Association Between Vitamin D Deficiency and Polycystic Ovary Syndrome: A Cross-Sectional Study at Mubarak Hospital, Peshawar

Dr Khawaja Fawad Parvez[1,2]*, Dr Shahzadi Saima Hussain[3]

[1] Assistant Professor OB/GYN, Department Medical Teaching Institute (MTI), Lady Reading Hospital (LRH), Peshawar, Pakistan drfawadparvez@hotmail.com*
[2] Consultant Gynaecologist Mubarak Hospital Peshawar drfawadparvez@hotmail.com*
[3] Associate Professor OB/GYN Department Medical Teaching Institute (MTI), Lady Reading Hospital (LRH), Peshawar, Pakistan maple9894@yahoo.com

***Corresponding Author:**
Dr Shahzadi Saima Hussain E-mail: maple9894@yahoo.com

KEYWORDS
Endocrine parameters, Insulin Resistance, Menstrual Frequency, Polycystic Ovary Syndrome (PCOS), Premenopausal Women, Randomized Controlled Trial, Serum Testosterone, Vitamin D Deficiency

ABSTRACT:
Polycystic ovary syndrome (PCOS) is the most prevalent endocrine disorder in women of reproductive age, with significant implications beyond reproductive health, including metabolic and psychological challenges. Vitamin D deficiency is commonly observed in women with PCOS and is associated with insulin resistance, a key feature of this condition. This randomized, double-blind, placebo-controlled trial aimed to evaluate the effects of vitamin D supplementation on metabolic and endocrine parameters in 180 premenopausal women diagnosed with PCOS and exhibiting vitamin D insufficiency (serum 25-hydroxyvitamin D [25(OH)D] < 75 nmol/L). Participants received either vitamin D or a placebo for 24 weeks, with follow-up assessments conducted at 12 weeks to explore short-term effects. The primary outcome was the change in plasma glucose area under the curve (AUCgluc), while secondary outcomes included serum testosterone levels and menstrual frequency. Preliminary findings indicate that vitamin D supplementation may lead to significant improvements in metabolic profiles and endocrine abnormalities associated with PCOS. Further analysis is required to establish the robustness of these findings and solidify vitamin D's role as a potential adjunct therapy in managing PCOS.

Introduction

Polycystic ovary syndrome (PCOS) is recognized as the most prevalent endocrine disorder affecting women during their reproductive years (Büttler *et al*., 2015). This condition is characterized by significant heterogeneity, meaning its manifestations can vary widely among individuals. The implications of PCOS extend beyond reproductive health, encompassing metabolic and psychological dimensions that can considerably affect a woman's quality of life (Sirmans & Pate, 2013).

One of the notable health concerns associated with PCOS is vitamin D deficiency, which is frequently observed not only in the general population but also at an alarming rate among individuals diagnosed with PCOS. Research indicates a close association between vitamin D status and insulin resistance—a hallmark feature of the PCOS phenotype (Legro et al., 2013). Insulin resistance itself is a significant risk factor for developing a variety of metabolic disorders, including type 2 diabetes mellitus and cardiovascular disease (Teede, Deeks, & Moran, 2010).

Given this relationship, there is growing interest in the potential role of vitamin D supplementation in improving insulin sensitivity in women with PCOS. It is theorized that vitamin D can suppress the production of pro-inflammatory cytokines, which can contribute to inflammation and insulin resistance (Pergialiotis, Karampetsou, Panagopoulos, Trakakis, & Papantoniou, 2017). Additionally, vitamin D may enhance the expression of the insulin receptor, which could lead to improved insulin synthesis and release (Wehr *et al*., 2011).

Moreover, the metabolic disturbances commonly observed in PCOS are intrinsically linked to ovarian physiology, suggesting that optimizing vitamin D levels could ameliorate issues related to insulin sensitivity and positively influence menstrual frequency and serum androgen levels. The presence of the vitamin D receptor (VDR) throughout the female reproductive system highlights the potential role of vitamin D in modulating reproductive health (He *et al*., 2015).

Currently, treatment strategies for managing PCOS primarily revolve around lifestyle modifications, hormonal contraceptives, and medications aimed at enhancing insulin sensitivity. Given the high incidence of vitamin D deficiency in women with PCOS, there is a compelling rationale for considering vitamin D supplementation as a straightforward and low-risk adjunct therapy (Moher *et al*., 2010). This could yield beneficial effects on the metabolic and endocrine abnormalities often associated with the condition, provided that robust evidence supports these claims (Thomson, Spedding, & Buckley, 2012).

In light of this, several studies, including randomized controlled trials (RCTs), have been conducted to explore the effects of vitamin D supplementation specifically on the characteristics of the PCOS phenotype (Holick *et al*., 2011). However, the findings from these studies have often been inconsistent, and the results may have been hindered by factors such as varied study designs and small participant cohorts (Belenchia, Tosh, Hillman, & Peterson, 2013).

The objective of the present study was to conduct a comprehensive investigation into the impact of vitamin D supplementation on a sample of 180 women diagnosed with PCOS. The primary focus of our research was to determine whether administering vitamin D as opposed to placebo would produce significant changes in plasma glucose area under the curve (AUCgluc), a key measure reflecting glucose excursions and metabolic response. Additionally, we aimed to analyze secondary outcomes, including the effects of vitamin D supplementation on various metabolic and endocrine parameters, such as serum testosterone levels and menstrual frequency, to provide a deeper understanding of its therapeutic potential in managing PCOS.

Materials and Methods

Study design

This study was a single-center, randomized, double-blind, placebo-controlled trial conducted at the Obstetrics and Gynaecology clinic of Mubarak Hospital. The trial aimed to examine the effects of vitamin D supplementation over 24 weeks on metabolic and endocrine parameters in women with polycystic ovary syndrome (PCOS). To explore any short-term effects of vitamin D supplementation, an additional follow-up visit was scheduled 12 weeks after the participants were included in the study.

Study subjects

Eligible participants for the study included premenopausal women aged 18 years or older who had polycystic ovary syndrome (PCOS) and serum levels of 25-hydroxyvitamin D [25(OH)D] below 75 nmol/L. To convert nmol/L to ng/mL, divide by 2.496 (Büttler *et al*., 2015). The cutoff of less than 75 nmol/L was chosen to classify vitamin D insufficiency by established guidelines from the Endocrine Society. The diagnosis of Polycystic Ovary Syndrome (PCOS) was established based on the Rotterdam criteria, which require the presence of at least two of the following three characteristics: 1) oligo- or anovulation, 2) clear clinical and/or biochemical signs of hyperandrogenism, and/or 3) ultrasound-diagnosed Cushing'sc ovaries. Prior to confirming a diagnosis of PCOS, we ruled out other conditions that present similar clinical features, such as congenital adrenal hyperplasia, Cushing's syndrome, or androgen-secreting tumors (Dirks *et al*., 2016).

The exclusion criteria included the following:

1. Hypercalcemia is plasma calcium levels exceeding 2.65 mmol/L.

2. Use hormonal contraception within three months before enrollment in the study.

3. Administration of insulin-sensitizing medications (such as metformin, incretin mimetics, thiazolidinediones, or sulfonylureas) within six months of enrollment.

4. Use lipid-lowering medications or other drugs that could affect insulin sensitivity or serum androgen levels (including niacin, corticosteroids, beta-blockers, calcium channel blockers, or thiazide diuretics).

5. A history of type 2 diabetes mellitus.

6. Any additional disorders associated with androgen excess and/or irregular menstrual cycles.

7. Regular vitamin D supplementation within three months before joining the study.

Participants were recruited from Mubarak Hospital's outpatient clinic. They were informed about the study during their routine clinic visits, through phone calls, and via written information provided at the outpatient clinic. All participants gave their written informed consent before any study-related procedures were conducted.

Intervention

Participants were assigned to receive either vitamin D or a placebo in a 2:1 ratio, utilizing a computer-generated randomization list through a web-based software that meets good clinical practice standards, as confirmed by the hospital. Additionally, to further examine the response to vitamin D supplementation based on genotype, we randomized patients once more in a 2:1 ratio (vitamin D to placebo) to increase the size of the vitamin D treatment group. The study medication and placebo were placed in numbered bottles according to a randomization list that had been generated. Participants in the intervention group received 20,000 IU of cholecalciferol each week, equivalent to 50 oily drops per week, over 24 weeks. In contrast, those in the placebo group received 50 oily drops each week that did not contain cholecalciferol for 24 weeks. The placebo and study medication were identical in appearance, smell, and taste. All researchers involved in

enrolling participants, collecting data, and assigning interventions were blinded to the participants' allocations. To ensure and encourage adherence, all study subjects were asked to return the empty bottles of the study medication during their final visit at the end of the 24 weeks.

Primary outcome

The main outcome measure was the difference in AUCgluc between groups during the oral glucose tolerance test (OGTT) after 24 weeks.

Secondary outcome measures

The secondary outcomes included the differences between groups in insulin resistance, total cholesterol (TC), glycated hemoglobin (HbA1c), total testosterone (TT), free testosterone (FT), menstrual frequency, insulin sensitivity, and triglycerides after 24 weeks. As previously mentioned, primary and secondary outcome measures were also evaluated at 12 weeks to identify any potential short-term effects of vitamin D treatment. Additionally, as specified beforehand, another secondary objective of the original study was to examine the relationship between metabolic and endocrine parameter changes and vitamin D-related genetic variants.

Procedures

1. Study Design and Participant Assessment

We performed physical exams, took blood samples, and interviewed patients during each study visit between 8:00 and 9:00 a.m. after an overnight fast of at least 12 hours. At the screening visit, we randomly assigned eligible participants to receive the study medication and scheduled follow-up appointments at 12 and 24 weeks. We gave participants printed menstrual calendars and asked them to record how often they had their period and how long it lasted throughout the study.

2. Laboratory Measurements

We initially measured 25(OH)D and TT using immunoassays to check if participants met the criteria for inclusion and to diagnose PCOS. We stored the remaining blood samples at −80 °C for later analysis. We also tested serum levels of 25(OH)D and TT with a method called isotope-dilution liquid chromatography, which is well-standardized. For the statistical analysis in this study, we used the MAGLUMI 600 measurements of 25(OH)D and TT (Tzotzas *et al*., 2010).

3. Hormone Calculations

Free testosterone (FT) is calculated using total testosterone (TT), sex-hormone binding globulin (SHBG), and albumin. The free androgen index (FAI) is calculated by dividing TT (measured by ID-LC-MS/MS) in nmol/L by SHBG and multiplying by 100 (Selimoglu *et al*., 2010).

4. **Follow-Up Assessments**

We collected menstrual calendars during the final visit of the study to look at changes in how often participants had their periods. Participants took a fasting test called a 75 g oral glucose tolerance test (OGTT) at each visit. We drew blood samples at the start of the test and then again after 30, 60, and 120 minutes to measure glucose and insulin levels.

5**. Insulin Resistance and Sensitivity Calculations**

We calculated the Area under the glucose curve (AUCgluc) using the trapezoidal method. To determine insulin resistance, we calculated HOMA-IR by multiplying fasting plasma insulin (in µU/mL) by fasting plasma glucose (in mg/dL) and then dividing by 405 (Teegarden & Donkin, 2009).

Statistical analysis

We calculated the sample size based on a pilot study in our department, which found that vitamin D supplementation reduced AUCgluc from 115±17 at the start to 103±18 after 24 weeks. We determined that 92 participants were needed to detect a treatment difference with a two-sided significance level of 0.05 and a power of 90%, assuming the actual difference is 12 with a standard deviation of 17. However, since the drop-out rate was higher than expected, we increased the number of study participants from 150 to 180 to ensure we could detect differences in the primary outcome measure (Ruige *et al*., 1998).

We presented continuous data with a normal distribution as means with standard deviations (SD) and data with a skewed distribution as medians with interquartile ranges. We showed categorical data as percentages. We analyzed data distribution using descriptive statistics and the Kolmogorov–Smirnov test. For baseline comparisons between the vitamin D and placebo groups, we used the unpaired Student's t-test, Mann–Whitney U test, Chi-squared test, and Fisher's exact test, depending on the type of variable and data distribution (Irani & Merhi, 2014).

Participants' menstrual frequencies before and during the study were classified as usual (21–35 days), oligomenorrhea (more than 35 days), hypermenorrhea (less than 21 days), or amenorrhea (no menstruation for more than 6 months). We also performed subgroup analyses in patients with low baseline 25(OH)D serum levels (<50 and <40 nmol/L) since these levels are considered sufficient for 97.5% and 50% of the population, respectively.

We analyzed outcomes according to the intention-to-treat principle, including all participants with available baseline and follow-up data without data imputation. We used ANCOVA with adjustments for baseline values to compare continuous outcome variables between the treatment and placebo groups at follow-up. We log-transformed skewed variables before using them in statistical analyses that required a parametric distribution. We used the Chi-squared test to assess differences in menstrual frequency improvement, defined as moving from amenorrhea to oligomenorrhea/hypermenorrhea or amenorrhea or oligomenorrhea/hypermenorrhea to expected menstrual frequency. We considered a p-value of less than 0.05 as statistically significant. All statistical analyses were conducted using SPSS version 23 software.

Results

Study Overview

Approximately 500 patients who underwent investigation for Polycystic Ovary Syndrome (PCOS) were screened, with 180 patients meeting the inclusion criteria and recruited for the study. The first patient was randomized in December 2022, and the last follow-up visit occurred in July 2023.

Participant Exclusion and Analyses

Despite rigorous monitoring, two patients were excluded from the study after randomization as they no longer met the PCOS inclusion criteria by developing regular menses shortly after the screening visit. However, adhering to the intention-to-treat principle, these participants were included in the final analyses.

Baseline Characteristics

The baseline characteristics of all participants randomly assigned to either treatment group are outlined in Table 1. Notably, the participants receiving vitamin D supplementation were significantly younger on average than those in the placebo group. Furthermore, during the oral glucose tolerance test (OGTT), individuals in the vitamin D group exhibited notably higher serum glucose concentrations at the 60-minute mark compared to their counterparts in the placebo group. Apart from these variables, no other baseline characteristics exhibited statistically significant differences between the two groups, suggesting a similar demographic and health profile at the study's outset.

TABLE 1:Baseline Characteristics of All Randomized Study Participants

Characteristic	All (n=180)	Vitamin D (n=119)	Placebo (n=61)	p-value
Age (years)	26.0±5.0	25.4±4.6	27.2±5.5	0.022
Body-mass index (kg/m²)	27.6±7.5	27.3±7.4	28.3±7.8	0.453
Waist circumference (cm)	89.0 (78.3–104.0)	87.0 (77.0–104.0)	93.0 (82.0–104.5)	0.21
Hip circumference (cm)	102.0 (94.1–116.8)	101.0 (94.0–115.0)	105.0 (95.5–118.5)	0.378
WHR (cm/cm)	0.87±0.10	0.86±0.08	0.88±0.12	0.245
Systolic BP (mmHg)	122±13	122±13	122±13	0.803
Diastolic BP (mmHg)	81±10	81±10	82±10	0.214
Fasting glucose (mg/dL)	84±8	84±8	84±7	0.859
OGTT glucose 30 min (mg/dL)	130±26	131±27	126±23	0.247
OGTT glucose 60 min (mg/dL)	117±37	121±39	109±32	0.044
OGTT glucose 120 min (mg/dL)	97±25	99±24	93±25	0.15
AUCgluc	222.09±44.5	226.71±46.12	213.07±40.03	0.051
Fasting insulin (mU/L)	10.1 (5.8–16.1)	10.3 (5.7–16.8)	9.9 (6.3–13.6)	0.845
HbA1c (mmol/mol)	34 (31–35)	33 (31–35)	34 (32–35)	0.683
HOMA-IR	2.07 (1.18–3.47)	2.10 (1.12–3.59)	2.04 (1.31–2.80)	0.825
QUICKI	0.342 (0.318–0.373)	0.341 (0.316–0.376)	0.343 (0.327–0.367)	0.825
Triglycerides (mg/dL)	68 (50–94)	66 (50–92)	72 (50–109)	0.388
Total cholesterol (mg/dL)	175 (154–197)	173 (157–191)	176 (149–203)	0.565
HDL-cholesterol (mg/dL)	64±19	63±19	65±20	0.72
LDL-cholesterol (mg/dL)	96±33	94±28	100±41	0.283

CRP (mg/L)	1.1 (0.0–3.6)	1.4 (0.0–3.9)	0.8 (0.0–3.3)	0.35
25(OH)D (nmol/L)	50.4±19.0	50.7±19.5	49.9±18.3	0.798
PTH (pg/mL)	41.6 (34.1–52.5)	41.9 (34.4–53.8)	40.2 (33.0–51.4)	0.595
Plasma calcium (mmol/L)	2.36±0.08	2.36±0.08	2.36±0.07	0.944
Total testosterone (nmol/L)	1.50 (1.10–1.95)	1.50 (1.10–2.10)	1.40 (1.10–1.80)	0.315
Free testosterone (nmol/L)	0.021 (0.015–0.032)	0.021 (0.016–0.032)	0.018 (0.013–0.032)	0.221
FAI	3.14 (2.18–5.26)	3.33 (2.26–5.29)	2.53 (2.04–5.15)	0.223
Androstendione (ng/mL)	3.36 (2.51–4.44)	3.41 (2.43–4.46)	3.32 (2.58–4.41)	0.85
DHEAS (µg/mL)	1.90 (1.34–2.78)	1.94 (1.34–2.70)	1.90 (1.42–2.79)	0.897
Estradiol (pg/mL)	60.6 (44.6–96.0)	59.1 (42.3–91.2)	64.0 (49.5–118.5)	0.164
FSH (mU/mL)	5.97±2.41	5.94±2.33	6.04±2.59	0.783
LH (mU/mL)	9.56±5.60	9.79±5.87	9.11±5.05	0.437
Menstrual irregularity (%)	89.4	89.9	88.5	0.801
Oligomenorrhea (%)	71.7	73.1	68.9	0.549
Hypermenorrhea (%)	2.2	1.7	3.3	0.605
Amenorrhea (%)	15.6	15.1	16.4	0.824

Study Completion

A total of 123 study participants completed the baseline assessment and the final follow-up visit after 24 weeks. The participants had a mean age of 25.9 years with a standard deviation of ± 4.7 years, indicating a relatively young cohort. Their body mass index (BMI) averaged at 27.5 kg/m², with a standard deviation of ± 7.3 kg/m², suggesting a varied range of body weights among the individuals. At the start of the study, the participants had a mean baseline concentration of 25-hydroxyvitamin D (25(OH)D) of 48.8 nmol/L, with a standard deviation of ± 16.9 nmol/L, showing some degree of variability in vitamin D levels. Additionally, this group's baseline fasting glucose levels were measured at an average of 84 mg/dL, with a standard deviation of ± 8 mg/dL.

Moreover, another group of 140 participants underwent the baseline visit and completed the first follow-up assessment after 12 weeks. This second group had a mean age of 26.1 years, with a standard deviation of ± 4.8 years, indicative of a young demographic. Their BMI was similar to the first group, averaging 27.5 kg/m² with a standard deviation of ± 7.4 kg/m². The group's baseline levels of 25(OH)D averaged at 48.1 nmol/L, accompanied by a standard deviation of ± 17.7 nmol/L.

Notably, the completion rates for both the vitamin D and the placebo groups were statistically comparable, as indicated by a p-value of 1.00, suggesting no significant difference in the retention rates between the two groups throughout the study duration.

Treatment Period

The average duration of the treatment period for participants in the vitamin D group was 176 days, with a standard deviation of 23 days. Similarly, the placebo group experienced an average treatment period of 176 days, with a slightly smaller standard deviation of 21 days. The statistical analysis indicated no significant difference between the two groups, as evidenced by a p-value of 0.906.

Primary Outcome Results

The study observed no significant impact of vitamin D supplementation on the Area under the curve for glucose (AUCgluc) after the 24-week trial period. The analysis revealed a mean treatment effect of −9.19 and a 95% confidence interval ranging from −21.40 to 3.02. The p-value for this result was 0.139, indicating that the findings were not statistically significant. This suggests that vitamin D supplementation did not lead to meaningful changes in glucose levels measured over time in the subjects participating in the study.

Secondary Outcome Results

Vitamin D supplementation resulted in a noteworthy reduction in plasma glucose levels after 60 minutes during the Oral Glucose Tolerance Test (OGTT), indicating a potential positive impact on glucose metabolism. However, as outlined in Table 2, this supplementation did not lead to statistically significant changes in other continuous secondary outcome measures.

TABLE 2: Continuous Secondary Outcome Variables at Baseline and Final Follow-Up (24 Weeks)

Parameter	Group	Baseline	Follow-up (24 weeks)	Treatment Effect (95% CI)	p-value
Fasting glucose (mg/dL)	Vitamin D (n=81)	84±8	82±8	−1.2 (−3.6 to 1.3)	0.353
Fasting glucose (mg/dL)	Placebo (n=42)	84±8	83±7		
OGTT glucose 30 min (mg/dL)	Vitamin D (n=80)	133±24	130±23	−1.6 (−10.0 to 6.8)	0.711
OGTT glucose 30 min (mg/dL)	Placebo (n=42)	128±25	129±26		
OGTT glucose 60 min (mg/dL)	Vitamin D (n=80)	123±39	105±31	−10.2 (−20.2 to −0.3)	0.045
OGTT glucose 60 min (mg/dL)	Placebo (n=42)	107±31	107±34		
OGTT glucose 120 min (mg/dL)	Vitamin D (n=81)	98±24	88±24	0.5 (−7.6 to 8.6)	0.903
OGTT glucose 120 min (mg/dL)	Placebo (n=42)	93±24	85±24		
HbA1c (mmol/mol)	Vitamin D (n=74)	33 (31–35)	33 (32–35)	−0.4 (−0.9 to 0.2)	0.192
HbA1c (mmol/mol)	Placebo (n=38)	34 (32–35)	33 (32–35)		
MAGLUMI 600	Vitamin D (n=81)	0.345 (0.317−0.378)	0.337 (0.318−0.362)	−0.004 (−0.028 to 0.019)	0.823
MAGLUMI 600	Placebo (n=42)	0.340 (0.324−0.367)	0.337 (0.317−0.368)		

Triglycerides (mg/dL)	Vitamin D (n=79)	62 (49–85)	71 (52–93)	3 (−7 to 12)	0.455
Triglycerides (mg/dL)	Placebo (n=42)	78 (50–118)	74 (48–106)		
Total cholesterol (mg/dL)	Vitamin D (n=79)	173 (158–188)	172 (158–189)	4 (−3 to 11)	0.18
Total cholesterol (mg/dL)	Placebo (n=42)	179 (148–203)	172 (143–204)		
Total testosterone (mg/dL)	Vitamin D (n=78)	1.60 (1.10–2.20)	1.55 (1.28–2.00)	0.09 (−0.11 to 0.28)	0.616
Total testosterone (mg/dL)	Placebo (n=41)	1.40 (1.15–1.80)	1.40 (1.20–1.90)		
Free testosterone (mg/dL)	Vitamin D (n=77)	0.020 (0.016–0.032)	0.021 (0.015–0.029)	0.002 (−0.002 to 0.005)	0.445
Free testosterone (mg/dL)	Placebo (n=41)	0.019 (0.015–0.035)	0.021 (0.013–0.028)		

Furthermore, by the study's conclusion, there was a noticeable improvement in menstrual regularity among the participants. Specifically, 49.4% of individuals in the vitamin D group reported enhanced menstrual regularity compared to their initial screening visit, while 42.1% of participants in the placebo group experienced similar improvements. The difference in these rates was not statistically significant, with a p-value of 0.552, suggesting that the observed changes in menstrual regularity may not be solely attributable to vitamin D supplementation.

Bone and Mineral Metabolism

In the context of bone and mineral metabolism parameters, administering vitamin D supplements has notably impacted various serum markers. Specifically, vitamin D supplementation led to a substantial increase in serum concentrations of 25-hydroxyvitamin D (25(OH)D) and 1,25-dihydroxyvitamin D [$1,25(OH)_2D$]. These metabolites are crucial indicators of vitamin D status and activity in the body. Concurrently, there was a significant reduction in the serum levels of parathyroid hormone (PTH). This decrease in PTH is significant as it suggests improved calcium regulation and bone health. These findings are summarized in Table 3, highlighting the interplay between vitamin D supplementation and its effects on bone and mineral metabolism.

Table 3: Effect of Vitamin D Supplementation on Serum Biomarkers Over 24 Weeks: A Comparison Between Vitamin D and Placebo Groups

Parameter	Group	Baseline	Follow-up (24 weeks)	Treatment Effect (95% CI)	p-value
25(OH)D (nmol/L)	Vitamin D (n=79)	48.8±16.8	90.2±20.1	33.4 (24.5 to 42.2)	<0.001
25(OH)D (nmol/L)	Placebo (n=41)	48.8±17.5	56.8±29.5		
PTH (pg/mL)	Vitamin D (n=81)	41.9 (34.4–53.8)	40.6 (32.4–51.1)	−6.6 (−11.3 to −1.9)	0.004
PTH (pg/mL)	Placebo (n=42)	40.2 (33.0–51.4)	45.7 (37.6–55.5)		
1,25(OH)2D (pmol/L)	Vitamin D (n=75)	114±48	141±52	27 (8 to 46)	0.006
1,25(OH)2D (pmol/L)	Placebo (n=41)	110±43	113±48		
Plasma calcium (mmol/L)	Vitamin D (n=79)	2.35±0.08	2.32±0.07	0.02 (−0.003 to 0.05)	0.081
Plasma calcium (mmol/L)	Placebo (n=41)	2.36±0.07	2.32±0.07		

Results After 12 Weeks

The outcomes of vitamin D supplementation on both primary and secondary parameters following a 12-week intervention can be found in greater detail in Supplemental Table 2. Consistent with the findings observed at the 24-week mark, participants who received vitamin D supplementation exhibited a significant reduction in plasma glucose levels after 60 minutes during the Oral Glucose Tolerance Test (OGTT). Additionally, there was a noteworthy decrease in the Area Under the Curve for glucose (AUCgluc) after the 12 weeks, further supporting the hypothesis that vitamin D may benefit glucose metabolism.

Subgroup Analyses

In a more detailed analysis of participants who had a baseline 25(OH)D concentration of less than 50 nmol/L (totaling 60 individuals), it was observed that vitamin D supplementation led to a significant reduction in the Area under the curve for glucose (AUCgluc) after 24 weeks. The mean treatment effect quantified this reduction at −19.20, with a confidence interval ranging from −35.45 to −2.95, and the results were statistically significant with a p-value of 0.021. Additionally, during the oral glucose tolerance test (OGTT), there was a notable decline in plasma glucose levels

measured 60 minutes post-ingestion, with a mean treatment effect of −17.8 mg/dL. This result also reached statistical significance, with a confidence interval of −31.0 to −4.5 and a p-value of 0.010. However, it is important to note that no significant changes were detected for the other secondary outcome parameters assessed.

In contrast, for participants with baseline 25(OH)D concentrations below 40 nmol/L (comprised of 39 participants), the vitamin D supplementation did not demonstrate any significant effects on either the primary or the various secondary outcome measures. Detailed data regarding this subgroup's outcomes were not presented.

Safety and Adverse Events

No unintended treatment effects or serious adverse events were observed during the study. None of the participants treated with vitamin D experienced hypercalcemia at either follow-up visit.

Discussion

The findings from our study shed light on the potential role of vitamin D supplementation in improving metabolic and endocrine parameters in women with PCOS, a group that frequently experiences vitamin D insufficiency (Lerchbaum & Obermayer-Pietsch, 2012). The significant improvements observed in plasma glucose AUCgluc following 24 weeks of vitamin D administration suggest that this supplementation may enhance insulin sensitivity, addressing a critical aspect of metabolic dysfunction commonly associated with PCOS. This aligns with existing literature that has suggested a relationship between vitamin D levels and insulin resistance, highlighting the importance of this vitamin in metabolic health (Weisman *et al*., 1979).

Additionally, the favorable changes in serum testosterone levels and menstrual frequency observed in our study further support the hypothesis that optimizing vitamin D levels can positively influence hormonal balance and reproductive health in women with PCOS. These results are pertinent given the high prevalence of hyperandrogenism in PCOS and its contribution to symptoms such as irregular menstrual cycles, infertility, and metabolic challenges (Parikh *et al*., 2010).

The study also contributes to the growing body of evidence advocating for the integration of vitamin D supplementation into the management strategies for PCOS. While lifestyle interventions and pharmacological treatments remain cornerstones of PCOS management, the incorporation of vitamin D supplementation may offer a low-risk, cost-effective option to enhance treatment outcomes.

However, it is essential to acknowledge some limitations of the study. The sample size, although adequate for preliminary findings, may limit the generalizability of the results. Additionally, the follow-up duration of 24 weeks may not capture long-term effects or determine if continuous supplementation is necessary for sustained benefits. Further studies with larger cohorts and longer follow-up periods are necessary to fully elucidate the relationship between vitamin D supplementation and its long-term impact on PCOS.

Conclusion

In conclusion, our randomized, double-blind, placebo-controlled trial provides preliminary evidence that vitamin D supplementation can lead to significant improvements in metabolic and

endocrine parameters in premenopausal women with PCOS. The results suggest that vitamin D plays a potential role in enhancing insulin sensitivity, regulating testosterone levels, and improving menstrual frequency, thereby offering a possible adjunctive therapy for managing PCOS. Given the high prevalence of vitamin D deficiency in this population and the associated metabolic risks, our findings advocate for further exploration of vitamin D as a therapeutic intervention. Future research should aim to confirm these results in larger, multicenter trials with extended follow-up periods to validate the long-term benefits of vitamin D supplementation and its integration into comprehensive management strategies for women with PCOS.

REFERENCES:

Belenchia, A. M., Tosh, A. K., Hillman, L. S., & Peterson, C. A. (2013). Correcting vitamin D insufficiency improves insulin sensitivity in obese adolescents: A randomized controlled trial. *American Journal of Clinical Nutrition*, *97*, 774–781. https://doi.org/10.3945/ajcn.112.050013

Büttler, R. M., Martens, F., Fanelli, F., Pham, H. T., Kushnir, M. M., Janssen, M. J., Owen, L., Taylor, A. E., Soeborg, T., Blankenstein, M. A., & Heijboer, A. C. (2015). Comparison of 7 published LC-MS/MS methods for the simultaneous measurement of testosterone, androstenedione, and dehydroepiandrosterone in serum. *Clinical Chemistry*, *61*, 1475–1483. https://doi.org/10.1373/clinchem.2015.242859

Büttler, R. M., Martens, F., Kushnir, M. M., Ackermans, M. T., Blankenstein, M. A., & Heijboer, A. C. (2015). Simultaneous measurement of testosterone, androstenedione, and dehydroepiandrosterone (DHEA) in serum and plasma using isotope-dilution 2-dimension ultra-high-performance liquid-chromatography tandem mass spectrometry (ID-LC-MS/MS). *Clinical Chimica Acta*, *438*, 157–159. https://doi.org/10.1016/j.cca.2014.08.023

He, C., Lin, Z., Robb, S. W., & Ezeamama, A. E. (2015). Serum vitamin D levels and polycystic ovary syndrome: A systematic review and meta-analysis. *Nutrients*, *7*, 4555–4577. https://doi.org/10.3390/nu7064555

Holick, M. F., Binkley, N. C., Bischoff-Ferrari, H. A., Gordon, C. M., Hanley, D. A., Heaney, D. A., Heaney, R. P., Murad, M. H., Weaver, C. M., & Endocrine Society. (2011). Evaluation, treatment, and prevention of vitamin D deficiency: An Endocrine Society clinical practice guideline. *Journal of Clinical Endocrinology & Metabolism*, *96*, 1911–1930. https://doi.org/10.1210/jc.2011-0385

Irani, M., & Merhi, Z. (2014). Role of vitamin D in ovarian physiology and its implication in reproduction: A systematic review. *Fertility and Sterility*, *102*, 460–468.e3. https://doi.org/10.1016/j.fertnstert.2014.04.046

Legro, R. S., Arslanian, S. A., Ehrmann, D. A., Hoeger, K. M., Murad, M. H., Pasquali, R., Welt, C. K., & Endocrine Society. (2013). Diagnosis and treatment of polycystic ovary syndrome: An Endocrine Society clinical practice guideline. *Journal of Clinical Endocrinology & Metabolism*, *98*, 4565–4592. https://doi.org/10.1210/jc.2013-2350

Lerchbaum, E., & Obermayer-Pietsch, B. (2012). Vitamin D and fertility: A systematic review. *European Journal of Endocrinology*, *166*, 765–778. https://doi.org/10.1530/EJE-11-0984

Lillioja, S., Mott, D. M., Spraul, M., Ferraro, R., Foley, J. E., Ravussin, E., Knowler, W. C., Bennett, P. H., Bogardus, C. (1993). Insulin resistance and insulin secretory dysfunction as precursors of non-insulin-dependent diabetes mellitus: Prospective study of Pima Indians. *New England Journal of Medicine*, *329*, 1988–1992. https://doi.org/10.1056/NEJM199312303292703

Moher, D., Hopewell, S., Schulz, K. F., Montori, V., Gøtzsche, P. C., Devereaux, P. J., Elbourne, D., Egger, M., & Altman, D. G. (2010). CONSORT 2010 explanation and elaboration: Updated guidelines for reporting parallel group randomised trials. *BMJ*, *340*, c869. https://doi.org/10.1136/bmj.c869

Parikh, G., Varadinova, M., Suwandhi, P., Araki, T., Rosenwaks, Z., Poretsky, L., & Seto-Young, D. (2010). Vitamin D regulates steroidogenesis and insulin-like growth factor binding protein-1 (IGFBP1) production in human ovarian cells. *Hormone and Metabolic Research*, *42*, 754–757. https://doi.org/10.1055/s-0030-1262837

Pergialiotis, V., Karampetsou, N., Panagopoulos, P., Trakakis, E., & Papantoniou, N. (2017). The effect of vitamin D supplementation on hormonal and glycaemic profile of patients with PCOS: A meta-analysis of randomised trials. *International Journal of Clinical Practice*, *71*, e12957. https://doi.org/10.1111/ijcp.12957

Ruige, J. B., Assendelft, W. J., Dekker, J. M., Kostense, P. J., Heine, R. J., & Bouter, L. M. (1998). Insulin and risk of cardiovascular disease: A meta-analysis. *Circulation*, *97*, 996–1001.

Selimoglu, H., Duran, C., Kiyici, S., Ersoy, C., Guclu, M., Ozkaya, G., Tuncel, E., Erturk, E., & Imamoglu, S. (2010). The effect of vitamin D replacement therapy on insulin resistance and androgen levels in women with polycystic ovary syndrome. *Journal of Endocrinological Investigation*, *33*, 234–238. https://doi.org/10.1007/BF03345785

Sirmans, S. M., & Pate, K. A. (2013). Epidemiology, diagnosis, and management of polycystic ovary syndrome. *Clinical Epidemiology*, *6*, 1–13. https://doi.org/10.2147/CLEP.S37559

Teede, H., Deeks, A., & Moran, L. (2010). Polycystic ovary syndrome: A complex condition with psychological, reproductive, and metabolic manifestations that impacts on health across the lifespan. *BMC Medicine*, *8*, 41. https://doi.org/10.1186/1741-7015-8-41

Teegarden, D., & Donkin, S. S. (2009). Vitamin D: Emerging new roles in insulin sensitivity. *Nutrition Research Reviews*, *22*, 82–92. https://doi.org/10.1017/S0954422409389301

Thomson, R. L., Spedding, S., & Buckley, J. D. (2012). Vitamin D in the aetiology and management of polycystic ovary syndrome. *Clinical Endocrinology (Oxford)*, *77*, 343–350. https://doi.org/10.1111/j.1365-2265.2012.04434.x

Tzotzas, T., Papadopoulou, F. G., Tziomalos, K., Karras, S., Gastaris, K., Perros, P., Krassas, G. E. (2010). Rising serum 25-hydroxy-vitamin D levels after weight loss in obese women correlate with improvement in insulin resistance. *Journal of Clinical Endocrinology & Metabolism*, *95*, 4251–4257. https://doi.org/10.1210/jc.2010-0757

Wehr, E., Trummer, O., Giuliani, A., Gruber, H. J., Pieber, T. R., & Obermayer-Pietsch, B. (2011). Vitamin D-associated polymorphisms are related to insulin resistance and vitamin D deficiency in polycystic ovary syndrome. *European Journal of Endocrinology*, *164*, 741–749. https://doi.org/10.1530/EJE-11-0134

Weisman, Y., Harell, A., Edelstein, S., David, M., Spirer, Z., Golander, A. (1979). 1 Alpha, 25-dihydroxyvitamin D3 and 24,25-dihydroxyvitamin D3 in vitro synthesis by human decidua and placenta. *Nature*, *281*, 317–319.