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Polycystic ovary syndrome: cardiovascular risk factors according to specific phenotypes

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Key words
Polycystic ovary syndrome, phenotypes, insulin resistance, cardiovascular disease, chronic low-grade inflammation, endothelial dysfunction

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Conflict of interest
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Abstract

Introduction. Polycystic ovary syndrome (PCOS) is associated with obesity and insulin resistance. The objective of this cross-sectional study was to investigate the impact of insulin resistance and body mass index (BMI) on inflammatory and hemostatic variables associated with long-term risk of cardiovascular disease in women with PCOS. *Material and methods.* 149 premenopausal women with PCOS were recruited consecutively from April 2010 to February 2012 at three Danish University Hospitals. The study was conducted at the Department of Gynecology and Obstetrics, Herlev University Hospital, Denmark. PCOS was diagnosed in accordance with the Rotterdam criteria and the women were classified into four phenotypes according to BMI and insulin resistance measured by the homeostasis model assessment of insulin resistance index. Body composition was determined by dual-energy X-ray absorptiometry. Main outcome measures were the biomarkers C-reactive protein (CRP), plasminogen activator inhibitor-1 (PAI-1), and von Willebrand factor antigen. *Results.* Normal weight insulin-resistant PCOS women were characterized by abdominal obesity and elevated levels of plasma PAI-1. Overweight/obese insulin-resistant PCOS women had increased levels of both PAI-1 and CRP. Of the three Rotterdam criteria, only hyperandrogenemia was significantly associated with the hemostatic risk marker of long-term cardiovascular disease risk. *Conclusions.* Surrogate risk markers for cardiovascular disease are elevated in women with PCOS, especially insulin-resistant and overweight/obese women.

Abbreviations: CRP, C-reactive protein; CV, coefficient of variance; CVD, cardiovascular disease; FSH, follicle stimulating hormone; HOMA-IR, homeostasis model assessment of insulin resistance; IR, insulin resistance; LH, luteinizing hormone; PAI-1, plasminogen activator inhibitor-1; PCOS, polycystic ovary syndrome; T, testosterone; vWF, von Willebrand factor; WC, waist circumference.

Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrinological abnormality in women of reproductive age. The prevalence of PCOS is dependent on ethnicity, lifestyle, age, body mass index (BMI) and the diagnostic criteria used. The diagnosis is currently based on the Rotterdam criteria, with a prevalence as high as 15% in the adult female population (1). The Rotterdam criteria

require two of three criteria fulfilled: anovulation, hyperandrogenism and/or polycystic ovaries, causing clinical and biochemical heterogeneous phenotypes.

Based on the higher rates of risk factors for cardiovascular disease (CVD) in women with PCOS, it has been assumed that the risk of long-term CVD is increased (2,3). The prognostic value of the Rotterdam criteria and different Rotterdam phenotypes regarding risk of CVD are currently being discussed (4,5). Insulin resistance (IR), obesity and hyperandrogenism are thought to play a pivotal role in the complex etiology of PCOS. IR is seen in 50–80% of the women with PCOS (6) and is partly independent of BMI (7). Approximately 60% of the women with PCOS are obese (8). IR and obesity are associated with chronic low-grade inflammation, endothelial dysfunction and hemostatic derangements, and precede development of type 2 diabetes and CVD (1,9).

In the general population, markers of inflammation and endothelial dysfunction such as plasma levels of C-reactive protein (CRP), plasminogen activator inhibitor-1 (PAI-1) and von Willebrand factor (vWF) have been validated as the most predictive for CVD (10–12). These risk markers have been studied in women with PCOS, but the studies are small and the results are conflicting (8,13).

Women with PCOS should be informed about the increased risk of CVD, according to the individual clinical phenotype. However, there is no evidence-based consensus about how to distinguish high-risk women with PCOS. The aim of the present study was therefore to evaluate whether four phenotypes of women with PCOS based on BMI and IR are associated with distinct metabolic profiles with special reference to markers of chronic low-grade inflammation, endothelial dysfunction as well as fat distribution. If so, BMI and IR should be included when CVD risk stratification is determined for PCOS women diagnosed according to the Rotterdam criteria. A secondary aim was to evaluate whether the Rotterdam criteria and various Rotterdam phenotypes are associated with the investigated risk markers of CVD.

Material and methods

The study was derived from the original Danish multicenter PCOS collaboration, the PICOLO study (14). This prospective cross-sectional clinical study comprised consecutively recruited premenopausal, 18- to 40-year-old women with PCOS, referred to three Danish university hospitals because of infertility or gynecological symptoms during the period April 2010 to February 2012.

At the first of three visits all participants were assessed using standardized baseline screening as described previously (14). PCOS was diagnosed according to the 2003 Rotterdam criteria. Whole body dual-energy X-ray absorptiometry was performed and blood samples were collected at the second visit after an overnight fast of at least 8 h. Blood was collected from women with regular menstrual cycles on the third to fifth cycle day and from women with irregular menstrual periods on a random day. All samples were collected between 8:00 and 9:30 h. Blood samples were drawn from an antecubital vein with a stasis of 40 mmHg, monitored by a manometer and a blood pressure-cuff. Samples related to PCOS screening and metabolism were analysed immediately (14). The other samples were centrifuged at 2000 ***g*** for 20 min, and stored in cryotubes at −80°C until analysis. At the third visit, a standard 2-h oral glucose tolerance test with a 75-g glucose load was performed. A 2-h plasma glucose concentration <7.8 mmol/L was considered to be normal glucose tolerance, ≥7.8 and <11 mmol/L impaired glucose tolerance and ≥11 mmol/L diabetic.

Oligomenorrhea was defined as menstrual cycle over 35 days and amenorrhea was defined as no menstrual cycle for 3–6 months. Clinical hyperandrogenism was quantified by a modified Ferriman–Gallwey score – a score ≥6 indicated clinically significant hirsutism (15). Biochemical hyperandrogenism was defined as total testosterone (total T > 1.8 nmol/L) and/or free testosterone (free T > 0.034 nmol/L) higher than the upper limit of the normal range for premenopausal women. Ovarian morphology was assessed by transvaginal ultrasound (Ultrasound Scanner, Class 1 type B, B-K Medical REF TYPE 2202. Bkmed.com). Antral follicles between 2 and 9 mm were counted; an ovary with an antral follicle count ≥12 was classified as polycystic. Waist circumference (WC) was measured at the level of umbilicus. Body weight was measured in light clothing and without shoes (OMRON BF 500. Omron-healthcare.com). Height was measured by a wall-mounted stadiometer to the nearest 0.5 cm. BMI was calculated as: body weight (kg)/[height (m)]^2. Blood pressure was measured by an electronic monitor (OSZ 5 Easy, Welch Allyn 2003, Dublin, Republic of Ireland; Intl.welchallyn.com). A larger cuff was used when measuring blood pressure in women with a BMI > 25.

Pregnant and breastfeeding women, as well as women with hyperprolactinemia, thyroid, renal or hepatic dysfunction, diabetes type 1 or 2, congenital adrenal hyperplasia and premature ovarian failure were excluded. A

Key Message

Evaluating the risk of cardiovascular disease in women with polycystic ovary syndrome should rely on determination of insulin resistance and body mass index rather than using the Rotterdam criteria.

wash-out period of at least 6 weeks before enrolment was required for women treated with insulin sensitizers, acetylsalicylic acid and/or hormonal treatment including oral contraceptives.

Body composition

In 100 of the 149 women body composition was estimated by whole body dual-energy X-ray absorptiometry (Hologic, Model Discovery 2009). Forty-nine of the PCOS women were recruited at the Fertility Clinic, Holbaek University Hospital; dual-energy X-ray absorptiometry was not performed for these women because of logistic problems. Lean mass, total fat mass, android and gynecoid fat mass were measured. Total fat mass was divided by height squared. The android and gynecoid regions of interest were predefined as a template overlay that could be adjusted to match the patient's anatomy.

Classification of the Rotterdam phenotypes

Combinations of the Rotterdam criteria resulted in four phenotypes: (i) anovulation + hyperandrogenism, (ii) anovulation + polycystic ovaries, (iii) hyperandrogenism + polycystic ovaries and (iv) anovulation + hyperandrogenism + polycystic ovaries.

The homeostasis model assessment of insulin resistance (HOMA-IR) index was used to estimate IR. HOMA-IR index is defined as: fasting insulin (μU/mL) × fasting glucose (mmol/L)/22.5 (16).

Classification of the BMI/IR-phenotypes

Women were divided into four clinical phenotypes according to BMI and IR. IR was defined as HOMA-IR index above the median value (0.9) in our population. A BMI of 24.9 kg/m^2 was used to distinguish between normal and overweight women. The classification resulted in four phenotypes: (i) BMI $\leq$ 24.9−IR (normal weight women without IR), (ii) BMI $\leq$ 24.9+IR (normal weight women with IR), (iii) BMI $\geq$ 25−IR (overweight/obese women without IR) and (iv) BMI $\geq$ 25+IR (overweight/obese women with IR).

Biochemical methodology

Total T was measured by tandem mass spectrometry after an extraction and column chromatography purification step (PerkinElmer CHS MSMS Steroids kit, Turku, Finland) with a detection limit of 0.1 nmol/L. The intra- and inter-assay coefficients of variance (CV) were 10.2 and 10%, respectively. Sex hormone-binding globulin (SHBG) was estimated by a chemiluminescent assay (Architect *i* 2000 system, Abbott, Europe). The intra- and inter-assay CV were 2.8 and 5.8%, respectively. Free T (testosterone not bound to SHBG and albumin) was calculated from total T and SHBG using Vermeulen's method (17). Plasma glucose was analysed by a colorimetric assay (Ortho-Clinical Diagnostics, Rochester, NY, USA). Serum insulin was measured by the chemiluminescent immunometric assay (Immulite 2000 Insulin, Siemens Healthcare, Lianberis, Gwynedd, UK) with a detection limit of 2 μU/mL. The intra- and inter-assay CV were 4.1 and 4.9%, respectively. CRP was analysed by CardioPhase hsCRP particle-enhanced immuno nephelometry (Siemens Healthcare, Marburg, Germany) with an intra- and inter-assay CV of 4.2 and 6.3%, respectively. Plasma PAI-1 concentrations were estimated by TriniLIZE PAI-1 Antigen (Trinity Biotech, Wicklow, Ireland) with a detection limit of 0.5 ng/mL and intra- and inter-assay CV of 2.7 and 4.6%, respectively. The plasma concentration of vWF was determined with an immunoturbidimetric assay, HemosIL von Willebrand factor Antigen (Instrumentation Laboratory, Milan, Italy). The intra- and inter-assay CV were 4.6 and 9.0%, respectively. The remaining analyses were performed using standardized routine methods at the Department of Clinical Biochemistry, Herlev University Hospital.

The study was approved by the Danish Data Protection Agency (J. nr. 2010-41-4331) and the Ethical Committee for the Capital Region of Denmark (protocol nr. H-4-2010-002). The protocol was conducted in accordance with the Helsinki Declaration. All participants provided an informed written consent.

Statistical analysis

C-reactive protein was our primary outcome as it is commonly used as a marker of inflammation and CVD. The power calculation has been reported previously (14). IBM Statistical Package for Social Sciences (SPSS) for Windows, version 19 (IBM Corp., Armonk, NY, USA) was used to analyse the data. The Kolmogorov–Smirnov test was used to assess the distribution of the data. Primarily, parametric statistics were used. Non-normal distributed data were log-transformed. Non-transformed values are shown in the tables. The Kruskal–Wallis and the Mann–Whitney *U*-test were used when data were not adequately normally distributed. One-way analysis of variance (ANOVA) was performed to compare the four phenotypes followed by post hoc comparisons using Bonferroni adjustment. Each Rotterdam criterion was evaluated both as continuous and categorical variables, categorical variables with the outcome, yes or no. A *t*-test was used to compare dichotomized categorical data against continuous variables.

Associations between variables were assessed by the Pearson correlation coefficient (*r*). Measurements of fat

distribution were strongly related to each other and BMI, therefore statistically significant measurements with the highest *r* coefficient with CRP and PAI-1 were selected for multiple regression analyses to explore independent predictors of CRP and PAI-1. CRP and PAI-1 were entered as dependent variables in the model and HOMA-IR, SHBG, free testosterone and total fat mass/H^2 as independent variables. Results are presented as standardized regression coefficients (β). Level of significance was determined by a two-sided value of $p < 0.05$.

Results

During the inclusion period, 149 women were eligible for enrolment. All of the women were diagnosed according to the Rotterdam criteria. The mean age of the total study population was 27 years (± 5 SD), mean BMI was 26 kg/m^2 (± 4 SD) and mean age of menarche was 13 years (SD ± 1.5). Oligo- or amenorrhea was present in 87% of the women ($n = 130$). Of the 149 women, 44.7% ($n = 68$) had hirsutism and 67.8% ($n = 103$) hyperandrogenemia. Polycystic ovaries were present in 90% of the women. All three Rotterdam criteria were fulfilled by 58% of the women (Table 1). In all, 54% of the population had BMI ≥25 and 70% of them were IR. Of the normal weight women, 24% were IR. All women had fasting plasma glucose <7.0 mmol/L except one. Five women had impaired glucose tolerance (one lean and four obese) and one had type 2 diabetes (excluded from the study).

Rotterdam phenotypes

Comparison among four Rotterdam phenotypes showed a significant difference in total and free T and serum luteinizing hormone/follicle stimulating hormone (LH/FSH) ratio which was highest in women fulfilling all three Rotterdam criteria ($p < 0.001$, $p < 0.001$ and $p = 0.002$, respectively). No other significant differences were observed across the groups, either in anthropometry, ovarian volume, metabolism and endocrinology or in body composition. Concentrations of CRP, PAI-1 and vWF were also not significantly different among the Rotterdam phenotypes (data not shown).

CVD risk markers and the Rotterdam criteria

There was a positive correlation between PAI-1 and free T, $r = 0.309$, $n = 149$, $p < 0.001$ (Table 2). No other associations were observed between Rotterdam criteria and the studied variables.

BMI/IR-phenotypes

The anthropometric, hormonal and metabolic profiles of the four phenotypes are shown in Tables 3 and 4. BMI did not differ in the two lean phenotypes but did differ significantly in the two obese phenotypes, with the IR women having the highest BMI ($p = 0.038$). The two normal weight phenotypes differed significantly in WC ($p = 0.023$), waist-hip ratio ($p = 0.028$), LH/FSH ratio ($p = 0.023$), total T ($p = 0.047$), serum insulin ($p < 0.001$) and HOMA-IR index ($p < 0.001$). All of the variables were highest in the IR phenotype. The two obese phenotypes

Table 2. Pearson correlation coefficients (*r*) between CRP and PAI-1, and clinical and biochemical variables in women with polycystic ovary syndrome.

	r (CRP)	*r* (PAI-1)
BMI	0.575[b]	0.546[b]
WC	0.525[b]	0.560[b]
WHR	0.226[b]	0.451[b]
Fasting insulin (μU/mL)[a]	0.423[b]	0.567[b]
HOMA-IR index[a]	0.408[b]	0.569[b]
Total T (nmol/L)	−0.065	−0.027
Free T (nmol/L)[a]	0.155	0.309[b]
SHBG (nmol/L)[a]	−0.28	−0.508[b]
Android fat (kg)	0.484[b]	0.543[b]
Android/gynoid fat ratio (kg/kg)[a]	0.346[b]	0.513[b]
Total fat mass/height^2 (kg/(m)^2)	0.639[b]	0.592[b]
PAI-1 (ng/mL)[a]	0.401[b]	

BMI, body mass index; CRP, C-reactive protein; HOMA-IR, homeostasis model assessment of insulin resistance; PAI-1, plasminogen activator inhibitor-1; SHBG, sex hormone-binding globulin; T, testosterone; WC, waist circumference; WHR, waist-hip ratio.

[a]Data were log-transformed to achieve normal distribution.

[b]Correlations were significant at the level of 0.01 (two-tailed) with $p < 0.001$.

Table 1. Distribution of the four Rotterdam and BMI/IR phenotypes.

	BMI ≤ 24.9−IR	BMI ≤ 24.9+IR	BMI ≥ 25−IR	BMI ≥ 25+IR	Total
Anov + HA	3	1	4	11	19 (13%)
Anov + PCO	12	1	6	6	25 (17%)
HA + PCO	8	3	0	8	19 (13%)
Anov + HA + PCO	29	11	14	32	86 (58%)
Total	52 (35%)	16 (11%)	24 (16%)	57 (38%)	149

Anov, anovulation; BMI, body mass index; HA, hyperandrogenism; IR, insulin resistance; PCO, polycystic ovaries.

Table 3. Anthropometric and hormonal characteristics of the four BMI/IR-phenotypes of polycystic ovary syndrome.

	BMI ≤ 24.9−IR	BMI ≤ 24.9+IR	BMI ≥ 25−IR	BMI ≥ 25+IR	*p*-value
n = 149	52 (35%)	16 (10%)	23 (16%)	57 (38.5%)	
Age (years)	27 ± 4.7	25 ± 4.2	29 ± 6.1	28 ± 4.9	0.105
BMI (kg/m^2)	21.7 ± 1.7	23 ± 1.9	28.5 ± 2.8[c,e]	30 ± 2.6[f,g,d]	<0.001
WC (cm)	75 ± 6.9	81 ± 8.1[b]	92 ± 9.6[c,e]	98 ± 9.0[d,f]	<0.001
WHR	0.78 ± 0.08	0.84 ± 0.07[b]	0.85 ± 0.07[c]	0.89 ± 0.06[d]	<0.001
BP systolic (mmHg)	116 ± 13	120 ± 9	127 ± 12[c]	127 ± 10[d]	<0.001
BP diastolic (mmHg)	74 ± 9	73 ± 6	73 ± 6	83 ± 9[d,f]	<0.001
Total T (nmol/L)	2.0 ± 1.0	2.8 ± 0.9[f,b]	2.1 ± 1.0	1.8 ± 0.9	0.008
Free T (nmol/L)[a]	0.027 (0.023–0.031)	0.048 (0.034–0.063)	0.050 (0.02–0.081)	0.048 (0.022–0.071)	0.261
Androstenedione (nmol/L)	6.96 (6.25–7.68)	10.5 (7.89–13.11)[b,e,f]	6.89 (5.45–8.33)	7.45 (6.64–8.26)	0.001
SHBG (nmol/L)[a]	79 (70–89)[d]	66 (40–92)	63 (43–82)	44 (38–51)	<0.001
LH/FSH (IU/L)[a]	1.58 (1.28–1.88)	2.61 (2.03–3.19)[b]	1.66 (1.12–2.19)	2.03 (1.67–2.39)	0.018

ANOVA, analysis of variance; BP, blood pressure; IR, insulin resistance; SHBG, sex hormone-binding globulin; T, testosterone; WC, waist circumference; WHR, waist-hip ratio.

P-values are significant at the level of 0.05. [a]Variables were log-transformed to achieve normal distribution. Mean values and 95% confidence intervals (CI) are presented. The remaining values are presented as mean ± SD or *n* (%).

P-values are based on significant differences between subgroups: [b]BMI ≤ 24.9−IR vs. BMI ≤ 24.9+IR; [c]BMI ≤ 24.9−IR vs. BMI ≥ 25−IR; [d]BMI ≤ 24.9−IR vs. BMI ≥ 25+IR; [e]BMI ≤ 24.9+IR vs. BMI ≥ 25−IR; [f]BMI ≤ 24.9+IR vs. BMI ≥ 25+IR; [g]BMI ≥ 25−IR vs. BMI ≥ 25+IR.

Table 4. Metabolic characteristics of the four BMI/IR-phenotypes of polycystic ovary syndrome.

	BMI ≤ 24.9−IR	BMI ≤ 24.9+IR	BMI ≥ 25−IR	BMI ≥ 25+IR	*p*-value
n = 149	52	16	24	57	
Fasting glucose (mmol/L)	4.9 ± 0.4	5.1 ± 0.4	5.0 ± 0.3	5.3 ± 0.6[e]	<0.001
Fasting insulin (μU/mL)[a]	2.25 ± 0.46	8.18 ± 3.11[c,f]	2.86 ± 0.75	10.0 ± 5.69[e,h]	<0.001
HOMA-IR index[a]	0.49 ± 0.11	1.89 ± 0.79[c,f]	0.64 ± 0.17	2.42 ± 1.58[e,h]	<0.001
2 h-OGTT (mmol/L)[b]	4.8 ± 1.2	4.1 ± 0.7	4.7 ± 0.6 [h]	5.8 ± 1.4[e]	<0.001
Total cholesterol (mmol/L)	4.5 ± 0.9	4.5 ± 0.9	4.8 ± 0.8	5.0 ± 0.8[e]	0.029
LDL cholesterol (mmol/L)	2.6 ± 0.7	2.5 ± 0.9	2.9 ± 0.6	3.2 ± 0.7[e,g]	<0.001
HDL cholesterol (mmol/L)[a]	1.6 ± 0.4	1.6 ± 0.6	1.5 ± 0.4	1.2 ± 0.3[e,g]	<0.001
Triglycerides (mmol/L)[a]	0.68 ± 0.28	0.82 ± 0.24	1.15 ± 1.30	1.26 ± 0.71[e]	<0.001
Fat mass/height^2 (kg/m^2)	6.25 ± 1.74	6.62 ± 1.40	10.5 ± 2.06[d,f]	11.4 ± 1.86[e,g]	<0.001
Android fat (kg)	1.2 ± 1.02	1.2 ± 0.39	2.3 ± 0.65[d,f]	2.8 ± 0.73[e,g]	<0.001
CRP (mg/L)[a]	0.50 (0.33–0.57)	0.86 (0.43–1.05)	1.25 (0.77–2.03)	2.59 (1.97–3.21)[e]	0.006
PAI-1 (ng/mL)[a]	9.7 (8.1–11.2)	21.6 (12.9–30.3)[c]	16.9 (12.9–20.9)	25.5 (21.4–29.7)[e,h]	<0.001
vWF[a]	1.17 (1.02–1.32)	1.45 (1.02–1.87)	1.08 (0.92–1.23)	1.22 (1.09–1.34)	0.176

ANOVA, analysis of variance; BMI, body mass index; CRP, C-reactive protein; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; IR, insulin resistance; LDL, low-density lipoprotein; OGTT, oral glucose tolerance test; PAI-1, plasminogen activator inhibitor -1; vWF, von Willebrand factor.

[a]Data were log-transformed to achieve normal distribution. *P*-values are significant at the level of 0.05. [b]*n* = 85. Data are presented as mean ± SD except for CRP, PAI-1 and vWF (mean and 95% CI).

P-values are based on significant differences between subgroups: [c]BMI ≤ 24.9−IR vs. BMI ≤ 24.9+IR; [d]BMI ≤ 24.9−IR vs. BMI ≥ 25−IR; [e]BMI ≤ 24.9−IR vs. BMI ≥ 25+IR; [f]BMI ≤ 24.9+IR vs. BMI ≥ 25−IR; [g]BMI ≤ 24.9+IR vs. BMI ≥ 25+IR; [h]BMI ≥ 25−IR vs. BMI ≥ 25+IR.

were comparable except for the level of serum insulin ($p < 0.001$), HOMA-IR index ($p < 0.001$) and PAI-1 ($p < 0.023$) being higher in the phenotype BMI ≥ 25+IR compared with the phenotype BMI ≥ 25−IR (Table 4).

The four phenotypes did not differ in oligo-/amenorrhea, the Ferriman–Gallwey score or AFC ($p = 0.682$, $p = 0.749$, $p = 0.504$, respectively). Markers of IR such as insulin and HOMA-IR index were significantly higher in the two IR phenotypes than in the non-IR women. LDL-cholesterol and triglycerides were higher and HDL-cholesterol lower in the phenotype BMI ≥ 25+IR than in the normal weight phenotypes (Table 4). The phenotype BMI ≤ 24.9+IR had significant higher total T than the phenotypes BMI ≤ 24.9−IR and BMI ≥ 25+IR ($p = 0.047$ and $p = 0.004$, respectively). All absolute and relative measurements of body composition were higher in the

phenotypes with BMI ≥ 25 than in phenotypes with BMI ≤ 24.9.

CVD risk markers and BMI/IR-phenotype

Significant differences were observed between the four BMI/IR phenotypes concerning the plasma levels of CRP and PAI-1, with an overall $p < 0.006$ and $p < 0.001$, respectively (Table 4). Serum CRP concentration was higher in phenotypes with BMI ≥ 25 than in phenotypes with BMI ≤ 24.9 ($p < 0.001$). Plasma PAI-1 levels were higher in the two IR phenotypes than in the non-IR phenotypes ($p < 0.001$). The levels of plasma vWF differed insignificantly among the four phenotypes.

Correlation analyses in the total study population

Highly significant and strong correlations between PAI-1, BMI and different body fat compartments, markers of IR and hyperandrogenemia were observed (Table 2). CRP had stronger positive correlations with BMI and body fat measurements than with the other variables investigated (Table 2). The plasma concentration of vWF was not associated to PAI-1, CRP or the parameters of body composition and IR.

Multiple regression analyses were performed to investigate whether obesity, IR or hyperandrogenemia were predictive of endothelial dysfunction and inflammation. Total fat mass/H^2 ($\beta = 0.358$, $p < 0.0005$), HOMA-IR ($\beta = 0.251$, $p = 0.009$) and SHBG ($\beta = -0.283$, $p = 0.003$) were predictors of PAI-1, whereas only total fat mass/H^2 ($\beta = 0.597$, $p < 0.0005$) was predictive of CRP. Similar results were obtained if WC or BMI was used instead of total fat mass but total fat mass/H^2 was the best significant predictor.

Discussion

The present study demonstrates the impact of IR and BMI in women with PCOS when evaluating risk factors of CVD. Significant differences between the four BMI/IR phenotypes were observed in measures of low-grade inflammation, endothelial dysfunction, hyperandrogenism and body fat distribution. The PCOS phenotype BMI ≥ 25+IR was characterized by increased levels of both plasma CRP and PAI-1, indicating low-grade inflammation and endothelial dysfunction. Furthermore, this phenotype displayed an atherogenic lipid profile and relatively increased blood pressure. Among normal weight PCOS women, the phenotype BMI ≤ 24.9+IR was characterized by increased WC, hyperandrogenemia and elevated levels of plasma PAI-1, suggesting coexisting endothelial dysfunction. Only 24% of the normal weight women with PCOS were insulin-resistant. Overall, obesity was the only predictor of CRP whereas both IR and obesity were predictors of PAI-1. No significant associations were observed between the Rotterdam phenotypes and the investigated biomarkers and the fat distribution.

Elevated high-sensitive CRP is a well-known independent predictive marker of risk of CVD in both apparently healthy individuals and individuals with established CVD (18). The observed level of CRP in obese women indicates an increased risk of CVD corresponding to an intermediate CV risk, whereas the CRP levels in normal weight women are in line with both an absolute and a relative low risk of CVD (19). The level of CRP in obese PCOS women in this study is in line with a previous Danish study. The CRP level in BMI and fat mass-matched controls was significantly lower (20). Another study showed higher levels of CRP in normal weight IR women than normal weight non-IR PCOS women (21). Our data did not show any difference in plasma CRP among normal weight IR and non-IR PCOS women, despite the significant difference in WC and waist-hip ratio besides IR. We observed a positive association between CRP and HOMA-IR in our total study population but this association was not found when women were divided in BMI/IR-phenotypes. The discrepancy between the studies may be explained by difference in the sample size, age and body composition. The observed association between CRP and obesity is in accordance with other studies demonstrating that the level of CRP is highest in individuals with obesity (22,23).

Elevated plasma concentrations of PAI-1 and vWF are indicators of endothelial dysfunction and impaired fibrinolysis, and are related to increased risk of CVD (12,24). We demonstrate that the two IR phenotypes, BMI ≤ 24.9+IR and BMI ≥ 25+IR, had the highest levels of plasma PAI-1, although a statistical significant difference between the phenotypes BMI ≤ 24.9+IR and BMI ≥ 25−IR was not observed. The lack of significance may be due to lack of power in our study, but the results are in line with experimental clinical studies demonstrating that hyperinsulinemia stimulates the production of PAI-1 (25). Other studies have demonstrated elevated levels of PAI-1 in women with PCOS, but the relation between PAI-1 and fat distribution was not evaluated (26,27). We found that IR and obesity are determinants of PAI-1 levels. Although the two non-IR phenotypes had lower levels of plasma PAI-1 compared with the IR phenotypes, there was a significant difference in PAI-1 level among the two non-IR phenotypes, probably explained by the difference in BMI and body fat mass. Thus PAI-1 seems driven by both IR and body fat mass, and possibly primarily by android fat.

von Willebrand factor is a biomarker of manifest endothelial cell injury. The association between plasma levels of vWF and CVD is stronger in patients with IR than in those without IR (12). We did not find any difference in the level of vWF among our four phenotypes despite differences in their BMI and IR profile. This observation is in agreement with some previous studies (26,28). As our study population consists of relatively young women, manifest endothelial damage may not yet have occurred.

Altered body composition with tendency to abdominal fat deposition, has been reported in normal weight women with PCOS (29). The difference in WC may be critical, resulting in IR in normal weight women and could also explain the high level of androgens. Another possible explanation could be that significant biochemical hyperandrogenism in the phenotype BMI $\leq$ 24.9+IR compared with BMI $\leq$ 24.9−IR, may predispose to android fat deposition, which in turn leads to IR, resulting in metabolic derangements and hemostatic stress including elevated PAI-1 (25). From a clinical point of view, measurement of WC (abdominal obesity) should be considered in normal weight women with PCOS.

This cross-sectional study has several specific advantages compared with other studies dealing with assessment of biomarkers of long-term CVD risk in women with PCOS. The PCOS diagnosis is established according to the most recent updated Rotterdam criteria. Our study population is larger than most other studies and for the first time the risk factors of CVD are analysed in PCOS women based upon clinical relevant subgroups defined by BMI and IR combined. The cross-sectional design of the study and lack of controls implies limitations to the interpretation of the results, as it remains unclear whether PCOS per se is associated with increased risk of CVD. The study shows that IR and abdominal obesity are associated with well-known risk markers for CVD but whether this association is stronger or more marked in women with PCOS than in non-PCOS women requires future studies. The chosen cut-off value for HOMA-IR index may seem low, but this cut-off point is representative for the Danish population, as a mean HOMA-IR index of 1.1 has been reported in a Danish PCOS population (30). Currently there is no validated clinical test for detecting IR in the general population. Measurement of insulin is subject to uncertainty because of pulsatile secretion of insulin, nocturnal variations and short half-life. Nonetheless the degree of IR varies among ethnicities and different populations.

In conclusion, IR, BMI and obesity are associated with well-known risk markers for CVD in women with PCOS and might be used to identify the women with PCOS who mostly need intervention. Whether the observed associations are stronger or more marked in women with PCOS than in other women remains elusive; prospective and controlled follow-up studies are required to determine whether the observed significant differences among the four BMI/IR-phenotypes have clinical implications.

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References

1. Fauser BC. Consensus on women's health aspects of polycystic ovary syndrome (PCOS). Hum Reprod. 2012;27:14–24.
2. Mani H, Levy MJ, Davies MJ, Morris DH, Gray LJ, Bankart J, et al. Diabetes and cardiovascular events in women with polycystic ovary syndrome: a 20 years retrospective cohort study. Clin Endocrinol (Oxf). 2013;78:926–34.
3. Wild RA. Long-term health consequences of PCOS. Hum Reprod Update. 2002;8:231–41.
4. Mohlig M, Spranger J, Osterhoff M, Ristow M, Pfeiffer AF, Schill T, et al. The polycystic ovary syndrome per se is not associated with increased chronic inflammation. Eur J Endocrinol. 2004;150:525–32.
5. Jovanovic VP, Carmina E, Lobo RA. Not all women diagnosed with PCOS share the same cardiovascular risk profiles. Fertil Steril. 2010;94:826–32.
6. Carmina E, Lobo RA. Use of fasting blood to assess the prevalence of insulin resistance in women with polycystic ovary syndrome. Fertil Steril. 2004;82:661–5.
7. Dunaif A, Segal KR, Futterweit W, Dobrjansky A. Profound peripheral insulin resistance, independent of obesity, in polycystic ovary syndrome. Diabetes. 1989;38:1165–74.
8. Mak W, Dokras A. Polycystic ovarian syndrome and the risk of cardiovascular disease and thrombosis. Semin Thromb Hemost. 2009;35:613–20.
9. Orio F, Vuolo L, Palomba S, Lombardi G, Colao A. Metabolic and cardiovascular consequences of polycystic ovary syndrome. Minerva Ginecol. 2008;60:39–51.
10. Kaptoge S, Di AE, Lowe G, Pepys MB, Thompson SG, Collins R, et al. C-reactive protein concentration and risk of coronary heart disease, stroke, and mortality: an individual participant meta-analysis. Lancet. 2010;375:132–40.

11. Vaughan DE, De Taeye BM, Eren M. PAI-1 antagonists: predictable indications and unconventional applications. Curr Drug Targets. 2007;8:962–70.
12. Frankel DS, Meigs JB, Massaro JM, Wilson PW, O'Donnell CJ, D'Agostino RB, et al. Von Willebrand factor, type 2 diabetes mellitus, and risk of cardiovascular disease: the Framingham offspring study. Circulation. 2008;118:2533–9.
13. Burchall G, Linden MD, Teede H, Piva TJ. Hemostatic abnormalities and relationships to metabolic and hormonal status in polycystic ovarian syndrome. Trends Cardiovasc Med. 2011;21:6–14.
14. Aziz M, Naver KV, Wissing ML, Mikkelsen AL, Nilas L, Skouby SO. Polycystic ovary syndrome: infertility, cardiovascular, metabolic and obstetrical risks, laboratory and clinical outcomes – the PICOLO Study. Gynecol Endocrinol. 2012;28:253–8.
15. Rosenfield RL. Clinical practice. Hirsutism. N Engl J Med. 2005;353:2578–88.
16. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28:412–9.
17. Vermeulen A, Verdonck L, Kaufman JM. A critical evaluation of simple methods for the estimation of free testosterone in serum. J Clin Endocrinol Metab. 1999;84:3666–72.
18. Ridker PM, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. N Engl J Med. 2000;342:836–43.
19. Pearson TA, Mensah GA, Alexander RW, Anderson JL, Cannon RO III, Criqui M, et al. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: a statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. Circulation. 2003;107:499–511.
20. Glintborg D, Hojlund K, Andersen M, Henriksen JE, Beck-Nielsen H, Handberg A. Soluble CD36 and risk markers of insulin resistance and atherosclerosis are elevated in polycystic ovary syndrome and significantly reduced during pioglitazone treatment. Diabetes Care. 2008;31:328–34.
21. Kim JW, Han JE, Kim YS, Won HJ, Yoon TK, Lee WS. High sensitivity C-reactive protein and its relationship with impaired glucose regulation in lean patients with polycystic ovary syndrome. Gynecol Endocrinol. 2012;28:259–63.
22. Tosi F, Dorizzi R, Castello R, Maffeis C, Spiazzi G, Zoppini G, et al. Body fat and insulin resistance independently predict increased serum C-reactive protein in hyperandrogenic women with polycystic ovary syndrome. Eur J Endocrinol. 2009;161:737–45.
23. Samy N, Hashim M, Sayed M, Said M. Clinical significance of inflammatory markers in polycystic ovary syndrome: their relationship to insulin resistance and body mass index. Dis Markers. 2009;26:163–70.
24. Meltzer ME, Lisman T, de Groot PG, Meijers JC, le Cessie S, Doggen CJ, et al. Venous thrombosis risk associated with plasma hypofibrinolysis is explained by elevated plasma levels of TAFI and PAI-1. Blood. 2010;116:113–21.
25. Carmassi F, De NF, Fioriti R, De GA, Giannarelli C, Fruzzetti F, et al. Insulin resistance causes impaired vasodilation and hypofibrinolysis in young women with polycystic ovary syndrome. Thromb Res. 2005;116:207–14.
26. Manneras-Holm L, Baghaei F, Holm G, Janson PO, Ohlsson C, Lonn M, et al. Coagulation and fibrinolytic disturbances in women with polycystic ovary syndrome. J Clin Endocrinol Metab. 2011;96:1068–76.
27. Tarkun I, Canturk Z, Arslan BC, Turemen E, Tarkun P. The plasminogen activator system in young and lean women with polycystic ovary syndrome. Endocr J. 2004;51:467–72.
28. Dahlgren E, Janson PO, Johansson S, Lapidus L, Lindstedt G, Tengborn L. Hemostatic and metabolic variables in women with polycystic ovary syndrome. Fertil Steril. 1994;61:455–60.
29. Carmina E, Bucchieri S, Esposito A, Del PA, Mansueto P, Orio F, et al. Abdominal fat quantity and distribution in women with polycystic ovary syndrome and extent of its relation to insulin resistance. J Clin Endocrinol Metab. 2007;92:2500–5.
30. Svendsen PF, Madsbad S, Nilas L. The insulin-resistant phenotype of polycystic ovary syndrome. Fertil Steril. 2010;94:1052–8.

