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Vitamin D deficiency is common and associated with metabolic risk factors in patients with polycystic ovary syndrome

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ABSTRACT

Both vitamin D deficiency and polycystic ovary syndrome (PCOS) are associated with aspects of metabolic syndrome, but it is unclear whether vitamin D deficiency contributes to the metabolic disturbances commonly found in women with PCOS. This study sought to investigate (1) the prevalence of vitamin D deficiency in PCOS women in Scotland and (2) the relationship between vitamin D status and metabolic risk factors. This was an observational study on 52 women (25 in PCOS group and 27 in control group). Serum 25-hydroxyvitamin D concentrations less than 25 nmol/L were classified as severe vitamin D deficiency and were found in 44.0% and 11.2% of subjects in the PCOS and control groups, respectively ($P = .047$). Among the PCOS subjects, 25-hydroxyvitamin D concentrations were negatively correlated with body mass index ($P = .033$), C-reactive protein ($P = .027$), and free androgen index ($P = .025$) and positively correlated with quantitative insulin sensitivity check index ($P = .035$), high-density lipoprotein cholesterol (HDL-C) ($P = .033$), and sex hormone binding globulin ($P = .038$). Associations of vitamin D deficiency with quantitative insulin sensitivity check index and HDL-C were independent of body mass index and waist-to-hip ratio. Vitamin D deficiency is highly prevalent in PCOS women in Scotland, and a larger proportion of PCOS patients than control women were found to be vitamin D deficient. We also demonstrate correlations of vitamin D status with insulin sensitivity, HDL-C, and C-reactive protein in PCOS patients, which support the increasing evidence that vitamin D deficiency is associated with multiple metabolic risk factors in PCOS women.

Author contributions : HWRL carried out the ultrasound examinations, performed the statistical analysis, and drafted the manuscript. REB helped in patient recruitment and blood taking, and took part in data management and analysis. RAA conceived the study, participated in its design and coordination, carried out the ultrasound examinations, and helped in data analysis and revision of the manuscript. AMW gave scientific input in the hormonal assays and participated in manuscript revision. CKMH conceived the study, participated in the study design, and helped in data analysis and manuscript writing. All authors read and approved the final manuscript.

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1. Introduction

It is well known that vitamin D is essential for skeletal growth and development and plays an important role in calcium metabolism. Besides osteomalacia and rickets, vitamin D deficiency is also implicated in the etiology of other conditions including cardiovascular disease, cancer, and diabetes mellitus [1].

Serum 25-hydroxyvitamin D (25[OH]D) concentration is widely accepted as the functional indicator of vitamin D status in the body [2]. At present, there is no general consensus on the minimum serum level of 25(OH)D that is optimal for health. A concentration of 25(OH)D less than 50 nmol/L (20 ng/mL) is generally considered as vitamin D deficiency, whereas serum 25(OH)D concentrations of 50 to 74 nmol/L (20-30 ng/mL) are considered as vitamin D insufficiency [2]. Serum 25(OH)D concentrations between 30 and 50 nmol/L (12-20 ng/mL) are frequently associated with biochemical abnormalities such as raised parathyroid hormone (PTH) levels, but not clinical symptoms [3]. Investigators in the field of vitamin D research generally agree that a serum 25(OH)D level of at least 50 to 80 nmol/L (20-32 ng/mL) is required for optimal bone health [4]. In the last National Diet and Nutrition Survey (NDNS) conducted in the United Kingdom, vitamin D deficiency (25[OH]D level <50 nmol/L) was very common and identified in 54% of women aged 19 to 64, whereas severe vitamin D deficiency (25[OH]D level <25 nmol/L) was detected in 15% of those women. Given its northerly latitudes, Britain normally enjoys only limited amount of sunshine especially in winter and spring months; vitamin D deficiency has been shown to be more widespread and severe in Scotland than the rest of the United Kingdom [5].

Polycystic ovary syndrome (PCOS) is characterized by hyperandrogenism, chronic anovulation, and abnormal development of ovarian follicles. In addition, insulin resistance and central obesity are common features found in PCOS patients [6]. Interestingly, in a nonrandomized observational study involving PCOS patients who had vitamin D insufficiency (25[OH]D <60 nmol/L), 7 of 9 developed normal menstrual cycles within 2 months of treatment with calcium 1500 mg daily and vitamin D 50 000 U weekly or biweekly [7]. Furthermore, in 3 recent studies conducted in Austria, Germany, and Turkey, serum 25(OH)D concentrations were negatively correlated with body mass index (BMI), body fat, homeostasis model assessment of insulin resistance (HOMA-IR), and hyperinsulinemia, suggesting a relationship between vitamin D status, obesity, and insulin resistance in PCOS patients [8-10]. It has also been demonstrated by previous studies that vitamin D deficiency is associated with other features of metabolic syndrome including glucose intolerance, hypertension, dyslipidemia, and chronic inflammation [11]. These metabolic risk factors are commonly found in women with PCOS, but a role for vitamin D deficiency in PCOS and metabolic syndrome is not entirely clear [6].

In this study, we sought to explore both the prevalence of vitamin D deficiency and its potential associations with hormonal and metabolic factors in a pilot group of PCOS women in Scotland.

2. Methods

2.1. Subjects

A total of 63 patients attending the infertility and reproductive endocrine clinics at the Royal Infirmary of Edinburgh from January to April 2009 were recruited into this study with written informed consent. A detailed history including past medical history, family history, current symptoms, and medications was taken from the participants. Body weight, height, waist-to-hip ratio (WTHR), and sitting blood pressure were measured. A blood sample was taken after at least 10 hours of fasting. Patients fulfilling at least 2 of the 3 criteria defined by the European Society of Human Reproduction and Embryology/American Society for Reproductive Medicine consensus 2003 [12]—that is, (1) oligo- or anovulation, (2) clinical and/or biochemical signs of hyperandrogenism, and (3) polycystic ovaries (by ultrasound measurement of ovarian volume and number of antral follicles)—were recruited into the PCOS group. Women who attended the infertility clinic and had regular ovulatory cycles were recruited as "controls." Exclusion criteria included known endocrine disorder such as diabetes mellitus, ovarian pathology, liver or renal diseases, and those who were on medications containing sex steroids within 3 months. This study was approved by the Lothian Research Ethics Committee, and informed written consent was obtained from all subjects recruited.

2.2. Laboratory investigations

Fasting serum lipid profile including total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), triglycerides, calcium, phosphate, C-reactive protein (CRP), and fasting plasma glucose were analyzed using an automated platform (Olympus, Southall, United Kingdom). Serum total testosterone and insulin were measured using immunoassays (Centaur; Bayer Healthcare, Berks, United Kingdom), and sex hormone binding globulin (SHBG) was measured by Immulite 2000 (DPC, Gwynedd, United Kingdom). Serum 25(OH)D was measured by liquid chromatography–tandem mass spectrometry as previously described [13]. Free androgen index (FAI) was calculated using the following formula: 100 × [total testosterone (nanomoles per liter)]/[SHBG (nanomoles per liter)] [14]. Insulin resistance was estimated by the HOMA-IR according to the following formula: [fasting insulin (milliunits per liter)] × [fasting glucose (millimoles per liter)]/22.5 [15]. Insulin sensitivity was calculated by the quantitative insulin sensitivity check index (QUICKI) according to the following formula: 1/{log [fasting insulin (milliunits per liter)] + log [fasting glucose (milligrams per deciliter)]}[16]. The HOMA-β, an index of β-cell function, was calculated using the following formula: 20 × [fasting insulin (milliunits per liter)]/[fasting glucose (millimoles per liter) – 3.5] [15]. Low-density lipoprotein cholesterol (LDL-C) (millimoles per liter) was calculated by the Friedewald equation: TC (millimoles per liter) – HDL-C (millimoles per liter) – triglyceride (millimoles per liter)/2.22. All assays were performed in accredited clinical laboratories. All PCOS patients underwent a 75-g oral glucose tolerance test as per standard clinical protocol to detect diabetes mellitus,

for which the subject would be excluded from this study. In our data analysis, subjects were classified into 2 subgroups: those with serum 25(OH)D levels less than 25 nmol/L (10 ng/mL), that is, "severe vitamin D deficiency," and those with 25(OH)D levels of at least 25 nmol/L.

2.3. Statistical analysis

The primary aim of this study was to determine the prevalence of vitamin D deficiency in a pilot group of PCOS women; exploratory analyses were also performed to investigate the characteristics of women with PCOS with and without severe vitamin D deficiency. Clinical and biochemical parameters between the 2 groups were compared using Mann-Whitney *U* test and χ^2 test for continuous and categorical variables, respectively. Correlation between variables was determined using linear regression analysis. To adjust for BMI and WTHR, analysis of covariance (ANCOVA) was used in the analysis of covariates. Given a previously reported 15% prevalence of severe vitamin D deficiency among British women in a national survey [17], a sample size of 16 to 25 PCOS patients was required to detect an increased prevalence (35%-40%) of severe vitamin D deficiency (type I error = 0.05, power = 0.80). Statistical tests were performed using SPSS 13.0 (SPSS, Chicago, IL). $P < .05$ was considered statistically significant.

3. Results

A total of 63 volunteers fulfilling the inclusion criteria were recruited into this study. Of these, 11 women were subsequently excluded from analysis because of concomitant use of hormonal medications (n = 8) or metformin (n = 1), the diagnosis of diabetes mellitus by glucose tolerance test (n = 1), or pregnancy (n = 1). Clinical and biochemical characteristics of the remaining 52 valid subjects (25 PCOS patients and 27 ovulatory controls) are summarized in Table 1. The PCOS patients were younger than the controls ($P < .001$); they also had higher BMI ($P < .005$) and WTHR ($P < .05$) than control women. In view of these differences in age and central obesity, correlations between biochemical parameters and vitamin D status in the 2 groups were analyzed separately (Table 2).

3.1. Vitamin D status

Serum 25(OH)D concentrations were highly variable in both PCOS patients and ovulatory controls, ranging from less than the detection limit (14 nmol/L) to 128 nmol/L. The majority of PCOS subjects (n = 18, 72%) were found to be vitamin D deficient, that is, 25(OH)D less than 50 nmol/L, of which 11 (44%) were severely deficient in vitamin D (ie, 25[OH]D <25 nmol/L), whereas a further 3 PCOS subjects (12%) had vitamin D insufficiency, that is, 25(OH)D between 50 and 74 nmol/L. The proportion of subjects classified as severely vitamin D deficient was considerably larger in the PCOS group (44.0%) than the ovulatory control group (11.2%) ($P < .05$, χ^2 test); however, medians of 25(OH)D levels were not statistically different between the 2 groups ($P > .05$, Mann-Whitney *U* test).

Table 1 – Clinical and biochemical characteristics of PCOS and ovulatory control subjects

	PCOS group (n = 25)	Ovulatory control group (n = 27)	*P*
Clinical parameters			
Age (y)	27.5 (18.6-35.9)	34.6 (27.3-40.4)	<.001*
BMI (kg/m^2)	30.8 (15.8-38.7)	23.5 (18.6-39.1)	.002*
WTHR	0.83 (0.66-0.99)	0.75 (0.67-0.91)	.017*
Systolic BP (mm Hg)	118 (97-155)	110 (90-138)	.006*
Diastolic BP (mm Hg)	80 (50-106)	75 (58-92)	.006*
Biochemical parameters			
25(OH)D (nmol/L)	27 (<14-101)	43 (<14-128)	.237
PTH (ng/L)	56 (26-124)	54 (19-80)	.190
Calcium (mmol/L)	2.43 (2.24-2.61)	2.42 (2.26-2.51)	.300
Fasting glucose (mmol/L)	5.0 (4.1-5.7)	4.8 (4.4-5.4)	.243
Fasting insulin (mU/L)	13.7 (4.6-51.0)	6.1 (4.0-31.9)	.001*
HOMA-IR	3.04 (0.90-12.69)	1.44 (0.78-6.95)	.001*
QUICKI	0.32 (0.27-0.39)	0.36 (0.29-0.40)	.001*
HOMA-β	184.4 (64.0-534.6)	92.3 (46.7-455.7)	<.001*
TC (mmol/L)	4.6 (3.5-7.9)	5.1 (3.7-7.6)	.378
LDL-C (mmol/L)	2.6 (1.8-5.7)	2.9 (1.8-4.8)	.720
HDL-C (mmol/L)	1.5 (0.9-2.0)	1.7 (1.2-2.6)	.009*
Fasting triglyceride (mmol/L)	1.2 (0.7-3.9)	0.8 (0.5-3.5)	.004*
TC/HDL-C ratio	3.3 (2.3-7.2)	2.8 (2.0-3.9)	.014*
CRP (mg/L)	2.2 (0.2-14.5)	0.8 (0.1-9.6)	.017*

Data are presented as median (range). BP indicates blood pressure.
* $P < .05$, Mann-Whitney *U* test.

Median concentrations of other calciotropic parameters including PTH and calcium were also not significantly different between the 2 groups (Table 1).

3.2. Vitamin D and obesity

In agreement with previous studies [8-10], levels of 25(OH)D were inversely associated with BMI in PCOS patients in our study (Table 2). In contrast, no such relationship between 25(OH)D and BMI was detectable in the control group. There was no statistically significant correlation of 25(OH)D levels with WTHR or age in either of the 2 groups.

3.3. Vitamin D and metabolic risk factors

Both insulin resistance and low HDL-C levels are cardinal features of metabolic syndrome. Among the PCOS subjects, we found a positive correlation of 25(OH)D levels with HDL-C concentrations and QUICKI, an index of insulin sensitivity. In comparison, there was a negative association between levels of 25(OH)D and CRP, which is a biomarker of inflammation, in PCOS women (Table 2).

The PCOS women with severe vitamin D deficiency were more insulin resistant (higher HOMA-IR, lower QUICKI) and had lower HDL-C than those with 25(OH)D of at least 25 nmol/L (Table 3). These associations of vitamin D status with HOMA-IR, QUICKI, and HDL-C were independent of BMI and WTHR. In contrast, whereas higher fasting insulin and CRP levels were detected in severely vitamin D–deficient PCOS women compared with those with 25(OH)D of at least 25 nmol/L, such

Table 2 – Correlation of 25(OH)D levels with clinical and biochemical parameters

	PCOS group (n = 25)		Ovulatory control group (n = 27)	
	R	P	R	P
Clinical parameters				
Age (y)	−0.064	.761	−0.046	.818
BMI (kg/m^2)	−0.428	.033*	−0.109	.588
WTHR	−0.328	.109	0.139	.490
Systolic BP (mm Hg)	−0.215	.303	0.155	.449
Diastolic BP (mm Hg)	−0.118	.573	−0.153	.455
Biochemical parameters				
PTH (ng/L)	−0.622	.001*	−0.474	.013
Calcium (mmol/L)	0.138	.509	0.331	.091
Fasting glucose (mmol/L)	−0.315	.125	0.002	.991
Fasting insulin (mU/L)	−0.347	.089	−0.367	.060
HOMA-IR	−0.354	.082	−0.354	.070
QUICKI	0.423	.035*	0.423	.028*
HOMA-β	−0.247	.235	−0.389	.045*
TC (mmol/L)	0.062	.770	−0.095	.636
LDL-C (mmol/L)	0.036	.865	−0.131	.515
HDL-C (mmol/L)	0.429	.033*	0.095	.638
TC/HDL-C ratio	−0.250	.228	0.019	.926
Fasting triglycerides (mmol/L)	−0.142	.500	−0.084	.678
CRP (mg/L)	−0.442	.027*	−0.072	.721
Total testosterone (nmol/L)	0.242	.243		
SHBG (nmol/L)	0.425	.038*		
FAI	−0.456	.025*		

* $P < .05$

differences became statistically insignificant after adjustment for BMI or WTHR (Table 3). Taken together, multiple well-recognized metabolic risk factors were found to be related to vitamin D status in PCOS women; among these, QUICKI and HDL-C were consistently associated with 25(OH)D levels, independent of BMI and WTHR.

Among the control subjects, similar associations were found between the severe vitamin D deficiency state and parameters of insulin metabolism, namely, fasting insulin level, HOMA-IR, and QUICKI. These associations remained significant after adjusting for WTHR, but became insignificant after adjusting for BMI, suggesting a BMI-dependent association between severe vitamin D deficiency and hyperinsulinemia/insulin resistance in the non-PCOS controls. The severe vitamin D deficiency state was also associated with higher LDL-C level, which was WTHR independent but BMI dependent (Table 4).

3.4. *Vitamin D and hyperandrogenemia*

Among PCOS women, 25(OH)D levels were associated positively with SHBG and negatively with FAI; there was no correlation between 25(OH)D and total testosterone (Table 2). The SHBG levels were lower in the severely vitamin D–deficient subgroup; but this difference was not statistically significant after adjusting for BMI and WTHR (Table 3), indicating obesity as a common determinant of both SHBG and 25(OH)D levels. Total testosterone and FAI were also not

Table 3 – Clinical and biochemical characteristics of PCOS subjects with and without vitamin D deficiency

	25(OH)D <25 nmol/L (n = 11)	25(OH)D ≥25 nmol/L (n = 14)	P value (Mann-Whitney U test)	P value adjusted by BMI (ANCOVA)	P value adjusted by WTHR (ANCOVA)
Clinical parameters					
Age (y)	25.4 (18.6-35.9)	27.7 (18.8-34.7)	.366		
BMI (kg/m^2)	33.6 (19.6-38.5)	28.8 (15.8-38.7)	.163		
WTHR	0.86 (0.66-0.90)	0.77 (0.68-0.99)	.208		
Systolic BP (mm Hg)	120 (104-140)	115 (97-155)	.826	.701	.786
Diastolic BP (mm Hg)	80 (50-106)	82 (64-93)	.238	.815	.687
Biochemical parameters					
PTH (ng/L)	60 (43-124)	49 (26-80)	.04*	.007*	.006*
Calcium (mmol/L)	2.43 (2.24-2.52)	2.47 (2.34-2.61)	.273	.261	.297
Fasting glucose (mmol/L)	5.0 (4.8-5.6)	4.8 (4.1-5.7)	.069	.141	.122
Fasting insulin (mU/L)	16.6 (4.8-51.0)	10. 0 (4.6-29.4)	.029*	.055	.054
HOMA-IR	3.91 (1.07-12.69)	2.15 (0.90-6.01)	.014*	.049*	.047*
QUICKI	0.31 (0.27-0.38)	0.34 (0.30-0.39)	.014*	.044*	.044*
HOMA-β	213.7 (64.0-485.7)	124.9 (78.8-534.6)	.075	.363	.421
TC (mmol/L)	4.5 (3.5-5.8)	5.0 (4.0-7.9)	.425	.186	.272
LDL-C (mmol/L)	2.6 (1.8-4.1)	2.6 (1.9-5.7)	.978	.621	.776
HDL-C (mmol/L)	1.1 (0.9-1.9)	1.6 (1.1-2.0)	.011*	.013*	.012*
TC/HDL-C ratio	4.1 (2.6-5.3)	3.2 (2.3-7.2)	.089	.370	.328
Triglycerides (mmol/L)	1.2 (0.8-2.0)	1.1 (0.7-3.9)	.294	.613	.601
CRP (mg/L)	3.2 (0.2-14.5)	1.6 (0.2-4.6)	.028*	.084	.081
Total testosterone (nmol/L)	2.1 (1.5-2.9)	2.4 (1.4-4.6)	.094	.141	.136
SHBG (nmol/L)	22 (12-121)	37 (20-91)	.017*	.714	.819
FAI	12 (1-17)	7 (2-13)	.137	.136	.204

Data are presented as median (range).
* $P < .05$.

Table 4 – Clinical and biochemical characteristics of non-PCOS controls with and without vitamin D deficiency

	25(OH)D <25 nmol/L (n = 5)	25(OH)D ≥25 nmol/L (n = 22)	*P* value (Mann-Whitney *U* test)	*P* value adjusted by BMI (ANCOVA)	*P* value adjusted by WTHR (ANCOVA)
Clinical parameters					
Age (y)	34.6 (27.3-39.9)	34.9 (29.2-40.4)	.786		
BMI (kg/m^2)	26.7 (23.5-39.1)	22.7 (18.6-37.9)	.064		
WTHR	0.79 (0.71-0.90)	0.75 (0.67-0.91)	.377		
Systolic BP (mm Hg)	120 (90-128)	108 (90-138)	.121	.785	.347
Diastolic BP (mm Hg)	84 (65-92)	74 (58-90)	.067	.307	.131
Biochemical parameters					
PTH (ng/L)	56 (44-64)	51 (19-80)	.411	.583	.424
Calcium (mmol/L)	2.41 (2.36-2.45)	2.43 (2.26-2.51)	.694	.846	.883
Fasting glucose (mmol/L)	4.9 (4.7-5.2)	4.8 (4.4-5.4)	.832	.767	.945
Fasting insulin (mU/L)	9.6 (5.5-31.9)	5.9 (4.0-14.2)	.033*	.091	.029*
HOMA-IR	2.09 (1.27-6.95)	1.25 (0.78-3.28)	.033*	.109	.034*
QUICKI	0.34 (0.29-0.37)	0.37 (0.32-0.40)	.033*	.141	.041*
HOMA-β	143.3 (64.7-455.7)	89.0 (46.7-215.0)	.055	.077	.027*
TC (mmol/L)	5.5 (4.5-7.6)	5.0 (3.7-6.2)	.086	.093	.057
LDL-C (mmol/L)	3.3 (2.6-4.8)	2.8 (1.8-4.1)	.033*	.084	.038*
HDL-C (mmol/L)	1.6 (1.2-2.3)	1.7 (1.3-2.6)	.447	.897	.641
TC/HDL-C ratio	3.0 (2.5-3.9)	2.8 (2.0-3.9)	.560	.618	.440
Triglycerides (mmol/L)	1.0 (0.5-3.5)	0.8 (0.5-1.6)	.739	.462	.202
CRP (mg/L)	1.7 (0.2-5.7)	0.7 (0.1-9.6)	.284	.380	.607

Data are presented as median (range).
* $P < .05$.

different between the 2 subgroups of PCOS women stratified according to vitamin D status.

4. Discussion

To our knowledge, this is the first study on the prevalence of vitamin D deficiency in PCOS women based in the United Kingdom. Because of the very low 25(OH)D concentrations commonly seen in the Scottish population, we made comparisons by dichotomizing our subjects with serum 25(OH)D less than 25 nmol/L (ie, severe vitamin D deficiency) vs those who were mildly deficient, insufficient, or sufficient in serum 25(OH)D (ie, serum concentration of 25 nmol/L or higher). A large proportion (44.0%) of PCOS women in our population was found to have 25(OH)D less than 25 nmol/L, compared with 11.2% of control women. In comparison, Wehr et al [8] reported that only 2.9% of PCOS women had 25(OH)D levels less than 25 nmol/L in Austria, whereas Hahn et al [9] detected very low 25(OH)D levels (<22.5 nmol/L) in 26.7% of PCOS women in Germany. Possible explanations for the large differences in vitamin D deficiency prevalence in these 3 studies include the northern latitudes and general lack of sunlight in Scotland, discrepancy in 25(OH)D assay performance (immunoassays in the previous 2 studies and tandem mass spectrometry in this study), seasonal variation of vitamin D status, and variable degree of obesity in the study populations. Although severe vitamin D deficiency was more prevalent in PCOS than control women in this study, the difference in median 25(OH)D concentrations between PCOS subjects (27 nmol/L) and ovulatory controls (43 nmol/L) did not reach statistical significance, possibly because of the relatively small sample size and the wide range of 25(OH)D values detected in both groups. Moreover, in comparing the proportions of severe vitamin D deficiency in PCOS and control groups, one must take into account the differences in BMI and age, both of which have been reported to affect serum 25(OH)D concentration.

Obesity is a well-recognized risk factor of vitamin D deficiency. An inverse correlation between BMI and serum 25(OH)D concentrations in PCOS women was demonstrated in this study and previous reports [8-10]. In obese individuals, a higher proportion of vitamin D, which is fat soluble, is sequestered in adipose tissues; and hence, bioavailability of the vitamin is lowered [18]. Alternatively, obese subjects may tend to spend less time outdoors exposed to sunlight, leading to insufficient vitamin D biosynthesis in skin. Dietary preference and vitamin D metabolism may also be different between obese and nonobese individuals. It is conceivable that the high prevalence of vitamin D deficiency in PCOS women in our study is to a large extent related to obesity.

Insulin resistance is common in women who have metabolic syndrome, PCOS, or both [19]. In line with other reports in both PCOS and non-PCOS individuals [8,9,20], our data show that severe vitamin D deficiency was associated with insulin resistance; this association was independent of BMI and WTHR in PCOS women but dependent of BMI in the non-PCOS controls. Our finding of higher insulin levels in non-PCOS subjects who were severely vitamin D deficient was also in agreement with a recent study [21]. Multiple cellular and molecular mechanisms have been proposed to explain this. The biologically active form of vitamin D, that is, 1,25-dihydroxyvitamin D, can directly produce several metabolic effects including (1) insulin release from β-cells, (2) expression of insulin receptor at the tissue level, and (3) suppression of the release of proinflammatory cytokines that are believed to mediate insulin resistance [20]. Vitamin D may also have a

beneficial effect on insulin action indirectly via its role in extracellular calcium regulation and normal calcium influx across cell membranes [22]. Besides being a cause of insulin resistance, a reduced circulating level of 25(OH)D could also be a consequence of insulin resistance. The interrelationship between vitamin D status and insulin resistance deserves further investigations. Interventional studies demonstrating improvement in glucose homeostasis and insulin sensitivity by vitamin D treatment were summarized in a recent literature review [20]. This association was BMI dependent in our non-PCOS controls but was independent of both BMI and WTHR in PCOS subjects; the reason for such a difference between the 2 groups is unclear and could be attributable to the small sample size and possible coexistence of other confounders.

Concerning the lipid profile, HDL-C was the only parameter found to have a significant correlation with 25(OH)D levels in PCOS women in our study. This finding was independent of BMI and concordant with results from 2 previous studies [8-9]. To date, a large body of evidence has accumulated linking low HDL-C levels with cardiovascular disease. It is therefore possible that by reducing serum HDL-C concentrations, vitamin D deficiency may indirectly contribute to increased cardiovascular risk in women with PCOS. Another important component of the metabolic syndrome is hypertriglyceridemia. Results from previous studies on a purported relationship between serum 25(OH)D and fasting triglyceride concentrations in PCOS women were conflicting [8-10]; our study detected no significant correlation between the 2 parameters in PCOS subjects. In a recent observational study, vitamin D (alfacalcidol) treatment slightly improved triglyceride and HDL-C levels in obese PCOS patients [23]; the magnitude of the reported changes was small, and the clinical significance remains to be elucidated.

One limitation of this study is the small sample size. Despite this, ours is the first report addressing the same issue in the United Kingdom that also confirmed the previously reported findings. This would form the basis for future studies. In the present study, a moderately large number of comparisons have been included; and our sample size was small, which might have led to some falsely significant findings. Hence, the genuine validity of our findings will need to be confirmed in larger replication studies in both Scotland and other countries where vitamin D deficiency is common. In particular, randomized controlled trials are warranted to explore the metabolic benefits of vitamin D therapy in PCOS patients. One recent Turkish study reported a significant reduction in HOMA-IR but not androgen indices after vitamin D replacement therapy in PCOS women [24], although this study was limited by its small sample size, and larger-scale trials are awaited.

5. Conclusions

The above results indicate that vitamin D deficiency is highly prevalent among women with PCOS in Scotland, more so than in ovulatory control women and the general UK population. We also demonstrate that vitamin D deficiency was associated with metabolic risk factors in PCOS women including insulin resistance and low HDL-C levels, independent of obesity measures. Metabolic benefits of vitamin D supplementation in PCOS women await to be investigated in future interventional studies.

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