



Metabolic syndrome and the risk of cardiovascular complications in young patients with different phenotypes of polycystic ovary syndrome

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Abstract

Purpose Insulin resistance is an important factor in the pathogenesis of polycystic ovary syndrome (PCOS), which is associated with higher risk of metabolic syndrome (MetS) and cardiovascular complications. Early atherosclerotic lesions may be diagnosed by ultrasonographic parameters: brachial artery flow-mediated dilation after reactive hyperaemia (FMD) and intima-media thickness of common carotid artery (IMT). The aim of the study was to assess the relation of IMT and FMD with clinical and laboratory parameters reflecting metabolic status in young women with different PCOS phenotypes.

Methods The study included 154 PCOS patients diagnosed with the Rotterdam criteria, divided into four phenotypes, and 113 healthy women. Laboratory analyses, transvaginal ultrasound, and IMT and FMD measurements were conducted. MetS was diagnosed with International Diabetes Federation/American Heart Association (IDF/AHA) consensus criteria.

Results MetS was more prevalent in PCOS patients than healthy women (14.29 vs. 5.31%; $p = 0.019$), with highest prevalence in phenotypes I and II ($p = 0.039$). IMT and FMD did not differ between PCOS patients and the controls, nor between the PCOS phenotypes. PCOS patients with MetS presented lower FMD than other PCOS patients ($p = 0.018$). In women with PCOS, FMD correlated with glucose and insulin concentrations in the fasting state ($R = -0.33$, $p = 0.002$; $R = -0.23$, $p = 0.026$) and at 2 h of OGTT ($R = -0.29$, $p = 0.006$; $R = -0.26$, $p = 0.014$). In patients with phenotype I, correlations were found between IMT and BMI ($R = 0.45$, $p = 0.006$) and between FMD and fasting glucose concentrations ($R = -0.46$, $p = 0.011$).

Conclusions Metabolic disturbances and the diagnosis of MetS in patients with PCOS, especially in hyperandrogenic phenotypes, might be associated with alterations in IMT and FMD.

Keywords Polycystic ovary syndrome · Metabolic syndrome · Atherosclerosis · Vascular stiffness · Cardiovascular disease

Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrinopathy in reproductive-age women, affecting from 6% to as much as 20% of this group, depending on the applied diagnostic criteria [1]. The currently used Rotterdam criteria were formulated by the ESHRE/ASRM PCOS Consensus Workshop Group in 2003 and include the following features: (1) clinical and/or biochemical hyperandrogenism (HA), (2) oligo/amenorrhoea (OA), (3) polycystic ovarian morphology (PCOM) on transvaginal ultrasound [2]. To establish the diagnosis of PCOS, any two of the criteria must be fulfilled, providing that other possible

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causes of the presented symptoms have been excluded. As a result, PCOS patients form a heterogeneous group, differing in terms of clinical presentation. Depending on the fulfilled criteria, they can be divided into four phenotypes: I – HA + OA + PCOM; II – HA + OA; III – HA + PCOM; IV – OA + PCOM.

Metabolic syndrome (MetS) is a complex of clinical and laboratory features that increase the risk of cardiovascular disease (CVD) and diabetes [3]. The most widely used definition of MetS was proposed in 2009 in a joint statement of several international scientific associations [3]. It includes central obesity diagnosed by waist circumference, increased triglyceride (TG) concentration, decreased high-density lipoprotein cholesterol (HDL-C) concentration, impaired fasting glucose, and hypertension, or pharmacological treatment of the disorders mentioned above. To diagnose MetS, any three of the presented criteria have to be fulfilled. It has been established that PCOS patients are at a higher risk of developing MetS comparing to healthy, BMI-matched controls [4], which was also confirmed in non-obese women with PCOS [5].

In spite of an adverse metabolic profile in PCOS patients, studies investigating the actual risk of CVD development in this group have brought conflicting results [6–8]. However, regardless of the equivocal data, the Androgen Excess-Polycystic Ovary Syndrome Society guidelines suggest treating PCOS patients with MetS as being at high CVD risk [9]. The development of clinically overt CVD is preceded by subclinical vascular changes, which may be non-invasively diagnosed and monitored by ultrasonographic parameters: brachial artery flow-mediated dilation after reactive hyperaemia (FMD) and intima-media thickness of common carotid artery (IMT). It has been demonstrated that both parameters are strong predictors of future cardiovascular events [10, 11]. Higher values of IMT and lower values of FMD were reported in PCOS patients, in comparison to age- and BMI-matched control women [12, 13].

It has been shown that metabolic risk varies between patients with different PCOS phenotypes. Higher prevalence of MetS and more adverse metabolic profile were observed in women with hyperandrogenic phenotypes, comparing to normoandrogenic patients [14–16]. However, there is scarce data regarding the indices of subclinical vascular disease in women with different PCOS phenotypes: to the best of our knowledge, there is no data regarding FMD and only one study examined the differences in IMT between the phenotypes [17]. Therefore, the aim of the present study was to assess the values of IMT and FMD in young women with different phenotypes of PCOS and to investigate the relations of IMT and FMD

with clinical and laboratory parameters reflecting metabolic status in this group of patients.

Materials and methods

Study participants

The study group comprised 154 reproductive-age patients with PCOS diagnosed on the basis of the Rotterdam criteria, recruited among the patients of the Department of Internal Medicine and Metabolic Diseases, the Department of Endocrinology, Diabetology and Internal Medicine, as well as the Endocrinology Outpatient Clinic of the Medical University of Bialystok. The control group comprised 113 healthy women with no history of reproductive disorders, recruited from students and staff of the Medical University of Bialystok. Both groups were similar in terms of age and body mass index (BMI). The exclusion criteria for both groups were: age <18 or >35 years; other possible causes of menstrual irregularity or androgen excess, i.e. Cushing's syndrome, hyperprolactinaemia, late-onset congenital adrenal hyperplasia; administration of hormonal contraception in any form or other treatment affecting sex hormone concentrations within the previous three months; administration of medications affecting glucose and/or lipid metabolism; administration of medications affecting cardiovascular system function; pregnancy and/or breastfeeding within the previous 12 months; infection within the previous 30 days. All women participated in the study voluntarily and gave written informed consent prior to the inclusion in the study.

Depending on the fulfilled criteria, patients with PCOS were divided into four phenotypes: phenotype I (HA + OA + PCOM) – 63 patients; phenotype II (HA + OA) – 30 patients; phenotype III (HA + PCOM) – 24 patients; phenotype IV (OA + PCOM) – 23 patients. Fourteen PCOS patients presented hyperandrogenism and menstrual dysfunction, but did not have transvaginal ultrasound performed, and therefore the phenotype could not be determined. These patients were only included in the analyses regarding the whole PCOS group.

In all subjects, physical examination was performed. Body weight in kg and height in cm were recorded, and BMI was calculated. Waist circumference was measured at the smallest circumference between the rib cage and the iliac crest. Hip circumference was measured at the maximum circumference at the level of the femoral trochanters. Waist-hip ratio (WHR) was calculated from the measurements. Body composition was analysed using bioelectrical impedance (InBody Co., Ltd., CA, USA). Hirsutism was assessed with modified Ferriman–Gallwey score, with eight

points or more indicating clinically relevant hirsutism [18, 19].

Laboratory analyses

Laboratory analyses were performed after an overnight fast, in early follicular phase in spontaneously menstruating women, and independently of the cycle phase, at least three months after the last spontaneous menses, in amenorrhoeic patients. Concentrations of serum total cholesterol, HDL-C, and TG were measured using enzymatic colorimetric method (Cobas c111, Roche Diagnostic Ltd., Switzerland). Serum low-density lipoprotein cholesterol (LDL-C) concentration was calculated with the Friedewald's formula [20]. Oral glucose tolerance test (OGTT) with 75 g of glucose was performed. Serum glucose concentrations were measured by hexokinase method (Cobas c111, Roche Diagnostic Ltd., Switzerland). Serum insulin concentrations during OGTT were assessed by immunoradiometric method (DIASource ImmunoAssays S.A., Belgium). Homeostasis model assessment for insulin resistance (HOMA-IR) and Matsuda index were calculated according to the published formulas [21, 22]. As there is no universal cut-off point of HOMA-IR for defining insulin resistance [23–25], the present study analysed the absolute values of HOMA-IR, without adopting any specific cut-off value.

Concentrations of prolactin (PRL), luteinizing hormone (LH) and follicle-stimulating hormone (FSH) were measured by immunoradiometric assay (DIASource ImmunoAssays S.A., Belgium). Serum sex hormone-binding globulin (SHBG) was measured by immunoradiometric assay (ZenTech, Belgium). Concentrations of testosterone and oestradiol were measured by radioimmunoassay (DIASource ImmunoAssays S.A., Belgium). Minimum detectable concentration for testosterone was 0.05 ng/mL; intra-assay coefficient of variation (CV) was 3.3%, and inter-assay CV was 4.8%. Free androgen index (FAI) was calculated as testosterone [nmol/l] \times 100/SHBG [nmol/l]. Metabolic syndrome was diagnosed on the basis of International Diabetes Federation/American Heart Association (IDF/AHA) consensus [3].

Ultrasonographic assessment

Transvaginal ultrasound was performed by the same gynaecologist in early follicular phase in spontaneously menstruating women, and independently of the cycle phase in amenorrhoeic patients. Polycystic ovarian morphology was defined as the volume of at least one ovary exceeding 10 cm³ and/or the presence of at least 12 follicles in at least one ovary [26].

The assessment of IMT was performed in 106 PCOS patients and 91 healthy women, and FMD was assessed in

90 PCOS patients and 84 healthy women. The measurements were performed in the morning, after an overnight fast. No intensive exercise was performed prior to the measurements. The thickness of intima-media complex of common carotid arteries was measured at the distance of more than 1 cm from the bifurcation, three times on each side, and the mean value was derived from the six measurements. Right brachial artery diameter was measured 2–10 cm above the elbow. Baseline diameter was assessed three times before the occlusion. Pneumatic cuff was positioned on the right forearm and inflated to the pressure of 210 mmHg for three minutes to induce ischaemia. Three post-ischaemic measurements of brachial artery diameter were performed in the reactive hyperaemia phase, between 45 and 60 sec after the deflation of the cuff. Flow-mediated dilation was defined as a percentage change in the diameter of brachial artery before and after ischaemia.

Statistical analyses

Statistical analyses were performed using Statistica 13.0 (Statsoft, OK, USA) and GraphPad Prism 8 (GraphPad Software, CA, USA). Due to non-normal distribution of data, non-parametric tests were used in the analysis. The PCOS group and the control group were compared using Mann–Whitney *U* test. The differences between the four PCOS phenotypes were assessed using non-parametric Kruskal–Wallis test with Dunn's post-hoc test. The differences in MetS prevalence between the groups were assessed with Chi-square test. The correlations between the studied parameters were assessed with Spearman's correlation coefficient. Statistical significance was considered at $p < 0.05$.

Results

The groups of PCOS patients and control subjects did not differ in terms of age and BMI. Women with PCOS presented higher WHR and fat mass assessed by bioelectrical impedance, as well as higher values of diastolic blood pressure in comparison to the healthy controls ($p = 0.017$; $p = 0.045$; $p = 0.020$, respectively). Concentrations of total cholesterol, LDL-C, and TG were also significantly higher in this group ($p = 0.023$; $p = 0.009$; $p = 0.016$, respectively). Glucose concentrations at 30 and 60 min of OGTT, insulin concentrations in the fasting state and at 60 and 120 min of OGTT, as well as mean glucose and insulin concentrations were higher in women with PCOS. Metabolic syndrome was more prevalent in PCOS patients, comparing to healthy subjects (14.29 vs. 5.31%; $p = 0.019$). The clinical and biochemical characteristics of PCOS and control groups are presented in Tables 1 and 2.

Table 1 Clinical and hormonal characteristics of the studied groups

| | PCOS group (n = 154) | Control group (n = 113) | <i>p</i> |
|------------------------------|-------------------------|----------------------------|------------------|
| Age (years) | 24 (22–28) | 24 (22–28) | 0.743 |
| BMI (kg/m ²) | 24.2 (21.6–28.8) | 22.7 (21.5–25.1) | 0.056 |
| WC (cm) | 84.0 (75.0–94.0) | 80.5 (74.5–87.0) | 0.071 |
| WHR | 0.83 (0.79–0.89) | 0.81 (0.77–0.85) | 0.017 |
| Fat mass (kg) | 22.0 (15.9–32.6) | 19.7 (15.4–24.8) | 0.045 |
| Fat-free mass (kg) | 45.6 (42.5–50.3) | 45.5 (42.8–49.2) | 0.747 |
| PBF (%) | 32.4 (26.4–39.0) | 30.2 (25.4–34.5) | 0.052 |
| SBP (mmHg) | 115 (107–123) | 113 (106–119) | 0.145 |
| DBP (mmHg) | 75 (69–82) | 74 (67–78) | 0.020 |
| Ferriman-Gallwey score (pts) | 7 (3–11) | 4 (2–6) | <0.001 |
| Testosterone (ng/ml) | 0.79 (0.60–0.92) | 0.54 (0.41–0.68) | <0.001 |
| Oestradiol (pg/ml) | 61.21 (45.26–87.15) | 65.17 (43.84–83.12) | 0.714 |
| LH (IU/l) | 4.24 (3.16–6.13) | 3.58 (2.69–4.61) | <0.001 |
| FSH (IU/l) | 4.83 (3.92–6.14) | 5.36 (4.27–6.44) | 0.140 |
| PRL (ng/ml) | 10.08 (7.02–16.41) | 9.46 (6.89–15.0) | 0.272 |
| SHBG (nmol/l) | 45.3 (30.2–63.2) | 60.5 (37.6–90.5) | <0.001 |
| FAI | 5.72 (3.48–8.99) | 3.07 (1.70–4.78) | <0.001 |
| O-V (cm ³) | 15.0 (11.3–19.1) | 10.6 (7.7–13.8) | <0.001 |
| O-FN (<i>n</i>) | 22 (17–28) | 13 (11–16) | <0.001 |

Data are presented as median (interquartile range). *p* values were derived from Mann–Whitney *U* test

BMI body mass index, *WC* waist circumference, *WHR* waist-hip ratio, *PBF* percentage of body fat, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *LH* luteinising hormone, *FSH* follicle-stimulating hormone, *PRL* prolactin, *SHBG* sex hormone-binding globulin, *FAI* free androgen index, *O-V* ovarian volume (left + right), *O-FN* ovarian follicle number (left + right)

Bold values identify statistical significance (*p* < 0.05)

No significant differences between PCOS patients and the control group were observed regarding IMT (0.454 mm vs. 0.448 mm; *p* = 0.517) or FMD (10.58 vs. 11.58%; *p* = 0.645). In women with PCOS, there were significant correlations between FMD and all the parameters included in the definition of MetS, absent in the control group (Table 3). Both IMT and FMD correlated with BMI (*R* = 0.29; *p* = 0.003 and *R* = -0.22; *p* = 0.034, respectively) and percentage of body fat assessed by bioelectrical impedance (*R* = 0.24; *p* = 0.014 and *R* = -0.23; *p* = 0.038, respectively) in PCOS patients. Among the parameters assessed in OGTT, FMD correlated with fasting glucose and insulin concentrations (*R* = -0.33; *p* = 0.002 and *R* = -0.23; *p* = 0.027), glucose and insulin concentrations at 120 min of OGTT (*R* = -0.29; *p* = 0.006 and *R* = -0.25; *p* = 0.015), HOMA-IR (*R* = -0.26; *p* = 0.014), and Matsuda index (*R* = 0.28; *p* = 0.007) in this group of patients. Carotid IMT correlated with glucose concentration at 60 min of OGTT (*R* = 0.31; *p* = 0.001) and mean glucose concentration during OGTT (*R* = 0.25; *p* = 0.010). No such correlations were observed in the control group.

In healthy women, IMT and FMD correlated positively with age (*R* = 0.27; *p* = 0.010 and *R* = 0.35; *p* = 0.001; respectively). There was also a positive correlation between

Table 2 Metabolic characteristics of the studied groups

| | PCOS group (n = 154) | Control group (n = 113) | <i>P</i> |
|---------------------------|-------------------------|----------------------------|------------------|
| MetS (<i>n</i> ; %) | 22; 14.29% | 6; 5.31% | 0.019 |
| Glucose 0' (mg/dl) | 92 (88–97) | 92 (88–97) | 0.713 |
| Glucose 120' (mg/dl) | 95 (81–109) | 94 (80–105) | 0.447 |
| Mean glucose (mg/dl) | 112.5 (95.8–126.5) | 107.5 (94.0–116.8) | 0.025 |
| Insulin 0' (uIU/ml) | 10.2 (7.6–14.7) | 9.1 (7.3–12.2) | 0.047 |
| Insulin 120' (uIU/ml) | 39.4 (24.1–62.9) | 30.0 (21.7–47.1) | 0.009 |
| Mean insulin (uIU/ml) | 51.2 (34.6–75.8) | 39.0 (30.0–57.2) | <0.001 |
| HOMA-IR | 2.35 (1.74–3.31) | 2.08 (1.59–2.91) | 0.065 |
| Matsuda Index | 4.31 (3.08–6.27) | 5.37 (3.97–7.15) | 0.002 |
| Total cholesterol (mg/dl) | 175 (159–198) | 168 (149–189) | 0.023 |
| HDL-C (mg/dl) | 64 (52–75) | 63 (57–75) | 0.545 |
| LDL-C (mg/dl) | 95.9 (81.3–114.2) | 87.6 (72.5–102.8) | 0.009 |
| TG (mg/dl) | 65 (52–99) | 63 (48–80) | 0.016 |
| IMT (mm) | 0.454 (0.412–0.525) | 0.448 (0.412–0.515) | 0.517 |
| FMD (%) | 10.58 (5.98–15.98) | 11.39 (7.32–16.76) | 0.645 |

Data are presented as median (interquartile range). *p* values were derived from Mann–Whitney *U* test or Chi-squared test

MetS metabolic syndrome, *HOMA-IR* homeostasis model assessment for insulin resistance, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *TG* triglycerides, *IMT* intima-media thickness, *FMD* flow-mediated dilation

Bold values identify statistical significance (*p* < 0.05)

Table 3 Correlations between flow-mediated dilation and the criteria for metabolic syndrome

| | PCOS group | | Control group | |
|---------------------|------------|--------------|---------------|----------|
| | <i>R</i> | <i>p</i> | <i>R</i> | <i>p</i> |
| Waist circumference | -0.21 | 0.045 | -0.02 | 0.878 |
| SBP | -0.27 | 0.020 | -0.10 | 0.428 |
| DBP | -0.33 | 0.004 | -0.10 | 0.404 |
| HDL-C | 0.28 | 0.007 | -0.01 | 0.916 |
| TG | -0.21 | 0.044 | <0.01 | 0.998 |
| Glucose 0' | -0.33 | 0.002 | 0.09 | 0.436 |

The correlations were assessed with Spearman's test

SBP systolic blood pressure, *DBP* diastolic blood pressure, *HDL-C* high-density lipoprotein cholesterol, *TG* triglycerides

Bold values identify statistical significance (*p* < 0.05)

IMT and heart rate in this group (*R* = 0.36; *p* = 0.002). The above correlations were not significant in the PCOS group.

In the group of women with PCOS, patients with MetS (PCOS + MetS) were older (27 years vs. 24 years; *p* = 0.019) and presented higher BMI (31.0 kg/m² vs. 23.3 kg/m²;

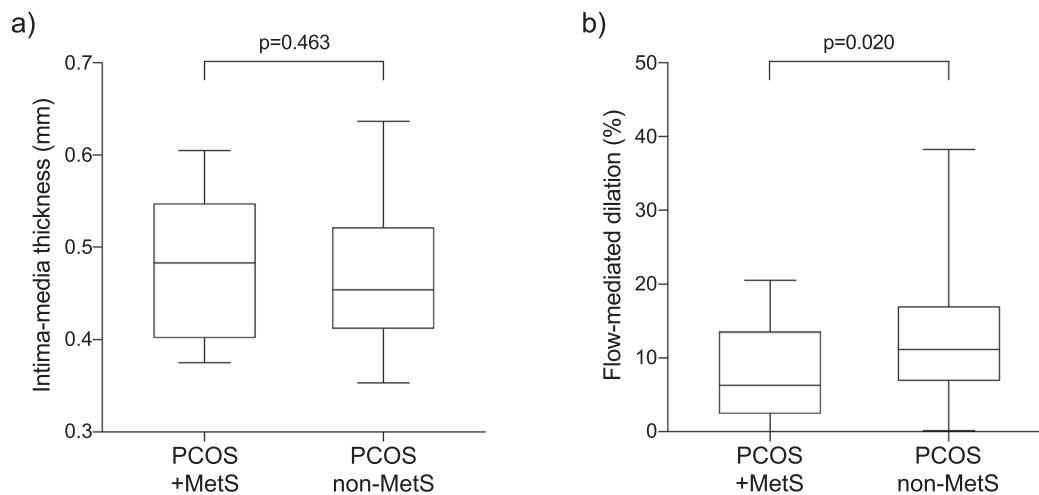


Fig. 1 The indices of subclinical vascular disease in PCOS patients with and without metabolic syndrome: **a** intima-media thickness of common carotid artery; **b** brachial artery flow-mediated dilation. The comparisons were made with Mann–Whitney *U* test

$p < 0.001$) in comparison to patients without MetS (PCOS/non-MetS). This group also presented higher values of FAI (9.51 vs. 5.23; $p < 0.001$) and lower concentrations of SHBG (23.59 nmol/l vs. 49.91 nmol/l; $p < 0.001$). No differences regarding IMT were observed between the two subgroups (0.483 mm vs. 0.454 mm; $p = 0.463$). Lower values of FMD were observed in PCOS + MetS, comparing to other PCOS patients (6.29 vs. 11.16%; $p = 0.020$) (Fig. 1). In women with PCOS + MetS, IMT correlated positively with BMI ($R = 0.78$; $p < 0.001$), waist circumference ($R = 0.74$; $p = 0.002$), hip circumference ($R = 0.56$; $p = 0.036$), and fat mass ($R = 0.76$; $p = 0.002$). No such correlations were observed in PCOS/non-MetS. No correlations were found between FMD and anthropometric parameters or the concentrations of sex hormones, lipids, or glucose in patients with PCOS + MetS.

Patients with the four PCOS phenotypes did not differ in terms of BMI. Women with phenotype I had higher WHR than phenotype IV ($p = 0.011$). Glucose and insulin concentrations during OGTT, but not fasting concentrations, differed between the phenotypes (all $p < 0.05$), with lowest mean glucose concentrations in phenotypes III and IV ($p < 0.001$). Matsuda index was higher in phenotype III in comparison to phenotype I ($p = 0.007$). The prevalence of MetS was the highest in PCOS patients with phenotypes I and II ($p = 0.039$). Clinical and biochemical characteristics of patients with different PCOS phenotypes are presented in Tables 4 and 5.

No differences in IMT and FMD were observed between the patients with different PCOS phenotypes ($p = 0.668$; $p = 0.268$) (Table 5). In PCOS patients with phenotype I, IMT correlated with BMI ($R = 0.45$; $p = 0.006$), waist circumference ($R = 0.39$; $p = 0.022$), diastolic blood pressure ($R = 0.60$; $p < 0.001$) and TG concentrations ($R = 0.35$;

$p = 0.034$). Significant correlations were also found between FMD and HDL-C ($R = 0.46$; $p = 0.011$) and fasting glucose concentrations ($R = -0.46$; $p = 0.011$). In phenotype II, significant correlations were observed between FMD and systolic and diastolic blood pressures ($R = -0.50$;

$p = 0.026$ and $R = -0.47$; $p = 0.038$). In phenotype III, FMD correlated negatively with BMI ($R = -0.61$; $p = 0.016$), diastolic blood pressure ($R = -0.81$; $p = 0.001$), and testosterone concentration ($R = -0.72$; $p = 0.002$). There was also a positive correlation between IMT and mean plasma glucose concentration ($R = 0.64$; $p = 0.003$). In phenotype IV, the only significant correlation was observed between FMD and diastolic blood pressure ($R = -0.66$; $p = 0.008$).

Discussion

The present study aimed to assess the indices of subclinical vascular disease in different phenotypes of PCOS and to investigate their relationship with clinical and laboratory parameters reflecting metabolic status. We demonstrated higher prevalence of MetS in women with PCOS in comparison to the control group. Among the patients with PCOS, the prevalence of MetS was the highest in phenotypes I and II. We also observed lower values of FMD in PCOS patients with MetS in comparison to PCOS patients without MetS. Both IMT and FMD were associated with individual criteria of MetS and the parameters of glucose metabolism only in the PCOS group. We did not observe the difference in IMT or FMD between the four PCOS phenotypes, despite significant differences in metabolic characteristics between the studied groups. In

Table 4 Clinical and hormonal characteristics of PCOS phenotypes

| | Phenotype I (n = 63) | Phenotype II (n = 30) | Phenotype III (n = 24) | Phenotype IV (n = 23) | p |
|------------------------------|---------------------------------|--------------------------------|-------------------------------|--------------------------|------------------|
| Age (years) | 25 (22–29) | 24 (23–28) | 24 (23–28) | 23 (22–27) | 0.662 |
| BMI (kg/m ²) | 24.2 (21.2–29.0) | 25.0 (22.0–29.8) | 23.4 (22.1–27.6) | 23.5 (19.9–27.1) | 0.439 |
| WC (cm) | 86.0 (75.0–98.0) | 82.0 (77.0–91.0) | 82.5 (74.5–91.5) | 83.0 (73.0–92.0) | 0.540 |
| WHR | 0.85 (0.81–0.91) ^c | 0.83 (0.79–0.90) | 0.80 (0.78–0.85) | 0.81 (0.77–0.84) | 0.011 |
| Fat mass (kg) | 22.7 (15.1–33.4) | 22.6 (16.6–35.0) | 21.8 (15.4–31.4) | 20.7 (16.3–28.1) | 0.661 |
| Fat-free mass (kg) | 45.4 (41.1–49.9) | 46.2 (42.8–49.0) | 45.1 (43.0–50.7) | 45.0 (42.6–48.5) | 0.949 |
| PBF (%) | 32.8 (25.7–41.0) | 32.1 (29.6–39.2) | 29.6 (24.8–36.4) | 32.6 (26.0–36.5) | 0.397 |
| SBP (mmHg) | 117 (110–128) | 114 (103–124) | 112 (106–122) | 115 (110–118) | 0.287 |
| DBP (mmHg) | 75 (69–82) | 75 (71–86) | 79 (73–88) | 71 (66–80) | 0.286 |
| Ferriman-Gallwey score (pts) | 6 (4–10) ^{a,c} | 11 (8–14) ^e | 7 (3–10) ^f | 2 (1–5) | <0.001 |
| Testosterone (ng/ml) | 0.88 (0.71–1.03) ^c | 0.78 (0.54–0.89) ^e | 0.83 (0.75–0.95) ^f | 0.59 (0.46–0.64) | <0.001 |
| Oestradiol (pg/ml) | 64.53 (47.00–96.80) | 58.97 (49.12–71.72) | 58.73 (46.82–70.90) | 54.58 (40.44–72.28) | 0.340 |
| LH (IU/l) | 4.76 (3.59–8.89) ^{a,b} | 3.75 (2.37–4.82) | 3.53 (2.95–4.62) | 4.47 (3.62–6.85) | 0.003 |
| FSH (IU/l) | 4.68 (3.72–6.07) | 4.81 (3.99–6.28) | 5.64 (4.80–6.24) | 4.66 (3.54–5.87) | 0.249 |
| PRL (ng/ml) | 9.47 (7.02–16.99) | 9.53 (6.77–13.58) | 12.8 (8.93–17.55) | 12.74 (8.08–20.13) | 0.190 |
| SHBG (nmol/l) | 44.5 (27.2–57.8) | 37.7 (30.2–63.0) | 48.8 (36.7–66.8) | 60.2 (34.8–79.7) | 0.308 |
| FAI | 6.86 (4.74–10.39) ^c | 5.76 (3.11–9.48) ^e | 6.33 (3.81–7.80) ^f | 2.91 (2.08–3.98) | <0.001 |
| O-V (cm ³) | 16.5 (13.6–21.0) ^a | 10.4 (7.5–14.8) ^{d,e} | 15.1 (12.0–19.8) | 15.8 (10.3–19.1) | <0.001 |
| O-FN (n) | 28 (21–33) ^{a,b} | 14 (13–17) ^{d,e} | 21 (18–25) | 24 (22–26) | <0.001 |

BMI body mass index, WC waist circumference, WHR waist-hip ratio, PBF percentage of body fat, SBP systolic blood pressure, DBP diastolic blood pressure, LH luteinising hormone, FSH follicle-stimulating hormone, PRL prolactin, SHBG sex hormone-binding globulin, FAI free androgen index, O-V ovarian volume (left + right), O-FN ovarian follicle number (left + right)

Data are presented as median (interquartile range). p values are derived from Kruskal–Wallis test

Bold values identify statistical significance ($p < 0.05$)

^a $p < 0.05$ phenotype I vs. II

^b $p < 0.05$ phenotype I vs. III

^c $p < 0.05$ phenotype I vs. IV

^d $p < 0.05$ phenotype II vs. III

^e $p < 0.05$ phenotype II vs. IV

^f $p < 0.05$ phenotype III vs. IV

hyperandrogenic PCOS phenotypes, IMT and FMD correlated significantly with metabolic parameters.

Polycystic ovary syndrome is widely recognised not only as a reproductive, but also a metabolic disorder. A number of studies demonstrated higher risk of MetS and its components in PCOS patients in comparison to the general population [4]. A recent meta-analysis revealed increased incidence of MetS, dyslipidaemia and glucose tolerance disturbances in non-obese PCOS patients [5]. Studies analysing different PCOS phenotypes showed higher prevalence of MetS and its components in women with phenotypes I and II, comparing to other PCOS patients [27–30]. Our results are in line with these findings – we demonstrated higher prevalence of MetS in patients with PCOS in comparison to healthy women, as well as its highest prevalence in PCOS patients with phenotypes I and II. Moreover, both groups analysed in the present study

included mainly lean patients and did not differ in BMI; despite that, higher values of diastolic blood pressure and higher concentrations of plasma lipids were already observed in PCOS patients in comparison to healthy women. Patients with the four PCOS phenotypes differed in terms of WHR, concentrations of fasting plasma glucose and LDL-C, and the prevalence of MetS, although they were comparable in terms of BMI. Similar results were obtained by other researchers, indicating that PCOS phenotypes are associated with different metabolic risk [14, 17].

Despite significant differences in metabolic profile between the PCOS patients and healthy women in our study, we observed no differences regarding IMT or FMD. It has been shown that both parameters are associated with higher risk of myocardial infarction or stroke [10, 11]. Two large meta-analyses demonstrated higher values of IMT and lower

Table 5 Metabolic characteristics of PCOS phenotypes

| | Phenotype I (n = 63) | Phenotype II (n = 30) | Phenotype III (n = 24) | Phenotype IV (n = 23) | P |
|---------------------------|------------------------------------|----------------------------------|---------------------------|--------------------------|------------------|
| MetS (n; %) | 12; 19.05% | 6; 20.00% | 0; 0.00% | 1; 4.35% | 0.039 |
| Glucose 0' (mg/dl) | 92 (89–98) | 95 (89–99) | 90 (84–95) | 90 (85–94) | 0.047 |
| Glucose 120' (mg/dl) | 101 (86–118) ^{a,b} | 98 (85–119) ^c | 84 (76–95) | 83 (78–97) | <0.001 |
| Mean glucose (mg/dl) | 119.1 (107.8–130.3) ^{a,b} | 118.0 (105.0–130.5) ^c | 99.8 (89.3–112.3) | 97.0 (90.5–118.5) | <0.001 |
| Insulin 0' (uIU/ml) | 11.3 (8.2–15.5) | 10.2 (6.9–14.5) | 8.56 (7.23–10.35) | 10.2 (7.7–14.7) | 0.219 |
| Insulin 120' (uIU/ml) | 48.8 (31.8–84.9) ^a | 32.4 (25.3–48.5) | 23.58 (15.36–41.67) | 33.6 (19.0–51.4) | 0.004 |
| Mean insulin (uIU/ml) | 58.2 (38.7–77.8) | 55.8 (33.0–75.7) | 40.13 (24.76–53.75) | 47.9 (34.3–57.6) | 0.141 |
| HOMA-IR | 2.55 (1.84–3.69) | 2.44 (1.44–3.22) | 1.85 (1.48–2.55) | 2.24 (1.68–3.31) | 0.224 |
| Matsuda Index | 3.66 (2.77–5.42) ^a | 4.35 (3.00–6.14) | 6.22 (4.30–7.84) | 5.26 (3.26–6.85) | 0.007 |
| Total cholesterol (mg/dl) | 180 (157–202) | 171 (162–198) | 168 (154–180) | 181 (164–203) | 0.122 |
| HDL-C (mg/dl) | 64 (49–74) | 62 (50–70) | 70 (58–78) | 66 (55–80) | 0.220 |
| LDL-C (mg/dl) | 103.7 (81.8–116.6) ^a | 92.2 (82.2–114.2) | 85.4 (67.6–91.0) | 103.5 (85.0–111.0) | 0.008 |
| TG (mg/dl) | 68 (52–111) | 64 (52–106) | 63 (48–89) | 60 (52–77) | 0.521 |
| IMT (mm) | 0.465 (0.413–0.535) | 0.463 (0.433–0.535) | 0.450 (0.411–0.508) | 0.443 (0.415–0.510) | 0.668 |
| FMD (%) | 10.71 (5.98–15.98) | 9.68 (5.36–12.05) | 14.45 (8.12–24.67) | 11.87 (5.23–15.73) | 0.268 |

MetS metabolic syndrome, HOMA-IR homeostasis model assessment for insulin resistance, QUICKI quantitative insulin check index, HDL-C high-density lipoprotein cholesterol, LDL-C low-density lipoprotein cholesterol, TG triglycerides, IMT intima-media thickness, FMD flow-mediated dilation

Data are presented as median (interquartile range). *p* values are derived from Kruskal-Wallis test or Chi-squared test

Bold values identify statistical significance ($p < 0.05$)

^a $p < 0.05$ phenotype I vs. III

^b $p < 0.05$ phenotype I vs. IV

^c $p < 0.05$ phenotype II vs. III

values of FMD in women with PCOS [12, 13], although the results obtained by individual researchers are equivocal. A number of studies reported comparable values of IMT and FMD between PCOS patients and controls [31–33]. The discrepancy may partially be explained by the characteristics of the examined populations of patients. In most studies, which found significant differences between women with PCOS and controls regarding IMT or FMD, mean BMI of patients was higher than in our study [34–36]. This might indicate that the influence of overweight or obesity on sub-clinical vascular changes might be greater in PCOS patients than in healthy women, highlighting the need for more strict metabolic control in this group. In line with that assumption, Dahan and Reaven observed significantly higher concentrations of insulin only in obese PCOS patients in comparison to obese control women, while no such differences were observed in the subgroups of lean and overweight subjects [37]. The authors of the aforementioned study therefore concluded that obese PCOS patients present hyperinsulinemia of a magnitude overcoming the effect of obesity alone and putting them at an increased metabolic risk, in comparison to BMI-matched control women [37]. Another factor contributing to discrepant results between the present study and the published reports may be the applied diagnostic criteria – in the studies by Luque-Ramírez et al. [35] and Alexandraki et al. [38], PCOS was diagnosed on the basis of National Institutes of Health (NIH) criteria, and it has been established that patients with the “classic” NIH phenotypes

present more adverse cardiometabolic profile [29]. Moreover, some studies included control groups that differed from the PCOS group in terms of BMI and therefore it was impossible to exclude the influence of body mass on the results [39]. In our study, both groups included mainly lean patients and did not differ in terms of BMI.

It is worth highlighting that PCOS patients presented higher WHR and fat mass than control women, despite no difference in BMI. In line with these findings, several studies reported higher values of waist circumference, waist/hip ratio, or visceral fat content in patients with PCOS in comparison to BMI-matched healthy women [40–42]. Apart from serving as energy storage, adipose tissue is responsible for synthesis and secretion of adipokines regulating a number of metabolic processes [43]. Visceral obesity is associated with higher risk of cardiovascular disease due to an increased proinflammatory activity, altered adipokine production, and promotion of insulin resistance [44]. The association of visceral adipose tissue with IMT was observed both in healthy subjects [45] and PCOS patients [42, 46]. The present study demonstrated significant correlations between IMT and FMD and the percentage of body fat assessed by bioelectrical impedance in the PCOS group. These findings, along with higher WHR and fat mass in this group, irrespective of BMI, might be associated with an increased cardiovascular risk.

In the present study, patients with PCOS and MetS presented lower concentrations of SHBG and higher values

of FAI, comparing to PCOS/non-MetS. Similar results were obtained by Tziomalos et al. [47] and Albu et al. [48], supporting the hypothesis that hyperandrogenism may exacerbate metabolic disturbances. It has been demonstrated that androgens increase visceral adiposity and affect adipokine secretion, thus exacerbating insulin resistance [49]. They also affect endothelial cells by inducing inflammation and oxidative stress, which results in the development of atherosclerosis [50]. Li et al. demonstrated that PCOS patients with FAI values ≥ 5 showed higher BMI, percentage of body fat and HOMA-IR, in comparison to women with PCOS and lower values of FAI [51]. In line with that, the present study demonstrated higher values of FAI in patients with PCOS and MetS, who also presented lower values of FMD, comparing to PCOS/non-MetS. A significant correlation was also found between testosterone concentration and FMD in PCOS patients with phenotype III. No correlations were observed between FMD and the concentrations of sex hormones in PCOS + MetS, although it might be associated with a small number of patients in this group. The difference in SHBG concentrations between PCOS patient with and without MetS observed in the present study was expected, as it has been previously reported that women with MetS present lower concentrations of SHBG in comparison to healthy women, regardless of the presence of PCOS [52].

It should be highlighted that PCOS patients with and without MetS differed in terms of age and BMI. However, MetS is defined by coexistence of clinical and laboratory features and its influence on FMD cannot be attributed solely to the presence of obesity. Cardiovascular consequences of MetS are a result of the effects exerted by all components and it is impossible to discern the impact of each individual factor. In the present study, in the whole PCOS group, significant correlations were observed between the values of FMD and each criterion included in the definition of MetS, which indicates that not only anthropometric parameters, but also other metabolic factors affect FMD. Moreover, although the groups of PCOS patients with and without MetS differed in age, they both included young women and the difference was not major (27 vs. 24 years). Parker et al. demonstrated an approximately two-fold decrease in FMD between young and elderly subjects, but the mean age of both groups was 22 years and 70 years, respectively [53]. The results from the Framingham Heart Study also confirmed an inverse relation between age and FMD, although the age-associated decrease in FMD was found to be 0.58% for each 10 years [54], which might suggest that the difference in FMD between our studied groups should not be significantly affected by the age difference.

In spite of the evidence from literature linking hyperandrogenism and metabolic disturbances, no significant

differences in IMT or FMD between normoandrogenic and hyperandrogenic PCOS phenotypes were observed in the present study. This might suggest that hyperandrogenaemia is not the sole factor influencing CVD risk in women with PCOS. One study investigating PCOS patients with different phenotypes actually showed lowest values of IMT, as well as highest concentrations of oestradiol in patients with ovulatory phenotype [17]. The authors suggested a putative protective effect of oestradiol on cardiovascular system [17]. In line with that, the association of testosterone, oestradiol, and oestradiol/testosterone ratio with CVD development later in life was observed in postmenopausal women [55]. Moran et al. observed that SHBG, but not testosterone concentration, was associated with MetS in overweight and obese PCOS patients, independently of BMI or glucose tolerance [56]. However, no correlations between SHBG concentrations and IMT or FMD were observed in the present study in patients with PCOS + MetS, probably due to a small sample size. These findings might suggest that not only hyperandrogenaemia, but the interplay between sex hormones, contributes to CVD risk.

It has also been shown that insulin could affect vascular function via multiple mechanisms, such as impairment of nitric oxide production, promotion of smooth muscle proliferation, increase in synthesis of proinflammatory and vasoconstrictive factors, or increase in the concentrations of circulating androgens [49, 57]. In line with that, the present study demonstrated correlations between indices of endothelial dysfunction and glucose metabolism parameters, which might indicate the role of hyperinsulinaemia and insulin resistance in the development of subclinical CVD.

Despite the fact that PCOS patients present more adverse metabolic profile in comparison to healthy women, the data regarding the actual risk of CVD are equivocal. In a recent Danish nationwide study, analysing a total number of over 70,000 women over a period of approximately 11 years, Glintborg et al. found that the incidence rate for CVD was higher in PCOS patients, comparing to the control group, and that the age at CVD diagnosis was significantly lower in PCOS [6], while other researchers reported comparable CVD risk ratios between PCOS women and control subjects [7, 8]. Regardless of the inconclusive results, the Androgen Excess-Polycystic Ovary Syndrome Society guidelines suggest treating PCOS patients with MetS as being at high CVD risk [9]. In line with that, the latest guidelines, developed by the Centre for Research Excellence in Polycystic Ovary Syndrome, European Society of Human Reproduction and Embryology and American Society of Reproductive Medicine, recommend screening for traditional CVD risk factors, i.e., overweight/obesity, hypertension and dyslipidaemia, in PCOS women regardless of age [58]. The studies which found significant differences in IMT or FMD between PCOS patients and healthy women

analysed mainly overweight or obese subjects [34–36], which might suggest that PCOS confers an additional CVD risk only in the presence of overweight or obesity. Additionally, in the present study, lower values of FMD were observed in women with PCOS and MetS in comparison to PCOS patients without MetS, whereas no difference was found in the control group. Therefore, the assessment of IMT and FMD should be considered particularly in PCOS patients with MetS or its components, as this group seems to present an increased CVD risk, higher than women with MetS but without PCOS.

A number of limitations of the present study have to be addressed. The main limitation is a small number of patients representing each PCOS phenotype. Additionally, a small number of patients with MetS in the control group did not allow to compare vascular parameters between MetS patients with and without PCOS. Another limitation is the assessment of testosterone concentrations with radioimmunoassay and not with liquid chromatography-mass spectrometry, which is a method recommended in the recent guidelines [58]. Moreover, the studied population included only young women, aged between 18 and 35 years, and it might be too early to assess the indices of cardiovascular dysfunction. A prospective study, analysing the actual development of CVD in PCOS patients with different phenotypes of the syndrome, would bring more information regarding the usefulness of IMT and FMD in predicting CVD.

In conclusion, young patients with PCOS are at an increased risk of developing metabolic abnormalities in comparison to the general population. It seems that the highest risk is present in patients with PCOS phenotypes associated with both hyperandrogenism and menstrual irregularity. The presence of metabolic syndrome or its components in PCOS patients, especially with phenotype I, may be associated with an increased cardiovascular risk. Central obesity, regardless of BMI, might constitute an additional risk factor. The assessment of IMT and FMD may be useful in the evaluation of early vascular changes in this group of patients. Other hormonal factors might also affect cardiovascular risk in PCOS patients, although further studies are required to verify this hypothesis.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethics approval This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of the Medical University of Białystok (approval No. R-I-002/221/2016).

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