Vitamin D Supplementation Decreases TGF-β1 Bioavailability in PCOS: A Randomized Placebo-Controlled Trial

Mohamad Irani, David B. Seifer, Richard V. Grazi, Nitasha Julka, Devika Bhatt, Bharati Kalgi, Sara Irani, Oded Tal, Geralyn Lambert-Messerlian, and Reshef Tal

Context: There is an abnormal increase in TGF-β1 bioavailability in women with polycystic ovary syndrome (PCOS), which might play a role in the pathophysiology of this syndrome. Vitamin D (VD) supplementation improves various clinical manifestations of PCOS and decreases TGF-β1 levels in several diseases including myelofibrosis.

Objective: The objective of the study was to determine the effect of VD supplementation on TGF-β1 bioavailability in VD-deficient women with PCOS and assess whether changes in TGF-β1/soluble endoglin (sENG) levels correlate with an improvement in PCOS clinical manifestations.

Design: This was a prospective, randomized, placebo-controlled trial.

Setting: The study was conducted at an academic-affiliated medical center.

Participants: Sixty-eight VD-deficient women with PCOS who were not pregnant or taking any exogenous hormones were recruited between October 2013 and January 2015.

Interventions: Forty-five women received 50 000 IU of oral vitamin D3 and 23 women received oral placebo once weekly for 8 weeks.

Main Outcomes Measures: Serum TGF-β1, sENG, lipid profile, testosterone, dehydroepiandrosterone sulfate, and insulin resistance were measured. The clinical parameters were evaluated before and 2 months after treatment.

Results: The VD level significantly increased and normalized after VD supplementation (16.3 $\pm$ 0.9 [SEM] to 43.2 $\pm$ 2.4 ng/mL; $P < .01$), whereas it did not significantly change after placebo. After the VD supplementation, there was a significant decrease in the following: the interval between menstrual periods (80 $\pm$ 9 to 60 $\pm$ 6 d; $P = .04$), Ferriman-Gallwey score (9.8 $\pm$ 1.5 to 8.1 $\pm$ 1.5; $P < .01$), triglycerides (138 $\pm$ 22 to 117 $\pm$ 20 mg/dL; $P = .03$), and TGF-β1 to sENG ratio (6.7 $\pm$ 0.4 to 5.9 $\pm$ 0.4; $P = .04$). In addition, the ΔTGF-β1 to sENG ratio was positively correlated with Δtriglycerides ($r = 0.59$; $P = .03$).

Conclusions: VD supplementation in VD-deficient women with PCOS significantly decreases the bioavailability of TGF-β1, which correlates with an improvement in some abnormal clinical parameters associated with PCOS. This is a novel mechanism that could explain the beneficial effects of VD supplementation in women with PCOS. These findings may support new treatment modalities for PCOS, such as the development of anti-TGF-β drugs. **(*J Clin Endocrinol Metab* 11: 0000–0000, 2015)**

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder among females affecting 5%–10% of reproductive-aged women (1). It is characterized by menstrual dysfunction, hyperandrogenism, hyperinsulinemia, and infertility (2). PCOS is also associated with an increased risk of hyperlipidemia, cardiovascular disease, type 2 diabetes mellitus, endometrial carcinoma, depression, and anxiety (2).

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Abbreviations: BMI, body mass index; BP, blood pressure; DBP, diastolic BP; DHEAS, dehydroepiandrosterone sulfate; FGS, Ferriman-Gallwey score; HDL, high-density lipoprotein; HOMA%B, homeostasis model assessment for β-cell function; HOMA-IR, homeostasis model assessment for insulin resistance; IR, insulin resistance; LDL, low-density lipoprotein; 25OH-D, 25 hydroxyvitamin D; PCOS, polycystic ovary syndrome; SBP, systolic BP; sENG, soluble endoglin.

Although the pathophysiology of PCOS is not well defined, there is compelling evidence suggesting a possible role for TGF-β dysregulation. Three TGF-β isoforms (TGF-β1, TGF-β2, and TGF-β3) have been identified in humans (3). They are members of the TGF-β superfamily of proteins, which includes antimullerian hormone, activins, and inhibins that have been implicated in the pathophysiology of PCOS (4–6). TGF-β regulates angiogenesis, fibroblast proliferation, and tissue fibrosis (3, 7, 8). The ovaries of women with PCOS manifest all the characteristics of TGF-β hyperactivity including increased vascularity and increased deposition of collagen in ovarian stroma and theca (9, 10). Moreover, there are genetic studies supporting the role of TGF-β dysregulation in PCOS and showing that allele 8 of D19S884 within intron 55 of the fibrillin-3 gene is associated with PCOS and increased insulin resistance (IR) in women with PCOS (11, 12). The fibrillins, which are matrix components of extracellular microfibrils, are regulated by TGF-β. The fibrillin-1 mutations in Marfan syndrome up-regulate TGF-β activity, leading to emphysema and cardiovascular and connective tissue disorders associated with this syndrome (13). Likewise, TGF-β dysregulation is associated with the allele 8 variant of fibrillin-3 gene, which may contribute to the metabolic disturbances in women with PCOS (14).

Endoglin is a part of TGF-β1 and TGF-β3 receptor complexes (15). It is highly expressed in fibroblasts and angiogenic endothelial cells playing an essential role as a mediator of tissue fibrosis and angiogenesis (15, 16). Soluble endoglin (sENG), the proteolytic product of endoglin, is a circulating receptor that binds TGF-β1 and decreases its bioavailability (17). Women with PCOS have abnormal increase in TGF-β1 bioavailability (TGF-β1/sENG) due to an increase in serum TGF-β1 and decrease in sENG levels (14, 18).

Women with PCOS tend to have decreased vitamin D levels (19), and vitamin D deficiency has been correlated with increased insulin resistance, body mass index (BMI), total testosterone, and dehydroepiandrosterone sulfate (DHEAS) in these women (20, 21). In addition, vitamin D treatment has been shown to improve various clinical parameters in vitamin D-deficient women with PCOS including glucose intolerance, hypertension, and androgen levels (22, 23). However, the mechanism/s mediating the beneficial effects of vitamin D in PCOS are unknown. Vitamin D administration has been shown to decrease TGF-β1 levels in several experimental fibrotic diseases in which TGF-β1 is elevated, including heart and kidney fibrosis (24, 25). Moreover, vitamin D has been recently shown to decrease TGF-β1 levels in rat ovaries (26). Taken together, the beneficial effects of vitamin D treatment in women with PCOS may be mediated via vitamin D's effects on TGF-β1 and/or sENG. Therefore, we hypothesized that vitamin D treatment of vitamin D-deficient women with PCOS could result in a decrease of TGF-β1 bioavailability (TGF-β1/sENG) concomitant with an improvement in clinical disease parameters. In addition, we hypothesized that improvement in clinical disease parameters may correlate with changes in TGF-β1 bioavailability.

Materials and Methods

Study subjects

This study was a randomized, single-blind, placebo-controlled trial designed to evaluate the effect of vitamin D supplementation in vitamin D-deficient women with PCOS. Ninety-three reproductive-aged women diagnosed with PCOS presenting to the Maimonides Women's Health Center for an annual check-up between October 2013 and March 2015 were screened for vitamin D deficiency (defined as serum 25 hydroxyvitamin D [25OH-D] levels <20 ng/mL). All participants signed the informed consent, and the study was approved by the institutional review board of the Maimonides Medical Center. PCOS was diagnosed according to the Rotterdam Consensus (European Society of Human Reproduction and Embryology/American Society for Reproductive Medicine criteria), ie, the presence of at least two of the following three criteria: oligo- or anovulation, signs of clinical hyperandrogenism and/or biochemical evidence of hyperandrogenism and polycystic ovaries on ultrasonography after exclusion of specific identifiable disorders (thyroid disorder, hyperprolactinemia, congenital adrenal hyperplasia, androgen secreting tumors, and Cushing's syndrome) (27). There were 20 participants who were diagnosed with PCOS prior to recruitment. The diagnosis of PCOS was reconfirmed immediately before the study for these 20 women. All other women were newly diagnosed during the recruitment process. We included women aged between 18 and 38 years who were not pregnant, postpartum, or breastfeeding or taking any vitamin D supplements, metformin, or any hormonal therapy within 6 months prior to recruitment. There were a total of three visits for each participant including the following: 1) venipuncture and workup for PCOS prior to recruitment, 2) venipunture within 2 weeks after completing the treatment, and 3) assessment of clinical parameters 2 months after completing the treatment.

Interventions and blood collection

Sixty-eight PCOS women diagnosed with vitamin D deficiency were enrolled. Participants were allocated to either a vitamin D or placebo treatment group according to a computer-

Departments of Obstetrics and Gynecology (M.I., N.J., D.B., B.K., S.I.) and Genesis Fertility and Reproductive Medicine (R.V.G.), Maimonides Medical Center, Brooklyn, New York 11219; Department of Reproductive Endocrinology and Infertility (D.B.S.), Oregon Health & Science University, Portland, Oregon 97239; School of Business and Hospitality (O.T.), Conestoga College, Kitchener, Ontario, Canada N2G 4M4; Division of Medical Screening and Special Testing (G.L.-M.), Women and Infants Hospital, Providence, Rhode Island 02905; and Division of Reproductive Endocrinology and Infertility (R.T.), Department of Obstetrics, Gynecology, and Reproductive Sciences, Yale University School of Medicine, New Haven, Connecticut 06520

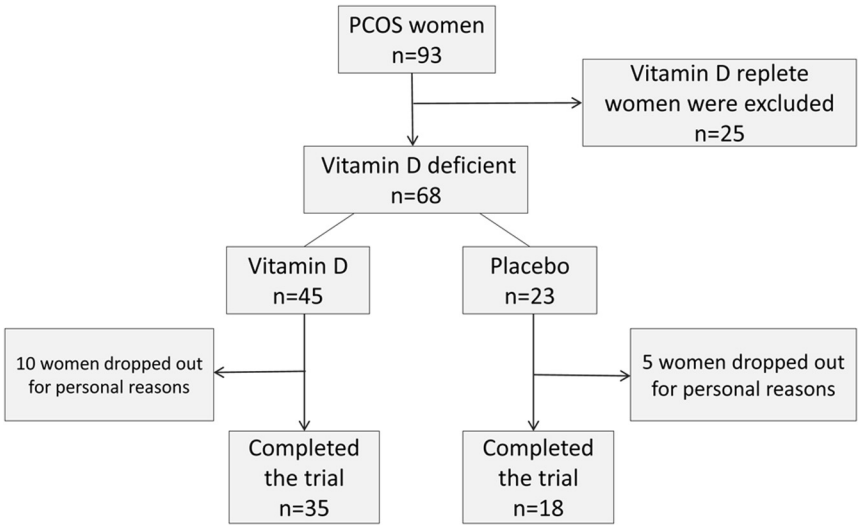

Figure 1. Schematic diagram representing recruitment, dropout, and follow-up of all study participants.

generated list using a ratio of 2:1 (vitamin D/placebo). Women allocated to the vitamin D group received one capsule of 50 000 IU of vitamin D3 once weekly for 8 weeks. The vitamin D supplementation regimen was according to the Endocrine Society guidelines (28). Women in the placebo group received one capsule of placebo once weekly for 8 weeks. The placebo was prepared at the Maimonides Medical Center's pharmacy: it had similar color to the vitamin D capsule but includes only lactose monohydrate powder (Gallipot Inc). To ensure compliance, each participant was called once weekly and reminded to take her pill. Fasting blood samples were collected by venipuncture before starting and within 2 weeks after completing the treatment (vitamin D or placebo). Blood samples were allowed to clot for 30 minutes at room temperature before centrifugation at 1200 rpm for 10 minutes. Serum was stored at −80°C in aliquots until assayed.

Assays of all measured hormones, 25OH-D, TGF-β1, and sENG

Serum 25OH-D levels were measured before and after completing the treatment. The levels were determined by the ADVIA Centaur vitamin D assay (Siemens Healthcare Diagnostics). DHEAS, testosterone, SHBG, TSH, FSH, and LH were measured using the IMMULITE 2000 immunoassay system (Siemens). Insulin and prolactin concentrations were quantified by a DXL 800 immunoassay analyzer according to manufacturer's protocols (Beckman Coulter). The homeostasis model assessment HOMA2 calculator version 2.2.3 (http://www.dtu.ox.ac.uk/homacalculator) was used to calculate insulin resistance (HOMA-IR) and pancreatic β-cell function (HOMA%B). The 17-hydroxyprogesterone level was determined by an ELISA assay (Eagle BioSciences). The Beckman Coulter DXC800I platform was used to measure the triglycerides and cholesterol levels. TGF-β1 concentration was measured using the human TGF-β1 quantikine ELISA kit according to the manufacturer's protocols (R&D Systems). The sENG levels were quantified by the human endoglin/CD105 quantikine ELISA kit (R&D Systems). The interassay and intraassay coefficients of variation for all assays were less than 10%.

Clinical parameters

All clinical parameters were evaluated before starting treatment and were reevaluated 2 months after the completion of treatment. These parameters included blood pressure (BP), Ferriman-Gallwey score (FGS) (hirsutism score), acne status, and interval between periods. The interval between periods was measured by calculating the average of the last two cycles. Acne was assessed during the first visit by examining the patient's face, chest, neck, back, and arms. After the vitamin D and placebo treatment, each patient was asked whether her acne became better, worse, or the same as before treatment.

Statistical analysis

Data were tested for normality. All values were expressed as mean ± SEM. A paired Student's t test was used to compare pre- and posttreatment serum levels and clinical parameters. Correlations between changes in serum TGF-β1/sENG and changes in clinical disease parameters were analyzed using a Pearson's test and linear regression. A χ^2 test was used to evaluate the changes in acne after treatment. SigmaPlot (Systat Software Inc) was used for statistical analysis. A value of $P < .05$ was considered to be statistically significant. Because our study is based on a clearly stated a priori hypothesis that TGF-β bioavailability (TGFβ to sENG ratio) may be decreased by vitamin D, we have not corrected for multiple testing (29).

Results

Demographics and changes in serum 25OH-D levels

A total of 53 participants completed the trial: 35 in the vitamin D group and 18 in the placebo group (Figure 1). The mean BMI in the vitamin D group and placebo group was 30 ± 1 kg/m^2 and 28 ± 1.6 kg/m^2, respectively (P = .33). There were no significant differences between the two groups in terms of age, ethnicity, skin color, daily milk consumption, smoking, and history of infertility ($P \geq .2$) (Table 1). Of note, women with a history of infertility are those who failed to achieve clinical pregnancy after 1 year or more of regular unprotected intercourse. None of the participants was seeking fertility treatment at the time of recruitment. The serum 25OH-D level significantly increased and normalized after vitamin D supplementation (16.3 ± 0.9 to 43.2 ± 2.4 ng/mL; $P < .01$), whereas it did not significantly change after placebo (17 ± 1.8 to 17.4 ± 1.9 ng/mL; $P = .85$).

Changes in PCOS clinical parameters after vitamin D supplementation

Women were re-evaluated two months after completing the treatment (4 months after starting treatment). Af-

Table 1. Demographics of the Participants

	Vitamin D-Deficient Women With PCOS		
	Vitamin D (n = 35)	Placebo (n = 18)	*P* Value
Age	30.5 ± 1	29.6 ± 1.7	.64
BMI	30 ± 1	28 ± 1.6	.33
Ethnicity			.76
Hispanic	69.4%	72.2%	
Asian	25%	22.2%	
White	0%	0%	
Black	5.5%	5.5%	
Skin color			.20
Dark	8.3%	5.5%	
Fair	52.7%	61.1%	
Light	38.8%	33.3%	
Daily milk consumption	48%	44.4%	.42
History of diabetes not using metformin	2.8%	5.5%	.27
History of infertility	57.1%	55.5%	.91
Smoking	13.8%	11.1%	.35
Interval between periods, d	80.2 ± 9.8	79 ± 9	.10
FGS	9.8 ± 1.5	8.1 ± 1.4	.93
SBP, mm Hg	112 ± 1.9	113 ± 2.8	.78
DBP, mm Hg	68.4 ± 1.1	69 ± 1.7	.58
Mean arterial pressure, mm Hg	83 ± 1.2	84 ± 1.9	.64
HOMA-IR	2.07 ± 0.37	1.58 ± 0.30	.31
HOMA%B	163.04 ± 17.27	136.96 ± 14.29	.16
Triglycerides, mg/dL	138 ± 22	113 ± 21	.52
Total cholesterol, mg/dL	183 ± 6.5	179 ± 9	.35
HDL, mg/dL	46 ± 2.4	47 ± 2.9	.91
LDL, mg/dL	106 ± 6.1	103 ± 7.7	.95
DHEAS, μg/dL	117 ± 16	140 ± 20	.30
Total T, ng/dL	30.4 ± 2.9	32.3 ± 3.4	.63
SHBG, nmol/L	32.9 ± 2.9	39 ± 4.9	.54
Free T, ng/dL	0.59 ± 0.06	0.61 ± 0.08	.58
FSH, mIU/mL	5.9 ± 0.6	6.8 ± 0.7	.38
LH, mIU/mL	9 ± 2.7	12 ± 2.8	.43
LH/FSH	1.4 ± 0.1	1.7 ± 0.2	.44
sENG, ng/mL	3 ± 0.14	3.5 ± 0.2	.09
TGF-β1, ng/mL	20 ± 1	18.2 ± 1.3	.32
TGF-β1/sENG	6.7 ± 0.4	5.6 ± 0.5	.14

Age and BMI are expressed as mean ± SEM. BMI was measured in kilograms per square meter. T, testosterone.

ter the vitamin D treatment, there was a significant decrease in the interval between menstrual periods (80 ± 9 to 60 ± 6 d; $P = .04$) and FGS (9.8 ± 1.5 to 8.1 ± 1.5; $P < .01$) (Table 2), whereas no significant changes in these parameters were observed after the placebo treatment. In addition, 28.6% of the women experienced an improvement in their acne 4 months after vitamin D treatment compared with 6.6% after placebo treatment ($P = .02$). Furthermore, whereas none of the study participants was undergoing fertility treatment, five women conceived after the vitamin D supplementation, whereas none did after placebo ($P = .09$). Four of these five women had history of secondary infertility but were not seeking any infertility treatment. There was no significant change in the systolic BP (SBP), diastolic BP (DBP), or mean arterial pressure after the vitamin D or placebo treatment (Table 2).

Changes in biochemical parameters and TGF-β1/sENG after vitamin D supplementation

In the group that received vitamin D supplementation, there was a significant decrease in fasting serum triglycerides (138 ± 22 to 117 ± 20 mg/dL; $P = .03$) and a nonsignificant trend toward decrease in total cholesterol (184 ± 6.5 to 166 ± 11 mg/dL; $P = .08$). There was also a significant increase in serum sENG (3 ± 0.1 to 3.3 ± 0.1 ng/mL; $P = .01$) and a significant decrease in TGF-β1 to sENG ratio (6.7 ± 0.4 to 5.9 ± 0.4; $P = .04$) after the vitamin D replacement (Table 2 and Figure 2). Serum low-density lipoprotein (LDL), high-density lipoprotein (HDL), HOMA-IR, HOMA%B, DHEAS, free testosterone, FSH, LH, LH/FSH, and TGF-β1 did not significantly change after vitamin D supplementation. None of the measured parameters significantly changed after the placebo treatment (Table 2).

Correlation between serum TGF-β1/sENG ratio and PCOS clinical and/or biochemical parameters

To evaluate for the presence of a relationship between the change in the serum TGF-β1 to sENG ratio and the change in clinical and/or biochemical parameters in PCOS women, linear correlation and regression analyses were used. The decrease in the serum TGF-β1 to sENG ratio in the vitamin D group positively correlated with the decrease in triglycerides ($r = 0.37$; $P = .03$) and total cholesterol ($r = 0.54$; $P = .04$) (Figures 3 and 4). No correlation was found between the change in the TGF-β1 to sENG ratio and any of the other clinical or biochemical parameters.

Discussion

This study examined the effect of vitamin D supplementation on serum TGF-β1 bioavailability (TGF-β1/sENG) and PCOS clinical and biochemical parameters in vitamin D-deficient women with PCOS. We found that vitamin D supplementation was associated with decreased acne, hirsutism, triglycerides, and the menstrual interval in women with PCOS. Furthermore, increased serum sENG and decreased TGF-β1 bioavailability were observed in PCOS women receiving vitamin D but not placebo. Notably, the decrease in TGF-β1 bioavailability significantly corre-

Table 2. Changes in the Clinical and Biochemical Parameters Following Placebo and Vitamin D3 Supplementation

	Before Placebo	After Placebo	*P* Value	Before Vitamin D	After Vitamin D	*P* Value
Interval between periods, d	79 ± 9	75 ± 9	.17	80.2 ± 9.8	60 ± 6.7	.04
FGS	8.1 ± 1.4	7.6 ± 1.54	.10	9.8 ± 1.5	8.1 ± 1.5	.003
SBP, mm Hg	113 ± 2.8	109 ± 2.3	.10	112 ± 1.9	108 ± 1	.13
DBP, mm Hg	69 ± 1.7	67 ± 1.6	.23	68.4 ± 1.1	67.9 ± 1.3	.79
Mean arterial pressure, mm Hg	84 ± 1.9	81 ± 1.7	.10	83 ± 1.2	81 ± 1.1	.30
HOMA-IR	1.58 ± 0.30	1.52 ± 0.24	.57	2.07 ± 0.37	2.03 ± 0.22	.89
HOMA%B	136.96 ± 14.29	130.50 ± 11.95	.37	163.04 ± 17.27	139.62 ± 15.76	.06
Triglycerides, mg/dL	113 ± 21	98 ± 13	.30	138 ± 22	117 ± 20	.03
Total cholesterol, mg/dL	179 ± 9	177 ± 6.3	.83	183 ± 6.5	166 ± 11	.08
HDL, mg/dL	47 ± 2.9	50 ± 3.2	.30	46 ± 2.4	48 ± 2.6	.20
LDL, mg/dL	103 ± 7.7	101 ± 5.1	.60	106 ± 6.1	100 ± 4.1	.20
DHEAS, μg/dL	140 ± 20	143 ± 18	.70	117 ± 16	121 ± 13	.60
Total T, ng/dL	32.3 ± 3.4	36.6 ± 4	.20	30.4 ± 2.9	38.4 ± 4.3	.10
SHBG, nmol/L	39 ± 4.9	43 ± 9.4	.60	32.9 ± 2.9	33.9 ± 3.6	.80
Free T, ng/dL	0.61 ± 0.08	0.68 ± 0.11	.35	0.59 ± 0.06	0.68 ± 0.06	.20
FSH, mIU/mL	6.8 ± 0.7	5.2 ± 0.6	.10	5.9 ± 0.6	4.5 ± 0.5	.10
LH, mIU/mL	12 ± 2.8	9.2 ± 1.6	.40	9 ± 2.7	8.3 ± 1.2	.70
LH/FSH	1.7 ± 0.2	1.7 ± 0.2	.90	1.4 ± 0.1	1.4 ± 0.1	.60
sENG, ng/mL	3.5 ± 0.2	3.6 ± 0.2	.30	3 ± 0.14	3.3 ± 0.16	.01
TGF-β1, ng/mL	18.2 ± 1.3	18.2 ± 1.3	.90	20 ± 1	19 ± 1	.39
TGF-β1 to sENG ratio	5.6 ± 0.5	5.5 ± 0.4	.32	6.7 ± 0.4	5.9 ± 0.4	.04

Data are expressed as mean ± SEM. Women allocated to the vitamin D group received one capsule of 50 000 IU of vitamin D3 once weekly for 8 weeks. Women in the placebo group received once capsule of placebo once weekly for 8 weeks. T, testosterone.

lated with the decrease in the triglyceride level and total cholesterol.

Vitamin D deficiency has been shown to correlate with some clinical and biochemical parameters of PCOS (21, 30). Vitamin D replacement has improved menstrual irregularity and pregnancy rates in women with PCOS (31, 32). Consistent with previous studies, our results show that the menstrual interval was decreased in vitamin D-treated PCOS women. Although our study was not designed to assess the impact of vitamin D on pregnancy rates and our population did not consist of women seeking fertility treatment, it is interesting to note that after the vitamin D supplementation, five pregnancies occurred in the vitamin D-treated group compared with none in the placebo group.

Serum 25OH-D level has been shown to be negatively correlated with serum androgen levels (DHEAS and testosterone) (33). Furthermore, hirsute women have significantly lower serum 25OH-D levels (34). Moreover, vitamin D treatment combined with calcium supplementation for 3 months has significantly decreased serum total testosterone levels ($P = .03$) (22). Although our data do not show that vitamin D supplementation significantly changed serum androgen levels, they demonstrate for the first time that hirsutism and acne improved 4 months after

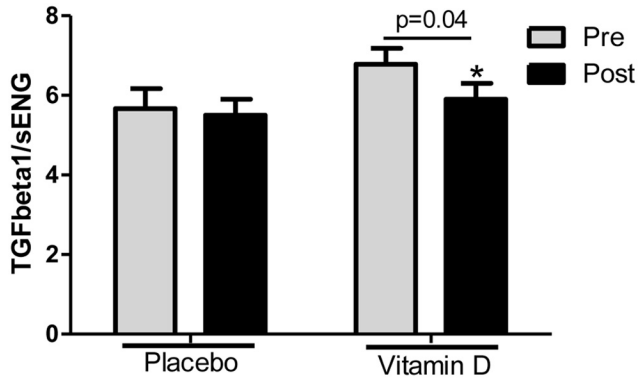

Figure 2. Changes in serum TGF-β1 to sENG ratio after placebo and vitamin D supplementation. Vitamin D supplementation significantly decreased the TGF-β1 to sENG ratio (6.7 ± 0.4 to 5.9 ± 0.4; $P = .04$) in vitamin D-deficient women with PCOS. There was no significant change in the TGF-β1 to sENG ratio after placebo.

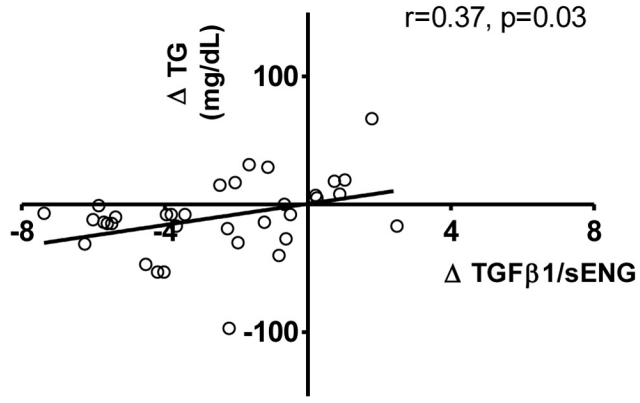

Figure 3. Correlation between ΔTGF-β1/sENG and Δtriglycerides after the vitamin D3 supplementation. After the vitamin D replacement, the decrease in TGF-β1 bioavailability (TGF-β1 to sENG ratio) positively correlated with the decrease in triglycerides (r = 0.59; $P = .03$) in vitamin D-deficient women with PCOS.

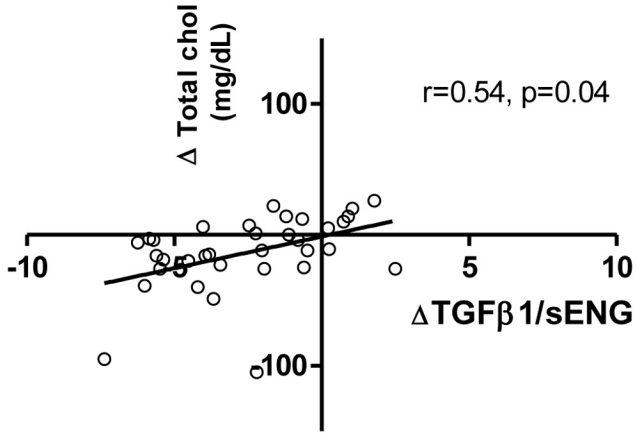

Figure 4. Correlation between ΔTGF-β1/sENG and Δtotal cholesterol after the vitamin D3 supplementation. After the vitamin D replacement, the decrease in TGF-β1 bioavailability (TGF-β1 to sENG ratio) positively correlated with the decrease in total cholesterol (r = 0.54; $P = .04$) in vitamin D-deficient women with PCOS.

normalizing serum 25OH-D levels. Of note, the vitamin D nuclear receptor is essential for hair cycling (35). Also, the inflammation that targets Propionibacterium acne and plays an important role in the pathogenesis of acne is modulated by vitamin D (36). Thus, vitamin D may improve acne and hirsutism through a direct effect on the pilosebaceous units and the immune system rather than through its influence on androgen levels.

Although still controversial, some clinical studies suggest a favorable role of vitamin D on cardiovascular health by lowering BP and reducing the incidence of cardiovascular events (37, 38). A daily vitamin D and calcium oral supplementation for 3 months significantly decreased SBP, DBP, and mean arterial pressure in women with PCOS (22). Our results show a nonsignificant trend toward a decrease in SBP (112 ± 1.9 to 108 ± 1 mm Hg; $P = .1$) but no significant changes in diastolic BP or mean arterial pressure. This discrepancy in the findings between studies may be explained by the differences in the participants' BMI, vitamin D regimen, and the use of calcium.

Wehr et al (39) have demonstrated that the serum 25OH-D level is positively correlated with insulin sensitivity and negatively correlated with IR in women with PCOS. They have also shown that a specific vitamin D receptor gene polymorphism (VDR Cdx-2 'AA') is associated with a significantly higher IR than other genotypes (39). Likewise, other studies described a similar correlation between vitamin D and IR (33, 40). However, the effect of vitamin D supplementation on IR has been inconsistent (22, 23). IR significantly decreased 3 weeks after a single oral administration of 300 000 IU of vitamin D3 (23). In contrast, 3 months of daily oral supplementation of vitamin D (8533 IU) and calcium (530 mg) did not influence IR (22). Our data demonstrated that normalizing serum 25OH-D levels did not affect IR. The differences in the vitamin D regimen, the interval from starting the vitamin D supplementation to measuring IR, and most importantly the method of calculating IR may explain the conflicting findings.

Vitamin D replacement has been shown in several studies to have a favorable impact on lipid profile in women with PCOS (32, 41, 42). In a study by Wehr et al (32), PCOS women receiving weekly 20 000 IU of vitamin D3 for 24 weeks demonstrated a significant decrease in triglycerides at 12 weeks and 24 weeks, whereas total cholesterol and LDL significantly increased. Similarly, Kotsa et al (41) showed that triglycerides significantly decreased and HDL significantly increased in 15 obese women with PCOS after they received alfacalcidol 1 μg/d for 3 months. Moreover, in a study that included 26 overweight and obese women with PCOS, Asemi et al (42) showed a significant decrease in triglyceride levels and very low density lipoprotein with no changes in total cholesterol, LDL, or HDL after treatment with calcium 1000 mg/d and vitamin D 50 000 IU/wk for 8 weeks. These trials showed a consistent decrease in triglycerides but variable effects on cholesterol (32, 41, 42). Our findings showing a significant decrease in triglyceride levels and a trend toward a decrease in total cholesterol with no changes in HDL or LDL after the administration of vitamin D3 50 000 IU/wk are in agreement with the literature.

Serum TGF-β1 has been previously shown to be elevated, combined with a decrease in its circulating receptor sENG, leading to increased TGF-β1 bioavailability in PCOS (14, 18). Increased TGF-β1 activity has been suggested to play an important role in the pathogenesis of PCOS (43, 44). First, TGF-β1 is a growth factor that plays a crucial role in angiogenesis, fibroblast activation, and tissue fibrosis (3, 7, 8) and may explain the increased collagen deposition in ovarian stroma and theca and increased vascularity noted in the ovaries of women with PCOS (9, 10, 44). Second, increased circulating TGF-β1 levels have been reported in various cardiometabolic complications such as hypertension (45), obesity (46), insulin resistance (46), diabetes (47), and coronary artery disease (48). The significant decrease in TGF-β1 bioavailability and its correlation with the improvement in the lipid profile in vitamin D-treated PCOS women in our study supports the notion that TGF-β1 dysregulation plays a role in the pathophysiology of PCOS. Moreover, this finding suggests a potential mechanism by which vitamin D exerts its beneficial effect on women with PCOS.

Vitamin D replacement improves many clinical and metabolic disturbances associated with PCOS (20, 21), although the mechanism mediating these effects is largely unknown. Previous studies have shown that vitamin D attenuates TGF-β1 in rat ovaries and decreases its levels in

the context of cardiac and renal fibrosis (24–26). To the best of our knowledge, this study is the first to report the effects of vitamin D on sENG, showing an increase in circulating sENG after vitamin D supplementation in women with PCOS, leading to a significant reduction in TGF-β1 bioavailability in these women. Such an increase in sENG may be beneficial in the setting of fibrosis because the systemic administration of sENG has been shown to inhibit the effect of TGF-β1 and attenuate cardiac fibrosis in mice (49). Importantly, the findings of our study suggest that a therapeutic intervention aimed at TGF-β1 may be of benefit in patients with PCOS. Further preclinical and experimental animal studies are indicated to test the potential utility of TGF-β1 inhibition in PCOS.

Some limitations of this study should be mentioned. We used HOMA-IR instead of the gold standard method (glucose clamp technique) to measure insulin resistance (50). The glucose clamp technique was not used in this study because many participants could not afford staying in the clinic for the duration of the test, which may have affected the recruitment and compliance. Also, this study did not use an objective quantitative acne scoring system to assess the changes in acne status after intervention. Additionally, it is a single blinded trial, which could theoretically affect the assessment of hirsutism. Although the FGS significantly decreased 4 months after starting therapy, it would have been ideal to reevaluate the FGS few months later because changes may take longer than 4 months to be observed.

In conclusion, we have demonstrated for the first time that vitamin D supplementation in vitamin D-deficient women with PCOS significantly decreases the bioavailability of TGF-β1, which correlates with an improvement in some abnormal clinical parameters associated with PCOS. This is a novel mechanism, which could explain the beneficial clinical effects of vitamin D supplementation in women with PCOS. These findings may support new treatment modalities for PCOS, such as the development of anti-TGFβ drugs.

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Address all correspondence and requests for reprints to: Mohamad Irani, MD, Department of Obstetrics and Gynecology, Maimonides Medical Center, 967 48th Street, Brooklyn, NY 11219. E-mail: mohamad.irani@hotmail.com.

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