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

Stavroula A Paschou1, Eleni Palioura1, Dimitrios Ioannidis2, Panagiotsis Anagnostis3, Argyro Panagiotakou4, Vasiliki Loi1, Georgios Karageorgos2, Dimitrios G Gouulis3 and Andromachi Vryonidou1

1Department of Endocrinology and Diabetes, Hellenic Red Cross Hospital, Athens, Greece
2Department of Endocrinology and Diabetes, Sismanoglio-Amalia Fleming Hospital, Athens, Greece
3Unit of Reproductive Endocrinology, First Department of Obstetrics and Gynecology, Medical School, Aristotle University of Thessaloniki, Thessaloniki, Greece

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, Δ4A, 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 Δ4A (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.

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...
Adrenal hyperandrogenism and PCOS

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 acne and/or hirsutism (modified Ferriman–Gallwey score >8). 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), Δ4-androstenedione (Δ4A), 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=glucose×insulin/405 (glucose in mg/dL) for the evaluation of insulin resistance (19). FAI was calculated by the formula: FAI=100×TT×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 inter assay coefficients of variation of 2.1% and 6.5%, respectively. Lipid concentrations were measured by automatic biochemical analyzers. SHBG concentrations 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 m^2). Clinical hyperandrogenism was assessed by the presence of acne and/or hirsutism (modified Ferriman–Gallwey score >8). 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).
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. Δ4A 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 μg/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, Düsseldorf, 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 ± standard deviation (S.D.) for continuous variables. Differences in categorical variables between patients and controls were tested using χ² 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 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/m²)     | 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 (μU/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 (μg/dL)  | 449.5±90.0      | 223.2±67.4      | <0.001  | <0.001                          |
| Δ4A (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; Δ4A, Δ4-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 × TT × 3.467/SHBG (TT in ng/mL); HOMA-IR = glucose × 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.

higher TT (0.96 ± 0.2 vs 0.8 ± 0.4 ng/mL, P = 0.05) and Δ4A (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, Δ4A 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 (30). 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.

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

References
1 Conway G, Dewailly D, Diamanti-Kandarakis E, Escobar-Morreale HF, Franks S, Gambineri A, Kelestimur F, Macut D, Micic D, Pasquali R, et al. The polycystic ovary syndrome: a position statement from the European Society of Endocrinology. European Journal of Endocrinology 2014 171 P1–P29. (doi:10.1530/EJE-14-0253)
2 Diamanti-Kandarakis E & Dunaif A. Insulin resistance and the polycystic ovary syndrome revisited: an update on mechanisms and implications. Endocrine Reviews 2012 33 981–1030. (doi:10.1210/er.2011-1034)
3 Wild RA. Dyslipidemia in PCOS. Steroids 2012 77 295–299. (doi:10.1016/j.steroids.2011.12.002)
4 Vyonidou A, Paschou SA, Muscojigi G, Orlo F & Goulias DG. Mechanisms in endocrinology: metabolic syndrome through the female life cycle. European Journal of Endocrinology 2015 173 R153–R163. (doi:10.1530/EJE-15-0275)
5 Goverde AJ, van Koert AJ, Eijkemans MJ, Knauff EA, Westerveld HE, Fauser BC & Broekmans FJ. Indicators for metabolic disturbances in anovulatory women with polycystic ovary syndrome diagnosed according to the Rotterdam consensus criteria. Human Reproduction 2009 24 710–717. (doi:10.1093/humrep/den433)
6 Castelo-Branco C, Steinvercel F, Ossorio A, Ros C & Balasch J. Atherogenic metabolic profile in PCOS patients: role of obesity and hyperandrogenism. Gynecological Endocrinology 2010 26 736–742. (doi:10.3109/09513590.2010.481025)
7 Yang R, Yang S, Li R, Liu P, Qiao J & Zhang Y. Effects of hyperandrogenism on metabolic abnormalities in patients with polycystic ovary syndrome: a meta-analysis. Reproductive Biology and Endocrinology 2016 14 67. (doi:10.1186/s12958-016-0203-8)
8 Yildiz BO & Azziz R. The adrenal and polycystic ovary syndrome. Reviews in Endocrine and Metabolic Disorders 2007 8 331–342. (doi:10.1007/s11154-007-9054-0)
9 Brennan K, Huang A & Azziz R. Dehydroepiandrosterone sulfate and insulin resistance in patients with polycystic ovary syndrome. Fertility and Sterility 2009 91 1848–1852. (doi:10.1016/j.fertnstert.2008.02.101)
10 Carmina E & Loboa RA. Prevalence and metabolic characteristics of adrenal androgen excess in hyperandrogenic women with different phenotypes. Journal of Endocrinological Investigation 2007 30 111–116. (doi:10.1007/s00125-007-9474-0)
11 Chen MJ, Chen CJ, Yang JH, Chen CL, Ho HN, Yang WS & Yang YS. High serum dehydroepiandrosterone sulfate is associated with phenotypic acne and a reduced risk of abdominal obesity in women with polycystic ovary syndrome. Human Reproduction 2011 26 227–234. (doi:10.1093/humrep/deq308)
12 Vryonidou A, Papaiothodorou A, Tavridou A, Terzi T, Loi V, Vatalas IA, Batakis N, Phenekos C & Dionysiou-Asteriou A. Association of hyperandrogenemic and metabolic phenotype with carotid intima-media thickness in young women with polycystic ovary syndrome. Journal of Clinical Endocrinology and Metabolism 2005 90 2740–2746. (doi:10.1210/jc.2004-2363)
13 Meyer C, McGrath BP, Cameron J, Kotsopoulos D & Teede HJ. Vascular dysfunction and metabolic parameters in polycystic ovary syndrome. Journal of Clinical Endocrinology and Metabolism 2005 90 4630–4635. (doi:10.1210/jc.2004-1487)
14 Brahimaj A, Muka T, Kavousi M, Laven JS, Dehghan A & Franco OH. Serum dehydroepiandrosterone levels are associated with lower risk of type 2 diabetes: the Rotterdam Study. Diabetologia 2017 60 98–106. (doi:10.1007/s00125-016-4136-8)
15 Manni T, Vigue J & Rossier MF. In vivo and in vitro evidences of dehydroepiandrosterone protective role on the cardiovascular system. International Journal of Endocrinology and Metabolism 2015 13 24660.
16 Alpañés M, Luque-Ramírez M, Martínez-García MÁ, Fernández-Durán E, Álvarez-Blasco F & Escobar-Morreale HF. Influence of adrenal hyperandrogenism on the clinical and metabolic phenotype of women with polycystic ovary syndrome. Fertility and Sterility 2015 103 795–801.
17 Schunkert H, Hense HW, Andus T, Riegger GA & Straub RH. Relation between dehydroepiandrosterone sulfate and blood pressure levels in a population-based sample. American Journal of Hypertension 1999 12 1140–1143.
18 Zawadski JK & Dunaif A. Diagnostic criteria for polycystic ovary syndrome: towards a rational approach. In Polycystic Ovary Syndrome, pp 377–384. Eds A Dunaif, JR Givens, FP Haseltine & GR Merriam. Boston, MA, USA: Blackwell Scientific Publications, 1992.
Received in final form 8 September 2017
Accepted 14 September 2017
Accepted Preprint published online 14 September 2017