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CLINICAL STUDY

# Association of hypovitaminosis D with metabolic disturbances in polycystic ovary syndrome

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## Abstract

*Objectives*: Women with polycystic ovary syndrome (PCOS) frequently suffer from metabolic disturbances, in particular from insulin resistance. Accumulating evidence suggests that vitamin D deficiency may contribute to the development of metabolic syndrome (MS). Hence, the aim of our study was to investigate the association of 25(OH)D levels and the components of the MS in PCOS women.

*Methods*: 25(OH)D levels were measured by means of ELISA in 206 women affected by PCOS. Metabolic, endocrine, and anthropometric measurements and oral glucose tolerance tests were performed.

*Results*: The prevalence of insufficient 25(OH)D levels ($<30$ ng/ml) was 72.8% in women with PCOS. PCOS women with MS had lower 25(OH)D levels than PCOS women without these features (17.3 vs 25.8 ng/ml respectively; $P<0.05$). In multivariate regression analysis including 25(OH)D, season, body mass index (BMI), and age, 25(OH)D and BMI were independent predictors of homeostatic model assessment-insulin resistance (HOMA-IR) and quantitative insulin sensitivity check index (QUICKI; $P<0.05$ for all). In binary logistic regression analyses, 25(OH)D (odds ratio, OR 0.86, $P=0.019$) and BMI (OR 1.28, $P<0.001$) were independent predictors of MS in PCOS women. We found significantly negative correlations of 25(OH)D levels with BMI, waist circumference, waist-to-hip ratio, systolic and diastolic blood pressure, fasting and stimulated glucose, area under the glucose response curve, fasting insulin, HOMA-IR, HOMA-β, triglycerides, and quotient total cholesterol/high-density lipoprotein (HDL) and positive correlations of 25(OH)D levels with QUICKI and HDL ($P<0.05$ for all).

*Conclusion*: We demonstrate that low 25(OH)D levels are associated with features of MS in PCOS women. Large intervention trials are warranted to evaluate the effect of vitamin D supplementation on metabolic disturbances in PCOS women.

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## Introduction

Polycystic ovary syndrome (PCOS) is the most common female endocrine disorder with a prevalence of ~5–10% in women of reproductive age (1–3). PCOS is characterized by increased ovarian and adrenal androgen secretion, hyperandrogenic symptoms such as hirsutism, acne and/or alopecia, menstrual irregularity, and polycystic ovaries. In addition, insulin resistance and hyperinsulinemia are common features in PCOS women (4), who are therefore at an increased risk of type 2 diabetes (5).

Accumulating evidence suggests that vitamin D deficiency might be a causal factor in the pathogenesis of metabolic syndrome (MS) in PCOS (6). This notion is supported by the fact that the vitamin D receptor gene regulates about 3% of the human genome, including genes that are crucial for glucose and lipid metabolism and blood pressure regulation (7–9). Clinical studies have largely but not consistently shown that type 2 diabetes and insulin resistance are associated with poor vitamin D status (10, 11). In addition, some studies suggest that vitamin D insufficiency is also associated with dyslipidemia (6) as well as arterial hypertension, which might be explained by the inhibition of the renin gene expression by vitamin D (9). Obesity is also linked with low 25(OH)D levels in PCOS cohorts (6, 12) and in large study cohorts including various groups of obese and normal weight women and men (13, 14), which might be a consequence of 25(OH)D deposition in the adipose tissue.

So far, the role of 25(OH)D in the development of MS in PCOS is largely unknown. Therefore, the aim of our study was to extend the currently rare knowledge about the association of vitamin D and MS in PCOS women.



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## Methods

### Subjects

We evaluated 206 women with PCOS aged 16–41 years, who were routinely referred to our outpatient clinic. The diagnosis was based on the Rotterdam criteria (15). Two out of three of the following are required to confirm the diagnosis: oligo- and/or anovulation; clinical and/or biochemical signs of hyperandrogenism; and polycystic ovaries (by ultrasound). Disorders with a similar clinical presentation, such as congenital adrenal hyperplasia, Cushing's syndrome, and androgen-secreting tumors, must be excluded. Oligo- and/or anovulation were defined by the presence of oligomenorrhea or amenorrhea. Hyperandrogenism was defined by the clinical presence of hirsutism (Ferriman–Gallwey score $\geq 6$), acne or alopecia, and/or elevated androgen levels. Polycystic ovarian morphology was examined by ultrasound. Polycystic ovaries were defined as the presence of 12 or more follicles in each ovary measuring 2–9 mm in diameter and/or increased ovarian volume ($>10$ ml; calculated using the formula $0.5 \times$length$\times$width $\times$thickness) (15). Hyperprolactinemia, Cushing's syndrome, congenital adrenal hyperplasia, and androgen-secreting tumors were excluded by specific laboratory analysis (cortisol, ACTH, 17αOH-progesterone, and DHEAS).

MS was defined by the National Cholesterol Education Program (NCEP) and the Adult Treatment Panel III (ATP III) in subjects presenting at least three of the following criteria: waist circumference $>88$ cm; high-density lipoprotein (HDL) cholesterol $<50$ mg/dl; triglyceride level $>150$ mg/dl; raised blood pressure (systolic $>130$ mmHg and diastolic $>85$ mmHg); and raised fasting glucose ($>110$ mg/dl) or impaired glucose tolerance (IGT) during oral glucose tolerance test (OGTT) (16). The study participants did not take any medication known to affect endocrine parameters, carbohydrate metabolism, or serum lipid profile for at least 3 months before entering the study.

The study protocol was approved by the local ethics committee, and written informed consent was obtained from each patient.

### Procedures

Standard anthropometric data (height, weight, and waist and hip circumference) were obtained from each subject. Blood pressure was measured after PCOS women had been seated for at least 5 min. The body mass index (BMI) was calculated as the weight in kilograms divided by the square of height in meters. Waist circumference was measured in a standing position midway between the lower costal margin and the iliac crest. Hip circumference was measured in a standing position at the maximum circumference over the buttocks. Hirsutism was quantified with the modified Ferriman–Gallwey score. Moreover, basal blood samples for hormonal (25(OH)D, parathyroid hormone (PTH), total testosterone, free testosterone, sex hormone-binding globulin (SHBG), androstenedione, DHEAS, free tri-iodothyronine, free thyroxine, TSH, 17αOH-progesterone, and cortisol) and metabolic (glucose, insulin, C-peptide, total cholesterol, HDL cholesterol, low-density lipoprotein cholesterol, and triglycerides) determinations were collected at 0800–0900 h after overnight fast. All participants underwent a fasting 75 g OGTT. Blood samples were drawn after 60 and 120 min for glucose, insulin, and C-peptide determination. Insulin resistance was estimated using the homeostatic model assessment-insulin resistance (HOMA-IR). HOMA-IR was calculated as the product of the fasting plasma insulin value (μU/ml) and the fasting plasma glucose value (mg/dl), divided by 405 (17). Quantitative insulin sensitivity check index (QUICKI) was used to estimate insulin sensitivity. QUICKI was calculated as 1/log fasting insulin (μU/ml) + log fasting glucose (mg/dl). To assess β-cell function, HOMA-β was calculated as (20$\times$fasting insulin (μU/ml))/(fasting glucose (mmol/l) $-$ 3.5). Hyperinsulinemia was assessed by calculating the area under the insulin response curve (AUCins). The free androgen index (FAI) was calculated as testosterone (nmol/l)/SHBG (nmol/l)$\times$100.

### Biochemical analysis

25(OH)D (normal range 30–60 ng/ml) was measured using a commercially available ELISA (IDS, Boldon, UK) with intra- and inter-assay coefficients of variation (CV) of 5.6 and 6.4% respectively. Insulin (2.0–25.0 μU/ml) and C-peptide (0.5–3.2 ng/ml) were measured by ELISA (DRG, Marburg, Germany) with intra- and inter-assay CV of 4.0 and 2.6, and 5.1 and 8.4% respectively. Fasting glucose ($\leq 115$ mg/dl), triglycerides ($\leq 150$ mg/dl), total cholesterol ($\leq 200$ mg/dl), HDL cholesterol ($>40$ mg/dl), and C-reactive protein (CRP) ($\leq 8$ mg/l) were determined using Modular Analytics SWA (Roche). Free testosterone (0.29–3.18 pg/ml) was determined using a RIA (DSL, Webster, TX, USA). SHBG (19–117 nmol/l), PTH (15–65 pg/ml; Roche), ACTH (10–51 pg/ml), cortisol (43.0–220.0 ng/ml), human growth hormone (HGH) (0.0–7.0 ng/ml; Siemens DPC Bühlmann, Salzburg, Austria), and insulin-like growth factor 1 (100–400 ng/ml), prolactin (2.8–29.2), total testosterone (0.14–0.77 ng/ml), and TSH (0.1–4.0 μU/ml; Bayer) were measured by luminescence immunoassay.

### Statistical analysis

For the purpose of this study and according to widely used cut-offs, subjects were divided into groups: vitamin D sufficiency (25(OH)D $\geq 30$ ng/ml); hypovitaminosis D (25(OH)D $<30$ ng/ml). Furthermore, we defined severe vitamin D deficiency (25(OH)D $<10$ ng/ml), moderate

vitamin D deficiency (25(OH)D 10–19.9 ng/ml), and vitamin D insufficiency (25(OH)D 20–29.9 ng/ml) (7, 18). Data are presented as means ± S.D., unless otherwise stated. Kolmogorov–Smirnov test was used to examine for normal distribution, and variables following a skewed distribution were logarithmically transformed before being used in correlation or regression analyses. Pearson correlations and partial correlation analyses were used to determine relationships between variables. Depending on the distribution of data, the Student's *t*-test for independent samples and the nonparametric Mann–Whitney *U*-test for independent samples were applied to test for differences between groups. CRP values within the normal range ($\leq$8 mg/l) were included in the analyses. To study seasonal variation, we subdivided the year into 4-month measurement periods: February–May (season 1); June–September (season 2); October–January (season 3) to address the seasonal changes in availability of sunlight (19). Multiple linear regression analyses were calculated with HOMA-IR and QUICKI as dependent variables and 25(OH)D, season, BMI, and age as independent variables. Binary logistic regression analyses were performed to examine the associations of MS (dependent variable) with 25(OH)D, season, BMI, and age (independent variables). Statistical analyses were performed by SPSS version 16.0 (SPSS Inc., Chicago, IL, USA). A $P$ value of $<0.05$ was considered statistically significant.

## Results

### *Findings for entire cohort*

Anthropometric and biochemical characteristics of PCOS women stratified by 25(OH)D levels are shown in Table 1. Hypovitaminosis D was present in 150 out of 206 PCOS women (72.8%); 6 out of 206 PCOS women (2.9%) had 25(OH)D levels $<$10 ng/ml; 74 out of 206 PCOS women (35.9%) had 25(OH)D levels between 10

**Table 1** Clinical and biochemical characteristics of polycystic ovary syndrome (PCOS) subjects based on 25(OH)D status.

| | **All PCOS** ($n$=206) | | **Hypovitaminosis D** ($<$30 ng/ml, $n$=150) | | **Vitamin D sufficiency** ($\geq$30 ng/ml, $n$=56) | | |
|---|---|---|---|---|---|---|---|
| | Mean | S.D. | Mean | S.D. | Mean | S.D. | ***P*** |
| Clinical characteristics | | | | | | | |
| Age (years) | 29 | 7 | 29 | 6 | 27 | 7 | 0.027 |
| Weight (kg) | 72.3 | 19.7 | 75.6 | 21.0 | 63.7 | 12.4 | 0.001 |
| Height (cm) | 166.1 | 6.4 | 166.0 | 6.4 | 166.2 | 6.5 | 0.980 |
| BMI (kg/m$^2$) | 26.2 | 6.9 | 27.4 | 7.4 | 23.0 | 4.1 | 0.001 |
| Waist circumference (cm) | 90 | 19 | 94 | 19 | 79 | 10 | $<$0.001 |
| Hip circumference (cm) | 106 | 13 | 108 | 13 | 98 | 10 | 0.001 |
| WHR | 0.85 | 0.10 | 0.86 | 0.09 | 0.81 | 0.10 | 0.005 |
| Systolic blood pressure (mmHg) | 113 | 15 | 114 | 16 | 108 | 12 | 0.026 |
| Diastolic blood pressure (mmHg) | 75 | 11 | 76 | 11 | 73 | 11 | 0.115 |
| Metabolic biochemical characteristics | | | | | | | |
| Fasting glucose (mg/dl) | 83 | 9 | 83 | 10 | 82 | 9 | 0.237 |
| 1-h glucose (mg/dl) | 115 | 40 | 120 | 43 | 103 | 28 | 0.003 |
| 2-h glucose (mg/dl) | 98 | 30 | 99 | 32 | 93 | 22 | 0.259 |
| AUCgluc | 102.5 | 27.8 | 105.5 | 29.9 | 94.6 | 18.9 | 0.046 |
| HbA1c (%) | 5.2 | 0.3 | 5.2 | 0.3 | 5.1 | 0.3 | 0.065 |
| HOMA-IR | 1.72 | 1.93 | 1.96 | 2.16 | 1.11 | 0.92 | 0.002 |
| HOMA-β | 160.2 | 122.7 | 173.3 | 131.1 | 126.7 | 90.9 | 0.014 |
| QUICKI | 0.39 | 0.09 | 0.38 | 0.10 | 0.40 | 0.05 | 0.002 |
| Fasting insulin (μU/ml) | 8.0 | 7.6 | 9.0 | 8.4 | 5.4 | 4.0 | 0.002 |
| 1-h insulin (μU/ml) | 58.0 | 44.1 | 61.7 | 45.0 | 48.9 | 40.6 | 0.049 |
| 2-h insulin (μU/ml) | 48.1 | 43.8 | 52.5 | 49.1 | 37.4 | 24.6 | 0.267 |
| AUCins | 42.8 | 31.5 | 45.9 | 33.3 | 35.2 | 25.3 | 0.074 |
| Total cholesterol (mg/dl) | 177.5 | 35.4 | 174.2 | 34.9 | 185.9 | 35.8 | 0.088 |
| Triglycerides (mg/dl) | 89.6 | 44.6 | 94.8 | 48.8 | 76.5 | 27.9 | 0.042 |
| HDL (mg/dl) | 64.1 | 16.2 | 61.7 | 15.7 | 70.0 | 16.3 | 0.002 |
| QChol/HDL | 2.9 | 0.8 | 3.0 | 0.8 | 2.8 | 0.7 | 0.106 |
| LDL (mg/dl) | 99.5 | 29.2 | 99 | 29 | 103.9 | 31.8 | 0.221 |
| CRP (mg/l) | 2.3 | 2.0 | 2.5 | 2.1 | 1.6 | 1.3 | 0.030 |
| Endocrine biochemical characteristics | | | | | | | |
| Testosterone (ng/l) | 0.66 | 0.28 | 0.65 | 0.28 | 0.69 | 0.28 | 0.243 |
| Free testosterone (pg/ml) | 2.79 | 1.19 | 2.80 | 1.25 | 2.76 | 0.99 | 0.788 |
| SHBG (nmol/l) | 51.6 | 27.8 | 50.0 | 29.1 | 55.7 | 23.9 | 0.038 |
| FAI | 5.8 | 4.3 | 6.1 | 4.7 | 5.2 | 3.2 | 0.549 |

Comparisons between PCOS women with hypovitaminosis D and sufficient 25(OH)D levels were performed by Student's *t*-test or Mann–Whitney U test as appropriate.

and 19.9 ng/ml; 70 women (34.0%) presented with 25(OH)D levels between 20 and 29.9 ng/ml; and 56 women (27.2%) showed sufficient 25(OH)D levels $\geq$30 ng/ml.

25(OH)D levels were determined between October and January (season 3) in the majority of PCOS women ($n$=93, 45.1%). Blood samples of 76 women (36.9%) were collected between February and May (season 1), and 37 women (18%) were examined between June and September (season 2). 25(OH)D levels were significantly higher in season 2 when compared with seasons 1 and 3 ($P<0.05$ for all).

25(OH)D levels correlation was significantly negative with BMI (Fig. 1a), weight, waist circumference, hip circumference, waist-to-hip ratio (WHR), systolic and diastolic blood pressure, fasting and stimulated glucose, area under the glucose response curve (AUCgluc; Fig. 1b), fasting and stimulated insulin, AUCins, PTH, triglycerides, quotient total cholesterol (QChol)/HDL, and CRP (Table 2). We found a significantly positive correlation between 25(OH)D levels and HDL cholesterol (Table 2). These latter results did not materially change when controlling for season and age in partial correlation analyses. However, the associations of 25(OH)D with anthropometric parameters, glucose, insulin, and triglycerides were no longer significant after additional adjustment for BMI. The positive correlation of 25(OH)D with HDL remained significant after adjustment for BMI ($r$=0.303, $P$=0.006).

In addition, we found a significant correlation between 25(OH)D and HOMA-IR (Table 2), QUICKI (Fig. 1c), as well as HOMA-β (Fig. 1d). To explore the association of 25(OH)D and HOMA-IR, we performed a multivariate regression analysis including HOMA-IR as dependent variable and BMI, age, 25(OH)D, and season as explanatory variables. In this analysis, 25(OH)D ($P$=0.036) was a significant and independent predictor for HOMA-IR, along with BMI ($P<0.001$). Furthermore, we performed a multivariate regression analysis including QUICKI as dependent variable and BMI, age, 25(OH)D, and season as explanatory variables. 25(OH)D ($P$=0.047) and BMI ($P<0.001$) were significant predictors of QUICKI, along with the other covariates explaining 18% of variation in QUICKI.

Thirty PCOS women (15%) presented with IGT. Women with IGT had significantly lower levels of 25(OH)D than women with normal glucose tolerance (20.2 and 25.6 ng/ml respectively; $P$=0.026). In PCOS women with vitamin D deficiency (<20 ng/ml), 16 women (20.0%) had IGT; in PCOS women with vitamin D insufficiency (20–29.9 ng/ml), 12 women (17.1%) showed IGT; and in PCOS women with sufficient 25(OH)D levels (>30 ng/ml), 2 women (3.6%) had IGT.

To address the heterogeneity of PCOS, subgroup analyses of PCOS women with and without a family history of type 2 diabetes were performed (20, 21). Data on family history of type 2 diabetes were available in 158 PCOS patients. Family history of type 2 diabetes

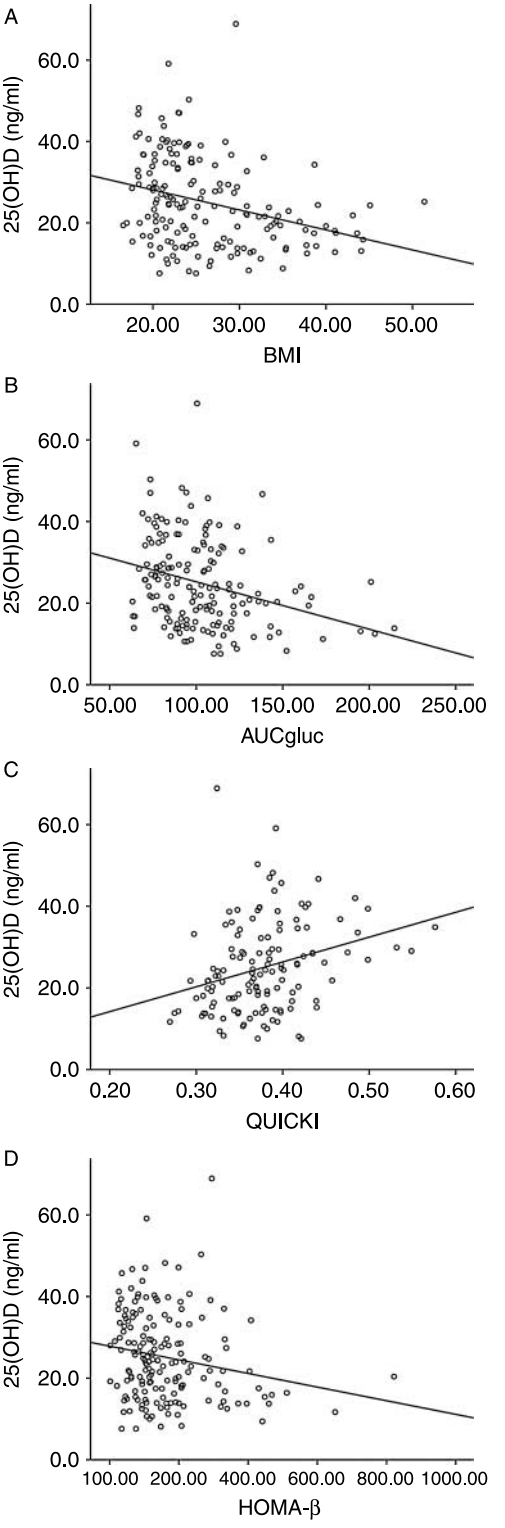


**Figure 1** Correlation between 25(OH)D and BMI, QUICKI, AUCgluc, and HOMA-β (Pearson correlation). (A) Correlation between 25(OH)D and BMI. $R=-0.346$, $P<0.001$. (B) Correlation between 25(OH)D and AUCgluc. $R=-0.333$, $P<0.001$. (C) Correlation between 25(OH)D and QUICKI. $R$=0.327, $P<0.001$. (D) Correlation between 25(OH)D and HOMA-β. $R=-0.212$, $P$=0.005.

**Table 2** Correlation of 25(OH)D levels with metabolic and endocrine parameters in polycystic ovary syndrome (PCOS) women ($n=206$).

| | 25(OH)D | |
|---|---|---|
| **Variable** | *r* | *P* |
| Clinical characteristics | | |
| Age (years) | −0.130 | 0.084 |
| Weight (kg) | −0.318 | <0.001 |
| Height (cm) | 0.021 | 0.789 |
| Waist circumference (cm) | −0.409 | <0.001 |
| Hip circumference (cm) | −0.378 | <0.001 |
| WHR | −0.316 | 0.023 |
| Systolic blood pressure (mmHg) | −0.197 | 0.029 |
| Diastolic blood pressure (mmHg) | −0.219 | 0.015 |
| Metabolic biochemical characteristics | | |
| Fasting glucose (mg/dl) | −0.210 | 0.005 |
| 1-h glucose (mg/dl) | −0.291 | <0.001 |
| 2-h glucose (mg/dl) | −0.230 | 0.003 |
| HOMA-IR | −0.327 | <0.001 |
| Fasting insulin (μU/ml) | −0.320 | <0.001 |
| 1-h insulin (μU/ml) | −0.215 | 0.007 |
| 2-h insulin (μU/ml) | −0.180 | 0.026 |
| AUCins | −0.227 | 0.005 |
| PTH (pg/ml) | −0.309 | 0.004 |
| Free testosterone (pg/ml) | 0.004 | 0.953 |
| FAI | −0.089 | 0.238 |
| SHBG (nmol/l) | 0.195 | 0.009 |
| Testosterone (ng/l) | 0.050 | 0.509 |
| Cholesterol (mg/dl) | 0.141 | 0.065 |
| Triglycerides (mg/dl) | −0.231 | 0.002 |
| HDL (mg/dl) | 0.278 | <0.001 |
| QChol/HDL | −0.231 | 0.002 |
| LDL (mg/dl) | 0.059 | 0.568 |
| CRP (mg/l) | −0.298 | 0.005 |
| Endocrine biochemical characteristics | | |
| Testosterone (ng/l) | 0.050 | 0.509 |
| Free testosterone (pg/ml) | 0.004 | 0.953 |
| SHBG (nmol/l) | 0.195 | 0.009 |
| FAI | −0.089 | 0.238 |

Data are given as Pearson's correlation coefficient *r*. Correlations of 25(OH)D levels with BMI, AUCgluc, QUICKI, and HOMA-β are shown in Fig. 1. BMI, body mass index; WHR, waist-to-hip ratio; HOMA-IR, homeostasis model assessment-insulin resistance; AUC, area under the curve; HDL, high-density lipoprotein; QChol/HDL, ratio of total cholesterol to HDL; LDL, low-density lipoprotein.

was present in 47 (29.7%) out of 158 PCOS patients, and these PCOS women had significantly lower 25(OH)D levels when compared with PCOS women without a family history of type 2 diabetes (21.9 vs 24.5, $P=0.049$). Moreover, PCOS women with a family history of type 2 diabetes had significantly higher BMI, waist and hip circumference, fasting insulin, HOMA-IR, and QChol/HDL, and significantly lower QUICKI and SHBG than PCOS women without a family history of type 2 diabetes ($P<0.05$ for all, data not shown).

No significant correlations were found between 25(OH)D levels and endocrine parameters such as testosterone, free testosterone, and FAI. Hirsutism score correlated as significantly negative ($r=-0.267$; $P=0.001$) and SHBG correlated as significantly positive (Table 2) with 25(OH)D. This correlation of 25(OH)D with SHBG was abolished, whereas the correlation of 25(OH)D with hirsutism score remained significant when controlling for BMI ($r=-0.367$; $P=0.002$). Furthermore, hirsute PCOS women had significantly lower 25(OH)D levels (21.4 ng/ml) than PCOS women without hirsutism (26.8 ng/ml; $P=0.001$).

Levels of PTH were significantly positive correlated with AUCgluc ($r=0.235$; $P=0.036$) and diastolic blood pressure ($r=0.278$; $P=0.006$).

## *Metabolic syndrome*

Presence of MS was analyzed in a subgroup of 174 out of 206 PCOS women (85.4%). MS was evident in 25 women (12.2%). Out of 25 PCOS women, 18 with MS (72%) presented with deficient 25(OH)D levels, 7 women with MS (28%) had insufficient 25(OH)D levels, and none had a sufficient 25(OH)D level (>30 ng/ml). PCOS women with the MS had significantly lower 25(OH)D levels than PCOS women without the MS (17.3 vs 25.8 ng/ml respectively; $P<0.001$).

In logistic regression analyses, MS was associated with BMI (odds ratio (OR) 1.28, 95% confidence interval (CI) (1.15–1.42), $P<0.001$) and 25(OH)D (OR 0.86, 95% CI (0.75–0.98), $P=0.019$).

## *Findings stratified by vitamin D sufficiency and hypovitaminosis D*

Clinical and biochemical characteristics of PCOS women with vitamin D sufficiency (≥30 ng/ml) and hypovitaminosis D (<30 ng/ml) are shown in Table 1. PCOS women with hypovitaminosis D had significantly higher age, weight, BMI, waist and hip circumference, WHR, and systolic blood pressure than women with vitamin D sufficiency ($P<0.05$ for all). Furthermore, the hypovitaminosis D group had significantly higher levels of 1 h glucose, AUCgluc, HOMA-IR, HOMA-β, fasting insulin, 1 h insulin, triglycerides, and CRP, and significantly lower levels of QUICKI, HDL, and SHBG than the vitamin D sufficient group ($P<0.05$ for all).

Furthermore, we performed subgroup analyses of lean (BMI≤25, $n=116$, 56.3%) and obese (BMI>25, $n=90$, 43.7%) PCOS women. When lean PCOS women were analyzed separately, we observed no significant differences for all parameters included in Table 1 between PCOS women with hypovitaminosis D and vitamin D sufficiency (data not shown). We found a significant positive correlation of 25(OH)D with HDL in lean PCOS women ($P<0.05$, data not shown), whereas all other correlations were not statistically significant. In the subgroup of obese PCOS women, we found significantly increased HbA1c levels and waist circumference in the hypovitaminosis D group when compared with the vitamin D sufficiency group ($P<0.05$ for all, data not shown), whereas all other parameters were not significantly different between groups. In obese PCOS women, there were significantly negative correlations

of 25(OH)D with 1-h glucose, 2-h glucose, AUCgluc, HbA1c, HOMA-β, fasting insulin, 1-h insulin, and AUCins, and significantly positive correlations of 25(OH)D with QUICKI and HDL ($P<0.05$ for all, data not shown).

## Discussion

Our results indicate that low 25(OH)D levels are significantly associated with components of the MS and insulin resistance in women with PCOS. 25(OH)D was an independent predictor of insulin resistance and insulin sensitivity in a multivariate regression analysis.

Low 25(OH)D levels have been linked to an increased risk for cancer (22), autoimmune diseases, diabetes, and cardiovascular diseases (7, 22, 23) indicating the importance of sufficient 25(OH)D levels. Although there is no consensus on optimal levels of 25(OH)D, a level of 30 ng/ml can be considered to indicate sufficient vitamin D status (7). In our study, 72.8% of PCOS women showed 25(OH)D values below this recommended level.

Our data demonstrate a significant association of low 25(OH)D levels with increased levels of fasting and stimulated glucose, AUCgluc, HOMA-IR, and fasting and stimulated insulin. Accordingly, Hahn *et al.* reported an association of low 25(OH)D levels with insulin resistance in 120 PCOS women (6). Apart from these cross-sectional findings, there is one small prospective intervention study with vitamin D supplementation, which demonstrates beneficial effects of vitamin D on insulin secretion and serum lipids in PCOS women (24). In nonPCOS cohorts including subjects with various BMI, vitamin D concentration was inversely related to the prevalence of diabetes (25), plasma concentrations of glucose (26), insulin resistance (10, 26), and the MS (8, 10). Besides, the risk of future hyperglycemia and insulin resistance was associated with hypovitaminosis D (27).

The mechanisms underlying the association of low 25(OH)D levels and insulin resistance are not fully understood. First, vitamin D may have a beneficial effect on insulin action by stimulating the expression of insulin receptor and thereby enhancing insulin responsiveness for glucose transport (8). Secondly, vitamin D regulates extracellular and intracellular calcium, which is essential for insulin-mediated intracellular processes in insulin-responsive tissues such as skeletal muscle and adipose tissue (8). Finally, as vitamin D has a modulating effect on the immune system (28), hypovitaminosis D might induce a higher inflammatory response, which is associated with insulin resistance (29). This hypothesis is supported by the results of our study indicating an association of low 25(OH)D with increased CRP levels.

In turn, an additional mechanism might be seen in impaired β-cell function in PCOS women. This is underlined by our finding of a negative association of 25(OH)D levels and HOMA-β and the inverse association of stimulated glucose levels and AUCgluc with 25(OH)D.

A new finding in our study was the association of increased triglycerides and QChol/HDL with low 25(OH)D levels. Moreover, we found low 25(OH)D levels associated with low levels of HDL, confirming previous results in PCOS women (6), but contrasting the lack of association in a recent study in a cohort of healthy young women (30). In a pilot study, Kotsa *et al.* showed an improvement of HDL and triglycerides after treatment with vitamin D in a small cohort of PCOS women (24). Since dyslipidemia should be considered as an additional therapeutic target in PCOS (31), vitamin D might be useful in the complex treatment of PCOS women.

These parameters, i.e. low HDL and elevated triglycerides, are central features of the MS. For the first time, we observed an association of low 25(OH)D levels with the MS independently from BMI, age, and seasonal variation of 25(OH)D in PCOS women.

Nevertheless, our data suggest a strong relationship of 25(OH)D and BMI in PCOS women, which is in agreement with previous studies (6, 12). So far, it is not clear whether vitamin D insufficiency results from obesity and/or whether obesity is a consequence of vitamin D insufficiency. On the one hand, obesity may contribute to low circulating vitamin D levels by trapping vitamin D in fat tissues. Wortsman *et al.* demonstrated that the increase in 25(OH)D levels 24 h after whole-body u.v. light exposure was 57% lower in obese than in nonobese subjects (32). On the other hand, obese patients may avoid sunlight, which is necessary for the synthesis of vitamin D in the skin (33). This might be especially the case in hirsute PCOS women, who tend to hide from the public due to their appearance. There is evidence that low vitamin D levels are associated with obesity (6) and vice versa low vitamin D intake might be an independent predictor of obesity (34).

Recent data from an overweight/obese women's study have suggested that women with high 25(OH)D levels respond more positively to hypocaloric diets and lose more body fat than women with low 25(OH)D levels (35). There is evidence that weight loss is probably the most effective treatment of PCOS women at the moment (36). Thus, vitamin D supplementation might be an element in the complex treatment of PCOS women. This hypothesis is supported by the findings of our study indicating the association of low 25(OH)D levels with obesity, insulin resistance, and MS in PCOS women.

In our study, there was no correlation of 25(OH)D levels with FAI, total testosterone, or free testosterone. Our observations are in line with a previous study that did not find any differences among PCOS and healthy control women with respect to 25(OH)D levels (37). One previous report on a correlation between vitamin D

levels and FAI (6) could be mediated through the obesity-induced reduction in SHBG. On this note, the significant correlation between SHBG and 25(OH)D in our patients was abolished when controlling for BMI. However, we found an inverse correlation of 25(OH)D with hirsutism score that was independent of BMI, which is in line with previous studies (6, 38). In addition, 25(OH)D levels were significantly lower in hirsute women, which is consistent with the results from a small study in hirsute women (38). This association might be caused by various mechanisms. First, the cosmetic distress may cause hypovitaminosis D due to the decreased sun exposure of hirsute women, as mentioned above. Secondly, the vitamin D receptor is found in keratinocytes of the outer root sheath as well as in cells of the bulge, indicating an important role of vitamin D in hair follicle cycling (28). However, the mechanism by which the vitamin D receptor regulates hair follicle cycling and its potential role in hirsutism remains unclear.

Our study has several limitations that should be noted. First, this study does not include a control group. Thus, we are not able to correlate the effects of low 25(OH)D levels with the metabolic profile of PCOS women specifically or with that of obese women in general. However, we performed subgroup analyses of lean and obese PCOS women with different 25(OH)D levels to address this limitation. Secondly, there was no information available with respect to calcium and carbohydrate intakes and exercise prior to biochemical measurements, which might influence our association of 25(OH)D levels with metabolic parameters (39, 40).

To the best of our knowledge, we are the first to describe an inverse association of low 25(OH)D levels with impaired β-cell function, IGT, and MS in PCOS women. Further, we confirm previous findings reporting the relationship of low 25(OH)D levels with obesity and insulin resistance women with PCOS. To prove these findings and to find new therapeutic approaches, large intervention trials with vitamin D supplementation are warranted in PCOS women.

## Declaration of interest

The authors declare no conflict of interest.

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