

Clinical Research Article

PCOS Features and Steroid Profiles Among Young Adult Women with a History of Premature Adrenarche

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Abbreviations: AGA, appropriate for gestational age; AMH, anti-Müllerian hormone; BMI, body mass index; CV, coefficient of variation; DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulfate; ELISA, enzyme-linked immunosorbent assay; FAI, free androgen index; FGS, Ferriman–Gallwey score; HOMA-IR, homeostasis model assessment for insulin resistance; LC-MS/MS, liquid chromatography–tandem mass spectrometry; PA, premature adrenarche; PCOS, polycystic ovary syndrome; PP, premature pubarche; SDS, standard deviation score; SGA, small for gestational age; SHBG, sex hormone-binding globulin.

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Abstract

Context: Premature adrenarche (PA) may increase the risk for polycystic ovary syndrome (PCOS).

Objective: To study features of PCOS in young adult women with a history of PA.

Methods: Thirty PA and 42 control females were followed from prepuberty to young adulthood (median age 18.1 years). The main outcome measures were ovarian function, the use of contraceptives, and clinical and biochemical indicators of hyperandrogenism.

Results: We found no differences in the use of hormonal contraceptives (50 vs 50%, PA vs controls, respectively; $P > .999$), indication for using contraceptives ($P = .193$), or in the history of oligo- (17 vs 26%, $P = .392$) and amenorrhea (0 vs 0%, $P > .999$). Among women not using hormonal contraceptives, those with a history of PA had a higher prevalence of hirsutism (27 vs 0%, $P = .023$) but not acne (87 vs 67%, $P = .252$). Steroid profiles were broadly comparable between the groups, but PA women had lower sex hormone-binding globulin (SHBG) concentrations (30.1 vs 62.4 nmol/L, $P < .001$) resulting in higher free androgen index (3.94 vs 2.14, $P < .001$). The difference in SHBG levels persisted

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through body mass index adjustment. SHBG correlated negatively with the homeostasis model assessment for insulin resistance ($r = -0.498$, $P = .003$). Anti-Müllerian hormone concentrations were comparable between the groups (39.3 vs 32.1 pmol/L, $P = .619$).

Conclusion: PA was not associated with evident ovarian dysfunction in young adult women. However, women with a history of PA had decreased SHBG levels and thus, increased bioavailability of circulating androgens.

Keywords: Adrenarche, polycystic ovary syndrome, dehydroepiandrosterone sulfate, sex hormone-binding globulin, ovarian function, hyperandrogenism

Adrenarche refers to reactivation of adrenal androgen production in childhood (1, 2). It is considered premature (PA), when there is evidence of increased adrenal androgen production for age, and at least 1 clinical sign of androgen action (pubic or axillary hair, acne, comedones, oily hair, or adult type body odor) is present before the age of 8 and 9 years in girls and boys, respectively. Other causes for androgen excess must be excluded. Premature pubarche (PP) refers to the presence of pubic hair before the above-mentioned ages. PA is the most common etiology for PP.

PA girls who are Tanner 1 for breast development tend to be taller and weigh more than healthy peers (1). By definition, children with PA have increased concentrations of adrenal androgen precursors (1). Later, they have more advanced pubertal development and earlier menarche (3). Risks for development of metabolic syndrome and polycystic ovary syndrome (PCOS) in girls with PA/PP remain controversial (1, 2).

PCOS is an etiologically and clinically heterogeneous syndrome, characterized by clinical and/or biochemical hyperandrogenism, altered ovarian morphology, and ovarian dysfunction (4-7). The Rotterdam criteria and the New International Guidelines (8, 9) define PCOS as the presence of 2 out of these 3 conditions. The most reliable sign of clinical hyperandrogenism is hirsutism (10) and ovarian dysfunction includes poly-, oligo-, and amenorrhea (11). Before PCOS diagnosis, other etiologies of hyperandrogenism and ovarian dysfunction need to be excluded.

The linkage between high levels of insulin and insulin-like growth factor-1, hyperandrogenism, dysmenorrhea, and PCOS is well known (5). Adolescents presenting with some of these features have been considered being at risk for PCOS (5-7), although diagnosing PCOS during adolescence is challenging because features of normal pubertal development overlap with adult PCOS (12, 13). Regardless, it has been postulated that PA is not a separate condition, but rather a phase in a sequence of events beginning with being born small for gestational age (SGA), continuing with

enhanced (early) growth, increased body mass index (BMI) and hyperinsulinemia in childhood, and hyperandrogenism, PCOS, and metabolic syndrome in adulthood (14, 15). Previous studies have found an increased prevalence of hirsutism, acne, and ovarian dysfunction in PA/PP (16-20) but, so far, ovarian function, clinical signs of hyperandrogenism, and steroid profiles in adult women with prior PA/PP have not been well characterized.

Previously, we have presented the long-term cardiometabolic outcome of PA in a prospective study, following previously characterized PA and age-matched control girls from prepubertal age until early adulthood (21). The same cohort was now examined to study if there are differences in ovarian function, clinical signs of hyperandrogenism or precise steroid profiles between women with a history of PA and their matched controls.

Subjects and Methods

Subjects and Design

In this follow-up study, we investigated 30 women with a history of PA and 42 healthy controls who had been followed from prepubertal stage to young adult age of 18 years (22, 23). All PA girls were originally recruited by clinical signs and they had presented with at least 1 sign of hyperandrogenism (pubic or axillary hair, acne, comedones, oily hair, or adult type body odor) before the age of 8 years. Sixteen (53%) of the PA girls had PP. Among the other 14 PA girls without PP at baseline, 57% presented with adult type body odor, 43% with oily hair, and 21% with acne/comedones. Children with a diagnosed endocrine disorder or long-term medication (including oral/inhaled corticosteroids) were excluded. All PA girls had increased serum dehydroepiandrosterone sulfate (DHEAS) concentration by age, and other sources of excessive androgen production (central puberty, congenital adrenal hyperplasia, external androgen exposure, androgen producing tumors) had been excluded. Most of the PA girls ($n = 25$, 83%) had serum DHEAS concentration $>1 \mu\text{mol/L}$ and in the other

5 PA girls, serum DHEAS concentrations were between 0.5 and 1 $\mu\text{mol/L}$. The control children were age-matched healthy girls without any clinical sign of androgen action, and they were recruited from the general community of the same Kuopio area as the PA girls. At young adult age, none of the PA or control women reported any significant health issues or the use of long-term medications which could have a significant impact on the results. All subjects were examined in the pediatric outpatient clinic of Kuopio University Hospital at the median age of 7.3 years and at a follow-up visit at the median age of 18.1 years. The study protocol was approved by the Research Ethics Committee of the Hospital District of Northern Savo. Informed consents were obtained from all subjects in accordance with the ethical principles stated in the Declaration of Helsinki.

Clinical Assessment

Data at birth (gestational age and birth weight and length) were collected from local electronic birth registers. Clinical examinations at the baseline (P.U.) and follow-up visits (J.L.) were performed by a single physician and a single trained research nurse. Height, as a mean of 3 repeated measurements, was measured with a calibrated Harpenden stadiometer (Holtain Ltd, Crymych, UK) and recorded to the nearest 0.1 cm. Weight was measured after an overnight fast using a calibrated electronic scale and recorded to the nearest 0.1 kg. BMI was calculated as weight in kilograms divided by the square of height in meters. Anthropometric measures at birth and at prepuberty were converted to standard deviation scores (SDSs) according to the recently updated Finnish references (24, 25). For those women who presented with terminal coarse body hair in a male-like pattern, namely in the face, chest, back, or abdomen, we calculated the modified Ferriman–Gallwey score (FGS) and defined hirsutism as FGS ≥ 6 , the cut-off generally used in Nordic countries (26). The presence and degree of acne was evaluated clinically, and divided into 3 categories: 1 no acne, 2 mild (comedones or small number of papules and pustules), and 3 severe (large number of papules and pustules with inflammation, or nodulocystic and conglobate acne with many large nodular or pustular lesions along with many smaller papules, pustules and comedones, and/or earlier postpubertal isotretinoin treatment).

Gynecological Data

At follow-up examination, our aim was to examine all women in the follicular phase of their menstrual cycle (1–14 days since the beginning of the last menstruation). Gynecological information was obtained by direct

structured interviews. Obtained information included menstrual history and the use of contraceptives (including the indication for contraceptives). Menstrual history (before using contraceptives if any) included menstrual cycle, menstrual duration, and history of oligo- and amenorrhea. Menstrual cycles of ≥ 35 days, or ≤ 10 total cycles in a year were recorded as oligomenorrhea. Primary amenorrhea was defined as failure to reach menarche before the age of 16, and absence of previously regular or irregular cycles for more than 3 months was considered secondary amenorrhea.

Biochemical Analyses

The biochemical results of this study are reported only for those women who did not use hormonal contraceptives. All blood samples were collected after an overnight fast between 9:00 and 10:00 AM (baseline) or 7:00 and 8:00 AM (follow-up visit). All serum samples were stored at -80°C until analyzed.

At baseline examination, serum DHEAS and testosterone concentrations were determined using radioimmunoassays (Diagnostic Products, Los Angeles, CA) (27), serum insulin and sex hormone-binding globulin (SHBG) concentrations using specific time-resolved fluoroimmunoassays (PerkinElmer Life and Analytical Sciences, Turku, Finland) (27), and serum anti-Müllerian hormone (AMH) concentration using enzyme-linked immunosorbent assay (ELISA) (Diagnostic Systems Laboratories Inc., Webster, TX) (28).

At the follow-up visit, serum DHEAS and AMH concentrations were analyzed using ELISA with an ELx808 microplate reader (Biotek Instruments Inc., Winooski, VT) and specific kits (DHEAS, cat 1950, Alpha Diagnostic International, San Antonio, TX; AMH, cat A79765, Beckman Coulter Diagnostics, Brea, CA) (29, 30). Sex hormone-binding globulin concentrations were analyzed using electrochemiluminescence immunoassays with Cobas e601 analyzer (Hitachi High Technology CO, Tokyo, Japan) and specific kits (cat 03052001 190, Roche Diagnostics GmbH, Mannheim, Germany) (31). Serum steroids other than DHEAS were measured using liquid chromatography–tandem mass spectrometry (LC-MS/MS) as previously described (32). Fasting plasma glucose was analyzed by the hexokinase method and serum insulin concentrations by an electrochemiluminescence immunoassay (33) using either the Cobas c501 (fasting glucose) or Cobas e601 (fasting insulin) analyzers (Hitachi High Technology CO, Tokyo, Japan).

In the DHEAS assay, the intra-assay coefficient of variation (CV) was 3.2% to 5.3% and the interassay CV was 5.5% to 11%. In the AMH assay, the intra-assay CV was 2.4% to 4.6% and the interassay CV was 4.8% to 8.0%.

The lower limits of detection for DHEAS and AMH assays were 0.25 $\mu\text{mol/L}$ and 1 pmol/L , respectively.

Free androgen index (FAI) was calculated with the following formula: (testosterone [nmol/L]/SHBG [nmol/L]) \times 100. Homeostasis model assessment for insulin resistance (HOMA-IR) was calculated with the following formula: (fasting glucose [mg/dL] \times fasting insulin [mU/L])/405. Insulin resistance was defined as HOMA-IR \geq 2.94 (34).

Statistical Analyses

All statistical analyses were performed with the SPSS 25.0 software (IBM Corp., Armonk, NY). All continuous variables are expressed as median (interquartile range) and were analyzed using the Mann–Whitney U-test. Categorical variables are expressed as n (%). With categorical variables, Pearson chi-squared or the Fischer's exact test was used when assessing differences between groups. When analyzing biochemical concentrations with BMI adjustment, we used 1-way analysis of covariance (after logarithmic transformation in case of non-normally distributed variables). For calculations of correlations, we used the Spearman correlation test. $P < .05$ was considered statistically significant.

Results

First, we analyzed clinical data for all 30 PA and 42 control women in this study. As the use of hormonal contraceptives has a significant impact on many studied variables, we then focused only on those 15 PA and 21 control women who were not using hormonal contraceptives.

Clinical Data of all 30 PA and 42 Control Women

There were no differences in birth measures between the study groups: the mean gestational age 40.1 vs 40.1 weeks ($P = .686$; PA and control women, respectively), mean birth weight SDS -0.17 vs 0.17 ($P = .204$), and mean birth length SDS -0.17 vs 0.25 ($P = .064$). Most women had been born at term (92/90 % of the PA/control females, respectively) and appropriate for gestational age (AGA, 96/98 %).

At presentation, all PA patients and control subjects were Tanner 1 for breast development with the median age of 7.6 years (interquartile range 6.8–8.0) for the PA girls and with the median age of 7.4 (6.8–8.0) for the control girls. At this prepubertal evaluation, the PA girls were taller (mean height SDS 0.92 vs -0.23 , $P < .001$) and had higher mean BMI SDS (0.77 vs 0.10 , $P = .020$) than the controls.

At the follow-up visit, all PA and control women were Tanner 5 for breast development with the median age of 18.1 years (interquartile range 17.8–19.6) for the PA women and with the median age of 18.1 (17.9–18.3) for the control women. PA and control women had comparable adult height ($P = .059$) and BMI ($P = .068$). The data illustrating menstrual history, the use of hormonal contraceptives, and the indications for using hormonal contraceptives are listed in Table 1. There were no statistically significant differences in the proportions of women using hormonal contraceptives or in the indications for the use of contraceptives between the study groups. We found no significant differences in the menstrual history between the study groups.

When we compared only PA women with PP ($n = 16$) to those without PP (non-PP, $n = 14$), we did not find significant differences in the proportions of women using hormonal contraceptives (57 vs 44%, $P = .715$, respectively) or in the indications for the use of contraceptives ($P > .999$).

Clinical Data of the 15 PA and 21 Control Women Who Did Not Use Hormonal Contraceptives

Gestational, prepubertal, and adulthood data of the 15 PA and 21 control women not using hormonal contraceptives are listed in Table 2. There were no differences

Table 1. Menstrual history and the use and indications for the use of hormonal contraceptives in young adult premature adrenarche (PA) and control women (median age 18.1 years)

	PA (n = 30)	Control (n = 42)	P
Menstrual history (before using contraceptives if any)			
Menstrual cycle, days ^a	30 (28–30)	29 (28–30)	.610
Menstrual duration, days ^a	6 (5–7)	5 (5–6)	.093
Oligomenorrhea	5 (17)	11 (26)	.392
Amenorrhea	0 (0)	0 (0)	>.999
Using hormonal contraceptives	15 (50)	21 (50)	>.999
Indications for using hormonal contraceptives ^b			.193
Acne	3 (20)	0 (0)	
Dysmenorrhea	4 (27)	6 (29)	
Irregular menstruation	2 (13)	3 (14)	
Birth control	6 (40)	12 (57)	

Variables are categorical, expressed as n (%), and analyzed with the Pearson chi-squared test unless noted otherwise.

^aContinuous variables, expressed as median (interquartile range) and analyzed using the Mann–Whitney U-test.

^bn = 15 (PA) and n = 21 (controls).

Table 2. Characteristics and clinical data of the premature adrenarche (PA) and control females not using hormonal contraceptives

	PA (n = 15)	Control (n = 21)	P
At birth			
Gestational age, weeks	40.0 (38.2-41.2)	40.1 (39.4-41.4)	.434
Birth length, cm	50.0 (48.8-51.3)	50.0 (50.0-51.5)	.727
Birth length, SD score	0.111 (-0.565-0.812)	0.171 (-0.229-0.695)	.803
Birth weight, g	3550 (3060-3750)	3550 (3290-3970)	.434
Birth weight, SD score	-0.160 (-0.755-0.565)	0.014 (-0.350-0.806)	.342
At prepuberty^a			
Age, years	7.32 (6.58-7.87)	7.32 (6.53-7.83)	.874
Height, cm	130 (125-138)	124 (120-127)	.005
Height, SD score	0.990 (0.536-1.76)	-0.342 (-0.945-0.340)	.001
Weight, kg	33.6 (26.0-37.7)	24.2 (22.2-27.7)	.001
BMI, SD score	0.712 (0.230-2.36)	0.031 (-0.488-0.573)	.021
DHEAS, $\mu\text{mol/L}$	2.05 (1.30-2.70)	0.85 (0.53-1.38)	<.001
Testosterone, nmol/L	0.460 (0.350-0.675)	0.350 (0.350-0.455)	.043
SHBG, nmol/L	66.0 (45.5-91.0)	106.0 (88.3-122.5)	.002
FAI ^b	0.809 (0.433-1.060)	0.340 (0.320-0.490)	<.001
fS-insulin, mU/L	6.10 (5.10-8.30)	3.50 (2.90-4.40)	.001
AMH, pmol/L	16.4 (12.9-28.6)	21.4 (13.2-31.1)	.699
At postpuberty			
Age, years	18.1 (17.7-18.3)	18.1 (17.7-18.3)	.751
Menarcheal age, years	11.5 (11.0-12.0)	13.0 (12.0-13.5)	.001
Height, cm	168 (163-171)	165 (161-167)	.089
Weight, kg	69.3 (59.7-89.4)	58.8 (55.0-66.1)	.004
BMI, kg/m^2	25.0 (22.0-30.8)	21.7 (19.3-25.5)	.083
fS-insulin, mU/L	11.4 (7.20-15.3)	6.90 (5.70-10.2)	.033
fP-glucose, mg/dL	95.5 (90.1-99.1)	91.9 (89.2-94.6)	.125
HOMA-IR ^c	2.74 (1.63-3.74)	1.87 (1.24-2.31)	.030
Insulin resistance ^{d,e}	7 (46.7)	1 (4.8)	.011
Acne ^e	13 (87)	14 (67)	.252
Degree of acne ^e			.262
Mild	13 (62)	14 (79)	
Severe	5 (38)	3 (21)	
Hirsutism ^{e,f}	4 (27)	0 (0)	.023

All variables are expressed as median (interquartile range) and analyzed using the Mann-Whitney U-test, unless noted otherwise. Bold values denote statistically significant values at $P < .05$.

Abbreviations: AMH, anti-müllerian hormone; PA, premature adrenarche; BMI, body mass index; DHEAS, dehydroepiandrosterone sulfate; SHBG, sex hormone-binding globulin; FAI, free androgen index; fP, fasting plasma; fS, fasting serum; HOMA-IR, homeostasis model assessment for insulin resistance.

^aTanner 1 for breast development;

^bFAI calculated as $100 \times (\text{testosterone (nmol/L)}/\text{SHBG (nmol/L)})$;

^cHOMA-IR calculated with the formula of $(\text{fasting glucose [mg/dL]} \times \text{fasting insulin [mU/L]})/405$;

^ddefined as HOMA-IR >2.94 ;

^ecategorical variables are expressed as n (%) and analyzed with the Fisher exact test;

^fmodified Ferriman-Gallwey score ≥ 6 .

in gestational age or birth size between the study groups. Only 1 PA woman was born preterm (29 weeks) and SGA (birth weight -2.12 SDS) while all other women in the PA and control groups were born full term and AGA. At presentation, the PA girls were taller and had higher BMI SDS. By the prepubertal examination, 9 of the 15 PA girls (60 %) had pubarche. The PA girls achieved menarche earlier than the controls (11.5 vs 13.0 years, $P = .001$).

At young adult age, median BMI values of the PA and control women not using contraceptives were comparable ($P = .083$). The PA women had higher prevalence of hirsutism compared to the controls (4 vs 0, $P = .023$), but there was no difference in the prevalence or severity of acne between the groups. Among the 4 women with hirsutism, modified FGs were 6, 10, 13, and 18. Altogether 20 (55.6%) women who did not use hormonal contraceptives

were in the follicular phase of their menstrual cycle at the time of examination (10 women in both groups, 66.7% in the PA group vs 47.6% in the control group, $P = .296$).

When analyzing only women with a history of PA and not using hormonal contraceptives, there was no significant difference between the PP ($n = 9$) and non-PP ($n = 6$) subgroups in the prevalence of hirsutism (2 women in both subgroups; 22 vs 33%, $P > .999$, respectively) or acne (9 vs 4 women; 100 vs 67%, $P = .273$, respectively).

Biochemical Data of the 15 PA and 21 Control Women Who Did Not Use Hormonal Contraceptives

At baseline, prepubertal PA girls had increased serum testosterone, DHEAS, and insulin concentrations, lower SHBG concentrations, and higher calculated FAI than controls. Prepubertal AMH concentrations were similar between the study groups (Table 2).

Table 3 depicts serum steroid and AMH concentrations and FAI, and Fig. 1 indicates the association between BMI and SHBG, testosterone, and FAI in the PA and control women without hormonal contraceptives at young adult age. While serum DHEAS concentrations did not differ significantly, the PA women had higher dehydroepiandrosterone (DHEA) and androstenedione concentrations than the controls, also when adjusted for BMI ($P = .006$ and 0.032 , respectively). Testosterone levels were similar, but SHBG concentrations were lower in the PA group, resulting in higher FAI in PA women than in controls. The difference in SHBG levels persisted also after BMI adjustment ($P = .004$). Although SHBG concentrations correlated negatively with BMI among all women (PA and control groups combined) without hormonal contraceptives ($r = -0.454$, $P = .005$), this correlation vanished when analyzing only the PA group (Fig. 2). There was also a moderate negative correlation between SHBG and HOMA-IR ($r = -0.498$, $P = .003$) and a positive correlation between BMI and HOMA-IR ($r = 0.486$, $P = .004$) among all women (PA and control groups combined) without hormonal contraceptives, but these correlations disappeared when analyzing the PA and control groups separately (Fig. 2). Otherwise, we found no statistically significant differences in steroid profiles of the 2 groups.

At young adult age, AMH concentrations were comparable between the study groups (Table 3), but the PA women had higher levels of fasting serum insulin ($P = .033$), resulting in increased HOMA-IR ($P = .030$) and a higher prevalence of insulin resistance ($P = .011$). Among the PA women, there were no significant differences in any biochemical parameter between the PP and non-PP subgroups ($P > .220$ for all).

Discussion

In this study, we followed a group of females with and without PA from childhood and investigated possible differences in the prevalence of biochemical and clinical signs of hyperandrogenism and ovulatory disturbances at young adult age. In addition, we measured steroid profiles using the sensitive LC-MS/MS method. We did not find evidence that the history of PA associates with an increased risk of ovulatory dysfunction or acne at young adult age. However, the prevalence of hirsutism was higher among PA women. While the androgen profiles were comparable between the study groups, the PA women had significantly lower SHBG concentrations leading to increased FAI. Surprisingly, lower SHBG concentrations in PA women were not associated with BMI. AMH levels did not differ between the 2 study groups.

Before starting contraceptives, the length of menstrual cycle and the prevalence of oligo- and amenorrhea in the PA women did not differ from those of the controls. There were no differences in the percentage of women using contraceptives or in the indications for contraceptive use in the 2 groups. These results suggest that a history of PA is not associated with an increased incidence of ovulatory dysfunction in early adult age. Our results are similar to previous studies which have found women with prior PA to have normal ovulatory function (19, 20). However, inconsistent results have also been found in some cross-sectional studies. In a study from Spain, late (>3 years) postmenarcheal girls (mean age 16.3 years) with prior PP had more anovulatory cycles compared to controls (16). In another more recent study from Brazil, a high prevalence (41.2%) of diagnosed PCOS among 34 prior PA subjects (age range 15.2-28.2 years) was reported, but this study had no controls (18).

In our current follow-up study on PA and control women, we found an increased prevalence of hirsutism among PA women. This result is consistent with prior studies investigating Spanish (mean age 15.4 years), American (mean age 14.6 years), French (mean age 17.4 years), and Brazilian postpubertal women with prior PP (median age 20.3, range 15.2-28.2 years) (17-20). The prevalence and severity of acne, however, were comparable between the PA and control groups in our current study. Similar results were found in the aforementioned French study (20), whereas others have found increased acne in addition to hirsutism (17-19). The inconsistency between our and previous studies could be explained by confounding factors, including ethnicity and birth size, which may play a substantial role in long term outcomes of PA (35, 36). Also, it may be explained by the differences in phenotypes of the study subjects, as prepubertal PA subjects with PP seem to have clinically and biochemically more advanced phenotype than those with other androgenic signs (37).

Table 3. Steroid profiles and AMH concentrations of the young adult premature adrenarche (PA) and control females without hormonal contraceptives (median age 18.1 years)

	PA (n = 15)	Control (n = 21)	P
DHEA (nmol/L)	59.3 (42.9-70.7)	34.1 (27.8-47.6)	.005
DHEAS (μmol/L) ^a	8.30 (6.30-15.0)	6.40 (4.55-8.90)	.067
Testosterone (nmol/L)	1.36 (0.93-1.99)	1.61 (1.09-1.82)	.849
11K-testosterone (nmol/L)	1.05 (0.678-1.90)	1.25 (1.02-1.91)	.409
11OH-testosterone (nmol/L)	0.340 (0.317-0.692)	0.487 (0.292-0.761)	.634
SHBG (nmol/L) ^a	30.1 (23.5-36.2)	62.4 (49.2-89.4)	<.001
FAI ^b	3.94 (2.68-6.85)	2.14 (1.42-3.22)	<.001
DHT (nmol/L)	0.403 (0.352-0.549)	0.411 (0.359-0.532)	>.999
Androstenedione (nmol/L)	6.99 (5.07-9.63)	6.68 (5.05-8.13)	.374
11K-androstenedione (nmol/L)	0.798 (0.641-1.18)	0.759 (0.644-1.100)	.612
11βOH-androstenedione (nmol/L)	5.71 (4.10-9.73)	5.39 (4.11-7.59)	.590
Androstanedione (nmol/L)	0.566 (0.377-0.752)	0.327 (0.254-0.437)	.025
Progesterone (nmol/L)	0.483 (0.255-9.840)	0.347 (0.268-1.100)	.505
17OH-progesterone (nmol/L)	1.94 (1.34-5.04)	2.16 (1.17-3.09)	.776
21OH-progesterone (nmol/L)	0.159 (0.109-0.303)	0.189 (0.125-0.286)	.680
Pregnenolone (nmol/L)	9.72 (5.24-14.5)	7.20 (5.06-9.86)	.309
17OH-pregnenolone (nmol/L)	17.4 (9.95-28.9)	11.4 (8.68-18.6)	.226
Estrone (pmol/L)	196 (141-421)	226 (145-371)	.899
AMH (pmol/L) ^a	39.3 (27.1-54.3)	32.1 (20.4-57.5)	.619

Measured by liquid chromatography–tandem mass spectrometry, unless noted otherwise. All variables are expressed as median (interquartile range) and analyzed using the Mann–Whitney U-test. Bold values denote statistically significant values at $P < .05$.

Abbreviations: DHEA, dehydroepiandrosterone; K, keto-; OH-, hydroxy-; FAI, free androgen index; AMH, anti-Müllerian hormone.

^aMeasured using enzyme-linked immunosorbent assay (ELISA).

^bcalculated with the following formula: [testosterone (nmol/L)/SHBG (nmol/L)] × 100.

Despite having similar levels of total testosterone and comparable androgen profiles, the PA women had decreased SHBG concentrations leading to higher FAI, which may be 1 factor explaining the increased prevalence of hirsutism. Earlier studies of PP women have yielded similar results (17, 20). Low SHBG and increased FAI are associated with a significantly increased risk for PCOS, type 2 diabetes, insulin resistance and hyperandrogenism (38). Multiple mechanisms by which low SHBG associates with PCOS have been described. These include obesity, and obesity-related dyslipidemia and low-grade inflammation, abnormal glucose metabolism and hyperinsulinemia, hyperandrogenism, hypothyroidism, and SHBG gene polymorphisms (39). In our previous studies with the same cohort, we found no difference in lipid profiles or low-grade inflammation markers between the PA and control groups but did observe a higher prevalence of insulin resistance in the PA group, and the difference was associated with central fat mass (21). Previous studies have found increased insulin levels among prepubertal (37, 40) and postpubertal PA/PP subjects (41). These results, combined with prior knowledge of childhood obesity significantly increasing the risk for obesity in adulthood (42), lead us to suggest that some subjects with former PA may be prone to adulthood metabolic disturbances due to childhood obesity, impaired

glucose metabolism and failure to maintain a healthy diet, hence leading to increased central adiposity and insulin resistance by adulthood (21). In the current study, PA women had lower SHBG levels, which correlated negatively with HOMA-IR but not with BMI. Obesity is the most common reason for insulin resistance, but insulin resistance occurs independently of obesity in PCOS women. The mechanism of obesity-independent insulin resistance in PCOS is not completely understood (43), but hyperinsulinemia associated with insulin resistance has been linked to decreased hepatic nuclear factor 4 alpha expression leading to lower hepatic SHBG gene expression (44). Decreased SHBG levels equal higher levels of free testosterone, which leads to elevated insulin concentrations hence resulting in a vicious circle (39, 45).

Novel data in the present study were obtained by the LC-MS/MS measurements of steroid profiles. There were no differences in testosterone, dihydrotestosterone, or androstenedione concentrations between the PA and control groups. Levels of 11-hydroxytestosterone and 11β-hydroxyandrostenedione, considered well suited for assessing adrenal hyperandrogenism because of their adrenal origin (46), were similar. Concentrations of 11-ketotestosterone, previously suggested to be the main mediator of many androgenic processes (47), were also

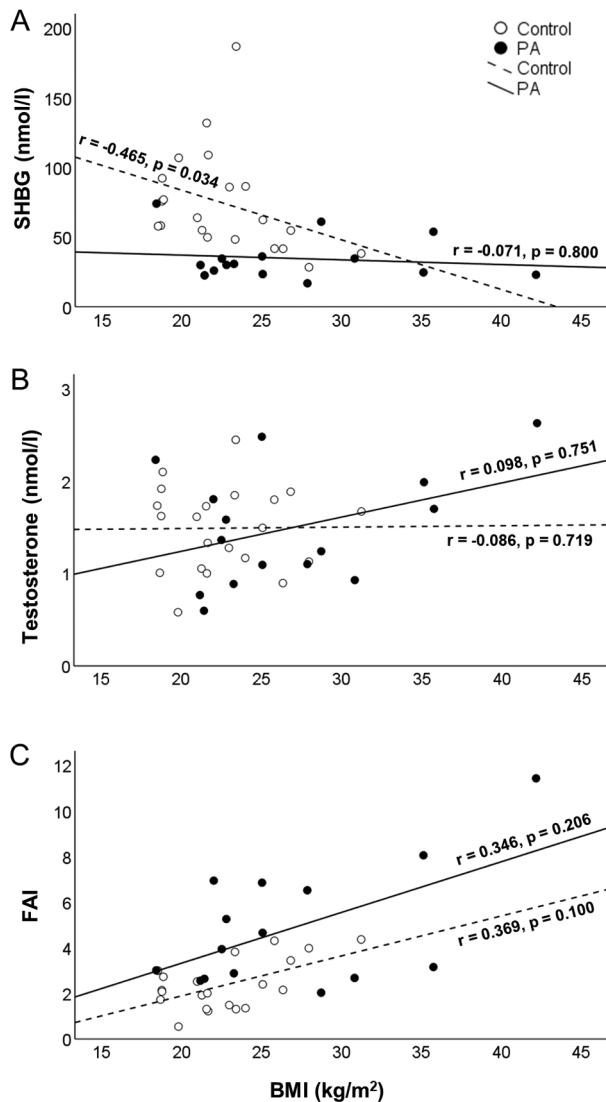


Figure 1. Associations between body mass index (BMI) and A) sex hormone-binding globulin (SHBG), B) testosterone, and C) free androgen index (FAI) in young adult premature adrenarche (PA) and control females (median age 18.1 years) without the use of hormonal contraceptives. Free androgen index (FAI) was calculated with the following formula: (testosterone/SHBG) \times 100. Solid and dotted lines indicate associations of the parameters in the PA and control groups, respectively.

similar. However, PA women had higher concentrations of DHEA, also after adjustment for BMI. Our steroid data suggest that adrenal androgen synthesis in PA girls attenuates during adolescence and that PA does not lead to ovarian hyperandrogenism by young adult age.

AMH levels of the subjects in this study have been measured twice. They were found to be lower in PA than control subjects in prepuberty when we previously analyzed the whole original cohort (28). In the current follow-up study, AMH concentrations in the PA women without hormonal contraceptives were similar to those in the control women without hormonal contraceptives. Previous studies

have shown that adult women with PCOS have typically elevated AMH levels, and AMH levels correlate positively with the antral follicle count, namely the presence of polycystic ovaries at ultrasound. Thus, AMH has been proposed as a substitute for antral follicle count in the diagnosis of PCOS (48). Moreover, AMH has been shown to correlate with the severity of PCOS phenotype (49). On the other hand, the recent International Guidelines for PCOS (9) concluded that serum AMH levels should not yet be used as an alternative for the detection of polycystic ovaries at ultrasound and do not recommend AMH measurement as a single test for the diagnosis of PCOS. In line with this, a previous Finnish study showed that AMH levels associated with PCOS-linked features (such as testosterone levels and oligo-amenorrhea) in adolescence (at age 16) but were not able to predict PCOS-associated features reliably in early adulthood at the age of 26 years (50).

The strength of this study lies in its prospective design, and steroid measuring technique (LC-MS/MS). However, a relatively small sample size, and the lack of maternal gynecological information diminish the strength of the study. We also aimed to perform gynecological ultrasounds for all women, but because of refusals and other study subject related reasons, too few of the women were ultimately examined and the results are thus unreliable and ineligible for presentation. However, according to the newest international guidelines (9), ultrasound should not be used for the diagnosis of PCOS in those with a gynecological age of <8 years (<8 years after menarche), due to the high incidence of multifollicular ovaries at this life stage. The median age of our patients was 18.1 years for both PA women and controls, and for most of them, menarche had occurred less than 8 years ago. Therefore, ovarian ultrasound examination would not be recommendable for the diagnosis of PCOS. Although we detected no differences in the clinical and biochemical parameters between the PP and non-PP subgroups among the adult PA women, it should be noted that the number of subjects in these subgroups was quite low, which hinders meaningful statistical analyses.

In conclusion, our study suggests that PA does not lead to unfavorable ovarian function, at least by early adult age, in PA women born AGA. Although young adult PA women have comparable serum androgen profiles with their peers, they tend to have lower SHBG levels leading to increased bioavailability of circulating free androgens. This may be explained by hyperinsulinemia associated with insulin resistance. Our findings should be confirmed by a larger prospective case-control study, preferably with the assessment of ovarian morphology.

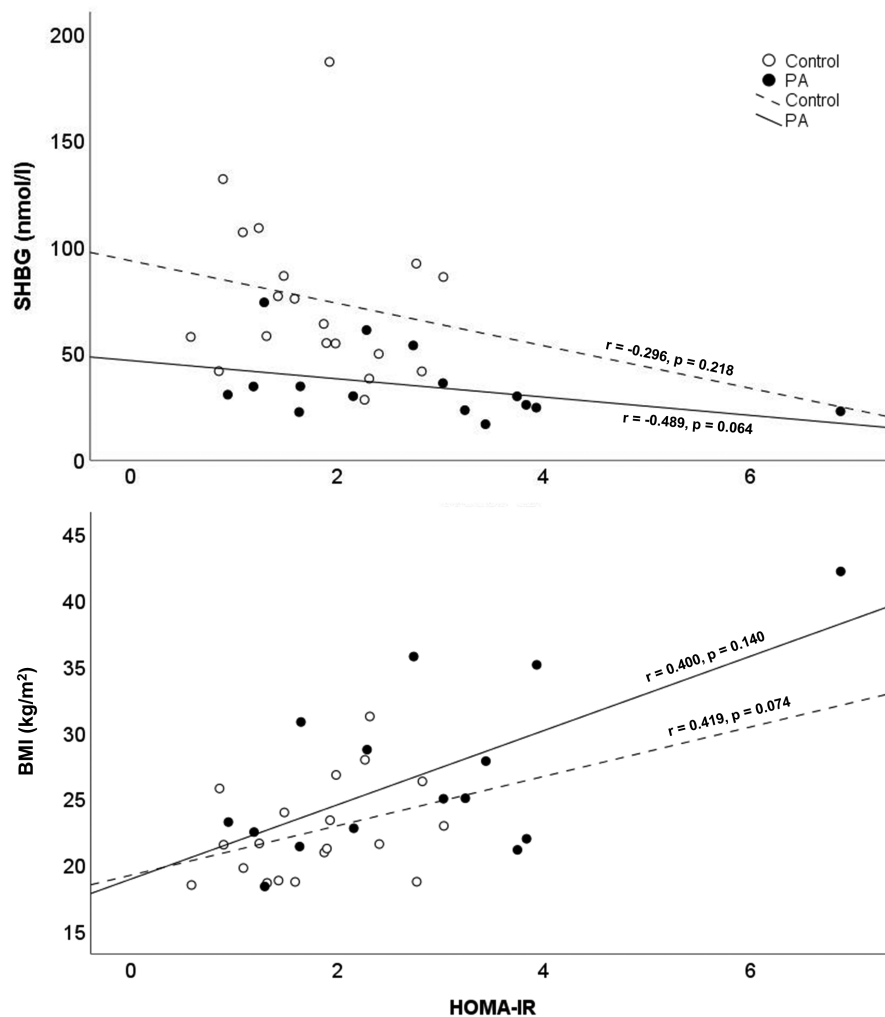


Figure 2. Associations of homeostasis model assessment for insulin resistance (HOMA-IR) with sex hormone-binding globulin (SHBG) and body mass index (BMI) in young adult premature adrenarche (PA) and control females (median age 18.1 years). Solid and dotted lines indicate associations of the parameters in the PA and control groups, respectively.

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