RESEARCH ARTICLE

Effect of Two Different Doses of Vitamin D Supplementation on Clinical, Metabolic, and Hormonal Profiles of Patients with Polycystic Ovary Syndrome (PCOS) with Insulin Resistance: A Randomized Controlled Trial

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Abstract

Objective: The rationale for using vitamin D in polycystic ovary syndrome (PCOS) with insulin resistance women is based on the fact that it reduces insulin resistance.

Materials and methods: This was a prospective, open-label, randomized controlled trial involving supplementation of Vitamin D in two different doses in insulin-resistant PCOS women to assess the effects on clinical, metabolic, and hormonal profiles. We recruited 72 women of PCOS aged 20–35 years with HOMA-IR >2.5, Vitamin D levels <20 ng/mL, and BMI <30 kg/m^2. Selected patients were randomized in two groups: Patients in group I received tablet Metformin 500 mg twice a day orally along with tablet vitamin D3 1000 IU orally per day for 3 months. Patients in group II received Metformin 500 mg twice a day orally along with tablet vitamin D3 4000 IU orally per day for 3 months.

Results: This study showed that vitamin D supplementation in dose of 4000 IU for 12 weeks to insulin-resistant women with PCOS had more beneficial effect on HOMA-IR, mFG score, global acne score, menstrual cycle regularity, BMI, LH levels, LH:FSH ratio, triglyceride levels, DHEAS levels, FBS, PPBS, fasting insulin, and postprandial insulin as compared to vitamin D supplementation in dose of 1000 IU daily for 3 months.

Conclusion: As it was a single-center study, results cannot be extrapolated to population as a whole. Major strength was that it was a randomized controlled trial comparing efficacy of both upper and lower limits of vitamin D supplementation in two groups having similar baseline characteristics. However, absence of consensus pertaining to optimal dose of vitamin D warrants need of further intervention trials with larger sample size.

Keywords: Polycystic ovary syndrome, Randomized clinical trial, Vitamin D.

Journal of South Asian Federation of Obstetrics and Gynaecology (2022): 10.5005/jp-journals-10006-2052

Introduction

Polycystic ovary syndrome (PCOS) is an important cause of menstrual irregularity and androgen excess affecting almost 4–8% of women in reproductive age-group.[1,2] Presence of two out of three Rotterdam's criteria establishes its diagnosis—oligo and/or anovulation, clinical and/or biochemical signs of hyperandrogenism, and polycystic ovarian morphology (on ultrasound).[3] Hyperandrogenism and insulin resistance remain key features in the pathogenesis of PCOS. However, exact mechanism for insulin resistance (IR) in PCOS women is not known but evidence suggests that this could be due to defect in postreceptor signaling pathways. Hyperinsulinemia due to insulin resistance affects about 65–70% of PCOS patients, of which 70–80% are obese patients.[4] Insulin sensitivity is decreased in both lean and obese PCOS women.[5] It has been observed that 67–85% of PCOS women are vitamin D deficient and deficiency correlates with insulin resistance and metabolic syndrome.[6–8]

The rationale for using vitamin D in PCOS women is based on the fact that it reduces insulin resistance. Vitamin D is able to modulate its effect on glucose-insulin homeostasis, through the action on vitamin D receptors (VDR) located on the skeletal muscle and pancreatic beta cells, that directly activates transcription of the human insulin receptor gene, stimulates the expression of insulin receptor, activates peroxisome proliferator activator receptor-d, and increases insulin-mediated glucose transport *in vitro*.[9] Evidence supports that 1,25-$(OH)_2$ D modulates calcium signaling in adipocyte, which in turn leads to increased lipogenesis and decreased lipolysis, possibly through inhibition of uncoupling protein-2 (UCP-2).[10] As there is evidence that intake of vitamin D supplements might improve lipid profile and insulin resistance, and may have antidiabetic effect, we hypothesized that vitamin D supplementation along with metformin may improve clinical, hormonal, and metabolic profiles of PCOS women. Metformin is

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How to cite this article: Bahadur A, Yadav A, Mundhra R, *et al.* Effect of Two Different Doses of Vitamin D Supplementation on Clinical, Metabolic, and Hormonal Profiles of Patients with Polycystic Ovary Syndrome (PCOS) with Insulin Resistance: A Randomized Controlled Trial. J South Asian Feder Obst Gynae 2022;14(3):307–312.

Source of support: Nil

Conflict of interest: None

one of the most time-tested insulin sensitizers prescribed in PCOS women.

Hence considering the beneficial effects of both metformin and vitamin D in improving insulin resistance in PCOS women, this study was planned to assess the effects of combining vitamin D with metformin on clinical, hormonal, and metabolic profile in PCOS women. We chose two different vitamin D doses based on the fact that a daily vitamin D intake of 1000–4000 IU or 25–100 µg ensures optimal blood levels in most people. As per recent consensus, adult consumption of vitamin D should be about 25 µg/day (1000 IU) for prevention of osteoporosis.[11] The upper limit considered as safe is 4000 IU/day for adults.[12,13] The purpose of this study was to compare metabolic and hormonal effects of metformin with vitamin D in 1000 IU/day dose vs combined effect of metformin with vitamin D in higher dose of 4000 IU/day in women with PCOS with insulin resistance.

Materials and Methods

Study Design, Sample Size, and Randomization

This prospective, open-label, randomized controlled trial was registered in Indian website for clinical trials as 2019/11/021926. This study was approved by Institutional Ethics Committee (IEC). We recruited 72 women with PCOS in age-group of 20–35 years with insulin-resistant (HOMA-IR >2.5), Vitamin D levels <20 ng/mL, and BMI <30 kg/m^2. Polycystic ovary syndrome women already on drug therapy, deranged kidney or liver function tests, uncontrolled thyroid disorders or hyperprolactinemia or on medications that interfere with vitamin D3 levels were excluded from the study sample. Sample size was calculated assuming type one alpha error as 0.05 and type two beta error as 80%. With reference to previous study[14] and considering difference in mean in homeostasis model of assessment-insulin resistance (HOMA-IR) in two groups as 1.12, sample size was calculated as 30 per group. Considering few drop-outs we recruited 36 patients in each group. The selected patients were randomized into two groups (group I and group II) according to computer-generated randomization table via random allocation software. Informed written consent was taken from the patients after explaining detailed plan, purpose, and duration of study in their own language.

Intervention

Patients in group I received tablet Metformin 500 mg twice a day orally along with tablet vitamin D3 1000 IU orally per day for 3 months. Patients in group II received Metformin 500 mg twice a day orally along with tablet vitamin D3 4000 IU orally per day for 3 months.

Clinical Assessment

A detailed clinical examination was done. Anthropometric measurements including height (in cms), weight (in kgs), hip circumference (widest part of hip), and waist circumference (horizontal at level umbilicus) were measured. Hirsutism scoring was done according to modified Ferriman-Gallaway (mFG) score. Nine areas of the body were assigned a score of 0 (none) to 4 (severe) according to the degree of hirsutism, with maximum possible score of 36. Polycystic ovary syndrome women who had mFG score ≥8 were considered to have clinical hyperandrogenism.[15] Severity of acne in patients was calculated using Global Acne Grading System;[16] six areas of the body were assigned a score of 1–4 according to degree of acne, with maximum possible score of 39.

Hormonal and Biochemical Assessment

Selected participants underwent biochemical and hormonal assessment on day 2 of their menstrual cycle. Ten milliliters of blood was taken for fasting and 2 hour postprandial (PP) blood glucose and serum insulin, respectively, vitamin D3 levels (done via chemiluminescence), lipid profile, follicle stimulating hormone (FSH), luteinizing hormone (LH), total testosterone, and DHEAS. HOMA-IR was calculated using fasting blood sugar and fasting serum insulin levels using the formula:[17]

$$\text{HOMA-IR} = \frac{\text{Fasting blood glucose (mg/dL)} \times \text{Fasting insulin (µIUL)}}{405}$$

Follow-up

Patients in both groups were reviewed after 3 months of therapy to see changes in clinical, hormonal, and biochemical parameters, and these were compared in both groups at baseline and at 3 months interval.

Compliance Assessment

Compliance was assessed by empty packets of Vitamin D and patients reporting >80% compliance were included in analysis.

Statistical Analysis

Categorical variables were presented in number and percentage (%) and continuous variables were presented as mean ± SD and median. Normality of data was tested by Kolmogorov-Smirnov test. If normality was rejected then nonparametric test was used. Quantitative variables were compared using Independent *t*-test/Mann-Whitney test (when data sets were not normally distributed) between the two groups. Paired *t*-test/Wilcoxon signed rank test was used for comparison between pre and post within group. Qualitative variables were correlated using Chi-square test/Fisher's exact test. Analysis was done using Statistical Package for Social Sciences (SPSS) version 21.0. *p*-value of <0.05 was considered statistically significant.

Results

One-hundred and ten patients with PCOS were assessed for eligibility. Twenty-three did not meet inclusion criteria and 15 were not willing to participate in study. A total of 72 patients were selected and randomized into two groups (Flowchart 1). Baseline characteristics in both groups were similar (Table 1). Statistically significant difference in BMI (p <0.021) was observed in group II over 3 months; however, no difference was seen in group I (p <0.21). We did not observe any significant effect on hip and waist circumference, and waist:hip ratio between baseline and at the end of 3 months in both the groups. There was statistically significant difference in mean global acne score, mFG score, and menstrual cycle irregularity between baseline and at 3 months in both groups (p-value <0.05) (Table 2).

Table 3 shows that two groups were similar at baseline in terms of hormonal and biochemical parameters. After intervention with low-dose vitamin D supplementation in group I and high dose in group II, we observed statistically significant improvement in levels of vitamin D, postprandial blood sugar, fasting insulin, HOMA-IR between baseline and at 3 months interval thereafter in both groups (p <0.05). No significant difference was seen in fasting blood sugar, postprandial insulin, triglycerides, HDL, LH,

Flowchart 1: Flowchart showing patient eligibility and randomization

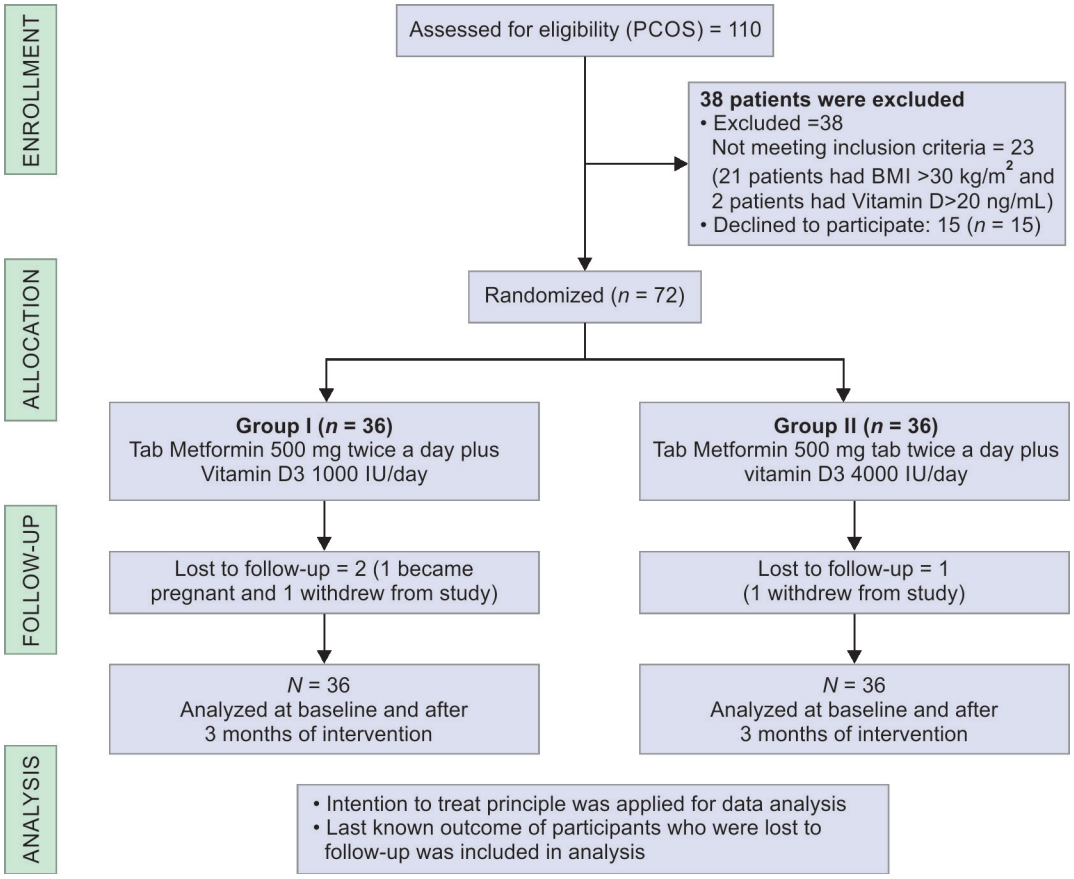

Table 1: Baseline characteristics of study population

Sl. No.	*Baseline characteristics*	*Group I (n = 36)*	*Group II (n = 36)*	*p-value*
1	Age*(years)	23.11 ± 3.98	23.97 ± 3.33	0.295
2	BMI*(kg/m^2)	25.64 ± 4.31	24.31 ± 4.74	0.217
3	Waist circumference* (cm)	86.11 ± 10.89	88.81 ± 7.45	0.225
4	Hip circumference* (cm)	101.61 ± 7.4	102.78 ± 4.97	0.435
5	W:H ratio*	0.85 ± 0.09	0.86 ± 0.07	0.359
6	mFG score**	6.97 ± 4.78	8.75 ± 4.53	0.11
7	Global acne score**	7.03 ± 3.75	6.78 ± 3.72	0.70
8	Irregular menstrual cycle	34 (94.4%)	31 (86.11%)	0.429
9	Serum vitamin D* (ng/mL)	14 ± 3.76	12.26 ± 3.79	0.054
10	HOMA-IR*	6.52 ± 0.67	6.72 ± 1.81	0.538

*Mean ± SD; **Percentage

LH:FSH ratio, and DHEAS levels between baseline and at 3 months in group I whereas group II showed statistically significant improvement between baseline and at 3 months in terms of these parameters.

Table 4 shows that at 3 months follow-up, statistically significant improvements were noted in group II as compared to group I in terms of mFG scores, Vitamin D levels, PPBS, Fasting Insulin, and HOMA-IR.

Discussion

Vitamin D deficiency was considered to affect bone metabolism. Evidence has emerged on its role in insulin resistance, insulin secretion, and metabolic syndrome. Data from Framingham study have shown an inverse association between 25-hydroxy vitamin D levels and insulin resistance.

Randomized controlled trials that studied the role of vitamin D supplementation on IR have reported either no effect or improvement in IR in those who received supplementation. In women of reproductive age group, PCOS is recognized as one of the most common endocrinopathies and these women are at higher risk for insulin resistance. Treatment modalities for PCOS mainly include lifestyle modification, hormonal contraceptives, and insulin sensitizers. Considering that these women have high prevalence of vitamin D deficiency, adding vitamin D supplementation could be a cost-effective add-on to this armamentarium.

Table 2: Comparison of clinical and anthropometric measurements between two groups at baseline and at 3 months follow-up

	Group I (metformin + vitamin D3 1000 IU)			*Group II (metformin + vitamin D3 4000 IU)*			
	Baseline	*3 months*	p^1	*Baseline*	*3 months*	p^2	p^3
BMI*	25.64 ± 4.31	24.35 ± 3.97	0.21	24.31 ± 4.74	21.83 ± 4.13	0.021	0.01
Waist circumference*	86.11 ± 10.89	85.36 ± 10.91	0.095	88.81 ± 7.45	87.28 ± 8.61	0.203	0.411
Hip circumference*	101.61 ± 7.4	100.75 ± 7.65	0.062	102.78 ± 4.97	102.11 ± 6.24	0.355	0.411
W:H ratio*	0.85 ± 0.09	0.85 ± 0.08	0.872	0.86 ± 0.07	0.86 ± 0.08	0.47	0.634
Global acne score*	7.03 ± 3.75	4.61 ± 3.10	<0.0001	6.78 ± 3.72	3.97 ± 2.68	<0.0001	0.36
mFG score*	6.97 ± 4.78	5.78 ± 4.09	0.011	8.75 ± 4.53	5.94 ± 3.15	0.0003	0.847
Menstrual cycle irregularity**	34 (94.4%)	25 (69.4%)	0.012	31 (86.11%)	21 (58.3%)	0.018	0.326

*Mean ± SD; **Percentage. p^1 value between baseline and at 3 months in group I; p^2 value between baseline and at 3 months in group II; p^3 value between both groups at 3 months

Table 3: Comparison of hormonal and biochemical parameters between two groups at baseline and at 3 months follow-up

	Group I (metformin + vitamin D3 1000 IU)			*Group II (metformin + vitamin D3 4000 IU)*				
	Baseline	*3 months*	p^1	*Baseline*	*3 months*	p^2	p^0	p^3
Vitamin D* (ng/mL)	14 ± 3.76	19.36 ± 2.84	<0.0001	12.26 ± 3.79	25.44 ± 3.34	<0.0001	0.054	<0.0001
FBS* (mg/dL)	96.78 ± 9.62	93.61 ± 5.93	0.084	100 ± 1.41	81.61 ± 6.07	<0.0001	0.812	<0.0001
PPBS* (mg/dL)	140.83 ± 21.84	107.94 ± 18.65	<0.0001	144.22 ±19.73	95.19 ± 16.51	<0.0001	0.398	0.02
Fasting	27.32 ± 1.38	19.95 ± 1.48	<0.000	27.2 ± 7.16	14.76 ± 6.17	<0.000	0.826	<0.0001
PP insulin* (mIU/mL)	90.82 ± 79.52	84.09 ± 77.28	0.875	83.96 ±46.21	43.25 ± 24.64	<0.0001	0.436	0.01
HOMA-IR*	6.52 ± 0.67	4.61 ± 0.41	<0.0001	6.72 ± 1.81	2.96 ± 1.26	<0.0001	0.538	<0.0001
TGL*(mg/dL)	92.33 ± 22.71	98.22 ± 31.31	0.791	114.25 ±70.98	95.19 ± 38.13	0.0003	0.201	0.40
HDL*(mg/dL)	40 ± 9.09	41.42 ± 8.64	0.666	41.28 ± 7.82	46.44 ± 15.87	0.007	0.41	0.122
LH*(IU/L)	9.22 ± 5.78	9.09 ± 4.53	0.423	8.54 ± 5.04	6.06 ± 2.24	0.0001	0.67	0.001
FSH*(IU/mL)	6.51 ± 2.3	6.43 ± 2.07	0.759	5.78 ± 1.73	5.64 ± 1.23	0.524	0.133	0.05
LH:FSHratio*	1.48 ± 0.94	1.39 ± 0.43	0.826	1.55 ± 0.96	1.11 ± 0.44	0.001	0.636	0.005
DHEAS*(µg/dL)	192.88 ± 98.59	183.97 ± 86.92	0.104	208.44 ± 98.83	165 ± 67.45	<0.0001	0.260	0.50
Total testosterone*(ng/dL)	49.68 ± 21.13	52.51 ± 18.92	0.2137	49.98 ± 19.82	47.13 ± 17.22	0.1498	0.95	0.21

*Mean ± SD; **Percentage. p^0 value between both groups at baseline; p^1 value between baseline and at 3 months in group I; p^2 value between baseline and at 3 months in group II; p^3 value between both groups at 3 months

Table 4: Comparison of improvements in both groups

	Change in parameter over 3 months		
Parameter	*Group I*	*Group II*	*p-value*
mGF score	−1.19 ± 2.67	−2.81 ± 4.15	0.027
Global acne score	−2.42 ± 1.25	−2.81 ± 2.63	0.218
Vitamin D levels	5.36 ± 2.79	13.19 ± 3.26	<0.0001
Postprandial blood sugar levels	−32.89 ± 28.37	−49.03 ± 24.41	0.005
Fasting insulin	−6.87 ± 11.73	−12.44 ± 10.8	0.040
HOMA-IR	−1.79 ± 2.8	−4.38 ± 2.86	0.0002

In this study, we assessed metabolic and hormonal effects of metformin along with vitamin D in two different doses of 1000 IU/day vs 4000 IU/day in insulin-resistant PCOS women.

Mean vitamin D3 level in group I was 14 ± 3.76 ng/mL and 12.26 ± 3.79 ng/mL in group II. Of the 110 patients screened for inclusion in our study, only 2 had vitamin D levels more than 20 ng/mL who were excluded from study showing that hypovitaminosis D is very common in patients with PCOS. Previous studies have shown that vitamin D deficiency is seen in 85% of patients with PCOS from developed countries.[18,19]

The results of our study indicate beneficial effects of high dose of vitamin D supplementation for 12 weeks in insulin-resistant women with PCOS were observed in levels of BMI, levels of LH, triglycerides, HDL, DHEAS, fasting blood sugar, and postprandial insulin as compared to low dose of vitamin D supplementation for similar duration. When we assessed other parameters like menstrual cycle regularity, HOMA-IR, mFG and global acne score, postprandial blood sugar, and fasting insulin levels at 3 months follow-up, both the groups showed significant improvement; however, these effects were greater with high dose as compared to low-dose

supplementation of vitamin D. Literature is sparse on the effect of different doses of vitamin D therapy along with metformin in PCOS patients.

Firouzabadi et al.[20] in their study on 100 infertile PCOS women found that group receiving calcium 1000 mg/day and Vitamin D 1,00,000 IU/month along with metformin 1500 mg/day had significant decrease in BMI (26.89 ± 2.11 to 25.49 ± 1.88) as compared to those receiving metformin 1500 mg/day (26.91 ± 2.35 to 26.28 ± 2.15) alone for 6 months (p-value 0.05). The results of this study were comparable to ours wherein improvement in BMI was noted in group receiving higher vitamin D supplementation. Similarly, in a prospective double-blinded randomized control trial done by Garg et al.,[21] it was observed that mean BMI changed significantly over 6 months in group receiving vitamin D 4000 IU daily along with metformin (p-value 0.05). Unlike our study, Firouzabadi et al.[20] did not find any significant improvement in menstrual cycle regularity.

Results from our study indicate statistically significant decrease in mFG scores in both groups ($p = 0.027$) with this decrease being observed more in group II compared to group I. Jamilian et al.[14] in their study of 90 vitamin D deficient PCOS patients observed that mFG score decreased from 12.3 ± 5.2 to 12.2 ± 5.1 in placebo group, decreased from 14.0 ± 3.9 to 13.1 ± 3.7 in low dose vitamin D group (group II), while in the high dose vitamin D group (group III), it decreased from 13.2 ± 5.7 to 12.1 ± 5.3. Difference in mFG score between the three groups was statistically significant (p <0.001).

Advani et al.[22] investigated the effect of high-dose vitamin D 4000 IU along with inositols and lycopene for 12 weeks in obese and lean PCOS patients and observed that global acne score decreased significantly in both obese and lean patients (p <0.01). Similar results of changes in global acne score were observed in our study.

Statistically significant difference was seen in HOMA-IR between baseline and at 3 months in both our study groups (p <0.0001) with higher improvement seen in group II (4.38 ± 2.86) as compared to group I (1.79 ± 2.8). Garg et al.[21] in their prospective double-blind randomized controlled trial assessed the effect of vitamin D on 36 women with PCOS. Serum HOMA-IR decreased from 3.8 ± 3.40 to 2.3 ± 1.32 in vitamin D group. The difference in HOMA-IR was statistically significant ($p = 0.003$), which was similar to our results. Selimoglu et al.[18] also observed significant decrease in HOMA-IR after administration of single oral dose of 3,00,000 IU cholecalciferol. HOMA-IR decreased significantly from 4.41 ± 1.38 to 3.67 ± 1.48 (p <0.043). We also had significant difference of serum triglycerides and fasting insulin levels between baseline and at 3 months in group receiving higher vitamin D levels in contrast to no difference observed by Garg et al.

Garg et al. analyzes eighteen studies evaluating the correlation between vitamin D status and IR in women affected by PCOS. They found that on univariate regression analysis rise in serum 25(OH)D levels was significantly associated with decline in HOMA-IR values in both PCOS and control women. The results indicated that for every 10 nmol/L rise in serum 25(OH)D levels HOMA-IR decreases by 0.27 in PCOS women and 0.19 in control women.[23]

Polycystic ovary syndrome women are at increased risk for metabolic syndrome and an interesting aspect noted in our sample was that none of the women had HDL more than 50 mg% similar to that seen by Madusudhanan et al.[24] This again highlights the importance of lifestyle modification as the first step in managing this group.

To conclude, the main limitation of this study was that being a single-center study, results cannot be extrapolated to population as a whole. Major strength was that it was randomized controlled trial comparing efficacy of both upper and lower limits of vitamin D supplementation in two groups having similar baseline characteristics. However, absence of consensus pertaining to optimal dose of vitamin D warrants need of further intervention trials with larger sample size.

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