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Effect of vitamin D supplementation on insulin kinetics and cardiovascular risk factors in polycystic ovarian syndrome: a pilot study

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Abstract

To assess the effect of vitamin D supplementation on parameters of insulin sensitivity/resistance (IS/IR) and insulin secretion in subjects with polycystic ovarian syndrome (PCOS). A prospective double-blind randomized control trial was conducted to assess the effect of vitamin D on insulin kinetics in women with PCOS. The trial was conducted in a tertiary care research hospital. A total of 36 subjects with PCOS, aged 18–35 years, were included in this study. Vitamin D3 4000 IU/day versus placebo was given once a month for 6 months and both groups received metformin. IS (by whole-body IS index or Matsuda index), IR (by homeostasis model assessment IR (HOMA-IR)), and insulin secretion (by insulinogenic index; II_{30}) were the main outcome measures. Secondary outcome included blood pressure (BP), lipid profile, disposition index (DI), and vascular stiffness. Out of 36 subjects who consented, 32 completed the study. Subjects were randomized into two groups: group A ($n=15$; metformin and vitamin D 4000 IU/day) or group B ($n=17$; metformin and placebo). Oral glucose tolerance tests with 75 g glucose were carried out at baseline and 6 months after supplementation. Hypovitaminosis D was observed in 93.8% of all subjects with mean serum 25 hydroxy vitamin D level of 7.30 ± 4.45 ng/ml. After 6 months of vitamin D supplementation, there was no significant difference in any of the parameters of IS/IR (area under curve (AUC)–glucose, AUC–insulin, insulin:glucose ratio, HOMA-IR, Matsuda index, insulinogenic index, and DI), II_{30}, and cardiovascular risk factors between the two groups. Supplementation of vitamin D, at a dose of 4000 IU/day for 6 months, did not have any significant effect on parameters of IS/IR and insulin secretion in subjects with PCOS.

Key Words
- PCOS
- insulin resistance
- insulin secretion
- vitamin D supplementation

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Introduction

Vitamin D deficiency (VDD) was once thought to exclusively affect bone metabolism. Currently, however, there is ample evidence of its role in many extra-skeletal conditions including metabolic syndrome, autoimmune diseases, and cancer (1). Vitamin D receptors are recognized to be present in numerous extra-skeletal tissues, including pancreas and muscle (2).

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Vitamin D has been reported to influence not only insulin sensitivity/resistance (IS/IR) but also insulin secretion (3, 4, 5). There are several cross-sectional studies, including data from the Framingham Heart Study (6), which have demonstrated an inverse association between serum 25 hydroxy vitamin D (S.25(OH)D) levels and IR (5). The randomized trials conducted to study the effect of vitamin D supplementation on IR demonstrate either no effect or improvement in IR with supplementation (5). Gedik & Akalin (7) reported significant improvements in insulin secretion following vitamin D supplementation. The most of the studies assessing effects of vitamin D supplementation on the insulin axis have assessed IS/IR only and that by using fasting blood samples that provide information about hepatic IS/IR with little or no information about peripheral insulin action. Vitamin D supplementation is more likely to influence peripheral IS, as demonstrated by Nagpal *et al.* (8), whereby three doses of 120 000 IU vitamin D every two weeks in comparison with placebo showed significant improvements in a 3 h oral glucose tolerance test (OGTT)-derived IS index, but not indices derived from fasting values, in Asian–Indian men. However, these studies have been limited by issues in design, including lack of randomization, absence of placebo control, use of indirect measures of insulin secretion and IS, small sample size, and inability to show relevant increases in serum vitamin D levels. The absence of consensus regarding optimal dosing of vitamin D and lack of definition for optimal therapeutic concentrations of S.25(OH)D level further inhibit the application of vitamin D intervention trials.

Polycystic ovarian syndrome (PCOS) is now recognized as one of the most common endocrinopathies in women of reproductive age with a prevalence of 4–10% (9). Women with PCOS are known to be at an increased risk for IR and present most frequently with complaints of infertility, menstrual irregularity, hirsutism, and/or other outward signs of androgen excess such as acne or alopecia (10). Prevalence rates of IR have been reported to range from 44% to 70%, though there have been methodological concerns about the assessment of IR in these reports (9).

Results of cross-sectional studies in subjects with PCOS have also indicated an inverse association between serum vitamin D level and IR (11, 12, 13, 14). There are few attempts to assess the effects of vitamin D supplementation on IR in subjects with PCOS; however, results are variable (15, 16, 17, 18, 19). In a recently published randomized controlled trial of high-dose vitamin D supplementation in PCOS, parameters of IS (QUICKI) and IR (HOMA-IR) (20) were assessed. In women with PCOS, especially with lean and normal BMI, hyperinsulinemia is often evident only in the postprandial but not in the fasted state, indicating that abnormalities of glucose metabolism are likely to be missed if only fasting values are considered (10, 21). We planned to assess effects of vitamin D supplementation on both IR (both hepatic as well as peripheral) and insulin secretion and cardiovascular risk factors in subjects with PCOS.

Material and methods

Study design

This study was conducted as a prospective randomized double-blind placebo controlled study after obtaining approval from the ethics committee of the institute and written informed consent was obtained from all subjects. The study subjects were selected from women attending the endocrinology and gynecology outdoor services.

Inclusion criteria

Subjects in the age group of 18–35 years diagnosed as having PCOS were included in this study.

Definition of PCOS ▸ PCOS was diagnosed as proposed by the European Society for Human Reproduction and Embryology and the American society for Reproductive Medicine (Rotterdam criteria, 2003), which includes presence of two out of the following three criteria: oligo- or chronic anovulation; clinical and/or biochemical signs of hyperandrogenism; and polycystic ovarian appearance on ultrasonography; with exclusion of other etiologies of androgen excess and anovulatory infertility (22).

Exclusion criteria

Subjects who were currently receiving or had received vitamin D supplementation or treatment for PCOS (metformin, spironolactone, oral contraceptives, or any other drugs) in the last 6 months were excluded from the study. Similarly, subjects with any known systemic disease (cardiac, hepatic, endocrine including diabetes or impaired glucose tolerance (IGT), gastrointestinal, and renal including stones) or either currently undergoing treatment with or having received treatment for at least 1 month in the past 6 months with medication known to interact with vitamin D metabolism (steroids, thiazide diuretics, phenytoin, phenobarbital, and antitubercular drugs) were excluded from the study. Subjects who were

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planning pregnancy in the next 6 months were also excluded. All married females underwent a urine pregnancy test at the time of screening to rule out pregnancy.

Methods ▸ Detailed history was obtained, and clinical and anthropometric examination was carried out. Blood samples were collected in the fasting state (minimum 8 h fasting), followed by an oral glucose tolerance test with sample collection at 0, 30, 60, 90, and 120 min after 75 g of glucose ingestion. All biochemical investigations were carried out at baseline and repeated after 6 months of supplementation. Corrected serum total calcium and urinary calcium:creatinine ratio were measured at baseline and then every 8 weeks till completion. As this study was designed as a pilot study, the sample size was fixed as 18 subjects in each group.

Intervention

Subjects were randomized (using computer generated simple random code) into two groups: group A (metformin and vitamin D) or group B (metformin and placebo) (Fig. 1). The dose of vitamin D supplementation (only cholecalciferol was used) in group A was calculated as 4000 IU/day but given once a month as a oral dose (120 000 IU, Cifrol; Brown Pharmaceuticals, Delhi, India, the monthly dose was chosen to maximize compliance), while inert granules of equal amount and similar appearance was used as an oral placebo. The duration of intervention was 6 months. Out of the total six doses, three doses were supervised in the hospital during monitoring visits, while three doses were taken on fixed days at home, which were confirmed by telephonic contact. Both groups received similar life style modification advice including consultation with a qualified dietician before randomization.

In both arms, metformin was initiated at a dose of 500 mg twice a day for the first 2 weeks followed by 500 mg three times a day, which was continued for 6 months without any change. Adherence to medication was assured by maintaining a diary to record daily timings of ingestion of metformin.

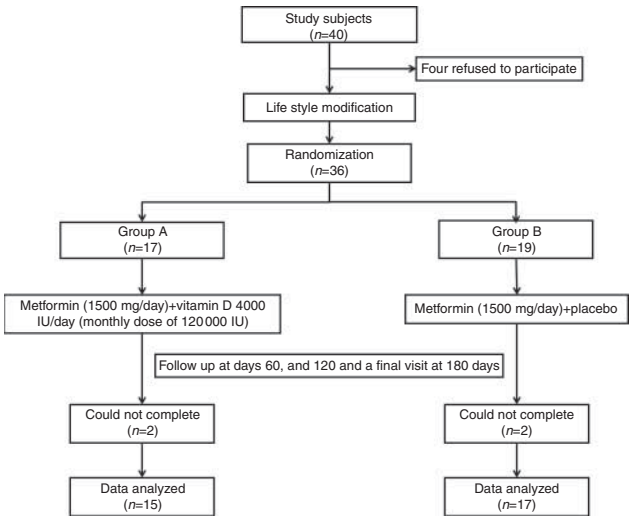

Figure 1
Flow diagram of the study, showing numbers of study subjects who were randomly assigned, received the intended vitamin D supplementation, and completed the study.

Safety of proposed intervention ▸ The two main safety issues of proposed intervention, hypercalcemia, and hypercalciuria were assessed by measurement of corrected serum total calcium and urinary calcium:creatinine ratio (non-fasting, second void sample) at baseline and then every 8 weeks and at the completion of intervention. Hypercalcemia was defined as serum calcium levels more than 10.4 mg/dl and hypercalciuria as urinary calcium:creatinine ratio more than 0.4 (mg/mg; (23)), which was considered as abnormal. Subjects with a urinary calcium:creatinine ratio of over 0.4 without hypercalcemia were re-evaluated with a timed 24 h urine calcium excretion (over 4 mg/kg excretion was considered as abnormal). If 24 h calcium excretion was within the normal limits, the subject continued in the study. Any subject, who developed both hypercalcemia and hypercalciuria, was excluded from further intervention and referred to the physician for standard management.

Anthropometric measurements ▸ All measurements were made with subjects dressed in minimal light clothing but without footwear. Height was measured with Holtain's stadiometer (Holtain, Inc., Crymych, Pembrokeshire, UK). Weight was measured with the same digital weighing machine. BP was measured in the right upper limb in the sitting position using a mercury sphygmomanometer after 5-min rest with an appropriate size cuff. BP was measured three times and the mean value of second and third recordings was considered.

Parameters of IR

i) IR – was calculated by a computer-based model called HOMA-IR utilizing fasting blood glucose and fasting serum insulin levels (24).

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ii) The whole-body IS index (WBISI) or Matsuda index – this composite WBISI was calculated by the formula suggested by Matsuda & DeFronzo (25).
iii) AUC – was calculated for blood glucose (0, 30, 60, 90, and 120 min) and serum insulin (0, 30, 60, 90, and 120 min) using the trapezoidal rule. Fasting srum insulin and blood glucose levels were used to calculate the insulin glucose ratio.
iv) Insulin secretion – was assessed by 'insulinogenic index' (II_{30}), which is the change in insulin levels divided by the change in glucose over the first 30 min of the OGTT (26) and was expressed as II_{30} (μIU/l per mg per dl) $=\Delta$ insulin 0–30/Δ glucose 0–30).
v) Disposition index (DI) – reflects the ability of the β-cell to compensate for IR and represent β-cell function (27). It is calculated by mathematical formula of insulin secretion$\times$IS (both these parameters were calculated separately).

Assessment of vascular stiffness

Vascular stiffness was assessed by pulse wave transit time analysis to determine aortic augmentation index (AIx) and pulse wave velocity (PWV) as suggested by international guidelines (28). All measurements were made on the right side and by a single observer using the SphygmoCors Cardiovascular Management System (AtCor Medical, Sydney, Australia). The AIx was calculated as augmented pressure (tonometrically derived) divided by central pulse pressure multiplied by 100 to give a percentage value for comparison. As AIx is a ratio of three aortic pressure values, the influence of any systematic errors in the estimation of aortic pulse pressure by the transfer function will be minimal (29). As AIx is influenced by heart rate, the corrected index for heart rate, 75 beats/min (AI at 75), was used. Carotid-femoral PWV was calculated by dividing distance (m) by transit time (s) and expressed as m/s. PWV is inversely related to vascular compliance. Hence, a stiffer vessel will conduct the pulse wave faster than a more distensible and compliant vessel.

Biochemical analysis ▸ Complete blood counts, liver and renal function tests, serum calcium (corrected calcium with serum albumin), phosphate, total alkaline phosphatase, and blood glucose were measured in all subjects using an automated chemistry analyzer (Roche Hitachi 912 Chemistry Analyzer, GMI, Inc., Ramsey, MN, USA). Serum insulin was measured on an auto analyzer (Roche Elecsys 2010) by electrochemiluminometric assay (CV for this method 2.1–2.8%). Serum total cholesterol, triglyceride, and HDL cholesterol (HDL-C) levels were estimated directly using an automated analyzer, while LDL cholesterol (LDL-C) was estimated using the Friedewald equation (30). Urinary calcium was measured by the colorimetric method and urinary creatinine by the liquid kinetic method. Serum intact parathyroid hormone (PTH) was measured using an electrochemiluminometric labeled tracer on a Roche-Cobas 411e auto-analyzer. S.25(OH)D was measured on the DiaSorin auto analyzer ('LIASON' DiaSorin, Inc., Stillwater, MN, USA) using a chemiluminescent label. The reproducibility of the assay ranged from 6% to 12% and was within the performance characteristics described by the manufacturer. Our laboratory is registered with the UK-DEQAS vitamin D assay external quality control assessment program and hence met their performance targets regularly (www.deqas.org).

VDD was defined as a S.25(OH)D level <20 ng/ml. This was further subdivided into severe, moderate, and mild VDD if S.25(OH)D levels were <5, 5–<10, and 10–<20 ng/ml respectively, while levels between 20 and 30 ng/ml were considered as insufficiency and >30 as vitamin D sufficient (31).

Statistical analysis

Baseline data are expressed as mean$\pm$S.D., or median and range, as appropriate. Continuous variables not showing normal distribution were log transformed for analysis. Comparison of means was performed using Student's *t*-test. Categorical variables were compared using the χ^2 test or Fisher's exact test as appropriate. For variables such as urinary calcium:creatinine ratio, serum DHEAS, HOMA-IR, and Matsuda index, log-transformed variables were used for analysis using Student's *t*-test for comparison between groups. For fasting plasma insulin, the Wilcoxon's rank-sum test was used for comparison between the groups. The outcome parameters were compared between the two groups as to their levels at 6 months after supplementation using Student's *t*-test/Wilcoxon's rank-sum test. For testing all hypotheses, tests were two-tailed and a P value of 0.05 was considered statistically significant. Analysis was performed using Stata 12.0 (College Station, TX, USA).

Results

Consecutive subjects attending the Gynecology and Endocrinology Outpatient Department were screened for PCOS. Forty subjects satisfying the inclusion criteria were

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Table 1 Baseline demographic characteristics of study subjects.

Characteristics	Group A ($n=15$)	Group B ($n=17$)	*P* value
Age (years)	22.0±4.61	22.8±4.56	0.64
BMI (kg/m^2)	26.8±4.56	26.7±6.11	0.96
History of menstrual irregularity (*n*)	15	14	0.23
Hirsutism (*n*)	5	10	0.18
Acne (*n*)	7	11	0.48
History of weight gain (*n*)	13	12	0.40
Polycystic appearance on USG (*n*)	13	16	0.58

USG, ultrasonography.

requested to participate in the study, of which 36 subjects provided consent for participation. Seventeen subjects were randomized to group A (metformin+vitamin D) and 19 to group B (metformin+placebo) (Fig. 1). Two subjects in each group were lost to follow up, leaving 32 subjects to complete the study (group A, $n=15$; group B, $n=17$). One subject in each of the two groups was more concerned about their irregular menstrual cycles and opted for treatment (started oral contraceptive pills) for regularization of cycles. One subject in group A started anti-androgen therapy for acne while one subject in group B moved out of the city and dropped out. All doses of intervention as well as placebo were taken on fixed days. Baseline demographic parameters of study subjects are shown in Table 1. Comparisons of biochemical parameters at baseline and 6 months after supplementation in both the groups are shown in Table 2.

Vitamin D deficiency

VDD (<20 ng/ml) was observed in 93.8% of all subjects, while vitamin D insufficiency was observed in the remaining 6.2% of subjects. Among 32 subjects who completed the study, 13 (40.3%) had severe VDD while 16 (50%) had moderate VDD. No significant difference in S.25(OH)D level or proportion of VDD was observed between the two groups at baseline.

Secondary hyperparathyroidism (S.iPTH >55 pg/ml) was observed in 53.3% of subjects in group A and in 41.1% of subjects in group B ($P=0.54$).

Impaired glucose tolerance

At baseline, three subjects in group A and one subject in group B had IGT but none of the study subjects had impaired fasting glucose. All three subjects in group A had normal glucose tolerance at 6 months of follow-up, while one subject in group B who had IGT at baseline continued having IGT at 6 months.

Correlation between serum vitamin D and parameters of IS/IR and insulin secretion at baseline

Parameters of IS/IR (AUC–glucose, AUC–insulin, insulin glucose ratio, HOMA-IR, Matsuda index, and DI) and insulin secretion (II_{30}) did not show any significant correlation (Spearman's rank test) with serum vitamin D level at baseline. Similarly, serum PTH also did not have any significant correlation with these parameters.

Safety of intervention

Three subjects in group A and one in group B had spot urine calcium:creatinine ratios exceeding 0.4 at 6 months, but all of these subjects had 24 h urinary calcium excretion in the normal range. None of the subjects developed hypercalcemia at any time point of the study. Metformin was well tolerated and no side effects were reported.

Follow-up after 24 weeks of intervention

There was no significant difference in any of the parameters of IS/IR and insulin secretion between the two groups at the end of 6 months. Similarly, there was no statistically significant difference between the two groups in BP, lipid profile, and parameters of vascular stiffness.

In group A, S.25(OH)D significantly increased ($P\leq0.001$) and serum PTH decreased ($P\leq0.001$) at 6 months in comparison to group B. As secondary analysis, a statistically significant decrease in serum insulin level and HOMA-IR was observed at 6 months in comparison to baseline values in group A but not in group B. Secondary hyperparathyroidism was present in 26.2% of subjects in group A and 29.4% in group B ($P=0.78$). The difference in change between the two groups (mean and CI) is shown in Table 2.

Similarly, there was no significant difference in serum total testosterone between the two groups at the end of 6 months. However, a significant decrease in serum total testosterone ($P=0.03$) was observed in group A at 6 months in comparison to baseline but was not observed in group B.

Discussion

In this study, we assessed the effects of vitamin D supplementation on parameters of IS/IR and insulin

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Table 2 Comparison of baseline and 6-month post supplementation characteristics in both groups.

Characteristics	Group A ($n=15$)			Group B ($n=17$)			$P^{\dagger}$	$P^{\ddagger}$	Mean (CI) difference in change between groups
	At baseline	At 6 months	P^{*}	At baseline	At 6 months	P^{*}			
BMI (Kg/m^2)	26.8±4.57	25.4±5.65	0.1	26.8±6.12	25.9±6.05	0.05	0.97	0.47	0.56 (−1.0, 2.14)
Serum testosterone (ng/ml)	0.47±0.19	0.35±0.12	0.03	0.55±0.18	0.52±0.11	0.51	0.19	0.17	0.08(−0.42, 0.21)
Serum DHEAS (μg/dl)	236.5±85.57	238.3±126	0.93	269.1±100.7	305±98.36	0.38	0.33	0.5	26.8 (−53.6, 07)
Serum calcium (mg/dl)	9.7±0.43	9.4±0.47	0.09	9.3±0.61	9.3±0.55	0.71	0.08	0.34	0.22 (−0.26, 0.7)
Serum phosphate (mg/dl)	3.7±0.57	3.8±0.63	0.2	3.3±0.74	3.5±0.53	0.06	0.12	0.71	0.06 (−0.2, 0.43)
Serum alkaline phosphatase (IU/l)	276±70	247±46	0.15	258±136	247±90	0.56	0.29	0.49	18.2 (−35.8, 72.2)
Serum albumin (mg/dl)	4.9±0.24	4.9±0.42	0.65	4.8±0.16	4.8±0.35	0.72	0.18	0.84	−0.03 (−0.33, 0.2)
Serum total cholesterol (mg/dl)	172±31	158±20	0.08	169±31	154±27	0.001	0.76	0.91	−0.9 (−16.9, 15.1)
Serum LDL cholesterol (mg/dl)	107±16	102±13	0.28	108±29	102±27	0.30	0.96	0.93	−0.62 (−16.1, 14.9)
Serum triglyceride (mg/dl)	116±102	81±42	<0.01	91±35	80±30	0.02	0.38	0.22	24.22 (−10.9, 59.4)
Serum HDL cholesterol (mg/dl)	42±3	43±6	0.36	40±6	42±6	0.30	0.27	0.91	0.23 (−4.3, 4.77)
Urinary calcium:creatinine ratio	0.10±0.05	0.11±0.06	0.31	0.10±0.06	0.09±0.06	0.57	0.91	0.25	−0.027 (−0.07, 0.02)
Serum intact PTH (pg/ml)	59.2±22.51	42.6±15.96	0.0008	53.5±23.65	52.7±18.58	0.83	0.49	0.007	15.84 (4.6, 27.02)
Serum 25 (OH) vitamin D (ng/ml)	7.7±6.05	31.5±13.88	<0.001	6.8±2.46	6.7±2.31	0.94	0.96	<0.001	−23.8 (−30, −16.9)
Fasting plasma glucose (mg/dl)	87.9±7.22	90±7	0.43	87.6±12.97	89±8	0.65	0.95	0.92	−0.5 (−10.9, 9.8)
Fasting plasma insulin (μU/ml)	17.3±15.28	10.3±5.92	<0.01	14.0±10.90	12.2±6.67	0.18	0.36	0.10	5.18 (−1.02, 11.39)
HOMA-IR	3.8±3.40	2.3±1.32	0.03	3.1±2.30	2.6±1.32	0.32	0.36	0.16	1.05 (−0.45, 2.56)
AUC–glucose	231.9±46.44	240.9±41.0	0.60	239.3±35.77	246.6±35.8	0.53	0.50	0.93	−1.72 (−42.6, 39.2)
AUC–insulin	191.0±142.91	138.5±54.55	0.12	187.9±106.77	181.4±151.00	0.76	0.49	0.64	46.0 (−29.8, 121.8)
Matsuda index	3.7±2.41	4.3±2.02	0.31	4.3±2.92	4.3±2.89	0.98	0.62	0.51	−0.64 (−2.67, 1.38)
Insulinogenic index	2.2±1.29	1.9±1.66	0.44	2.6±3.19	1.8±1.53	0.33	0.65	0.30	−0.52 (−2.41, 1.36)
Disposition index	7.7±5.48	8.1±7.79	0.86	8.4±10.08	6.0±3.93	0.79	0.99	0.95	−2.9 (−10.39, 4.63)
AoPWV (m/s)	5.6±1.30	6.2±1.32	0.22	6.5±1.25	6.3±1.04	0.61	0.08	0.16	−0.70 (−1.70, 0.31)
Augmentation index	11.9±10.72	10.6±10.50	0.89	12.1±7.73	11.4	0.94	0.98	0.78	1.23 (−7.84, 10.3)

*P, difference between baseline and 6 months; $^{\dagger}P$, difference between the two groups at baseline; $^{\ddagger}P$, difference between two groups at the end of 6 months; AUC, area under the curve; AoPWV, aortic pulse wave velocity.

secretion in subjects with PCOS. The results of our study indicate that vitamin D supplementation given at doses of 4000 IU/day (given once a month) for 6 months to vitamin D deficient/insufficient subjects with PCOS did not have any significant effects on parameters of IS/IR and insulin secretion despite normalization of s.25(OH)D levels.

A high prevalence of VDD has been reported all over India for all age groups including neonates, infants, school-going children, adolescents, adults, pregnant and lactating women, and senior citizens. This is probably a result of poor sun exposure, dark skin complexion, atmospheric pollution, vegetarian food habits, absence of food fortification with vitamin D, and poor intake of vitamin D supplements (32). Consistent with these observations, VDD was observed in 93.8% of all subjects while the rest had vitamin D insufficiency. In our study, subjects in group A received supplementation with 4000 IU of vitamin D/day, resulting in a significant increase in mean S.25(OH)D levels from 7.73 to 31.54 ng/ml at 6 months with a significant fall in PTH (mean change of 16.62 pg/ml) from baseline. However, mean S.25(OH)D levels and PTH levels did not change significantly in group B.

The effects of vitamin D supplementation on IR and IS in subjects with PCOS have been investigated in a small number of studies (15, 16, 17, 18, 19). All studies except one have failed to find a significant effect of vitamin D supplementation on IS parameters. However, it would be difficult to compare results with these studies as the dose and duration of vitamin D supplementation were quite variable among the studies and, only in three studies (15, 16, 20), subjects were randomized. In a recently published randomized placebo controlled trial, Raja-Khan *et al.* (20), using a high dose of vitamin D (12 000 IU/day) for 12 weeks, also did not find any significant difference in IS.

In the only study where vitamin D supplementation resulted in a decrease in IR, the results were of borderline significance ($P=0.043$). In this study, Selimoglu *et al.* (18) administered a single oral dose of 300 000 IU of vitamin D3 to 11 women with PCOS (mean age 23.6 ± 5.7 years and BMI 33.9 ± 5.1 kg/m^2). Three weeks after administration of vitamin D, while IR (as measured by HOMA-IR) decreased significantly ($P=0.043$), there was no significant difference in fasting insulin and glucose levels.

A significant decrease in fasting serum insulin levels in group A was observed after vitamin D supplementation ($P=0.009$), which resulted in borderline decrease in HOMA-IR (0.03). A similar decrease in insulin levels after vitamin D supplementation has also been reported by Raja-Khan *et al.* (20). Although it would be difficult to identify from studies of small sample size, a direct effect of vitamin D on insulin secretion is possible. Both of these studies are similar in design, but there were some important noticeable differences. The mean S.25(OH)D level was much lower in our study with the presence of VDD in 93% study subjects. Similarly, BMI was also hugely different in both these studies with a much lower BMI in our study (mean BMI was 37.20 ± 4.53 and 35.09 ± 9.81 kg/m^2 versus 26.8 ± 4.56 and 26.7 ± 4.56 kg/m^2 in the vitamin D and placebo groups respectively).

The reports of the few studies that have measured AUC for glucose and insulin (17, 19), in addition to calculating IR parameters from fasting serum insulin and blood glucose, did not describe any significant effect. In our study, both AUC–glucose and AUC–insulin were not different between the two groups after intervention.

Although results from our study indicate an improvement in DI after vitamin D supplementation in group A, it was not statistically significant. Our study being a pilot project was not powered enough to detect a significant difference in the parameters of insulin kinetics.

PCOS has been reported to be associated with factors of increased cardiovascular risk, such as IR, dyslipidemia, and type 2 diabetes (33, 34, 35). PCOS has also been suggested to be associated with increased arterial stiffness, although whether this association is independent of obesity or not is not certain (36). In our study, we did not find any significant difference between lipid profile and parameters of vascular stiffness between the two groups after vitamin D supplementation. Similar results of changes in lipid parameters were also reported by Raja-Khan *et al.* (20).

Thus, in our study, like previous studies in subjects with PCOS, there was no significant effect of vitamin D supplementation on parameters of IS/IR and insulin secretion.

Although there was no significant difference in serum testosterone between the two groups at baseline or at 6 months, a significant fall in serum testosterone was observed in group A at 6 months in comparison with the baseline in the same group. A similar result has also been described in a study by Pal *et al.* (17). However, others (19, 37) have observed no effect on serum testosterone levels. A direct effect of vitamin D on the steroidogenesis pathway (ovarian and/or adrenal) has been proposed to explain the observed reduction in circulating androgens (17).

None of the study subjects developed clinical or biochemical evidence of hypercalcemia at any time

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during supplementation While three subjects in group A and one in group B had spot urine calcium:creatinine ratios exceeding 0.4, none of them had elevated 24 h urinary calcium excretion or hypercalcemia. This indicates that the doses used in our study are safe and effective for improving S.25(OH)D levels. Wehr *et al.* (19), using vitamin D dose of 20 000 IU once a week for 24 weeks, reported that, in one subject at 12 weeks and in another subject at 24 weeks, S.25(OH)D levels were found to be in excess of 100 ng/ml. However, both these subjects remained normocalcemic throughout the study. The mean S.25(OH)D levels of study subjects were 28 ± 11 ng/ml, which were higher than the mean S.25(OH)D levels of our subjects.

To the best of our knowledge, this is the first study evaluating effects of vitamin D supplementation on both IS/IR and insulin secretion parameters derived from both fasting and post-glucose challenge and cardiovascular risk factors in subjects with PCOS. The double-blind placebo controlled design, use of both fasting- and OGTT-derived parameters, and 6 months of supplementation duration are the main strengths of our study, but being a pilot study in design, it was not powered to detect a significant difference in outcome measures. Hence, small sample size and lack of power were major limitations. Another limitation was the use of metformin in both study groups along with vitamin D/placebo, which might have overruled a small positive effect of vitamin D on IS. As all subjects were symptomatic (oligomenorrhea and hirsutism), it was decided to treat them along with administering vitamin D/placebo.

Although our results indicate no significant difference in outcomes between the two groups they need to be interpreted cautiously in view of the small sample size and the study power for comparison of outcomes being meager, the maximum being only 27% for comparison of HOMA-IR. Another important limitation was very few subjects with IGT (three in group A and one in group B).

In conclusion, we did not find any beneficial effects of vitamin D supplementation on parameters of IS in women with PCOS. However, studies with larger sample sizes and adequate power may detect possible therapeutic benefits of vitamin D supplementation on IR and β-cell function in subjects with PCOS.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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