Divergences in Insulin Resistance Between the Different Phenotypes of the Polycystic Ovary Syndrome

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Context/Objective: Current diagnostic criteria for polycystic ovary syndrome (PCOS) have generated distinct PCOS phenotypes, based on the different combinations of diagnostic features found in each patient. Our aim was to assess whether either each single diagnostic feature or their combinations into the PCOS phenotypes may predict insulin resistance in these women.

Patients/Design: A total of 137 consecutive Caucasian women with PCOS, diagnosed by the Rotterdam criteria, underwent accurate assessment of diagnostic and metabolic features. Insulin sensitivity was measured by the glucose clamp technique.

Results: Among women with PCOS, 84.7% had hyperandrogenism, 84.7% had chronic oligoanovulation, and 89% had polycystic ovaries. According to the individual combinations of these features, 69.4% of women had the classic phenotype, 15.3% had the ovulatory phenotype, and 15.3% had the normoandrogenic phenotype. Most subjects (71.4%) were insulin resistant. However, insulin resistance frequency differed among phenotypes, being 80.4%, 65.0%, and 38.1%, respectively, in the 3 subgroups ($P < .001$). Although none of the PCOS diagnostic features per se was associated with the impairment in insulin action, after adjustment for covariates, the classic phenotype and, to a lesser extent, the ovulatory phenotype were independently associated with insulin resistance, whereas the normoandrogenic phenotype was not. Metabolic syndrome frequency was also different among phenotypes ($P = .030$).

Conclusions: There is a scale of metabolic risk among women with PCOS. Although no single diagnostic features of PCOS are independently associated with insulin resistance, their combinations, which define PCOS phenotypes, may allow physicians to establish which women should undergo metabolic screening. In metabolic terms, women belonging to the normoandrogenic phenotype behave as a separate group. **(*J Clin Endocrinol Metab* 98: E628–E637, 2013)**

Polycystic ovary syndrome (PCOS) is a common condition of women in the reproductive age, with a number of potential clinical consequences, including an increased risk for infertility, dysfunctional bleeding, endometrial hyperplasia, obesity, and insulin resistance with the associated metabolic alterations. Moreover, these subjects have an increased risk of endometrial carcinoma and possibly cardiovascular disease later in life (1, 2).

Unfortunately, there are no unequivocal criteria for diagnosing PCOS, and its definition remains controversial.

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Abbreviations: AE-PCOS, Androgen Excess and PCOS Society; BP, blood pressure; CV, coefficient of variation; ESHRE/ASRM, European Society of Human Reproduction and Embryology/American Society for Reproductive Medicine; OR, odds ratio; PCO, polycystic ovary; PCOS, polycystic ovary syndrome.

According to the 2003 Rotterdam European Society of Human Reproduction and Embryology/American Society for Reproductive Medicine (ESHRE/ASRM) consensus workshop (3), PCOS may be diagnosed by the presence of at least 2 of 3 cardinal features: hyperandrogenism, chronic oligoanovulation, and polycystic ovary (PCO) morphology, after exclusion of secondary causes. This definition widened the previous diagnostic boundaries of the syndrome, set out in 1990 by a National Institutes of Health consensus conference (4), with two major consequences: (1) PCOS prevalence has risen substantially, from 6–8% to 12–20% (5, 6); and (2) according to the various possible combinations of the 3 above-mentioned diagnostic features in each individual subject, adoption of the Rotterdam criteria has introduced different PCOS phenotypes, subsequently named classic (characterized by hyperandrogenism and oligoanovulation, with or without PCO morphology, and corresponding to the previous National Institutes of Health definition), ovulatory (hyperandrogenism and PCO), and normoandrogenic (oligoanovulation and PCO).

In 2006, the Rotterdam criteria were brought into question by the Androgen Excess and PCOS Society (AE-PCOS). The document summarizing this discussion confirmed that PCOS diagnosis should be based on the 3 clinical features indicated by the ESHRE/ASRM consensus. However, it established a hierarchical order of these features, in that hyperandrogenism was considered fundamental, combined with oligoanovulation and/or PCO morphology (1). As a consequence, the AE-PCOS position excluded the possibility of a normoandrogenic PCOS phenotype. The debate on these issues is still ongoing.

One major implication of the increased clinical heterogeneity underpinning adoption of the Rotterdam diagnostic criteria comes from the hypothesis that the different PCOS phenotypes could diverge in terms of insulin resistance (7). If confirmed, this hypothesis suggests that these patients should be screened differently and possibly treated differently for the metabolic dysfunction. On the other hand, these findings may actually reflect disparities in obesity, which seems to be associated differently with the various PCOS phenotypes (8).

A better understanding of these issues may also have significant theoretical implications, because it was argued that if the various PCOS phenotypes have the same overall morbidity in terms of insulin resistance, then the conclusion that they all really reflect the same overall syndrome would be strongly supported (1).

Insulin resistance is not a disease per se, but rather it is a common physiological abnormality increasing the likelihood that several alterations may occur (9). In this regard, it is noteworthy that insulin resistance probably plays a pathogenetic role in PCOS (10). Moreover, it is a forerunner of several metabolic alterations, which also occur frequently in these subjects. It has been estimated that about 70% of women with PCOS are insulin resistant (11, 12). However, the true prevalence of this phenomenon in subjects with PCOS and it stratification among the different PCOS phenotypes remain unclear issues, because interpretation of available studies is constrained by several limitations, particularly with regard to methods used to assess insulin sensitivity.

The hyperinsulinemic-euglycemic clamp is the best available technique for measurement of in vivo insulin sensitivity (13). However, it is complex and expensive. Therefore, epidemiological studies generally rely on surrogate indices, based on measurement of insulin and glucose levels (14). Unfortunately, although all these indirect estimates correlate with the direct measures of insulin action, the degree of correlation is limited (15–18). Moreover, most of these studies used routine testosterone assays. These methods generally have poor sensitivity and accuracy in the female range (19, 20), making assessment of hyperandrogenism and, consequently, of PCOS phenotypes imprecise.

The aim of this study was to assess whether either the different features used for diagnosing PCOS or their combinations into the PCOS phenotypes may be helpful in predicting insulin resistance in these women. To answer this question, 137 women with PCOS were carefully characterized, using state-of-the-art methods.

Subjects and Methods

Subjects

A total of 137 consecutive Caucasian women with PCOS, recruited in the Verona PCOS Pathophysiology and Phenotype (Verona 3P) Study, an ongoing project with the aim of building a detailed resource for assessing the relationships between the different features of these women, were studied. All of them had been referred to the Division of Endocrinology, Diabetes and Metabolism of Verona Hospital, Verona, Italy, for oligoamenorrhea and/or hyperandrogenism. Inclusion criteria were a confirmed diagnosis of PCOS and age 18 to 40 years. Exclusion criteria were diabetes mellitus or other diseases or use of medications that could potentially interfere with the evaluations carried out in the study. In particular, patients should not have received oral contraceptives, insulin-sensitizing agents, antiandrogens, or glucocorticoids in the past 6 months.

PCOS was diagnosed according to criteria indicated by the Rotterdam Workshop (3), ie, the presence of at least 2 of the following 3 features: clinical and/or biochemical hyperandrogenism, chronic oligoanovulation, and PCO morphology, after exclusion of secondary causes. Hyperandrogenism was defined by presence of hirsutism and/or increased free testosterone. Acne, alopecia, and serum androgens other than free testosterone were not used to diagnose hyperandrogenism, because they

are less specific and add a limited incremental amount to diagnosis of PCOS (1). Chronic oligoanovulation was diagnosed by presence of either oligoamenorrhea (fewer than 9 cycles per year) or a luteal phase serum progesterone level less than 12 nmol/L. In most cases, progesterone measurement was repeated in 2 subsequent menstrual cycles. PCO was diagnosed according to ESHRE/ASRM recommendations (3). Secondary causes of PCOS were ruled out by systematic 17-hydroxyprogesterone, prolactin, and TSH assays.

All the participants gave their written informed consent before the study, which was conducted according to the Declaration of Helsinki and approved by our institutional ethics committee.

Protocol

Subjects included in the study underwent complete medical examination, assessment of endocrine and metabolic features and glucose tolerance, ultrasound evaluation of ovarian morphology, and measurement of insulin sensitivity.

Medical examination comprised measurement of body weight and height, waist circumference, blood pressure (BP), and hirsutism score. Elevated BP values were confirmed on 2 occasions. Hirsutism was assessed by the modified Ferriman-Gallwey score, the gold standard for clinical evaluation of hirsutism (21), with a cutoff point of ≥ 8.

Subsequently, in the early follicular phase of a spontaneous menstrual cycle, or, in women with severe menstrual alterations, after at least 3 months of amenorrhea, a fasting venous blood sample was drawn for metabolic and hormonal profile assessment. A standard 75-g oral glucose tolerance test was also performed, with measurement of plasma glucose and insulin at fasting and every 30 minutes for 2 hours.

Ovarian morphology was evaluated by 1 of 2 experienced gynecologists, by a transvaginal approach whenever possible, with a Voluson 730 PRO device (GE Healthcare, Milwaukee, Wisconsin), equipped with volumetric 3-dimensional probes. Ovary volume was calculated using the ellipsoid formula (length · height · width · $\pi/6$).

Insulin sensitivity was assessed by the glucose clamp technique as described previously (22), using a primed insulin infusion rate of 80 mU/m^2 · min. The duration of the clamp was 120 minutes, and the glucose disposal rate was calculated during the last 30 minutes, with standard equation (23). Because muscle is responsible for most insulin-induced glucose metabolism (24), data are expressed per fat-free mass (FFM). Body composition was assessed by bioelectrical impedance (Bia-103; Akern, Florence, Italy) (25).

Metabolic syndrome was diagnosed according to the 2009 Joint Interim Statement of the International Diabetes Federation (IDF) and other societies (26). This document revised the Adult Treatment Panel III (ATP III) diagnostic criteria for metabolic syndrome (27), concluding that the cutoff point for waist circumference to define abdominal obesity should be $\geq$ 80cm in women of European origin, as were the women recruited in the present study.

In 4 women with PCOS (3 with the classic phenotype and 1 with the ovulatory phenotype), glucose clamp studies were not completed, because of technical problems. Therefore, clamp data refer to 133 subjects. Metabolic syndrome could be diagnosed as present or absent in 134 women. Diagnosis in 3 subjects with the classic phenotype was not possible because of missing lipid data.

A historical sample of 24 healthy nonhirsute women, with regular menstrual cycles and normal ovarian morphology, served as the reference cohort for clamp data, whereas 51 women with the same characteristics served to define the reference interval for serum testosterone.

Assays

Plasma glucose was measured by the glucose-oxidase method, using a glucose analyzer (YSI-2300 Stat Plus; YSI Inc, Yellow Springs, Ohio).

Serum total testosterone was measured by liquid chromatography-mass spectrometry using a Micromass Quattro Premier XE Mass Spectrometer (Waters Corporation, Milford, Massachusetts). The limit of quantification was 2.5 ng/dL and the intraassay coefficient of variation (CV) was 9.1% at concentration 14 ng/dL, and interassay CV was 9.3% at concentration 26 ng/dL. The free testosterone fraction was estimated by equilibrium dialysis (28, 29); the interassay CV was $<8\%$.

SHBG and insulin were assayed by immunoradiometric methods (Orion Diagnostica, Espoo, Finland; and Biosource, Fleurus, Belgium, respectively) and gonadotropins by an automated chemiluminescent method (Advia Centaur XP; Siemens, Tarrytown, New York). Serum lipids were determined by standard laboratory procedures.

Statistical analysis

Continuous variables are described by median and interquartile range, because most of them (except for the M-clamp value, total cholesterol, and BP) were not normally distributed. Categorical variables are summarized by percentages. Nonnormally distributed variables were log- or square root−transformed before analysis.

Comparisons between phenotypes were performed using 1-way ANOVA. Because triglyceride distribution could not be normalized, values were compared by a nonparametric Kruskal-Wallis ANOVA. Multiple post hoc comparisons were performed using Bonferroni correction.

Multiple regression was used to assess the relationship between the M-clamp value as a dependent continuous variable and the clinical features used in diagnosis of PCOS as independent variables. Multiple regression analysis was also used to compare M-clamp values in subjects belonging to the different PCOS phenotypes vs the historical sample of healthy women, which was included as the reference group, to establish whether insulin sensitivity was abnormal in each PCOS phenotype. The same analyses were performed with adjustment for age and fat mass (analysis of covariance).

Logistic regression analyses were performed to investigate the associations between the dichotomized M-clamp variable (insulin resistance present or absent) as a dependent categorical variable and either the PCOS diagnostic features or the PCOS phenotypes as independent variables. In a second step, adjustments for age and fat mass were performed. In these analyses, insulin resistance was defined by a M-clamp value below the 25th percentile (11.75 mg/kg FFM · min) of the distribution in healthy women, according to the World Health Organization and the European Group for the Study of Insulin Resistance (EGIR) definitions of insulin resistance (30, 31).

Percentages of women with metabolic syndrome in the different PCOS phenotype groups were compared using the Fisher exact test.

For power calculation, the primary endpoint was risk of insulin resistance in each PCOS phenotype. From the limited available literature, the prevalence of insulin resistance in these women is about 70% (11, 12). To detect a difference with respect to the reference population, in which this prevalence is by definition 25%, with an α error = .05 and power = .80 and a 2-sided test, 19 subjects per group are required.

Analyses were performed using STATA version 10.1 (StataCorp, College Station, Texas).

Results

In the whole sample of 137 women with PCOS included in the study, 84.7% had hyperandrogenism, 84.7% had chronic oligoanovulation, and 89% had PCO morphology. According to the individual combinations of these features, 95 (69.4%) of these women had the classic phenotype, whereas 21 (15.3%) had the ovulatory phenotype, and the remaining 21 (15.3%) had the normoandrogenic phenotype.

Table 1 shows the main characteristics of the whole cohort of women with PCOS. The median body mass index was 28.5 kg/m^2. Medians of metabolic features were in the normal range, except for fasting insulin, which was increased, and high-density lipoprotein (HDL) cholesterol, which was marginally decreased. As expected, serum free testosterone was above and SHBG below the limits of the respective reference range.

Table 1. Main characteristics of the cohort of women with PCOS included in the study and corresponding reference intervals

Characteristic	Women with PCOS (n = 137)	Reference Interval
Age, y	23.0 (19.0–28.0)	
Body mass index, kg/m^2	28.5 (23.2–34.9)	18.5–24.9
Waist circumference, cm	92.0 (78.0–106.0)	<80
Fat mass, kg	26.8 (18.3–38.4)	
Fat mass, %	35.3 (29.5–40.7)	
FFM, kg	48.3 (42.8–54.8)	
Ferriman-Gallwey score	9.0 (4.0–15.0)	<8
Systolic BP, mm Hg	120 (110–130)	<130
Diastolic BP, mm Hg	80 (70–86)	<85
Fasting glucose, mg/dL	85.0 (79.0–92.0)	70–99
Fasting insulin, mU/L	13.3 (7.2–23.3)	<9
Total cholesterol, mg/dL	161 (143–182)	<200
HDL cholesterol, mg/dL[a]	48.0 (40.0–58.0)	≥50
Triglycerides, mg/dL[b]	67.5 (48.5–108.0)	<150
SHBG, nmol/L	29.7 (19.7–45.0)	39–121
LH, IU/L	7.7 (4.7–10.9)	1.0–25.0
FSH, IU/L	5.4 (4.3–6.2)	1.5–11.0
Total testosterone, ng/dL	34.5 (25.3–46.2)	<41
Free testosterone, ng/dL	0.60 (0.49–0.93)	<0.50
Ovarian follicles, n	13.0 (12.0–16.0)	<12
Ovarian volume, mL	10.7 (8.1–13.7)	≤10

Data are shown as median (interquartile range).

[a] Available for 133 women

[b] Available for 136 women.

The comparison of women belonging to the different PCOS phenotypes revealed several differences (Table 2). In particular, compared with the classic phenotype subgroup, the normoandrogenic subgroup was leaner and had lower insulin and triglyceride and higher HDL cholesterol and SHBG levels. Moreover, as expected according to phenotype definition, the Ferriman-Gallwey score and serum testosterone level also differed between these subgroups. Conversely, compared with the classic phenotype subgroup, the ovulatory subgroup had lower free testosterone and triglyceride levels. Comparison of these latter subgroups also showed notable differences in terms of anthropometric features, although these did not reach statistical significance.

Glucose utilization during the clamp (M-clamp value) was reduced in this cohort of women with PCOS (10.0 ± 3.3 mg/kg FFM · min; reference value in healthy women >11.75 mg/kg FFM · min), showing that most of them (71.4%) were insulin resistant. The frequency of insulin resistance was 80.4%, 65.0%, and 38.1%, respectively, in the classic, ovulatory, and normoandrogenic phenotype subgroups ($P < .001$).

When the relationship between the M-clamp value and the presence or absence in these women of each specific PCOS diagnostic feature was analyzed (Table 3, model 1), insulin-stimulated glucose utilization was associated with hyperandrogenism ($P < .0001$) but not with oligoanovulation ($P = .21$) or PCO morphology ($P = .15$). However, none of the features of PCOS was independently associated with insulin action after inclusion of fat mass and age in the analysis (Table 3, model 2): only these latter variables were associated (fat mass negatively and age positively) with insulin sensitivity.

On the other hand, when subjects with PCOS were categorized into phenotypes and compared with healthy women, insulin action was significantly impaired in patients with either the classic or the ovulatory phenotype, but not in those with the normoandrogenic phenotype (Table 4, univariable analysis, and Figure 1). The inclusion of fat mass and age in the analysis did not substantially affect these conclusions: the classic phenotype was still a predictor of impaired insulin action ($P = .003$), whereas the ovulatory phenotype showed borderline significance ($P = .051$) (Table 4, multivariable analysis). With regard to this latter finding, statistical significance is fully reached by using a 1-sided test ($P = .026$).

When considered as a categorical variable, the presence of insulin resistance was associated with hyperandro-

Table 2. Main characteristics of the women with PCOS subdivided according to the PCOS phenotypes

	Classic (n = 95)	Ovulatory (n = 21)	Normoandrogenic (n = 21)	*P* by ANOVA
Age, y	23.0 (19.0–28.5)	24.0 (19.7–27.0)	26.0 (23.5–29.0)	.340
Body mass index, kg/m^2	30.4 (25.5–36.5)	26.1 (22.9–33.4)	21.6 (20.4–27.2)[a]	**<.001**
Waist circumference, cm	96.0 (78.0–106.0)	85.0 (77.0–101.6)	76.0 (68.7–85.5)[a]	**<.001**
Fat mass, kg	28.9 (21.8–40.7)	25.6 (17.8–35.6)	17.4 (14.0–23.8)[a]	**<.001**
Fat mass, %	36.4 (32.0–42.1)	33.6 (29.7–40.4)	29.0 (24.7–34.3)[a]	**.001**
FFM, kg	50.3 (44.9–56.8)	47.3 (42.0–52.1)	42.6 (39.7–44.8)[a]	**<.001**
Ferriman-Gallwey score	11.0 (5.0–16.0)	9.0 (7.7–15.6)	3.0 (2–4)[a, b]	**<.001**
Systolic BP, mm Hg	120 (110–130)	120 (114–128)	118 (110–126)	.355
Diastolic BP, mm Hg	80 (70–88)	80 (73–85)	75 (67–84)	.368
Fasting glucose, mg/dL	86.0 (81.0–94.0)	83.0 (79.0–90.5)	83.0 (77.7–88.7)	.269
Fasting insulin, mU/L	16.1 (9.0–25.8)	11.8 (7.4–19.4)	7.3 (5.3–10.0)[a]	**<.001**
Total cholesterol, mg/dL	161 (149–188)	150 (136–162)	166 (149–178)	.131
HDL cholesterol, mg/dL[c]	47.0 (38.0–54.2)	50.0 (42.5–54.5)	61.5 (49.5–70.0)[a]	**.002**
Triglycerides, mg/dL[d]	80.5 (53.0–113.0)	54.0 (44.2–73.0)[e]	53.0 (39.0–64.0)[e]	**.007**
SHBG, nmol/L	25.7 (18.2–37.9)	28.8 (20.4–44.4)	47.5 (35.7–66.9)[a, f]	**<.001**
LH, IU/L	7.8 (4.7–12.0)	5.6 (3.5–8.6)	8.7 (5.3–14.6)[f]	**.034**
FSH, IU/L	5.3 (4.3–6.0)	5.8 (3.8–6.2)	5.9 (4.8–6.9)	.073
Total testosterone, ng/dL	39.1 (27.7–48.0)	30.4 (23.5–37.2)	28.5 (22.6–38.1)[e]	**.028**
Free testosterone, ng/dL	0.74 (0.54–1.02)	0.56 (0.50–0.66)[e]	0.43 (0.33–0.46)[a, b]	**<.001**
Ovarian follicles, n	13.0 (12–15)	12.0 (8.5–13.0)	14.0 (12.7–18.2)	.085
Ovarian volume, mL	11.5 (8.6–13.8)	8.4 (6.5–12.7)	10.1 (7.8–13.3)	.158

Data are shown as median (interquartile range). Statistically significant *P* values are in bold.

[a] $P < .001$ vs classic PCOS.

[b] $P < .001$ vs ovulatory PCOS.

[c] Not available in 3 subjects with the classic phenotype and 1 subject with the ovulatory phenotype.

[d] Not available in 1 subject with the classic phenotype.

[e] $P < .05-.01$ vs classic PCOS.

[f] $P < .05-.01$ vs ovulatory PCOS.

genism (odds ratio [OR] = 6.03, $P = .001$) and only to a minor extent (OR = 3.12, $P = .053$) with oligoanovulation, but not with PCO morphology (Supplemental Table I published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org). However, after inclusion of fat mass and age in the model, only body fat was a significant predictor of insulin resistance in these women (Supplemental Table I).

On the other hand, when subjects with PCOS were categorized into phenotypes, insulin resistance proved to be a feature of both the classic subgroup (OR = 13.98, $P < .001$) and the ovulatory subgroup (OR = 6.31, $P = .008$), but not of the normoandrogenic subgroup (Supplemental Table II). Notably, after inclusion of fat mass and age in the model (Supplemental Table II), the classic phenotype was still associated with insulin resistance (OR = 5.42, $P = .007$), whereas the ovulatory phenotype was not (OR = 3.43, $P = .098$). However, this latter association maintained statistical significance in a 1-sided test ($P = .049$).

It is noteworthy that 32.8% of these young women with PCOS met the criteria for diagnosis of metabolic syndrome according to the 2009 International Diabetes Federation statement (Table 5). Among the components contributing to metabolic syndrome diagnosis, increased waist circumference (73.7%) and low HDL cholesterol (54.1%) were the most common alterations. Adoption of Adult Treatment Panel III criteria, which use a higher waist circumference cutoff value, reduced the frequency of abdominal obesity (57.7%) in our cohort, but only marginally lowered the prevalence of metabolic syndrome (31.3%).

The metabolic syndrome frequency was higher in women with the classic phenotype, intermediate in the ovulatory subgroup, and lower in the normoandrogenic women (39.1%, 28.6%, and 9.5%, respectively, $P = .030$). However, these differences were no longer statistically significant after adjustment for fat mass and age (data not shown).

Discussion

This study characterized accurately and comprehensively a large sample of women with PCOS, using state-of-the-art methods, to assess which features predict insulin resistance in these subjects. This is a relevant issue, because PCOS is a heterogeneous condition, and it remains unclear

Table 3. Multiple regression analysis for the association between the different clinical elements used in diagnosis of PCOS and insulin sensitivity

Feature[a]	b Coefficient	SE	*P*
Model 1			
Hyperandrogenism	−2.920	0.78	**<.001**
Oligoanovulation	−1.135	0.91	.213
PCO morphology	1.303	0.90	.150
Model 2			
Hyperandrogenism	−1.179	0.68	.084
Oligoanovulation	−0.049	0.75	.948
PCO morphology	1.060	0.74	.153
Age in y	0.120	0.04	**.005**
Fat mass in kg	−0.128	0.02	**<.001**

Insulin sensitivity was measured by M-clamp (insulin-stimulated glucose utilization during the clamp, mg/kg FFM · min) and considered as a continuous variable. Statistically significant *P* values are in bold.

[a] Model 1 includes only PCOS-specific clinical elements. Model 2 includes PCOS-specific clinical elements adjusted by age and fat mass.

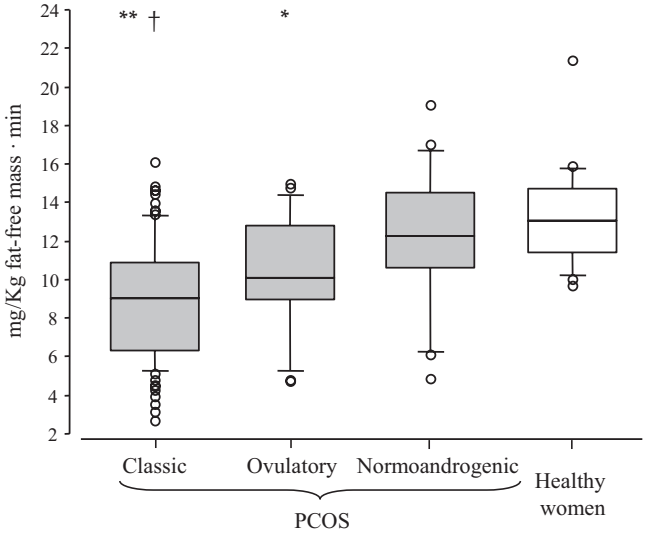

Figure 1. Box plots of the M-clamp values measured in the women with PCOS included in the study, subdivided into the PCOS phenotypes. Data are compared with reference values for healthy control subjects. *$P = .013$ vs healthy control subjects; **$P < .001$ vs healthy control subjects; †$P < .001$ vs normoandrogenic phenotype.

how the metabolic risk stratifies among the subjects who are affected. In particular, we investigated whether insulin resistance is associated either with each single PCOS diagnostic feature, as currently defined by the Rotterdam workshop (3), or with their aggregation into the PCOS phenotypes, which originate from the different possible combinations of these features.

Our data confirmed that insulin resistance is a common characteristic of women with PCOS, present in about 70% of our study population. However, the novel finding was that after adjustment for relevant confounding factors, such as body fat and age (32, 33), insulin resistance appeared to be a specific feature of the classic phenotype and to a lesser extent of the ovulatory phenotype, but not of the normoandrogenic phenotype. On the other hand, the impairment in insulin action was not independently associated with any single diagnostic feature of PCOS. It is noteworthy that similar conclusions were reached when the clamp-derived measure of insulin sensitivity was analyzed either as a categorical or as a continuous variable, excluding a possible pitfall due to the arbitrary definition of an insulin resistance cutoff point.

According to the conclusions of both the ESHRE/ASRM Rotterdam workshop (3) and the AE-PCOS consensus statement (1), PCOS remains a syndrome and, therefore, no single diagnostic feature is sufficient per se for clinical diagnosis. Notably, our data now show that this concept also applies to the association between diagnostic features of PCOS and insulin resistance. It is noteworthy that similar conclusions were reached previously regarding blood concentrations of advanced glycation end-products, which are potential links between the metabolic and reproductive abnormalities of women with PCOS and were found to be higher in these subjects than in those with isolated components of the syndrome and healthy control subjects (34).

Table 4. Regression analysis for the association between the different phenotypes of PCOS and insulin sensitivity

	Univariable Analysis			Multivariable Analysis		
	b Coefficient	SE	*P*	b Coefficient	SE	*P*
PCOS phenotype vs healthy women						
Classic	−4.31	0.73	**<.001**	−2.13	0.70	**.003**
Ovulatory	−2.97	0.95	**.002**	−1.66	0.84	.051
Normoandrogenic	−1.22	0.94	.197	−0.62	0.81	.451
Age in y	0.15	0.05	**.003**	0.10	0.04	**.016**
Fat mass in kg	−0.14	0.02	**<.001**	−0.11	0.02	**<.001**

Insulin sensitivity was measured by M-clamp (insulin-stimulated glucose utilization during the clamp, mg/kg FFM · min) and considered as a continuous variable. Statistically significant *P* values are in bold.

Table 5. Number (and percentage) of women with PCOS with metabolic syndrome and with each clinical components contributing to metabolic syndrome diagnosis in the entire cohort of subjects and in women subdivided according to their PCOS phenotypes

	All Women with PCOS	PCOS Phenotypes		
		Classic	Ovulatory	Normoandrogenic
Metabolic syndrome[a]	44 (32.8)	36 (39.1)	6 (28.6)	2 (9.5)
Fasting glucose ≥100 mg/dL	15 (10.9)	10 (10.5)	3 (14.3)	2 (9.5)
HDL cholesterol <50 mg/dL	72 (54.1)	58 (62.4)	9 (45.0)	5 (25.0)
Triglycerides ≥150 mg/dL	17 (12.5)	13 (13.8)	1 (4.8)	3 (14.3)
Waist ≥80 cm	101 (73.7)	80 (84.2)	15 (71.4)	6 (28.6)
BP ≥130/85 mm Hg	49 (36.0)	38 (40.4)	6 (28.6)	5 (23.8)

[a] Because of missing values for some subjects (see footnotes to Tables 1 and 2), 134 of 137 women with PCOS have been included in the analysis of metabolic syndrome frequency. All missing subjects had the classic phenotype.

Our findings provide useful information with regard to 2 relevant, still open questions: first, whether nonclassic phenotypes really merit inclusion in the PCOS diagnosis; and second, whether all women with PCOS should be uniformly screened for metabolic abnormalities.

With regards to the first question, because there is no unequivocal method to diagnose PCOS, any criteria are questionable. Nonetheless, if the metabolic dysfunction and the associated risks are considered central issues, then our findings suggest that the so-called normoandrogenic phenotype should be considered a separate condition.

With regard to the second question, our data support the conclusion that phenotype distinction may guide physicians in designing cost-effective strategies for screening of metabolic risk upon diagnosis of PCOS and may possibly also guide treatments aimed to prevent the metabolic complications of this condition.

This issue has been investigated in a body of literature. With regard to the single diagnostic features, many studies reported an association between hyperandrogenism and insulin resistance (35–38), whereas data regarding oligoanovulation were more contradictory (38, 39). On the other hand, the studies that assessed the relationship between insulin resistance and ovarian morphology generally did not support the existence of increased metabolic dysfunction among women with polycystic ovaries (35, 38, 40–44). Nonetheless, some authors reported an association between PCO morphology and either reduced (39, 45) or increased insulin sensitivity (46, 47).

With regard to PCOS phenotypes, most previous studies reported that women with the classic phenotype were more insulin resistant than those with either the ovulatory (43, 48–53) or the normoandrogenic phenotype (35, 37, 41, 43, 47, 50, 52, 54). However, several studies did not confirm these findings (55–63). The comparison of the ovulatory and the normoandrogenic phenotypes gave even more discordant results (43, 50, 52–54, 61, 63). Moreover, in the comparisons with control women without PCOS, although the classic phenotype subgroup generally appeared to be insulin resistant (41, 43, 48–50, 52, 56, 58, 61, 63, 64), discrepancies were still found with regard to both the ovulatory (43, 48–54, 59, 61, 63) and the normoandrogenic (41, 43, 49, 50, 52–54, 56, 58, 61, 63) phenotype subgroups.

Unfortunately, interpretation of all these studies is constrained by several limitations. In particular, all of them used surrogate measures of insulin sensitivity, which do not predict accurately the gold standard measurement of insulin action obtained by the glucose clamp (15–18, 65). Many reports were also flawed by the inaccuracy of routine platform assays for testosterone and other androgens (19, 20). The imprecise assessment of one of the cardinal features on which diagnosis of PCOS is based may have affected classification of subjects into the PCOS phenotypes or even caused inappropriate inclusion or exclusion of some women into the PCOS diagnosis (1). Moreover, several studies did not take into account differences in body composition among subjects. This is a crucial issue in women with PCOS, because hyperandrogenism appears to be linked to an excess of body fat (8). Finally, none of these studies included all diagnostic features of PCOS together in the analysis. These limitations weaken the conclusions of previous research and may also account for the inconsistencies among studies.

Notably, in our cohort of PCOS women, the relationship between age and insulin sensitivity showed an unexpected positive direction, in contrast with previous findings in the general population (32, 66). A possible explanation for this unique phenomenon is attenuation with age of all diagnostic features of PCOS, as reported by several authors (67–71). We can thus speculate that our findings may be linked to less severe PCOS clinical features in older women. If this hypothesis is true, a primary role for one or more diagnostic features of PCOS in the impairment of insulin action would be supported.

The major strength of this study is the careful characterization of women with PCOS, in particular the use of state-of-the-art methods to robustly assess insulin sensi-

tivity and hyperandrogenism. This assessment adds more validity to the argument that there is a scale of metabolic risk among PCOS phenotypes. Although, to the best of our knowledge, this is the largest published study to use the gold standard technique to measure insulin sensitivity in these women, the number of subjects with the nonclassic phenotypes was still limited. Moreover, extrapolation of these findings to non-Caucasian women with PCOS should be undertaken with caution.

In conclusion, insulin resistance is a common feature in women with PCOS. However, there is a scale of metabolic risk in these subjects. Although no single diagnostic feature of PCOS is independently associated with impaired insulin action, the combinations of these features, which define PCOS phenotypes, may allow physicians to establish which women merit screening for metabolic dysfunction. From a metabolic point of view, women with the normoandrogenic phenotype behave as a separate group.

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