

# The Effects of Vitamin D Supplementation on Metabolic Status of Patients with Polycystic Ovary Syndrome: A Randomized, Double-Blind, Placebo-Controlled Trial

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## ABSTRACT

Data on the effects of vitamin D supplementation on metabolic status of patients with polycystic ovary syndrome (PCOS) are scarce. The current study was conducted to evaluate the effects of vitamin D supplementation on metabolic status of patients with PCOS. This randomized double-blind, placebo-controlled trial was performed on 70 vitamin D-deficient (serum concentrations < 20 ng/ml) women with phenotype B-PCOS according to the Rotterdam criteria aged 18–40 years old. Participants were randomly allocated into 2 groups to take either 50 000 IU vitamin D (n = 35) or placebo (n = 35) every 2 weeks for 12 weeks. Metabolic, endocrine, inflammation, and oxidative stress biomarkers were quantified at the beginning of the study and after 12-week intervention. After the 12-week intervention, compared to the placebo, vitamin D supplementation significantly decreased fasting plasma glucose (FPG) ( $-3.1 \pm 7.3$  vs.  $+0.5 \pm 6.3$  mg/dl,  $p = 0.02$ ), insulin ( $-1.4 \pm 3.6$  vs.  $+2.6 \pm 7.0$   $\mu$ U/ml,  $p = 0.004$ ), homeostasis model of assessment-estimated insulin resistance ( $-0.3 \pm 0.8$  vs.  $+0.6 \pm 1.6$ ,  $p = 0.003$ ), homeostasis model of assessment-estimated B cell function ( $-4.9 \pm 13.4$  vs.  $+9.9 \pm 26.9$ ,  $p = 0.005$ ), and increased quantitative insulin sensitivity check index ( $+0.01 \pm 0.01$  vs.  $-0.02 \pm 0.05$ ,  $p = 0.007$ ). Supplementation with vitamin D also led to significant reductions in serum high-sensitivity C-reactive protein (hs-CRP) ( $-0.7 \pm 1.4$  vs.  $+0.5 \pm 2.1$   $\mu$ g/mL,  $p = 0.009$ ) and plasma malondialdehyde (MDA) levels ( $-0.1 \pm 0.5$  vs.  $+0.9 \pm 2.1$   $\mu$ mol/l,  $p = 0.01$ ) compared to the placebo. Overall, vitamin D supplementation for 12 weeks in vitamin D-deficient women with phenotype B-PCOS had beneficial effects on glucose homeostasis parameters, hs-CRP, and MDA.

## Abbreviations

DHEAS	dehydroepiandrosterone sulfate
FPG	Fasting plasma glucose
FAI	Free androgen index
GSH	Total glutathione
HOMA-IR	Homeostasis model of assessment-estimated insulin resistance

HOMA-B	Homeostasis model of assessment-estimated B cell function
hs-CRP	High-sensitivity C-reactive protein
mF-G	Modified Ferriman–Gallwey
MDA	Malondialdehyde
NO	Nitric oxide
PCOS	Polycystic ovary syndrome
QUICKI	Quantitative insulin sensitivity check index

SHBG	Sex hormone-binding globulin
TAC	Total antioxidant capacity

## Introduction

Polycystic ovary syndrome (PCOS) is a common heterogeneous disorder that is associated with disturbances of reproductive and metabolic function such as hyperinsulinemia, impaired glucose metabolism, and hyperandrogenism [1]. It affects approximately 6–10% of women of child-bearing age [2]. It is still unclear whether milder phenotypes have the same metabolic and reproductive outcomes as the more severe forms. In a study, Tehrani et al. [3] reported that the metabolic syndrome was more prevalent among phenotypes B. However, in another study, there were no significant differences in insulin resistance, metabolic syndrome and/or glucose intolerance between the 4 PCOS phenotypes [4].

Some studies have indicated that vitamin D deficiency is a problem in patients with PCOS [5, 6], although results from randomized trials evaluating the effect of vitamin D supplementation on metabolic profiles among women with PCOS are conflicting. In a pilot study, Wehr et al. [7] demonstrated that vitamin D treatment at a dosage of 20 000 IU weekly for 24 weeks improved glucose metabolism and menstrual frequency in PCOS women. However, no significant change in fasting serum insulin and glucose concentrations, insulin sensitivity, and homeostasis model assessment of insulin resistance was observed following supplementation with 50 000 IU vitamin D3 for 2 months among patients with PCOS [8]. Data on the effects of vitamin D supplementation on biomarkers of inflammation and oxidative stress are also scarce. Our previous study among patients with diabetic foot ulcer indicated that 50 000 IU vitamin D administration every 2 weeks after 12 weeks decreased serum high sensitivity C-reactive protein (hs-CRP) and plasma malondialdehyde (MDA), but did not influence plasma nitric oxide (NO) and or total antioxidant capacity (TAC) levels [9].

The beneficial effects of vitamin D administration on markers of insulin metabolism, lipid profiles, biomarkers of inflammation, and oxidative stress may be mediated by its impact on activating insulin receptor expression, the downregulation of cytokine generation [10], decreasing the production of reactive oxygen species, and pro-inflammatory markers [11]. To the best of our knowledge, limited data are available assessing the effects of vitamin D supplementation on glucose homeostasis parameters, hormonal status, lipid concentrations, markers of inflammation, and/or oxidative stress of vitamin D-deficient patients with phenotype B-PCOS. The aim of the present study was to assess the effects of vitamin D supplementation on metabolic status in these patients.

## Subjects and Methods

### Trial design

This study was a prospective randomized double-blind placebo-controlled clinical trial.

### Participants

In the current study, 70 vitamin D-deficient (serum concentrations <20 ng/ml) patients with phenotype B (oligo-anovulation and

hyperandrogenism) of PCOS aged 18–40 years old who were referred to the Kosar Clinic in Arak, Iran, between April 2016 and June 2016 were included. Diagnosis of PCOS was made according to the Rotterdam criteria [12]. Oligo- and/or anovulation was defined as delayed menses >35 days or <8 spontaneous hemorrhagic episodes/year [12]. Hyperandrogenism was defined as clinical [hirsutism using modified Ferriman–Gallwey score (mFG) of  $\geq 8$ ] [12] and/or biochemical signs of hyperandrogenism [12]. We excluded pregnant women, women with endocrine diseases including diabetes or impaired glucose tolerance from the study.

### Ethics statements

This study was done in accordance with the Declaration of Helsinki and signed informed consent was obtained from all subjects. The research was approved by the ethics committee of Arak University of Medical Sciences (AUMS).

### Study design

First, all subjects were matched for age and BMI. Participants were then randomly divided into 2 groups to be given either vitamin D supplements (n = 35) or placebo (n = 35) for 12 weeks. Participants were requested not to change their ordinary physical activity and not to take any nutritional supplements during the 12-week trial. All patients completed 3-day food records and 3 physical activity records at baseline and at weeks 3, 6, and 9 of the intervention and at the end-of-trial. Daily macro- and micro-nutrient intakes were analyzed by nutritionist IV software (First Databank, San Bruno, CA, USA). In the current study, physical activity was described as metabolic equivalents (METs) in hours per day. To determine the METs for each patient, we multiplied the times (in hour per day) reported for each physical activity by its related METs coefficient by using standard tables.

### Intervention

In the intervention group, subjects received 50 000 IU vitamin D supplements orally, every 2 weeks for 12 weeks. Due to the lack of evidence about the appropriate dosage of vitamin D for vitamin D-deficient patients with phenotype B of PCOS, we used the above-mentioned dose of vitamin D based on a previous study in vitamin D-deficient PCOS subjects [13]. Quality control of vitamin D was performed in the laboratory of Food and Drug Administration in Tehran, Iran by high-performance liquid chromatography (HPLC) method. Vitamin D supplements and placebo capsules were similar in shape and size and manufactured by Zahravi (Tabriz, Iran).

### Treatment adherence

Every 4 weeks, participants were given enough supplements to last until 3 days after their next scheduled visit and were instructed to return all the unused supplements at each visit. Persons were scheduled for the follow-up visits every 4 weeks for an intermediate evaluation. To evaluate compliance the remaining supplements were counted and subtracted from the amount of supplements provided to the participants. To increase compliance, all participants received short messages on their cell phones every 2 weeks to remind them about taking the capsules.

## Assessment of anthropometric measures

Weight and height of participants were determined in an overnight fasting status using a standard scale (Seca, Hamburg, Germany) at baseline and after the 12-week intervention. BMI was calculated as weight in kg divided by height in meters squared. All anthropometric measures were made by a trained midwife.

## Assessment of outcomes

In our study, markers of insulin resistance and androgens were considered as the primary outcomes measurements and lipid profiles, and biomarkers of inflammation and oxidative stress were considered as the secondary outcomes measurements.

## Clinical assessments

In the current study, clinical assessments included determinations of hirsutism using a mFG scoring system [14], of acne score [15] and of alopecia, based on assessment guidelines collated by Olsen et al. [16]. Acne was scored using a 4-point scale: 0, no acne; 1, minor acne on face; 2, moderate acne on face only; and 3, severe acne, face, and back or chest [15].

## Biochemical assessment

Ten ml fasting blood samples were collected at the onset and the end of the study at Arak reference laboratory in a fasting status and centrifuged to separate serum, which were stored at  $-80^{\circ}\text{C}$  before analysis. Serum insulin concentrations were evaluated by the use of available ELISA kit (DiaMetra, Milano, Italy) with inter- and intra-assay coefficient variances (CVs) of 3.4 to 5%, respectively. The homeostasis model of assessment-insulin resistance (HOMA-IR),  $\beta$ -cell function (HOMA-B) and the quantitative insulin sensitivity check index (QUICKI) were determined according to the suggested formulas [17]. Serum total testosterone with inter- and intra-assay CVs of 3.9 to 5.8%, sex hormone-binding globulin (SHBG) with inter- and intra-assay CVs of 3.6 to 5.2%, free testosterone with inter- and intra-assay CVs of 4.0 to 5.8% and dehydroepiandrosterone (DHEAS) concentrations with inter- and intra-assay CVs of 4.2 to 6.4% were evaluated by using commercial kits (DiaMetra, Milano, Italy). Free androgen index (FAI) was calculated as the ratio of total testosterone to SHBG. Enzymatic kits of Pars Azmun (Tehran, Iran) were used to determine fasting plasma glucose (FPG), serum triglycerides, VLDL-, total-, LDL- and HDL-cholesterol concentrations. All inter- and intra-assay CVs for FPG and all lipid concentrations were less than 5%. Serum hs-CRP concentrations were quantified by commercial ELISA kit (LDN, Nordhorn, Germany) with inter- and intra-assay CVs of 4.4 to 6.6%, respectively. The plasma NO concentrations were assessed using Griess method [18]. Plasma TAC concentrations were determined by the method of ferric reducing antioxidant power developed by Benzie and Strain [19], total glutathione (GSH) were determined using the method of Beutler et al. [20] and MDA concentrations were determined by the thiobarbituric acid reactive substances spectrophotometric test [21]. All inter- and intra-assay CVs for NO, TAC, GSH, and MDA concentrations were less than 5%.

## Sample size

In this study, we used a randomized clinical trial sample size calculation formula where type one ( $\alpha$ ) and type 2 errors ( $\beta$ ) were 0.05,

and 0.20 (power = 80%), respectively. According to the previous trial [22], we used 1.41 as the SD and 1.05 as the change in mean (d) of HOMA-IR as a primary outcome. Based on the formula, we needed 30 subjects in each group; after following for 5 dropouts in each group, the final sample size was 35 subjects in each group.

## Randomization

Randomization assignment was conducted using computer-generated random numbers. Randomization and allocation were concealed from the researchers and subjects until the final analyses were completed. The randomized allocation sequence, enrolling participants and allocating them to interventions were conducted by trained staff at the clinic.

## Statistical methods

To evaluate whether the study variables were normally distributed or not, we used the Kolmogorov-Smirnov test. To detect differences in anthropometric measures as well as in macro- and micro-nutrient intakes between the 2 groups, we applied Student's t-test to independent samples. The Pearson Chi-square test was used to compare categorical variables. To determine the effects of vitamin D supplementation on glucose homeostasis parameters, hormonal and lipid profiles, biomarkers of inflammation, and oxidative stress, we used one-way repeated measures analysis of variance. Adjustment for changes in baseline values of biochemical variables, age and BMI at baseline was performed by analysis of covariance (ANCOVA) using general linear models. A p-value of  $<0.05$  were considered statistically significant. All statistical analyses were conducted using the Statistical Package for Social Science version 18 (SPSS Inc., Chicago, Illinois, USA).

## Results

In the current study, all 70 participants [vitamin D ( $n = 35$ ) and placebo ( $n = 35$ )] completed the trial (► Fig. 15). On average, the rate of compliance in the present study was high, such that higher than 90% of capsules were taken throughout the study in both groups. No side effects were reported following the supplementation of women with vitamin D in women with PCOS throughout the 12-week study.

Mean age, height, and weight, BMI and METs at baseline and end-of-trial were not statistically different between the 2 groups (► Table 15). Alopecia (4.3 vs. 12.0%,  $p = 0.33$ ) and acne (6.3 vs. 14.8%,  $p = 0.39$ ) were unchanged following the consumption of vitamin D supplements compared with the placebo.

Based on the 3-day dietary records obtained at the baseline, end-of-trial and throughout the trial, we found no significant difference in mean dietary macro- and micro-nutrient intakes between the 2 groups (data not shown).

After 12 weeks of intervention, compared to the placebo, vitamin D supplementation significantly decreased FPG ( $-3.1 \pm 7.3$  vs.  $+0.5 \pm 6.3$  mg/dl,  $p = 0.02$ ), insulin ( $-1.4 \pm 3.6$  vs.  $+2.6 \pm 7.0$   $\mu\text{IU/ml}$ ,  $p = 0.004$ ), HOMA-IR ( $-0.3 \pm 0.8$  vs.  $+0.6 \pm 1.6$ ,  $p = 0.003$ ), HOMA-B ( $-4.9 \pm 13.4$  vs.  $+9.9 \pm 26.9$ ,  $p = 0.005$ ) and increased QUICKI ( $+0.01 \pm 0.01$  vs.  $-0.02 \pm 0.05$ ,  $p = 0.007$ ) (► Table 1). Supplementation with vitamin D led to significant reductions in serum hs-CRP ( $-0.7 \pm 1.4$  vs.  $+0.5 \pm 2.1$   $\mu\text{g/mL}$ ,  $p = 0.009$ ) and plasma MDA levels

► **Table 1** Metabolic profiles, and biomarkers of inflammation; and oxidative stress at the baseline and after 12 weeks of the intervention in patients with polycystic ovary syndrome.

	Placebo group (n = 35)			Vitamin D group (n = 35)			P*
	Baseline	End-of-trial	Change	Baseline	End-of-trial	Change	
	Vitamin D (ng/ml)	14.5±5.1	14.4±5.2	-0.1±4.3	12.8±4.5	27.5±9.8	
FPG (mg/dl)	93.8±7.8	94.3±9.8	0.5±6.3	91.0±6.1	87.8±7.6	-3.1±7.3	0.02
Insulin (μU/ml)	9.1±7.3	11.7±6.5	2.6±7.0	9.6±4.5	8.2±2.8	-1.4±3.6	0.004
HOMA-IR	2.1±1.7	2.7±1.6	0.6±1.6	2.2±1.1	1.8±0.6	-0.3±0.8	0.003
HOMA-B	31.6±28.6	41.5±25.2	9.9±26.9	34.1±16.5	29.2±11.3	-4.9±13.4	0.005
QUICKI	0.36±0.05	0.34±0.04	-0.02±0.05	0.34±0.02	0.35±0.02	0.01±0.01	0.007
Total testosterone (ng/ml)	1.5±0.7	1.6±0.6	0.1±0.5	1.3±0.6	1.3±0.7	-0.04±0.5	0.15
SHBG (nmol/l)	42.4±38.3	46.4±20.0	4.0±40.6	42.4±13.3	47.9±17.0	5.5±12.2	0.84
FAI	0.20±0.22	0.14±0.10	-0.05±0.22	0.12±0.07	0.11±0.09	-0.007±0.07	0.25
mF-G scores	10.1±6.3	10.1±6.1	-0.02±0.5	10.6±4.9	10.5±4.8	-0.1±0.5	0.34
Free testosterone (pg/ml)	4.9±2.7	4.8±2.5	-0.1±1.8	4.5±3.8	4.7±3.7	0.2±1.2	0.32
DHEAS (μg/ml)	3.4±2.2	2.9±1.8	-0.5±0.9	2.8±1.1	2.3±1.0	-0.5±0.8	0.57
Triglycerides (mg/dl)	112.0±39.3	118.9±48.9	6.9±23.6	98.4±55.6	97.8±40.9	-0.5±46.4	0.39
VLDL-cholesterol (mg/dl)	22.4±7.9	23.8±9.8	1.4±4.7	19.7±11.1	19.6±8.2	-0.1±9.3	0.39
Total cholesterol (mg/dl)	160.1±30.4	163.5±32.1	3.4±27.3	151.2±22.5	144.5±22.7	-6.7±28.7	0.13
LDL-cholesterol (mg/dl)	87.6±25.8	90.8±28.7	3.2±26.8	86.1±20.9	79.8±26.3	-6.3±24.1	0.12
HDL-cholesterol (mg/dl)	50.1±8.5	48.9±8.4	-1.2±5.7	45.4±5.7	45.2±6.4	-0.2±6.5	0.51
hs-CRP (μg/ml)	2.9±2.5	3.5±2.9	0.5±2.1	2.6±2.8	1.9±1.7	-0.7±1.4	0.009
NO (μmol/l)	50.0±13.1	50.9±12.8	0.8±16.3	48.8±4.9	52.0±4.3	3.2±5.4	0.42
TAC (mmol/l)	841.7±179.1	846.2±169.3	4.5±151.3	849.7±114.9	843.1±108.0	-6.6±100.6	0.71
GSH (μmol/l)	481.6±108.2	528.6±144.4	47.1±130.1	577.8±90.5	592.9±130.7	15.2±104.6	0.26
MDA (μmol/l)	2.1±0.8	3.0±1.7	0.9±2.1	2.2±0.4	2.1±0.4	-0.1±0.5	0.01

All values are means ± SDs; \* p-Values represent the time × group interaction (computed by analysis of the one-way repeated measures ANOVA); For abbreviations, see the abbreviation list in the text

( $-0.1 \pm 0.5$  vs.  $+0.9 \pm 2.1$   $\mu\text{mol/l}$ ,  $p=0.01$ ) compared to the placebo. We did not observe any significant effect of vitamin D supplementation on hormonal and lipid profiles, or on other biomarkers of inflammation and oxidative stress.

There was a significant difference in the baseline levels of HDL-cholesterol ( $p=0.008$ ) and GSH ( $p<0.001$ ) between the 2 groups. Therefore, we adjusted the analysis for baseline of biochemical variables, age and baseline BMI. When we controlled the analysis for baseline values of biochemical parameters, age and baseline BMI, the difference in changes in QUICKI ( $p=0.19$ ) between the 2 groups became non-significant, while difference in changes in total-cholesterol ( $p=0.04$ ) (► **Table 25**). Other findings did not alter significantly after this adjustment.

## Discussion

In this study, we evaluated effects of vitamin D supplementation on glucose homeostasis variables, hormonal status, lipid concentrations, and biomarkers of inflammation and oxidative stress among vitamin D-deficient women with phenotype B-PCOS. We demonstrated that vitamin D supplementation for 12 weeks in vitamin D-deficient women with phenotype B-PCOS had beneficial effects of glucose homeostasis variables, hs-CRP and MDA, but did not affect hormonal and lipid profiles, and on other biomarkers of inflammation and oxidative stress.

Patients with PCOS are susceptible to several metabolic disorders including insulin resistance, inflammation, and oxidative stress [23, 24]. Previous studies have reported that vitamin D deficiency is a problem in subjects with PCOS [5, 6]. A large number of observational studies have demonstrated an association between low levels of 25(OH)D3 and features of the metabolic syndrome in PCOS women [25, 26]. One theory relies on the regulatory effect of vitamin D on the intracellular and extracellular calcium levels that is essential for insulin-mediated intracellular processes, and may have impact on insulin secretion and insulin sensitivity [10]. Another hypothesis involves the stimulatory effect of vitamin D on the expression of insulin receptors [27]. In addition, vitamin D affects the immune system and can cause a higher inflammatory response associated with insulin resistance [28]. The current study demonstrated that taking vitamin D supplementation for 12 weeks in vitamin D-deficient women with phenotype B-PCOS led to significant decreases in FPG, serum insulin levels, HOMA-IR, and HOMA-B, and a significant increase in QUICKI compared with the placebo, but did not influence hormonal and lipid profiles. Results of cross-sectional studies in patients with PCOS have indicated an inverse relation between serum vitamin 25(OH)D concentrations and insulin resistance [26, 28]. There have been few attempts to evaluate the effects of vitamin D administration on insulin resistance in women with PCOS, but the results have been controversial. In a study by Selimoglu et al. [29], it was seen that the administration of a single oral dose of 300 000 IU of vitamin D3 to women with PCOS after 3 weeks decreased HOMA-IR, but did not change fasting insulin and glucose levels, and hormonal profiles. However, supplementation with 50 000 IU of vitamin D3 for 2 months among patients with PCOS did not affect fasting serum insulin and glucose concentrations, QUICKI, and HOMA-IR [8]. Different study designs, from those used in our study, or in duration, along with character-

istics of study patients might provide some reasons for discrepant findings. Hyperinsulinemia and insulin resistance are important factors associated with the typical clinical signs and hormonal disorders in of subjects with PCOS, and this trait has cause-consequence relationships with increased risk of coronary heart diseases (CHD) [30], gestational diabetes (GDM) and type 2 diabetes mellitus (T2DM) [31]. Increased vitamin D intake might improve glucose homeostasis variables by its effect on calcium and phosphorus metabolism, the upregulation of the insulin receptor genes, and increased transcription of insulin receptor genes [27].

We found that taking vitamin D supplements for 12 weeks in vitamin D-deficient women with phenotype B-PCOS decreased serum hs-CRP and plasma MDA concentrations compared with the placebo, but did not influence other biomarkers of inflammation and oxidative stress. However, observational and interventional studies have reported that supplemental vitamin D may have anti-inflammatory and antioxidant effects; findings across all completed randomized trials have been inconsistent. Supporting our findings, the administration of 60 000 IU/month vitamin D in rheumatoid arthritis patients with vitamin D deficiency for 3 months decreased CRP concentrations [32]. In addition, our previous study among patients with diabetic foot ulcer showed that taking 50 000 IU vitamin D supplements every 2 weeks for 12 weeks decreased serum hs-CRP and plasma MDA, but did not affect plasma NO, TAC and GSH values [9]. Unlike, in a study by Ferozghi et al. [33], who had observed that supplementation with 50 000 IU vitamin D per week for 12 weeks among subjects with nonalcoholic fatty liver disease (NAFLD) had no effect on CRP concentrations. Less production of parathyroid hormone [34], decreasing the production of reactive oxygen species and pro-inflammatory markers by vitamin D supplements [11] may explain its beneficial effects on biomarkers of inflammation and oxidative stress.

The current study had few limitations. First, due to shortage of funding, we did not evaluate other biomarkers of inflammation and oxidative stress including interleukin 6 (IL-6), tumor necrosis factor alpha (TNF- $\alpha$ ), catalase, and superoxide dismutase (SOD). In addition, we did not assess the expressed levels of variables relevant to insulin resistance and lipid profiles.

Overall, vitamin D supplementation for 12 weeks in vitamin D-deficient women with phenotype B-PCOS had beneficial effects of glucose homeostasis parameters, hs-CRP and MDA, but did not affect hormonal and lipid profiles, and other biomarkers of inflammation and oxidative stress.

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## Conflict of Interest

The authors declare that they have no conflict of interest.

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### Note of Concern

Since publication of this article, [serious concerns](#) have been raised about the integrity of the reported methods, results and analysis. Responses by the leading author and ethics committees have been unsatisfactory and inconclusive; we advise readers to interpret the information presented in the article with due caution.