Associations of vitamin D concentration with metabolic and hormonal indices in women with polycystic ovary syndrome presenting abdominal and gynoidal type of obesity

Zależności między stężeniem witaminy D a wskaźnikami metabolicznymi i hormonalnymi u kobiet z zespołem policystycznych jajników prezentujących brzuszny i gynoidalny typ otyłości

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Abstract

Goal: *The aim of the study was to estimate potential associations of vitamin D concentration with metabolic and hormonal indices in women with polycystic ovary syndrome (PCOS) presenting abdominal and gynoidal type of obesity.*

Material and methods: *Twenty-six women with PCOS (19-49 years old, BMI: 26.8-53.8 kg/m2), presenting predominantly abdominal and gynoidal type of obesity, were recruited. Anthropometric measures, body composition using dual-energy absorptiometry, fasting serum 25-hydroxyvitamin D, leptin, glucose, insulin, homeostatic model of assessment (HOMA), lipids, androgens and sex hormone-binding globulin (SHGB) were estimated.*

Results: *Vitamin D insufficiency was found in 2, and deficiency or deep deficiency in 12 patients. Levels of vitamin D were lower in obese than non-obese women, and in patients with abdominal as compared to gynoidal obesity (9.60±3.7 vs. 16.02±3.3 ng/mL, p<0.04). In obese women, vitamin D correlated negatively with all, except for gynoidal fat, measures of obesity, fasting glucose levels, and HOMA. No correlations with androgens were found. In women with abdominal obesity, vitamin D correlated with luteinizing hormone/follicle-stimulating hormone ratio (LH/FSH) and SHBG.*

Conclusions: *We demonstrated that women with PCOS are often vitamin D deficient. Its concentration was lower in patients with predominantly abdominal obesity as compared to subjects with gynoidal fat excess. In overweight/obese subjects with PCOS, vitamin D correlated with fasting glucose and HOMA. The correlation with LH/FSH suggests that vitamin D status may contribute to hormonal dysregulation. Further studies are needed to elucidate a potentially different impact of abdominal and subcutaneous fat on vitamin D metabolism.*

Key words: **vitamin D** / **abdominal obesity** / **gynoidal obesity** / / **polycystic ovary syndrome** /

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Streszczenie

Cel pracy: *Ocena potencjalnych zależności między stężeniem witaminy D a wskaźnikami metabolicznymi i hormonalnymi u kobiet z zespołem policystycznych jajników (PCOS) i otyłością brzuszną lub gynoidalną.*

Materiał i metody: *26 kobiet (19-49 lat, BMI 26,8-53,8 kg/m2) z PCOS, z przewagą otyłości brzusznej lub gynoidalnej. Oceniono pomiary antropometryczne, skład ciała metodą DEXA, 25-hydroksywitaminę D, leptynę, glukozę, insulinę, wskaźnik insulinooporności HOMA, lipidy, androgeny i globulinę wiążącą hormony płciowe (SHBG) w surowicy.*

Wyniki: *U dwóch badanych stwierdzono obniżone stężenie, a u dwunastu niedobór lub głęboki niedobór witaminy D. Stężenie tej witaminy było niższe u otyłych w porównaniu do nieotyłych kobiet z PCOS, a także u badanych z dominacją otyłości brzusznej w porównaniu do pacjentek z przewagą otyłości gynoidalnej (9,60±3,7 vs 16,02±3,3 ng/mL, p<0,04). U otyłych badanych stężenie witaminy D korelowało ze wszystkimi, z wyjątkiem tłuszczu gynoidalnego wskaźnikami otyłości, ze stężeniem glukozy i HOMA. Nie stwierdzono korelacji między witaminą D i androgenami. U badanych z dominacją otyłości brzusznej witamina D korelowała ze wskaźnikiem hormon luteinizujący/hormon stymulujący pęcherzyki (LH/FSH) i SHBG.*

Wnioski: *U kobiet z PCOS niedobór witaminy D jest częsty. Stężenie tej witaminy jest niższe u pacjentek z przewagą otyłości brzusznej w porównaniu do chorych z dominacją tłuszczu gynoidalnego i w obu grupach koreluje z BMI. U kobiet z nawagą/otyłością, stwierdzono korelację między witaminą D a glukozą na czczo i HOMA. Korelacja ze wskaźnikiem LH/FSH wskazuje na możliwy udział witaminy D w mechanizmie zaburzeń hormonalnych. Potrzebne są dalsze badania, które wyjaśnią wpływ tłuszczu brzusznego i gynoidalnego na metabolizm witaminy D.*

Słowa kluczowe: **witamina D** / **otyłość brzuszna** / **otyłość gynoidalna** / / **zespół policystycznych jajników** /

Introduction

Vitamin D is a prohormone produced mainly in skin through ultraviolet irradiation of 7-dehydrocholesterol. After vitamin D is metabolized to 25-hydroxyvitamin D and, in second step, to its active form - 1α,25-dihydroxyvitamin D, it acts through a specific nuclear receptor [1, 2]. Many genes encoding proteins that regulate cell proliferation, differentiation, and apoptosis are modulated, at least in part, by this vitamin. The majority of cells have vitamin D receptors, and some convert 25(OH)D to $1\alpha,25(OH)_2D$ [3].

Vitamin D is thought to influence the development of PCOS through gene transcription and hormonal modulation that influences insulin metabolism and fertility regulation [4, 5]. Inverse associations between vitamin D and indices of obesity in women with PCOS have been reported [6-8]. In several trials, low levels of vitamin D in women with PCOS, with average 25-hydroxyvitamin D levels between 11 and 31 ng/ml [6], with the majority having values of <20 ng/ml (67–85%), were found [7, 8].

Deficiency of this vitamin is associated with insulin resistance, signs of hyperandrogenemia, ovulatory and menstrual disturbances, lower pregnancy success, and elevated cardiovascular risk factors [7-10]. On the other hand, there are also evidences for beneficial effects of vitamin D supplementation on insulin resistance and menstrual dysfunction in women with PCOS [9, 11, 12].

In our study, we estimated potential associations of vitamin D with metabolic and hormonal indices in women with PCOS presenting abdominal and gynoidal type of obesity.

Material and methods

We evaluated 26 overweight and obese women, aged 19-49 years (28.4 ± 7.4), with BMI of 26.8 - 53.8 kg/m2 (35.7 ± 6.7). All subjects were enrolled from the population of out-patient clinics of gynecological endocrinology and endocrinology in Żoliborz, Warsaw. PCOS was diagnosed on the basis of the Rotterdam consensus criteria [13]. Other conditions: hyperprolactinemia, Cushing's syndrome, non-classical congenital adrenal hyperplasia were excluded. Also, current or previous (within the last 3 months) use of oral contraceptives and other hormonal drugs excluded from the study.

All patients were classified on the basis of measurements of body composition to the group with predominantly abdominal (group I) or gynoidal obesity (group II).

Subjects were studied after an overnight fast. Body height and weight were measured, and the body mass index (BMI) was calculated. Blood was collected at about 08^{00} h for vitamin 25(OH) D, glucose, lipids, (total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol and triglycerides), leptin, insulin, LH, FSH, testosterone, dehydroepiandrosterone-sulfate (DHEA-S), 17-hydroxyprogesterone and sex hormone-binding globulin (SHBG), through an iv catheter placed in the forearm. Homeostasis Model of Assessment - Insulin Resistance (HOMA) was calculated with the formula: fasting plasma insulin (microinternational units per milliliter) x fasting plasma glucose (millimoles per liter) /22.4. Free androgen index (FAI) was calculated as testosterone (nmol/l)/SHBG (nmol/l) levels. FAI >5 indicated hyperandrogenemia.

All of the investigated subjects underwent transvaginal ultrasonography (TV-US) and US of the abdomen to exclude adrenal pathology. Body composition was determined by dual-energy absorptiometry (DEXA). The same two operators performed all TV-US and DEXA measurements, respectively.

Levels of 25(OH)D were interpreted as follows: ≥30 ng/mL: norm; 21-29 ng/mL: insufficiency; 11-20 ng/mL: deficiency; ≤10 ng/ml: deep deficiency.

The local ethics committee approved the study and an informed consent was obtained from all participants.

Assays

Vitamin 25(OH)D was measured by a chemiluminescent immunoassay method with LIAISON® analyzer (DiaSorin, Italy), with the detection limit of <4.0 nmol/L (<10 nmol/l). Glucose, cholesterol and triglycerides were measured with Cobas integra 400 plus analyzer (Roche Diagnostics Ltd, Switzerland). Insulin was measured by an immunoradiometric method (Insulin IRMA – Immunotech SA, France); sensitivity was 2.0 mIU/ml. Leptin was measured by RIA (Linco Res. Inc, USA), using rabbit antibodies against human leptin. The sensitivity for this assay was 0.5 ng/ml. LH, FSH, estradiol, DHEA-S and total testosterone were measured by immunechemiluminescence method with IMMULITE 2000 (Siemens Healthcare Diagnostics, Inc); sensitivity for LH, FSH, estradiol, DHEA-S and testosterone were: 0.1 mIU/ml, 0.05 mIU/ml, 15 pg/ml, 30 ng/ml and 15 ng/ml, respectively. The results of testosterone were then multiplied by factor 3.46 to obtain nmol/L. 17-hydroxyprogesterone was measured by 17OH-progesterone-RIA-CT Kit (DIAsource ImmunoAssays SA, Belgium), with the detection limit of 0.02 ng/ml. SHBG was measured by IRMA method (Orion Diagnostics Oy, Finland), detection limit 1.3 nmol/l. Urinary 17-ketosteroids were measured by the Zimmermann method.

BMI was calculated as the body weight (kg)/height (m2). Measures of the fat mass were performed by the method in which abdominal fat is estimated in the region between the upper part of the pelvis with the upper margin 96 mm superior to the lower part of this region. The lateral part of this region is defined by the lateral part of the thorax. The upper part of the gynoid fat region is defined by the superior part of trochanter major, with the lower margin 96 mm inferior to the upper part of the trochanter major. The lateral part of this region is defined by the subcutaneous tissue on the hip, which can be visualized using the Image Values option [14, 15]. We used Lunar Prodigy (GE Lunar, Madison, WI, USA) equipment, which was calibrated each day with a standardized phantom and serviced regularly. The coefficient of variation for measurements of body composition with this method is about 2%.

Statistical analyses

All data are presented as the mean ± SD. The distribution of continuous variables was tested for normality by the Kolmogorov-Smirnov test. Comparisons between groups with normal distribution of the data were performed by unpaired Student's t-test. Pearson correlation analysis was used to examine bivariate relationships between data. For all analyses, a two-tailed $P \leq 0.05$ was considered as statistically significant. All calculations were performed with the Statistica 8.0 software package (StatSoft Inc, Tusla, OK, USA).

Results

Characteristics of patients with predominantly abdominal and gynoidal obesity are presented in Table 1. Six patients (23.1%) were overweight, thirteen were obese (50.0%) and the remaining seven (26.9%) were considered as morbidly obese. Fourteen subjects (53.8%) were insulin resistant according to HOMA, ten from group I and four from group II. All subjects were hyperandrogenic, although in five of them only clinical symptoms of hyperandrogenemia were found.

Two patients from group II were vitamin D insufficient, whereas deficiency and deep deficiency were found in ten (six from group I and four from group II) and two (from group I), respectively.

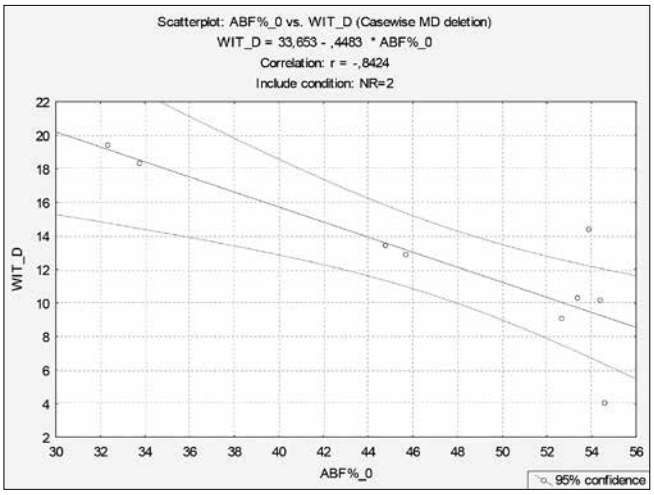

Figure 1. Negative correlation between serum 25-hydroxyvitamin D levels (ng/mL) and abdominal fat (%) in women with PCOS.
ABF – abdominal fat mass; ABF – masa tłuszczu brzusznego.

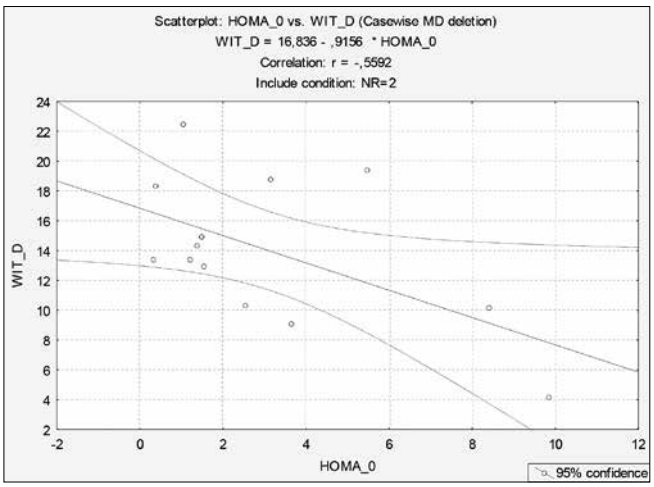

Figure 2. Negative correlation between serum 25-hydroxyvitamin D levels (ng/mL) and HOMA in overweight/obese women with PCOS.

Table II presents correlations between vitamin D and indices of fatness. In all of our patients vitamin D correlated negatively with all, except for gynoid fat, indices of obesity. Calculations performed separately for group I and II showed negative correlations between vitamin D and BMI. Figure 1 illustrates the correlation between vitamin D and abdominal fat.

An additional analysis revealed vitamin D levels to be significantly lower in women with higher abdominal fat mas (above medium) in comparison to patients with lower (below medium) abdominal fat: 9.6 ± 3.7 ng/mL vs. 16.40 ± 3.7 ng/mL, respectively (p=0.01). On the other hand, patients with higher vitamin D (above medium) in comparison to women with lower levels of this vitamin (below medium) had significantly lower abdominal fat mass and BMI: 39.9 ± 12.1% vs. 52.0 ± 12.1%, p<0.05, and 30.3 ± 3.4 kg/m2 vs. 37.4 ± 3.4 kg/m2, p<0.02, respectively.

Table I. Age, measures of fatness, metabolic parameters and hormonal results in patients with predominantly abdominal (n= 13, group I) and gynoidal (n=13, group II) obesity.

Parameter	Group I (n=13)	Group II (n=13)	P
	Mean ± SD	Mean ± SD	
Age (y)	30.2 ± 8.8	26.8 ± 6.2	NS
Body weight (kg)	104.1 ± 14.5	86.2 ± 16.6	<0.02
Body mass index (kg/m^2)	36.6 ± 4.8	33.6 ± 6.5	NS
Fat mass (kg)	50.1 ± 9.1	37.7 ± 9.9	0.005
Fat mass of the trunk (kg)	26.7 ± 4.1	18.9 ± 3.9	<0.001
Abdominal fat mass (%)	53.7 ± 2.3	43.2 ± 6.2	0.0001
Gynoidal fat mass (%)	54.3 ± 1.6	50.5 ± 2.0	<0.001
TC (mmol/L)	4.98 ± 0.9	5.46 ± 1.2	NS
LDL (mmol/L)	3.05 ± 0.8	3.39 ± 1.0	NS
HDL (mmol/L)	1.18 ± 0.3	1.28 ± 0.2	NS
TG (mmol/L)	1.62 ± 0.5	1.69 ± 0.9	NS
Glucose (mmol/L)	5.35 ± 0.8	4.69 ± 0.5	<0.03
Insulin (μIU/mL)	16.91 ± 8.1	11.15 ± 8.9	0.1
HOMA	4.22 ± 2.5	2.36 ± 1.9	<0.05
Leptin (ng/mL)	48.31 ± 23.2	44.01 ± 30.6	NS
Testosterone (mmol/l)	2.63 ± 1.3	3.82 ± 2.1	NS
DHEA-S (ng/ml)	2288.0 ± 1033.9	3032.0 ± 994.7	NS
17-KS (μg/24 h)	16.1 ± 4.6	22.1 ± 11.6	NS
Free androgen index (FAI)	10.41 ± 9.4	17.57 ± 11.1	NS
LH/FSH	1.22 ± 0.7	1.29 ± 0.6	NS
SHBG (nmol/L)	35.5 ± 22.8	28.3 ± 16.2	NS
25-hydroxyvitamin D (ng/mL)	9.60 ± 3.7	16.02 ± 3.3	<0.04

TC, total cholesterol; LDL, low density lipoprotein cholesterol; HDL, high density lipoprotein cholesterol; TG, triglycerides; HOMA, homeostatic model of assessment; DHEA-S, dehydroepiandrosterone-sulfate; 17-KS, 17-ketosteroids; LH/FSH, luteinizing hormone/follicle-stimulating hormone ratio; SHBG, sex hormone-binding globulin; NS, not significant
TC, cholesterol całkowity; LDL, cholesterol o małej gęstości; HDL, cholesterol o dużej gęstości; TG, triglicerydy; HOMA, *homeostatic model of assessment*; DHEA-S, siarczan dehydroepiandrosteronu; 17-KS, 17-ketosteroidy; LH/FSH, hormon luteinizujący/hormon stymulujący pęcherzyki; SHBG, globulina wiążąca hormony płciowe NS, brak znamienności statystycznej

Correlations of 25-hydroxyvitamin D with serum lipids and glucose metabolism indices are presented in Table 3. Significant negative correlations between vitamin D and glucose levels as well as with HOMA index (Figure 2) were found in all patients. Also, serum insulin levels correlated nearly significantly with vitamin D (r=-0.49, p=0.08)

Serum vitamin D levels correlated neither with serum testosterone and DHEA-S levels, nor with daily urine 17-ketosteroids excretion, but correlated significantly with the LH/FSH ratio (r=0.68, p=0.04). This correlation persisted when calculations were made separately for patients with predominantly abdominal type of obesity (Table IV).

Discussion

In our study, we attempted to investigate potential associations of vitamin D with metabolic and hormonal indices in women with PCOS, presenting abdominal and gynoidal type of obesity. We divided the overweight and obese patients on the basis of regional fat mass measures into groups with predominantly abdominal or gynoidal types of obesity.

Insufficiency in vitamin D was found in two subjects, ten patients were vitamin D-deficient, and in another two (26 and 41 years old) even deep deficiency was demonstrated. Levels of this vitamin were lower in the studied overweight/obese subjects as compared to lean women with PCOS (unpublished data), and correlated negatively with all, except for gynoidal fat, measures of obesity. However, only correlations with BMI persisted when calculations were performed separately for both groups. It could be speculated that small sample size and high SD contributed to lack of statistical significance in these cases.

Negative associations between serum vitamin D and BMI, body fat and waist circumference in women with PCOS have been reported [8, 16-18]. This vitamin is fat soluble and may be sequestered in adipose tissue with lower bioavailability, as a consequence [19]. Another explanation might be less time spent outdoors by obese subjects, with subsequent insufficient biosynthesis of this vitamin in the skin. Further, we demonstrated that women with PCOS with predominantly abdominal type of obesity have significantly lower levels of vitamin D in comparison to subjects with predominantly gynoidal fat. Additional analysis showed that levels of this vitamin are significantly lower in women with higher abdominal fat mass (above medium) in comparison to patients with lower abdominal obesity. In previously published studies in women with PCOS, vitamin D levels were associated with higher visceral fat surrounding vital organs and with subcutaneous fat [20, 21]. However, regional fat mass has not been taken into consideration in these trials. All these findings may suggest that the relationship between vitamin D and adiposity may not be as simple as increased sequestering of this vitamin just below the skin surface. In fact, they may indicate that greater amounts of visceral relative to subcutaneous fat could have an impact on vitamin D levels. Alternatively, greater relative amount of visceral fat and decreased vitamin D levels may be markers of the same condition. On the other hand, The Fourth Korea National Health and Nutrition Examination Survey (IV-2) revealed that waist circumference was not significantly associated with vitamin D. Also, a significant inverse in the association of this vitamin with fasting insulin and HOMA in both, normal and abdominally obese, groups was observed [22]. We found negative correlations between vitamin D, serum fasting glucose levels and HOMA. However, it was suggested previously that obesity may have a confounding role in this relationship. Multivariate regression analysis revealed that vitamin D level was a significant and independent predictor for HOMA, along with BMI [17]. Further studies will be needed to demonstrate conclusively whether vitamin D levels in patients with PCOS depend on the type of obesity, and to elucidate the potentially different role of abdominal and subcutaneous fat on vitamin D metabolism.

Table II. Correlations between serum 25-hydroxyvitamin D levels and indices of fatness in the studied women with PCOS. Pearson's correlation coefficients r(X,Y) are shown.

Parameter	All patients (n=26)	Group I (n=13)	Group II (n=13)
	r (X,Y)	r (X,Y)	r (X,Y)
Body weight (kg)	-0.93[c]	-0.85	-0.99
Body mass index (kg/m^2)	-0.97[c]	-0.92[a]	-0.99[a]
Fat mass (g)	-0.95[c]	-0.84	-0.97
Fat mass of the trunk (g)	-0.93[c]	-0.79	-0.99
Abdominal fat mass (%)	-0.84[b]	-0.28	-0.99
Gynoidal fat mass (%)	-0.37	0.83	-0.96

a – p< 0.05; b – p<0.01; c – p< 0.001

Table III. Correlations between serum 25-hydroxyvitamin D levels, serum lipids and indices of glucose metabolism in the studied women. Correlation coefficients r(X,Y) are shown.

Parameter	All patients (n=26)	Group I (n=13)	Group II (n=13)
	r (X,Y)	r (X,Y)	r (X,Y)
TC (mmol/L)	0.08	-0.40	-0.21
LDL (mmol/L)	0.16	-0.42	-0.42
HDL (mmol/L)	0.21	0.65	0.33
TG (mmol/L)	-0.46	-0.97[b]	-0.19
Glucose (mmol/L)	-0.67[a]	-0.69	0.34
Insulin (μIU/mL)	-0.49	-0.77	0.57
HOMA	-0.56[a]	-0.78	0.57

a – p<0.05; b – p<0.01; TC – total cholesterol; LDL – low density lipoprotein cholesterol; HDL – high density lipoprotein cholesterol; TG – triglycerides; HOMA – homeostatic model of assessment

Table IV. Correlations between serum 25-hydroxyvitamin D levels, serum androgens, 17-ketosteroids excretion, LH/FSH ratio and SHBG in overweight and obese women with a PCOS. Correlation coefficients r(X,Y) are shown.

Variable	All patients (n=26)	Group I (n=13)	Group II (n=13)
	r (X,Y)	r (X,Y)	r (X,Y)
Testosterone (nmol/L)	-0.01	-0.26	we-0.86
DHEA-S (ng/mL)	-0.46	-0.67	-0.18
17-ketosteroids (μg/24 h)	0.17	0.08	-0.87
Free androgen index	-0.10	-0.59	-0.44
LH/FSH	0.68[a]	0.98[b]	-0.49
SHBG (nmol/L)	0.17	0.98[a]	1.00

a – p<0.05; b – p<0.01

We did not find any correlations between vitamin D and androgens in our patients, neither in women with abdominal obesity nor in subjects with predominantly gynoidal fat. However, in women with predominantly abdominal obesity vitamin D correlated with LH/FSH index and with SHBG levels. Significant correlation with LH/FSH suggests that vitamin D status may contribute to hormonal dysregulation in women with PCOS. In a study that included 120 PCOS patients, an association of vitamin levels with free androgen index and SHBG, but not with total testosterone, androstenedione, DHEA-S, estradiol, or LH/FSH ratio, was found [8]. Our results are consistent with data on positive associations between vitamin D and SHBG levels [7, 8, 17]. It has been suggested that correlations between vitamin D status and hyperandrogenism may be due to the reduction in SHBG that results from obesity [7, 8].

There are several limitations to our study. First of all, the sample size was relatively small and the results are often accompanied by high SD. In our opinion, this may be the main cause of lack of statistical significance in some cases. Secondly, it was impossible to classify patients to the group

of purely abdominal or gynoidal obesity so patients with only predominantly abdominal or gynoidal fat excess were compared. Finally, not all subjects were able to complete all the tests, what can also cause errors.

Conclusion

In conclusion, we demonstrated that women with PCOS are often vitamin D deficient. Levels of this vitamin were lower in patients with predominantly abdominal obesity in comparison to subjects with gynoidal fat excess. In overweight/obese subjects with PCOS, vitamin D correlated with fasting glucose and HOMA index. Correlations of vitamin D with LH/FSH ratio and SHBG may suggest that vitamin D status may contribute to hormonal dysregulation, at least in patients with abdominal fat excess. Further studies are needed to elucidate the potentially different impact of abdominal and subcutaneous fat on vitamin D metabolism.

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