Fertility biomarkers to estimate metabolic risks in women with polycystic ovary syndrome

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Abstract

Purpose We sought to evaluate the relationship between the polycystic ovary syndrome (PCOS)-defining characteristics and the risk of developing metabolic complications in women presenting with complaints of infertility and/or menstrual irregularities and subsequently diagnosed with PCOS.

Methods This was a cross-sectional study. Women presenting with complaints of infertility and/or irregular menses and diagnosed with PCOS by the Rotterdam criteria, underwent endocrine, metabolic, and ultrasound assessment in the early follicular phase. Reproductive and metabolic parameters were included in regression analysis models with the PCOS-defining characteristics; ROC curves were calculated for the significant predictors.

Results Three hundred and seventy-four women with PCOS were included in our study. Oligo-anovulation, menstrual irregularities, and hirsutism were not predictive of any of the variables. Ovarian volume, follicle count, and biochemical hyperandrogenism were predictors for hormonal, metabolic, and endometrial complications. The relationships were independent of age and body mass index. ROC curves identified lower cut-off values of the PCOS-defining characteristics to

predict patients' risks of hyperinsulinemia, dyslipidemia, and glucose intolerance.

Conclusions Adverse metabolic effects of PCOS are already present in women at the time they present complaining of infertility and/or irregular menses. Hyperandrogenism and ultrasound can assist in predicting the patients' concomitant metabolic abnormalities and can aid physicians in tailoring counseling for effective preventive strategies.

Keywords PCOS · Metabolic · Hyperandrogenism · Hyperinsulinemia · Glucose intolerance · Cross-sectional

Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in reproductive age women, with a prevalence of 6.6 to 15 %, depending on the classification system used [1, 2]. Diagnosis is made on the clinical findings of menstrual irregularities, signs and symptoms of hyperandrogenism, and/or polycystic ovaries on ultrasound, after ruling out other disease processes. However, its pathophysiology and long-term complications still evade us, as PCOS patients frequently develop metabolic complications, such as obesity, hyperinsulinemia, glucose intolerance, and dyslipidemia. These abnormalities put patients with PCOS at risk for metabolic syndrome, type 2 diabetes, and cardiovascular disease at a young age.

During their reproductive years, patients with PCOS usually present to medical care providers because of hirsutism, irregular menses, infertility, or obesity. Although 36–90 % of women with PCOS are reported to be overweight or obese [3, 4], lean women with PCOS may also develop dyslipidemia, insulin resistance, and glucose intolerance [5, 6]. For this reason, guidelines from the American Society for Reproductive

Capsule Hyperandrogenism and ultrasound can assist in predicting metabolic abnormalities in women with PCOS.

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Medicine and the European Society of Human Reproduction and Embryology [7], the American College of Obstetricians and Gynecologists [8], and Androgen Excess/PCOS Society suggested screening all PCOS patients for glucose intolerance with a 2-h glucose tolerance test [9] as well as for dyslipidemia with a lipid profile. Recent studies on PCOS and its relationship with metabolic dysfunction have prompted discussion about separating the syndrome into two phenotypes identified by the NIH and the Rotterdam classification systems. The NIH criteria would identify women at risk for metabolic consequences, while the Rotterdam criteria, which include the polycystic appearance of the ovaries, would identify women with infertility from anovulation, but with a lesser risk of metabolic sequelae [10]. With this new stratification, women with predominant ovarian irregularities and infertility would not be identified as having an increased risk for metabolic abnormalities such as insulin resistance and dyslipidemia. This system may prevent screening and treatment of metabolic abnormalities from PCOS, which have long-term consequences on overall health.

Women with PCOS may also be at an increased risk for other endocrine derangements such as subclinical thyroid dysfunction, which can have long-term consequences on cardiovascular and overall health. Hashimoto's thyroiditis has been reported to be threefold higher in women with PCOS than in the general female population [11–13]. Subclinical hypothyroidism, defined as having a serum thyroid stimulating hormone (TSH) greater than 2.4 mIU/ml with normal levels of T3 and T4 in the absence of anti-thyroid antibodies, has to be aggressively treated in pregnant women [14]. For this reason, it has become common practice to treat a TSH greater than 2.4 mIU/ml in women with infertility and/or recurrent pregnancy loss.

We tested the hypothesis that young PCOS women with complaints of menstrual irregularities and/or infertility would already show metabolic abnormalities associated with the long-term complications of PCOS. We sought to evaluate the relationship between the PCOS-defining characteristics identified in the Rotterdam classification as menstrual irregularities (cycle length), clinical or laboratory signs of hyperandrogenism (hirsutism/acne, total testosterone, and free androgen index), and ultrasound appearance of the ovaries (ovarian volume and follicle count) with the metabolic abnormalities of PCOS (such as hyperinsulinemia, dyslipidemia, and glucose intolerance) in women presenting to our center with complaints of infertility, and/or menstrual irregularities, subsequently diagnosed with PCOS.

Materials and methods

This was a cross-sectional study of a cohort of PCOS patients. We studied patients who presented to our clinic complaining

of menstrual irregularities and/or infertility and were diagnosed with PCOS by the Rotterdam criteria. The study was approved by the University of Tennessee Health Science Center Human Institutional Review Board. Patients had a complete history, physical exam, and testing on cycle-day 3 (range, day 2–5). Patients with menstrual cycles longer than 45 days were given a progesterone challenge test with 10 mg medroxyprogesterone acetate for 10 days in order to induce a withdrawal bleed and perform cycle-day 3 testing.

The diagnosis of PCOS was based on the presence of the following: (1) oligo- and/or anovulation, (2) clinical and/or biochemical signs of hyperandrogenism, (3) polycystic ovaries, and (4) exclusion of other etiologies (congenital adrenal hyperplasia, androgen-secreting tumors, Cushing's syndrome) [1].

Oligo-ovulation or anovulation was diagnosed when the length of the menstrual cycle was less than 26 and/or greater than 35 days and/or if no ovulation was detected by ovulation predictor kits or cycle-day 21 serum progesterone levels were less than 3.0 ng/ml. Hyperandrogenism was diagnosed as *biochemical* when the serum testosterone level was greater than 78 ng/dl (upper limit of normal range in our laboratory) or $\text{FAI} > 4.97$ (free androgen index is serum testosterone (ng/dl): sex hormone-binding globulin (SHBG) mmol/ml $\times 3.47$) [15], or *clinical* when signs of hyperandrogenism were present during the physical exam, such as hirsutism (diagnosed with facial, abdominal, or chest hair) and/or acne. We did not use a dedicated scoring system for hirsutism, as this was not the main reason for presentation to our clinic and no therapy was implemented for this complaint. Ultrasound diagnosis of polycystic ovaries was made when 12 or more antral follicles and/or an ovarian volume greater than 10 ml were present in at least one ovary.

Obesity was defined as a body mass index (BMI) greater than 29 kg/m², and being overweight as a BMI between 25 and 29 kg/m². Clinical diagnosis of hyperinsulinemia was made when obesity or acanthosis nigricans (in non-friction areas) were present [16]. Biochemical diagnosis was made when the glucose/insulin ratio was less than 4.5, the fasting insulin level was greater than 20 IU/ml, the 2-h insulin level was greater than 200 IU/ml, or the homeostatic model assessment for insulin resistance, HOMA1, was greater than 1.775. This cut-off value was used to define insulin resistance in non-diabetic individuals and previously described by Esteghamati et al. [17]. HOMA1 has been shown to closely mirror the insulin sensitivity results obtained by the glycemic clamp technique and is calculated as follows: fasting serum glucose in mmol/l $\times$ fasting serum insulin in $\mu\text{IU/ml} / 22.5$ [18].

Dyslipidemia was diagnosed, based on the 2012 American Association of Clinical Endocrinologists guidelines, when triglycerides were greater than 150 mg/ml, total cholesterol was greater than 200 mg/ml, LDL-cholesterol (LDL-C) was

greater than 99 mg/ml, and/or HDL-cholesterol (HDL-C) was lower than 50 mg/ml [19].

Glucose intolerance was diagnosed when a fasting glucose was greater than or equal to 100 mg/dl, a 2-h glucose was 145–199 mg/dl, or a hemoglobin A1c was 5.7–6.4 %, based on the American Diabetes Association guidelines [20].

No single test can confidently diagnose luteal phase defect (LPD): we diagnosed the condition when either spotting for more than 1 day before starting menses or a short luteal phase (calculated using LH-surge kits), or both, were present.

Endometrial morphology was assessed by ultrasound. The presence of endometrial polyps and/or endometrial hyperplasia was confirmed by pathologic analysis on the surgical specimens and recorded in our database.

In addition to standard descriptive statistics, we calculated 95 % confidence intervals to define internal estimates associated with each probability, and prevalence, for all the variables under investigation. Regression analysis assessed which of the Rotterdam diagnostic criteria of PCOS had a relationship with the metabolic abnormalities and hormonal and cycle characteristics present in our study population. PCOS-defining, or independent, variables were as follows: antral follicular count (AFC), average ovarian volume, cycle length, oligo-/anovulation, biochemical hyperandrogenism (FAI, testosterone), and clinical hyperandrogenism (hair on face, abdomen, or chest, and acne). Linear regressions were performed for the continuous dependent variables: serum levels of triglycerides, total cholesterol, LDL-C, HDL-C, fasting glucose, 2-h glucose, thyroid stimulating hormone (TSH), luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol (E2), fasting insulin, 2-h insulin, hemoglobin A1c (HgbA1c), fasting glucose/fasting insulin ratio, HOMA1, prolactin, 17-OH-progesterone (17-OH-P), systolic blood pressure (SBP), diastolic blood pressure (DBP), and cycle-day 3 endometrial echo thickness (CD3-EM). Logistic regressions were performed for the categorical dependent variables: acanthosis nigricans, LPD, EM polyps, and/or hyperplasia (confirmed at pathology). Since age and BMI influence changes in all the variables, in our analyses, we controlled for both, which were forced into the model at step one as continuous variables. Because of the number of analyses, in our regression model, we lowered the value of significance to ≤ 0.003 from < 0.05 .

To further understand the relationship between the PCOS-defining characteristics and PCOS' metabolic complications, we built receiver operator characteristic (ROC) curves with the significant variables at regression analyses. The ROC curves would identify objective cut-off points for the PCOS-defining characteristics that define the metabolic complications under investigation. The ROC curve data was not further

corrected for age and BMI. We used SPSS v21 for Windows (SPSS, Chicago, IL).

Results

We studied 374 women with PCOS. Their demographic, clinical, and metabolic characteristics are reported in Table 1. Obesity and/or being overweight were present in 65.8 % of patients, and the average BMI was 30.9 ± 8.3 kg/m². All the patients who experienced a previous weight gain reported their periods were more irregular after the weight gain. Systolic and diastolic blood pressures were above the recommended limits of normal in 23.8 and 44.1 % of the patients, respectively. Almost 50 % of the patients in our study had a cycle length shorter than 35 days (46.8 %). However, oligo-/anovulation was very common, being present in 74.6 % of the patients, with LPD present in 21.1 %.

Thirty-nine percent of them (144/374 women) conceived in the years preceding their presenting to the clinic. The ones who conceived had a total of 252 pregnancies (average 1.8 pregnancies per patient): 134/252 (53 %) ended in livebirth, 108/252 (43 %) in miscarriage, 8/252 (3 %) in elective abortion, and 3/252 (1 %) in ectopic pregnancy. Age at the last pregnancy averaged 26.1 ± 5.4 years: all women reported a lower BMI at that time.

The prevalence of a LH/FSH ratio greater than two was seen in only 14.5 % of the patients, while clinical hyperandrogenism defined by hirsutism and acne was the most common finding in our cohort (Table 2). Biochemical hirsutism defined by increased testosterone was present in only 9.5 %, while it was present in 33.8 % of the patients when identified by FAI. Overt hypothyroidism from Hashimoto's thyroiditis or post-treatment of Grave's disease was present in 2.9 % of the patients, but subclinical hypothyroidism with a TSH > 2.4 mIU/l was present in 27 % of the patients, whether or not they were on replacement with levothyroxine. Hyperinsulinemia identified by a HOMA1 greater than 1.775 was present in 70.2 % of patients, while fasting glucose/insulin ratio less than 4.5 identified the condition in only 19.0 % of the population. With 65.8 % of our patients having a BMI greater than 25 kg/m² (Table 1) and identifying hyperinsulinemia with being overweight or obese [16], HOMA1 appears the most sensitive means to diagnose hyperinsulinemia. Acanthosis nigricans, a clinical sign of hyperinsulinemia, was present in 20.0 % of patients (81/374). Glucose intolerance was diagnosed by HbA1c in 37.3 % by fasting glucose in only 7.4 %, and by 2-h glucose in 2.7 % of our patients. This finding makes HbA1c the most sensitive means to diagnose glucose intolerance. An HDL-C less than 50 mg/dl and an LDL-C greater than 100 mg/dl were the most represented lipid abnormalities in our study population (45.1 and 44.4 % of the patients, respectively), while the

Table 1 Demographics and clinical characteristics of 374 consecutive women diagnosed with PCOS by the Rotterdam criteria [206 (55 %) Caucasian; 123 (32.8 %) African-American; 12 (3.2 %) Hispanic; 6 (1.6 %) Arabic; 10 (2.7 %) Indian; 17 (4.5 %) Asian]

Characteristics	Average±SD	95 % CI	Prevalence	% (No.)
Age (years)	29.5±5.6	28.9–30.1		
BMI (kg/m ²)	30.9±8.3	29.9–31.9	BMI>25	65.8 % (246/374)
SBP (mmHg)	120±11	119–122	SBP ≥130 mmHG	23.8 % (89/374)
DBP	78±8	77–79	DBP ≥80 mmHG	44.1 % (165/374)
Weight gain (lbs.)	24.2±26.9	24.2–27.2		
Wt gain interval (years)	3.2±2.9	2.9–3.5		
Menarche (age in years)	12.5±1.7	12.3–12.7	Menarche ≥14 years	21.7 % (81/374)
Cycle length (days)	61.3±64.0	54.8–67.8	Cycle length >35 days	53.2 % (199/374)
			Oligo-/anovulation	74.6 % (279/374)
			LPD	37.7 % (141/374)

SBP systolic blood pressure, DBP diastolic blood pressure, LPD luteal phase defect

prevalence of total cholesterol greater than 200 mg/dl and triglycerides greater than 150 mg/dl were 18.7 and 18.2 % of the patients, respectively.

The average endometrial echo thickness on cycle-day 3 was 6.5±3.3 mm (95 % CI 6.2–6.9 mm), and the endometrium was abnormal in 20.1 % of the patients, with endometrial polyps being present in 63/353 patients and endometrial hyperplasia in 8 patients.

Linear and hierarchical logistic regression analyses of the PCOS-defining variables (oligo-/anovulation, oligomenorrhea, clinical and biochemical hyperandrogenism, AFC, ovarian volume) and their relationship with the various metabolic characteristics are reported in Table 3. All regression analyses were controlled for age, and BMI and significance was reached with a $p \leq 0.003$. Increased ovarian volume was a positive predictor of elevated systolic blood pressure and

Table 2 Endocrine and metabolic variables and their prevalence in the study population

Characteristics	Average±SD	95 % CI	Prevalence	% (No./total)
CD3-FSH (mIU/ml)	5.8±1.8	5.6–6.0		
CD3-LH (mIU/ml)	6.7±4.0	6.3–7.1		
LH/FSH ratio	1.2±0.7	1.1–1.3	LH/FSH ≥2.0	14.8 % (48/324)
CD3-Estradiol (pg/ml)	41.1±17.4	39.3–42.9		
Testosterone (ng/dl)	45.3±25.5	42.7–47.9	Testosterone ≥78 ng/dl	9.5 % (29/305)
			Hirsutism	62.0 % (232/374)
			Acne	47.6 % (178/374)
TSH (mIU/l)	2.02±1.38	1.88–2.16	TSH>2.4 mIU/ml ^a	27 % (90/333)
Prolactin (ng/dl)	13.5±8.1	12.6–14.3		
FAI	4.7±4.6	4.2–5.2	FAI>4.97	33.8 % (94/278)
Fasting glucose (FG)	88.3±16.3	86.6–89.9	FG ≥100 mg/dl	7.4 % (21/283)
2-h glucose	103.5±33.6	100.1–107.0	2-h ≥145	2.7 % (4/146)
HgbA1-c	5.6±0.5	5.6–5.7	5.7 HgbA1-c –6.4 %	37.3 % (76/204)
			Diabetes ^b	2.0 % (4/204)
Fasting insulin	13.7±10.5	12.6–14.8	Fasting ≥19 IU/l	18.4 % (36/196)
2-h-insulin	78.0±64.1	71.4–84.5	2-h >200 IU/l	6.1 % (8/132)
HOMA1	3.1±2.5	2.8–3.3	HOMA1>1.775	70.2 % (134/191)
Glucose/insulin ratio	10.8±13.1	9.5–12.1	Gluc/insul <4.5	19.0 % (36/189)
Total cholesterol (mg/dl)	173.9±31.8	170.6–177.1	≥200 mg/dl	18.7 % (51/273)
HDL-cholesterol (mg/dl)	51.1±13.2	49.8–52.5	≤50 mg/dl	45.1 % (123/273)
LDL-cholesterol (mg/dl)	100.7±27.2	98.0–103.5	≥100 mg/dl	44.4 % (120/270)
Triglycerides (mg/dl)	106.1±67.7	102.6–118.3	≥150 mg/dl	18.2 % (50/274)

^a Hashimoto's or post-Grave's 2.9 % 11/374

^b Diagnosed as 2-h glucose >200 and/or HgbA1-c >6.4 %

Table 3 Linear (1) and logistic (2) regression analysis for predictors of adverse metabolic and ultrasonographic parameters, controlling for age and BMI

Dependent variables:	Average follicle number (1) R^2 (p value)	Average ovarian volume (1) R^2 (p value)	FAI (1) R^2 (p value)	Testosterone (1) R^2 (p value)	Cycle length >35 D (2) OR (95 % CI; p value)	Oligo-ovulation (2) OR (95 % CI; p value)	Clinical Hirsutism (2) OR (95 % CI; p value)	Acne (2) OR (95 % CI; p value)
Cardiovascular								
Systolic blood pressure (mmHg)		0.025 (0.001) n		0.027 (0.003) p				
Diastolic blood pressure (mmHg)				0.051 (<0.001) p				
Dyslipidemia								
Triglycerides (mg/dl)			0.040 (0.002) p					
Total cholesterol (mg/dl)								
HDL-cholesterol (mg/dl)			0.090 (<0.001) n					
LDL-cholesterol (mg/dl)								
Hyperinsulinemia								
Fasting insulin (IU/l)								
2-h insulin (IU/l)								
Fasting glucose/insulin ratio			0.086 (<0.001) p					
HOMA1								
Glucose intolerance								
Fasting glucose (mg/dl)			0.037 (0.002) p					
2-h glucose (mg/dl)			0.143 (<0.001) p					
HgbA1-c (%)			0.173 (<0.001) p					
Acanthosis nigricans							2.41 (1.11–5.2; 0.026)	
Ultrasonographic								
Endometrial polyps/hyperplasia								1.82 (1.03–3.21; 0.038)
Cycle-day 3 endometrial thickness (mm)		0.053 (<0.001) p						0.61 (0.39–0.95; 0.029)

Blanc cells signify non-significance; p=positive relationship; n=negative relationship

endometrial irregularities. Clinical hyperandrogenism with hirsutism increased the risk of acanthosis nigricans 2.4 times (sensitivity 84.0 %, specificity 39.2 %). Acne, instead, was a significant predictor of endometrial abnormalities: PCOS women with acne have a 39 % decreased probability of a cycle-day 3 endometrium thicker than 5 mm (sensitivity 82.7 %, specificity 72.4 %; $p=0.029$) but a 1.8 times increased probability of endometrial polyps/hyperplasia (sensitivity 41.3 % and specificity 54.7 %; $p=0.038$). Oligomenorrhea and oligo-ovulation did not predict any metabolic abnormalities, probably because so represented in our population. Biochemical, but not clinical, hyperandrogenism, with increased total testosterone and FAI, was a significant predictor of increased systolic and diastolic blood pressure. In addition, a higher FAI was a positive predictor of all the different metabolic

abnormalities measured in our study, except for endometrial abnormalities.

To narrow our findings from the linear regression analysis and find new cut offs for the PCOS-defining variables that predict metabolic complications (AFC, average ovarian volume, FAI, total testosterone), we built ROC curves plotting the significant predictor individual values with the diagnostic thresholds for the metabolic conditions under investigation (Table 4). For systolic and diastolic blood pressure, testosterone was the only predictor with an ideal cut-off value of 62.0 ng/dl for systolic and 50.5 ng/dl for diastolic. FAI predicted dyslipidemia with cut-off values of 2.55 for HDL-C less than 50 mg/dl and 5.95 for triglycerides greater than 150 mg/dl. FAI was the only predictor of a fasting glucose/insulin ratio less than 4.5, but the ROC curve was not significant. For the diagnosis of glucose intolerance, FAI was the

Table 4 ROC curves representing the significant variables from linear regression analyses. New cut-off points for the PCOS-defining characteristics define the metabolic complications under investigation

Dependent variable	Independent variable	Ideal cut-off	Sensitivity	Specificity	AUC ^a	<i>p</i> value
Cardiovascular						
Systolic blood pressure >130 mmHg	Testosterone	62.0 ng/dl	39 %	83 %	0.596	0.018
Diastolic blood pressure >80 mmHg	Testosterone	50.5 ng/dl	45 %	78 %	0.623	0.001
Dyslipidemia						
HDL-cholesterol <50 mg/dl	Free androgen index	2.55	78 %	59 %	0.731	<0.001
Triglycerides >150 mg/dl	Free androgen index	5.95	59 %	79 %	0.677	<0.001
Hyperinsulinemia						
Fasting glucose insulin ratio <4.5	Average ovarian volume	11.0 cm ³	55 %	66 %	0.404	ns
Glucose intolerance						
HbA1c >5.6 %	Free androgen index	4.65	49 %	69 %	0.606	0.018
Endocrine parameters						
Presence of luteal phase defect	Average follicle count	17.5	74 %	55 %	0.663	<0.000
	Free androgen index	4.05	55 %	75 %	0.660	<0.001
Ultrasonographic						
Cycle-day 3 endometrial thickness >5 mm	Average ovarian volume	14.8 cm ³	30 %	92 %	0.575	0.018

*Area under the curve

only predictor with a cut-off value of 4.65 for a HbA1c greater than 5.6 %.

Among the reproductive hormones entered in the regression analysis model, CD3-prolactin, FSH, and TSH were not predicted by any of the PCOS-defining variables. AFC, $R^2=0.112$, and testosterone, $R^2=0.063$, were positive predictors of CD3-LH ($p<0.001$). In addition, testosterone positively predicted CD3-estradiol, $R^2=0.41$, and CD3-17-OH-progesterone, $R^2=0.087$ ($p=0.002$). LPD was predicted by a lower AFC (odds ratio, OR=0.96, 95 % CI 0.92–0.99; $p=0.044$) as well as less severe oligomenorrhea (OR=0.03, 95 % CI 0.15–0.63; $p<0.001$) and oligo-anovulation (OR=0.12, 95 % CI 0.07–0.21; $p<0.001$). The ROC curve identified an AFC less than 17.5, a FAI less than 4.05, and a cycle length less than 32.8 days as new cut offs to predict LPD.

Discussion

The prevalence of obesity, metabolic complications, and endometrial abnormalities in young women diagnosed with PCOS while presenting with complaints of menstrual irregularities and/or infertility was high in our cohort of women with PCOS. Of the different laboratory testing used to diagnose the clinical conditions in our cohort, FAI best diagnosed clinical hirsutism, HOMA1 best diagnosed hyperinsulinemia, and HbA1c best diagnosed glucose intolerance. Menstrual irregularities/oligo-ovulation and hirsutism/acne were the most common manifestations of PCOS but were predictors of a minority of its complications. Of the PCOS-defining

criteria, FAI was a predictor of all the metabolic and hormonal characteristics evaluated in this study and it was the only predictor of glucose intolerance, hyperinsulinemia, and dyslipidemia. The FAI cut-off points identified by the ROC curves were for the most part lower than the ones that define hyperandrogenism (FAI>4.97). Ovarian volume and hyperandrogenism were the best predictors of increased systolic and diastolic blood pressure, while AFC only predicted LPD. Importantly, the PCOS-related complications, believed to appear only at an older age, were present in younger PCOS patients presenting to our clinic with complaints of infertility and/or menstrual irregularities. Their relationship with the PCOS-defining characteristics were independent of the patient's age and BMI.

HOMA-1 identified insulin resistance in obese, as well as lean, women with PCOS, and a fasting glucose/insulin ratio less than or equal to 4.5 was the second best diagnostic test to identify insulin resistance/hyperinsulinemia, but was far less precise than HOMA1. We found an association between increased FAI and hyperinsulinemia. The source of this relationship could be the recognized action of insulin on IGF1 receptors in the ovary, which leads to decreased aromatase activity with accumulation of androgens and stalling of follicular development and estrogen production [21]. This results in an increased stromal component and polycystic appearance of the ovaries, well characterized on ultrasound. Once androgens enter the blood stream, peripheral conversion into estrogens by the adipose tissue's aromatase activity is responsible for the altered LH/FSH feedback, which produces anovulation [22]. The androgen-dependent accumulation of adiposity characterizes women with PCOS, with increased fat deposition in the

abdominal, chest, and shoulder areas. The obesity resulting from hyperinsulinemia perpetrates the vicious cycle of insulin resistance/hyperinsulinemia, hyperandrogenism, and anovulation [22]. In addition, the insulin-dependent decrease in serum SHBG is responsible for an increased bioavailability of circulating androgens, which further increases the androgenic effects in PCOS women, like acne, hirsutism, and male-pattern obesity [23].

Development of glucose intolerance, hypertriglyceridemia, and type 2 diabetes in women with PCOS is dependent upon the pancreatic insulin production and its inability to sustain the increasing insulin demand from peripheral resistance. The mechanism to explain the PCOS-related dyslipidemia, characterized by low HDL-C and high LDL-C is unclear and thought to be due to the development of obesity [24]. However, in our study dyslipidemia was present also in women who were not overweight or obese and was directly associated with the degree of hyperandrogenism. In light of our results, we propose a distinct mechanism for the development of PCOS-related dyslipidemia: the vicious cycle of hyperinsulinemia/insulin resistance/obesity is responsible for the increased androgen production, and the increased circulating androgens are responsible for the development of the male-pattern fat distribution, increased total cholesterol and decreased HDL-C. In our interpretation, hyperandrogenism, and not obesity, is responsible for the increased cardiovascular risk in women with PCOS [9, 25].

Cycle-day 3 hormonal levels were predicted by AFC and total testosterone: these results are explained by, and account for, the increased androgenic ovarian production. We did not find a high prevalence of Hashimoto's thyroiditis in our study population, which deviates from findings in previous reports [11–13]. However, we found a high prevalence of subclinical hypothyroidism, defined as a TSH level greater than 2.4 ng/ml with normal levels of T3 and T4, independently from the presence of anti-thyroid antibodies. Even though the lowered threshold to diagnose hypothyroidism has not been associated with decreased pregnancy rates in women undergoing in vitro fertilization (IVF) [26], it does hinder the ability to carry a pregnancy to term [14] and we believe it might contribute to infertility by possibly worsening oligo-/anovulation and endometrial receptivity in women not undergoing IVF. In fact, although the role of thyroid hormone receptors in the ovarian follicles and endometrium remains to be elucidated, we previously found the changes in TSH-receptor and DIO2 (iodothyronine deiodinase, the most abundant enzyme that converts T4 produced in the thyroid gland to the more potent T3) in the endometrium to be inversely correlated with serum estrogen levels [27]. In our study population, subclinical hypothyroidism was not linked to any of the predicting variables, but we speculate that the concurrent presence of subclinical hypothyroidism in women with PCOS worsens the sporadic ovulation and infertility.

The presence of acne predicts a decreased CD-3 endometrial thickness, but also the presence of endometrial polyps/hyperplasia, while an increased ovarian volume predicts an increased CD-3 endometrial thickness (Table 3). We are unsure of these findings' meaning, but with ovarian volume being associated with hyperinsulinemia, we hypothesize a possible insulin and androgen-dependent mechanism.

Despite the fact that FAI and testosterone levels were mostly below the current cut-off values, these variables were found to influence the metabolic parameters more consistently than clinical hyperandrogenism, oligo-anovulation, and menstrual irregularities. In addition, the ultrasound measurements of ovarian volume and follicular count identified metabolic complications as well as clinical hyperandrogenism. The ROC curves' lower cut-off values for the significant predictors of metabolic parameters narrowed our results.

Our study is novel in several ways, such as the assessment of reproductive, hormonal, metabolic, and ultrasonographic parameters during the follicular phase of the menstrual cycle, which aids reproducibility; evaluation of the relationship between PCOS-defining criteria and metabolic parameters; and, detection of new cut-off points for the PCOS characteristics to diagnose concurrent metabolic complications. We believe this study adds to the accuracy in assessing the prevalence of metabolic variables by decreasing the inconsistency brought about by menstrual cycle changes. Limitations of our study include the limited number of highly selected patients, the cross-sectional design and the lack of a control group, which limits the identification of cause-and-effect relationships. However, even though cross-sectional studies may suffer from prevalence/incidence bias, they are the design of choice to understand the burden of a particular disease and are useful for hypothesis generation.

In conclusion, our results show that women complaining of infertility and/or menstrual irregularities and subsequently diagnosed with PCOS already have metabolic complications and that these complications can be predicted by quantification of the PCOS-defining characteristics. Of these, an increased FAI predicts the presence of all metabolic complications analyzed in this study, while hirsutism predicted none. We confirm the pivotal role of insulin in the development of the clinical characteristics and metabolic implications of PCOS, which is independent of age and BMI. In addition, we identified a new role for hyperandrogenism in the development of male-pattern truncal obesity and dyslipidemia, and pointed out the negligible accountability of oligo-/anovulation and menstrual irregularities in the occurrence of adverse metabolic outcomes. Finally, our results showed that the initial ultrasound assessment is not just for fertility appraisal, but is fundamental in the identification of women at risk for metabolic complications. Our findings challenge the opinion that using the Rotterdam criteria for diagnosis of PCOS would be useful only for identification of PCOS women with infertility.

[10]. Thus, the Rotterdam, rather than the NIH, classification system may add value in diagnosing PCOS, as the ultrasound assessment of the ovaries allows for better fertility assessment for early identification of women at risk for PCOS-related adverse metabolic outcomes and for tailoring counseling for effective preventive and health promotion strategies such as starting lifestyle modifications and/or pharmacologic therapy.

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References

1. Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. *Fertil Steril*. 2004;81:19–25.
2. Azziz R, Carmina E, Dewailly D, Diamanti-Kandarakis E, Escobar-Morreale HF, Futterweit W, et al. Positions statement: criteria for defining polycystic ovary syndrome as a predominantly hyperandrogenic syndrome: an Androgen Excess Society guideline. *J Clin Endocrinol Metab*. 2006;91:4237–45.
3. Knochenhauer ES, Key TJ, Kahsar-Miller M, Waggoner W, Boots LR, Azziz R. Prevalence of the polycystic ovary syndrome in unselected black and white women of the southeastern United States: a prospective study. *J Clin Endocrinol Metab*. 1998;83:3078–82.
4. Nandi A, Chen Z, Patel R, Poretsky L. Polycystic ovary syndrome. *Endocrinol Metab Clin North Am*. 2014;43:123–47.
5. Dunaif A, Segal KR, Futterweit W, Dobrjansky A. Profound peripheral insulin resistance, independent of obesity, in polycystic ovary syndrome. *Diabetes*. 1989;38:1165–74.
6. Carmina E. Cardiovascular risk and events in polycystic ovary syndrome. *Climacteric*. 2009;12 Suppl 1:22–5.
7. Fauser BC, Tarlatzis BC, Rebar RW, Legro RS, Balen AH, Lobo R, et al. Consensus on women's health aspects of polycystic ovary syndrome (PCOS): the Amsterdam ESHRE/ASRM-Sponsored 3rd PCOS Consensus Workshop Group. *Fertil Steril*. 2012;97:28–38.e25.
8. American College of Obstetricians and Gynecologists Committee on Practice Bulletins—Gynecology. ACOG practice bulletin no. 108: polycystic ovary syndrome. *Obstet Gynecol*. 2009;114:936–49.
9. Wild RA, Carmina E, Diamanti-Kandarakis E, Dokras A, Escobar-Morreale HF, Futterweit W, et al. Assessment of cardiovascular risk and prevention of cardiovascular disease in women with the polycystic ovary syndrome: a consensus statement by the Androgen Excess and Polycystic Ovary Syndrome (AE-PCOS) Society. *J Clin Endocrinol Metab*. 2010;95:2038–49.
10. Dunaif A, Fauser BC. Renaming PCOS—a two-state solution. *J Clin Endocrinol Metab*. 2013;98:4325–8.
11. Gleicher N, Barad D, Weghofer A. Functional autoantibodies, a new paradigm in autoimmunity? *Autoimmun Rev*. 2007;7:42–5.
12. Janssen OE, Mehlmauer N, Hahn S, Offner AH, Gartner R. High prevalence of autoimmune thyroiditis in patients with polycystic ovary syndrome. *Eur J Endocrinol*. 2004;150:363–9.
13. Ott J, Aust S, Kurz C, Nouri K, Wirth S, Huber JC, et al. Elevated antithyroid peroxidase antibodies indicating Hashimoto's thyroiditis are associated with the treatment response in infertile women with polycystic ovary syndrome. *Fertil Steril*. 2010;94:2895–7.
14. Abalovich M, Amino N, Barbour LA, Cobin RH, De Groot LJ, Glinier D, et al. Management of thyroid dysfunction during pregnancy and postpartum: an Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab*. 2007;92:S1–47.
15. Hahan S, Kuehnel W, Tan S, Kramer K, Schmidt M, Roesler S, et al. Diagnostic value of calculated testosterone indices in the assessment of polycystic ovary syndrome. *Clin Chem Lab Med*. 2007;45:202–7.
16. Mayfield J. Diagnosis and classification of diabetes mellitus: new criteria. *Am Phys Ass*. 1998;58:1355–62. **1369–70**.
17. Esteghamati A, Ashraf H, Khalilzadeh O, Zandieh A, Nakhjavani M, Rashidi A, et al. Optimal cut-off of homeostasis model assessment of insulin resistance (HOMA-IR) for the diagnosis of metabolic syndrome: third national surveillance of risk factors of non-communicable diseases in Iran (SuRFNCD-2007). *Nutr Metab*. 2010;7:7–26.
18. Bonora E, Targher G, Alberiche M, Bonadonna RC, Saggiani F, Zenere MB, et al. Homeostasis model assessment closely mirrors the glucose clamp technique in the assessment of insulin sensitivity: studies in subjects with various degrees of glucose tolerance and insulin sensitivity. *Diabetes Care*. 2000;23:57–63.
19. Jellinger PS, Smith DA, Mehta AE, Ganda O, Handelsman Y, Rodbard HW, et al. American association of clinical endocrinologists' guidelines for management of dyslipidemia and prevention of atherosclerosis. *Endocr Pract*. 2012;18 Suppl 1:1–78.
20. ADA practice Guidelines: <http://mcintranet.musc.edu/agingq3/calculationswesbite/ADA%20Guidelines/ADA%20Binder.pdf>; accessed on January 31, 2015.
21. Dunaif A. Insulin resistance and the polycystic ovary syndrome: mechanisms and implications for pathogenesis. *Endocrin Rev*. 1997;18:774–800.
22. Ehmman DA, Barnes RB, Rosenfield RL. Polycystic ovary syndrome as a form of functional ovarian hyperandrogenism due to dysregulation of androgen production. *Endocr Rev*. 1995;16:322–53.
23. Nestler JE, Powers LP, Matt DW, Steingold KA, Plymate SR, Ritmaster RS, et al. A direct effect of hyperinsulinemia on serum sex hormone-binding globulin levels in obese women with the polycystic ovary syndrome. *J Clin Endocrinol Metab*. 1991;72:83–9.
24. Wild RA, Rizzo M, Clifton S, Carmina E. Lipid levels in polycystic ovary syndrome: systematic review and meta-analysis. *Fertil Steril*. 2011;95:1073–9.e1–11.
25. Christian RC, Dumesic DA, Behrenbeck T, Oberg AL, Sheedy 2nd PF, Fitzpatrick LA. Prevalence and predictors of coronary artery calcification in women with polycystic ovary syndrome. *J Clin Endocrinol Metab*. 2003;88:2562–8.
26. Reh A, Grifo J, Danoff A. What is a normal thyroid-stimulating hormone (TSH) level? Effects of stricter TSH thresholds on pregnancy outcomes after in vitro fertilization. *Fertil Steril*. 2010;94:2920–2.
27. Detti L, Uhlmann RA, Fletcher NM, Diamond MP, Saed GM. Endometrial signaling pathways during ovarian stimulation for assisted reproductive technology. *Fertil Steril*. 2013;100:889–94.