CLINICAL STUDY

The role of vitamin D in metabolic disturbances in polycystic ovary syndrome: a systematic review

Y H M Krul-Poel¹, C Snackey¹, Y Louwers², P Lips³, C B Lambalk⁴, J S E Laven² and S Simsek^{1,3}

¹Department of Internal Medicine, Medical Centre Alkmaar, PO Box 501, 1800 AM Alkmaar, The Netherlands, ²Division of Reproductive Medicine, Department of Obstetrics and Gynaecology, Erasmus Medical Centre, PO Box 2040, 3000 CA Rotterdam, The Netherlands, Departments of ³Internal Medicine/Endocrinology and ⁴Obstetrics, Gynaecology and Reproductive Medicine, VU University Medical Centre, PO Box 7057, 1007 MB Amsterdam, The Netherlands

(Correspondence should be addressed to S Simsek at Department of Internal Medicine, Medical Centre Alkmaar; Email: s.simsek@mca.nl)

Abstract

Context: Metabolic disturbances, in particular, insulin resistance (IR) and dyslipidemia, are common in women suffering from polycystic ovary syndrome (PCOS). Evidence is accumulating that vitamin D status may contribute to the development of metabolic disturbances in PCOS.

Objective: The aim of this study was to carry out a systematic review addressing the association between vitamin D status, vitamin D receptor polymorphisms, and/or polymorphisms related to vitamin D metabolism and metabolic disturbances in women with PCOS.

Design and methods: A systematic search of electronic databases was carried out up to January 2013 for observational studies and clinical trials in women suffering from PCOS with outcome measures that were related to vitamin D status. We conducted univariate and multivariate regression analyses of the weighted means to gain insights into the association between vitamin D, BMI, and IR based on existing literature.

Results: We found 29 eligible trials with inconsistency in their results. One well-designed randomized controlled trial has been carried out until now. Univariate regression analyses of the weighted means revealed vitamin D to be a significant and independent predictor of IR in both PCOS and control women. The significance disappeared after adjustment for BMI in PCOS women.

Conclusions: Current evidence suggests an inverse association between vitamin D status and metabolic disturbances in PCOS. Owing to the heterogeneity of the studies, it is hard to draw a definite conclusion. The causal relationship between vitamin D status and metabolic disturbances in PCOS remains to be determined in well-designed placebo-controlled randomized clinical trials.

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Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women of reproductive age, with a prevalence of 6–10% in the general population. PCOS is characterized by the following: i) ovulatory dysfunction resulting in oligo- and/or anovulation, ii) hyperandrogenism and/or hirsutism, and iii) the presence of polycystic ovarian morphology (1). PCOS is by far the most common cause of anovulatory infertility and has been reported to be associated with insulin resistance (IR), hyperinsulinemia, dyslipidemia, and central obesity (2, 3, 4), which are all risk factors for the metabolic syndrome, type 2 diabetes mellitus, and cardiovascular disease. Metabolic disturbances are common in women suffering from PCOS: 30–40% have impaired glucose tolerance and IR with compensatory hyperinsulinemia, and as many as 10% will have type 2 diabetes mellitus by their fourth decade (5, 6, 7). A recent meta-analysis has revealed that dyslipidemia

occurs more frequently in PCOS women than in control women: women with PCOS had higher LDL-cholesterol and non-HDL-cholesterol levels, regardless of BMI (4).

Current evidence suggests that IR has a central role in the pathogenesis of PCOS, contributing to both metabolic and reproductive disturbances (3).

Many studies have been conducted to clarify the mechanism of metabolic disturbances, in particular IR, in women affected by PCOS. In part, IR might be due to obesity. However, a substantial number of lean women affected by PCOS have IR as well, independent of obesity (8, 9). Recently, vitamin D deficiency has been proposed as the possible missing link between IR and PCOS. This assumption is supported by the finding that the active vitamin D–vitamin D receptor (VDR) complex regulates over 300 genes, including genes that are important for glucose and lipid metabolism as well as blood pressure regulation (10). Moreover, there is an association between poor vitamin D status and IR in patients with type 2 diabetes mellitus (11, 12, 13, 14, 15). Still, it

remains unclear whether vitamin D and IR are causally interrelated or whether they constitute two independent characteristics in women with PCOS. A recently published review carried out by Thomson *et al.* (16) about the role of vitamin D in the etiology and management of PCOS suggests that there is an association between vitamin D status and hormonal and metabolic dysfunctions in PCOS. However, literature about the association between vitamin D status and metabolic and hormonal disorders in women suffering from PCOS is scarce, and it has provided the scientific community with conflicting results. Therefore, we carried out this systematic review to examine the following: i) the association between vitamin D status and metabolic disturbances and/or endocrine parameters in women with PCOS; ii) the effect of vitamin D supplementation on metabolic disturbances and/or endocrine parameters in PCOS; and iii) the influence of VDR polymorphisms in women with PCOS.

Subjects and methods

Search strategy

Two authors independently carried out a formal computer-assisted search for observational studies and clinical trials that investigated the association between vitamin D status or VDR polymorphisms and endocrine or metabolic parameters in PCOS women. This search was confined to the MEDLINE database, Embase, and The Cochrane Library and included English-language literature up to January 2013. The following search terms were used: polycystic ovary syndrome, polycystic ovaries, vitamin D, cholecalciferol, 25-hydroxyvitamin D (25OHD), vitamin D deficiency, vitamin D receptor, vitamin D receptor polymorphisms, and related terms. The detailed search strategy is given in [Supplementary information](#), see section on [supplementary data](#) given at the end of this article.

Outcome of interest and criteria for inclusion

The primary objective of this systematic review was to examine the role of vitamin D in metabolic disturbances in women affected by PCOS. Studies that met the following criteria were included in this systematic review: i) inclusion of women with PCOS, with or without a control group and ii) inclusion of vitamin D status or VDR polymorphisms or polymorphisms related to vitamin D metabolism and their correlation with metabolic and/or endocrine parameters as the outcome of interest. We excluded letters, abstracts, and conference proceedings that were not published in fully peer-reviewed journals. For study inclusion, PCOS was defined by the presence of a combination of oligo- or anovulation, PCO morphology, and hyperandrogenism, according to the National Institutes of Health (NIH) criteria or the Rotterdam criteria (17).

Data extraction

The following data were extracted (in case they were available): author name, study design, publication year, study location, sample size, criteria used for the diagnosis of PCOS, inclusion and exclusion criteria for cases and controls, characteristics of the study population, anthropometric parameters, metabolic parameters, endocrine parameters, serum 25-hydroxyvitamin D (25OHD), and method used for the measurement of serum 25OHD levels and IR (defined as homeostasis model assessment-IR (HOMA-IR)). To avoid multiple-publication bias, we excluded publications with overlapping patient populations in the regression models.

Statistical analysis

Statistical analyses were carried out using the SPSS Software (version 20.0, SPSS, Inc.). Mean values of serum 25OHD, HOMA-IR, and BMI were employed if available in the reviewed articles. These data were weighted by the number of participants included in the study. In case the mean values were not reported, the median was used if available. Linear regression models of the weighted means were used to assess the independent relationships between serum vitamin D, BMI, and IR in women affected by PCOS and controls. First, univariate analyses and subsequently multivariate analyses with HOMA-IR as an independent variable and serum 25OHD and BMI as dependent variables were carried out. A *P* value <0.05 was considered statistically significant.

Results

Identified studies

The initial literature search yielded 114 hits, 25 of which were duplicates, resulting in 89 non-duplicated publications. Of these, 52 studies were excluded based on the title or abstract and eight studies were excluded based on the lack of inclusion criteria ([Fig. 1](#)). In total, 29 studies were included in this systematic review.

The association between vitamin D status and metabolic disturbances in PCOS in observational studies

Twelve studies had an observational design (18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29), employing a cross-sectional or case-control design, with vitamin D status as one of the preliminary outcomes. A summary of these studies is given in [Table 1](#). The studies included premenopausal women (14–50 years) of various ethnicities, with the number of participants ranging from 45 to 400. Different stratifications were used: seven studies

Figure 1 Flow chart for systematic review.

compared PCOS women with controls (19, 20, 22, 23, 24, 27, 28), three studies compared lean vs obese PCOS women (18, 21, 26), one study compared PCOS women with and without the metabolic syndrome (25), and one study solely included PCOS women (29). Large differences were found in the prevalence of vitamin D deficiency (defined as serum 25OHD levels < 50 nmol/l), varying from 37% in a study carried out in Italy (21) to 72% in a study carried out in Scotland (19).

The studies that compared serum 25OHD levels between PCOS and control women yielded conflicting results. In detail, three studies demonstrated a significantly lower serum 25OHD level in PCOS women: 32.4 vs 73.7 nmol/l in 90 PCOS women and 47 control women (24), 30.0 vs 43.7 nmol/l among 103 PCOS women and their controls (28), and 17.7 vs 79.2 nmol/l in 30 PCOS women and 15 control women (27). By contrast, another study among 291 PCOS women and 109 control women demonstrated a lower serum 25OHD level in control women than in PCOS women (53.5 vs 73.7 nmol/l) (23).

Eleven of all the included observational studies investigated the correlation between vitamin D status and IR (18, 19, 21, 22, 23, 24, 25, 26, 27, 28, 29). Most of these studies used HOMA-IR as an indicator of IR. Five studies reported insulin sensitivity using the quantitative insulin-sensitivity check index (18, 19, 22, 25, 27). In one study carried out by Muscogiuri *et al.* in 23 obese and 15 lean PCOS women, IR was evaluated using the hyperinsulinemic-euglycemic clamp (HEC) method, the gold standard for the determination of IR. The authors found a positive correlation between serum 25OHD levels and glucose uptake during HEC ($r=0.33$; $P=0.03$). Serum 25OHD levels were inversely correlated with BMI ($r=-0.49$; $P=0.04$), waist circumference ($r=-0.41$; $P=0.008$), and total

fat mass ($r=-0.47$; $P=0.02$). In a multivariate analysis, the authors reported that only total fat mass was an independent predictor of serum 25OHD (21).

Nine studies found an inverse correlation between serum 25OHD levels and IR and/or insulin sensitivity in PCOS women (18, 19, 22, 23, 24, 25, 26, 27, 29). However, in one of these studies, this correlation was BMI dependent (23), and four studies did not adjust for BMI or obesity (18, 22, 24, 26). Two studies carried out a multivariate analysis including serum 25OHD as an explanatory variable regarding IR (22, 25). Both the studies demonstrated serum 25OHD to be an independent predictor of IR ($P=0.007$, $P=0.036$). In the latter study, 206 PCOS women were stratified by hypovitaminosis D (< 75 nmol/l, $n=150$) and vitamin D sufficiency (≥ 75 nmol/l, $n=56$). The authors reported that the hypovitaminosis D group had a significantly higher HOMA-IR (1.96 vs 1.11, $P=0.002$) than the vitamin D-sufficient group. However, after a subsequent analysis in lean and obese PCOS women stratified by hypovitaminosis D and sufficient vitamin D, no significant difference was found in HOMA-IR (25).

The studies that compared serum 25OHD levels between obese and lean women suffering from PCOS observed a significantly lower serum 25OHD level in obese PCOS women (18, 21, 23, 24, 26).

The effect of vitamin D supplementation in women with PCOS

Ten intervention trials had been carried out and published until our search in January 2013 (30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40). Among these, four had a randomized controlled trial (RCT) design (30, 35, 36, 40), five an open-labeled single-arm design (32, 33, 37, 38, 39), and one a case-control study design (31). One RCT carried out by Ardabili *et al.* had a randomized placebo-controlled double-blind study design in which the effect of vitamin D3 supplementation on metabolic parameters and cardiovascular risk factors was evaluated in 50 PCOS women. This trial resulted in two articles included in this review (30, 34). In their RCT, Sathyapalan *et al.* (36) examined the effect of atorvastatin vs placebo on serum vitamin D and subsequently studied the effect of an increase in serum vitamin D levels on metabolic and endocrine parameters in PCOS women. The third RCT carried out by Rashidi *et al.* (35) was a pilot study in which 60 infertile PCOS women were randomized into groups treated with: i) calcium and vitamin D3; ii) calcium, vitamin D3, and metformin; or iii) metformin. They assessed effects on folliculogenesis and menstrual cycle. Bonakdaran *et al.* (40) carried out a RCT among 51 women in which active vitamin D (calcitriol) was administered and compared with metformin treatment and placebo.

The number of participants included in the intervention trials ranged from 11 to 100, and all were premenopausal PCOS women. Different treatment protocols

Table 1 Summary of the observational studies included in the review.

References	Location	25OHD assay	Groups	No. of study participants	Mean BMI (kg/m ²)	Mean HOMA-IR	Mean serum 25OHD (nmol/l)	Prevalence ^a of 25OHD deficiency ^b (%)	Main results regarding vitamin D and metabolic disturbances	Adjustments
(18)	Germany	Immunoassay (DiaSorin)	PCOS Obese Lean	120 98 32	37.1 22.2	5.7 1.7	37.4 53.2	67.5	25OHD was inversely correlated with BMI, body fat, HOMA-IR, and insulin levels. There was a positive association between 25OHD and QUICKI	Season
(27)	Egypt	Immunoassay (IDS)	PCOS Obese Control	30	29.3 25.8	7.9 2.1	17.7 79.2		25OHD was inversely correlated with BMI, HOMA-IR, QUICKI, and fasting insulin levels	BMI
(19) ^c	Scotland	LC-MS	PCOS Control	25 15	30.8 23.5	3.0 1.4	27 43	72	25OHD was inversely correlated with BMI and CRP levels and positively correlated with QUICKI and HDL-C levels in PCOS women	BMI and waist:hip ratio
(20)	Iran	Immunoassay	PCOS Control	85 27	27.6 26.3	4.0 2.9	73.2 48.4		PCOS had significantly positive effects on serum 25OHD and insulin levels and HOMA-IR	Age and BMI
(28)	Iran	ELISA (DRG)	PCOS Control	103 115	27.7 26.2	2.3 1.5	30 43.7		PCOS women had significantly lower 25OHD and adiponectin levels. 25OHD was inversely correlated with BMI. 25OHD was positively correlated with adiponectin levels. There was no association between 25OHD and HOMA-IR	BMI and waist circumference
(21)	Italy	Immunoassay (DiaSorin)	PCOS Obese Lean Control	38 23 15 103	25.1 (all)	NR	51.9 75.4	37	25OHD was inversely correlated with BMI, waist:hip ratio, and total fat mass. There was a positive association between 25OHD and glucose uptake during HEC	BMI and season
(22)	Australia	Immunoassay (IDS)	PCOS Control	27 20	26.9 26.3	2.1 1.4	79.3 60.5		25OHD was positively correlated with QUICKI in the PCOS group	
(23)	Greece	Immunoassay (BioSource Europe)	PCOS Control	291	25.5 26.2	2.8 2.6	73.7 53.5		25OHD was inversely correlated with BMI, insulin levels, and HOMA-IR; however, all were BMI dependent	Age and BMI
(29)	India	ELISA (immundiag-nostik)	PCOS Control	60 109	27.1	5.5	52.3		25OHD was inversely correlated with HOMA-IR and fasting plasma glucose levels	BMI and waist:hip ratio
(24)	Italy	Immunoassay (DiaSorin)	PCOS Control	90	28.0 28.5	3.1 1.8	32.4 73.7		25OHD was inversely correlated with BMI, HOMA-IR, insulin levels, PED/PEA15, and L:A ratio in women affected by PCOS	

Table 1 Continued

References	Location	25OHD assay	Groups	No. of study participants	Mean BMI (kg/m ²)	Mean HOMA-IR	Mean serum 25OHD (nmol/l)	Prevalence ^a of 25OHD deficiency ^b (%)	Main results regarding vitamin D and metabolic disturbances	Adjustments
(25)	Austria	Immunoassay (IDS)	PCOS MS ^d Non MS Control ^e	206	26.2 (all)	1.7 (all)	43.2 64.4	38.8	25OHD was inversely correlated with HOMA-IR, HOMA- β , QUICKI, and BMI, and there was a positive correlation with HDL levels	Age, BMI, and season
(26)	Turkey	HPLC	PCOS Obese Lean	100 57 43	32.8 22.1	4.6 2.2	31.9 73.1	67	25OHD was inversely correlated with BMI, WHR, HOMA-IR, and TG levels	

25OHD, 25-hydroxy vitamin D; FAI, free androgen index; HEC, hyperinsulinemic–euglycemic clamp; HOMA-IR, homeostasis model assessment–insulin resistance; L:A ratio, leptin:adiponectin ratio; LC–MS, liquid chromatography–tandem mass spectrometry; I, lean; MS, metabolic syndrome; NIH, National Institute of Health; NR, not reported; o, obese, defined as a BMI ≥ 30 kg/m²; o/o, overweight/obese, defined as a BMI ≥ 25 kg/m²; PCOS, polycystic ovary syndrome; PED/PEA15, phosphoprotein enriched in diabetes gene product; PTH, parathyroid hormone; QUICKI, quantitative insulin-sensitivity check index; SHBG, sex hormone-binding globulin.

^aWhere reported.

^bVitamin D deficiency defined by serum 25OHD levels < 50 nmol/l.

^cMedian values.

^dMS defined by the National Cholesterol Education Program and the Adult Treatment Panel III.

^eBMI-matched control group.

of vitamin D supplementation were used in the trials varying from 400 IU a day in the trial carried out by Rashidi *et al.* (35) to a single oral dose of 300 000 IU cholecalciferol in that carried out by Selimoglu *et al.* (37). The follow-up duration ranged from 3 weeks to 6 months. A summary of the studies is given in Table 2. In detail, the first study carried out in 1999 by Thys-Jacobs *et al.* (38) among 13 PCOS women, who were treated with 50 000 IU ergocalciferol weekly or biweekly to achieve a target serum 25OHD level of 75–100 nmol/l, demonstrated an improvement in menstrual regularity and acne, and two women became pregnant in a follow-up period of 6 months. In addition, a more recent study carried out in Iran including 60 infertile PCOS women has found an improvement in menstrual regularity after 3 months of supplementation with 1000 mg/day calcium and 400 IU/day vitamin D (35).

Seven intervention trials examined the effect of vitamin D supplementation on IR and other metabolic characteristics (30, 32, 33, 36, 37, 39, 40). The only randomized placebo-controlled trial that administered vitamin D3 and examined metabolic disturbances as primary prespecified outcomes did not indicate a significant effect on IR after the administration of three oral doses of vitamin D3 in 2 months. The increase in serum 25OHD levels was 40 nmol/l in the vitamin D group (30). The authors did find a significant increase in insulin secretion in the vitamin D group, but compared with the placebo group, this was not significant. Total cholesterol, triglyceride, and very LDL (VLDL) levels were decreased in the intervention group after vitamin D supplementation, but no effect on BMI and LDL and HDL levels was observed. The RCT carried out by Bonakdaran *et al.* (40), in which calcitriol was administered, also failed to find an effect on IR. In the calcitriol group, serum 25OHD levels increased from 29 to 51 nmol/l. HOMA-IR decreased from 4.2 to 2.7; however, this was not significant.

The other intervention trials yielded conflicting results: Selimoglu *et al.* (37) observed a significant decrease in HOMA-IR after the administration of a single oral dose of 300 000 IU cholecalciferol, whereas the others failed to find a positive effect on IR after the use of different regimens of vitamin D replacement (32, 33, 36, 39).

The influence of VDR polymorphisms and polymorphisms related to vitamin D metabolism in women with PCOS

Literature assessing VDR polymorphisms and/or polymorphisms related to vitamin D metabolism in women suffering from PCOS in relation to vitamin D status and metabolic disturbances is scarce. Six genetic linkage studies examined VDR and related polymorphisms in relation to PCOS (41, 42, 43, 44, 45, 46). The number of participants ranged from 56 to 690. None of the

Table 2 Summary of the intervention studies included in this review.

References ^a	Location	25OHD assay	Number of study participants	Duration (months)	Main outcome	Study design/groups	Intervention	Mean 25OHD (nmol/l)		Main results
								Before treatment	After treatment	
(30) ^b	Iran	Immunoassay (DiaSorin)	50 ^c	2	Metabolic parameters	RCT	Vit D3: 50 000 IU/20 days Placebo	17.2	58.4	↑ HOMA-B
(40)	Iran	Immunoassay	51	3	Endocrine and metabolic parameters	RCT	Metformin: 1000 mg/day Calcitriol: 0.5 µg/day Placebo	19.5	NR	= HOMA-IR, insulin, glucose, QUICKI, and BMI
(31)	Iran	Immunoassay	100 ^d	6	Reproductive and anthropometric parameters	G1 G2 G3 Case-control	Metformin: 1000 mg/day Calcitriol: 0.5 µg/day Placebo	70.4 28.5 49.7	66.6 50.9 47.4	Gr 2: ↓ PTH = BMI, BP, HOMA-IR, insulin, and glucose
(32)	Greece	Immunoassay (RIA)	15 ^e	3	Glucose metabolism measured by IVGTT	Single-arm	Metformin: 1500 mg/day Ca: 1000 mg/day. Vit D3: 50 000 IU/week	33.8	71.4	↑ Menstrual regularity, follicle maturation, and pregnancy (6 vs 9) ↓ BMI and hyperandrogenism
(33)	USA	Immunoassay (RIA)	12 ^c	3	Endocrine and metabolic parameters	Single-arm	Vit D2: 50 000 IU/month, Vit D3: 2000 IU/day, and Ca 530 g/day	43.9	71.8	NS difference between both groups ↑ Insulin secretion and HDL-cholesterol ↑ TG = Serum glucose and insulin ↓ BP, total testosterone, and androstenedione = Insulin resistance, insulin, and glucose
(34)	Iran	Immunoassay (DiaSorin)	50 ^c	2	Cardiovascular risk factors	RCT	Vit D3: 50 000 IU/20 days Placebo	17.5	57.2	↓ TC, TG and VLDL, and PTH
(35)	Iran	NR	60 ^d	Treatment: 3	Folliculogenesis and menstrual regularity	G1 G2 RCT (pilot)		19.5	NR	= HDL, LDL, hs-CRP, APO-A1, and BMI ↑ Menstrual regularity and folliculogenesis in the metformin, calcium, and vitamin D groups

Table 2 Continued

References ^a	Location	25OHD assay	Number of study participants	Duration (months)	Main outcome	Study design/groups	Intervention	Mean 25OHD (nmol/l)		Main results
								Before treatment	After treatment	
(36)	UK	LC-MS	37	6 Follow-up: 6	Metabolic and endocrine parameters	G1 G2 G3 RCT	Ca: 1000 mg/day, Vit D3: 400 IU/day Ca: 1000 mg/day, Vit D3: 400 IU/day, metformin: 1500 mg/day Metformin: 1500 mg/day			
(37)	Turkey	Immunoassay	11 ^e	3 weeks	Endocrine and glucose parameters	G1 G2 Single-arm	Atorvastatin: 20 mg/day Placebo Vit D3: 300 000 IU/day as a single, oral dose	45.9 44.8 42.2	61.9 42.1 92.6	↑ Serum 25OHD = HOMA-IR and serum lipids ↓ HOMA-IR
(38)	USA	Radioligand-binding assay	13	6	Reproductive and menstrual dysfunction	Single-arm	Ca: 1500 mg/day; ergocalciferol: 50 000 IU weekly or biweekly	28.0	NR (target 75–100)	= Endocrine parameters, insulin, and glucose ↑ Menstrual regularity and acne, two women became pregnant
(39)	Austria	Immunoassay (IDS)	52	24 weeks	Metabolic and endocrine parameters	Single-arm	Vit D3: 20 000 IU/week	69.9	130.8	↑ TC, LDL, and menstrual frequency ↓ Fasting and stimulated glucose, C-peptide, TG, PTH, estradiol, and hip circumference = HOMA-IR

G1, Group 1; G2, Group 2; G3, Group 3; 25OHD, 25-hydroxy vitamin D; APO-A1, apolipoprotein A1; BP, blood pressure; HOMA-B, homeostasis model assessment-β-cell function; HOMA-IR, HOMA-insulin resistance; hs-CRP, high-sensitive C-reactive protein; IVGTT, intravenous glucose tolerance test; LC-MS, liquid chromatography-tandem mass spectrometry; NR, not reported; NS, no significant; OGTT, oral glucose tolerance test; PCOS, polycystic ovary syndrome; PTH, parathyroid hormone; TC, total cholesterol; TG, triglycerides; QUICKI, quantitative insulin-sensitivity check index; ↑, increase; ↓, decrease; =, unchanged.

^aYear of publication.

^bOverlap in study population, only one study was used in the analysis.

^cVitamin D-deficient (<50 nmol/l) PCOS women.

^dInfertile PCOS women.

^eInsulin-resistant PCOS women.

studies included a replication cohort. Besides single-nucleotide polymorphisms (SNPs) related to *VDR* genotype, SNPs in the insulin receptor (*INSR*), calcium-sensing receptor (*CASR*), parathyroid hormone (*PTH*) receptor, adiponectin (*ADIPOQ*) receptor, genes involved in cholesterol synthesis (*DHCR7*) and hydroxylation (*CYP2R1*), and genes involved in vitamin D transport (*GC*) were examined. A summary of the studies is given in Table 3.

Five studies determined the association between *VDR* polymorphisms and metabolic disturbances in women with PCOS (41, 42, 43, 45, 46).

In a cohort of 162 PCOS women and 162 control women, variants of *VDR* were found to be associated with an increase in insulin levels and IR: the *VDR* Apa-I genotype was found to be associated with the prevalence of PCOS (42). A study carried out by Wehr *et al.* (45) among 545 PCOS women and 145 control women found that the *VDR* Cdx2 'AA' polymorphism was associated with lower fasting insulin levels ($P=0.039$) and HOMA-IR ($P=0.041$) compared with the other polymorphisms. These authors reported that the variants of both the *GC* gene, which encodes vitamin D-binding globulin, and *DHCR7* gene, which

encodes the enzyme 7-dehydrocholesterol reductase interacting with the synthetic pathway of vitamin D3, were associated with a significantly higher risk of vitamin D deficiency (OR 2.53 (1.27–5.06), $P=0.009$ and OR 2.66 (1.08–6.55), $P=0.033$). Ranjzad *et al.* (43) found that the *CASR* 'TG' polymorphism was associated with IR.

Another study carried out in 181 Iranian PCOS women and 181 control women examined whether different polymorphisms in *VDR*, *ADIPOQ*, *PTH*, and *INSR* genes were associated with PCOS. The authors found no difference in *VDR* gene polymorphisms between PCOS and control women (44). This result is in line with the results of the studies carried out by Lin *et al.* (41) and Zadeh-Vakili *et al.* (46).

The correlation between vitamin D status, BMI, and IR in women with PCOS

Vitamin D status and IR In order to provide an overview of the relationship between vitamin D status and IR in women affected by PCOS compared with healthy control women, we carried out a regression analysis including serum 25OHD as an independent

Table 3 Summary of the genetic linkage studies.

References ^a	Location	No. of study participants	SNPs	Main results
(42)	Iran	PCOS: 162 Control: 162	<i>VDR</i> (Bsm-I, Fok-I, Apa-I, and Taq-I) polymorphisms	<i>VDR</i> Apa-I was associated with PCOS <i>VDR</i> Fok-I 'FF' independently increased the risk for insulin levels and insulin resistance in PCOS
(45)	Austria	PCOS: 545	<i>VDR</i> (Cdx2, Bsm-I, Fok-I, Apa-I, and Taq-I), <i>GC</i> ^a , <i>DHCR7</i> ^b , and <i>CYP2R1</i> ^c polymorphisms	In PCOS women, <i>VDR</i> Cdx2 'AA' was associated with lower fasting insulin levels and HOMA-IR and higher QUICKI. <i>GC</i> 'GG' and <i>DHCR7</i> 'GG' were associated with a higher risk of vitamin D deficiency
(43)	Iran	PCOS: 181 Control: 145	<i>INSR</i> , <i>ADIPOQ</i> , <i>PTH</i> , and <i>VDR</i> polymorphisms (SNPs ^d)	No significant difference was observed in genotype and allele frequencies between women with PCOS and control women
(44)	Iran	PCOS: 56 Control: 181	<i>VDR</i> (Bsm-I, Fok-I, Apa-I, Taq-I, and Tru9-I), <i>PTH</i> , <i>CASR</i> , <i>INSR</i> , and <i>ADIPOQ</i> polymorphisms ^e	<i>CASR</i> 'TG' was associated with higher HOMA-IR <i>VDR</i> Taq-I was associated with elevated serum LH levels <i>VDR</i> Bsm-I and <i>ADIPOQ</i> (rs1501299) were associated with decreased SHBG levels
(46)	Iran	PCOS: 260 Control: 221	<i>VDR</i> (Tru9-I)	No significant difference was observed in genotype and allele frequencies between women with PCOS and control women

ADIPOQ, adiponectin; CaSR, calcium-sensing receptor; HOMA-IR, homeostasis model assessment–insulin resistance; INSR, insulin receptor; NR, not reported; PCOS, polycystic ovary syndrome; PTH, parathyroid hormone; QUICKI, quantitative insulin-sensitivity check index; SHBG, sex hormone-binding globulin; SNPs, single-nucleotide polymorphisms; VDR, vitamin D receptor.

^aEncodes vitamin D-binding globulin.

^bEncodes the enzyme 7-dehydrocholesterol reductase, interacting with the synthetic pathway of vitamin D3.

^cEncodes a hepatic microsomal enzyme, hypothetically involved in the 25-hydroxylation of vitamin D in the liver.

^d*INSR* gene (rs2059806 and rs1799817), *ADIPOQ* gene (rs2241766 and rs1501299), *PTH* gene (rs6256), and *VDR* gene (rs757343).

^e*PTH* (rs6256), *CASR* (rs1801725), *INSR* (rs2059806 and rs1799817), and *ADIPOQ* (rs2241766 and rs1501299).

Figure 2 Association between vitamin D status and insulin resistance in PCOS women (18, 19, 20, 22, 23, 24, 26, 27, 28, 29, 30, 33, 37, 39, 40, 41, 43, 45) and control women (19, 20, 22, 23, 24, 27, 28, 41, 45). The circles and unbroken line represent PCOS women and the triangles and broken line represent control women. In the univariate regression analysis, the slope of the line was -0.03 for PCOS women and -0.02 for control women. The size of the symbols indicates the number of participants included in the corresponding study.

variable and HOMA-IR as a dependent variable. Of the 29 reviewed studies, 18 examined this correlation and reported the mean and/or median values and were therefore included in this analysis (18, 19, 20, 22, 23, 24, 26, 27, 28, 29, 30, 33, 37, 39, 40, 41, 43, 45). Nine studies reported these values also for control women (19, 20, 22, 23, 24, 27, 28, 41, 45). The data were weighted by the number of participants in each study, resulting in a total of 1893 PCOS women and 717 control women. The overall mean of serum 25OHD was 61.2 nmol/l in PCOS women and 67.1 nmol/l in control women. The overall mean of HOMA-IR was 2.71 in PCOS women and 1.8 in control women. Univariate regression analysis revealed that an increase in serum 25OHD levels was significantly associated with a decrease in HOMA-IR in both PCOS women ($B_1 = -0.027$; CI -0.030 to -0.024 ; $P \leq 0.001$) and control women ($B_1 = -0.019$; CI -0.021 to -0.017 ; $P \leq 0.001$) (Fig. 2). The results indicate that every 10 nmol/l increase in serum 25OHD levels decreases HOMA-IR by 0.27 in PCOS women and 0.19 in control women. After multivariate regression analysis with serum 25OHD and BMI as independent variables, serum vitamin D was found to no longer be an independent predictor of IR in PCOS women. The result remained significant for control women.

Vitamin D status and BMI Data on the association between anthropometric parameters and serum 25OHD were provided in 23 of the 29 reviewed studies (18, 19, 20, 21, 22, 23, 24, 26, 27, 28, 29, 30, 31, 32, 33, 34, 36, 37, 38, 39, 41, 43, 45), and nine studies reported

these data also for control women (19, 20, 22, 23, 24, 27, 28, 41, 45). After regression analyses of the weighted means including a total of 2045 PCOS women and 717 control women, BMI appeared to be an independent predictor of serum 25OHD in both PCOS and control women ($B_1 = -4.5$, $P < 0.001$ and $B_1 = -8.4$, $P < 0.001$; Fig. 3). The overall mean of BMI in these studies was 26.6 kg/m² in PCOS women and 25.1 kg/m² in control women.

BMI and IR Nineteen of the included studies investigated the correlation between BMI and HOMA-IR in PCOS women (18, 19, 20, 22, 23, 24, 25, 26, 27, 28, 29, 30, 33, 37, 39, 41, 43, 45, 46) and nine in control women (19, 20, 22, 23, 24, 27, 28, 41, 45). All the studies revealed a significant association between BMI and HOMA-IR. A total of 2311 PCOS women and 717 control women participated in these studies. Multivariate regression analyses with serum 25OHD and BMI as independent variables and HOMA-IR as a dependent variable revealed that BMI was an independent predictor of HOMA-IR in PCOS and control women ($B_1 = 0.41$, $P < 0.001$ and $B_1 = 0.10$, $P < 0.001$ respectively; Fig. 4).

Discussion

This systematic review was carried out to examine the role of vitamin D status and polymorphisms in metabolic disturbances, in particular IR, in women suffering from PCOS. There was inconsistency in the

Figure 3 Association between BMI and vitamin D status in PCOS women (18, 19, 20, 21, 22, 23, 24, 26, 27, 28, 29, 30, 31, 32, 33, 34, 36, 37, 38, 39, 41, 43, 45) and control women (19, 20, 22, 23, 24, 27, 28, 41, 45). The circles and unbroken line represent PCOS women and the triangles and broken line represent control women. In the univariate regression analysis, the slope of the line was -4.5 for PCOS women and -8.4 for control women. The size of the symbols indicates the number of participants included in the corresponding study.

Figure 4 Association between BMI and insulin resistance in PCOS women (18, 19, 20, 22, 23, 24, 25, 26, 27, 29, 30, 33, 37, 39, 41, 42, 43, 45, 46) and control women (19, 20, 22, 23, 24, 27, 28, 41, 45). The circles and unbroken line represent PCOS women and the triangles and broken line represent control women. In the univariate regression analysis, the slope of the line was 0.41 for PCOS women and 0.10 for control women. The size of the symbols indicates the number of participants included in the corresponding study.

results of the studies analyzing the relationship between vitamin D status and metabolic disturbances in PCOS women. These conflicting findings might be due to the small sample sizes, the lack of adjustments for confounders, the use of different definitions for PCOS, the use of different assays for serum 25OHD measurement, the duration of intervention, the use of different amounts of vitamin D supplementation in the intervention trials, and the lack of an optimal serum 25OHD level in the general population. Only one well-designed randomized placebo-controlled trial demonstrating no effect of vitamin D3 supplementation on IR has been carried out until now (30). The small sample size and the relatively short follow-up duration could account for the lack of an effect. Another RCT carried out among 51 PCOS women by Bonakdaran *et al.* (40) did report a decrease in HOMA-IR after 3 months of calcitriol supplementation. However, this decrease was not significant probably due to the small sample size.

The relationship between obesity, vitamin D status, and IR has not been clarified yet. There is an ongoing discussion on whether vitamin D deficiency and IR are causally interrelated or whether they are both BMI dependent. In this review, we provided an overview of the correlation between these variables by carrying out a linear regression analysis. The univariate linear regression analysis revealed both serum vitamin D and BMI to be independent predictors of IR. However, in the multivariate analysis, serum 25OHD was no longer an independent predictor of IR in PCOS women. A strong independent relationship was observed between BMI as an explanatory variable and serum 25OHD as an outcome variable. A limitation of the data

analyses conducted in this review is that the weighted means of the reviewed studies instead of the original data were used. Another important point to be considered is the different methodologies used for vitamin D measurement in the reviewed studies. At present, many discussions are going on as to which test is the most reliable one for vitamin D measurement. Owing to the increasing recognition of the high prevalence and diverse consequences of vitamin D deficiency, a massive increase in vitamin D testing is being observed worldwide. A recent trial has demonstrated the limitation of different assays (47). Both could have influenced the results.

The demonstrated inverse relationship between BMI and vitamin D status has been established in earlier reports (48, 49). It has been shown that in obese individuals a higher proportion of vitamin D, which is fat soluble, is sequestered in adipose tissue and thereby the bioavailability of vitamin D is low. Alternatively, obese individuals tend to spend less time outdoors exposed to sunlight, leading to insufficient biosynthesis of vitamin D generated through the skin. Moreover, obesity is highly prevalent in women affected by PCOS, with the highest prevalence being reported in studies conducted in the USA and Australia, with 61–76% of women with PCOS being considered obese and 85% considered overweight or obese (1). In addition, obesity has a strong correlation with IR and type 2 diabetes mellitus (50). This association is in line with our results. Despite the strong association between obesity and IR, multiple studies have demonstrated that women affected by PCOS have more severe IR than expected on the basis of their body weight (51, 52, 53). In this regard, vitamin D status has more than once been designated as a possible contributing factor for IR in women affected by PCOS. This hypothesis is supported by animal studies with *Vdr* null mice and by human models (10). The largest cross-sectional study (NHANES data) has demonstrated an inverse correlation between serum 25OHD concentrations and diabetes prevalence. After multivariate adjustment, serum 25OHD concentrations were found to be negatively correlated with IR (15). Pittas *et al.* (54) confirmed these results in a 3-year follow-up study in which participants with impaired fasting glucose levels who used cholecalciferol plus calcium supplementation had a smaller increase in IR compared with controls who received a placebo.

The potential mechanisms by which vitamin D can affect glucose metabolism could be the result of direct and indirect actions of serum 25OHD: i) direct stimulation of insulin release through the expression of VDR as well as the enzyme 1α -OHase in the pancreatic β -cells; ii) through the binding of the $1,25(\text{OH})_2\text{D}$ -VDR complex to the vitamin D response element of the INSR at the tissue level and thereby enhancing insulin responsiveness for glucose transport; and iii) suppression of the release of proinflammatory cytokines that are believed to mediate IR (11). The latter

mechanism is supported by studies showing an association between low serum 25OHD levels and increased C-reactive protein levels (25, 55). Vitamin D may play an indirect role through its influence on the extracellular and intracellular calcium regulation, which is essential for the mediation of glucose transport in target tissues.

Nine of the reviewed studies compared PCOS women with control women. An important observation is that vitamin D status did not markedly differ between both the groups (overall mean 61.2 nmol/l in PCOS women vs 67.1 nmol/l in control women). It is remarkable that despite a slight difference in mean BMI between both the groups, HOMA-IR differed considerably, suggesting that PCOS *per se* is a risk factor for the development of IR. This is in line with some but not all earlier results (8, 9, 53). By contrast, PCOS *per se* does not seem to be a risk factor for vitamin D deficiency. The studies carried out by Ngo *et al.*, Panidis *et al.*, and Mahmoudi *et al.* even found a higher serum 25OHD level in PCOS women than in control women (20, 22, 23). However, these data were not adjusted for the season of measurement and diet.

Another important note is that the inconsistent results in the reviewed articles may be explained by the hypothesis that vitamin D supplementation may only be effective on IR in case a significant IR exists. As the majority of the reviewed studies did not include diabetic PCOS women, this could be a possible explanation why an effect of vitamin D supplementation on IR was not evident in all studies. However, the RCT carried out by Ardabili *et al.* did not report an effect of vitamin D supplementation, despite HOMA-IR of 3.17 in PCOS women (30).

Another hypothesis for the lack of an effect may be that baseline serum 25OHD is already sufficient. It seems plausible that in case of an adequate vitamin D status no effect can be observed after vitamin D supplementation. In this regard, it should be noted that the optimal serum 25OHD level is not clear until now. Although there is no consensus on optimal serum 25OHD levels, frequently vitamin D deficiency is defined by a serum 25OHD level <25 nmol/l (10 ng/ml). Serum 25OHD levels between 25 and 50 nmol/l (10–20 ng/ml) are considered to indicate vitamin D insufficiency, and serum 25OHD levels >50 nmol/l are considered to indicate vitamin D sufficiency. Recently, the Institute of Medicine, reviewing all the evidence, has set the required serum 25OHD levels at 50 nmol/l (56), but expert opinions remain divided. In addition, no optimal treatment regimen has been established yet for the treatment of vitamin D deficiency in different populations. In this regard, this may influence the study results, as all the reviewed intervention studies used different amounts and durations of vitamin D supplementation.

In conclusion, the relationship between vitamin D status and metabolic disturbances in women with PCOS remains unclear. Current literature suggests an inverse correlation between vitamin D status and IR in women

with PCOS. However, due to the heterogeneity of the studies, it is hard to draw an informed conclusion. Future research with adequately powered randomized placebo-controlled double-blind studies of vitamin D supplementation in women affected by PCOS is warranted. Until then, screening women who are at risk of vitamin D deficiency and supplementation with vitamin D could be considered.

Supplementary data

This is linked to the online version of the paper at <http://dx.doi.org/10.1530/EJE-13-0617>.

Declaration of interest

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