects of vitamin D supplementation on total testosterone (TT).

quality assessment projects of 6 studies (19,23,24,29,30,31) were all low-risk, and 7 quality assessment projects of 3 studies (18,22,33) were low-risk. On the whole, the quality of literature included in this meta-analysis was relatively high.

### Meta-analysis

#### Effects of vitamin D supplementation on testosterone

Data on the effect of vitamin D supplementation on TT were reported in eleven studies involving 567 subjects (16,17,20-22,25-27,29,31,33). There was no statistical heterogeneity among the studies (P=0.75, I<sup>2</sup>=0%). Fixed effects model analysis demonstrated that vitamin D supplementation could significantly reduce serum TT (Figure 2, SMD =-0.24, 95% CI: -0.40, -0.07, P=0.006). Subgroup analysis showed that 12 weeks of vitamin D supplementation, vitamin D dose less than 1,000 IU/day, vitamin D combination therapy and daily intake could reduce TT in PCOS patients (Table 3).

#### Effects of vitamin D supplementation on high sensitivity C-reactive protein

Nine studies involving 559 subjects examined the effect of vitamin D supplementation on hs-CRP (19,23-27,29,30,32). There was no statistical heterogeneity among the studies (P=0.11, I<sup>2</sup>=36%). Fixed effects model analysis demonstrated that vitamin D supplementation could significantly reduce serum hs-CRP (Figure 3, SMD =-0.56, 95% CI: -0.73, -0.38, P<0.00001). Different course of treatment, type of vitamin D intervention, frequency and dose of vitamin D supplementation can reduce hs-CRP in patients with PCOS (Table 3).

#### Effects of vitamin D supplementation on TAC

Meta-analysis of nine studies (18,19,23,24,26-29,32) demonstrated that vitamin D supplementation could significantly increase serum TAC levels compared with the control group (Figure 4A, SMD =0.42, 95% CI: 0.01, 0.83, P=0.04). As there was high statistical heterogeneity (P<0.00001, I<sup>2</sup>=83%), random effects model analysis was used. Following removal of the two studies with high heterogeneity (18,28), there was less statistical heterogeneity between studies (P=0.10, I<sup>2</sup>=40). After using the fixed effects model, the difference was still statistically significant (Figure 4B, SMD =0.61, 95% CI: 0.42, 0.80, P<0.00001). Subgroup analysis showed that 12 weeks of vitamin D supplementation and daily administration frequency improved serum TAC in patients with PCOS (Table 3).

#### Effects of vitamin D supplementation on MDA

Nine studies reported the effect of vitamin D supplementation on MDA (18,19,23,24,26-29,32), with statistical heterogeneity among the studies (P=0.002, I<sup>2</sup>=63%). The results showed that compared with the control group, vitamin D supplementation treatment could significantly reduce serum MDA (Figure 5A, SMD =-0.59, 95% CI: -0.87, -0.32), P<0.00001). After removing the one study with high heterogeneity (18), there was no statistical heterogeneity among the studies (P=0.46, I<sup>2</sup>=0%). In the fixed effects model analysis, the results demonstrated that vitamin D supplementation significantly reduced serum MDA (Figure 5B, SMD =-0.72, 95% CI: -0.90, -0.54, P<0.00001). Subgroup analysis revealed that different courses of treatment, type of vitamin D intervention, frequency and dose of vitamin D supplementation could reduce MDA in PCOS patients (Table 3).

Table 3 Subgroup analysis

Variable	Intervention duration			Vitamin D dosage		Type of supplementation		Supplementation manner	
	8 week	12 week	24 week	≤1,000 IU/day	>1,000 IU/day	Vitamin D alone	Co-supplement	Daily intake	Weekly intake
TT	N=1, I <sup>2</sup> =– 0.16 (–0.40, 0.73) P=0.57	N=7, I <sup>2</sup> =0% –0.35 (–0.56, –0.14) P=0.001	N=3, I <sup>2</sup> =– –0.12 (–0.43, 0.19) P=0.45	N=3, I <sup>2</sup> =6% –0.43 (–0.81, –0.06) P=0.02	N=8, I <sup>2</sup> =0% –0.19 (–0.38, 0.00) P=0.05	N=9, I <sup>2</sup> =0% –0.17 (–0.36, 0.02) P=0.07	N=2, I <sup>2</sup> =0% –0.47 (–0.83, –0.10) P=0.01	N=6, I <sup>2</sup> =0% –0.41 (–0.68, –0.13) P=0.003	N=5, I <sup>2</sup> =0% –0.13 (–0.35, 0.08) P=0.22
FT	N=2, I <sup>2</sup> =93% –0.78 (–2.42, 0.86) P=0.35	N=2, I <sup>2</sup> =45% –0.04 (–0.61, 0.54) P=0.90	N=2, I <sup>2</sup> =54% 0.20 (–0.45, 0.85) P=0.55	N=2, I <sup>2</sup> =94% –0.51 (–2.74, 1.72) P=0.66	N=4, I <sup>2</sup> =0% 0.02 (–0.23, 0.27) P=0.89	N=5, I <sup>2</sup> =1% 0.05 (–0.19, 0.29) P=0.68	N=1, I <sup>2</sup> =– –1.63 (–2.25, –1.00) P<0.00001	N=3, I <sup>2</sup> =89% –0.49 (–1.79, 0.82) P=0.46	N=3, I <sup>2</sup> =0% 0.07 (–0.19, 0.33) P=0.60
DHEA-S	N=2, I <sup>2</sup> =79% –0.43 (–1.30, 0.43) P=0.33	N=3, I <sup>2</sup> =0% –0.08 (–0.40, 0.23) P=0.61	N=2, I <sup>2</sup> =0% –0.21 (–0.75, 0.32) P=0.43	N=3, I <sup>2</sup> =45% –0.48 (–0.86, –0.09) P=0.02	N=4, I <sup>2</sup> =0% –0.09 (–0.36, 0.19) P=0.54	N=6, I <sup>2</sup> =0% –0.09 (–0.34, 0.15) P=0.46	N=1, I <sup>2</sup> =– –0.87 (–1.43, –0.31) P=0.002	N=4, I <sup>2</sup> =22% –0.43 (–0.77, –0.10) P=0.01	N=3, I <sup>2</sup> =0% –0.05 (–0.35, 0.25) P=0.75
SHBG	N=1, I <sup>2</sup> =– –0.11 (–0.68, 0.46) P=0.71	N=5, I <sup>2</sup> =0% 0.12 (–0.12, 0.35) P=0.33	N=1, I <sup>2</sup> =– –0.33 (–1.16, 0.49) P=0.43	N=2, I <sup>2</sup> =21% 0.07 (–0.36, 0.50) P=0.75	N=5, I <sup>2</sup> =0% 0.05 (–0.19, 0.29) P=0.66	N=5, I <sup>2</sup> =0% –0.02 (–0.28, 0.24) P=0.9	N=2, I <sup>2</sup> =0% –0.20 (–0.16, –0.56) P=0.27	N=3, I <sup>2</sup> =0% 0.02 (–0.34, 0.38) P=0.93	N=4, I <sup>2</sup> =0% 0.08 (–0.18, 0.34) P=0.55
FAI	– – –	N=4, I <sup>2</sup> =0% 0.01 (–0.25, 0.27) P=0.94	N=1, I <sup>2</sup> =– –0.13 (–0.94, 0.69) P=0.76	N=2, I <sup>2</sup> =0% 0.01 (–0.42, 0.44) P=0.95	N=3, I <sup>2</sup> =0% –0.01 (–0.32, 0.30) P=0.94	N=2, I <sup>2</sup> =0% –0.03 (–0.32, 0.26) P=0.86	N=3, I <sup>2</sup> =0% 0.07 (–0.44, 0.57) P=0.80	N=3, I <sup>2</sup> =0% –0.05 (–0.41, 0.30) P=0.77	N=2, I <sup>2</sup> =34% 0.05 (–0.31, 0.40) P=0.79
Hs-CRP	N=3, I <sup>2</sup> =0% –0.51 (–0.87, –0.15) P=0.005	N=8, I <sup>2</sup> =52% –0.56 (–0.85, –0.27) P=0.0001	– – –	N=3, I <sup>2</sup> =1% –0.80 (–1.13, –0.47) P<0.00001	N=8, I <sup>2</sup> =36 –0.47 (–0.67, –0.26) P<0.00001	N=6, I <sup>2</sup> =35 –0.36 (–0.60, –0.12) P=0.004	N=5, I <sup>2</sup> =0% –0.76 (–1.01, –0.52) P<0.00001	N=5, I <sup>2</sup> =44% –0.63 (–0.90, –0.36) P<0.00001	N=6, I <sup>2</sup> =39% –0.50 (–0.73, –0.27) P<0.0001
NO	N=3, I <sup>2</sup> =0% –0.10 (–0.46, 0.25) P=0.56	N=6, I <sup>2</sup> =0% 0.17 (–0.05, 0.38) P=0.13	– – –	N=3, I <sup>2</sup> =0% 0.00 (–0.32, 0.32) P=1	N=6, I <sup>2</sup> =0% 0.14 (–0.09, 0.37) P=0.22	N=4, I <sup>2</sup> =0% 0.11 (–0.18, 0.39) P=0.48	N=5, I <sup>2</sup> =0% 0.09 (–0.15, 0.33) P=0.49	N=4, I <sup>2</sup> =0% 0.02 (–0.26, 0.30) P=0.89	N=5, I <sup>2</sup> =0% 0.15 (–0.10, 0.39) P=0.23
TAC	N=4, I <sup>2</sup> =75% 0.50 (–0.08, 1.08) P=0.09	N=7, I <sup>2</sup> =68% 0.48 (0.12, 0.85) P=0.009	– – –	N=4, I <sup>2</sup> =60% 0.41 (–0.02, 0.85) P=0.06	N=7, I <sup>2</sup> =89% 0.44 (–0.18, 1.06) P=0.17	N=4, I <sup>2</sup> =72% 0.57 (–0.01, 1.15) P=0.05	N=7, I <sup>2</sup> =88% 0.34 (–0.22, 0.91) P=0.24	N=5, I <sup>2</sup> =68% 0.56 (0.11, 1.01) P=0.02	N=6, I <sup>2</sup> =89% 0.31 (–0.34, 0.96) P=0.35
GSH	N=3, I <sup>2</sup> =56% 0.48 (–0.07, 1.03) P=0.09	N=7, I <sup>2</sup> =68% 0.11 (–0.25, 0.47) P=0.54	– – –	N=4, I <sup>2</sup> =69% 0.31 (–0.19, 0.80) P=0.23	N=6, I <sup>2</sup> =65% 0.15 (–0.24, 0.54) P=0.46	N=4, I <sup>2</sup> =0% –0.04 (–0.33, 0.25) P=0.52	N=5, I <sup>2</sup> =79% 0.40 (–0.14, 0.95) P=0.14	N=5, I <sup>2</sup> =61% 0.26 (–0.14, 0.66) P=0.2	N=5, I <sup>2</sup> =72% 0.17 (–0.30, 0.65) P=0.47
MDA	N=4, I <sup>2</sup> =62% –0.46 (–0.92, 0.00) P=0.05	N=7, I <sup>2</sup> =25% –0.71 (–0.94, –0.47) P<0.00001	– – –	N=4, I <sup>2</sup> =21% –0.72 (–1.03, –0.41) P<0.00001	N=7, I <sup>2</sup> =72% –0.54 (–0.93, –0.14) P=0.008	N=4, I <sup>2</sup> =0% –0.44 (–0.73, –0.15) P=0.003	N=7, I <sup>2</sup> =76% –0.70 (–1.11, –0.29) P=0.0009	N=5, I <sup>2</sup> =23% –0.65 (–0.94, –0.36) P<0.0001	N=6, I <sup>2</sup> =76% –0.57 (–1.03, –0.12) P=0.01

TT, total testosterone; FT, free testosterone; DHEA-S, dehydroepiandrosterone sulfate; SHBG, sex hormone binding globulin; FAI, free androgen index; hs-CRP, high sensitivity C-reactive protein; NO, nitric oxide; TAC, total antioxidant capacity; GSH, glutathione; MDA, malondialdehyde.

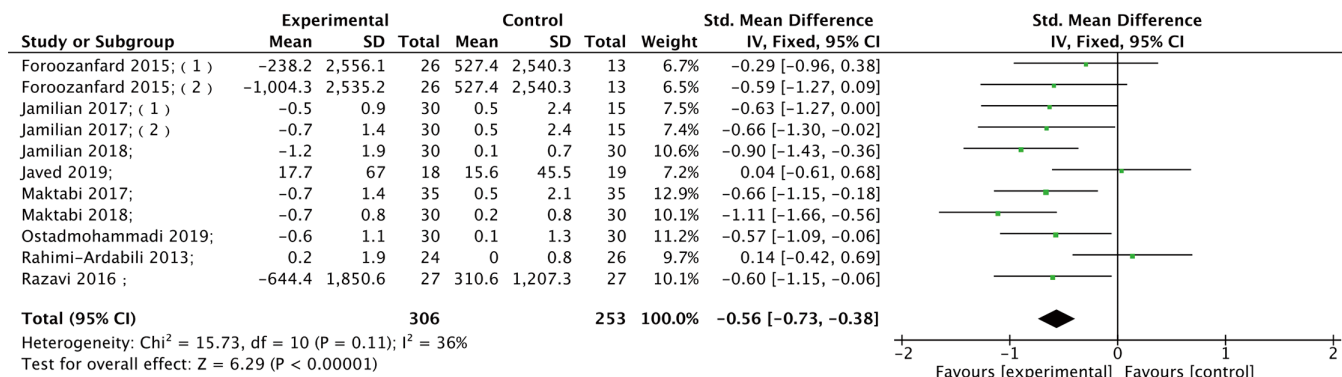


Figure 3 Effects of vitamin D supplementation on high-sensitivity C-reactive protein (hs-CRP).

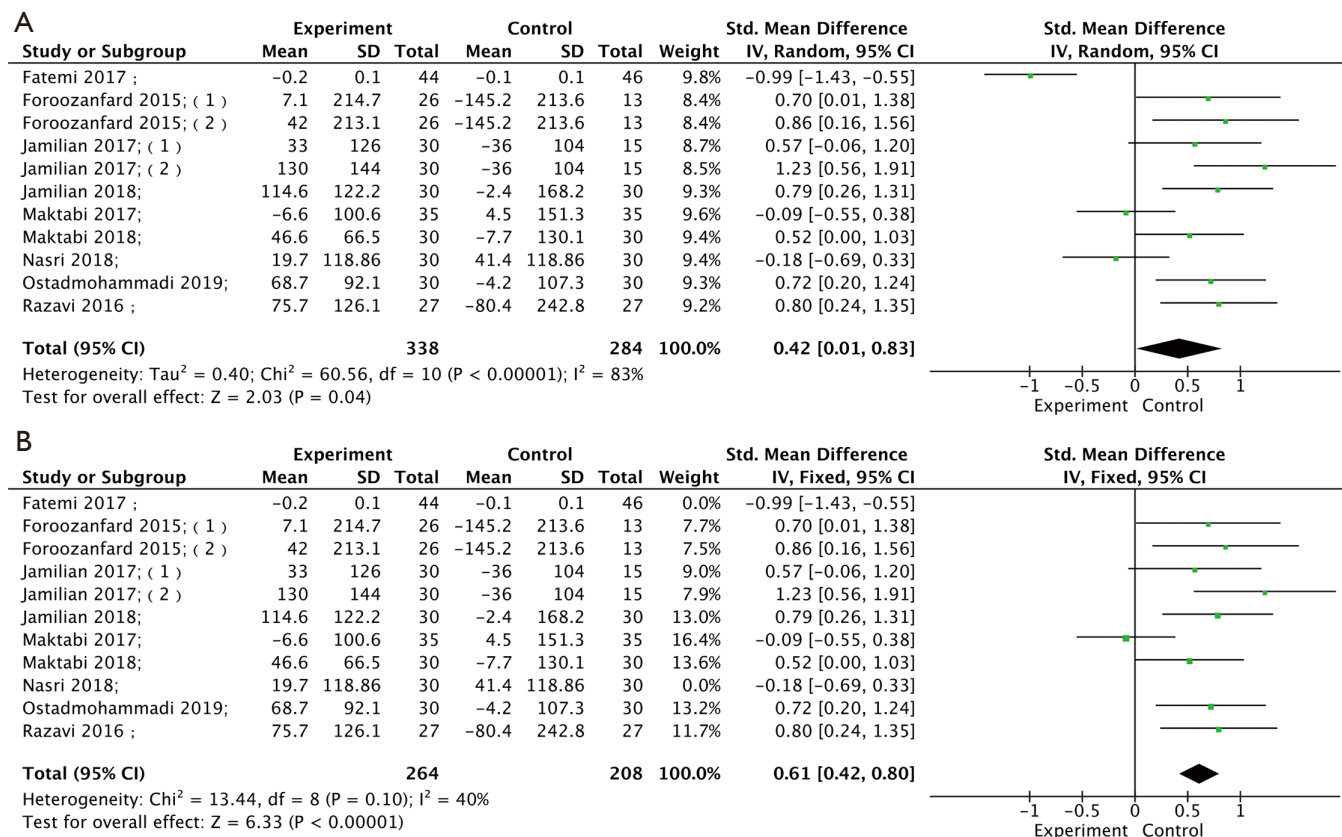


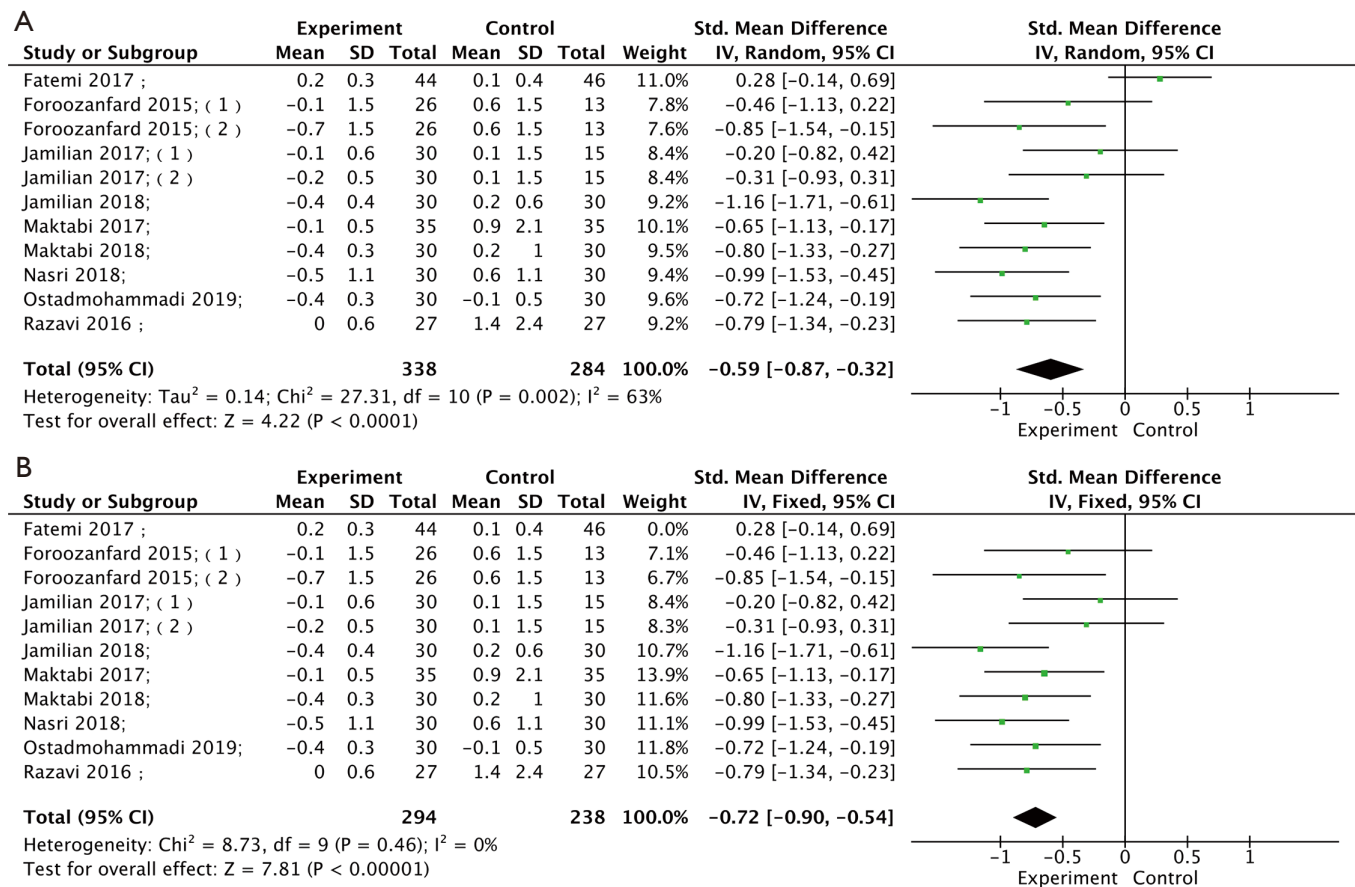
Figure 4 Effects of vitamin D on total antioxidant capacity (TAC). (A) Effects of vitamin D supplementation on TAC and (B) re-analyzed data of the effect of vitamin D supplementation on TAC.

**Effects of vitamin D supplementation on levels of FT, dehydroepiandrosterone sulphate, sex hormone binding globulin, FAI, NO and GSH**

As shown in Table 4, the effect of vitamin D on FT was evaluated in six studies (17,21,26,31-33). There was

no significant difference in FT between the vitamin D treatment and the control group (P=0.47). Subgroup analysis showed that vitamin D combined with calcium supplementation could reduce FT in PCOS patients (Table 3). The effect of vitamin D supplementation on





**Figure 5** Effects of vitamin D supplementation on malondialdehyde (MDA). (A) Effects of vitamin D supplementation on MDA and (B) re-analyzed data of the effect of vitamin D supplementation on MDA.

DHEA-S was reported in seven studies (16,17,20,22,26,32), and the effect on SHBG was also reported in seven studies (17,21,22,25,27,29). The fixed effects model analysis showed that compared with the control group, serum DHEA-S sulfate treatment demonstrated a downward trend, but there was no statistical significance (P=0.06). Vitamin D supplementation had no significant effect on improving SHBG (P=0.59). Subgroup analysis showed that vitamin D dose less than 1,000 IU/day, vitamin D combined with supplement therapy and daily administration frequency could reduce the level of DHEA-S in PCOS patients (Table 3). Five studies reported the effect of vitamin D supplementation on FAI (17,22,25,26,27). The combined results demonstrated that vitamin D supplementation had no significant effect on FAI (P=0.99). Seven studies evaluated the effect of vitamin D on NO (19,23,24,26,27,29,32). Again, vitamin D supplementation had no significant effect on NO levels (P=0.32). Eight

studies reported the effect of vitamin D supplementation on GSH (19,23,24,26-29,32). The random effects model analysis showed no significant difference in GSH between the vitamin D treatment group and the control group (P=0.16).

### Heterogeneity analysis

There is some heterogeneity in the analysis of FT, TAC, GSH and MDA. The sensitivity analysis of the sources demonstrated that the heterogeneity mainly derived from the studies by Fatemi (18), Nasri (28) and Razavi (32). Therefore, the data was re-analyzed after excluding these major heterogeneity studies. The results of FT, TAC, GSH and MDA analysis before and after the removal of major heterogeneity sources did not change (TAC: Figure 4B; MDA: Figure 5B; FT and GSH: Table 4). In the above three studies vitamin D treatment was combined with a co-

**Table 4** Effects of vitamin D supplementation on FT, DHEA-S, SHBG, FAI, NO and GSH levels

Variable	Model for meta-analysis	Subject (n)	No. of trials	Effect size (95% CI) mg/dL	P value	I <sup>2</sup> (%)	Q-statistics (P)
Total results							
FT	RE	6	347	0.20 (-0.76, 0.35)	0.47	82	<0.0001
DHEA-S	FE	7	317	-0.22 (-0.44, 0.01)	0.06	13	0.330
SHBG	FE	7	357	0.06 (-0.15, 0.27)	0.59	0	0.900
FAI	FE	5	244	0.00 (-0.25, 0.25)	0.99	0	0.680
NO	FE	7	472	0.09 (-0.09, 0.28)	0.32	0	0.950
GSH	RE	8	532	0.21 (-0.09, 0.51)	0.16	65	0.002
Results after removing the main heterogeneity sources							
FT	FE	5	293	0.05 (-0.19, 0.29)	0.68	0	0.400
GSH	FE	7	472	0.08 (-0.11, 0.26)	0.43	45	0.070

FE, fixed effect model; RE, random effect model; FT, free testosterone; DHEA-S, dehydroepiandrosterone sulfate; SHBG, sex hormone binding globulin; FAI, free androgen index; NO, nitric oxide; GSH, glutathione.

supplement (18,28,32). The vitamin D dose administered fluctuated greatly from 400 to 7,143 IU/day. Also, subgroup analysis demonstrated that the heterogeneity of most subgroups was significantly reduced (*Table 3*). Therefore, we infer that the heterogeneity may come from different vitamin D supplements, treatment courses, intervention types and frequency. Also, differences in age, sex ratio, patient health status (such as baseline vitamin D levels, comorbidities, etc.) and study quality may also be additional sources of heterogeneity.

### Publication bias

We evaluated the publication bias of the outcome indicators with statistical significance. The funnel plot results are shown in *Figure 6*. Egger's and Begg's tests showed that vitamin D supplementation had no publication bias on TT (Begg's:  $P=0.533$  and Egger's:  $P=0.270$ ), hs-CRP (Begg's:  $P=0.276$  and Egger's:  $P=0.360$ ) and MDA (Begg's:  $P=0.640$  and Egger's:  $P=0.094$ ). However, publication bias was found on TAC (Begg's:  $P=0.020$ ). After adjusting for the bias by the trim-and-fill method, the total results of TAC did not change (before:  $SMD = 0.42$ , 95% CI: 0.01, 0.83,  $P=0.04$ ; after:  $SMD = 0.64$ , 95% CI: 0.39, 0.89,  $P<0.00001$ ).

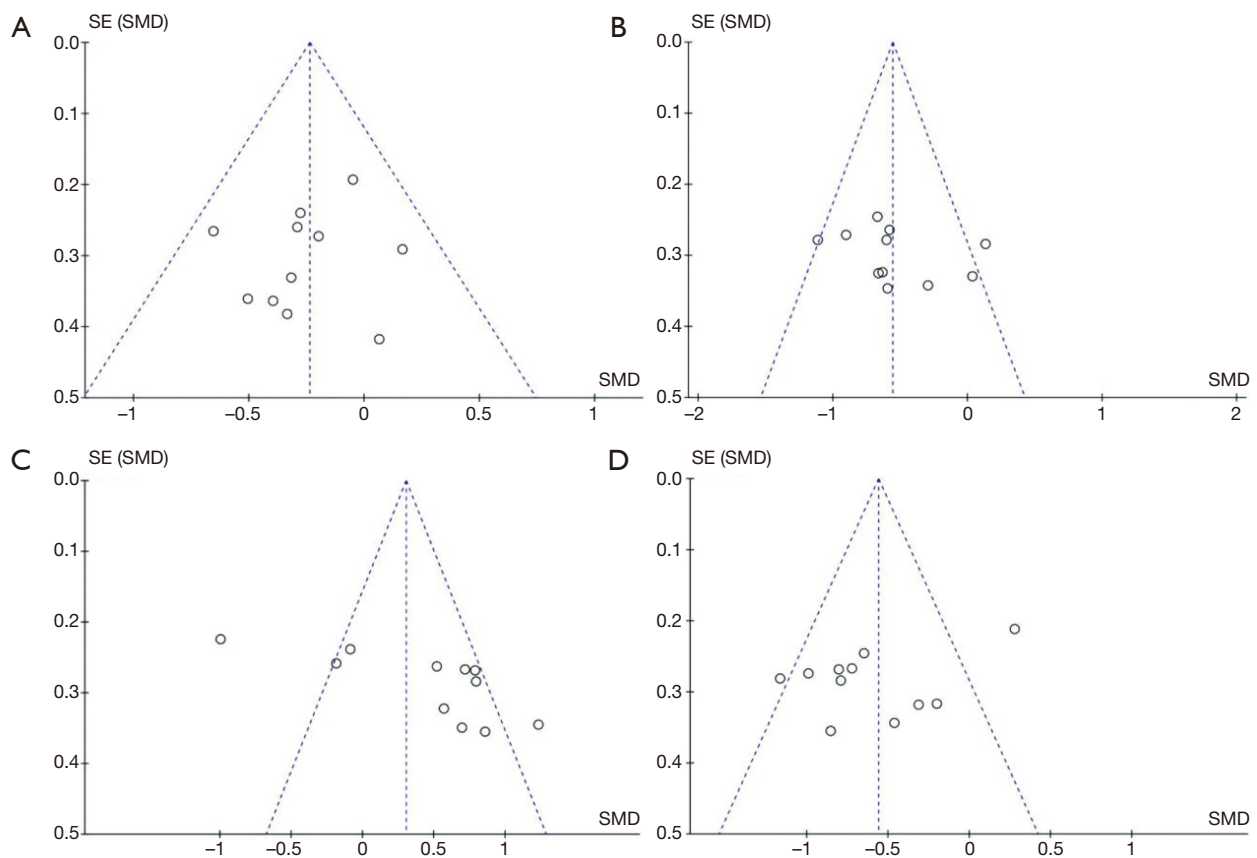
### Discussion

This meta-analysis of eighteen RCTs studies involving

1,060 subjects was conducted to assess the effects of vitamin D supplementation on hormones, oxidative stress, and inflammatory markers in women with PCOS. The current research demonstrated that vitamin D supplementation could improve TT, hs-CRP, TAC, and MDA in PCOS patients, but there was no significant difference in FT, DHEA-S, SHBG, FAI, NO, and GSH levels. Subgroup analysis showed that different courses of treatment, type of vitamin D intervention, frequency, and dose of vitamin D supplementation could reduce hs-CRP and MDA in PCOS patients. Vitamin D supplementation for 12 weeks can improve TT and TAC, low-dose vitamin D ( $\leq 1,000$  IU/day) can improve TT and DHEA-S. Vitamin D combined with a co-supplement, can improve TT, FT, and DHEA-S, while daily administration frequency can improve TT, DHEA-S, and TAC.

### Effect of vitamin D supplementation on hormone

HA is an important pathophysiological factor in PCOS. High androgen levels in local and circulating ovaries can cause ovarian dysfunction, resulting in abnormal ovulation, irregular menstruation, and even amenorrhea (1). Also, hyperandrogen-like changes, such as hirsutism, acne, and skin seborrhea, are some of the characteristics of PCOS. Our results suggest that vitamin D supplementation improves TT levels in women with PCOS. This is consistent with the results of Pal and colleagues (38).



**Figure 6** Funnel plot of standard error by standard differences in the means of plasma (A) total testosterone (TT); (B) high-sensitivity C-reactive protein (hs-CRP); (C) total antioxidant capacity (TAC); and (D) malondialdehyde (MDA).

In their single arm open label trial, twelve overweight women with VDD who received vitamin D and calcium for three months showed decreased TT ( $P=0.036$ ) compared with baseline. Also, a study by Firouzabadi and colleagues demonstrated that vitamin D combined with calcium supplement therapy can reduce weight, promote follicular maturation, and improve menstrual disorders and hyperandrogen related symptoms in PCOS patients (39). Observational studies demonstrated that 25(OH)D levels in PCOS patients were negatively correlated with TT, DHEA-S, and SHBG (40,41). Also, Muscogiuri and colleagues showed that vitamin D might reduce PCOS androgen by participating in the synthesis of steroids (11).

#### *Effects of vitamin D supplementation on inflammation and oxidative stress*

Insulin resistance and hyperinsulinemia are important characteristics of PCOS patients. Chronic inflammatory

reactions and, in turn, inflammatory mediators play an important role in the occurrence and development of insulin resistance. These inflammatory mediators directly affect the phosphorylation and activation process of IRS-1 and IRS-2, thereby blocking the transmission of insulin biological signals and causing insulin resistance (42). A key transcription factor regulating the inflammatory response is the nuclear transcription factor kappa-B (NF- $\kappa$ B) (42). Rasheed and others have shown that vitamin D can inhibit NF- $\kappa$ B, reducing the production of free radicals and pro-inflammatory cytokines (43). Our meta-analysis showed that serum hs-CRP levels decreased after vitamin D supplementation in patients with PCOS. This was consistent with the study by Ngo and colleagues (44), which found a negative correlation between serum 25(OH)D level and hs-CRP. Compared with non-PCOS patients, the level of oxidative stress in PCOS patients is elevated, and the proportion of oxidants is higher than that of antioxidants (45). Hyperinsulinemia can stimulate

the production of reactive oxygen species (ROS) in local tissues and cells, leading to oxidative stress (46,47). Also, PCOS induced ovulation treatment can cause an increase in oxidative stress (46). Inflammation and oxidative damage play key roles in the pathophysiology of diseases (4,5). As MDA is a product of local tissue reaction with ROS, it can be used to reflect the production of ROS. In ROS generation, the antioxidant enzyme (superoxide dismutase, SOD) in the body is continuously consumed, leading to a decrease in TAC levels, and both MDA and TAC are related to the degree of oxidative stress reaction (48,49). Our meta-analysis demonstrated that vitamin D supplementation could improve TAC and MDA levels in PCOS patients. This is consistent with the results of Asemi and colleagues (50), showing that the markers of oxidative stress were significantly improved after six weeks of vitamin D and calcium supplementation in patients with gestational diabetes mellitus. Cavalcante and others found that after taking 200,000 IU vitamin D for 4 weeks, TAC increased, but MDA concentration was not affected (51). Studies by Jain and colleagues suggested that vitamin D may improve oxidative stress by increasing intracellular GSH levels and reducing ROS and proinflammatory factors (52).

### *Subgroup analysis*

Subgroup analysis found that vitamin D combined with supplementation appeared to be more effective than vitamin D alone in PCOS patients. Foroozanfard and colleagues treated 104 overweight PCOS women with VDD with 50,000 IU vitamin D per week and 1,000 mg calcium daily for 8 weeks (19). They observed that compared with placebo, vitamin D combined with calcium significantly improved hs-CRP, MDA, TAC, and GSH. Tabesh and others found that compared with vitamin D alone, co-supplementation with calcium can improve the chronic inflammation of diabetic patients with VDD by reducing the concentration of interleukin-6 and tumor necrosis factor- $\alpha$  (53). Among the included studies in this meta-analysis, three studies co-administered vitamin D with calcium (19,27,32), one with vitamin E (18), one with unsaturated fatty acids (24), one with grass oil (28), and one with probiotics (29). However, due to the small number of relevant studies, it could not be determined, which co-supplement combination with vitamin D was most effective. Further RCT studies and mesh meta-analysis will be required.

In the subgroup of vitamin D supplementation alone, TT also showed a downward trend, but it was not statistically

significant ( $P=0.07$ ). It cannot be ruled out that the sample size included in the subgroup analysis was too small.

Our subgroup analysis also demonstrated that low-dose vitamin D supplementation ( $\leq 1,000$  IU/day) was sufficient to produce significant benefits in the treatment of PCOS. This finding is consistent with the meta-analysis results of Xue (54) and Lagowska (55). They demonstrated that low-dose vitamin D supplementation was sufficient to reduce triglycerides and (homeostasis model assessment of insulin resistance, HOMA-IR) levels in PCOS patients, while high-dose vitamin D supplementation did not result in any benefit (54,55). Also, Sanders and colleagues noted that high-dose vitamin D ( $>500,000$  IU) might increase fracture risk and cause tolerance problems (56). But it cannot be excluded that low-dose vitamin D supplementation may result in better patient compliance which may influence the results of those studies. Daily administration may be more beneficial to the treatment of PCOS than weekly or fortnightly frequency. Increased frequency of administration may lead to improved absorption of vitamin D in the intestinal tract. A smaller dose of administered daily may also improve tolerance. Most of the studies in this meta-analysis had an intervention time of 12 weeks, with only a few studies using 8 or 24 weeks. Therefore, due to the small sample size, we could not draw any conclusions on the effect of intervention time on vitamin D supplementation in PCOS patients.

Considering the limitations of the whole subgroup analysis, such as the differences in treatment drugs and the difference is administration time, further high-quality prospective studies are required to verify our results.

In this current meta-analysis, English RCTs were selected through mainstream medical databases using a detailed retrieval strategy. The latest research was included and collected. Eighteen studies were RCT studies, seventeen of which were placebo-controlled, with high quality and reliable data. However, this study was limited by the large variations in vitamin D supplementation doses, the relatively small sample size, and the variations in co-supplement drugs and administration regimes employed. All these factors likely played a role in increasing the heterogeneity of this meta-analysis.

### **Conclusions**

This meta-analysis data demonstrated that vitamin D supplementation could improve TT, hs-CRP, TAC, and MDA in PCOS patients. Vitamin D combined

with supplementary therapy, vitamin D dose less than 1,000 IU/day, and daily dose frequency appeared to be more conducive to improving hormone, inflammation, and oxidative stress in patients with PCOS. Considering the limitations of the current study, further large-scale and high-quality RCT studies are still necessary to better assess the impact of vitamin D supplementation on women with PCOS.

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## Footnote

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*Conflicts of Interest:* All authors have completed the ICMJE uniform disclosure form (available at <http://dx.doi.org/10.21037/apm-20-2201>). The authors have no conflicts of interest to declare.

*Ethical Statement:* The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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