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Insulin resistance and endocrine characteristics of the different phenotypes of polycystic ovary syndrome: a prospective study

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BACKGROUND: Polycystic ovary syndrome (PCOS) is a heterogeneous disorder characterized by oligo- or anovulation (ANOV), biochemical or clinical manifestations of hyperandrogenemia (HA) and PCOs. Four phenotypes of PCOS exist [phenotype 1 (ANOV + HA + PCO), phenotype 2 (ANOV + HA), phenotype 3 (HA + PCO) and phenotype 4 (ANOV + PCO)] but the differences between them are not well studied. We compared markers of insulin resistance (IR) and endocrine characteristics between the different PCOS phenotypes.

METHODS: We prospectively studied 1212 consecutive women with PCOS and 254 BMI-matched healthy women.

RESULTS: Phenotypes 1–4 were present in 48.2, 30.7, 9.7 and 11.4% of patients, respectively. BMI did not differ between the four phenotypes and controls. Both normal weight and overweight/obese women with phenotypes 1 and 2 were more insulin resistant than controls. Overweight/obese, but not normal weight, women with phenotype 4 were more insulin resistant than controls, while IR in women with phenotype 3 did not differ from controls regardless of obesity. In normal weight subjects, women with phenotypes 1 and 2 were more insulin resistant than women with phenotype 4. In overweight/obese subjects, women with phenotype 1 were more insulin resistant than women with phenotypes 2 and 3 and women with phenotype 4 were more insulin resistant than those with phenotype 3. Circulating androgens were higher in normal weight and overweight/obese PCOS patients with phenotypes 1–3 compared with those with phenotype 4, and higher in normal weight PCOS patients with phenotype 1 than in those with phenotype 2.

CONCLUSIONS: Phenotype 1 is associated with more IR and more pronounced HA than phenotype 2. Phenotypes 2 and 4 with obesity, are also characterized by IR. In contrast, phenotype 3 is not associated with IR.

Key words: polycystic ovary syndrome / phenotypes / insulin resistance / hyperandrogenemia / obesity

Introduction

Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders in women of reproductive age (Norman *et al.*, 2007). PCOS is a heterogeneous disorder and several criteria have been proposed for its diagnosis (Zawadski and Dunaif, 1992; Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2003). According to the 1990 National Institutes of Health (NIH) criteria, the diagnosis of PCOS requires the presence of both oligo- or anovulation (ANOV) and biochemical hyperandrogenemia (HA) or clinical manifestations of HA, regardless of the presence of polycystic ovaries (PCOs) on ultrasound (Zawadski and Dunaif, 1992). According to the European Society for Human Reproduction and Embryology (ESHRE) and the American Society for Reproductive Medicine (ASRM) definition introduced in Rotterdam in 2003, PCO is also considered a diagnostic criterion for PCOS along with ANOV and HA; PCOS is diagnosed when at least two of the three criteria (ANOV, HA and PCO) are present (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2003). Based on these newer criteria, two additional phenotypes of PCOS arise,

Table I Definition of the phenotypes of the PCOS based on the 2003 Rotterdam criteria (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2003).

PCOS phenotype	Anovulation	Hyperandrogenemia	Polycystic ovaries in transvaginal ultrasonography
1 (severe PCOS)	+	+	+
2 (anovulation and hyperandrogenemia)	+	+	−
3 (ovulatory PCOS)	−	+	+
4 (mild PCOS)	+	−	+

Phenotypes 1 and 2 were also included in the National Institutes of Health 1990 criteria (Zawadski and Dunaif, 1992).

which were not included in the NIH definition of PCOS [HA and PCO without ANOV (phenotype 3) and ANOV and PCO without HA (phenotype 4)] (Table I).

The 2003 Rotterdam criteria are the subject of ongoing controversy (Azziz, 2006; Franks, 2006; Azziz *et al.*, 2009). Some studies suggest that the additional PCOS phenotypes introduced by the 2003 Rotterdam criteria, particularly phenotype 4, are characterized by less severe endocrine and metabolic abnormalities (Azziz *et al.*, 2009; Moran and Teede, 2009). However, others reported that these differences are mainly due to the higher prevalence of obesity in women diagnosed with PCOS according to the NIH criteria (Moran and Teede, 2009). Nevertheless, most studies that have compared the different PCOS phenotypes have been small, have not controlled for the differences in BMI between phenotypes and have not analyzed overweight/obese and normal weight women separately (Moran and Teede, 2009).

The aim of the present study was to compare insulin resistance (IR) and endocrine characteristics of the different PCOS phenotypes in a large cohort of PCOS patients and BMI-matched healthy women. We also aimed to analyze whether obesity contributes to the differences in PCOS phenotypes by analyzing normal weight and overweight/obese women with PCOS separately.

Materials and Methods

Patients

All women who were diagnosed with PCOS between May of 2004 and May of 2011 at the Gynecological Endocrinology Infirmary of the Second Department of Obstetrics and Gynecology, Aristotle University of Thessaloniki, Greece, were included in the study. A total of 1212 consecutive women with PCOS were studied (age 24.1 $\pm$ 5.7 years, BMI 26.7 $\pm$ 6.9 kg/m^2). During the same period, 254 BMI-matched healthy women were also studied (age 31.3 $\pm$ 5.6 years, BMI 25.7 $\pm$ 6.4 kg/m^2) (control group). Women of the control group were healthy volunteers with normal ovulatory cycles (28 $\pm$ 2 days, blood progesterone levels $>$10 ng/ml in two consecutive cycles), no signs of hyperandrogenism and normal sonographic appearance of the ovaries.

Diagnosis of PCOS was based on the revised criteria of Rotterdam (Table I) (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2003). Patients with phenotype 1 ('severe PCOS') had ANOV ($<$8 spontaneous hemorrhagic episodes/year), biochemical HA (early follicular phase testosterone levels $>$60 ng/dl, corresponding to the mean $\pm$ 2 SD of 200 control subjects measured in our laboratory) or clinical manifestations of HA (Ferriman–Gallwey score $\geq$8), and PCO ($\geq$12 follicles with a diameter of 2–9 mm in at least one ovary and/or ovarian volume $>$10 cm^3). Patients with phenotype 2 had ANOV, HA and normal sonographic appearance of the ovaries. Patients with phenotype 3 ('ovulatory' PCOS) had HA and PCO without ANOV. Patients with phenotype 4 ('mild' PCOS) had ANOV and PCO without HA.

None of the women studied had galactorrhea or any endocrine or systemic disease that could possibly affect reproductive physiology. No woman reported use during the last semester of any medication that could interfere with the normal function of the hypothalamic-pituitary-gonadal axis. When basic 17α-hydroxyprogesterone (17α-OHP) levels were $>$4.5 nmol/l, the Synacthen test (0.25 mg/1 ml; Novartis Pharma S.A., Rueil-Malmaison, France) was performed to rule out congenital adrenal hyperplasia. Other causes of HA, including prolactinoma, Cushing's syndrome and androgen-secreting tumors, were also excluded.

Informed consent was obtained from all women, and the study was approved by the Ethics Committee of the Medical School of the Aristotle University of Thessaloniki. The study met the requirements of the 1975 Helsinki guidelines.

Study protocol

In all women, body weight, height and waist circumference (*W*) were measured. Body weight was measured with analog scales and in light clothing; height was measured barefoot with a stadiometer. The BMI was calculated by dividing weight (in kg) by height squared (in m) to assess obesity. The *W* was obtained as the smallest circumference at the level of the umbilicus.

Baseline blood samples were collected between Days 3 and 7 of the menstrual cycle in the control group and between 3 to 7 days after a spontaneous bleeding episode in patients with PCOS, after an overnight fast. In women with PCOS who did not have a spontaneous bleeding episode for $>$90 days, 100 mg of micronized progesterone (Utrogestan, Faran Laboratories S. a., Athens, Greece) was administered to induce a bleeding episode and blood samples were collected afterwards. The circulating levels of FSH, LH, prolactin (PRL), testosterone, Δ_4-androstenedione (Δ_4-A), dehydroepiandrosterone sulfate (DHEA-S), 17α-OHP, sex hormone-binding globulin (SHBG), glucose, insulin, thyroid-stimulating hormone (TSH) and free thyroxin (FT4) were measured. Immediately after the baseline blood sampling an oral glucose tolerance test (OGTT) was performed; 75 g of glucose was administered orally and serum glucose levels were determined after 30, 60, 90 and 120 min. On the same day, transvaginal ultrasonography was performed and the volume of each ovary was determined, as well as the number of follicles in each ovary.

Patients with PCOS and controls were divided according to BMI in normal weight (BMI $<$25 kg/m^2; $n = 639$ and $n = 150$, respectively) and overweight/obese (BMI $\geq$25 kg/m^2; $n = 573$ and $n = 104$, respectively).

Measurements

Plasma glucose, insulin, FSH, LH, PRL, androgens, 17α-OHP, TSH and FT4 concentrations were measured as previously described (Piouka *et al.*, 2009). Free androgen index (FAI) was determined as follows: FAI = testosterone (nmol/l) × 100/SHBG (nmol/l) (Carter *et al.*, 1983). The homeostasis model assessment of IR (HOMA-IR) index was calculated as follows: HOMA-IR = fasting insulin (mIU/l) × fasting glucose (mmol/l)/22.5 (Matthews *et al.*, 1985). The quantitative insulin sensitivity check index (QUICKI) was calculated according to the following formula: QUICKI = 1/[log fasting insulin (mIU/l) + log fasting glucose (mg/dl)] (Katz *et al.*, 2000). The area of glucose levels under the OGTT curve (AUCgluc-OGTT) was calculated with the trapezoidal method.

Transvaginal ultrasonography

Transvaginal ultrasonography was performed by an experienced operator in all women. Ovarian volume was calculated as follows: Ovarian volume = (π/6) × ovarian length × ovarian height × ovarian width. To calculate the mean number of follicles in the two ovaries, we measured the number of follicles in the entire left and right ovary. The sum of the follicles in the left and right ovary was then divided by two.

Statistical analysis

Data analysis was performed with the statistical package SPSS (version 17.0; SPSS Inc., Chicago, IL). Data are reported as mean ± SD. Because plasma LH, PRL, FAI, SHBG, 17α-OHP, insulin and TSH levels as well as the glucose/insulin and the HOMA-IR did not follow normal distribution as assessed with the Kolmogorov–Smirnov test, these parameters were log transformed prior to analysis. For these parameters, non-transformed values are shown in the Tables. Differences between groups were assessed with one-way analysis of variance (ANOVA) with the Holm-Sidak method for multiple comparison testing. In all cases, a $P < 0.05$ was considered significant.

Results

Among the 1212 women with PCOS in our study, 584 presented phenotype 1 (48.2%), 372 presented phenotype 2 (30.7%), 118 presented phenotype 3 (9.7%) and 138 presented phenotype 4 (11.4%). The BMI did not differ between the four phenotypes and controls (Table II). Comparisons between the different PCOS phenotypes and controls in the total study population are shown in Table II. Circulating androgens were higher in women with phenotypes 1–3 than in controls. In contrast, only plasma Δ_4-A, FAI and 17α-OHP were higher in women with phenotype 4 than in controls ($P = 0.009$, $P < 0.001$ and $P < 0.001$, respectively). Women with phenotypes 1 and 2 were more insulin resistant than controls (i.e. higher plasma insulin levels, AUCgluc-OGTT and HOMA-IR and lower glucose/insulin and QUICKI). In contrast, women with phenotype 3 did not differ from controls in any marker of IR, whereas women with phenotype 4 had marginally higher HOMA-IR and marginally lower QUICKI index than controls ($P = 0.016$ and $P = 0.034$, respectively).

Comparisons between the different PCOS phenotypes in the total study population are shown in Table II. Circulating androgens were higher in women with phenotypes 1–3 compared with women with phenotype 4 and were also higher in women with phenotype 1 than in women with phenotypes 2 and 3. Markers of IR did not differ between the different PCOS phenotypes.

Comparisons between normal weight women with PCOS and normal weight controls are shown in Table III. Circulating androgens were higher in women with phenotypes 1–3 than in controls. In contrast, only plasma Δ_4-A, FAI and 17α-OHP were higher in women with phenotype 4 than in controls ($P = 0.023$, $P = 0.013$ and $P < 0.001$, respectively). Women with phenotypes 1 and 2 were more insulin resistant than controls (i.e. had higher plasma insulin levels, HOMA-IR and AUCgluc-OGTT than the latter and lower glucose/insulin and QUICKI). In contrast, women with phenotypes 3 and 4 did not differ from controls in any marker of IR.

Comparisons between the different PCOS phenotypes in normal weight subjects are also shown in Table III. Circulating androgens were higher in women with phenotypes 1–3 compared with women with phenotype 4 and were also higher in women with phenotype 1 than in women with phenotype 2. Plasma Δ_4-A levels were marginally higher in women with phenotype 1 than in women with phenotype 3 ($P = 0.046$) but other circulating androgens did not differ between these phenotypes. Women with phenotype 1 were more insulin resistant than women with phenotype 4 (i.e. had greater AUCgluc-OGTT than the latter; $P = 0.013$). Women with phenotype 2 were also more insulin resistant than women with phenotype 4 (i.e. had lower glucose/insulin than the latter; $P = 0.043$). Markers of IR did not differ between phenotypes 1 and 2, phenotypes 1 and 3, phenotypes 2 and 3 or phenotypes 3 and 4.

Comparisons between overweight/obese women with PCOS and overweight/obese controls are shown in Table IV. Circulating androgens were higher in women with phenotypes 1–3 than in controls, whereas only the FAI was higher in women with phenotype 4 than in controls ($P < 0.001$). Women with phenotypes 1 and 4 were more insulin resistant than controls (i.e. had higher plasma insulin levels and HOMA-IR than the latter and lower glucose/insulin and QUICKI). Women with phenotype 2 were also more insulin resistant than controls (i.e. had lower glucose/insulin levels; $P = 0.033$). In contrast, women with phenotype 3 did not differ from controls in any marker of IR.

Comparisons between the different PCOS phenotypes in overweight/obese subjects are also shown in Table IV. Circulating androgens were higher in women with phenotypes 1– 3 compared with women with phenotype 4. Plasma Δ_4-A levels were higher in women with phenotype 1 than in women with phenotype 2 ($P = 0.004$) and the FAI was higher in women with phenotype 1 than in women with phenotype 3 ($P = 0.019$). Women with phenotype 1 were more insulin resistant than women with phenotype 2 (i.e. had lower QUICKI than the latter; $P = 0.039$) and women with phenotype 3 (i.e. had higher plasma insulin levels and HOMA-IR than the latter and lower glucose/insulin and QUICKI; $P = 0.003$, $P = 0.003$, $P = 0.005$ and $P = 0.003$, respectively). Women with phenotype 4 were also more insulin resistant than women with phenotype 3 (i.e. had higher plasma insulin levels and HOMA-IR than the latter and lower glucose/insulin and QUICKI; $P = 0.008$, $P = 0.003$, $P = 0.038$ and $P = 0.007$, respectively). Markers of IR did not differ between phenotypes 1 and 4, phenotypes 2 and 3 or phenotypes 2 and 4.

Discussion

This is the largest study that has compared IR and endocrine characteristics of the four different phenotypes of PCOS. In agreement with

Table II Comparison between the different phenotypes of PCOS and the controls.

	PCOS (total population) (n = 1212)				Controls (total population) (n = 254)	*P*-value (overall)	*P* (*post hoc* tests between the different phenotypes of PCOS)					
	Phenotype 1 (n = 584)	Phenotype 2 (n = 372)	Phenotype 3 (n = 118)	Phenotype 4 (n = 138)			1 versus 2	1 versus 3	1 versus 4	2 versus 3	2 versus 4	3 versus 4
Age (years)	23.5 ± 5.2[a]	24.3 ± 6.0[a]	25.3 ± 5.7[a]	24.8 ± 6.5[a]	31.3 ± 5.6	<0.001	NS	0.016	NS	NS	NS	NS
BMI (kg/m^2)	26.7 ± 6.8	26.9 ± 7.4	26.2 ± 5.4	26.4 ± 7.7	25.7 ± 6.4	NS	NA	NA	NA	NA	NA	NA
Waist (cm)	84.2 ± 15.8	83.5 ± 15.7	81.2 ± 11.5	82.4 ± 16.4	82.4 ± 13.6	NS	NA	NA	NA	NA	NA	NA
W/H	0.79 ± 0.07	0.78 ± 0.07	0.77 ± 0.06	0.77 ± 0.07	0.78 ± 0.06	0.006	NS	0.015	NS	NS	NS	NS
FSH (IU/l)	5.7 ± 1.7[a]	5.9 ± 1.8[a]	6.3 ± 1.8[b]	6.0 ± 2.0[a]	7.1 ± 2.3	<0.001	NS	0.006	NS	NS	NS	NS
LH (IU/l)	8.9 ± 6.0[a]	7.0 ± 5.2	6.2 ± 4.0	6.7 ± 4.5	5.9 ± 2.7	<0.001	<0.001	<0.001	<0.001	NS	NS	NS
Testosterone (nmol/l)	2.9 ± 1.0[a]	2.6 ± 0.9[a]	2.6 ± 0.9[a]	1.4 ± 0.4	1.3 ± 0.4	<0.001	<0.001	0.022	<0.001	NS	<0.001	<0.001
Δ_4-A (nmol/l)	10.8 ± 4.2[a]	9.1 ± 3.1[a]	9.8 ± 3.8[a]	7.3 ± 2.4[c]	5.9 ± 1.7	<0.001	<0.001	NS	<0.001	NS	<0.001	<0.001
DHEA-S (μg/l)	309.2 ± 127.6[a]	304.7 ± 128.0[a]	318.6 ± 131.7[a]	204.9 ± 88.7	190.4 ± 77.6	<0.001	NS	NS	<0.001	NS	<0.001	<0.001
FAI	10.25 ± 7.94[a]	8.82 ± 7.12[a]	7.69 ± 4.51[a]	3.93 ± 3.44[a]	2.59 ± 1.72	<0.001	0.002	0.017	<0.001	NS	<0.001	<0.001
17α-OHP (nmol/l)	3.9 ± 1.8[a]	3.3 ± 1.5[a]	3.6 ± 1.8[a]	3.0 ± 1.8[a]	2.4 ± 1.2	<0.001	<0.001	NS	<0.001	NS	NS	NS
SHBG (nmol/l)	39.1 ± 23.7[a]	41.9 ± 26.2[a]	43.8 ± 24.1[a]	53.7 ± 31.0[a]	65.6 ± 35.6	<0.001	NS	NS	<0.001	NS	<0.001	NS
Glucose (mmol/l)	5.4 ± 0.9	5.3 ± 0.7	5.4 ± 0.6	5.6 ± 0.7	5.4 ± 0.6	0.006	NS	NS	NS	NS	0.002	NS
Insulin (pmol/l)	96.1 ± 100.4[a]	87.5 ± 73.2[a]	73.9 ± 45.2	91.1 ± 82.5	69.6 ± 71.0	<0.001	NS	NS	NS	NS	NS	NS
Glucose/insulin	0.087 ± 0.057[a]	0.088 ± 0.053[a]	0.099 ± 0.060	0.106 ± 0.089	0.111 ± 0.065	<0.001	NS	NS	NS	NS	NS	NS
AUCgluc-OGTT	881.5 ± 191.9[a]	859.8 ± 171.1[d]	848.6 ± 146.7	834.5 ± 202.5	807.9 ± 162.9	<0.001	NS	NS	NS	NS	NS	NS
HOMA-IR	3.33 ± 3.85[a]	2.94 ± 2.49[b]	2.47 ± 1.61	3.28 ± 3.41[d]	2.40 ± 2.79	<0.001	NS	NS	NS	NS	NS	NS
QUICKI	0.336 ± 0.034[a]	0.339 ± 0.032[a]	0.344 ± 0.029	0.340 ± 0.039[d]	0.351 ± 0.033	<0.001	NS	NS	NS	NS	NS	NS
Ovarian volume (cm^3)	9.2 ± 3.6[a]	5.6 ± 1.9	8.1 ± 3.5[a]	8.8 ± 4.2[a]	5.3 ± 1.8	<0.001	<0.001	0.011	NS	<0.001	<0.001	NS
Ovarian follicles	13.3 ± 4.7[a]	6.9 ± 2.1	11.9 ± 3.9[a]	12.4 ± 4.4[a]	6.3 ± 1.9	<0.001	<0.001	0.002	NS	<0.001	<0.001	NS

PCOS, polycystic ovary syndrome; NS, not significant; NA, not applicable; BMI, body mass index; W/H, waist to hip ratio; FSH, follicle-stimulating hormone; LH, luteinizing hormone; T, testosterone; Δ_4-A, Δ_4-androstenedione; DHEA-S, dehydroepiandrosterone sulfate; FAI, free androgen index; 17α-OHP, 17α-hydroxyprogesterone; SHBG, sex hormone-binding globulin; AUCgluc-OGTT, area of serum glucose levels under the oral glucose tolerance test curve; HOMA-IR, homeostasis model assessment of IR; QUICKI, quantitative insulin sensitivity check index.
Significant differences in the *post hoc* comparisons between PCOS phenotypes and controls: [a]$P < 0.001$; [b]$P < 0.005$; [c]$P < 0.01$; [d]$P < 0.05$.

Table III Comparisons between normal weight women with different phenotypes of PCOS and normal weight controls.

	PCOS (BMI <25) (n = 639)				Controls (BMI <25) (n = 150)	P-value (overall)	P [*post hoc* tests between the different phenotypes of PCOS (BMI <25)]					
	Phenotype 1 (n = 303)	Phenotype 2 (n = 194)	Phenotype 3 (n = 60)	Phenotype 4 (n = 82)			1 versus 2	1 versus 3	1 versus 4	2 versus 3	2 versus 4	3 versus 4
Age (years)	22.7 ± 4.3[a]	23.3 ± 5.1[a]	24.8 ± 5.5[a]	24.7 ± 6.5[a]	30.8 ± 5.5	<0.001	NS	0.038	0.012	NS	NS	NS
BMI (kg/m^2)	21.5 ± 1.9	21.6 ± 1.9	22.0 ± 19.9	21.6 ± 1.9	21.8 ± 1.9	NS	NA	NA	NA	NA	NA	NA
Waist (cm)	72.3 ± 5.7	72.2 ± 5.9	73.2 ± 5.7	72.3 ± 5.5	73.8 ± 5.8	NS	NA	NA	NA	NA	NA	NA
W/H	0.75 ± 0.05	0.74 ± 0.05	0.74 ± 0.05	0.75 ± 0.05	0.76 ± 0.05	NS	NA	NA	NA	NA	NA	NA
FSH (IU/l)	5.7 ± 1.6[a]	6.2 ± 1.9[a]	6.7 ± 1.9	6.4 ± 2.1[b]	7.1 ± 2.2	<0.001	NS	0.003	NS	NS	NS	NS
LH (IU/l)	10.0 ± 7.0[a]	7.6 ± 5.8	6.9 ± 4.3	7.4 ± 4.8	6.5 ± 2.9	<0.001	<0.001	0.010	0.002	NS	NS	NS
Testosterone (nmol/l)	2.9 ± 1.0[a]	2.6 ± 0.9[a]	2.6 ± 0.9[a]	1.4 ± 0.4	1.3 ± 0.4	<0.001	0.001	NS	<0.001	NS	<0.001	<0.001
Δ_4-A (nmol/l)	11.2 ± 3.8[a]	9.1 ± 3.5[a]	9.8 ± 3.8[a]	7.3 ± 2.4[b]	5.9 ± 1.4	<0.001	<0.001	0.046	<0.001	NS	0.001	<0.001
DHEA-S (μg/l)	300.7 ± 117.3[a]	296.9 ± 118.7[a]	319.4 ± 121.5[a]	196.7 ± 77.9	188.9 ± 76.1	<0.001	NS	NS	<0.001	NS	<0.001	<0.001
FAI	7.79 ± 6.19[a]	6.45 ± 4.89[a]	6.59 ± 4.17[a]	2.85 ± 1.93[b]	2.06 ± 1.15	<0.001	0.003	NS	<0.001	NS	<0.001	<0.001
17α-OHP (nmol/l)	3.6 ± 1.5[a]	3.3 ± 1.8[a]	3.3 ± 1.8[a]	3.3 ± 1.5[a]	2.1 ± 1.2	<0.001	0.003	NS	0.028	NS	NS	NS
SHBG (nmol/l)	48.2 ± 26.3[a]	52.7 ± 30.4[a]	51.9 ± 27.0[a]	65.5 ± 31.6	75.8 ± 35.1	<0.001	NS	NS	<0.001	NS	0.004	NS
Glucose (mmol/l)	5.3 ± 0.7	5.3 ± 0.6	5.4 ± 0.7	5.5 ± 0.5	5.3 ± 0.6	0.042	NS	NS	0.044	NS	0.049	NS
Insulin (pmol/l)	71.0 ± 115.5[b]	66.0 ± 50.9[c]	58.8 ± 30.1	56.7 ± 38.0	50.9 ± 27.3	0.001	NS	NS	NS	NS	NS	NS
Glucose/insulin	0.112 ± 0.063[b]	0.104 ± 0.051[c]	0.115 ± 0.066	0.135 ± 0.098	0.126 ± 0.058	0.001	NS	NS	NS	NS	0.043	NS
AUCgluc-OGTT	853.9 ± 172.0[a]	844.9 ± 166.8[a]	827.8 ± 127.5	787.2 ± 168.8	766.9 ± 139.4	<0.001	NS	NS	0.013	NS	NS	NS
HOMA-IR	2.32 ± 3.78[b]	2.17 ± 1.66[c]	1.94 ± 1.09	1.94 ± 1.32	1.67 ± 0.99	0.005	NS	NS	NS	NS	NS	NS
QUICKI	0.353 ± 0.031[b]	0.350 ± 0.028[c]	0.354 ± 0.027	0.357 ± 0.033	0.362 ± 0.027	0.006	NS	NS	NS	NS	NS	NS
Ovarian volume (cm^3)	9.0 ± 3.6[a]	5.6 ± 2.1	7.9 ± 4.1[a]	8.1 ± 3.7[a]	5.2 ± 1.7	<0.001	<0.001	NS	NS	<0.001	<0.001	NS
Ovarian follicles	13.2 ± 5.0[a]	6.9 ± 1.9	11.6 ± 3.6[a]	12.8 ± 4.4[a]	6.1 ± 1.9	<0.001	<0.001	NS	NS	<0.001	<0.001	NS

NS, not significant; NA, not applicable. Other abbreviations are defined in Table II.
Significant differences in the *post hoc* comparisons between PCOS phenotypes and controls: [a]$P < 0.001$; [b]$P < 0.05$, [c]$P < 0.005$.

Table IV Comparisons between overweight/obese women with different phenotypes of PCOS and overweight/obese controls.

	PCOS (BMI ≥25) (n = 573)				Controls (BMI ≥25) (n = 104)	P–value (overall)	P [*post hoc* tests between the different phenotypes of PCOS (BMI ≥25)]					
	Phenotype 1 (n = 281)	Phenotype 2 (n = 178)	Phenotype 3 (n = 58)	Phenotype 4 (n = 56)			1 versus 2	1 versus 3	1 versus 4	2 versus 3	2 versus 4	3 versus 4
Age (years)	24.4 ± 5.9[a]	25.4 ± 6.7[a]	25.9 ± 5.9[a]	24.9 ± 6.5[a]	32.0 ± 5.7	<0.001	NS	NS	NS	NS	NS	NS
BMI (kg/m^2)	32.2 ± 5.8	32.8 ± 6.8	30.5 ± 4.3	33.4 ± 7.6	31.5 ± 6.2	NS	NA	NA	NA	NA	NA	NA
Waist (cm)	96.7 ± 13.1	95.9 ± 13.5	89.6 ± 9.9	96.7 ± 16.2	93.5 ± 12.6	0.004	NS	0.004	NS	0.027	NS	NS
W/H	0.83 ± 0.07	0.82 ± 0.07	0.79 ± 0.07	0.81 ± 0.07	0.81 ± 0.06	0.001	NS	0.001	NS	NS	NS	NS
FSH (IU/l)	5.6 ± 1.7[a]	5.8 ± 1.7[a]	5.9 ± 1.4[b]	5.5 ± 1.9[a]	7.1 ± 2.5	<0.001	NS	NS	NS	NS	NS	NS
LH (IU/l)	7.6 ± 4.5[a]	6.5 ± 4.4	5.3 ± 3.6	5.8 ± 3.8	4.9 ± 2.0	<0.001	0.011	<0.001	0.012	NS	NS	NS
Testosterone (nmol/l)	2.9 ± 1.1[a]	2.7 ± 1.0[a]	2.6 ± 0.9[a]	1.5 ± 0.4	1.3 ± 0.4	<0.001	NS	NS	<0.001	NS	<0.001	<0.001
Δ_4-A (nmol/l)	10.1 ± 4.2[a]	9.1 ± 3.1[a]	9.8 ± 3.8[a]	6.6 ± 2.4	5.9 ± 1.7	<0.001	0.004	NS	<0.001	NS	<0.001	<0.001
DHEA-S (μg/l)	318.4 ± 137.5[a]	313.1 ± 137.3[a]	317.7 ± 142.6[a]	217.1 ± 102.1	192.6 ± 80.1	<0.001	NS	NS	<0.001	NS	<0.001	<0.001
FAI	12.91 ± 8.74[a]	11.39 ± 8.21[a]	8.82 ± 4.59[a]	5.51 ± 4.44[a]	3.37 ± 2.09	<0.001	NS	0.019	<0.001	NS	<0.001	<0.001
17α-OHP (nmol/l)	3.6 ± 1.8[a]	3.0 ± 1.2[a]	3.6 ± 1.8[a]	2.7 ± 1.8	2.4 ± 1.2	<0.001	NS	NS	0.003	NS	NS	0.008
SHBG (nmol/l)	29.3 ± 15.3[a]	30.3 ± 13.1[a]	35.3 ± 17.2[a]	36.5 ± 20.5[a]	50.9 ± 31.1	<0.001	NS	0.045	0.038	NS	NS	NS
Glucose (mmol/l)	5.6 ± 1.1	5.4 ± 0.7	5.4 ± 0.6	5.8 ± 0.8	5.6 ± 0.7	0.027	NS	NS	NS	NS	0.047	NS
Insulin (pmol/l)	124.1 ± 72.5[a]	111.2 ± 85.4	89.7 ± 53.1	141.3 ± 102.6[b]	97.6 ± 101.2	<0.001	NS	0.003	NS	NS	NS	0.008
Glucose/insulin	0.061 ± 0.034[a]	0.071 ± 0.049[c]	0.082 ± 0.049	0.063 ± 0.049[a]	0.089 ± 0.068	<0.001	NS	0.005	NS	NS	NS	0.038
AUCgluc-OGTT	912.7 ± 208.2	877.9 ± 175.1	871.2 ± 163.1	907.3 ± 228.8	867.7 ± 176.7	NS	NA	NA	NA	NA	NA	NA
HOMA-IR	4.41 ± 3.63[a]	3.77 ± 2.94	3.02 ± 1.86	5.22 ± 4.46[b]	3.47 ± 3.99	<0.001	NS	0.003	NS	NS	NS	0.003
QUICKI	0.318 ± 0.027[a]	0.327 ± 0.031	0.335 ± 0.029	0.315 ± 0.034[b]	0.334 ± 0.035	<0.001	0.039	0.003	NS	NS	NS	0.007
Ovarian volume (cm^3)	9.3 ± 3.5[a]	5.6 ± 1.7	8.3 ± 2.8[a]	9.8 ± 4.6[a]	5.4 ± 1.8	<0.001	<0.001	NS	NS	<0.001	<0.001	NS
Ovarian follicles	13.4 ± 4.4[a]	7.0 ± 2.2	12.1 ± 4.3[a]	11.8 ± 4.4[a]	6.5 ± 1.8	<0.001	<0.001	NS	0.022	<0.001	<0.001	NS

NS, not significant; NA, not applicable. Other abbreviations are defined in Table II.
Significant differences in the *post hoc* comparisons between PCOS phenotypes and controls: [a]$P < 0.001$; [b]$P < 0.005$ [c]$P < 0.05$; [d]$P < 0.01$.

previous reports, phenotype 1 was the most prevalent PCOS phenotype (48.2%) (Dewailly *et al.*, 2006; Shroff *et al.*, 2007; Guastella *et al.*, 2010). Both phenotypes 1 and 2 were more insulin resistant than controls, in agreement with other reports (Legro *et al.*, 2005; Dewailly *et al.*, 2006; Hahn *et al.*, 2006; Shroff *et al.*, 2007; Guastella *et al.*, 2010). In addition, overweight/obese women with phenotype 1 had higher circulating androgens than women with phenotype 2 and appeared to be more insulin resistant than the latter as evidenced by the lower QUICKI. Previous studies in overweight/obese women with PCOS did not detect any differences in circulating androgens or markers of IR between these phenotypes (Legro *et al.*, 2005; Dewailly *et al.*, 2006; Hahn *et al.*, 2006; Shroff *et al.*, 2007; Guastella *et al.*, 2010). However, the latter studies were smaller and phenotype 2 was considerably less frequent (6.7–14.3% versus 30.7% in our study) (Dewailly *et al.*, 2006; Shroff *et al.*, 2007; Guastella *et al.*, 2010). Therefore, they might have lacked the statistical power to identify differences. In addition, we assessed a variety of markers of IR and only the QUICKI differed between phenotypes 1 and 2. Previous studies evaluated a limited number of indices of IR and this might also explain their discrepant results. It has been reported that PCO *per se*, in the absence of ANOV and HA, is associated with increased androgen levels and IR; even though this association is not consistent across studies, it might explain the difference between phenotypes 1 and 2 (Adams *et al.*, 2004; Carmina *et al.*, 2005; Azziz, 2006). Of note, circulating androgens were higher in normal weight women with phenotype 1 than in women with phenotype 2 but insulin sensitivity was comparable in the two groups. It is therefore possible that obesity, by aggravating IR, allows subtle differences in IR between these two phenotypes to become more apparent. However, we are not aware of any other studies that compared normal weight women with phenotypes 1 and 2 of PCOS and more data are needed to confirm or refute our observations.

An important finding of our study is that neither normal weight nor overweight/obese women with phenotype 3 differed from controls in markers of IR. This is in agreement with previous studies in overweight/obese women with PCOS (Welt *et al.*, 2006; Carmina *et al.*, 2009; Wiltgen and Spritzer, 2010), whereas in previous smaller cohorts of normal weight subjects, phenotype 3 showed similar or more pronounced IR than BMI-matched controls (Carmina *et al.*, 2005; Barber *et al.*, 2007). However, the *W* was not reported in the single study that found higher insulin levels and lower QUICKI in normal weight women with phenotype 3 than in controls (Carmina *et al.*, 2005) and it is possible that the former women had more pronounced abdominal adiposity despite the comparable BMI (Carmina *et al.*, 2009). Most scientific societies concur that the diagnosis of PCOS is justified in women with phenotype 3 of PCOS, despite the presence of ovulatory cycles (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2003; Azziz *et al.*, 2009). Indeed, in our study, circulating androgens did not differ between phenotype 3 and the 'classic' PCOS phenotype 2. In addition, there were marginally significantly higher plasma Δ_4-A levels in normal weight women with phenotype 1 than in women with phenotype 3, whereas only overweight/obese women with phenotype 1 had more severe HA than women with phenotype 3. These findings support previous reports that suggest that HA is comparable in phenotype 3 and in the 'classic' phenotypes 1 and 2 (Carmina *et al.*, 2005, 2006; Welt *et al.*, 2006). Therefore, phenotype 3 appears to be part of the PCOS spectrum but it might be related with lower cardiovascular risk because of the lack of IR. Interestingly, it was recently reported that women with phenotype 3 have lower carotid intima-media thickness, a marker of subclinical atherosclerosis, compared with BMI-matched women with phenotypes 1, 2 and 4 (Dilbaz *et al.*, 2011). This favorable cardiovascular profile of phenotype 3 has important implications, since approximately 1 in 10 PCOS patients belonged to this group in our study and this proportion appears to be even higher in other populations (up to 28.8%) (Dewailly *et al.*, 2006; Shroff *et al.*, 2007; Guastella *et al.*, 2010).

Whether phenotype 4 belongs to PCOS is the subject of ongoing debate (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2003; Azziz, 2006; Franks, 2006; Azziz *et al.*, 2009). The androgen excess and PCOS Society suggested that PCOS should be first considered a disorder of androgen excess or hyperandrogenism and does not consider women with phenotype 4 to have PCOS (Azziz *et al.*, 2009). However, in our study, overweight/obese women with phenotype 4 were more insulin resistant than BMI-matched controls; moreover, the former did not differ in markers of IR from women with phenotypes 1 and 2. Previous smaller studies reported similar findings (Welt *et al.*, 2006; Shroff *et al.*, 2007), whereas others, who did not identify differences in IR between phenotype 4 and controls, evaluated only a limited number of markers of IR (Broekmans *et al.*, 2006; Barber *et al.*, 2007). On the other hand, normal weight women with phenotype 4 did not differ from controls in markers of IR, in support of previous reports (Dewailly *et al.*, 2006; Guastella *et al.*, 2010). Therefore, our results suggest that overweight/obese women with phenotype 4 have similarly increased IR as women with the NIH phenotypes.

Women with PCOS were younger than controls and this represents a limitation of our study. In healthy women of reproductive age, circulating androgens progressively decline with aging (Davison *et al.*, 2005; Spencer *et al.*, 2007). Therefore, the differences in circulating androgens between women with PCOS and controls in our study might have been smaller if the two groups were age-matched. However, we consider it unlikely that the highly significant differences in circulating androgens that we observed between women with PCOS and controls ($P < 0.001$ in all comparisons) would not be significant if age did not differ between the two groups. On the other hand, IR worsens with aging in healthy women of reproductive age (Ferrannini *et al.*, 1996). Since the controls were older than women with PCOS in our study, the difference in markers of IR might have been even greater if age did not differ between the two groups. In addition, when we performed an ANOVA with age as a covariate, all differences in markers of IR and circulating androgens between PCOS patients and controls persisted in both the total population and in overweight/obese subjects (data not shown). When we performed a similar analysis in normal weight subjects, insulin levels, HOMA and QUICKI were no longer significantly different between PCOS patients and controls; however, the differences in other markers of IR (AUCgluc-OGTT, glucose/insulin and glucose levels) and in circulating androgens between PCOS patients and controls persisted (data not shown). Finally, and perhaps more importantly, age did not differ between the different phenotypes of PCOS and therefore we believe that our findings regarding the differences in IR and endocrine characteristics between the different PCOS phenotypes are valid.

In conclusion, women with phenotype 1 of PCOS appear to be more insulin resistant and to have more pronounced HA than women with phenotype 2. Phenotype 4 is also characterized by IR, when obesity is present, despite the absence of HA. In contrast, women with phenotype 3 do not appear to differ in markers of IR from BMI-matched controls. It remains to be established in long-term studies whether these differences in endocrine features and IR between phenotypes will also translate into different cardiovascular outcomes.

Authors' roles

D.P. and G.M. contributed to the conception and design of the study, to the acquisition, analysis and interpretation of data and to drafting the article. K.T. and D.M. contributed to the conception and design of the study, to the analysis and interpretation of data and to drafting the article. E.P., G.B. and I.L. contributed to the acquisition of data and revised the article critically for important intellectual content. All authors gave their final approval of the version to be published.

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Conflict of interest

None declared.

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