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Improvement of Symptoms in Patients With Polycystic Ovarian Syndrome by Vitamin D and Calcium Supplementation

Abstract

Background: Polycystic ovarian syndrome (PCOS) is a common female endocrine disorder with unknown etiology. Many treatments are available to treat the symptoms associated with this condition including obesity, insulin resistance, abnormal glucose metabolism, infertility, menstrual irregularity, hirsutism, and acne vulgaris. More recently, it has been found that women with PCOS, particularly if obese, are more likely to be vitamin D deficient. It is hypothesized that vitamin D and calcium metabolism can also affect symptoms associated with PCOS as it influences many physiologic processes within the body. Does vitamin D and calcium supplementation improve symptoms of PCOS?

Methods: An exhaustive medical literature search was conducted using Medline-OVID, CINAHL, EBMR Multifile, GoogleScholar and Web of Science using keywords: polycystic ovarian syndrome and vitamin D. The search was further narrowed to include articles with English language and humans only. For included articles, bibliographies were screened for relevant articles. Included articles were evaluated using GRADE.

Results: For this review, the final number of relevant, primary articles meeting inclusion criteria was a total of 6 articles. These articles included two randomized- controlled trials, three observational studies, and one case-control study. These studies vary in sample size, measurement of outcomes, population selection, and significance in results. Multiple studies suggest improvement in menstruation regularity, fertility, BMI, insulin resistance, glucose metabolism, and hyperandrogenism; but the studies evaluated have many limitations and yield insignificant observations.

Conclusion: It is possible that vitamin D and calcium supplementation yields a good outcome in a variety of symptoms common in PCOS, but evidence is lacking in quality. A weak recommendation for vitamin D and calcium supplementation can be made as vitamin D is relatively inexpensive and safe. A stronger recommendation is made if PCOS patients are vitamin D deficient. Long-term, randomized clinical studies need to be conducted to determine whether vitamin D and calcium supplementation is necessary in the treatment of PCOS and to demonstrate the benefits of supplementation in relation to the general population with this condition.

Degree Type

Capstone Project

Degree Name

Master of Science in Physician Assistant Studies

Keywords

polycystic ovarian syndrome, vitamin D, calcium

Subject Categories

Medicine and Health Sciences

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Improvement of Symptoms in Patients With Polycystic Ovarian Syndrome by Vitamin D and Calcium Supplementation

Alyssa M. Galusha



A Clinical Graduate Project Submitted to the Faculty of the

School of Physician Assistant Studies

Pacific University

Hillsboro, OR

For the Masters of Science Degree, August 10th, 2013

Faculty Advisor: Dr. Rob Rosenow, Pharm.D., O.D.

Clinical Graduate Project Coordinator: Annjanette Sommers, PA-C, MS

Biography

Alyssa M. Galusha is a native of Oregon where she majored in Biology with Health Science emphasis from the Oregon Institute of Technology in 2010. While completing her undergraduate degree, Alyssa worked as a Certified Nurse Assistant in two different hospitals within underserved communities. Most of her experience included post-surgery, pediatrics and labor and delivery. She was also able to complete training as a scrub technician for cesarean sections. After completion of her undergraduate degree, she moved to Portland and worked at the Center for Health Research performing clinical work for influenza studies. She hopes to work in primary care in an underserved community, moonlight in urgent care, and become a diabetes educator.

Abstract

Background: Polycystic ovarian syndrome (PCOS) is a common female endocrine disorder with unknown etiology. Many treatments are available to treat the symptoms associated with this condition including obesity, insulin resistance, abnormal glucose metabolism, infertility, menstrual irregularity, hirsutism, and acne vulgaris. More recently, it has been found that women with PCOS, particularly if obese, are more likely to be vitamin D deficient. It is hypothesized that vitamin D and calcium metabolism can also affect symptoms associated with PCOS as it influences many physiologic processes within the body. Does vitamin D and calcium supplementation improve symptoms of PCOS?

Methods: An exhaustive medical literature search was conducted using Medline-OVID, CINAHL, EBMR Multifile, GoogleScholar and Web of Science using keywords: polycystic ovarian syndrome and vitamin D. The search was further narrowed to include articles with English language and humans only. For included articles, bibliographies were screened for relevant articles. Included articles were evaluated using GRADE.

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Keywords: *polycystic ovarian syndrome, vitamin D, calcium*

Acknowledgements

To *my parents*: Thank you, Mark and Patricia, for helping me succeed and for supporting me when I felt I had nothing left in me to move forward.

To *my classmates*: Without the support of my peers, I would not have been able to survive the life stressors and challenges of this program.

To *my friends*: Thank you for your patience and support as I worked on my goals and myself.

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TABLE I: Characteristics of Reviewed Studies and Summary of Findings, GRADE profile

List of Abbreviations

PCOS.....	Polycystic Ovarian Syndrome
IR.....	Insulin Resistance
SHBG.....	Sex Hormone-Binding Globulin
LH.....	Luteinizing Hormone
FSH.....	Follicle Stimulating Hormone
BMI.....	Body Mass Index
GRADE.....	Grading of Recommendations, Assessment, Development and Evaluation
RCT.....	Randomized Clinical Trial
ANOVA.....	Analysis of Variance
OR.....	Odds Ratio
NNT.....	Number Needed to Treat
NIH.....	National Institute of Health
ACTH.....	Adrenocorticotrophic Hormone
DHEAS.....	Dihydroxyepiandrosterone Sulfate
PTH.....	Parathyroid Hormone
25(OH)D.....	Vitamin D
HOMA.....	Homeostasis Model Assessment
AUC.....	Area Under the Curve
FG.....	Ferriman Galleway Score

Improvement of Symptoms in Patients With Polycystic Ovarian Syndrome by Vitamin D and Calcium Supplementation

BACKGROUND

Polycystic ovarian syndrome (PCOS) is a frustrating condition for affected women and one that is found particularly hard to treat by providers. An estimated 4 to 12 percent of women in the United States have PCOS.¹ The Rotterdam criteria define the syndrome in patients who have two of three of the following: chronic oligomenorrhea or amenorrhea, evidence of androgen excess or polycystic ovaries.² Classical features of PCOS include, but are not limited to; infertility, hyperandrogenism, truncal obesity, abnormal glucose metabolism, insulin resistance (IR), hirsutism, and acne vulgaris. Symptoms typically manifest in early puberty and worsen with maturation. Nearly 80 percent of patients affected present with more than one symptom.³

The primary etiology of PCOS has not yet been determined, but it is known that many metabolic disturbances occur within this disease and cause associated symptoms. It is hypothesized that dysfunction of glucose and insulin metabolism leads to increasing symptoms of androgen excess, which in turn, results in worsening of metabolic function and obesity. Increased weight gain is attributed to IR and induces more hyperandrogenic effects.⁴ Insulin resistance leads to hyperinsulinemia,⁵ more consistently in obese PCOS patients.⁴ As a result, increased insulin promotes secretion of androgens from the ovaries and decreases the amount of serum sex hormone-binding globulin (SHBG); therefore, serum free testosterone is elevated.³ Other hormones such as luteinizing hormone (LH), follicle stimulating hormone (FSH), estrogen and other androgenic derivatives are

negatively affected.³ Ovarian follicle growth and maturation is altered by hyperinsulinemia and androgen excess,⁵ causing menstrual irregularity and infertility.

It is newly accepted that these metabolic changes are related to dysfunction of vitamin D and calcium metabolism, which is also important in follicular development⁶ and normal glucose metabolism.⁷ Multiple studies have shown that PCOS patients, particularly if obese, have lower serum vitamin D levels.^{4,8-10} Because vitamin D is fat soluble, it is likely that a larger than normal percentage of available vitamin D is requisitioned into adipose tissue, causing decreased serum levels in obese patients.¹¹ Lower vitamin D levels have been associated with hyperandrogenism,^{4,10,11} metabolic syndrome,⁹ insulin resistance,^{4,9,10,12} and increased body mass index (BMI).^{4,10,12,13} Physiologic functions of active vitamin D that affect glucose and insulin metabolism include producing insulin from pancreatic beta cells, augmenting insulin sensitivity by increasing expression of the insulin receptor, and decreasing release of cytokines that facilitate IR.¹⁴ One study found significant correlation between hirsute women with PCOS and lower vitamin D levels.⁹ Other studies have not agreed with these findings, suggesting lack of research and validity in regards to the results. Limited evidence has shown that vitamin D and calcium metabolism affects oocyte maturation and production of androgens, but one research study revealed that 1,25-dihydroxyvitamin D mediates an enzyme known as aromatase which converts androgen derivatives to estrogen in granulosa cells of the ovaries.¹⁵ Therefore, dysfunction in this mechanism affects the menstrual cycle and ovulation by altering the hormone levels mediated and suppressed by estrogen, LH, and FSH.

To validate some of these outcomes, researchers have experimented in treating PCOS patients with vitamin D and calcium supplementation. This systematic review is to evaluate the current evidence and make recommendations as to whether vitamin D and calcium supplementation improves symptoms in PCOS patients.

METHODS

An exhaustive medical literature search was conducted using Medline-OVID, CINAHL, EBMR Multifile, GoogleScholar and Web of Science using keywords: polycystic ovarian syndrome and vitamin D. The search was further narrowed to include articles with English language and humans only, as well as articles published from January 1990 to August 2012. Articles using vitamin D supplementation with or without calcium supplementation were included. Primary articles evaluating vitamin D in addition to fertility treatment (i.e. Clomiphene) were excluded from review. For included articles, bibliographies were screened for additional relevant resources. Relevant articles were appraised for quality, validity, and risk of bias using the Grading of Recommendations, Assessment, Development and Evaluation (GRADE).¹⁶

RESULTS

The preliminary search throughout all databases yielded 45 articles for review. The final number of relevant, primary articles meeting inclusion criteria was a total of 6 articles. These articles included two randomized- controlled trials (RCT),^{17,18} three

observational studies,^{6,19,20} and one case-control study.⁷ Results and summaries of these studies are also demonstrated on Table I.

Rashidi et al

This randomized clinical trial (pilot study)¹⁷ evaluated the effects of calcium and vitamin D supplementation in PCOS on Metformin therapy. For this study, all patients were referred to the Vali-e-Asr Reproductive Research Center. Sixty Iranian, infertile PCOS patients aged 20 to 40, were selected to participate if they met 2 of 3 of the Rotterdam criteria for PCOS. Primary outcomes included BMI, menstruation regularity, follicular size, and pregnancy rates. Secondary outcomes included review of dietary consumption questionnaires and patient recall of diet intake, particularly information of dairy, protein, and fat consumption. Changes in serum vitamin D levels were not evaluated.¹⁷

Notable exclusion criteria included history of endocrine disorders, primary hyperparathyroidism or hyperprolactinemia, hormone secreting tumor of androgenic nature, prior history of abdominal or pelvic surgery, coexisting male factor infertility, or history of abnormal ultrasound of the uterus and salpinges. All participants completed physicals including transvaginal ultrasound to determine the presence of polycystic ovaries and follicle diameter and questionnaires regarding diet consumption. Biochemical markers such as serum and urine calcium, FSH, LH, prolactin, and serum magnesium were evaluated in all patients for homogeneity.¹⁷

Patients were randomly separated into three groups by a random number table with 20 patients in each group. A statistical method known as analysis of variance

(ANOVA) determined no significant difference in characteristics amongst participants within the selected groups. Group 1 received 1 000 mg of calcium and 400 IU vitamin D daily (Calcium-D tab 500 mg 200 IU; Tehran Darou, Iran). Group 2 was similar to Group 1, except for the addition of 1 500 mg daily of Metformin (Metformin tab 500 mg; Mino Darou, Iran). Group 3 members were given Metformin 1 500 mg daily without other supplementation. All treatments were received for 3 months, and follow up included an additional 3 months after therapy.¹⁷

Results demonstrated an increase in size and number of follicles ≥ 14 mm after follow up in patients who had received calcium and vitamin D supplementation (Group 2) in addition to Metformin, compared to other groups (Group 2 vs Group 1, $P = 0.0372$, Odds Ratio (OR) = 2.01; Group 3 vs Group 1, $P = 0.2896$; No OR provided). Six months after therapy, Group 2 showed continued treatment response compared to other groups (number needed to treat (NNT) = 9.5). Chi-squared tests showed no significance in positive changes in menstruation ($P = 0.400$, NNT = 6.7) and follicular stimulation ($P = 0.2896$). A subgroup analysis of three BMI groups (<25 , $25-27$, >27), showed no significant difference in menstruation regularity and BMI. No pregnancy occurred in the three study groups. Analysis of diet through Kruskal-Wallis test revealed that the majority of participants (60%) were below recommendations for dairy intake.¹⁷

The authors recognized limitations in sample size and suggest a larger sample size may prove results to be more significant. A small loss to follow up was noted in each study group with no reasons provided by the authors. The conclusion of this study was that vitamin D and calcium supplementation in addition to Metformin had a more successful treatment effect on menstrual regulation and follicular development.¹⁷

Thys-Jacobs et al

This observational study⁶ investigated whether diminished calcium and vitamin D regulation affects follicular development in 13 premenopausal women with PCOS. There is no documentation of location and setting to which the study takes place. Patients were ages 21 to 41 and included in the study if they met the National Institute of Health (NIH) Consensus Criteria of PCOS: chronic oligomenorrhea/anovulation and signs of hyperandrogenism; including acne vulgaris, hirsutism, and alopecia. Two patients were Caucasian and 11 patients were Hispanic. All patients presented with hirsutism; 5 patients had acanthosis nigricans, 2 with alopecia, and 3 with acne vulgaris. Nine of thirteen participants had polycystic ovaries. Primary outcomes included follicular development, menstruation regularity, and androgenic changes.⁶

Exclusion criteria included any androgen secreting tumors, Cushing's syndrome, hyperprolactinemia, primary hyperparathyroidism, cancer, history of gastrectomy, renal failure, inflammatory bowel disease, use of anti-seizure medications and mental retardation. If adrenal hyperplasia was detected later in the study and by an adrenocorticotrophic hormone (ACTH) suppression test, patients were excluded from the study. It is not described whether or not patients were on or excluded for concomitant medications. Participants underwent history and physical evaluations and laboratory analysis for prolactin, dihydroxyepiandrosterone sulfate (DHEAS), 17-hydroxyprogesterone, androstenedione, AM cortisol, total testosterone, LH and FSH. Other laboratory tests for baseline included total calcium, intact parathyroid hormone (PTH), 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D. A transvaginal sonogram was obtained for each patient to evaluate for polycystic ovaries.⁶

All patients were placed on 1 500 mg daily of calcium carbonate and ergocalciferol (vitamin D) 50 000 units weekly or biweekly for 6 months. Vitamin D levels improved in all patients within 3 months of therapy. Within two months of therapy, 9 of 13 patients had normalized their menstrual cycles, defined as 27 to 32 days of cycle length. Two patients had resolution of dysfunction uterine bleeding and two other women became pregnant. After 6 months of therapy, acne presentation improved in affected patients but no other improvements related to androgen excess were observed.⁶

No limitations of this study were discussed. This study concluded that calcium and vitamin D supplementation enhances menstruation regularity and elucidates the possibility that this therapy could correct abnormal follicular development.⁶

Firouzabadi et al

One-hundred Iranian, infertile PCOS patients, aged 20 to 40, were enrolled in this case-control study.⁷ Participants were randomized into 2 groups (n=50) via random number table. All portions of the study were conducted at Shahid Sadoughi University of Medical Sciences, Department of Obstetrics and Gynecology, Research and Clinical Center for Infertility. All patients were selected to participate if they met 2 of 3 of the Rotterdam criteria for PCOS. Evaluated outcomes included menstruation regularity, changes in BMI, follicular size, pregnancy rate, and vitamin D levels. There were no significant differences of patient characteristics between groups.⁷

Patients with Cushing's syndrome, hyperprolactinemia, renal failure, any type of androgen secreting tumor, primary hyperparathyroidism, cancer, history of gastrectomy, inflammatory bowel disease, anti-seizure medications, and mental retardation were

excluded from the study. Patients underwent history and physical examination, transvaginal ultrasound, and diagnostic lab analysis of hormones including LH, FSH, serum calcium, and vitamin D levels (25(OH)D).⁷

Group 1 received 1 500 mg daily of Metformin (Metformin tab, 500 mg; Osvah Pharmaceutical Company), and Group 2 was treated with 1 000 mg daily of calcium (Tablet 500 mg; Sobhandarou Pharmaceutical Company) and vitamin D (Pearl Vitamin D3, 50 000 IU/week; Zahravi Pharmaceutical Company) 100 000 IU monthly in addition to 1 500 mg daily of Metformin for 6 months.⁷

The study revealed a more significant decrease in BMI in the treatment group (Group 2, $P = 0.054$). A notable improvement in menstrual regularity after 6 months of therapy in Group 1 (58%) vs Group 2 (70%) was observed, but Chi-square analysis proved this outcome insignificant between study groups ($P = 0.211$, $NNT = 8.3$). Less significant changes in follicular maturation were noted between study groups at three and six months after therapy ($P = 0.759$ and $P = 0.698$, respectively). Although more pregnancies occurred in Group 2 than Group 1 (18% vs 12%), there was no significance in pregnancy rates between the two groups ($P = 0.401$, $NNT = 16.7$). A Chi-square analysis within each group comparing before and after treatment showed significant effect on menstrual regularity, BMI, and follicular response ($P = 0.001$). Vitamin D levels improved substantially and were significant when comparing Group 2 to Group 1, which did not receive vitamin supplementation ($P \leq 0.001$).⁷

The authors noted lack of a control group as a limitation in the study and discussed the fact that Iranian women are at increased risk for vitamin D deficiency due to cultural and location factors: clothing, environment, prevention of sun exposure and/or

increased approaches to avoid sun exposure.¹⁸ This study states vitamin D and calcium supplementation can improve weight reduction, follicular development, improvements of androgen excess symptoms, and regularity of menses in vitamin D deficient PCOS patients. The authors also suggested that vitamin D supplementation could be a potential treatment in improving IR and infertility associated with PCOS.⁷

Ardabili et al

This double-blinded, placebo-controlled randomized clinical trial¹⁹ evaluated the effects of vitamin D supplementation on glucose levels and insulin resistance. Sixty vitamin D deficient, PCOS patients recruited from an outpatient clinic at Alzahra Hospital of Tabriz University of Medical Sciences in Iran were included in the study. Patients must to have been diagnosed with PCOS as characterized by having met 2 of 3 symptoms of the Rotterdam criteria, vitamin D deficient (<20 ng/mL), and between 20 and 40 years of age. The primary outcomes of the study were glucose metabolism, insulin resistance, and insulin sensitivity. A secondary outcome was assessment of dietary intake through a 3-day recall from each participant.¹⁹

This study's exclusion criteria consisted of any patients with an androgen secreting tumor, Cushing's syndrome, congenital adrenal hyperplasia, hyperprolactinemia, and/or virilism. Other characteristics were also considered: history of impaired fasting glucose or diabetes, thyroid disorders, liver disease, renal disease, and cardiovascular disease. Any patients that took medications that affect the hormone system, such as metformin or corticosteroids, or taking vitamin D and calcium supplementation, were excluded from the study. All participants had examinations and

evaluation of height and body weight. Also, patients gave blood samples to evaluate PTH, glucose, insulin, vitamin D (25(OH)D) levels before and after treatment.¹⁹

Patients were randomized into two groups, receiving 3 doses of 50 000 units vitamin D3 (D-Vitin 50 000 IU; Zahravi Pharm Co, Tabriz, Iran) or placebo (Zahravi Pharm Co) in 2 months. Covariance analysis showed no significant difference between study groups. The appearance of treatment and placebo, including packaging, were identical to ensure blinding. Follow up consisted of assessment of compliance with interviews, pill monitoring, and telephone calls. Noncompliant patients were excluded from the study. With loss to follow up and noncompliance, 24/30 participants completed the study in the treatment group and 26/30 participants completed in the placebo group.¹⁹

This study demonstrated improvements in vitamin D levels, PTH levels, and insulin secretion in the experimental group, but it was not significant to placebo. Homeostasis Model of Assessment (HOMA)-IR, a measurement for IR, did not change significantly before and after treatment between groups (3.17 +/- 4.08 to 3.21 +/- 2.59, P = 0.42). HOMA-B, an assessment of beta cell function, showed an increase in the treatment group, but it was not significant when compared to placebo (120.5 +/- 120.4 to 152.5 +/-97.49, P = 0.01). Insulin sensitivity measures, HOMA-S, was not significant in the treatment group (0.79 +/- 1.49 to 0.59 +/- 0.91, P = 0.26). A quantitative insulin sensitivity check index (QUICKI) also revealed no significant impact on insulin sensitivity in the treatment group (0.347 +/- 0.059 to 0.337 +/- 0.046, P = 0.14). Serum vitamin D levels increased in the treatment group (6.9 +/- 2.8 to 23.4 +/- 6.1 ng/mL, P < 0.0001) and serum PTH levels decreased (70.02 +/- 43.04 to 50.033 +/- 21.99 microIU/mL, P = 0.02). The result of this analysis concluded there was no correlation

between vitamin D levels and IR or insulin sensitivity. Significance of the results did not change with loss to follow up.¹⁹

Noted limitations by the authors include short follow up duration, not using the glucose clamp method to accurately evaluate insulin sensitivity and secretion, and form and low dosage of vitamin D supplementation. The study concluded that vitamin D may not have a significant impact on insulin resistance and sensitivity in PCOS patients with vitamin D deficiency.¹⁹

Kotsa et al

This observational study²⁰ assessed the effect of vitamin D supplementation on glucose metabolism in obese PCOS patients. Patients were recruited from an outpatient clinic in Greece and included participants who were aged 20 to 40 with history of PCOS and IR. In this study, PCOS is defined as chronic anovulation (fewer than 6 cycles in 12 months) and clinical or/and laboratory finding of androgen excess. Other inclusion criteria included a family history of diabetes mellitus, type II and BMI>30. Patients were to refrain from hormone altering medications for two months prior to enrollment. To enroll into the study, patients also had to complete a one-month diet containing 1 500 mg of calcium daily. Primary outcomes included peripheral insulin resistance and increased insulin effectiveness. Secondary outcomes were BMI and PTH levels.²⁰

Exclusion criteria included any other condition causing hyperandrogenism, menstrual dysfunction, and virilism. All participants had laboratory analysis of glucose, insulin, PTH, calcium, phosphorus, vitamin D (25(OH)D) level, triglycerides, high density lipoprotein (HDL), and low density lipoprotein (LDL) at baseline and after

treatment completion. Transvaginal sonography was not conducted to evaluate presence or absence of polycystic ovaries or follicle size.²⁰

All fifteen patients received 1 microgram of Alphacalcidol (1-alpha-hydroxyvitamin D3) daily for 3 months. No significant changes in serum glucose and insulin levels were observed after therapy. Serum 25(OH)D level improved significantly with treatment (15.15 +/- 1.84 to 28.62 +/- 1.65 ng/mL, $P < 0.0001$). Serum PTH levels decreased significantly after therapy (40.2 +/- 3.92 to 32 +/- 1.99, $P < 0.006$). First phase area under the curve (AUC) increased significantly with treatment (763 +/- 60.89 to 834.67 +/- 71.99 microU/mL, $P < 0.0001$). Also, first phase AUC for glucose decreased substantially (2,298.8 +/- 71.99 to 2,182.73 +/- 73.53 mg/dL, $P < 0.05$). Although there was an observed increase in insulin sensitivity, it was not significant ($P = 0.1$); but there was notable significance in insulin effectiveness ($P < 0.01$). Vitamin D levels were inversely correlated with insulin effectiveness ($P = 0.09$) and insulin secretion ($P < 0.0001$). No change in BMI was observed in the study.²⁰

Notable limitations to this study include small sample size, short follow up duration, and no control group. All study participants were obese, so there is a potential for bias as the study participants are more likely to have glucose and insulin metabolism dysfunction. Baseline information for one participant was not included in the study data. The conclusion of this study was that vitamin D supplementation has beneficial effects in insulin sensitivity and effectiveness, and overall, improves glucose metabolism.²⁰

Selimoglu et al

In this randomized, observational study²¹, the researchers investigated the effects of vitamin D supplementation on IR, glucose metabolism, and hyperandrogenic features associated with PCOS. A total of 11 insulin resistant, obese PCOS patients were enrolled into the study. Patients were recruited from the Endocrinology and Metabolism Division of Medical School of Uludag University Bursa, in Turkey. For inclusion in the study, PCOS was defined as clinical and/or laboratory diagnosis of hyperandrogenism, oligomenorrhea or amenorrhea (cycles longer than 35 days or less than 2 cycles in 6 months, respectively), and polycystic ovaries. Study participants also had to be between ages 18 to 35 and have a BMI greater than 25.²¹

Any patients with other causes of hyperandrogenism or hormone dysfunction or virilism were excluded from the study. Other exclusion criteria included history of diabetes mellitus type II, thyroid disease, major organ dysfunction (i.e. liver, heart, kidney) or intake of hormone altering medications that might affect vitamin D metabolism or glucose metabolism. All participants had laboratory analysis of serum glucose, insulin, 25(OH)D, androgen biomarkers, LH, FSH, estrogen and progesterone levels, and calcium and phosphorus before and after therapy. A Ferriman – Gallwey score (FG) for hirsutism was assessed by two endocrinologists, and patients were considered hirsute if the score was ≥ 6 . Each patient was also evaluated with pelvic ultrasound.²¹

All 11 PCOS patients received one dose of 300 000 units of vitamin D (Devit 3 amp 300,000 IU, Deva Iiac, Turkey) to determine the effects of supplementation on glucose metabolism, hyperandrogenism, and insulin resistance. Three weeks after therapy, analysis showed improvement in serum 25(OH)D levels (16.9 +/- 16.0 to 37.1

+/- 14.6 ng/mLm, $P= 0.027$), decreased in HOMA-IR (4.41 +/- 1.38 to 3.67 +/- 1.48, $P = 0.043$). Although there were decreases in serum insulin and glucose, the results were not significant. There were also small decreases in free testosterone levels, but they were also not significant (3.10 +/- 1.64 to 1.96 +/- 1.49 pg/mL, $P = 0.09$). No changes in other androgen levels or relationship between vitamin D and HOMA were noted.²¹

The limitations of this study included small sample size, short follow up duration, and short duration for treatment effect or lack of treatment. The conclusion of this study is vitamin D supplementation improves insulin resistance in obese PCOS patients with vitamin D deficiency, and recommends administration of vitamin D supplementation to this population of patients to prevent onset of diabetes mellitus type II or IR development and progression. They also inquire about the possibility of vitamin D supplementation to improve hirsutism.²¹

DISCUSSION

The combined vitamin D and calcium supplementation shows potential in improving various symptoms and features associated with PCOS. Positive effects were demonstrated in fertility, menstruation regulation, BMI, IR, and glucose metabolism, and less so in hyperandrogenic characteristics; but quality of evidence is still lacking and results between studies appear inconsistent. This may be due to population selection, small sample size, differences in measuring outcomes, duration of treatment, differences in treatment, and differences in study methodology.

Various studies have shown an increased proportion of PCOS patients with vitamin D deficiency, particularly if obese. There is no current recommendation for

vitamin D supplementation in this patient population and it is not common practice to evaluate patients for vitamin D deficiency, which should also be considered in the management of this condition.

In current practice, PCOS treatment involves weight management, correction of menstrual irregularity, monitoring and preventing of glucose metabolism and insulin resistance, and if necessary, fertility treatment. Weight loss has been associated with improvement of insulin resistance and menstrual regularity. Adipose tissue has the capacity to hold on to additional estrogen, so a decrease in weight and adipose loss results in alteration of hormones that affects the menstrual cycle and improves menstrual regularity. Oral contraceptives, more specifically medications with anti-androgenic properties, are chosen to improve menstrual regulation and hirsutism. Unlabeled use of Metformin, a diabetes medication used to increase insulin sensitivity, improves IR and ovulation. As a result, it affects androgenic hormones and can also improve clinical and biological signs of hyperandrogenism. Fertility agents that induce ovulation are another option for those seeking pregnancy. Due to its physiological function, it is possible that vitamin D supplementation may have a beneficial treatment effect in improving symptoms of PCOS.

Fertility is a critical patient outcome that is commonly addressed in managing PCOS patients. Vitamin D supplementation has potential to alter folliculogenesis and response of the developing follicle,^{6,7,17} as observed in studies within this review. Several studies suggest calcium metabolism dysfunction upsets oocyte development and therefore, fertility⁶. As a result, vitamin D deficiency may alter the availability and function of calcium, suggesting that vitamin D supplementation could correct this

imbalance. Menstrual irregularity improved with vitamin D and calcium supplementation.^{6,7,17} Across studies within this review, there are inconsistent findings regarding insulin resistance, insulin sensitivity, and glucose metabolism.^{7,19-21} Evidence suggests that lower vitamin D levels are inversely correlated with higher BMI,^{4,10} so vitamin D supplementation could potentially assist in weight management in PCOS.

There is limited evidence that vitamin D supplementation improves symptoms of androgen excess.^{6,7,21} Although there is beneficial potential to vitamin D supplementation, current evidence is lacking in reproducibility and quality. A summary of findings demonstrates important flaws and limitations noted amongst each study within this review (Table I).

In general, multiple studies within this review were conducted in the Middle East with participants being from Iran,^{7,17,19} Greece,²⁰ and Turkey.²¹ These populations have very large cultural influences on environment, activity, food, and clothing; which could impact the severity of vitamin D deficiency and PCOS. Other differences between studies include type of vitamin D supplementation and whether participants were on other medications like Metformin or calcium supplements. Each study has been downgraded quality of evidence and therefore, the quality of evidence is graded as very low by GRADE criteria.

Rashidi et al¹⁷ has no details of blinding and allocation and used a surrogate outcome to correlate with pregnancy rates not observed in results. There was a lack of treatment and follow up for the outcome of pregnancy, which was not discussed by the authors. The small loss to follow up may have affected the results of this study as the population sample size was small. In this study menstrual regulation, diet, and pregnancy

had objective measurements, so it is downgraded for recall bias. As with multiple studies assessing fertility,^{6,7,17} a possible confounder is no recording of sexual activity for the outcome of pregnancy. If patients are not sexually active or are limited in sexual activity, then pregnancy rates are likely affected and variation would be noted between participants.

Thys-Jacobs et al⁶ has no control population and flawed measurement of hyperandrogenism due to objectivity, therefore indicating recall bias. Other studies used more specific evaluations and scoring criteria to evaluate for clinical and biochemical androgen excess. Menstrual regularity was also a patient-related outcome that was assessed by patients objectively and short length for this outcome, also illustrating recall bias and lack of treatment for this outcome. Other limitations in this study include small population size and use of NIH criteria rather than Rotterdam criteria; although they share similar characteristics that diagnose PCOS. In this study, patients did not receive Metformin in conjunction with vitamin D and calcium supplementation like other studies included in this review.¹⁹⁻²¹

Firouzabadi et al⁷ is a case-control study without blinding, has large confidence intervals (CI), and incomplete account for outcome. In the baseline evaluation, one patient was not shown to have baseline results. Similar to Thys-Jacobs et al⁶, this study did not include a quality measurement for hyperandrogenic features. The population selected was also varied with no control group to compare for treatment effect. The sample size may have been too small to complete a subgroup analysis for varied ethnicity within the study.

Ardabili et al¹⁹ was a double-blinded RCT, but had loss to follow up without intention-to-treat analysis, underpowered outcomes due to small treatment effect (i.e. loss to follow up and small sample size), and large CI for results. The loss to follow up was accounted for by authors with 5/60 patients unwilling to continue with participation, 2/60 loss to follow up, 2/60 noncompliant, and 1/60 moving from the study parameters.¹⁹ Still, loss to follow up for this study was 17 percent and is likely had a significant impact on results. The dietary intake from patients was a form of recall bias, as the study required participants recall what they ingested for 3 days in order to determine if confounders were present.¹⁹ Although all patients were Iranian women, the authors of this study suggested that all patients located in the same area, 39 degrees latitude, would not have ultraviolet radiation as a potential confounder in the study¹⁹. Again, it is variation in culture and environment within this population that may have affected the results in this study as well as others. Participants did not receive Metformin or calcium supplements.

Kotsa et al²⁰ has no control population, missing data from one participant, and flawed measurement for the primary outcome of IR. Missing data is a question of validity and could potentially underestimated or overestimate the results. Surrogate outcomes such as lipid profile were also used to measure glucose metabolism. The flawed measurement for IR was not using HOMA-IR, rather; insulin secretion and insulin sensitivity is measured and evaluated as AUC to make suggestions regarding IR and insulin function.²⁰ The CI was large and insignificant for glucose metabolism and IR. This study does not describe BMI as a primary outcome, but reported no change in BMI as a result of the study. All participants were from Greece, so there is potential for

population bias and confounders within this study. This study did not allow participants to take Metformin or calcium supplementation.

Selimoglu et al²¹ has no control population, short follow up duration, small sample size, and does not describe hyperandrogenism as a primary outcome. This study measured primary outcomes with HOMA-IR for insulin resistance, but poorly measured insulin secretion for glucose metabolism. Androgen levels were discussed in the results of this study, but it is not indicated as a primary outcome or found to be correlated with vitamin D levels.²¹ Participants in this study only received one dose of vitamin D supplementation, without Metformin or calcium supplements, which other studies have allowed of their study participants. Authors described Turkish women as being more vitamin D deficient,²¹ which is potential for population bias. As Rashidi et al¹⁷ had shown, vitamin D in conjunction with Metformin may prove to augment the benefits of Metformin or calcium activity in PCOS patients, which is yet to be determined. Therefore, results of this study¹⁷ and others without Metformin or calcium may be insignificant or show that vitamin D has an insignificant effect. A longer study with increased vitamin D treatment may show more consistent results with other studies and improve treatment effect for IR and glucose metabolism, as well as observing more significant improvement in androgenic characteristics.

CONCLUSION

It is possible that vitamin D and calcium supplementation yields a positive outcome in a variety of symptoms common in PCOS, including fertility, menstruation regularity, weight, glucose metabolism, insulin resistance, and hyperandrogenism. The

studies evaluated in this review all had small sample sizes and short duration of therapy, so it is difficult to determine the significance of each study outcome.

Further long-term, RCT, prospective studies are necessary to determine if this supplementation is beneficial to PCOS patients. If larger studies were conducted, multiple subgroups analyses may be necessary to remove confounders associated with environment (i.e. sunlight exposure), family history, ethnicity, diet, sexual activity, and use of other medications. Vitamin D supplementation should be considered as a safe and easily accessible therapy that is relatively inexpensive in the treatment of patients with PCOS and vitamin D deficiency, but more studies are necessary to assess its effectiveness on associated PCOS symptoms and whether vitamin D supplementation should be administered concurrently with Metformin and/or calcium supplementation.

Based on the GRADE criteria, the evidence in this review is of very low quality. Because most of the study population patients selected across these studies was vitamin D deficient, it is recommended they receive vitamin D supplementation. A further inquiry is whether serum vitamin D level needs monitoring while managing PCOS patients.

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TABLE I. Characteristics of Reviewed Studies and Summary of Findings, GRADE profile

Quality Assessment							Summary of Findings					Importance	
		Downgrade Criteria					Study	Number of Patients		Effect			Quality
No. of Studies	Design	Limitations	Indirectness	Imprecision	Inconsistency	Publication Bias		Treatment (total)	Placebo or no treatment (total)	Relative Risk	NNT		
Fertility													
3	1 RCT 1 Observational 1 Case Control	Very Serious limitations ^a	Serious indirectness ^b	No serious imprecision	Some inconsistencies ^c	No bias likely	Rashidi et al	5/19	14/19	1.66	NNT = 9.5	Very Low	Critical
							Thys-Jacobs et al	2/13	n/a	n/a	n/a		
							Firouzabadi et al	9/50	41/50	1.50	NNT = 16.7		
Menstruation irregularity													
3	1 RCT 1 Observational 1 Case Control	Very Serious limitations ^a	Some indirectness ^b	No serious imprecision	Some inconsistencies ^c	No bias likely	Rashidi et al	10/20	10/20	1.42	NNT = 6.7	Very Low	Important
							Thys-Jacobs et al	7/13	n/a	n/a	n/a		
							Firouzabadi et al	35/50	15/50	1.21	NNT = 8.3		
Glucose metabolism													
3	1 RCT 2 Observational	Very Serious limitations ^d	Serious indirectness ^e	Serious imprecision ^f	No serious inconsistencies	No bias likely	Ardabili et al	n/a	n/a	n/a	n/a	Very Low	Critical
							Kotsa et al	n/a	n/a	n/a	n/a		
							Selimoglu et al	n/a	n/a	n/a	n/a		
Insulin resistance													
3	1 RCT 2 Observational	No serious limitations ^d	Serious indirectness ^e	Serious imprecision ^f	No serious inconsistencies	No bias likely	Ardabili et al	n/a	n/a	n/a	n/a	Very Low	Critical
							Kotsa et al	n/a	n/a	n/a	n/a		
							Selimoglu et al	n/a	n/a	n/a	n/a		
Weight/BMI													
2	1 Observational 1 Case Control	Very Serious limitations ^g	Serious indirectness ^h	Serious imprecision ⁱ	No serious inconsistencies	No bias likely	Kotsa et al	n/a	n/a	n/a	n/a	Very Low	Critical
							Firouzabadi et al	n/a	n/a	n/a	n/a		
Hyperandrogenism (i.e. Androgen Excess)													
3	2 Observational 1 Case Control	Very Serious limitations ^j	Serious indirectness ^k	No serious imprecision	No serious inconsistencies	No bias likely	Selimoglu et al	n/a	n/a	n/a	n/a	Very Low	Important
							Thys-Jacobs et al	3/13	n/a	n/a	n/a		
							Firouzabadi et al	n/a	n/a	n/a	n/a		

^a Rashidi et al has no details of blinding and allocation, Thys-Jacobs et al has no control population, Firouzabadi et al is a case-control study without blinding.

^b Rashidi et al used surrogate outcome to correlate with possible pregnancy rates not seen in the study, therefore, follicular size/growth shown in Summary of Findings. Note: Pregnancy rate was zero among all three groups.

^c Rashidi et al lack of treatment and follow up for outcome.

^d Ardabili et al has loss of follow up without intention-to-treat analysis, Kotsa et al has no control population, missing data from participant and flawed measurement for outcome, Selimoglu et al has no control population and short follow up duration.

^e Ardabili et al has underpowered outcome due to small treatment effect (i.e. loss of follow up and small sample size), Kotsa et al used surrogate outcome for glucose metabolism.

^f Ardabili et al has large CI, Kotsa et al shows CI as insignificant for effect, Selimoglu et al has large CI.

^g Kotsa et al has no control population, Firouzabadi et al is a case-control without blinding.

^h Kotsa et al does not describe BMI as primary outcome.

ⁱ Firouzabadi et al has large CI.

^j Selimoglu et al has no control population and short follow up duration, Thys-Jacobs et al has no control population and flawed measurement for hyperandrogenism (objective), Firouzabadi et al is a case-control study without blinding and incomplete account for outcome.

^k Selimoglu et al does not describe hyperandrogenism as a primary outcome.