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Metabolically healthy polycystic ovary syndrome (MH-PCOS) and metabolically unhealthy polycystic ovary syndrome (MU-PCOS): a comparative analysis of four simple methods useful for metabolic assessment

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STUDY QUESTION: Is it possible to distinguish metabolically healthy polycystic ovary syndrome (MH-PCOS) from metabolically unhealthy PCOS (MU-PCOS) by simple diagnostic tools such as body mass index (BMI), waist/hip ratio (WHR), at-risk category suggested by Androgen Excess Society (AES) and visceral adiposity index (VAI)?

SUMMARY ANSWER: VAI could be an easy and useful tool in clinical practice and in population studies for assessment of MU-PCOS.

WHAT IS KNOWN ALREADY: VAI is a good indicator of insulin sensitivity and cardiometabolic risk in oligo-ovulatory women with PCOS.

STUDY DESIGN, SIZE, DURATION: We conducted a cross-sectional study of 232 women with PCOS in a university hospital setting.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Anthropometric, hormonal and metabolic parameters were evaluated. An oral glucose tolerance test measured areas under the curve (AUC) for insulin ($AUC_{2h\ insulin}$) and for glucose ($AUC_{2h\ glucose}$). Homeostasis model assessment of insulin resistance (HOMA2-IR), the Matsuda index of insulin sensitivity (ISI), the oral dispositional index (DIo) and VAI were determined.

MAIN RESULTS AND THE ROLE OF CHANCE: The prevalence of MU-PCOS according to the different criteria was: BMI, 56.0%; WHR, 18.1%; at-risk criteria of AES, 72.0% and VAI, 34.5%. The likelihood that a woman would exhibit MU-PCOS (except when diagnosed by the WHR criterion) showed a significant positive association with high HOMA2-IR [BMI criterion: (odds ratio (OR): 1.86; 95% confidence interval (CI): 1.43–2.41); risk criteria of AES (OR: 1.86; 95% CI: 1.36–2.56); VAI criterion (OR: 1.45; 95% CI: 1.17–1.80)] and a significant negative association with low ISI Matsuda [BMI criterion: (OR: 0.81; 95% CI: 0.72–0.91); risk criteria of AES (OR: 0.78; 95% CI: 0.69–0.89); VAI criterion (OR: 0.82; 95% CI: 0.71–0.94)]. Only MU-PCOS according to the VAI criterion showed a significant association with low DIo (OR: 0.85; 95% CI: 0.75–0.96); these women also showed a significant association with low luteal progesterone levels (OR: 0.97; 95% CI: 0.95–0.99).

LIMITATIONS, REASONS FOR CAUTION: The analysis is limited by the lack of a gold standard definition of metabolic health that would have allowed the execution of a receiver operator characteristic analysis of the four proposed criteria.

WIDER IMPLICATIONS OF THE FINDINGS: The results will facilitate the early recognition of cardiometabolic risk in women with PCOS before they develop overt metabolic syndrome.

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Key words: PCOS / visceral adiposity index / metabolic assessment

Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder among women of reproductive age, with a prevalence of between 5 and 10% (Diamanti-Kandarakis *et al.*, 1999; Asuncion *et al.*, 2000). Recently, however, the variability in the prevalence data was found to depend on the diagnostic criteria used; the prevalence reported being lower when the NIH criteria were used (March *et al.*, 2010) but higher reaching 19.9 and 15.3% using the Rotterdam and Androgen Excess and Polycystic Ovary Syndrome Society criteria, respectively (Yildiz *et al.*, 2012)]. The prevalence of PCOS is also linked to the body mass index (BMI) of the study population (Boyle *et al.*, 2012). In addition to chronic anovulation and hyperandrogenism (clinical and biochemical), PCOS is also associated with an increased risk of glucose intolerance and type 2 diabetes mellitus (DM), dyslipidemia, subclinical atherosclerosis and vascular dysfunction, independently of BMI (Dunaif, 1997). Although there are few large prospective longitudinal studies of cardiovascular event rates and mortality in PCOS, an increased prevalence of classical and non-classical cardiovascular risk factors and changes in potential modulators of cardiometabolic risk have been well documented (Amato *et al.*, 2008; Galluzzo *et al.*, 2008; Hoffman and Ehrmann, 2008; Diamanti-Kandarakis and Dunaif, 2012; Randeva *et al.*, 2012). Today the influence of insulin resistance (IR) in the genesis of PCOS and its associated cardiometabolic risk are well known. Although in 2006 the Androgen Excess Society (AES) declared that 'PCOS should be first considered a disorder of androgen excess or hyperandrogenism' (Azziz *et al.*, 2006), it has recently issued a position statement highlighting the primary importance of assessment of cardiometabolic risk and its prevention in women with PCOS (Wild *et al.*, 2010). In particular, considering the risk categories for women provided by the American Heart Association (Mosca, 2007), the committee recommends that PCOS-related cardiovascular disease (CVD) risk be categorized as follows: at-risk [PCOS women with any of the following risk factors: obesity (especially increased abdominal adiposity), cigarette smoking, hypertension, dyslipidemia (increased low-density lipoprotein (LDL)-cholesterol and/or non-high-density lipoprotein (HDL) cholesterol), subclinical vascular disease, impaired glucose tolerance (IGT), family history of premature CVD (<55 years of age in male relative, <65 years of age in female relative)] or at high risk [PCOS women with metabolic syndrome (MetS), type 2 diabetes, overt vascular or renal disease]. Given the frequent presence of IR in patients with PCOS and its association with adverse metabolic consequences, it is important to identify women with PCOS who are insulin resistant; regrettably, the gold standard hyperinsulinemic euglycemic clamp and the minimal model analysis of intravenous glucose tolerance test data, while being very reliable indexes (Bergman *et al.*, 1985), involve high costs and difficulties of execution, making them inapplicable in the routine diagnostic phase of PCOS. For these reasons the use of a simple homeostatic model assessment (HOMA-IR) has spread both in research and in clinical practice (Matthews *et al.*, 1985). However, this has considerable limitations, especially in normal weight women with PCOS, where it is unable to investigate the compensatory hyperinsulinism secondary to IR (Fulghesu *et al.*, 2006). A reasonable alternative is to use one of the indexes derived from glucose and insulin values during the oral glucose tolerance test (OGTT), which have proved to exhibit satisfactory accuracy (Belfiore *et al.*, 1998; Mari *et al.*, 2001); but given the high prevalence of PCOS to run OGTTs on a sufficiently large scale would entail considerable costs for the public health-care system.

In the current study, we wanted to verify whether it was possible to distinguish women with metabolically healthy PCOS (MH-PCOS) from women with metabolically unhealthy PCOS (MU-PCOS) through the use of simple diagnostic tools such as BMI, waist to hip ratio (WHR), the at-risk category suggested by AES (Wild *et al.*, 2010) and the visceral adiposity index (VAI) (Amato *et al.*, 2010, 2011a,b).

Materials and Methods

Subjects

This study was approved by the Institutional Review Board at the Faculty of Medicine of the University of Palermo. At the time of observation in our outpatients clinic, all patients regularly signed an informed consent for the scientific use of their data. It involved a cross-sectional study of 302 consecutive Caucasian women of reproductive age [mean: 24.09 years; standard deviation (SD): 6.03; range: 14–43] with mean BMI values of 29.68 kg/m^2 (SD: 6.91; range: 18–50) followed up in our outpatients clinic (from 1 January 2004 to 31 December 2011) for hirsutism and/or other signs and symptoms of hyperandrogenism (acne/seborrhea, alopecia) and/or irregular cycles (oligo-amenorrhea). By applying the diagnostic criteria of the AES (Azziz *et al.*, 2006), we retrospectively selected 232 of these cases (76.8%) with PCOS.

The following subjects were excluded from the study: women with amenorrhea at the moment of observation (absence of vaginal bleeding >6 months) as there were very few of these (22/302 cases) and those with various progesterone (Pg) bleeding induction schemes, potentially interfering with sex hormone patterns; women treated with clomiphene citrate, oral contraceptives, antiandrogens and drugs to control their appetite or insulin-sensitizing drugs (metformin, pioglitazone and rosiglitazone) during the 6 months prior to the first examination; women with hyperprolact inemia (4/302 cases); patients with 17OH-progesterone (17OH-pg) levels >6.05 nmol/l who, after 250 mg Synacthen (synthetic analog of adrenocorticotrophic hormone), showed 17OH-pg >30.26 nmol/l at 60 min (3/302 cases); women with dehydroepiandrosterone sulfate (DHEA-S) >16.32 mmol/l, who, when screened with a computerized axial tomography scan, presented adrenal hyperplasia or adenoma or virilizing androgen-secreting neoplasias (1/302 cases); women whose clinical and hormone evaluation (phenotype, increased 24 h free urinary cortisol, high cortisol levels after 1 mg of overnight dexamethasone) suggested Cushing's syndrome (3/302 cases); all women with hypo- or hyperthyroidism were not taken into consideration for the study.

The following relevant data were obtained from our database: possible family history of diabetes, premature CVD (<55 years of age in male relative and <65 years of age in female relative), oligo-amenorrhea, hirsutism, acne and age of menarche; weight, BMI, waist circumference (WC), blood pressure and Ferriman–Gallwey (FG) score. It is routine practice in our outpatients clinic to evaluate the degree of hirsutism with the use of the FG map scoring system, which divides the body up into 11 domains. Hirsutism was defined as the FG score >8 (Ferriman and Gallwey, 1961). During the follow-up in our outpatients clinic, patients were generally tested for FSH, LH, 17-b-estradiol (E2), 17OH-pg, basal prolactin, total testosterone, DHEA-S, Δ4androstenedione and sex hormone-binding globulin (SHBG), during the follicular phase (Day 7 from the beginning of the last period). On the same day, we also tested for total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, glutamic–pyruvic transaminase (GPT), glutamic-oxaloacetic transaminase (GOT) and uric acid and performed an OGTT (75 g glucose) and measured glycemia and insulinemia at 0, 30, 60, 90 and 120 min. Serum Pg level was determined between Days 20 and 24 of the menstrual cycle and chronic oligo-anovulation was established if two consecutive cycles were anovulatory [Pg level <3 ng/ml (international system (SI): <9.54 nmol/l)] (Azziz *et al.*, 2009). Biochemical hyperandrogenism was diagnosed when androgen levels were as follows: total testosterone >2.84 nmol/l, DHEA-S >12.14 mmol/l and Δ4androstenedione >10.72 nmol/l [calculated on the basis of the 95th percentile upper limits of basal serum androgen concentrations in 144 healthy normal Sicilian eumenorrheal women without hirsutism and with no family history of PCOS (used as a control group in a previous study (Amato *et al.*, 2011a,b)]. Transvaginal ovarian ultrasound scanning was performed between Days 5 and 10 from the beginning of the last period using a 7.5-MHz vaginal probe transducer (General Electric LOGIQ 400MD, Milwaukee, WI, USA). Both ovaries were measured in the sagittal, transverse and coronal planes. Ovaries were classified as polycystic if 12 or more follicles measuring 2–8 mm in diameter were present in each ovary, and/or there was an increase in ovarian volume (<10 ml) (Christensen *et al.*, 1997; The Rotterdam ESHRE/ASRM-sponsored PCOS Consensus Workshop Group, 2004; Carmina *et al.*, 2005).

MetS was diagnosed according to the National Cholesterol Education Program Adult Treatment Panel (NCEP-ATP) III definition [Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults, 2001] and diagnosis of DM according to the American Diabetes Association criteria (Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 1997).

Assays

All hormones were measured in our laboratory using commercial kits. These included ELISA (DRG Diagnostics, DRG Instruments GmbH, Germany) for FSH (mUI/ml), LH (mUI/ml), 17-β-E2 (pg/ml), 17OH-Pg (ng/ml), Pg (ng/ml), PRL (ng/ml), total testosterone (ng/ml), Δ4androstenedione (ng/ml; Arnika, Milan, Italy) and insulin (mUI/l; the intra- and inter-assay CVs were ≤4% and ≤3.6%, respectively). Chemiluminescence assays were used for DHEA-S (μg/dl; Immulite, Diagnostic Products, Genoa, Italy) and serum SHBG (nmol/l; Immulite, Diagnostic Products, Genoa, Italy). Blood glucose levels (mg/dl) were measured using an electrochemical system (Glucocard, Menarini Diagnostics, Italy). Total cholesterol, HDL, triglycerides, GOT, GPT and uric acid were measured in our laboratory using standard assays. LDL cholesterol levels were calculated with Friedewald's formula. The conversion factors for the SI were the following: glucose (mg/dl versus mmol/l: 0.0555), insulin (mUI/l versus pmol/l: 6.945), total cholesterol (mg/dl versus mmol/l: 0.0259), total testosterone (ng/ml versus nmol/l: 3.467), free testosterone (pg/ml versus pmol/l: 3.47), DHEA-S (μg/dl versus mmol/l: 0.0272), Δ4androstenedione (ng/ml versus nmol/l: 3.492), 17-β-E2 (pg/ml versus pmol/l: 3.671), 17-OHPg (ng/ml versus nmol/l: 3.026), Pg (ng/ml versus nmol/l: 3.180), PRL (ng/ml versus μg/l: 1), FSH (mUI/ml versus IU/l: 1) and LH (mUI/ml versus IU/l: 1).

Free androgen index (FAI) was calculated as the ratio of total testosterone levels in nmol/l to SHBG levels in nmol/l × 100 (%) (Vermeulen *et al.*, 1999). Insulin sensitivity was estimated indirectly using fasting plasma insulin and fasting plasma glucose to calculate the homeostatic model of insulin resistance (HOMA2-IR) [calculations were performed using free software provided by the University of Oxford Diabetes Trial Unit (http://www.dtu.ox.ac.uk/homacalculator)] and using glucose and insulin values during the OGTT to calculate the Matsuda index of insulin sensitivity (ISI Matsuda) (Matsuda and De Fronzo, 2001) (10 000/glucose (mg/dl) × insulin (μU/ml) × glucose mean × insulin mean). A composite measure of β-cell function relative to insulin sensitivity, assessed by oral disposition index (DIo) (Utzschneider *et al.*, 2009), was calculated as ($\Delta Insulin_{0-30}/\Delta Glucose_{0-30}$) × (1/fasting insulin). The trapezoidal method was used for the calculation of the areas under the curves for insulin ($AUC_{2h\ insulin}$) and glucose ($AUC_{2h\ glucose}$).

VAI was calculated as described previously (Amato *et al.*, 2010) using the following sex-specific equations, where TG is the triglyceride concentration expressed in mmol/l and HDL is the HDL cholesterol concentration expressed in mmol/l:

$$\text{Females: VAI} = \left(\frac{\text{WC}}{36.58 = (1.89 \times \text{BMI})}\right) \times \left(\frac{\text{TG}}{0.81}\right) \times \left(\frac{1.52}{\text{HDL}}\right).$$

Statistical methods

The Statistical Packages for Social Sciences SPSS version 17 and MedCalc version 11.3 were used for data analysis. Baseline characteristics were presented as the mean ± SD and range for continuous variables; rates and proportions were calculated for categorical data. Normality of distribution for quantitative data was assessed by the Kolmogorov–Smirnov test. Because total cholesterol did not present normal distribution, before statistical analysis with parametric tests, it was log-transformed. Differences between two groups in univariate analysis were detected by the unpaired Student's *t*-test for continuous variables (after testing for equality of variance: Levene test) and by the χ^2 test and Fisher's exact test (when appropriate) for categorical variables.

A case–control comparison was made between women with MH-PCOS and MU-PCOS, distinguished using the following four criteria: (i) BMI ≤ versus >27 kg/m^2 [since some authors believe that this cutoff identifies IR (Garca-Estévez *et al.*, 2004; Stepto *et al.*, 2013)], (ii) WHR < versus ≥0.85 (http://whqlibdoc.who.int/publications/2011/9789241501491_eng.pdf), (iii) the presence of metabolic risk as defined by 2010-AES Consensus Statement (Wild *et al.*, 2010), (iv) VAI ≤ versus >1.675 [the cutoff point of VAI previously found to identify an ISI Matsuda value of <25th percentile in 144 healthy women (Amato *et al.*, 2011a,b)]. The agreement (Cohen's kappa value) between the four criteria was calculated. The kappa value ranges from 0 (full disagreement) to 1 (full agreement). Values in the range of 0–0.20, 0.21–0.40, 0.41–0.60, 0.61–0.80 and 0.81–1.00 were considered poor, fair, moderate, good and very good concordance (Altman, 1991).

A multiple logistic regression analysis was performed to determine the association of MU-PCOS with hormonal and metabolic parameters. Variables significantly associated with the dependent variable on univariate analysis (probability threshold, $P \leq 0.050$) were corrected for age and $AUC_{2h\ insulin}$ (in the case of hormonal parameters), or for age and androgen levels (in the case of metabolic parameters). HOMA2-IR, ISI Matsuda, $AUC_{2h\ insulin}$, $AUC_{2h\ glucose}$ and DIo were analyzed separately in the multivariate models, because they are collinear variables. A *P* value of <0.05 was considered statistically significant.

Table I Clinical, anthropometric and biochemical characteristics of the 232 women with PCOS.

	All PCOS women (n = 232)	
	Mean ± SD	**Range**
Age	24.2 ± 6.05	14–43
BMI (kg/m^2)	30.1 ± 6.98	18–50
Age of menarche	12.0 ± 1.39	9–17
WC (cm)	92 ± 17.7	54–138
WHR	0.8 ± 0.09	0.59–1.17
FG score	13.7 ± 6.88	1–35
Classes of obesity[a]	Subjects (No)	Subjects (%)
Underweight	3	1.3
Normal weight	66	28.4
Overweight	64	27.6
Obese class I	42	18.1
Obese class II	37	15.9
Obese class III	20	8.6
Cigarette smoking	41	17.7
Dyslipidemia (increased LDL-C and/or non-HDL-C)[b]	79	34.1
Subclinical vascular disease[b]	2	0.9
Family history of premature CVD (<55 years of age in male relative, <65 years of age in female relative)[b]	24	10.3
Metabolic syndrome[c]	39	16.8
Diabetes or fasting glucose ≥5.6 mmol/l	35	15.1
High blood pressure (≥130/85 mmHg)	14	6
High triglycerides (≥1.7 mmol/l)	36	15.5
Low HDL cholesterol (<1.04 mmol/l)	96	41.4
Increased WC (>88 cm)	127	54.7
IFG	28	12.1
IGT	9	3.9
IFG + IGT	5	2.2
DM	2	0.9
Hormonal profiles	Mean ± SD	Range
FSH (IU/l)	6.6 ± 2.82	1–21.70
LH (IU/l)	8.5 ± 4.58	0.30–24
17-β-E2 (pmol/l)	190 ± 98. 8	30.95–587
17OH-pg (nmol/l)	4.4 ± 2.94	0.51–20.58
Pg (nmol/l)	11.943 ± 16.50	0.29–89.68
Total testosterone (nmol/l)	2.3 ± 1.44	0.07–11.09
SHBG (nmol/l)	69 ± 43.0	10–200
FAI [100 × (total testosterone/SHBG)] (%)	5.4 ± 6.05	0.18–42.49
DHEA-S (μmol/l)	8.1 ± 4.52	1.33–23.61
Δ4androstenedione (nmol/l)	9.9 ± 6.99	1.05–45.40
Metabolic profiles	Mean ± SD	Range
HOMA2-IR	2.3 ± 1.35	0.40–7.20
ISI Matsuda	3.9 ± 3.05	0.25–23.00
$AUC_{2h\ insulin}$ (pmol/l 120 min)	11 000 ± 6800	762–36 540
$AUC_{2h\ glucose}$ (mmol/l 120 min)	770 ± 151	477–1401
Dio	4.4 ± 11.68	0.05–96.10
VAI	1.9 ± 1.64	0.19–19.87
Total cholesterol (mmol/l)	5.2 ± 1.20	2.94–11.56
HDL cholesterol (mmol/l)	1.3 ± 0.28	0.17–2.19
Calculated LDL cholesterol (mmol/l)	3.0 ± 1.07	0.95–8.85
Non-HDL cholesterol (mmol/l)	3.9 ± 1.22	1.60–10.58
Triglycerides (mmol/l)	1.29 ± 0.56	0.29–4.35
Uric acid (μmol/l)	253 ± 100.5	107.06–571
GOT (U/l)	21.2 ± 7.81	11–68
GPT (U/l)	24.8 ± 15.04	7–129

IFG, impaired fasting glucose; IGT, impaired glucose tolerance; DM, diabetes mellitus
[a]WHO classification.
[b]According to the Consensus Statement by the Androgen Excess and PCOS (AE-PCOS) Society (2010).
[c]According to the ATP III criteria.

Results

The demographic, clinical and biochemical characteristics (including anthropometric measurements, hormonal profile and metabolic profile) of the 232 PCOS women are presented in Table I. Of the entire cohort of patients, only 28.4% had a normal weight and 1.3% were underweight; the remaining women had a ponderal excess (70.3%). Subdividing the 232 women with PCOS on the basis of the four criteria described in the statistical methods, the prevalence of MU-PCOS was 130/232 (56.03%) according to the BMI criterion, 42/232 (18.10%) according to the WHR criterion, 167/232 (71.98%) according to the risk criteria of AES and 80/232 (34.48%) according to the VAI criteria. Agreement between the BMI criterion and WHR, AES and VAI criteria was poor ($K = 0.152$; $P = 0.001$), fair ($K = 0.354$; $P < 0.001$) and fair ($K = 0.285$; $P < 0.001$), respectively; the agreement between the WHR and AES and VAI criteria was poor ($K = 0.105$; $P = 0.003$) and poor ($K = 0.183$; $P = 0.002$), respectively; the agreement between the AES and VAI criteria was fair ($K = 0.204$; $P < 0.001$).

MU-PCOS according to BMI > 27 kg/m^2

Applying this criterion, women with MU-PCOS showed no phenotypic differences from MH-PCOS, as regards oligo-menorrhea, polycystic ovarian morphology (PCOM), hirsutism, biochemical hyperandrogenism and acne/seborrhea (Table II). The MU-PCOS women showed a significantly higher age than the MH-PCOS ones (25.2 ± 6.52 versus 23.0 ± 5.16 years; $P = 0.006$). The prevalence of MetS and of all its

components was significantly higher in the MU-PCOS women, while there were no significant differences for the categories of glucose tolerance except for a higher prevalence of impaired fasting glucose (IFG) (Table II). A high-risk condition according to the AES definition was more prevalent in MU-PCOS than in MH-PCOS (29.2 versus 2.9%; $P < 0.001$). Multivariate logistic regression analyses showed that high HOMA2-IR, $AUC_{2h\ insulin}$, $AUC_{2h\ glucose}$, VAI, triglycerides, GOT, GPT, uric acid, low HDL cholesterol and ISI Matsuda were significantly associated with MU-PCOS. Multivariate analysis showed no association between hormonal profiles and MU-PCOS (Supplementary data, Table SI; Fig. 1).

MU-PCOS according to WHR ≥ 0.85

Women with MU-PCOS (identified by applying the WHR criterion) showed no phenotypic differences compared with those with MH-PCOS, as regards oligo-menorrhea, PCOM, hirsutism, biochemical hyperandrogenism and acne/seborrhea (Table II). MU-PCOS and MH-PCOS women had similar ages (23.7 $\pm$ 5.95 versus 24.3 $\pm$ 6.08 years; $P = 0.525$). The prevalence of MetS was significantly higher in women with MU-PCOS, but only a significant increase in the prevalence of the 'high triglycerides' and 'increased WC' components was found; no significant differences were found for the categories of glucose tolerance (Table II). A high-risk condition according to the AES definition was more prevalent in MU-PCOS compared with MH-PCOS (31.0% versus 14.7%; $P = 0.013$). Multivariate logistic regression analyses showed that high VAI, triglycerides and low HDL cholesterol were significantly associated with MU-PCOS. Multivariate analysis showed no association between hormonal profiles and MU-PCOS (Supplementary data, Table SI; Fig. 1).

MU-PCOS according to the presence of metabolic risk as defined by the 2010-AES Consensus Statement

Applying this criterion, women with MU-PCOS showed no phenotypic differences from ones with MH-PCOS, as regards oligo-menorrhea, PCOM, hirsutism, biochemical hyperandrogenism and acne/seborrhea (Table II). MU-PCOS women showed a comparable age to MH-PCOS ones (24.5 $\pm$ 6.40 versus 23.4 $\pm$ 4.93 years; $P = 0.151$). The prevalence of MetS and of all its components (except the diabetes or fasting glucose $\geq$5.6 mmol/l) was significantly higher in MU-PCOS women, while there were no significant differences for the categories of glucose tolerance (Table II). A high-risk condition according to the AES definition was more prevalent in MU-PCOS compared with MH-PCOS (24.0% versus 1.5%; $P < 0.001$). Multivariate logistic regression analyses showed that the status of MU-PCOS has a significant positive association with HOMA2-IR, $AUC_{2h\ insulin}$, VAI, total cholesterol, LDL cholesterol, triglycerides, TGO, TGP, uric acid and a significant association negative with ISI Matsuda and HDL cholesterol. Moreover women with MU-PCOS showed lower levels of SHBG and higher levels of total testosterone and FAI (Supplementary data, Table SII; Fig. 1).

MU-PCOS according to VAI >1.675

Applying this criterion, women with MU-PCOS showed phenotypic differences from ones with MH-PCOS, meaning a higher prevalence of oligo-menorrhea (88.8 versus 69.7%; $P < 0.001$) and hirsutism (91.3 versus 72.4%; $P < 0.001$) (Table I). MU-PCOS women showed a comparable age to MH-PCOS women (25.2 $\pm$ 6.65 versus 23.7 $\pm$ 5.66 years; $P = 0.083$). The prevalence of MetS and of all its components (except the diabetes or fasting glucose $\geq$5.6 mmol/l) was significantly higher in MU-PCOS women, while there were no significant differences for the categories of glucose tolerance (Table II). A high-risk condition according to the AES definition was more prevalent in MU-PCOS than in MH-PCOS (45.0 versus 3.3%; $P < 0.001$). Multivariate logistic regression analyses showed that the status of MU-PCOS has a significant positive association with HOMA2-IR, $AUC_{2h\ insulin}$, total cholesterol, LDL cholesterol, uric acid and a significant negative association with ISI Matsuda and DIo. Regarding associations with hormonal profiles, women with MU-PCOS showed no association with the androgenic quota, but showed a significant association with low Pg levels (Supplementary data, Table SII; Fig. 1).

Phenotypes of PCOS according to AES and MU-PCOS

According to the AES classification of PCOS, nine different phenotypes of the condition are possible (Supplementary data, Table SIII). Using the different phenotypes described no significant differences are found in the prevalence of MU-PCOS, for all four of the used criteria, except for the *risk according to the AES criterion* [significant lower prevalence of MU-PCOS in D phenotype (1.2 versus 9.2%; $P = 0.006$) and significant higher prevalence of MU-PCOS in I phenotype (7.8 versus 0%; $P = 0.021$) and VAI > 1.675 criterion [significant lower prevalence of MU-PCOS in H phenotype (2.5 versus 15.8%; $P = 0.001$) and in I phenotype (0 versus 8.6%; $P = 0.005$)] (Supplementary data, Table SIII).

Discussion

Insulin resistance is a common but not universal feature of PCOS, and it is not always associated with an increased BMI. Indeed, many studies have shown that both lean and obese women with PCOS have IR (Dunaif, 1997); although, recently it has been shown that in PCOS there is an intrinsic IR that is further worsened with increasing BMI (Stepto *et al.*, 2013). It is also known that obesity, in the general population, is not necessarily an expression of cardiometabolic risk, given that there exists 'Metabolically Healthy Obesity' in which the particular gynoid distribution of fat does not confer a cardiometabolic risk (Després, 2012).

Our study has proved that it is possible to identify the MU-PCOS phenotype using simple and practical diagnostic tools. Women with the MU-PCOS, beyond the criterion used to define them, showed a high prevalence of MetS (risk according to AES: 22.8; WHR $\geq$ 0.85: 31%; BMI > 27 kg/m^2: 27.7%; VAI > 1.675: 43.8%) and high prevalence of high risk according to AES (risk according to AES: 24.0%; WHR $\geq$ 0.85: 31%; BMI > 27 kg/m^2: 29.2%; VAI > 1.675: 45.0%). The four criteria used showed fair-to-moderate agreement, except that WHR $\geq$ 0.85 showed that a poor agreement with risk according to AES and VAI > 1.675.

Among the four criteria VAI > 1.675, not only presented the highest prevalence of MetS and high risk according to AES but identified women with MU-PCOS that stood out from those with

Table II **Comparison of clinical characteristics between MH-PCOS and MU-PCOS, according to four different approaches [BMI > 27 kg/m^2, WHR ≥0.85, presence of cardiovascular risk according to the 2010-AES Consensus Statement, VAI >1.675 (cutoff point able to identify in 144 healthy women an ISI Matsuda value of <25th percentile)].**

	BMI > 27 kg/m^2			WHR ≥ 0.85			Risk according to AES[a]			VAI > 1.675		
	MH-PCOS (No 102)	MU-PCOS (No 130)		MH-PCOS (No 190)	MU-PCOS (No 42)		MH-PCOS (No 65)	MU-PCOS (No 167)		MH-PCOS (No 152)	MU-PCOS (No 80)	
	Mean ± SD	Mean ± SD	*P*	Mean ± SD	Mean ± SD	*P*	Mean ± SD	Mean ± SD	*P*	Mean ± SD	Mean ± SD	*P*
Age (years)	23.0 ± 5.16	25.2 ± 6.52	0.006	24.3 ± 6.08	23.7 ± 5.95	0.525	23.4 ± 4.93	24.5 ± 6.40	0.151	23.7 ± 5.66	25.2 ± 6.65	0.083
BMI (kg/m^2)	23.8 ± 2.59	34.9 ± 5.25	<0.001	29.4 ± 7.10	32.9 ± 5.70	0.004	25.0 ± 3.08	32.0 ± 7.11	<0.001	28.4 ± 6.72	33.3 ± 6.36	<0.001
Age of menarche (years)	12.2 ± 1.41	11.8 ± 1.35	0.017	12.0 ± 1.38	112.0 ± 1.46	0.944	12.2 ± 1.24	11.9 ± 1.44	<0.001	12.1 ± 1.49	11.8 ± 1.19	0.205
WC (cm)	76.6 ± 9.76	104 ± 12.5	<0.001	89.5 ± 17.57	103 ± 13.8	<0.001	80.2 ± 10.25	96.5 ± 17.88	<0.001	86.6 ± 16.60	102 ± 5.3	<0.001
FG score	12.6 ± 6.36	14.5 ± 7.18	0.032	13.8 ± 6.94	13.2 ± 6.70	0.611	12.8 ± 6.57	14.0 ± 6.99	0.222	13.2 ± 6.87	14.6 ± 6.86	0.152
	Subjects (%)	Subjects (%)	*P*	Subjects (%)	Subjects (%)	*P*	Subjects (%)	Subjects (%)	*P*	Subjects (%)	Subjects (%)	*P*
Diagnostic criteria of PCOS[b]												
Oligo-menorrhea	77 (75.5)	100 (76.9)	0.799	141 (74.2)	36 (85.7)	0.113	51 (78.5)	126 (75.4)	0.628	106 (69.7)	71 (88.8)	0.001
PCOM	80 (78.4)	94 (72.3)	0.285	142 (74.7)	32 (76.2)	0.844	51 (78.5)	123 (73.65)	0.554	115 (75.7)	59 (73.8)	0.750
Hirsutism	78 (76.5)	105 (80.8)	0.426	150 (78.9)	33 (78.6)	0.957	50 (76.9)	133 (79.6)	0.649	110 (72.4)	73 (91.3)	0.001
Biochemical hyperandrogenism	57 (55.9)	65 (50.0)	0.373	102 (53.7)	20 (47.6)	0.476	32 (49.2)	90 (53.9)	0.523	78 (51.3)	44 (55.0)	0.593
Acne/seborrhea	46 (45.1)	54 (41.5)	0.587	84 (44.2)	18 (42.9)	0.873	33 (50.8)	69 (41.3)	0.193	72 (47.4)	30 (37.5)	0.150
Chronic oligo-anovulation[e]	69 (67.6)	87 (66.9)	0.907	128 (69.2)	26 (65.0)	0.605	42 (65.6)	112 (69.6)	0.566	100 (69)	54 (67.5)	0.821
Metabolic syndrome[c] (according to NCEP-ATP III criteria)	3 (2.9)	36 (27.7)	<0.001	26 (13.7)	13 (31.0)	0.007	1 (1.5)	38 (22.8)	<0.001	4 (2.6)	35(43.8)	<0.001
Diabetes or fasting glucose ≥5.6 mmol/l	6 (5.9)	24 (18.5)	0.005	25 (13.2)	5 (11.9)	0.827	5 (7.7)	25 (15.0)	0.138	17 (11.2)	13 (16.3)	0.274
High blood pressure	—	14 (10.8)	<0.001	12 (6.3)	2 (4.8)	1	—	14 (8.4)	0.016	4 (2.6)	10 (12.5)	0.006
High triglycerides	8 (7.8)	28 (21.5)	<0.001	21 (11.1)	15 (35.7)	<0.001	3 (4.6)	33 (19.8)	0.004	—	36 (45.0)	<0.001
Low HDL cholesterol	25 (24.5)	71 (54.6)	<0.001	76 (40)	20 (47.6)	0.364	15 (23.1)	81 (48.5)	<0.001	35 (23.0)	61 (76.3)	<0.001
Increased WC	13 (12.7)	114 (87.7)	<0.001	92 (48.4)	35 (83.3)	<0.001	16 (24.6)	111 (66.5)	<0.001	61 (40.1)	66 (82.5)	<0.001

Impaired fasting glucose (IFG)	6 (5.9)	22 (16.9)	0.010	23 (12.1)	5 (11.9)	0.971	5 (7.7)	23 (13.8)	0.202	16 (10.5)	12 (15)	0.320
IGT	3 (2.9)	6 (4.6)	0.735	7 (3.7)	2 (4.8)	0.668	—	9 (5.4)	0.065	4 (2.6)	5 (6.3)	0.175
IFG + IGT	2 (2.0)	3 (2.3)	1	5 (2.6)	—	0.588	1 (1.5)	4 (2.4)	1	2 (1.3)	3 (3.8)	0.343
DM	—	2 (1.5)	0.505	2 (1.1)	—	1	—	2 (1.2)	1	1 (0.7)	1 (1.3)	1
High risk according to AES[d]	3 (2.9)	38 (29.2)	<0.001	28 (14.7)	13 (31.0)	0.013	1 (1.5)	40 (24.0)	<0.001	5 (3.3)	36 (45.0)	<0.001

IGT, impaired glucose tolerance; DM, diabetes mellitus
[a]PCOS women with any of the following risk factors: obesity, cigarette smoking, hypertension, dyslipidemia [increased LDL-C (>3.37 mmol/l) and/or non-HDL-C (>4.14 mmol/l)], subclinical vascular disease, IGT, family history of premature CVD (<55 years of age in male relative, <65 years of age in female relative).
[b]According to the AES.
[c]According to the ATP III criteria.
[d]Women with metabolic syndrome and/or DM and/or overt vascular or renal disease.
[e]Pg level <9.54 nmol/l between Days 20 and 24 of the menstrual cycle. Student's *t*-test or or χ^2 test/Fisher's exact test.

MH-PCOS by having a higher prevalence of oligo-menorrhea and hirsutism. However, a significant association between MU-PCOS and the hormonal profile was not observed, except with the low Pg levels in the luteal phase. These data are supported by other studies showing that patients with PCOS and oligo-menorrhea/amenorrhea had more severe IR and a worse metabolic profile than patients with PCOS and regular cycles (Robinson *et al.*, 1993; Welt *et al.*, 2006; Norman *et al.*, 2007; Goodarzi *et al.*, 2011; Panidis *et al.*, 2012). Moreover, our previous study (Amato *et al.*, 2011a,b) found that the oligo-menorrhoic phenotypes of PCOS (applying the Rotterdam criteria) are characterized by a high VAI and a condition of cardiometabolic risk. More difficult to interpret is the fact that women with PCOS and VAI > 1.675, despite exhibiting a significant increase in the prevalence of hirsutism, do not show an increased androgen levels. We may hypothesize that the insulin/IGF system plays a role in stimulating hair follicle growth acting together with androgens and so the increased responsiveness of the pilo-sebaceous unit to androgens seems to be influenced by IR or compensatory hyperinsulinism (Philpott *et al.*, 1994; Itami *et al.*, 1995; Su *et al.*, 1999; Amato *et al.*, 2006).

Three of the four criteria examined (BMI > 27 kg/m^2, risk according to AES and VAI > 1.675) showed a good association with metabolic parameters (insulin sensitivity, insulin secretion, lipid pattern, transaminase and uric acid), but only VAI > 1.675 was significantly associated with a reduction in insulin secretion related to insulin sensitivity expressed by DIo. However, while the *risk according to the AES* criterion highlights the association with hyperandrogenemia, the VAI > 1.675 criterion highlights the association with a luteal phase defect (low Pg). In our opinion, the *risk according to AES* criterion, despite being a good criterion in identifying women with MU-PCOS, is a very broad criterion (presence of any of the following risk factors: obesity, cigarette smoking, hypertension, dyslipidemia, subclinical vascular disease, IGT, family history of premature CVD) and therefore may tend to overestimate the prevalence of MU-PCOS (71.9% with *risk according to the AES* criterion and 34.8% with VAI > 1.675 criterion). In addition, some of the risk factors provided for AES, though important cardiovascular risk factors do not play any role in the pathogenesis of PCOS. VAI being an index of impaired fat distribution and function, in addition to indirectly expressing altered production, release and/or function of adipocytokines and inflammatory factors, involved in the genesis of PCOS (Gambineri *et al.*, 2002), also expresses a condition of cardiometabolic risk (Amato *et al.*, 2010, 2011a,b).

As is well known, when applying the AES-PCOS criteria, nine different phenotypes of the condition are possible. As confirmed by other literature data (Cupisti *et al.*, 2011), our study showed that using the different phenotypes described in the AES classification there is no advantage in identifying women with metabolic risk profile.

A strong correlation between VAI and rate of peripheral glucose utilization (*M* value) has also been demonstrated in the general population during the gold standard euglycemic–hyperinsulinemic clamp (Amato *et al.*, 2010). Recently, this aspect has been addressed in a population of young women with PCOS, in which the VAI can replace visceral computed tomography scanning as a marker for visceral adiposity and predicting IR (Oh *et al.*, 2012).

Among the four criteria tested, VAI > 1.675 was the only one that showed a significant association between MU-PCOS and a reduction in DIo. This is a very interesting datum, because if we consider the low prevalence of diabetes (0.9%) and prediabetes (IFG: 12.1%, IGT 3.9%, IFG + IGT: 2.2%) in the 232 studied women, mainly due to

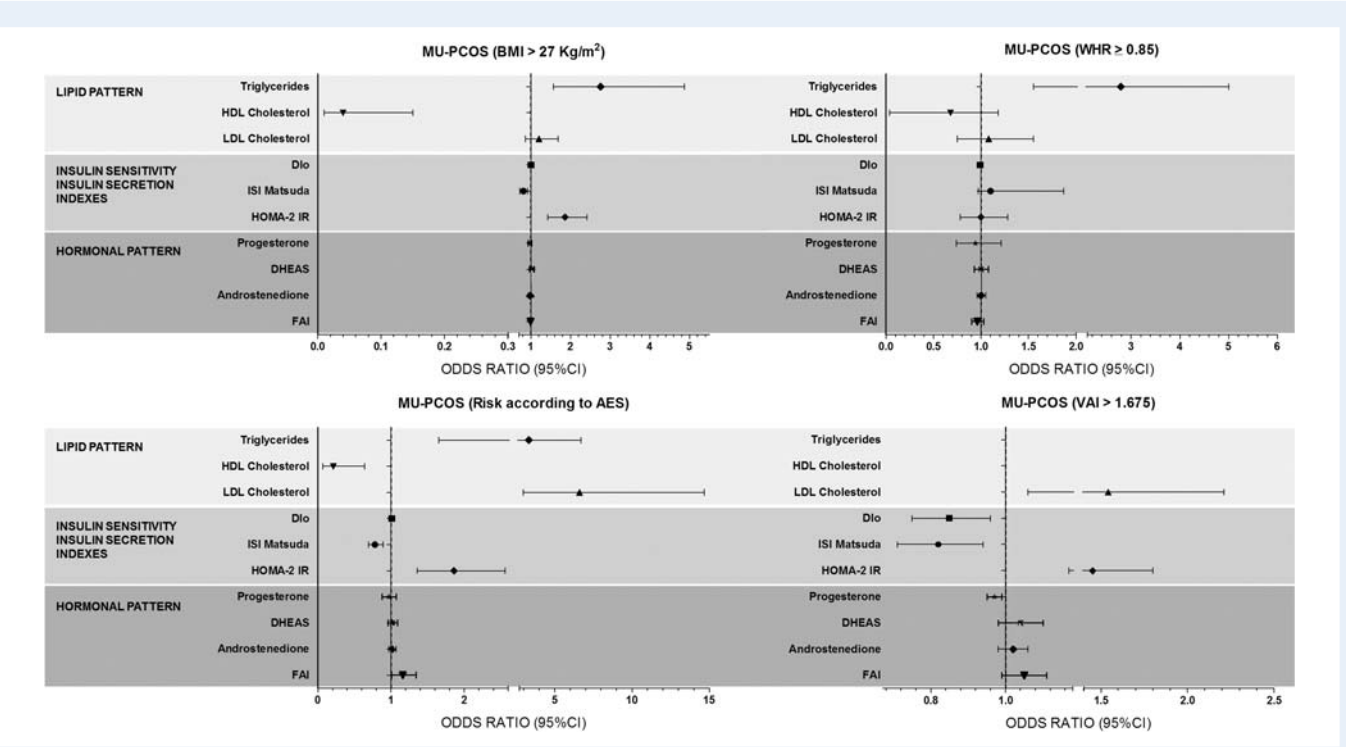

Figure 1 Associations of MU-PCOS (according to BMI > 30 kg/m^2, WHR ≥ 0.85, risk AES and VAI > 1.675) with lipid pattern, insulin sensitivity indexes, DIo and hormonal pattern.

the young age of patients, DIo could provide interesting data on the diabetes risk linked to the condition of MU-PCOS. Indeed, DIo, which expresses the ability of β-cells to adequately compensate IR through increased insulin secretion, has been shown to predict the development of diabetes in adults (Utzschneider *et al.*, 2009).

In conclusion, risk according to AES, BMI > 27 kg/m^2 and VAI > 1.675 have similar diagnostic value in detecting adverse metabolic profile in PCOS patients (MU-PCOS), although we believe that the risk according to AES and BMI > 27 kg/m^2 criteria tends to overestimate the problem; given the simplicity of WC and BMI measurement and TG and HDL assessment, we suggest that VAI could be an easy and useful tool for the assessment of MU-PCOS in daily clinical practice and in population studies for the assessment of cardiometabolic risk associated with PCOS and for taking appropriate preventive and therapeutic measures. This interesting data, however, need further confirmation by appropriate prospective studies.

Supplementary data

Supplementary data are available at http://humrep.oxfordjournals.org/.

Authors' roles

M.C.A. was involved in study design, execution, statistical analysis, manuscript drafting and critical discussion; V.G. was involved in execution; D.F. was involved in execution; M.D. contributed to participation in study design; S.D. contributed to execution; C.G. contributed to study design, manuscript drafting and critical discussion.

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Conflict of interest

None declared.

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