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Elevated coagulation and inflammatory markers in adolescents with a history of premature adrenarche

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Abstract

Females with a history of premature adrenarche are at high risk of developing polycystic ovary syndrome (PCOS) and features of the metabolic syndrome later in life. Coagulation disorders, subclinical inflammation, and oxidative stress have been reported in patients with PCOS and metabolic syndrome. These factors were studied in a group of adolescents with a history of premature adrenarche. This is a cross-sectional study that determined the biochemical-hormonal profile and indices of inflammation, coagulation, and oxidative stress in 45 adolescent girls with a history of premature adrenarche and 19 age- and body mass index–matched controls. Girls with premature adrenarche had hyperandrogenism and higher indices of insulin resistance than controls. They also had significantly higher C-reactive protein (0.76 ± 0.65 vs 0.41 ± 0.31 mg/L, $P = .0001$) and plasminogen activator inhibitor 1 (37.6 ± 24.7 vs 24.47 ± 4.6 ng/mL, $P = .034$), and lower tissue plasminogen activator values in comparison with controls (3.5 ± 1.5 vs 5.2 ± 2.12 ng/mL, $P = .0019$). Both C-reactive protein($r = 0.545$, $P = .0001$) and plasminogen activator inhibitor 1 ($r = 0.36$, $P = .04$) were positively correlated with oxidative stress, whereas tissue plasminogen activator was positively correlated ($r = 0.37$, $P = .02$) with total antioxidant status. None of these factors was correlated with androgens or indices of insulin resistance. Adolescent girls with a history of premature adrenarche display metabolic deviations usually encountered in subjects with PCOS and metabolic syndrome, such as subclinical inflammation and fibrinolytic abnormalities.

1. Introduction

Premature adrenarche (PA) denotes earlier maturation of the zona reticularis of the adrenal cortex. Premature adrenarche is clinically characterized by earlier appearance of pubic hair (pubarche) and is biochemically detected from elevated adrenal androgen concentrations. Although PA was originally thought to represent a benign chronologic deviation of a normal component of the pubertal process, accumulating evidence suggests that PA should be considered as a prodromal stage of the developmental sequela of polycystic ovary syndrome (PCOS) [1].

Indeed, most girls with a history of PA develop later in life features of PCOS, such as hyperandrogenism, insulin resistance, and anovulatory menstrual cycles [2]. The connection between PCOS and metabolic syndrome is direct and based on strong evidence [3-5]. Nevertheless, the recent finding by Coviello et al [6] who reported that teenagers with PCOS developed a significantly higher proportion of metabolic syndrome components in comparison with their healthy peers is alarming.

The search for metabolic pathways linking PCOS and metabolic syndrome has revealed the pivotal role of insulin resistance, subclinical inflammation, and oxidative stress in the development of these disorders [7-14]. In addition, aberrations of the blood coagulation system are usually found in patients with either of these syndromes [15-18]. Because PA most likely represents a prodromal stage of

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PCOS and/or metabolic syndrome [19], we propose that studies in adolescents with PA might disclose primary, rather than secondary, pathogenic factors, leading to the developmental process of these metabolic entities.

Therefore, in the present study, we investigated concurrent alterations in oxidative stress, hormonal, metabolic, inflammatory, coagulation, and endothelial dysfunction markers in a group of 45 girls with a history of PA; and these findings were compared with data from 19 age- and body mass index (BMI)–matched controls.

2. Subjects and methods

Sixty-four adolescent girls of Greek origin were recruited from the Endocrinology Unit of the First Department of Pediatrics, Athens University Medical School, and enrolled in the study: 45 girls had a history of PA, and 19 were healthy controls. The girls with a history of PA initially presented to the endocrine clinic for early growth of pubic hair without breast enlargement. They represented sequential cases with no selection related to the density of pubic hair. None of the girls had signs of virilization or other underlying diseases; and none was taking hormonal medications, including oral contraceptives. Growth records were thoroughly reviewed for each studied subject, and only 1 girl in the PA group had a history of intrauterine growth retardation. The control group included healthy girls with normally timed adrenarche and puberty, matched for age and BMI. Family history for diabetes, PCOS, metabolic syndrome, dyslipidemia, and cardiovascular disease was obtained for each studied subject.

In each subject, height, weight, and waist-to-hip ratio were assessed. Body mass index was calculated as the weight in kilograms divided by the square of height in meters. The BMI standard deviation score (SDS) was calculated using recent data from healthy Greek children [20]. Pubertal development (breasts and pubic hair) was assessed according to the Tanner and Whitehouse [21] criteria. The presence of excess body and facial hair was assessed by the use of the Ferriman-Gallwey [22] index. Each of the subjects studied was clinically assessed by the same author (SL), and all subjects have been under our care.

2.1. Ethics

The purpose of the study was explained to all subjects before participation. The study was performed in accordance with the Helsinki Declaration of 1964 (as amended in 1983 and 1989) and approved by the Ethics Committee of the "Aghia Sophia" Children's Hospital, Athens. Informed consent was obtained from a parent, and assent was obtained from each underage subject.

2.2. Biochemical and hormonal evaluation

Blood samples were obtained in the morning (8:00 AM-9:00 AM) after an overnight fasting by venipuncture; serum and plasma (citrate) were separated by centrifugation within 1 hour and stored at −70°C until analyzed. In menstruating girls, blood samples were obtained between the fourth and eighth day of the menstrual cycle. Blood chemistry, including determinations of serum total cholesterol, high-density lipoprotein (HDL), triglycerides, and glucose, was performed using the Bayer ADVIA 1650 clinical chemistry analyzer (Bayer, Tarrytown, NY). Cholesterol bound to low-density lipoprotein (LDL) was estimated by the Friedewald equation. Internal quality control of the lipids was carried out according to the laboratory manual of the Lipid Research Clinics Program. Serum insulin was measured by radioimmunoassay (RIA) (Sorin Biomedica, Saluggia, Italy). The sensitivity of the assay was 2.5 μU/mL; and the intra- and interassay coefficients of variation (CVs) were 7.5% and 8.9%, respectively.

Luteinizing hormone (LH) and follicle-stimulating hormone (FSH) were determined by chemiluminescence using the Chiron (Emeryville, CA) diagnostics kit. For the LH and FSH assay, intra- and interassay CVs were 4.2% and 5.1%, and 3.8% and 4.9%, respectively. Estradiol was measured by RIA using commercially available reagents from Immunometrics (London, UK); intra- and interassay CVs were 4.2% and 7%, respectively. Cortisol was measured by chemiluminescence (Bayer ACS 180); and the intra- and interassay CVs were 5.5% and 6.05%, respectively. Dehydroepiandrosterone sulfate (DHEAS) and androstenedione (Δ4) were measured by RIA (Diagnostic Systems Laboratories, Webster, TX). For the DHEAS and Δ4 assay, intra- and interassay CVs were, respectively, 3.03% and 5.06%, and 3.96% and 4.54%. 17OH progesterone (17OHP) was determined by RIA (ICN Pharmaceuticals, Costa Mesa CA); and intra- and interassay CVs were 9.1% and 13.6%, respectively. Testosterone and sex hormone–binding globulin (SHBG) were measured using a chemiluminescent enzyme immunometric assay (IMMULITE; DPC Systems, Los Angeles, CA). The intra- and interassay CVs were 5.5% and 6.3%, and 4.1 and 5.1%, respectively. Free androgen index (FAI) was calculated by the following formula: FAI = [testosterone (in nanomoles per liter)/SHBG (in nanomoles per liter)] × 100.

High-sensitivity C-reactive protein (CRP) assay was performed by fully mechanized latex-particle–enhanced immunonephelometric assays on the BN ProSpec nephelometer (Dade Behring, Liederbach, Germany). The intra- and interassay CVs were 4.2% and 5.25%, respectively. Enzyme-linked immunosorbent assay was used to determine plasma concentrations of von Willebrand factor (vWF), tissue plasminogen activator (t-PA) antigen, and plasminogen activator inhibitor 1 (PAI-1) (Asserachrom; Diagnostica Stago, Asnières, France). The intra- and interassay CVs were 3.1% and 3.5%, 4.5% and 5%, and 5.48% and 6.5% for vWF, t-PA, and PAI-1, respectively. Interleukin 6 (IL-6), tumor necrosis factor α (TNF-α), and adhesion molecules vascular cell adhesion molecule–1 and intercellular cell adhesion molecule–1 were also measured by a high-sensitivity enzyme-linked immunoassay (R&D Systems Europe, Abingdon, United Kingdom). The intra- and interassay CVs were 6.9% and 7.4%, 5.9% and 10.6%, 2.3% and 5.5%,

and 4.8% and 4.6%, respectively. Endothelin 1 was measured by chemiluminescence by commercially available reagents from Biomedica Gruppe (Vienna, Austria). The intra- and interassay CVs were 4.5% and 4.4%, respectively.

Oxidative stress was assessed by measuring the total lipid peroxides in plasma. Serum total peroxide concentrations were determined photometrically by the serum peroxide concentration assay (Immunodiagnostik, Bensheim, Germany), which is based on the reaction of a peroxidase with peroxides in the sample using tetramethylbenzidine as a chromogen substrate (450-nm wavelength). The intra- and interassay CVs were 3.1% and 5.1%, respectively; the detection limit was 7 mmol/L.

Serum total antioxidant status (TAS) was measured using a colorimetric test system (ImAnOxassay, Immunodiagnostik). This photometric test, which reflects the sum of all antioxidant components, is based on the reaction of antioxidants in the sample with a defined amount of exogenously provided H_2O_2. The antioxidants in the sample eliminate a certain amount of the provided H_2O_2; and the residual H_2O_2 is determined colorimetrically by an enzymatic reaction, which involves the conversion of tetramethylbenzidine to a colored product measured at 450 nm. The intraassay CV was 1.6%, the interassay CV was 2.0%, and the detection limit was 130 mmol/L [23].

2.2.1. Insulin resistance indices

In this study, several insulin resistance indices were estimated. Specifically, homeostasis model assessment for insulin resistance (HOMA-IR) was calculated using the following formula: fasting plasma glucose (in millimoles per liter) × fasting serum insulin (in microunits per milliliter)/22.5. The G/I ratio was calculated by dividing fasting glucose (in milligrams per deciliter) with insulin (in microunits per milliliter); this marker has been validated as a useful marker of insulin resistance in girls with PA [24,25]. Furthermore, from oral glucose tolerance test (OGTT) data, insulinogenic index (IGI) was calculated using the formula δ insulin (30 − 0 minute) in microunits per milliliter divided by δ glucose (30 − 0 minute) in milligrams per deciliter; and Matsuda index was calculated using the formula $10000/\sqrt{(G \times I) \times (MG \times MI)}$, where G and I denote fasting glucose and insulin values, whereas MG and MI denote mean glucose and insulin values during OGTT, respectively.

2.3. Statistics

All continuous variables showed normal distribution as it was documented by the use of the Kolmogorov-Smirnov test. Data are presented as means and standard deviations/standard errors. The 2-tailed unpaired t test was used to evaluate the differences in normally distributed variables between the 2 groups; and the Bonferroni correction, for post hoc comparisons. The Pearson correlation coefficient was applied to assess the correlation between the variables. Linear regression analysis was applied to assess factors potentially affecting CRP, PAI-1, and t-PA values; and analysis of covariance, especially for BMI SDS, was applied to assess the significance of differences between the study groups. Statistical analysis was performed using the statistical package SPSS (Chicago, IL) version 13.00. Statistical significance was set at 95%.

3. Results

The 2 groups did not differ when considering family history for diabetes, PCOS, dyslipidemia, metabolic syndrome, and cardiovascular disease. Considering anthropometric characteristics, the 2 did not differ in chronologic age (13.1 ± 3.4 vs 12.8 ± 4.5 years), BMI values (22.6 ± 4 vs 21.6 ± 3.5 kg/m^2), BMI SDS (1.1 ± 1 vs 1.1 ± 0.9), waist-to-hip ratio (0.8 ± 0.1 vs 0.82 ± 0.5), and pubertal status (breast, 4 ± 1.2 vs 3.8 ± 1; pubic hair, 4.1 ± 0.7 vs 3.5 ± 0.8). Considering pubertal status distribution, in 11 (25%) girls with PA, the breast stage ranged from I to III according to the Tanner and Whitehouse criteria; and in the remaining 34 (75%), it ranged from IV to V, whereas 26 (57%) girls were postmenarcheal. Analogous distribution was noticed in the control group (30%, 70%, and 45%, respectively). However, PA girls had a higher Ferriman-Gallwey index (9.7 ± 2.2 vs 4.2 ± 1.5, $P < .04$).

Considering features of PCOS and/or metabolic syndrome in PA group, 14 (31%) and 10 (22%) girls had developed characteristics of PCOS and metabolic syndrome, respectively, whereas 6 (13%) girls had developed characteristics of both PCOS and metabolic syndrome. On the other hand, none of the subjects in the control group had characteristics of PCOS and/or metabolic syndrome.

Biochemical and hormonal results are shown in Table 1. In brief, the levels of lipids, gonadotropins, estradiol, and cortisol were comparable in the 2 groups, whereas androgen levels were significantly higher in PA girls than in controls. In addition, PA girls were more insulin resistant than controls as indicated by lower G/I ratio (4.6 ± 0.8 vs 11.9 ± 2.65, $P < .0001$) and IGI (1.87 ± 1.29 vs 3.2 ± 1.4, $P < .0001$), and higher HOMA-IR (3.7 ± 1.7 vs 1.5 ± 0.6, $P < .0001$) and Insulin Sensitivity Index (2.76 ± 0.65 vs 1.8 ± 0.4, $P < .0001$) after adjustment for BMI SDS.

The CRP values were significantly higher in PA girls than in controls (0.76 ± 0.65 vs 0.41 ± 0.31 mg/L, $P < .0001$) and were related to BMI SDS (r = 0.397, P = .007). After adjustment for BMI SDS, CRP values remained significantly higher in the PA group. In addition, PA girls showed defects in blood coagulation, as they had significantly higher PAI-1 (37.6 ± 24.7 vs 24.47 ± 4.6 ng/mL, $P < .03$) and lower t-PA (3.5 ± 1.5 vs 5.2 ± 2.12 ng/mL, $P < .002$) after adjustment for BMI SDS (Fig. 1).

A positive correlation was found in the total group between CRP ($r = 0.545$, $P = .0001$) and PAI-1 ($r = 0.36$, $P = .04$) values with oxidative stress. On the other hand, t-PA was positively correlated with TAS (r = 0.37, P = .02). These correlations were also found in the 2 subgroups studied

Table 1
Pertinent biochemical data in girls with PA and in controls (mean ± SD)

	PA (n = 45)	Controls (n = 19)	*P*
Glucose (mmol/L)	4.6 ± 0.3	4.7 ± 0.4	.53
Insulin (pmol/L)	129 ± 57	50.9 ± 20	.0001
G/I ratio	4.6 ± 0.8	11.9 ± 2.65	.0001
HOMA-IR	3.7 ± 1.7	1.5 ± 0.66	.0001
IGI	1.87 ± 1.29	3.2 ± 1.4	.0001
ISI (Matsuda index)	2.76 ± 0.65	1.8 ± 0.4	.0001
Testosterone (nmol/L)	1.9 ± 0.9	1 ± 0.6	.041
SHBG (nmol/L)	37 ± 17	68 ± 26	.038
FAI	5.1 ± 1.3	1.4 ± 0.7	.04
Δ4 (pmol/L)	54.1 ± 28	27.5 ± 10	.031
DHEAS (μmol/L)	6.5 ± 3	3.7 ± 0.6	.021
17OHP (nmol/L)	2.6 ± 1.4	1.3 ± 0.8	.024
CRP (mg/L)	0.76 ± 0.65	0.41 ± 0.31	.0001
PAI-1 (ng/mL)	37.6 ± 24.7	24.47 ± 4.6	.034
t-PA (ng/mL)	3.5 ± 1.5	5.2 ± 2.12	.0019
vWF (%)	34 ± 13	42.1 ± 10.8	.28
Cholesterol (mmol/L)	4.3 ± 0.4	4 ± 0.5	.27
Triglycerides (mmol/L)	0.68 ± 0.29	0.65 ± 0.21	.56
HDL (mmol/L)	1.3 ± 0.2	1.5 ± 0.5	.32
LDL (mmol/L)	2.6 ± 0.4	2.5 ± 0.3	.41
LH (IU/L)	5.8 ± 10.5	6.9 ± 8.2	.52
FSH (IU/L)	5.3 ± 2.8	4.1 ± 3.2	.49
E_2 (pmol/L)	161.8 ± 108	154 ± 69	.6
Cortisol (nmol/L)	337 ± 160	282 ± 124	.51
IL-6 (pg/mL)	1.21 ± 0.48	0.92 ± 0.35	.18
TNF-α (pg/mL)	2.3 ± 1.2	1.3 ± 0.23	.24
E_1 (fmol/mL)	0.83 ± 0.36	0.75 ± 0.27	.56
VCAM-1 (ng/mL)	639.9 ± 1531	584.7 ± 1021	.23
ICAM-1 (ng/mL)	164.6 ± 45	102.3 ± 58	.45
TOS (μmol/L)	327 ± 132	317 ± 147	.51
TAS (μmol/L)	273 ± 51	258 ± 84	.32

ISI indicates Insulin Sensitivity Index; E_2, estradiol; E_1, estrone; VCAM-1, vascular cell adhesion molecule 1; ICAM-1, intercellular cell adhesion molecule 1; TOS, total oxidative stress.

separately. No correlation was observed between CRP, PAI-1, and t-PA with any of insulin resistance indices or circulating androgen levels.

4. Discussion

In the present study, we found significantly higher CRP levels and fibrinolytic aberrations in adolescents with a history of PA. This study was conducted based on the fact that PA represents a prodromal stage in the development of PCOS and metabolic syndrome [1,2]. Hence, recognition of early markers in adolescence might reveal primary pathogenetic alterations predictive of latter development of PCOS and metabolic syndrome.

Based on this idea, several studies have been carried out in subjects with PA. However, excluding the universal finding of hyperandrogenism, the available data reported are controversial. Specifically, considering lipids, studies from Ibanez et al [26] and Guven et al [27] have reported an unfavorable lipid profile in subjects with a history of PA, although this finding was not confirmed by other investigators [24,28]. In our study, we have not found any differences in cholesterol, triglycerides, HDL, and LDL concentrations between PA and controls.

Insulin resistance has been detected in subjects with a history of PA in studies from Spain, United States, and Switzerland [26,29,30], whereas it was not found in studies from France and Lithuania [28,31]. In our study, we have found significantly higher insulin resistance in the PA group based on indices obtained from basal and OGTT data. The discrepancies described above are showing the heterogeneity of girls with a history of PA and may be attributed to genetic, constitutional, and environmental differences or to differences in the selection of the subjects.

Two recent and very important observations in PA girls that have not been thoroughly studied yet are subclinical inflammation and coagulation disorders. Subclinical inflammation has been investigated only by Ibanez et al [32] who

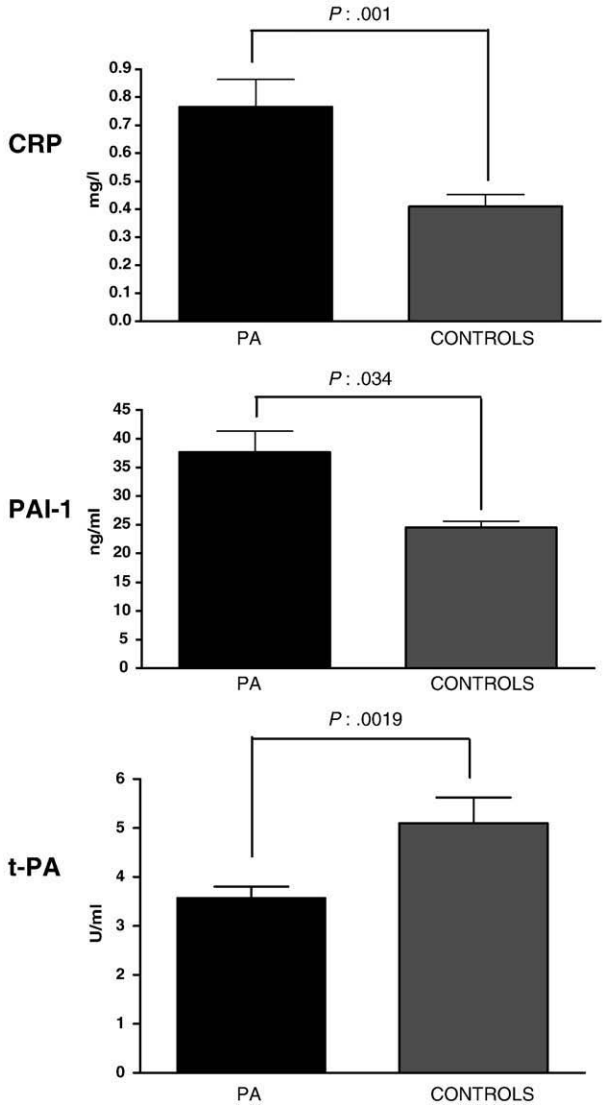

Fig. 1. Concentrations of CRP, PAI-1, and t-PA in adolescents with PA and in controls.

reported increased values of CRP, TNF-α, and IL-6 values in PA girls. However, in the present study, only CRP values were significantly higher in girls with a history of PA in comparison with controls, whereas TNF-α and IL-6 values were not significantly different between the 2 groups, despite a trend toward higher values of both cytokines in PA girls. This difference could be attributed to ethnicity or to the fact that, in the present study, the girls were younger than the aforementioned one.

Large epidemiologic studies have proven that CRP is considered as a prognostic factor of the latter development of metabolic syndrome in adult population [7,10]. In addition, CRP values are elevated in nonobese young women with PCOS, as subclinical inflammation is considered to be one of the key players in the evolution of the syndrome [33-35]. The fact that elevated CRP values were detected in our PA group is interesting and demands further analysis. The well-known link between adiposity and CRP production [36] has to be ruled out in our case because CRP values remained significantly higher in the PA group after adjustment for BMI SDS. Another possible mechanism leading to higher CRP concentrations could be insulin resistance, as several studies have disclosed reciprocal changes of insulin resistance and CRP during various therapeutic interventions in adolescents with a history of PA or in women with PCOS [37,38]. However, in our PA subjects, CRP values were not related to insulin resistance indices.

In contrast, at the present study, CRP values were significantly related to oxidative stress, a finding not previously reported in PA girls. In the last few years, a great amount of data emerged showing the integration of oxidative stress in the pathogenesis of PCOS. Data from in vitro studies have demonstrated that the ovarian steroidogenic enzymes responsible for androgen production were stimulated by oxidative stress [39], whereas in vivo studies reported that oxidative stress could induce insulin resistance and hyperandrogenemia in women with PCOS [12,13]. Moreover, women with PCOS have increased generation of reactive oxygen species after glucose loading in comparison with controls [40]. Finally, Gonzalez et al [14] showed that oxidative stress induced an inflammatory state in PCOS women, which contributed to the development of insulin resistance and hyperandrogenemia. Kelishadi et al [41] have detected in 512 healthy children a significant positive association between CRP and oxidative stress markers. Based on this observation, the authors concluded that oxidative stress and CRP may interact in the early inflammatory processes of atherosclerosis in healthy adolescents. The fact that oxidative stress correlates with CRP in both PA subjects and controls, and given that PA is a prelude of PCOS, indicates that oxidative stress might have a role in the pathogenesis of morbidities related to PCOS at later life.

Another interesting finding in the present study was the coagulation disorders of PA girls, as they had significantly higher PAI-1 and lower t-PA values than controls. The available data considering aberrations in fibrinolysis in PA girls are limited [42], although it has been reported that, in this particular group, PAI-1 concentrations before menarche are higher and they are related to postmenarcheal development of insulin resistance [43]. Because PAI-1 production is known to be up-regulated by insulin, triglycerides, cortisol, and TNF-α [44-46], we analyzed these factors in an attempt to explain our findings. However, in our group, this did not seem to be the case of PAI-1 elevation because no correlation was documented between PAI-1 concentrations and any of the above-mentioned factors. On the other hand, PAI-1 levels were strongly related to oxidative stress, suggesting an important connection between oxidative stress and PAI-1 production. These results are supported by the findings of Vulin and Stanley [47] who demonstrated that PAI-1 promoter is activated by oxidative stress. In addition to PAI-1, we also found that t-PA values were lower in our group of PA girls than in controls and that t-PA concentrations were correlated positively with TAS. These observations might be without importance, given that the same correlations were observed in controls. However, it is the first time that such an association is observed in subjects with a history of PA; therefore, it might be useful in the understanding of mechanisms regulating the evolution from PA to PCOS and/or metabolic syndrome.

In conclusion, adolescent girls with a history of PA display metabolic deviations usually encountered in subjects with PCOS and metabolic syndrome, such as subclinical inflammation and fibrinolytic abnormalities.

References

[1] Dacou-Voutetakis C, Livadas S, Voutetakis A, et al. Adrenarche, premature. In: Martini L, editor. Encyclopedia of endocrine diseases, vol 1. Elsevier Inc.; 2004. p. 99-105.
[2] Ibanez L, Dimartino-Nardi J, Potau N, et al. Premature adrenarche—normal variant or forerunner of adult disease? Endocr Rev 2000;21: 671-96.
[3] Talbott EO, Zborowski JV, Rager JR, et al. Evidence for an association between metabolic cardiovascular syndrome and coronary and aortic calcification among women with polycystic ovary syndrome. J Clin Endocrinol Metab 2004;89:5454-61.
[4] Ehrmann DA, Liljenquist DR, Kasza K, et al. Prevalence and predictors of the metabolic syndrome in women with polycystic ovary syndrome. J Clin Endocrinol Metab 2006;91:48-53.
[5] Kousta E, Tolis G, Franks S. Polycystic ovary syndrome. Revised diagnostic criteria and long-term health consequences. Hormones (Athens) 2005;4:133-47.
[6] Coviello AD, Legro RS, Dunaif A. Adolescent girls with polycystic ovary syndrome have an increased risk of the metabolic syndrome associated with increasing androgen levels independent of obesity and insulin resistance. J Clin Endocrinol Metab 2006;91:492-7.
[7] Haffner SM. Insulin resistance, inflammation, and the prediabetic state. Am J Cardiol 2003;92:18-26.
[8] Sinaiko AR, Steinberger J, Moran A, et al. Relation of body mass index and insulin resistance to cardiovascular risk factors, inflammatory factors and oxidative stress during adolescence. Circulation 2005;111: 1985-91.
[9] Stephens JW, Gable DR, Hurel SJ, et al. Increased plasma markers of oxidative stress are associated with coronary heart disease in males with diabetes mellitus and with 10-year risk in a prospective sample of males. Clin Chem 2006;52:446-52.

[10] Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. N Engl J Med 2005;352:1685-95.
[11] Couillard C, Ruel G, Archer WR, et al. Circulating levels of oxidative stress markers and endothelial adhesion molecules in men with abdominal obesity. J Clin Endocrinol Metab 2005;90:6454-9.
[12] Fenkci V, Fenkci S, Yilmazer M, et al. Decreased total antioxidant status and increased oxidative stress in women with polycystic ovary syndrome may contribute to the risk of cardiovascular disease. Fertil Steril 2003;80:123-7.
[13] Sabuncu T, Vural H, Harma M, et al. Oxidative stress in polycystic ovary syndrome and its contribution to the risk of cardiovascular disease. Clin Biochem 2001;34:407-13.
[14] Gonzalez F, Rote NS, Minium J, et al. Reactive oxygen species–induced oxidative stress in the development of insulin resistance and hyperandrogenism in polycystic ovary syndrome. J Clin Endocrinol Metab 2006;91:336-40.
[15] Mertens I, Verrijken A, Michiels JJ, et al. Among inflammation and coagulation markers, PAI-1 is a true component of the metabolic syndrome. Int J Obes (Lond) 2006;30:1308-14.
[16] Glueck CJ, Sieve L, Zhu B, et al. Plasminogen activator inhibitor activity, 4G5G polymorphism of the plasminogen activator inhibitor 1 gene, and first-trimester miscarriage in women with polycystic ovary syndrome. Metabolism 2006;55:345-52.
[17] Glueck CJ, Wang P, Fontaine RN, et al. Plasminogen activator inhibitor activity: an independent risk factor for the high miscarriage rate during pregnancy in women with polycystic ovary syndrome. Metabolism 1999;48:1589-95.
[18] Orio Jr F, Palomba S, Cascella T, et al. Is plasminogen activator inhibitor-1 a cardiovascular risk factor in young women with polycystic ovary syndrome? Reprod Biomed Online 2004;9:505-10.
[19] Auchus RJ, Rainey WE. Adrenarche—physiology, biochemistry and human disease. Clin Endocrinol (Oxf) 2004;60:288-96.
[20] Chiotis D, Krikos X, Tsiftsis G, et al. Body mass index and prevalence of obesity in subjects of Hellenic origin aged 0-18 years, living in the Athens area. Ann Clin Pediatr Univ Atheniensis 2004; 51:139-54.
[21] Tanner JM, Whitehouse RH. Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. Arch Dis Child 1976;51:170-9.
[22] Ferriman D, Gallwey J. Clinical assessment of body hair growth in women. J Clin Endocrinol Metab 1961;21:1440-7.
[23] Schimke I, Muller J, Priem F, et al. Decreased oxidative stress in patients with idiopathic dilated cardiomyopathy one year after immunoglobulin adsorption. J Am Coll Cardiol 2001;38:178-83.
[24] Vuguin P, Saenger P, Dimartino-Nardi J. Fasting glucose insulin ratio: a useful measure of insulin resistance in girls with premature adrenarche. J Clin Endocrinol Metab 2001;86:4618-21.
[25] Silfen ME, Manibo, McMahon DJ, et al. Comparison of simple measures of insulin sensitivity in young girls with premature adrenarche: the fasting glucose to insulin ratio may be a simple and useful measure. J Clin Endocrinol Metab 2001;86:2863-8.
[26] Ibanez L, Potau N, Chacon P, et al. Hyperinsulinaemia, dyslipaemia and cardiovascular risk in girls with a history of premature pubarche. Diabetologia 1998;41:1057-63.
[27] Guven A, Cinaz P, Bideci A. Is premature adrenarche a risk factor for atherogenesis? Pediatr Int 2005;47:20-5.
[28] Meas T, Chevenne D, Thibaud E, et al. Endocrine consequences of premature pubarche in post-pubertal Caucasian girls. Clin Endocrinol (Oxf) 2002;57:101-6.
[29] Dimartino-Nardi J. Premature adrenarche: findings in prepubertal African-American and Caribbean-Hispanic girls. Acta Paediatr Suppl 1999;88:67-72.
[30] l'Allemand D, Schmidt S, Rousson V, et al. Associations between body mass, leptin, IGF-I and circulating adrenal androgens in children with obesity and premature adrenarche. Eur J Endocrinol 2002;146:537-43.
[31] Zukauskaite S, Seibokaite A, Lasas L, et al. Serum hormone levels and anthropometric characteristics in girls with hyperandrogenism. Medicina (Kaunas) 2005;41:305-12.
[32] Ibanez L, Valls C, De Zegher F. Discontinuous low-dose flutamide-metformin plus an oral or a transdermal contraceptive in patients with hyperinsulinaemic hyperandrogenism: normalizing effects on CRP, TNF-alpha and the neutrophil/lymphocyte ratio. Hum Reprod 2006;21: 451-6.
[33] Kravariti M, Naka KK, Kalantaridou SN, et al. Predictors of endothelial dysfunction in young women with polycystic ovary syndrome. J Clin Endocrinol Metab 2005;90:5088-95.
[34] Kelly CC, Lyall H, Petrie JR, et al. Low grade chronic inflammation in women with polycystic ovarian syndrome. J Clin Endocrinol Metab 2001;86:2453-5.
[35] Tarkun I, Arslan BC, Canturk Z, et al. Endothelial dysfunction in young women with polycystic ovary syndrome: relationship with insulin resistance and low-grade chronic inflammation. J Clin Endocrinol Metab 2004;89:5592-6.
[36] Moran A, Steffen LM, Jacobs Jr DR, et al. Relation of C-reactive protein to insulin resistance and cardiovascular risk factors in youth. Diabetes Care 2005;28:1763-8.
[37] Diamanti-Kandarakis E, Paterakis T, Alexandraki K, et al. Indices of low-grade chronic inflammation in polycystic ovary syndrome and the beneficial effect of metformin. Hum Reprod 2006;21:1426-31.
[38] Ibanez L, Valls C, Cabre S, et al. Flutamide-metformin plus ethinylestradiol-drospirenone for lipolysis and antiatherogenesis in young women with ovarian hyperandrogenism: the key role of early, low-dose flutamide. J Clin Endocrinol Metab 2004;89:4716-20.
[39] Kwintkiewicz J, Spaczynski RZ, Foyouzi N, et al. Insulin and oxidative stress modulate proliferation of rat ovarian theca-interstitial cells through diverse signal transduction pathways. Biol Reprod 2006; 74:1034-40.
[40] Dinger Y, Akcay T, Erdem T, et al. DNA damage, DNA susceptibility to oxidation and glutathione level in women with polycystic ovary syndrome. Scand J Clin Lab Invest 2005;65:721-8.
[41] Kelishadi R, Sharifi M, Khosravi A, et al. Relationship between C-reactive protein and atherosclerotic risk factors and oxidative stress markers among young persons 10-18 years old. Clin Chem 2007;53: 456-64.
[42] Andiran N, Yordam N, Kirazli S. Global fibrinolytic capacity is decreased in girls with premature adrenarche: a new pathological finding? J Pediatr Endocrinol Metab 2005;18:1373-81.
[43] Ibanez L, Aulesa C, Potau N, et al. Plasminogen activator inhibitor-1 in girls with precocious pubarche: a premenarcheal marker for polycystic ovary syndrome? Pediatr Res 2002;51:244-8.
[44] Morange PE, Aubert J, Peiretti F, et al. Glucocorticoids and insulin promote plasminogen activator inhibitor 1 production by human adipose tissue. Diabetes 1999;48:890-5.
[45] Mussoni L, Mannucci L, Sirtori M, Camera M, Maderna P, Sironi L, et al. Hypertriglyceridemia and regulation of fibrinolytic activity. Arteriosclerosis and Thrombosis 1992;12:19-27.
[46] Swiatkowska M, Szemraj J, Cierniewski CS. Induction of PAI-1 expression by tumor necrosis factor alpha in endothelial cells is mediated by its responsive element located in the 4G/5G site. FEBS J 2005;272:5821-31.
[47] Vulin AI, Stanley FM. Oxidative stress activates the plasminogen activator inhibitor type 1 (PAI-1) promoter through an AP-1 response element and cooperates with insulin for additive effects on PAI-1 transcription. J Biol Chem 2004;279:25172-8.