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Effect of Vitamin D_3 on Polycystic Ovary Syndrome Prognosis, Anthropometric and Body Composition Parameters of Overweight Women: A Randomized, Placebo-Controlled Clinical Trial

Nahla Al-Bayyari, Rae'd Hailat

Abstract**—**Vitamin D deficiency and overweight are common in women suffering from polycystic ovary syndrome (PCOS). Weight gain in PCOS is an important factor for the development of menstrual dysfunction and signs of hyperandrogenism and alopecia. Features of PCOS such as oligomenorrhea can be predicted by anthropometric measurements as body mass index (BMI). Therefore, the aim of this trial was to study the effect of 50,000 IU/week of vitamin D_3 supplementation on the body composition and on the anthropometric measurements of overweight women with PCOS and to examine the impact of this effect on ovaries ultrasonography and menstrual cycle regularity. The study design was a prospective randomized, double-blinded placebo-controlled clinical trial conducted on 60 overweight Jordanian women aged (18-49) years with PCOS and vitamin D deficiency. The study participants were divided into two groups; vitamin D group (n = 30) who were assigned to receive 50,000 IU/week of vitamin D_3 and placebo group (n = 30) who were assigned to receive placebo tablets orally for 90 days. The anthropometric measurements and body composition were measured at baseline and after treatment for the PCOS and vitamin D deficient women. Also, assessment of the participants' picture of ovaries by ultrasound and menstrual cycle regulatory were performed before and after treatment. Results showed that there were no significant ($p > 0.05$) differences between the placebo and vitamin D group basal 25(OH)D levels, body composition and anthropometric parameters. After treatment, vitamin D group serum levels of 25(OH)D increased (12.5 ± 0.61 to 50.2 ± 2.04 ng/mL, ($p < 0.001$), and decreased (50.2 ± 2.04 to 48.2 ± 2.03 ng/mL, $p < 0.001$) after 14 days of vitamin D_3 treatment cessation. There were no significant changes in the placebo group. In the vitamin D group, there were significant ($p < 0.001$) decreases in body weight, BMI, waist, and hip circumferences and fat mass. In addition, there were significant increases ($p < 0.05$) in fat free mass and total body water. These improvements in both anthropometric and body composition as well as in 25(OH)D concentrations, resulted in significant improvements in the picture of PCOS women ovaries ultrasonography and in menstrual cycle regularity, where nearly most of them (93%) had regular cycles after vitamin D_3 supplementation. In the placebo group, there were only significant decreases ($p < 0.05$) in waist and hip circumferences. It can be concluded that vitamin D supplementation improving serum 25(OH)D levels and PCOS prognosis by reducing body weight of overweight PCOS women and regulating their menstrual cycle.

Keywords**—**Anthropometric, overweight, polycystic ovary syndrome, vitamin D_3.

Nahla Al-Bayyari is with the Department of Nutrition and Food Technology, Faculty of Al-Huson University College, Al-Balqa Applied University, Al-Salt 19117, Jordan (corresponding author, phone: 00962795767524; e-mail: n.bayyari@bau.edu.jo).

Rae'd Hailat is with the Department of Epidemiology, Rollins School of Public Health, Emory University, Atlanta, Georgia, USA.

I. Introduction

PCOS is considered as one of the most frequent endocrine disorders that causes infertility in women of reproductive age and affects 5–10% of them globally [1]. It is characterized by hyperandrogenism and chronic anovulation [2]. It is associated with obesity [3], insulin resistance (IR) [4] and features of metabolic syndrome [5]. Obesity may be a major factor that exacerbates the symptoms and signs of PCOS rendering them more susceptible to type 2 diabetes mellitus (T2DM), cardiovascular diseases, and reproductive problems [5]. In addition, obesity seems to play a pathogenetic role in the development of PCOS [6] and it is usually associated with IR [7]. Moreover, obesity induces the genetic predisposition to hormonal anovulatory disorders in PCOS [8], metabolic disturbances, and possible development of estrogen-dependent tumors such as endometrial carcinoma are more common in obese women with PCOS than in lean women with PCOS [9].

Weight gain in PCOS is an important factor for the development of menstrual dysfunction such as dysfunctional bleeding, and signs of hyperandrogenism as hirsutism, acne, and alopecia [8]. BMI may be used as a modest predictor of oligomenorrhea and associated features of PCOS [10]. A wide range 38% to 88% of obesity (BMI > 30 kg/m^2) or overweight (BMI > 25 kg/m^2) is presented in women with PCOS [11].

Disorders in vitamin D metabolism and serum parathyroid hormone (PTH) concentrations are closely related to obesity and to clinical features of PCOS [1]. Vitamin D deficiency occurs commonly in obese women diagnosed with PCOS [12]; this supports the hypothesis that, vitamin D might be trapped by adipose tissue [13], which provides an evidence that vitamin D deficiency and IR could be two independent features in PCOS. Several research studies have reported inverse associations between body weight (BMI, body fat and waist measurements) and serum 25(OH)D levels in women with PCOS [14], and levels of 25(OH)D were reported to be 27-56% lower in obese women with PCOS compared with non-obese women with PCOS [14]. Because of these associations between overweight, 25(OH)D levels and PCOS, it is essential to study the effect of improving serum 25(OH)D levels on anthropometric indices and body composition among PCOS women. Also, most of the small number of vitamin D_3

intervention trials on PCOS women concentrate on the changes in BMI after intervention and ignored the changes in other anthropometric indices and body composition. Subsequently, we hypothesized that improving the 25(OH)D levels will decrease the body weight and other anthropometric and body composition parameters such as BMI, waist and hip circumferences and fat mass of the overweight reproductive women with PCOS and thus their ovaries ultrasonography picture will improve, and their menstrual cycle will be regulated. The main objective of this interventional trial was to study the effect of vitamin D_3 supplementation on anthropometric measurements and body composition of overweight women with PCOS and the impact of this intervention on ovaries ultrasonography and menstrual cycle.

II. Methods and Materials

A. Study Participants and Design

This study is a prospective randomized double-blind placebo-controlled clinical trial conducted on overweight Jordanian women aged 18-49 years old who attended the obstetrics and gynecology clinics at King Abdullah University Hospital (KAUH) in the North of Jordan. This study was approved by the Institutional Research Board (IRB) committee at KAUH and by Jordan Food and Drug Administration (JFDA). Also, this study was registered at the clinical trials database for all clinical trials conducted around the world [15] and an informed consent was obtained from each woman participating in the study.

B. Sample Size Determination

The required sample size was determined using [16]:

$$n = 2* (Z_\alpha + Z_\beta)^2 * SD^2 \div \delta^2$$

where n is the required number of participants in treatment and placebo groups, Z_α and Z_β are the values of the standard normal distribution at specific levels of confidence, 95%, given a two-sided α of 0.05 = 1.96 and a power of 80%, $Z_{0.20}$ = 0.84. SD is the standard deviation (pooled), and δ is the estimation of the difference between the treatment and placebo groups. According to the above equation, the sample size calculation showed that approximately 24 subjects were required for each arm of the trial to detect a change of 1.2 in the homeostasis model assessment of insulin resistance (HOMAIR) between the treatment and placebo groups with 80% power and 5% significance. The standard deviation (SD) was assumed to be 1.48 [17].

$$n = 2* (1.96 + 0.84)^2 * (1.48)^2 \div (1.2)^2 \approx 24 \text{ participants per group.}$$

To increase the power of the analysis and to take into consideration the dropout of study subjects, the number of participants was increased to 60 women per group.

C. Diagnosis of PCOS

Diagnosis of PCOS was carried out by the gynecologist based on the Rotterdam criteria [18], which necessitated the presence of two of the following three features: oligo-ovulation and anovulation, biochemical signs of hyperandrogenism; with an exclusion of related disorders such as hyperthyroidism, congenital adrenal hyperplasia, androgen secreting tumor, Cushing syndrome and hyperprolactinemia, and polycystic ovaries on ultrasound examination (defined as the presence of 12 follicles measuring 2-9 mm in diameter and/or an ovarian volume > 10 cm^3).

D. Inclusion and Exclusion Criteria

Overweight (BMI 25-29.9 kg/m^2) women diagnosed with PCOS, having a serum 25(OH)D level < 20 ng/ml, inadequate dietary intake of vitamin D (<600 IU/day or < 15 μg/day), normal complete blood count (CBC), aspartate and alanine aminotransferase (AST and ALT), urea and creatinine and who are able and willing to comply with the study rules, and to sign an informed consent were included in the study. Meanwhile, participants were excluded from the study if any of these conditions applied; pregnancy, lactation, women aged < 18 years or > 49 years, underweight, normal body weight and obese, diagnosed with diabetes, hypothyroidism, hyperthyroidism, liver disease, renal dysfunction, and cardiovascular diseases, presence of food allergies or intolerance, drug or alcohol abuse, smoking of 10 cigarettes or more or smoking of hookah, adequate dietary intake of vitamin D (600 IU/day or 15 μg/day), participants who are on medications known to affect metabolic parameters, such as metformin, vitamin D, calcium and corticosteroids, serum 25(OH)D level > 20 ng/ml, abnormal laboratory results of CBC, AST, ALT, urea and creatinine and participation in another clinical or bioequivalence study within 90 days prior to the start of this trial.

E. Assessment of Body Composition and Anthropometry

Height was measured using same stadiometer and women were barefooted and having their heels together, arms to the side, legs straight, shoulders relaxed and the head in the Frankfort horizontal plane. The measurement was recorded to the nearest 0.5 cm [19]. Weight was measured using the same beam scale (Seca 700 physicians beam scale) with minimum clothing and without shoes and looking straight ahead. The measurement was recorded to the nearest 0.1 kg [19]. To obtain waist to hip ratio (WHR), waist and hip circumferences were measured using a non-elastic measuring tape. Waist circumference (WC) was measured at the narrowest level between the lowest rib and the iliac crest at the end of normal expiration. Hip circumference (HC) was obtained when the tape meter was positioned horizontally around the maximum circumference of the buttocks. Measurements were recorded to the nearest 0.1 cm [19]. BMI was calculated according to Quetelet's formula: BMI= weight (kg)/height $(m)^2$ [19]. However, assessments of body composition, particularly body fat, including fat mass (FM), fat free mass (FFM), dry lean weight (DLW) and total body water (TBW) were measured using the Bioelectrical Impedance (Bodystate 1500 MDD, Body Composition/Wellness Monitoring Unit) analyzer. All anthropometric measurements were assessed before and after

intervention, measured twice and the average of the two readings was used.

F. Treatment Allocation and Determination of 25(OH)D Levels

Participating women were insured against civil claims of clinical trials, randomly allocated and assigned either to the placebo (n = 30) group or to the vitamin D_3 (n = 30) group using the computer generator random numbers in SPSS. Randomization for placebo or vitamin D_3 was done using the manufacturing batch numbers of placebo and vitamin D_3 tablets. Women in the Vitamin D group were supplemented with 50,000 IU vitamin D_3 every week for 90 days, while women in the placebo group received placebo capsules which were manufactured to be identical to vitamin D_3 capsules in color, shape, size, and packaging. Each participant received one bottle that contains four tablets every four weeks according to her randomization. Blood samples for the determination of serum 25(OH)D were obtained before seven days from the initiation of the study (-7 days), at the beginning of study (0 day or basal), after 30 days, 60 days and 90 days of taking vitamin D_3 or the placebo to monitor the changes in serum 25(OH)D levels over the intervention period. The final blood sample was obtained from each participant after 14 days from taking the last dose of vitamin D_3 or placebo (day 104 of the initiation of the study).

To determine the 25(OH)D levels, a morning venous blood sample (approximately 5 ml) was drawn after at least 8-12-hours overnight fasting using 10 ml Vacutainer (VACUETTE) plain tubes containing clot activator (Z-serum clot activator) and stored at room temperature for 30 minutes before centrifugation at 4000-rpm and 23°C for 5 minutes using an Eppendorf Centrifuge 5810 R; then, it was transferred into endocrine automated analyzer (Beckman Coulter, Access 2 immuno assay system, USA).

F. Statistical Analyses

Collected data were entered in data sheets twice and checked and analyzed using the SPSS statistical package (IBM, SPSS version 22, 2013). All continuous variables were examined for normal distribution by the nonparametric Kolmogorov–Smirnov test. Descriptive statistics were performed using means and standard error of the means (SEM) to describe continuous variables while, frequencies and percentages were used to describe categorical variables. Frequencies and percentages were calculated and compared using Mantel–Haenszel $\chi 2$ and Fisher exact tests. The differences between the means of the normally distributed variables including the participants' basal 25(OH)D levels, anthropometric measurements and body composition were examined using Student t test for independent samples between the placebo and vitamin D groups. In addition, the paired-sample t test was used to test the differences between the means of the normally distributed variables before and after intervention among the placebo and vitamin D groups. The medians were compared when variables followed a significantly skewed distribution using the Mann-Whitney U test for the independent samples and the Wilcoxon signed rank test for paired samples. All reported P values are 2-tailed, and $P \leq 0.05$ was statistically significant.

III. Results

A. Characteristics of the Study Participants

Seventy overweight women with PCOS were assessed based on serum 25(OH)D concentrations. Out of them, 64 women had serum 25(OH)D level < 20 ng/ml and four of them dropped out after screening. Therefore, only 60 women participated in the intervention trial and two of them were lost to follow up on day 90 and day 104 of the study. Thereby, 29 women were included in statistical analysis from each group (Fig.1). The participants' mean age was (23.56±0.58) years and the mean values of their anthropometric parameters and body composition were (68.92±1.26) kg for body weight, height (158.86±0.96) cm, BMI (27.2±0.21) kg/m^2, WC (84.0±1.05) cm, HC (100.7±1.17) cm, WHR (0.83±0.01), FM (34.30±0.36) %, FFM (65.69+0.36) %, DLW (13.64±0.34) and TBW was (46.05±0.32) %. More than the half (64.3%) were single and around (48.6%) of them were university students. Also, 94.3% were covering their heads, and 83% of them do not expose directly to sunlight.

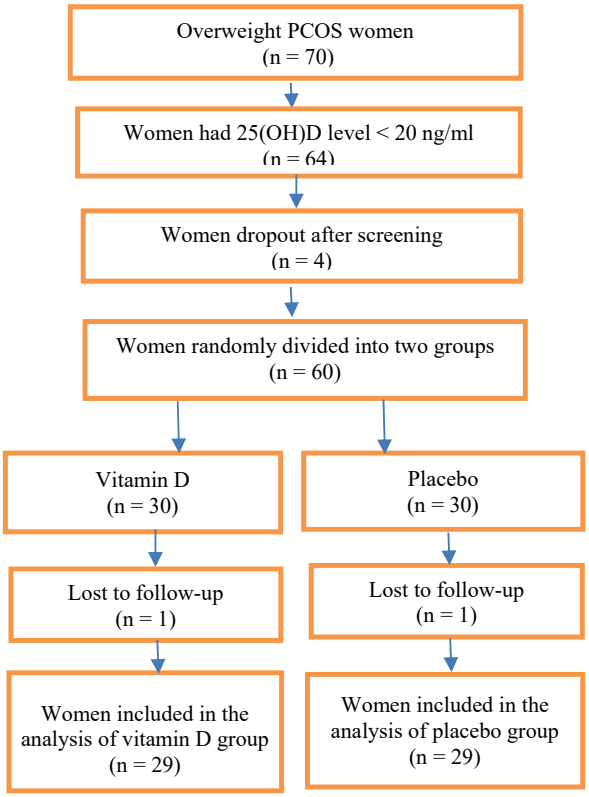

Fig. 1 Selection of participants included in the study analysis

Overweight PCOS women (n = 70) were divided into two categories according to their BMI and into three strata according to their 25(OH)D levels. Results revealed that more

than 90% of the screened women were vitamin D deficient (Table I).

TABLE I
FREQUENCY DISTRIBUTION OF BMI AND VITAMIN D LEVELS AMONG PCOS WOMEN

	25(OH)D levels			
	Deficient < 20 ng/ml n (%)[a]	Insufficient 20-29 ng/ml n (%)[a]	Sufficient >29 ng/ml n (%)[a]	Total n (%)[a]
BMI (kg/m^2)				
25-27.9	45 (70.3)	1(100)	2 (40.0)	48 (68.6)
28-29.9	19 (29.7)	0 (0.0)	3 (60.0)	22 (31.4)
Total n (%)[a]	64 (100)	1 (100)	5 (100)	70 (100)

[a] Values are presented as frequencies (n) and percentages (%).

Table II shows that there were no significant differences between the baseline means of age, body composition and anthropometric characteristics of the placebo and the vitamin D groups.

TABLE II
SERUM 25 (OH)D LEVELS, BODY COMPOSITION AND ANTHROPOMETRIC CHARACTERISTICS OF PLACEBO AND VITAMIN D GROUPS [A]

Variable	Placebo (n = 29) Mean± S.E.M [b]	Vitamin D (n = 29) Mean± S.E.M [b]	P [c]
25(OH)D (ng/ml)	12.4±0.52	12.5±0.60	0.876
Age (years)	23.8±1.08	23.5±0.76	0.841
Body weight (kg)	67.1±1.67	69.3±1.97	0.304
Height (cm)	157.1±1.40	158.9±1.41	0.351
BMI (kg/m^2)	26.9±0.30	27.3±0.35	0.371
WC (cm)	82.5±1.41	85.0±1.80	0.178
HC (cm)	98.9±1.90	101±1.75	0.428
WHR	0.83±0.01	0.84±0.01	0.440
FM (%)	34.1±0.67	34.5±0.50	0.681
FFM (%)	66.0±0.67	65.5±0.50	0.681
TBW (%)	46.5±0.52	45.9±0.46	0.347
DLW (kg)	13.1±0.50	13.7±0.52	0.304

[a] Means and standard errors of the means of all study participants (N = 58) for 25(OH)D levels, age, body composition, and anthropometric characteristics were compared according to randomization using the independent sample t-test for normally distributed continuous variables. Medians were compared when variables followed a significantly skewed distribution using the Mann-Whitney-U-test for the independent samples.
[b] Values are presented as the means±SEMs.
[c] *p-value ≤ 0.05 is statistically significant.

B. Changes in Means of Serum 25(OH)D Levels Overtime

Changes in means of serum 25(OH)D for vitamin D and placebo groups showed highly significant ($p < 0.001$) increases in serum 25(OH)D overtime among the vitamin D group compared to the placebo group, except at the baseline levels. However, there were minor fluctuations in the means of serum 25(OH)D levels overtime in the placebo group. Also, among the vitamin D group, there was a significant decrease in 25(OH)D levels after 14 days (day 104) of vitamin D_3 termination (Fig. 2).

C. Mean Comparisons among Placebo and Vitamin D Groups

Table III shows the mean comparisons before and after intervention among the placebo and vitamin D groups. There were only statistically significant ($P < 0.05$) differences noticed in the mean values of WC, HC and DLW after placebo administration. In the vitamin D group, there was a statistically significant ($P < 0.05$) decrease in the mean value of body weight, BMI, WC, HC, FM, and DLW after treatment with vitamin D_3. There was also a statistically significant ($P \leq 0.05$) increase in FFM, and TBW levels.

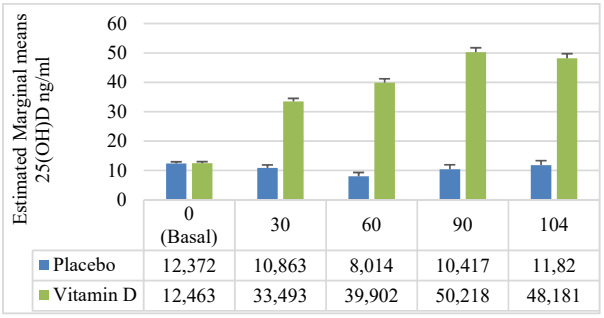

Fig. 2 Estimated marginal means of 25(OH)D for placebo and vitamin D groups overtime

D. Comparisons of Frequencies of Ultrasonography and Menstrual Regulation among Placebo and Vitamin D Groups

Table IV shows that there were significant ($P \leq 0.05$) changes in the ovaries ultrasonography and menstrual regulation in the vitamin D group. After treatment, 24% of PCOS patients have normal ovaries and 93% have regular menstrual cycle. In the placebo group, there was only a statically significant ($P \leq 0.05$) difference in ovaries ultrasonography. Although, 62% of placebo group still have both ovaries polycystic and 86% still have irregular menstrual cycle.

IV. DISCUSSION

Comparing the results of this study with other studies conducted in different parts of the world should be done with caution due to many factors such as differences in study design, criteria used for PCOS diagnosis, cut-off points of biochemical and clinical parameters and indices used to determine signs and symptoms of PCOS. In addition, there are other factors that should be taken into consideration, such as differences in socio-demographic characteristics, culture, dietary habits, ethnicity, geographical locations, religious rites and lifestyle characteristics. Even though Jordan is a sunny country, except during the winter season, the prevalence of vitamin D deficiency (25-OH-D < 20 ng/ml) among the study population was 90%. This rate agrees with the rate found in a previous study conducted among reproductive age Jordanian women, which showed that the prevalence of vitamin D deficiency (<12 ng/ml) was 60.3% and 95.7% for insufficiency (<20 ng/ml) [20]. Moreover, most women in this study were either covering their heads (94.3%) and/or veiled covering their faces for religious or cultural reasons and 83% not exposed directly to sunlight. This might support the hypothesis raised previously which claimed that "covering heads and/or wearing veil" may affect serum 25(OH)D levels

in Jordanian women [20], [21]. In this interventional trial a highly significant increase ($p < 0.001$) in the means of serum 25(OH)D levels over the study period was found compared to the basal levels among the vitamin D group. Meanwhile, a significant decrease was found in the serum 25(OH)D level among the placebo group compared to the basal level. Also, no significant differences were found between the basal levels of the placebo and vitamin D groups. The most significant increase in the means of serum 25(OH)D was observed on day 90 of the intervention period, which was expected since the day 90 is the last day of taking vitamin D_3 treatment. There was a significant reduction in serum 25(OH)D levels on day 104; after 14 days from the last week of the intervention period, which was not expected since vitamin D is a fat-soluble vitamin stored in the liver. Similar significant increases in serum 25(OH)D levels were reported in previous clinical intervention trials [4], [22]-[24]. Unfortunately, there were not any vitamin D_3 intervention clinical trial that followed the participants after two weeks or more of the intervention period to study the changes in serum 25(OH)D levels. In addition, controlling for the vitamin D_3 treatment residue showed significant increase in serum 25(OH)D levels within the days of interventions except on day 104 compared to day 90. Rahimi-Ardabili et al. [23] and Khan et al. [24] recently reported similar results. Significant changes in mean values of WC, HC and DLW were noticed before and after placebo treatment. These changes could be due to psychological satisfaction through taking medication and we found some of the placebo participants were followed dietary programs and properly increased their level of activity. Unfortunately, most of the little published placebo controlled clinical trials on PCOS did not measure HC, WC and DLW. Meanwhile, in the vitamin D group, significant differences were found in all anthropometric characteristics (body weight, BMI, WC, HC, FM, FFM, TBW, and DLW) except for WHR between the mean values before and after treatment with vitamin D. Most of the small number of vitamin D_3 intervention trials concentrate on the changes in BMI after intervention and ignored the changes in other anthropometric indices except for a recently published article in 2018, which studied the effect of vitamin D_3 on weight, BMI, fat mass, waist and hip circumference and waist-to-hip ratio and it showed that vitamin D_3 treatment for 12 weeks combined with weight reducing diet significantly decreased weight, BMI, WC, HC and WHR [25]. That study's findings were somehow similar to the findings of the current study; although, a weight reducing diet was not used in combination with the vitamin D_3 treatment dose in this research.

Al-Daghri et al. [26] studied the differences in BMI, HC, WC and WHR on overweight women without PCOS and vitamin D_3 supplements, just advised the participants to regularly exposed to sunlight. Their findings were like those of the current study regarding the significant decrease in body weight, BMI, HC and WC, and to the non-significant change in WHR.

TABLE III
BODY COMPOSITION AND ANTHROPOMETRIC CHARACTERISTICS BEFORE AND AFTER INTERVENTION AMONG PLACEBO AND VITAMIN D GROUPS [A]

Variable	Placebo (n = 29)			Vitamin D (n = 29)		
	Before Mean±S.E.M [b]	After Mean±S.E.M [b]	P [c]	Before Mean±S.E.M	After Mean±S.E.M	P [c]
Body weight (Kg)	67.1±1.67	66.2±1.73	0.14	69.3±1.97	65.0±1.86	0.01
BMI (kg/m^2)	26.9±0.30	26.5±0.37	0.13	27.3±0.35	25.6±0.37	0.01
WC (Cm)	82.5±1.41	77.9±1.43	0.01	85.0±1.80	77.7±1.67	0.01
HC (Cm)	98.9±1.90	94.2±1,67	0.01	101±1.75	93.0±1.49	0.01
WHR	0.83±0.01	0.82±0.01	0.31	0.84±0.01	0.83±0.01	0.45
FM (%)	34.1±0.67	33.7±0.82	0.46	34.5±0.50	31.5±0.59	0.01
FFM (%)	66.0±0.67	66.3±0.80	0.46	65.5±0.50	68.5±0.59	0.01
TBW (%)	46.5±0.52	47.0±0.65	0.30	45.9±0.46	48.5±0.50	0.01
DLW (Kg)	13.1±0.50	12.9±0.50	0.01	13.7±0.52	13.1±0.52	0.01

[a] Means and standard errors of the means of body composition and anthropometric characteristics were compared before and after intervention among placebo (n=29) and vitamin D groups (n=29) using paired sample t-test for normally distributed continuous variables. Medians were compared when variables followed a significantly skewed distribution using Wilcoxon Signed Rank test for the independent samples.
[b] Values are presented as the means±SEMs.
[c] *p-value ≤ 0.05 is statistically significant.

TABLE IV
OVARIES ULTRASONOGRAPHY AND MENSTRUAL CYCLE REGULARITY BEFORE AND AFTER INTERVENTION AMONG PLACEBO AND VITAMIN D GROUPS [A]

Variable	Placebo (n = 29)		P [c]	Vitamin D (n = 29)		P [c]
	Before n (%) [b]	After n (%) [b]		Before n (%) [b]	After n (%) [b]	
Ovaries ultrasonography						
Both normal	0	0		0	7 (24.2)	
Left or right normal	0	11 (37.9)	0.01	0	5 (17.2)	0.001
Both polycystic	29 (100)	18 (62.1)		29(100)	17 (58.6)	
Menstrual Cycle						
Regular	4 (13.8)	4 (13.8)	1	3 (10.3)	27 (93.1)	0.001
Irregular	25 (86.2)	25 (86.2)		26 (89.7)	2 (6.9)	

[a] Frequencies and percentages of ovaries ultrasonography and menstrual cycle regularity were compared before and after intervention among placebo (n=29) and vitamin D groups (n=29) using Mantel–Haenszel $\chi 2$ and Fisher exact tests.
[b] Values are presented as frequencies (n) and percentages (%).
[c] *p-value ≤ 0.05 is statistically significant.

Wehr et al. [27] also found a significant decrease in HC among PCOS women supplemented with 20,000 IU/week. In a recent placebo-controlled pilot clinical trial [24], the researchers reported no significant differences in BMI changes after 12-week intervention with 12,000 IU vitamin D or placebo daily, which is inconsistent with the findings of this study. Furthermore, a randomized, placebo-controlled, double-blinded trial conducted on 50 women with PCOS aged 20-40 years with vitamin D deficiency, assigned to receive three oral treatments consisting of 50,000 IU of vitamin D_3 or a placebo (one every 20 days) for two months found a decrease in vitamin D group BMI compared to the placebo group; although, this decrease was not significant [4]. On the other hand, many epidemiological studies reported negative associations between serum 25(OH)D and weight, BMI, HC, WC and WHR [14], [28], [29]. Overall results of studying the differences in the mean values of the anthropometric characteristics between both groups after treatment showed that, only FM and FFM had significant differences. This means, vitamin D supplementation (50,000 IU/week) decreased FM and increased FFM. No published results from clinical trials regarding FM and FFM have been found except a recent study in 2018 which found that vitamin D_3 supplementation in treatment dose of 50,000 IU for 12 weeks in combination with a weight reducing diet resulted in a significant decrease in fat mass [25]. In addition, another study examined the associations between serum 25(OH)D, FM and FFM using Dual Energy X-ray Absorptiometry (DEXA) and revealed that there was a strong association between 25(OH)D and total fat mass [14]. Since BMI includes both total fat mass and fat free mass, it does not provide a reliable measure as bioelectrical impedance (bodystate) or DEXA, thus FM and FFM were better predictors than BMI [14].

The impact of vitamin D_3 treatment was also investigated on the clinical features and PCOS prognosis through the ovaries ultrasonography and regulation of the menstrual cycle. In the vitamin D group, we found that around 25% of PCOS women have a normal picture of their both ovaries and 93% have regular menstrual cycles after treatment. Similarly, significant improvements in menstrual frequency were observed in a recent study but they did not report any changes in ovaries ultrasonography [25]. However, beneficial effects on menstrual regularity and ovulation may have resulted from vitamin D treatment along with calcium supplementation and metformin therapy in women with PCOS [30]. In general, most of the interventional trial results were consistent with the researcher's expectations; thus, the study hypotheses are accepted. Nevertheless, the findings of this study could not be generalized to the whole population, as they have some drawbacks such as a somewhat small sample size and body composition was determined using the bioelectrical impedance, and it might be better to use DEXA, as well, the findings were only applicable in overweight PCOS women of a reproductive age.

It can be concluded that vitamin D supplementation improving serum 25(OH)D levels and reducing body weight, BMI, HC, WC and FM and increasing FFM and TBW of overweight PCOS women. In addition to modulating the clinical features and PCOS prognosis through normalizing the ovaries ultrasonography and regulating ovulation and menstrual cycle.

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