Metabolic Inflexibility Is a Feature of Women With Polycystic Ovary Syndrome and Is Associated With Both Insulin Resistance and Hyperandrogenism

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Context: Metabolic inflexibility, ie, the impaired ability of the body to switch from fat to carbohydrate oxidation under insulin-stimulated conditions, is associated with insulin resistance. This alteration in metabolic plasticity can lead to organ dysfunction and is considered a key issue among the abnormalities of the metabolic syndrome. It is still unknown whether this phenomenon occurs in women with polycystic ovary syndrome (PCOS).

Objective: Our objective was to examine whether metabolic inflexibility is a feature of PCOS women and whether hyperandrogenism may contribute to this phenomenon.

Design and Patients: Eighty-nine Caucasian women with PCOS were submitted to hyperinsulinemic-euglycemic clamp. Respiratory exchange ratios were evaluated at baseline and during hyperinsulinemia by indirect calorimetry to quantify substrate oxidative metabolism. Total testosterone was measured by liquid chromatography mass spectrometry and free testosterone by equilibrium dialysis.

Setting: Outpatients were seen in a tertiary care academic center.

Main Outcome Measure: Metabolic flexibility was assessed by the change in respiratory quotient upon insulin stimulation.

Results: Sixty-five of the 89 PCOS women (73%) had increased serum free testosterone, 68 (76%) were insulin resistant, and 62 (70%) had an impaired metabolic flexibility. Comparison of hyperandrogenemic and normoandrogenemic women showed that the 2 subgroups were of similar age but differed in terms of several anthropometric and metabolic features. In particular, hyperandrogenemic women had greater body mass index (32.9 $\pm$ 1.0 vs 24.7 $\pm$ 0.9 kg/m^2, $P < .001$) and lower glucose utilization during the clamp (9.2 $\pm$ 0.4 vs 10.9 $\pm$ 0.7 mg/kg fat-free mass $\cdot$ min, $P = .023$) and metabolic flexibility (0.09 $\pm$ 0.06 vs 0.12 $\pm$ 0.01, $P = .014$). In univariate analysis, metabolic flexibility was associated with several anthropometric, endocrine, and metabolic features. In multivariate analysis, this feature was directly associated with baseline respiratory quotient and insulin sensitivity and inversely with free testosterone and free fatty acids concentrations under insulin suppression ($R^2 = 0.634$, $P < .001$).

Conclusions: Metabolic inflexibility is a feature of PCOS women. Both insulin resistance and androgen excess might contribute to this abnormality. **(*J Clin Endocrinol Metab* 98: 2581–2588, 2013)**

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Abbreviations: BMI, body mass index; FFA, free fatty acid; FFM, fat-free mass; HDL, high-density lipoprotein; LDL, low-density lipoprotein; npRQ, nonprotein RQ; OGTT, oral glucose tolerance test; PCOS, polycystic ovary syndrome; RQ, respiratory quotient.

Metabolic flexibility is the capacity for the body to rapidly switch from predominant lipid oxidation with high rates of fatty acid uptake in low insulin conditions to predominant glucose oxidation and storage with suppression of lipid oxidation in high insulin conditions (1). This physiological adaptive metabolic change is reflected by an increase in the respiratory quotient (RQ), ie, in the CO_2 produced to O_2 consumed ratio (1). The RQ is 0.7 when only fat is burned, whereas it rises to 1.0 when energy balance relies entirely on glucose oxidation (2). Intermediate values indicate that both sources are used.

Previous research showed that obese and/or diabetic individuals, as compared with healthy lean individuals, have an impaired metabolic flexibility (3). Interestingly, a similar finding was reported for insulin-resistant nondiabetic individuals with a family history of type 2 diabetes (4), suggesting this phenomenon may be directly related to the impaired insulin action.

Insulin resistance and the associated metabolic abnormalities are frequent findings in women with polycystic ovary syndrome (PCOS) (5). However, until now, no studies have assessed whether these women have an altered metabolic flexibility. Moreover, it is unknown whether androgen excess, which is associated with insulin resistance and may affect adipose tissue metabolism, may contribute to this alteration.

The aims of this study were to assess 1) whether metabolic flexibility is impaired in PCOS women, and 2) if so, whether hyperandrogenism may contribute to this phenomenon.

To answer these questions, respiratory exchange ratios were assessed by indirect calorimetry at baseline and during a hyperinsulinemic-euglycemic clamp in 89 Caucasian PCOS women. Serum testosterone was measured by liquid chromatography mass spectrometry, and free testosterone fraction was estimated by equilibrium dialysis, the gold standard for this assay.

Subjects and Methods

Subjects

Eighty-nine nondiabetic, Caucasian women with PCOS were examined. Mean age was 23.6 ± 0.6 years and body mass index (BMI) was 30.7 ± 0.9 kg/m^2. They were among the patients recruited in the Verona PCOS Pathophysiology and Phenotype (Verona 3P) Study, an ongoing project aimed at building a comprehensive database for exploring the relationships between the different features of these women. All these subjects were referred to the outpatient clinic of the Division of Endocrinology, Diabetes, and Metabolism of Verona Hospital, Italy, for hirsutism and/or menstrual alterations, and PCOS diagnosis was established according to the Rotterdam criteria (6). In particular, 66 of these 89 women had the classic PCOS phenotype (ie, they showed both clinical and/or biochemical hyperandrogenism and chronic oligo-anovulation), whereas 23 had either the ovulatory or the normoandrogenic phenotypes. Further details on this cohort have been reported elsewhere (7). Inclusion criteria were a confirmed PCOS diagnosis and age 18 to 40 years. Exclusion criteria were diabetes mellitus or other diseases or medications potentially interfering with the evaluations carried out in the study. For PCOS diagnosis, hyperandrogenism was defined by the presence of hirsutism and/or increased serum free testosterone. In accordance with the Androgen Excess and PCOS Society guidelines for diagnosis of PCOS, other clinical features, such as acne or alopecia, and serum androgens other than free testosterone were not used, because they are less specific and add a limited incremental amount to PCOS diagnosis (8). Consistent with this statement, in our sample, only a single (nonhirsute) PCOS patient among those with normal serum free testosterone showed a mild increase in serum androstenedione. Chronic oligoanovulation was diagnosed by the presence of either oligoamenorrhea (fewer than 9 cycles per year) or luteal-phase serum progesterone below 12 nmol/L. Specific endocrine disorders were excluded by assessment of serum 17-hydroxyprogesterone, TSH, and prolactin, whereas other investigations were carried out when indicated on clinical grounds.

Data previously obtained in a historical sample of 24 healthy volunteers, with regular menses, normal ovarian morphology and no clinical evidence of hyperandrogenism, served to define the reference intervals for insulin sensitivity and metabolic flexibility. Fifty-one women with the same characteristics served to define the reference interval for serum free testosterone. The two groups of healthy women were similar in terms of both age (26.6 ± 3.6 vs 28.3 ± 6.0 years) and BMI (21.9 ± 2.0 vs 21.1 ± 2.0 kg/m^2).

All subjects gave their informed written consent before the study, which was conducted in accordance with the Declaration of Helsinki and approved by the institutional ethics committee.

Protocol

All subjects underwent a complete physical examination, including assessment of height, body weight, waist circumference, and blood pressure (BP). Body fat and fat-free mass (FFM) were measured by bioelectrical impedance (BIA 103; Akern, Florence, Italy) (9).

After an overnight fast, blood samples for serum testosterone and metabolic parameters were collected. The metabolic assessment included an oral glucose tolerance test (OGTT) with measurement of plasma glucose and insulin at baseline and every 30 minutes for 2 hours.

On a separate day, a hyperinsulinemic-euglycemic clamp was carried out to assess whole-body insulin-stimulated glucose utilization (10). In the basal, non–insulin-stimulated period and under steady-state hyperinsulinemic clamp conditions, indirect calorimetry was performed to measure the RQ and quantify substrate oxidation (2).

Hyperinsulinemic-euglycemic clamp and indirect calorimetry

After overnight fasting, iv catheters were inserted in an antecubital vein of an arm for infusions and in a dorsal hand vein of the contralateral arm for arterialized blood sampling. After baseline sampling, a primed-continuous insulin infusion (Humulin R; Eli Lilly & Co, Indianapolis, Indiana) was started and

maintained for 2 to 3 hours at a constant rate of 80 mU/m^2/min. Euglycemia (serum glucose concentrations at approximately 90 mg/dL) was concurrently maintained throughout the clamp by a variable infusion of 20% dextrose, adjusted by monitoring plasma glucose levels in arterialized venous blood, approximately every 5 to 10 minutes. Sampling for assessing serum insulin and other parameters (free fatty acids [FFAs]) was made at baseline and in the last 30 minutes of the clamp at 10-minute intervals. We previously found that in nondiabetic hyperandrogenic subjects and control women, endogenous glucose production was negligible at this insulin infusion rate (11). Therefore, the amount of glucose infused can be considered equivalent to whole-body insulin-stimulated glucose utilization. Because muscle is responsible for most insulin-stimulated glucose metabolism (12), the mean glucose infusion rate during the steady-state period (last 30 minutes) of the clamp was normalized for FFM.

Resting RQ and glucose and lipid oxidation rates were measured over 30 minutes at baseline and at steady state during the clamp by indirect calorimetry using a Quark RMR instrument (Cosmed, Cernusco sul Naviglio, Italy) equipped with a ventilated hood.

Assays

Plasma glucose was assayed by a glucose analyzer (YSI 2300 STAT PLUS, Yellow Springs, Ohio). Serum total testosterone was measured by liquid chromatography mass spectrometry, using a Micromass Quattro Premier XE mass spectrometer from Waters Corporation (Milford, Massachusetts). The limit of quantification was 2.5 ng/dL, and the intra-assay coefficient of variation was 9.1% at a concentration of 14 ng/dL and the interassay coefficient of variation was 9.3% at a concentration of 26 ng/dL. Free testosterone fraction was estimated by equilibrium dialysis (13, 14) with an interassay coefficient of variation $<8\%$.

SHBG and insulin were assayed by immunoradiometric methods (Orion Diagnostica, Espoo, Finland; and Biosource, Fleurus, Belgium, respectively). Serum FFAs were measured in a sample of 54 women by an enzymatic colorimetric assay (Wako Chemicals GmbH, Neuss, Germany).

Calculations

Glucose and lipid oxidation rates were calculated using standard formulas (2). In this analysis, the protein oxidation rate was assumed to be equal to 31.2 mg/min, the mean value previously obtained in our lab by measuring nitrogen urinary excretion rate in a sample of PCOS subjects submitted to the same protocol. By including this figure, it is possible to calculate the nonprotein RQ (npRQ), which reflects glucose and lipid oxidation only (2). Metabolic flexibility was calculated as the difference between the npRQ value obtained in the steady-state period of the clamp and the fasting npRQ. Nonoxidative glucose metabolism was calculated as the difference between total glucose utilization and glucose oxidation rate. Low-density lipoprotein (LDL)-cholesterol was estimated by the Friedewald formula (15).

Statistical analysis

Data were summarized by means and SEs. Normality of the distribution of the variables was assessed by the Shapiro-Wilk test. Skewed variables were log or square root transformed before analysis.

The Student's *t* test for unpaired data was used to compare groups of hyperandrogenemic and normoandrogenemic PCOS women, and 1-way ANOVA was used to compare subgroups of these women divided into tertiles of metabolic flexibility. Associations between metabolic flexibility and each variable of interest were assessed by Pearson correlation coefficients (*r*). The Mann-Whitney *U* test and Spearman rank correlations were used when variables could not be normalized.

To assess predictors of metabolic flexibility, multiple regression analysis was performed, using metabolic flexibility as the dependent variable and baseline npRQ, age, total fat mass, waist circumference, glucose tolerance (2-hour OGTT glucose), insulin sensitivity (glucose disposal rate during clamp), serum FFAs under hyperinsulinemia, and free testosterone as independent variables. In these analyses, independent variables were chosen on the basis of associations in univariate analyses with the dependent variable and/or of biological plausibility.

P values $< .05$ were considered statistically significant. Analyses were carried out using STATA version 12.1 (StataCorp, College Station, Texas).

Results

Table 1 shows the main characteristics of the entire sample of PCOS women included in the study, either as a whole or stratified into hyperandrogenemic and normoandrogenemic subgroups. In the whole sample, mean BMI was 30.7 kg/m^2. Among the metabolic features, mean glucose levels at fasting and after OGTT were within the reference intervals, whereas plasma insulin concentrations were increased. A reduction in serum high-density lipoprotein (HDL)-cholesterol was the only abnormality in the lipid profile.

Sixty-five of the 89 PCOS women (73%) had increased serum free testosterone levels. Comparison of hyperandrogenemic and normoandrogenemic women showed that the 2 subgroups differed in terms of a number of anthropometric and metabolic features (Table 1). In particular, total body fat, waist circumference, and fasting glucose and insulin levels were significantly higher in hyperandrogenemic subjects.

Table 2 shows the measures of insulin action and substrate utilization obtained by the glucose clamp procedure combined with indirect calorimetry. In the whole sample, the mean glucose disposal rate during clamp (M-clamp value) was below the lower limit of the reference interval for our lab (11.75 mg/kg FFM · min), and most subjects (n = 68, 76%) were insulin-resistant. The metabolic flexibility, measured by the increase in npRQ from baseline to insulin-stimulated conditions, was also reduced in PCOS women (reference value in our healthy women >0.12). In particular, 62 (70%) of these patients showed impaired metabolic flexibility.

Table 1. Main Characteristics of the Whole Cohort of PCOS Women Included in the Study and of Subgroups of Hyperandrogenemic and Normoandrogenemic Subjects, With the Corresponding Reference Intervals[a]

Features	All n = 89	Hyperandrogenemic n = 65	Normoandrogenemic n = 24	Reference Interval
Age, y	23.6 ± 0.6	23.4 ± 0.7	24.3 ± 1.1	NA
BMI, kg/m^2	30.7 ± 0.9	32.9 ± 1.0	24.7 ± 0.9[e]	18–25
Waist circumference, cm	95.9 ± 2.0	100.9 ± 2.4	82.2 ± 2.4[e]	<80
Fat mass, kg	30.9 ± 1.5	34.3 ± 1.9	21.8 ± 1.6[e]	
FFM, kg	50.9 ± 1.1	53.1 ± 1.3	44.9 ± 1.1[e]	
Ferriman-Gallwey score	10.6 ± 0.8	11.0 ± 0.9	9.3 ± 1.6	<8
Systolic BP, mm Hg	121 ± 2	122 ± 2	118 ± 3	<130
Diastolic BP, mm Hg	79 ± 1	79 ± 1	78 ± 2	<85
Fasting glucose, mg/dL	87 ± 1	89 ± 1	82 ± 1[d]	70–99
Fasting insulin, mU/L	18 ± 1.4	20.5 ± 1.7	11.0 ± 1.4[e]	<9.0
2-h glucose OGTT, mg/dL	103 ± 4	106 ± 4	93 ± 6	<140
Total cholesterol, mg/dL	165 ± 3	167 ± 4	159 ± 6	<200
HDL-cholesterol, mg/dL[b]	48.4 ± 1.3	46.3 ± 1.5	54.0 ± 2.1[e]	≥50
LDL-cholesterol, mg/dL[b]	100 ± 2.7	103 ± 3.1	91 ± 5.0[d]	<130
Triglycerides, mg/dL[c]	85.1 ± 6.4	91.4 ± 8.2	68.2 ± 7.5	<150
SHBG, nmol/L	32.5 ± 1.9	29.2 ± 2.0	41.4 ± 3.9[e]	39–121
Total testosterone, ng/dL	37.6 ± 1.6	42.1 ± 2.2	25.4 ± 2.2[e]	<41
Free testosterone, ng/dL	0.76 ± 0.04	0.90 ± 0.04	0.39 ± 0.02[e]	<0.50
Ovarian follicles, n	13 ± 1	13 ± 1	13 ± 1	<12
Ovarian volume, mL	11.4 ± 0.5	11.5 ± 0.5	11.2 ± 1.1	≤10

Abbreviation: NA, not applicable.

[a] Data are shown as mean ± SE.

[b] Not available in 2 subjects.

[c] Not available in 1 subject.

[d] $P < .05$–$.01$ vs hyperandrogenemic patients.

[e] $P < .01$–$.001$ vs hyperandrogenemic patients.

Comparison of hyperandrogenemic and normoandrogenemic PCOS subjects showed that the impairment in insulin action was more severe in the former (Table 2). Differences between these subgroups in nonoxidative glucose metabolism accounted for most of the reduction in insulin-stimulated glucose utilization found in hyperandrogenemic women. Metabolic flexibility was impaired to a greater extent in hyperandrogenemic than in normoandrogenemic PCOS subjects (Table 2 and Figure 1). This difference was confirmed also after adjusting for glucose disposal rate and fat mass.

Conversely, serum free testosterone levels significantly differed between subgroups of PCOS women divided into tertiles of metabolic flexibility ($P = .005$). Interestingly, in post hoc analysis, serum free testosterone levels of subjects in the lower tertile of metabolic flexibility differed from those of subjects in both the upper ($P = .006$) and the intermediate ($P = .038$) tertiles, whereas the latter had similar free testosterone concentrations ($P = .520$). These results were maintained after controlling for glucose disposal rate and fat mass. Three normoandrogenemic vs 27 hyperandrogenemic subjects (12% vs 42% of the respec-

Table 2. Substrate Metabolism at Baseline and During the Clamp in the Whole Cohort of PCOS Women Included in the Study and in the Subgroups of Hyperandrogenemic and Normoandrogenemic Patients[a]

Features	All	Hyperandrogenemic	Normoandrogenemic
Baseline npRQ	0.726 ± 0.006	0.732 ± 0.007	0.709 ± 0.010
Metabolic flexibility (ΔnpRQ)	0.098 ± 0.005	0.091 ± 0.060	0.120 ± 0.010[b]
Glucosal disposal rate, mg/kg FFM/min	9.63 ± 0.34	9.16 ± 0.39	10.91 ± 0.68[b]
Glucose oxidation (baseline), mg/kg FFM/min	0.53 ± 0.08	0.60 ± 0.10	0.31 ± 0.10
Glucose oxidation (clamp), mg/kg FFM/min	2.20 ± 0.11	2.19 ± 0.14	2.30 ± 0.18
Nonoxidative glucose metabolism (clamp), mg/kg FFM/min	7.41 ± 0.31	7.0 ± 0.35	8.61 ± 0.63[b]
Lipid oxidation (baseline), mg/kg FFM/min	1.83 ± 0.05	1.78 ± 0.06	1.96 ± 0.10
Lipid oxidation (clamp), mg/kg FFM/min	1.24 ± 0.05	1.25 ± 0.06	1.21 ± 0.08

[a] Data are shown as mean ± SE.

[b] $P < .05$–$.01$ vs hyperandrogenemic patients.

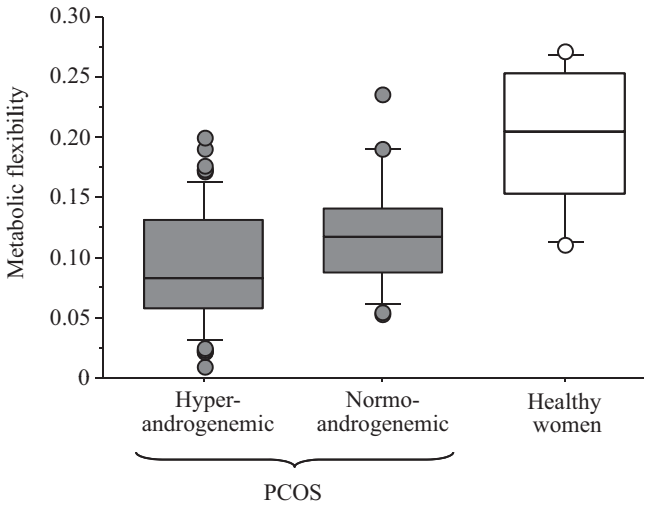

Figure 1. Box plots of metabolic flexibility in hyperandrogenemic and normoandrogenemic PCOS women. Data are compared with reference values of healthy controls. $P = .014$ between subgroups of PCOS women; $P < .001$ between healthy women and both PCOS subgroups.

tive subgroups, $P = .011$) were in the lower tertile of metabolic flexibility.

Serum FFA levels at baseline and during the clamp were similar in both hyperandrogenemic and normoandrogenemic PCOS women (607 ± 25 vs 566 ± 39 μEq/L, $P = .381$; and 74.5 ± 4.2 vs 65.6 ± 7.2 μEq/L, $P = .189$, respectively).

As shown in Table 3, metabolic flexibility was inversely associated with the basal npRQ value and with a number of anthropometric, endocrine, and metabolic features (BMI, waist circumference, total-body fat mass, BP, serum free testosterone, insulin, triglycerides, 2-hour OGTT plasma glucose, and FFA levels during the clamp). Conversely, it was directly associated with glucose disposal rate and serum HDL-cholesterol.

Table 3. Associations (Pearson's *r*) Between Metabolic Flexibility (ΔnpRQ) and Anthropometric, Metabolic, and Hormonal Parameters in the Whole Population of PCOS Women

	r	*P*[a]
Baseline npRQ	−0.464	**<.001**
Age	0.164	.124
BMI	−0.406	**<.001**
Waist circumference	−0.356	**<.001**
Fat mass	−0.406	**<.001**
FFM[b]	−0.355	**<.001**
Systolic BP	−0.313	**.003**
Diastolic BP	−0.272	**.010**
Free testosterone	−0.341	**.001**
Fasting insulin	−0.464	**<.001**
Fasting glucose	−0,173	.104
2-h glucose OGTT	−0.234	**.030**
Glucose disposal rate	0.453	**<.001**
Total cholesterol	−0.200	.062
HDL-cholesterol	0.331	**.002**
Triglycerides	−0.248	**.020**
LDL-cholesterol	−0.267	**.012**
FFA, baseline	−0.033	.784
FFA, clamp	−0.432	**.001**

[a] Statistically significant *P* values are in bold.

[b] *ρ*-Value, by nonparametric Spearman correlation.

In multiple regression analysis, metabolic flexibility was independently associated with baseline npRQ value, glucose disposal rate, free testosterone levels, and FFA concentrations under insulin suppression (Table 4; overall $R^2 = 0.634$, $P < .001$). When free testosterone was replaced by total testosterone and SHBG in the multiple regression analysis, total testosterone ($P = .031$) but not SHBG ($P = .127$) was associated with metabolic flexibility.

Discussion

This is the first study to investigate metabolic flexibility of PCOS women and to explore which features are associated with this phenomenon. Our data showed that PCOS women have an impaired ability to shift their substrate metabolism from a condition of predominant lipid oxidation at low (fasting) insulin levels toward a condition of predominant glucose oxidation at high (clamp) insulin levels. Metabolic plasticity is fundamental in adaptation and the inability to adjust to variations in energy demand and substrate fluxes can lead to organ dysfunction and, eventually, disease. In this regard, it is noteworthy that a number of data point to metabolic inflexibility as a key dysfunction among the abnormalities featuring the metabolic syndrome (16). Moreover, it was hypothesized that metabolic inflexibility may lead to ectopic fat accumulation in insulin-sensitive tissues, such as liver and skeletal muscle (17). In turn, this phenomenon may cause hepatic/systemic insulin resistance and atherogenic dyslipidemia as well as inflammation and fibrosis in the liver, being also linked to an increased risk of cardiovascular events (18).

Interestingly, in our study, metabolic flexibility was independently associated with baseline npRQ values and metabolic and endocrine parameters such as whole-body insulin-stimulated glucose metabolism, FFA concentrations under insulin suppression, and androgen levels.

The association between metabolic inflexibility and insulin resistance was previously reported in other conditions characterized by an impaired insulin action, such as obesity and type 2 diabetes (19, 20). Galgani et al (20) suggested that the altered sensitivity of tissues to insulin action on glucose metabolism may explain most if not all of this phenomenon. However, the same group subsequently reported that metabolic flexibility was higher in African-Americans than in Caucasians as well as in non-

Table 4. Predictors of Metabolic Flexibility (Δ npRQ) by Multiple Regression Analysis in the Whole Group of PCOS Subjects ($R^2 = 0.634$, $P < 0.001$).

Features	β-Coefficient	SE	P[a]
Baseline npRQ	−0.586	0.110	**<.001**
Glucose disposal rate, mg/kg FFM/min	0.007	0.002	**.002**
FFA (clamp),[b] μEq/L	−0.574	0.218	**.012**
Free testosterone, ng/dL	−0.047	0.019	**.017**
Waist circumference,[c] cm	0.006	0.003	.090
2-h glucose OGTT,[d] mg/dL	−0.001	0.002	.689
Fat mass, kg	−0.001	0.001	.911
Age, y	−0.001	0.001	.986

[a] Statistically significant *P* values are in bold.

[b] Available in 54 subjects.

[c] β-Coefficient was calculated on the basis of an increase of 5 cm.

[d] β-Coefficient was calculated on the basis of an increase of 10 mg/dL.

diabetic than in diabetic subjects even after adjusting for glucose disposal rate, suggesting that factors different from insulin resistance may independently contribute to this metabolic abnormality (21). Yet it was reported that metabolic inflexibility may precede insulin resistance of glucose metabolism in prediabetic subjects (22), consistent with the conclusion that this phenomenon is not simply a manifestation of insulin resistance. Although the mechanisms underlying the association between insulin resistance and metabolic inflexibility are not fully understood, a body of literature indicates that insulin-resistant subjects have a mitochondrial dysfunction caused by a reduction in the number of mitochondria in skeletal muscle and/or an intrinsic functional defect of them (23, 24). It was hypothesized that the mitochondrial defect may primarily induce impaired fat oxidation and increased fat accumulation in the muscle (25). In turn, this phenomenon may impair insulin signaling, thus inducing a defect in both oxidative and nonoxidative glucose metabolism (24, 26, 27). Interestingly, when this alteration occurs, mitochondria oxidative pathways seem to prefer FFAs to glucose (25), thus explaining the finding of an altered metabolic flexibility.

Another alteration previously linked to metabolic inflexibility is the impaired suppression by insulin of FFA release (ie, lipolysis) (16, 20). It is noteworthy that impaired serum fatty acid reduction during insulin infusion is another manifestation of abnormal insulin action, occurring at the adipose tissue level. However, the independent association between metabolic inflexibility and this phenomenon, in a model also including a measure of insulin action on glucose metabolism, indicates that serum FFA concentrations during hyperinsulinemia carry supplemental information. It is noteworthy that similar findings were previously reported in obese diabetic and nondiabetic subjects (20). This is consistent with a tissue-specific sensitivity (or resistance) to insulin, which accounts for the poor correlation between the effects of the hormone on muscle and adipose tissue (28). In pathophysiological terms, increased serum FFA levels may contribute to impaired glucose oxidation (25). This phenomenon may explain the observation of Randle et al (29) who proposed a glucose-fatty acid cycle to describe the dynamic interactions between these substrates. In addition, the increased exposure of tissues to FFAs can induce inhibition of insulin signal, thus contributing to the further worsening of insulin resistance (22, 30, 31).

Interestingly, a novel and intriguing finding of this study is that serum free testosterone levels were an additional predictor of metabolic inflexibility in these women, independent of muscle and adipose tissue insulin resistance and body fat. These data give further support to the hypothesis that androgens may play a direct role in the regulation of metabolic processes. Interestingly, a change in capillary density and fiber composition, with reduction in type 1 oxidative and increase in type 2 glycolytic fibers, was previously reported in ovariectomized rats exposed to a moderate androgen excess (32). Moreover, testosterone administration may increase FFA oxidation and decrease glucose oxidation, independently of changes in whole-body insulin-stimulated glucose metabolism (33). Consistently, testosterone increased palmitate oxidation while increasing nonoxidative but not oxidative metabolism of glucose in isolated myotubes from postmenopausal women (34).

The main strengths of this study are the relatively large sample size, the comprehensive assessment of subjects, and the measurement of several parameters (e.g., insulin sensitivity, free testosterone, etc.,) by state-of-the-art techniques.

We also acknowledge some limitations in the study. The first limitation is intrinsic to the different characteristics of normoandrogenic and hyperandrogenic PCOS women. It is well known that these subgroups of PCOS

women differ in terms of body fat mass, fat distribution, and metabolic features (7, 35). Therefore, heterogeneity between groups is unavoidable when samples of unselected consecutive PCOS women are recruited, as was the case in our study. The mechanisms underlying the association between obesity and hyperandrogenism remain largely unknown, and it was hypothesized that this relationship could be bidirectional. To overcome this problem, we performed multivariable analysis in which several features that differed between groups were included as independent variables to assess the specific role of androgen excess in the impaired metabolic flexibility. An alternative approach could be the comparison of BMI-matched women. However, obesity is uncommon in our normoandrogenic PCOS subjects. Moreover, recruitment of hyperandrogenic and normoandrogenic PCOS women with a similar BMI would necessarily result in samples that are not fully representative of their respective populations. In addition, it is likely that fat mass excess in obese normoandrogenic and hyperandrogenic PCOS women may differ in terms of origin, distribution, and pathophysiology.

Second, our sample was constituted entirely of Caucasian women. Therefore extrapolation of these results to other ethnic groups should be undertaken with caution. Finally, in our sample of historical healthy controls used to define the reference interval for metabolic flexibility, measurement of serum testosterone by mass spectrometry and equilibrium dialysis was not available, precluding assessment of any role of androgens in metabolic flexibility of healthy women. This would be an interesting point. However, it is likely that the sample size required to address this issue would need to be very large.

Overall, our findings demonstrate for the first time that metabolic inflexibility is a feature of PCOS women and that both insulin resistance and hyperandrogenism may contribute to impaired insulin-stimulated glucose oxidation in these subjects. Future prospective studies are needed to assess to what extent these findings may account for the striking metabolic differences between hyperandrogenic and normoandrogenic PCOS women and for the increased visceral and ectopic fat accumulation in these subjects.

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