



Adrenal hyperandrogenism does not deteriorate insulin resistance and lipid profile in women with PCOS

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Abstract

Objective: The aim of this study was to investigate the impact of adrenal hyperandrogenism on insulin resistance and lipid profile in women with polycystic ovary syndrome (PCOS).

Patients and methods: We studied 372 women with PCOS according to the NIH criteria. 232 age- and BMI-matched women served as controls in order to define adrenal hyperandrogenism (DHEA-S >95th percentile). Then, patients with PCOS were classified into two groups: with adrenal hyperandrogenism (PCOS-AH, $n=108$) and without adrenal hyperandrogenism (PCOS-NAH, $n=264$). Anthropometric measurements were recorded. Fasting plasma glucose, insulin, lipid profile, sex hormone-binding globulin (SHBG) and androgen (TT, $\Delta 4A$, DHEA-S) concentrations were assessed. Free androgen index (FAI) and homeostatic model assessment-insulin resistance (HOMA-IR) index were calculated.

Results: Women with PCOS-AH were younger than PCOS-NAH ($P<0.001$), but did not differ in the degree and type of obesity. No differences were found in HOMA-IR, total cholesterol, HDL-c, LDL-c and triglyceride concentrations (in all comparisons, $P>0.05$). These metabolic parameters did not differ between the two groups even after correction for age. Women with PCOS-AH had lower SHBG (29.2 ± 13.8 vs 32.4 ± 11.8 nmol/L, $P=0.025$) and higher TT (1.0 ± 0.2 vs 0.8 ± 0.4 ng/mL, $P=0.05$) and $\Delta 4A$ (3.9 ± 1.2 vs 3.4 ± 1.0 ng/mL, $P=0.007$) concentrations, as well as FAI (14.1 ± 8.0 vs 10.2 ± 5.0 , $P<0.001$). These results were confirmed by a multiple regression analysis model in which adrenal hyperandrogenism was negatively associated with age ($P<0.001$) and SHBG concentrations ($P=0.02$), but not with any metabolic parameter.

Conclusions: Women with PCOS and adrenal hyperandrogenism do not exhibit any deterioration in insulin resistance and lipid profile despite the higher degree of total androgens.

Key Words

- ▶ PCOS
- ▶ adrenal
- ▶ DHEA-S
- ▶ lipids
- ▶ insulin resistance

Endocrine Connections
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Introduction

Polycystic ovary syndrome (PCOS) is characterized by chronic anovulation and hyperandrogenism (1). It is often accompanied by insulin resistance (2) and abnormal

lipid profile (3, 4). Several studies have shown that insulin resistance is positively associated with the degree of hyperandrogenism (5, 6) and this was confirmed by a



meta-analysis including 32 observational studies and 9556 women with PCOS in total. The higher the levels of androgens, the higher the degree of insulin resistance (7). Androgens are mainly of ovarian origin but in 30% of the patients, they originate from the adrenal glands (8), the main representative being dehydroepiandrosterone sulfate (DHEA-S). The effect of this specific (mainly adrenal) androgen on the metabolic profile of women with PCOS has not yet been clarified.

The presence of adrenal androgen excess, expressed by elevated DHEA-S concentrations, has been postulated to contribute to a favorable metabolic (9, 10, 11) and cardiovascular profile (12, 13) in women with PCOS, as well as in the general population (14, 15). However, scientific evidence is controversial, as other studies suggest a negative impact of adrenal hyperandrogenism on metabolic disturbances, including insulin resistance and hypertension in both PCOS (16) and in middle-aged individuals (17). Therefore, the exact influence of adrenal androgens on the metabolic aspects of PCOS remains inconclusive and requires further investigation.

The aim of this study was to investigate the impact of adrenal hyperandrogenism on insulin resistance and lipid profile in women with PCOS and its correlation with clinical and laboratory metabolic parameters.

Subjects and methods

Patients and controls

The study recruited 372 women with PCOS and 232 healthy controls. Patients with PCOS were selected from the outpatient clinics of two endocrine centers (Hellenic Red Cross Hospital and 'Sismanoglio-Amalia Fleming' Hospital). The enrolled control population consisted of medical or dietology students or hospitals' personnel. All participants provided written informed consent. Institutional Review Boards of the Hellenic Red Cross Hospital and 'Sismanoglio-Amalia Fleming' Hospital approved research procedures, while clinical investigations have been conducted according to the principles of the Declaration of Helsinki.

The National Institutes of Health (NIH) diagnostic criteria for PCOS were used, determined as the presence of less than eight menses per year and a free androgen index (FAI) greater than 5 and/or clinical hyperandrogenism (presence of acne and/or hirsutism) (18). Other causes of anovulation and hyperandrogenism were excluded. The control subjects had no history of menstrual irregularities

and no clinical evidence of hyperandrogenism. Diabetes, hypertension, dyslipidemia and any other medical or psychiatric illness were excluded both in patients and controls. The use of oral contraceptives, anti-androgens or metformin was not reported for at least three months prior to the study.

Control group served as a means to define adrenal hyperandrogenism. The threshold of the 95th percentile of DHEA-S concentrations (334 µg/dL) in women with regular menstruation and no clinical hyperandrogenism was applied. According to this, patients with PCOS were divided into two groups: group A ($n=108$) with adrenal hyperandrogenism (PCOS-AH) and group B ($n=264$) without adrenal hyperandrogenism (PCOS-NAH).

Study protocol

Medical history of patients and controls was obtained and physical examination was performed by endocrinologists. Anthropometric measurements including weight, height and waist circumference (WC) were recorded. Body mass index (BMI) was calculated by the formula: (weight in kg)/(height in m²). Clinical hyperandrogenism was assessed by the presence of acne and/or hirsutism (modified Ferriman–Gallwey score >8). Morning blood samples were drawn from all participants, after an overnight fast. Plasma glucose, insulin, total testosterone (TT), $\Delta 4$ -androstenedione ($\Delta 4A$), dehydroepiandrosterone sulfate (DHEA-S) and sex hormone-binding globulin (SHBG) were assessed in the early follicular phase of the menstrual cycle. Homeostatic model assessment-insulin resistance (HOMA-IR) index was calculated by using the mathematic model: $HOMA-IR = \text{glucose} \times \text{insulin} / 405$ (glucose in mg/dL) for the evaluation of insulin resistance (19). FAI was calculated by the formula: $FAI = 100 \times TT \times 3.467 / SHBG$ (TT in ng/mL).

Assays

Assays were performed as previously described (20). Glucose concentrations were measured in plasma by an enzymatic, colorimetric method in a Cobas Integra/400/700/800 autoanalyzer (Roche Laboratory Systems). Insulin concentrations were measured in serum by an immunoradiometric assay (IRMA, DIASource Immunoassays S.A.) with a sensitivity of 1 µIU/mL and intra- and interassay coefficients of variation of 2.1% and 6.5%, respectively. Lipid concentrations were measured by automatic biochemical analyzers. SHBG concentrations



were measured in serum by an immunoradiometric assay (IRMA, Immunotech s.r.o.) with a sensitivity of 0.4 nmol/L and intra- and interassay coefficients of variation of 6.1 and 8.3%, respectively. TT concentrations were measured by radioimmunoassay (RIA, Cisbio Bioassays) with a sensitivity of 0.086 ng/mL and intra- and interassay coefficients of variation of 6% and 8.5%, respectively. Δ 4A concentrations were measured by radioimmunoassay (RIA, DIASource Immunoassays S.A.) with a sensitivity of 0.03 ng/mL and intra- and interassay coefficients of variation of 4.5% and 9%, respectively. DHEA-S concentrations were measured by radioimmunoassay (RIA, Immunotech s.r.o.) with a sensitivity of 2.64 μ g/dL and intra- and interassay coefficients of variation of 4.93% and 9.32%, respectively.

Statistical analysis

The study was powered to detect a 1.0 difference in HOMA-IR index, given a series of assumptions (HOMA-IR in PCOS-AH group: 4.0 ± 2.5 ; HOMA-IR in PCOS-NAH group: 3.0 ± 2.5 ; α error probability: 0.05, β error probability: 0.05 (power: 0.95), allocation ratio: 1/2 (PCOS-AH/PCOS-NAH)). According to these assumptions, 368 women had to be recruited (PCOS-AH: $n=123$; PCOS-NAH: $n=245$). Study power calculations were performed using the G*Power, version 3.1.9.2 (Heinrich Heine University, Dusseldorf, Germany).

Distribution of continuous parameters was tested by the Kolmogorov–Smirnov Test. Results are presented as

absolute numbers (percentage) for categorical variables, while as mean \pm standard deviation (s.d.) for continuous variables. Differences in categorical variables between patients and controls were tested using χ^2 test with Yates Correction. Differences in continuous variables between patients and controls were tested using the non-parametric Mann–Whitney U test. Univariate Analysis of Variance was used to correct for age (age set as covariate). A multiple regression analysis model was used to evaluate the relationship between adrenal hyperandrogenism (DHEA-S/TT ratio being the dependent variable) and metabolic parameters. A P value of <0.05 was considered statistically significant. All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS 16.0, Inc).

Results

The metabolic and hormonal characteristics of two groups of women with PCOS are shown in Table 1. Women with PCOS-AH were younger than PCOS-NAH ($P<0.001$), but did not differ in the degree and type of obesity (assessed by BMI and WC, $P>0.05$). No differences were found in HOMA-IR (3.4 ± 2.3 vs 3.0 ± 1.7), total cholesterol (183 ± 37 vs 185 ± 34 mg/dL), HDL-c (51.3 ± 13.0 vs 53.8 ± 14.0 mg/dL), LDL-c (112 ± 35 vs 111 ± 32 mg/dL) and triglyceride (83.1 ± 35.0 vs 87.3 ± 49.0 mg/dL) concentrations (in all comparisons, $P>0.05$). Women with PCOS-AH had lower SHBG (29.2 ± 13.8 vs 32.4 ± 11.8 nmol/L, $P=0.025$) and

Table 1 Metabolic and hormonal characteristics of two groups of women with PCOS.

Characteristics	PCOS-AH ($n=108$)	PCOS-NAH ($n=264$)	P value	P value after correction for age
Age (years)	23.5 ± 4.9	26.3 ± 6.3	<0.001	–
BMI (kg/m^2)	27.9 ± 6.8	27.8 ± 7.1	0.831	0.322
WC (cm)	88.2 ± 12.8	87.0 ± 12.0	0.576	0.239
Glu (mg/dL)	83.1 ± 7.7	83.1 ± 7.8	0.948	0.718
Ins ($\mu\text{IU}/\text{mL}$)	16.5 ± 5.9	14.6 ± 4.1	0.117	0.133
HOMA-IR	3.4 ± 2.3	3.0 ± 1.7	0.104	0.113
Total cholesterol (mg/dL)	183.0 ± 37.0	185.0 ± 34.0	0.752	0.795
HDL cholesterol (mg/dL)	51.3 ± 13.0	53.8 ± 14.0	0.319	0.388
LDL cholesterol (mg/dL)	112.0 ± 35.0	111.0 ± 32.0	0.889	0.807
Triglycerides (mg/dL)	83.1 ± 35.0	87.3 ± 49.0	0.592	0.541
TT (ng/mL)	1.0 ± 0.2	0.8 ± 0.4	0.052	0.196
DHEA-S ($\mu\text{g}/\text{dL}$)	449.5 ± 90.0	222.3 ± 67.4	<0.001	<0.001
Δ 4A (ng/mL)	3.9 ± 1.2	3.4 ± 1.0	0.007	0.099
SHBG (nmol/L)	29.2 ± 13.8	32.4 ± 11.8	0.025	0.021
FAI	14.1 ± 8.0	10.2 ± 5.0	<0.001	<0.001

BMI, body mass index; Δ 4A, Δ 4-androstenedione; DHEA-S, dehydroepiandrosterone sulfate; FAI, Free Androgen Index; HDL, high density lipoprotein; HOMA-IR, homeostatic model assessment-insulin resistance; LDL, low density lipoprotein; PCOS, polycystic ovary syndrome; PCOS-AH, polycystic ovary syndrome with adrenal hyperandrogenism; PCOS-NAH, polycystic ovary syndrome without adrenal hyperandrogenism; SHBG, sex hormone-binding globulin; TT, total testosterone; WC, waist circumference.

FAI = $100 \times \text{TT} \times 3.467/\text{SHBG}$ (TT in ng/mL); HOMA-IR = $\text{glucose} \times \text{insulin}/405$ (glucose in mg/dL).

Table 2 Multiple regression analysis model for women with PCOS and dependent variable the DHEA-S/TT ratio.

Independent variables	Beta coefficient	P value*
Age	−0.320	<0.001
BMI	0.211	0.214
WC	−0.143	0.392
HOMA-IR	−0.178	0.075
Total cholesterol	−0.221	0.272
HDL cholesterol	0.008	0.943
LDL cholesterol	0.186	0.343
Triglycerides	−0.035	0.726
SHBG	−0.242	0.010

*The same pattern was observed, when DHEA-S was set as the dependent variable.

BMI, body mass index; DHEA-S, dehydroepiandrosterone-sulfate; HDL, high density lipoprotein; HOMA-IR, homeostatic model assessment-insulin resistance; LDL, low density lipoprotein; PCOS, polycystic ovary syndrome; SHBG, sex hormone-binding globulin; TT, total testosterone; WC, waist circumference.
HOMA-IR, glucose × insulin/405 (glucose in mg/dL).

higher TT (0.96 ± 0.2 vs 0.8 ± 0.4 ng/mL, $P=0.05$) and $\Delta 4A$ (3.9 ± 1.2 vs 3.4 ± 1.0 ng/mL, $P=0.007$) concentrations, as well as FAI (14.1 ± 8.0 vs 10.2 ± 5.0 , $P<0.001$). As serum DHEA-S concentrations are heavily influenced by age in humans, we corrected the aforementioned comparisons for age and the P value after this correction is presented in Table 1. The metabolic parameters did not differ between the two groups even after correction for age.

A multiple regression analysis followed, setting the DHEA-S/TT ratio as the dependent variable. We used this ratio in order for adrenal hyperandrogenism to be also adjusted for the ovarian hyperandrogenism. Age ($P<0.001$) and SHBG concentrations ($P=0.02$) were demonstrated to be negative predictors of adrenal hyperandrogenism. No association with any metabolic parameters was concluded from the multiple regression model (Table 2). The same pattern was observed, when DHEA-S was set as the dependent variable.

Discussion

This study provided evidence that women with PCOS and adrenal hyperandrogenism do not exhibit any additional deterioration of insulin resistance and lipid profile compared with women without adrenal hyperandrogenism.

Androgen excess, a cardinal feature of PCOS, has been experimentally incriminated as a potential developmental contributor to syndrome pathogenesis during fetal life (20, 21), but also as an independent aggravating factor of

the metabolic disturbances during adolescent and adult life (3, 4, 5, 6, 7, 8, 22). The co-existence of elevated adrenal and ovarian androgen production shown in the present study is in accordance with previous studies (11, 12, 13) and may mirror the participation of the adrenal steroidogenesis to the total circulating steroid pool (23).

In the present study, when a cohort of women with PCOS was classified into two subgroups according to DHEA-S concentrations, no significant difference was observed between them in insulin resistance index and lipid profile, despite the higher concentrations of TT, $\Delta 4A$ and FAI in the group with elevated DHEA-S concentrations. As serum DHEA-S concentrations are heavily influenced by age in humans, the aforementioned comparisons were corrected for age. Insulin resistance and lipid profile did not differ between the two groups even after this correction.

These data may demonstrate an independent effect of adrenal androgens that prevent further exacerbation of metabolic abnormalities in women with PCOS and high androgen concentrations. Previous studies have suggested that there is a beneficial impact of adrenal androgens on the metabolic phenotype in women with PCOS. Elevated DHEA-S concentrations have been inversely correlated with insulin resistance, estimated by HOMA-IR, in a PCOS cohort of over 350 women; this relationship was stronger than that of free testosterone or SHBG in the multivariate analysis (9). Furthermore, increased DHEA-S concentrations have been associated with a favorable lipid profile along with improved insulin sensitivity (assessed by quantitative insulin sensitivity check index – QUICKI) in a group of women with PCOS and hyperandrogenemia when compared to similar age and body weight patients with normal adrenal androgens (10). Another study including 318 untreated consecutive women with PCOS from Taiwan resulted again in inverse correlation of DHEA-S levels with the WC, waist-to-hip ratio, BMI, insulin resistance, LDL and triglycerides levels (11). Of great importance, DHEA-S concentrations were shown to be inversely correlated with the carotid intimal media thickness in earlier studies, suggesting not only favorable metabolic effects but also cardioprotective ones for endogenous DHEA-S in women with PCOS (12, 13).

The underlying pathogenic mechanisms of these findings remain unclear, but may reflect a direct effect of DHEA-S on insulin (24) and lipid metabolism (25). Dehydroepiandrosterone (DHEA) concentrations have been positively correlated to insulin binding activity



(24), suggesting a direct impact on insulin physiology. On the other hand, insulin was experimentally reported to enhance DHEA-S production through a direct effect on the adrenal gland itself (26). Interestingly, in the present study, a negative correlation between adrenal hyperandrogenism and SHBG concentrations was demonstrated. Given that low SHBG concentrations are associated with insulin resistance (27), the elevated DHEA-S concentrations could reflect an adaptive mechanism to this metabolic disturbance, implying further complexity in the interplay between hormones and energy homeostasis (28). Furthermore, the negative correlation between adrenal androgens with age demonstrated in the present study is in accordance with previous findings of an age-related reduction in DHEA-S concentrations in the general population (29) as well as in women with PCOS (30).

The strengths of the study include the use of NIH criteria, as well as the well-defined population of Caucasian women only with similar socio-economic status. A limitation of the study could be the sample size from an epidemiological point of view. However, it is one of the largest in this research topic.

In conclusion, this study provided evidence that the presence of adrenal hyperandrogenism, defined by elevations in DHEA-S concentrations, may constitute a factor that prevents further deterioration of metabolic profile (insulin resistance, lipid abnormalities) in women with PCOS. It remains to be clarified whether higher DHEA-S concentrations are an adaptive mechanism to insulin resistance or they exert a protective role on the metabolic profile of women with PCOS.

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.

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