CLINICAL STUDY

Spectrum of metabolic dysfunction in relationship with hyperandrogenemia in obese adolescent girls with polycystic ovary syndrome

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Abstract

Objective: Polycystic ovary syndrome (PCOS) in adult women is associated with increased risk of metabolic syndrome (MS) and atherosclerosis. We evaluated the spectrum of metabolic dysfunction in relationship with hyperandrogenemia (HA) in adolescent girls with PCOS.

Materials and methods: Ovulatory function, acne, hirsutism (HS), body mass index (BMI), body composition, fasting lipids, glucose, insulin, free testosterone (FT), high-sensitivity C-reactive protein (hs-CRP), and HbA1c were evaluated in 103 girls. The homeostatic assessment model equations (HOMA-IR and HOMA-%B) were used for determination of insulin resistance and β -cell function respectively.

Results: The oligo-ovulation (Oligo)+HA+HS ($n=44$), Oligo+HA ($n=28$), and Oligo+HS ($n=31$) phenotypes had similar BMI. However, hyperandrogenemic phenotypes had higher prevalence of acanthosis nigricans (AN) and acne ($P<0.01$) and higher insulin, HOMA-IR, HOMA-%B, HbA1c, and hs-CRP levels than Oligo+HS group ($P<0.01$). Serum FT was correlated with HOMA-IR ($r=0.38$, $P<0.01$), HOMA-%B ($r=0.49$, $P<0.01$), hs-CRP ($r=0.42$, $P<0.01$), AN ($r=0.39$, $P<0.01$), and HbA1c ($r=0.27$, $P<0.01$). Furthermore, 34% of girls met diagnostic criteria for MS displaying higher BMI, FT, HOMA-%B, HOMA-IR, hs-CRP, and HbA1c than subjects without MS ($P<0.01$). Using combined HOMA-IR ≥ 4.0 and hs-CRP > 3.0 cut-off values, 71.4% of MS versus 23.5% non-MS group were considered at risk of diabetes and atherosclerosis ($P<0.0001$).

Conclusions: Hyperandrogenemic PCOS phenotypes have greatest degree of insulin resistance and inflammation. The use of insulin resistance and inflammatory markers may help identify adolescent girls with PCOS at risk of cardiometabolic syndrome.

European Journal of Endocrinology 162 1093–1099

Introduction

Polycystic ovary syndrome (PCOS) is a common endocrine disorder, occurring in $\sim 6\%$ of women at reproductive age (1, 2). This endocrinopathy is characterized by oligomenorrhea and/or anovulation, clinical and/or biochemical hyperandrogenemia (HA) with or without ultrasonographic evidence of polycystic ovaries after exclusion of other disorders such as hyperprolactinemia, thyroid dysfunction, and nonclassical adrenal hyperplasia (3, 4). This hyperandrogenemic state with chronic ovulatory dysfunction is a heterogeneous disorder with a broad spectrum of phenotypes (5). These clinical phenotypes are defined by their clinical, hormonal, metabolic, and reproductive features of PCOS in adult women and adolescents (5, 6). PCOS is accompanied by an enhanced risk of insulin

resistance and hyperinsulinemia, type 2 diabetes mellitus (T2DM), dyslipidemia, and atherosclerosis (7, 8), implying that diagnosis of PCOS in young women increases the likelihood of life-long risks of menstrual dysfunction, infertility, T2DM, and premature heart disease (7–9).

Insulin directly stimulates ovarian androgen production through the stimulation of 17α -hydroxylase activity at the ovarian theca and reduces $17,20$ -lyase responsiveness in women with PCOS (10, 11). Similarly, hyperinsulinemia has been shown to be associated with excess adrenal androgen synthesis (11, 12). Recent studies have not demonstrated any effect of insulin on adrenal androgen production (12, 13).

The diagnosis of PCOS might be difficult since some features of the syndrome are physiologic during adolescence (14). Almost 60% of the menstrual cycles

are anovulatory, and enlarged ovaries may be common findings, representing features of the physiologic processes of maturation during the first few years after menarche (15, 16). During puberty, there is a physiologic increase in insulin resistance, and circulating insulin and androgen levels, resulting from the increase in GH levels, but a reduction in sex hormone-binding globulin levels (17, 18). Accurate diagnosis of PCOS may be difficult using current criteria, which might overestimate the diagnosis and interfere with the clinical management of adolescent oligomenorrhea. Among postmenarchal obese girls who subsequently develop glucose intolerance, PCOS has become a prevalent cause of HA, defined as free testosterone (FT) > 35 pmol/l (15, 16) and ovulatory dysfunction. In adult women, three clinical phenotypes of PCOS, with varying degrees of metabolic dysfunction influencing the development of HA and hirsutism (HS), have been described (5). We evaluated the spectrum and prevalence of metabolic dysfunction in relationship with HA, indices of insulin resistance and low-intensity inflammation in a group of adolescent females with three PCOS phenotypes.

Materials and methods

Subjects

One hundred and three female adolescents (aged 13.2–19.9 years) who met the criteria for PCOS and obesity (BMI > 95 th percentile for age) were included in the study. All subjects were evaluated at the Children's Hospital of Wisconsin (CHW; affiliated with the Medical College of Wisconsin) Endocrine Clinic for oligomenorrhea or secondary amenorrhea between September 2005 and February 2008. Race/ethnicity was self-assigned: Caucasian (C, $n = 56$; 54.4%), Mexican American (Hispanic (H), $n = 26$; 25.2%), and African American (AA, $n = 21$; 20.4%). All patients were postmenarchal for a minimum of 2 years. Individuals with hyperprolactinemia, hypo- or hyperthyroidism, congenital adrenal hyperplasia (17-hydroxyprogesterone (17-OHP) < 200 ng/ml (6.0 nmol/l)) (19), Cushing's syndrome, androgen-secreting tumors, and primary or secondary ovarian failure, and on hormonal treatment for at least 3 months prior to their evaluation were excluded (2). In compliance with the recommendations of the Declaration of Helsinki, the CHW Institutional Review Board approved the retrospective review of participants' clinical charts; therefore, informed consent was waived.

Methods

Clinical evaluation Patients and/or their guardians completed a standard questionnaire detailing their menstrual history, HS, acne, gynecologic and obstetric

history, medications, self-declared ethnicity, and family history of PCOS and T2DM diagnosis. A positive family history of HA was defined as a history of PCOS or of HS and oligomenorrhea in the first-degree female relatives of the patient. Oligomenorrhea was defined as menstrual periods occurring at intervals of > 35 days, with only four to nine periods in a year. Amenorrhea was defined as complete absence of menstruation for 6 months or more, after having established menstrual bleeding for a minimum of 2 years. All subjects were evaluated during the follicular phase of their cycle.

On physical examination, height, weight, BMI, blood pressure (BP), and body composition analysis by bioelectrical impedance (BIA) (TANITA-TBF-410, TANITA Corporation of America Inc., Arlington Heights, IL, USA) (20) were obtained. The presence of terminal hair growth in a male-like pattern was scored using Ferriman–Gallwey (FG) method (21); HS was defined as a score > 8 (16). The presence or absence of acanthosis nigricans (AN) and acne was noted, but not quantified. Two well-trained clinicians determined pubertal maturation (Tanner stage). Fasting serum samples were obtained for glucose, insulin, and lipid profile, high-sensitivity C-reactive protein (hs-CRP) along with HbA1c, LH, FSH, DHEAS, 17-OHP, androstenedione, total testosterone, and FT. Subjects were classified into three phenotype groups: Oligo + HA + HS, Oligo + HA, and Oligo + HS as previously described in adolescents and adults (5, 6).

Laboratory studies and calculations All blood samples were obtained between 0800 and 1100 h. Serum glucose was measured by an autoanalyzer (Orthodiagnosics Fusion 5.1, Ortho-Diagnostics, Rochester, NY, USA). The hs-CRP assays were carried out at Quest Diagnostics (San Jose, CA, USA) using a polystyrene particle-enhanced immunonephelometric method (Dade Behring BNII). The detection limit of this assay was 0.20 mg/l with measuring range of 0.18–1150 mg/l with intra-assay and inter-assay coefficients of variance of 2.65 and 3.6% respectively. The hs-CRP value > 3.0 mg/l was considered as an index of high cardiovascular risk (22), but hs-CRP values > 10 mg/l were excluded to avoid influence of acute infection. HbA1c was determined by the Bayer DCA (Bayer Diagnostics Inc.) 2000 instrument (nondiabetic range of 4.5–5.7%).

Fasting serum insulin was measured by Nichols RIA (Nichols Institute, San Clemente, CA, USA) with intra-assay and inter-assay coefficients of variation (CV) of 2.4–6.3 and 5.2–13.0% respectively. The homeostatic model assessment estimates for insulin resistance (HOMA-IR) and percent β -cell function (HOMA-%B) were calculated as previously described (23): $\text{HOMA-IR} = (\text{blood glucose (mmol/l)} \times \text{insulin } (\mu\text{U/ml})) / 22.5$ and $\text{HOMA-\%B} = 20 \times \text{insulin } (\mu\text{U/ml}) / (\text{glucose (mmol/l)} - 3.5)$. A cut-off HOMA-IR value

≥ 4.0 mol μ U/ml (>95 th percentile (HOMA-IR = 3.99)) was used to define insulin-resistant state (24, 25).

Serum LH and FSH concentrations were determined by chemiluminescent (Johnson & Johnson S.p.A-Otho Clinical Inc., Rochester, NY, USA). Serum DHEAS level was determined by a chemiluminescent assay (Quest Diagnostics) with intra-assay and inter-assay CV of 6.2–9.6 and 7.3–11.3% respectively. Serum 17-OHP concentrations were determined by high-pressure liquid chromatography–mass spectrometry (HTLC–MS) (Quest Diagnostics) with relative s.d. (RSD) range of 4.8–14.0%. Serum androstenedione concentrations were determined by HTLC–MS (Quest Diagnostics) with RSD range of 4.3–14.0%. Total testosterone level was determined by HTLC–MS (Quest Diagnostics) with RSD range of 7.1–10.8%. Serum FT was determined by an equilibrium dialysis method (Quest Diagnostics) with intra-assay and inter-assay CV of 4.5–11.8 and 4.2–11.3% respectively.

Total cholesterol, high-density lipoprotein cholesterol (HDL-C), and triglycerides (TGs) were determined by colorimetric methods (Beckman spectrophotometer, Fullerton, CA, USA). Low-density lipoprotein cholesterol (LDL-C) was calculated using Friedewald's equation (26).

BP measurements were taken twice with the patient in sitting position. Elevated systolic or diastolic pressure was defined as a value above the 95th percentile for age and gender (27).

Modified National Cholesterol Education Program criteria (28) for diagnosis of metabolic syndrome (MS) were defined as the presence of three or more of the following: age-adjusted BMI >95 th percentile, age-adjusted systolic or diastolic BP >90 th percentile, age-adjusted TG >90 th percentile, age-adjusted HDL-C <5 th percentile, and impaired fasting glucose >5.6 mmol/l.

Statistical analysis

Statistical analyses were carried out using SPSS (version 14.0). Data are expressed as mean $\pm$ s.d. unless otherwise specified. Body mass index (BMI) values were converted into SDS, which were normalized for age and gender based on 2000 Center for Disease control (CDC) growth charts. The natural logarithmic transformation of the variables was used in the correlation and regression analyses when they were found to be skewed. Differences between PCOS + MS and PCOS only groups were estimated using unpaired Student's *t*-tests. Then, the differences among PCOS phenotypes were estimated by one-way ANOVA; Bonferroni's *post hoc* test was applied whenever appropriate. The χ^2 analyses were used to compare prevalence of AN, acne, oligomenorrhea, and amenorrhea in PCOS subgroups. Spearman's correlations were performed to examine the associations between FT and HOMA-IR, HOMA-%B, HbA1c, hs-CRP and TG, while controlling for fat mass (FM). Bivariate correlations and

Table 1 Comparison of clinical and biochemical characteristics among adolescent polycystic ovary syndrome phenotypes.

Characteristics	All	Oligo + HA + HS	Oligo + HA	Oligo + HS	P
Number (%)	103	44 (42.7)	28 (27.2)	31 (30.1)	NS
Age (years)	15.5 $\pm$ 1.3	15.5 $\pm$ 1.2	15.6 $\pm$ 1.6	15.3 $\pm$ 1.1	NS
Race/ethnicity					
Caucasian (%)	56 (54.4)	27 (61.4)	14 (50.0)	15 (48.4)	NS
Hispanics (%)	26 (25.2)	7 (15.9)	8 (28.6)	11 (35.5)	$<0.01^{\dagger}$
African Americans (%)	21 (20.4)	10 (22.7)	6 (21.4)	5 (16.1)	NS
BMI (kg/m ²)	36.2 $\pm$ 8.4	37.8 $\pm$ 9.4	36.9 $\pm$ 7.9	33.6 $\pm$ 6.8	NS
BMI SDS	2.12 $\pm$ 0.47	2.20 $\pm$ 0.42	2.16 $\pm$ 0.48	1.98 $\pm$ 0.52	NS
Acanthosis nigricans present (%)	63 (61.2)	32 (72.7)	21 (75.0)	10 (32.3)	$<0.01^{\ddagger}$
Acne present (%)	34 (33.1)	18 (40.9)	13 (46.4)	6 (19.4)	$<0.01^{\ddagger}$
FG score	8.9 $\pm$ 3.3	11.2 $\pm$ 2.5	4.9 $\pm$ 0.9	9.2 $\pm$ 2.2	$<0.0001^{\dagger\ddagger}$
Free testosterone (pmol/l)	41.8 $\pm$ 17.1	48.1 $\pm$ 13.7	48.0 $\pm$ 17.6	26.2 $\pm$ 5.7	$<0.001^{\ddagger}$
Testosterone (nmol/l)	2.4 $\pm$ 0.8	2.6 $\pm$ 0.8	2.6 $\pm$ 0.7	1.8 $\pm$ 0.3	$<0.001^{\ddagger}$
DHEAS (μ mol/l)	5.9 $\pm$ 3.3	6.8 $\pm$ 3.9	6.5 $\pm$ 3.1	4.5 $\pm$ 1.8	$<0.01^{\ddagger}$
17-Hydroxyprogesterone (nmol/l)	3.4 $\pm$ 1.6	3.6 $\pm$ 1.6	3.3 $\pm$ 1.7	3.3 $\pm$ 1.6	NS
Androstenedione (nmol/l)	7.7 $\pm$ 3.3	8.1 $\pm$ 3.2	7.2 $\pm$ 3.0	7.3 $\pm$ 3.4	NS
Fasting glucose (mmol/l)	4.9 $\pm$ 0.4	4.8 $\pm$ 0.4	5.1 $\pm$ 0.4	4.9 $\pm$ 0.3	NS
Fasting insulin (pmol/l)	181.5 $\pm$ 99.6	205.1 $\pm$ 110.2	201.8 $\pm$ 83.5	132.5 $\pm$ 80.1	$<0.01^{\ddagger}$
HOMA-IR (mol μ U/ml)	6.6 $\pm$ 3.7	7.3 $\pm$ 3.8	7.6 $\pm$ 3.4	4.8 $\pm$ 3.2	$<0.01^{\ddagger}$
HOMA- β -cell (%)	495.8 $\pm$ 406.9	613.9 $\pm$ 499.4	484.2 $\pm$ 371.9	329.7 $\pm$ 184.8	$<0.01^{\S}$
HbA1c (%)	5.2 $\pm$ 0.4	5.2 $\pm$ 0.3	5.3 $\pm$ 0.4	5.0 $\pm$ 0.3	$<0.01^{\ddagger}$
hs-CRP (mg/l)	3.0 $\pm$ 1.5	3.2 $\pm$ 1.4	3.3 $\pm$ 1.5	2.4 $\pm$ 1.3	$<0.025^*$
Triglycerides (mmol/l)	1.6 $\pm$ 0.9	1.7 $\pm$ 1.0	1.6 $\pm$ 1.0	1.4 $\pm$ 0.9	NS
Cholesterol (mmol/l)	4.5 $\pm$ 1.0	4.5 $\pm$ 1.1	4.5 $\pm$ 0.9	4.4 $\pm$ 1.0	NS

* $P < 0.025$, Oligo + HA + HS and Oligo + HA groups versus Oligo + HS group. $^{\dagger}P < 0.01$, for comparison of Oligo + HA + HS group versus Oligo + HA and Oligo + HS groups. $^{\ddagger}P < 0.01$, for comparison of Oligo + HA + HS and Oligo + HA groups versus Oligo + HS group. $^{\S}P < 0.01$, for comparison of Oligo + HA + HS group versus Oligo + HS group. $^{\parallel}P < 0.001$, for comparison of Oligo + HA + HS and Oligo + HA groups versus Oligo + HS group. $^{\dagger\ddagger}P < 0.0001$, for comparison of Oligo + HA + HS and Oligo + HS groups versus Oligo + HA group.

a stepwise regression analysis between potential predictor variables and the dependent variable (FT) were performed while controlling for the demographic covariates of the entire cohort. $P < 0.05$ was considered significant.

Results

Table 1 summarizes the clinical and biochemical characteristics of entire cohort of obese adolescent girls with oligo-ovulation and three groups of PCOS phenotypes. Three groups were similar in age, BMI, BMI SDS, FM, fat-free mass, and total body water. While Caucasian and African American subjects were observed to have similar representations of three phenotypes, the percentages of Hispanic girls with Oligo + HA and Oligo + HS were higher than those with Oligo + HA + HS. Also, hyperandrogenemic groups (FT > 35 pmol/l) had higher frequencies of acne and AN than the Oligo + HS group. However, the frequencies of amenorrhea were not statistically different among three phenotypes (36.4 vs 35.7 vs 22.6%, $P = \text{NS}$).

While there were no differences in LH, FSH, LH:FSH ratio, 17-OHP, and androstenedione values among groups, serum testosterone, FT, and DHEAS were higher in Oligo + HA + HS and Oligo + HA compared with Oligo + HS ($P < 0.01$). There were no significant differences in hormonal and metabolic parameters in Oligo + HA + HS versus Oligo + HA. However, the hyperandrogenemic groups had higher insulin, HOMA-IR,

HbA1c, and hs-CRP. All three groups displayed marked hyperinsulinemia with both hyperandrogenemic groups displaying similar β -cell function values; however, HOMA-%B values were found to be only higher in Oligo + HS + HA group compared with Oligo + HS group. Furthermore, three groups displayed similar fasting lipid profiles (data not shown).

Table 2 summarizes the clinical and biochemical characteristics of females stratified into PCOS + MS and PCOS only subgroups. In our cohort, 34% of females met diagnostic criteria for MS displaying higher BMI, FT, HOMA-%B, HOMA-IR, hs-CRP, and HbA1c and frequency of AN than PCOS only subjects ($P < 0.05$) without significant differences in relationship with other androgens (data not shown). There were no differences between the groups in relationship with positive family history of T2DM and PCOS, frequencies of acne, amenorrhea (40 vs 27.9%, $P = \text{NS}$), and FG score. A cut-off HOMA-IR ≥ 4.0 identified 77.4% of the entire cohort as insulin resistant with 94.3% of PCOS + MS compared with 72.1% of PCOS only groups as insulin resistant ($P < 0.01$). A cut-off hs-CRP > 3.0 identified 41.7% of entire cohort with low-grade chronic inflammation with 74.3% of PCOS + MS group compared with 25.0% of PCOS only group at increased risk of atherosclerosis ($P < 0.0001$). Using combined HOMA-IR ≥ 4.0 and hs-CRP > 3.0 cut-off values, 71.4% of PCOS + MS compared with 23.5% PCOS only group were deemed at risk of diabetes and atherosclerosis ($P < 0.0001$).

Table 2 The comparison of polycystic ovary syndrome (PCOS) + metabolic syndrome (MS) and PCOS only subgroups.

Characteristics	PCOS + MS	PCOS only	P
Number (%)	35 (34.0)	68 (66)	NA
Age (years)	15.5 $\pm$ 1.3	15.4 $\pm$ 1.3	NS
Race/ethnicity			
Caucasian (%)	16 (45.7)	40 (58.8)	NS
Hispanics (%)	10 (28.6)	16 (23.5)	NS
African Americans (%)	9 (25.7)	12 (17.7)	NS
BMI SDS	2.30 $\pm$ 0.47 [†]	2.04 $\pm$ 0.46	<0.01
FM (kg)	49.7 $\pm$ 18.3 [†]	39.4 $\pm$ 14.9	<0.01
Acanthosis nigricans present (%)	25 (71.4) [*]	38 (55.9)	<0.05
Acne present (%)	16 (45.7)	21 (30.9)	NS
FG score	9.7 $\pm$ 3.6	8.5 $\pm$ 3.1	NS
Amenorrhea (%)	14 (40.0%)	19 (27.9%)	NS
Oligomenorrhea (%)	21 (60%)	49 (72.1%)	NS
Systolic BP	132.4 $\pm$ 16.1 [†]	123.5 $\pm$ 11.3	<0.01
Diastolic BP	72.1 $\pm$ 9.3 [†]	67.1 $\pm$ 8.5	<0.01
Free testosterone (pmol/l)	48.6 $\pm$ 17.7 [†]	38.5 $\pm$ 15.9	<0.01
Fasting glucose (mmol/l)	4.9 $\pm$ 0.5	4.9 $\pm$ 0.4	NS
Fasting insulin (pmol/l)	226.8 $\pm$ 115.2 [†]	158.2 $\pm$ 82.0	<0.001
HOMA-IR (mol μ U/ml)	8.2 $\pm$ 4.1 [†]	5.8 $\pm$ 3.2	<0.01
HOMA- β -cell (%)	638.2 $\pm$ 475.1 [†]	422.5 $\pm$ 348.5	<0.01
HbA1c (%)	5.3 $\pm$ 0.4 [†]	5.1 $\pm$ 0.3	<0.01
hs-CRP (mg/l)	3.8 $\pm$ 1.6 [§]	2.6 $\pm$ 1.2	<0.0001
Triglycerides (mmol/l)	2.3 $\pm$ 1.0 [§]	1.2 $\pm$ 0.6	<0.0001
Cholesterol (mmol/l)	4.9 $\pm$ 1.0 [†]	4.3 $\pm$ 1.0	<0.01
Cholesterol:HDL-C	5.7 $\pm$ 1.4 [§]	4.1 $\pm$ 1.2	<0.0001
Triglycerides:HDL-C	2.7 $\pm$ 1.3 [§]	1.2 $\pm$ 0.8	<0.0001

* $P < 0.05$, [†] $P < 0.01$, [‡] $P < 0.001$, and [§] $P < 0.0001$; not applicable (NA) and not significant (NS).

Table 3 Bivariate correlations between predictor and dependent variables, while controlling for covariates in the entire cohort.

	FT	AN	TG:HDL-C	HOMA-IR	HOMA-%B	hs-CRP	HbA1c
FT (pmol/l)	1.0	0.33 [†]	0.18	0.32 [†]	0.44 [†]	0.36 [†]	0.24*
Acanthosis nigricans		1.0	0.13	0.43 [†]	0.30 [†]	0.41 [†]	0.45 [†]
TG:HDL-C			1.0	0.28 [†]	0.29 [†]	0.54 [†]	0.08
HOMA-IR (mol μ U/ml)				1.0	0.65 [†]	0.42 [†]	0.33 [†]
HOMA- β -cell (%)					1.0	0.41 [†]	0.14
hs-CRP (mg/l)						1.0	0.25*
HbA1c (%)							1.0

* $P < 0.05$, [†] $P < 0.01$.

Correlation analyses were conducted with all the potential predictor variables (HOMA-IR, HOMA-%B, hs-CRP, HbA1c, TG, TG:HDL-C, AN, DHEAS, and Chol:HDL) and the dependent variable (FT). Serum FT was correlated with HOMA-IR, HOMA-%B, hs-CRP, HbA1c, TG:HDL-C, and AN. Correlation analyses were then conducted with these predictor and/or the dependent variables and demographic variables (ethnicity, BMI, FM, and age). Serum FT was related to BMI and FM. BMI and ethnicity were correlated with HOMA-IR, HOMA-%B, hs-CRP, HbA1c, and AN. FM was correlated with HOMA-IR, HOMA-%B, hs-CRP, TG:HDL-C, and AN (data not shown).

Table 3 summarizes the partial correlations for the related predictor variables (HOMA-IR, HOMA-%B, hs-CRP, HbA1c, TG:HDL-C, and AN) and the dependent variable (FT), while controlling for the covariates (ethnicity, BMI, and FM) within the entire cohort. Serum FT remained correlated with AN, HOMA-IR, HOMA-%B, hs-CRP, and HbA1c, but not with TG:HDL-C.

Using a stepwise regression analysis, the covariates were entered first (ethnicity, BMI, and FM), and the predictor variables that were significantly related to the dependent variable (FT) were entered next (AN, HOMA-IR, HOMA-%B, hs-CRP, and HbA1c). BMI accounted for a significant proportion of the variance ($\beta = 0.26$, $t = 2.73$, $P < 0.01$). HOMA-%B and AN were the only predictor variables that emerged with significant main effects for FT ($\beta = 0.48$, $t = 4.82$, $P < 0.01$ and $\beta = 0.21$, $t = 2.09$, $P < 0.01$ respectively).

Discussion

In our study, subjects with hyperandrogenemic phenotypes displayed the greatest degree of hyperinsulinemia, β -cell function, and chronic inflammation, whereas females with HS but no evident HA had mildest degrees of androgen elevation, hyperinsulinemia, insulin resistance, and inflammation. Females with only HA seemed to have intermediate degrees of HA and metabolic dysfunction. The three PCOS phenotypes displayed varying degrees of metabolic dysfunction.

The rise in serum androgens is accompanied by excess insulin secretion, suggesting that insulin directly

stimulates ovarian androgen production as previously shown (29). The degree of hyperinsulinemia does not seem to be directly correlated with the development of HS. The observed difference in the degree of HS (FG score) between hirsute females with and without HA implies that higher androgen and insulin levels stimulate hair growth (30, 31). However, higher activity of androgen receptors has been associated with preferential expression of androgen receptors, implying that genetic and epigenetic changes may be involved in the pathogenesis of PCOS (32). In this cohort, degree of hyperinsulinemia and insulin resistance determined the severity of the phenotype with HS only demonstrating lesser β -cell function and insulin resistance than hyperandrogenemic phenotypes (4). While there was no significant difference in body adiposity among the groups, hyperandrogenemic groups had higher prevalence of acne and AN than hirsute group without excess androgens. The frequencies of oligomenorrhea and amenorrhea among three phenotypes were similar to those previously reported in women and adolescents (6, 33). Although higher prevalence of amenorrhea has been associated with a greater degree of metabolic dysfunction (34), this is likely due to increased body adiposity, and not insulin resistance, across all phenotypes (35, 36). In our cohort, frequency of amenorrhea was not higher in PCOS+MS groups compared with PCOS only group, despite higher insulin resistance and androgen levels in the former. Indeed, biochemical HA did not correlate consistently with this menstrual disorder, and is likely due to variable tissue-specific end-organ sensitivity to androgens (32).

HA has been identified as an important risk factor for MS and dyslipidemias in premenopausal women and adolescents (5, 6, 37). According to the HOMA, 77.4% of our subjects were insulin resistant which is higher than observed by Valerio *et al.* (41.2%) in obese children and adolescents (38); yet, only one-third of our cohort met diagnostic criteria for MS (28) compared with 53% of a group of obese adolescents with PCOS (39). It has been suggested that the metabolic impairment is not evident to the same degree in all PCOS phenotypes in adolescent females (6). While obesity is the most common cause of insulin resistance and dyslipidemias

(40), the metabolic differences cannot be explained by the degree of obesity alone. In our study, HA was found to be an independent risk factor for MS as previously reported by Coviello *et al.* (41).

It has been reported that HA is an important risk factor for the development of dyslipidemia, especially low HDL-C and high cholesterol and TG levels (6, 42). Treatment of PCOS women with antiandrogen flutamide reduced total and LDL-C and increased HDL-C (43). While our study found no relationship between FT and lipid profile indices after correcting for ethnicity, BMI, and FM, the positive relationship between FT and hs-CRP implies that androgens contribute to low-intensity inflammatory state and development of MS (44). The degree of obesity and threshold in the degree of low-intensity inflammation, as measured by hs-CRP, appear to set the ground for the development of dyslipidemias and atherosclerosis (22, 45). In our study, about one-third of the subjects displayed evidence of chronic inflammation (46) with almost 75% of subjects with MS at high risk of atherosclerosis and 71.4% at risk of both diabetes and atherosclerosis. These findings suggest that obesity and HA, and not insulin resistance, are the major determinants of chronic inflammation and risk of atherosclerosis in adolescents with PCOS (47). The use of an inflammation marker may help identify high-risk females with PCOS.

Limitations to this study include retrospective design, relative under-representation of African American and Hispanic subjects, and lack of oral glucose tolerance data to assess glucose homeostasis and β -cell function in relationship with androgen levels and lipid profile. Also, the accuracy of BIA for the assessment of body composition has been questioned because of larger errors in individual estimates of body fat compared with dual-energy x-ray absorptiometry (DXA) method (44). However, BIA has been deemed accurate for assessing body composition in large groups of normal-weight or obese pediatric subjects compared with DXA (20). Another limitation to the study is that there were no age- and sex-matched normal-weight and obese controls for each racial/ethnic group. Inclusion of adolescent females without PCOS may have yielded stronger results evaluating the degrees of metabolic dysfunction in relationship with androgen levels when subgroups of the sample were analyzed.

In conclusion, hyperandrogenemic PCOS phenotypes have greatest degree of hyperinsulinemia, insulin resistance, and inflammation. The screening evaluation of insulin resistance and inflammation markers may identify hyperandrogenemic PCOS patients at risk of developing diabetes and atherosclerosis.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

Acknowledgements

Presented at 91st Annual Meeting of the Endocrine Society, Washington, DC, June 9–13, 2009.

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Received 31 March 2010

Accepted 5 April 2010