Adrenal Function during Childhood and Puberty in Daughters of Women with Polycystic Ovary Syndrome

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Context: In some patients, PCOS may develop as a consequence of an exaggerated adrenarche during pubertal development.

Objective: The aim of the study was to assess adrenal function during childhood and pubertal development in daughters of women with PCOS (PCOSd).

Design: We included 98 PCOSd [64 during childhood (ages 4–8 yr) and 34 during the peripubertal period (ages 9–13 yr)] and 51 daughters of control women (Cd) [30 during childhood and 21 during the peripubertal period]. In both groups, an acute ACTH-(1–24) stimulation test (0.25 mg) and an oral glucose tolerance test were performed. Bone age and serum concentrations of cortisol, androstenedione, 17-hydroxyprogesterone, dehydroepiandrosterone (DHEA), DHEA sulfate (DHEAS), glucose, and insulin were determined.

Results: PCOSd and Cd were similar in age and body mass index. During the peripubertal period, basal and poststimulated DHEAS concentrations were higher in PCOSd compared to Cd. Among PCOSd, 12.5% of girls in childhood and 32.4% in peripuberty presented biochemical evidence of exaggerated adrenarche. Stimulated insulin was higher in PCOSd compared to Cd during childhood ($P = 0.03$) and peripuberty ($P = 0.03$). An advancement of 8 months between bone and chronological age was observed in peripubertal PCOSd compared to Cd.

Conclusions: In PCOSd, basal and stimulated DHEAS concentrations were higher during the onset of puberty. Around 30% of the PCOSd demonstrated an exacerbated adrenarche, which may reflect increased P450c17 activity. In addition, a modest advance in bone age was observed, probably secondary to the hyperinsulinemia and/or adrenal hyperandrogenism. (***J Clin Endocrinol Metab* 94: 3282–3288, 2009**)

The polycystic ovary syndrome (PCOS) is an extremely common endocrine-metabolic disorder, affecting approximately 5–10% of reproductive-age women. It is characterized by ovulatory/menstrual irregularity, polycystic ovaries, and hyperandrogenism (1–4). Although the ovary is the principal source of androgen excess in most of these patients, 40–70% of them also exhibit elevated levels of adrenal androgens (AAs), particularly dehydroepiandrosterone sulfate (DHEAS) (5–7). In addition, most women with PCOS also demonstrate peripheral insulin resistance, which plays a key role in the pathogenesis of the syndrome (3, 8, 9) and contributing, in part, to the ovarian and/or adrenal hyperandrogenism (10, 11).

Adrenarche is the maturation of the zona reticularis of the adrenal gland, which leads to an increase in AAs to levels usually seen in early puberty (12). Biochemically,

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Abbreviations: A4, Androstenedione; AA, adrenal androgen; BMI, body mass index; Cd, control daughters; DHEA, dehydroepiandrosterone; DHEAS, DHEA sulfate; HOMA-IR, homeostasis model assessment index for insulin resistance; ISI, insulin sensitivity index; OGTT, oral glucose tolerance test; 17-OHP, 17-hydroxyprogesterone; PCOS, polycystic ovary syndrome; PCOSd, PCOS daughters; SDS, SD score; SGA, small for gestational age.

adrenache is characterized by increases in dehydroepiandrosterone (DHEA) and its sulfate, DHEAS (13). The role of adrenarche in human physiology is relatively unknown (13, 14) and the activation of the gonadal axis is not required for adrenal activation. Moreover, although normal gonadarche apparently does not require the presence of the adrenal glands (15), it is possible that adrenarche in some manner acts to modulate the maturation of the reproductive axis (16).

Premature adrenarche is characterized by an early increase in AA production that results in the development of pubic hair before the age of 8 yr in girls with or without axillary hair, and with no other signs of sexual development (12). It has been suggested that premature adrenarche is associated with ovulatory dysfunction and functional ovarian hyperandrogenism (17–19) and an increased risk of developing metabolic abnormalities (20–22). Moreover, premature adrenarche causes a transient acceleration in growth and bone maturation (23–25). Premature adrenarche has also been associated with lower birth scores, compared with control girls of similar gestational ages (26, 27). Ibañez *et al.* (26, 28) have suggested that adolescent girls who are small for gestational age (SGA) demonstrate exaggerated adrenarche, characterized by high levels of DHEAS and hyperinsulinism.

We have reported that PCOS mothers demonstrate significantly higher prevalence of SGA newborns (29), and that a subgroup of peripubertal and pubertal daughters born to PCOS mothers exhibit altered ovarian follicular development (30, 31) and metabolic derangements (32). Moreover, we have observed that hyperinsulinemia and increased ovarian volume are present in daughters of PCOS women before the onset of puberty and persist during pubertal development; alternatively, ovarian hyperandrogenism appears to be present only during late puberty (33). Overall, daughters of PCOS women constitute a group of patients at high risk for the development PCOS.

We have hypothesized that adrenal function is altered in daughters of PCOS women, primarily demonstrating premature or exacerbated adrenarche. To test this hypothesis, we studied 98 daughters of women with PCOS and 51 controls, assessing their response to acute adrenocortical stimulation and an oral glucose tolerance test (OGTT) and determining their bone age and hormonal profile during childhood and pubertal development.

Subjects and Methods

Subjects

We studied 64 girls during childhood (ages 4 to 8 yr) and 34 girls during the peripubertal period (ages 9–13 yr) born to mothers with PCOS [PCOS daughters (PCOSd)]. As a control group, we studied 30 girls during childhood and 21 girls during the peripubertal period born to mothers with regular menses and without hyperandrogenism [control daughters (Cd)]. All PCOSd were born at term from singleton pregnancies. Birth weight was obtained from the medical records. SGA infants were defined as having birth weight below the 5th percentile according to local normative data for newborns (34).

PCOS mothers were recruited from patients attending the Unit of Endocrinology and Reproductive Medicine, University of Chile. This group of PCOS mothers is part of an unselected group of patients that has attended our clinic since they were diagnosed with PCOS. Diagnosis of PCOS was made according to the 1990 National Institutes of Health criteria (2). PCOS mothers were evaluated before pregnancy; they exhibited chronic oligomenorrhea or amenorrhea, hirsutism (modified Ferriman-Gallway score >8) (35), serum total testosterone concentrations above 0.6 ng/ml, and/or free androgen index (total testosterone/SHBG) above 5.0 or androstenedione (A4) concentration above 3.0 ng/ml. Normative data were based on hormonal values of healthy Chilean women, as previously described (36). In addition, PCOS women demonstrated the characteristic polycystic ovarian morphology according to the criteria described by Adams *et al.* (37) on ultrasonography.

All PCOS mothers were normoglycemic, with different degrees of hyperinsulinemia determined by a 75-g 2-h OGTT. All patients also had a waist-to-hip ratio greater than 0.85. We excluded patients with hyperprolactinemia, thyroid dysfunction, androgen-secreting neoplasms, Cushing's syndrome, and 21-hydroxylase deficient non-classic adrenal hyperplasia by clinical and hormonal testing as previously described (38).

As control mothers, we selected women of similar socioeconomic level to that of the PCOS patients and with a history of singleton pregnancies, regular 28- to 32-d menstrual cycles, absence of hirsutism and other manifestations of clinical hyperandrogenism, and no history of infertility or pregnancy complications.

The protocol was approved by the institutional review boards of the San Juan de Dios and San Borja Arriarán hospitals and the University of Chile. All parents signed informed consents before entering the study.

Study protocol

Girls were admitted with their mothers to the pediatric unit of our Clinical Research Center at approximately 0830 h. We performed a complete physical examination in each girl, including the following anthropometric measurements: weight, height, weight and height SD scores (SDS), waist and hip circumferences, waist-to-hip ratio, body mass index (BMI), and BMI SDS calculated by the Growth Analyzer Program using the USA Growth Charts BMI for Age (39). Pubertal development was assessed according to the criteria of Tanner by the same observer (V.P.). Skeletal maturation was determined by the standards of Greulich and Pyle by one observer (A.L.d.G.) (40).

Both groups of girls underwent a standard acute ACTH-(1–24) stimulation test. The test was started between 0800 and 0900 h, an iv bolus of Cortrosyn (0.25 mg; Organon, East Orange, NJ) was administered, and cortisol, 17-hydroxyprogesterone (17-OHP), DHEA, A4, and DHEAS were assessed at baseline and 60 min. Exaggerated adrenarche was defined by a value of serum DHEAS (28, 41) greater than the mean plus 2 SD of the

control group (*i.e.* 64.7 μg/dl for childhood and 93.3 μg/dl for peripuberty). Subsequently, in all children we performed an OGTT (1.75 g/kg, up to a maximum of 75 g glucose in 250 ml water) after a 12-h overnight fast. Serum glucose and insulin were determined in each sample. Postmenarchal girls were studied during the early follicular phase of the menstrual cycle (d 3–7 of the cycle). In premenarchal girls, the study was performed whenever feasible. The insulin resistance index was estimated by the homeostasis model assessment (HOMA-IR) (42), and the whole-body insulin sensitivity index (ISI) composite was calculated (43).

Assays

Serum cortisol (Diagnostic Systems Laboratories, Webster, TX), 17-OHP (Diagnostic Products Corp., Los Angeles, CA), A4 (Diagnostic Systems Laboratories), DHEA (Diagnostic Systems Laboratories), and DHEAS (Diagnostic Products Corp.) were assayed by RIA. For cortisol, 17-OHP, A4, DHEA, and DHEAS, the assay sensitivities were 1.0 μg/dl, 0.1 ng/ml, 0.1 ng/ml, 0.1 ng/ml, and 5.0 μg/dl, respectively; intra- and interassay coefficients of variation were 4.2 and 5.2% for cortisol, 4.3 and 6.0% for 17-OHP, 5.0 and 5.0% for A4, 3.8 and 8.6% for DHEA, and 5.1 and 11.0% for DHEAS.

Serum glucose was determined by the glucose oxidase method (Photometric Instrument 4010; Roche, Basel, Switzerland). The intraassay coefficient of variation of this method was less than 2.0%. Serum insulin was assayed by RIA (Diagnostic Systems Laboratories). The intra- and interassay coefficients of variation were 5 and 8%, respectively.

Statistical and data analysis

Data are expressed as median and range. Normal distribution of data were assessed by Kolmogorov-Smirnov test. Differences among study groups were assessed with the Student *t* test when data were normally distributed or by the Mann-Whitney test when not normally distributed. The DHEAS values were log-transformed to ensure that the values were normally distributed. Categorical data were analyzed using χ^2 or Fisher's exact test. Statistical analysis was performed with STATA 7.0 package (StataCorp, College Station, TX). A *P* value of less than 0.05 was considered to be statistically significant.

Results

As expected, PCOS mothers had increased serum testosterone, insulin concentrations, free androgen index, BMI, waist circumference, and ovarian volume. In addition, they had decreased levels of SHBG. The frequency of obesity, defined by BMI greater than 30 kg/m^2, was 52.0%. Moreover, by design, all PCOS mothers presented hirsutism (modified Ferriman-Gallway score >8) and oligoanovulation. Table 1 depicts the clinical and endocrine characteristics of the PCOS mothers, compared with a group of 71 normal women studied (36).

Table 2 depicts the clinical characteristics of Cd and PCOSd cross-sectionally during childhood and peripuberty. During both periods, age, weight, weight SDS, height, height SDS, BMI, BMI SDS, waist circumference, waist-to-hip ratio, and birth weight did not differ significantly between Cd and PCOSd. The prevalence of SGA was slightly higher in PCOSd compared with Cd, although the difference did not reach statistical significance ($P = 0.264$). During childhood, all Cd demonstrated Tanner I breasts, and only one girl in the PCOSd group demonstrated Tanner II breasts. Alternatively, peripubertal PCOSd demonstrated Tanner IV and V breast and pubic hair more often than Cd. In the peripubertal period, 9.5% of Cd and 20.5% of PCOSd were postmenarchal ($P = 0.224$).

TABLE 1. Clinical and endocrine characteristics of PCOS mothers before pregnancy

	PCOS mothers	Normative values[a]
Age (yr)	29.6 (20.0–35.0)	25.0 (16.0–36.0)
BMI (kg/m^2)	31.2 (20.5–42.7)	27.1 (19.0–41.9)
Waist circumference (cm)	92.0 (74.0–113.0)	78 (68.0–88.0)
Testosterone (ng/ml)	1.07 (0.60–2.05)	0.35 (0.15–0.57)
SHBG (nmol/liter)	32.12 (11.37–95.16)	61.2 (20.5–138.6)
Free androgen index	13.2 (5.2–22.1)	1.98 (0.8–4.8)
Fasting glucose (mg/dl)	76.1 (60.0–102.6)	78.0 (51.0–107.0)
Fasting insulin (μIU/ml)	15.3 (5.0–42.6)	8.5 (3.0–19.7)
2-h glucose (mg/dl)	107.0 (94.6–138.0)	83.0 (50.0–139.0)
2-h insulin (μIU/ml)	101.3 (63.8–235)	43.4 (6.4–194.0)
Ovarian volume (cm^3)	12.6 (9.0–21.8)	8.1 (5.6–10.0)

Values depicted are median (range).

[a] See Ref. 36.

Basal and stimulated cortisol and steroid levels in Cd and PCOSd are depicted in Table 3. During childhood, basal and ACTH-stimulated cortisol, 17-OHP, A4, DHEA, and DHEAS levels were similar in both groups. During the peripubertal period, basal and stimulated DHEAS were significantly higher in PCOSd compared with Cd; however, the other hormones were comparable in both groups.

Table 4 depicts the metabolic parameters of Cd and PCOSd. During childhood, fasting glucose, insulin, and HOMA-IR were not significantly different between the two groups. The 2-h glucose and ISI composite levels were also similar between both groups. However, the 2-h insulin levels were significantly higher. During the peripubertal period, fasting glucose and insulin, 2-h glucose levels, HOMA-IR, and ISI composite were similar between both groups. However, 2-h insulin levels were significantly higher in PCOSd, compared with Cd.

Figure 1 depicts the discrepancies between the chronological and bone ages of Cd and PCOSd. During childhood, no differences between groups were observed. However, in the peripubertal period an advance of 8 months in bone age compared with chronological age in PCOSd and of 2 months in Cd was observed. Moreover, in the peripubertal period, 23.8% of Cd and 53.0% of

TABLE 2. Clinical characteristics of Cd and PCOSd

	Childhood		Peripuberty	
	Cd	PCOSd	Cd	PCOSd
n	30	64	21	34
Age (yr)	5.9 (4.0 to 7.6)	5.7 (3.9 to 7.6)	10.6 (8.7 to 12.9)	10.6 (8.2 to 13.0)
Weight (kg)	23.4 (14.8 to 35.5)	23.0 (15.5 to 38.4)	42.9 (26.0 to 63.2)	40.2 (25.5 to 82.5)
Weight SDS	0.7 (−0.7 to 2.4)	0.8 (−1.4 to 2.6)	0.7 (−1.4 to 2.3)	0.4 (−1.8 to 2.8)
Height (m)	1.1 (1.0 to 1.3)	1.1 (1.0 to 1.3)	1.4 (1.3 to 1.6)	1.4 (1.2 to 1.6)
Height SDS	0.1 (−2.0 to 2.1)	0.0 (−4.0 to 2.2)	0.2 (−1.4 to 1.8)	0.1 (−1.9 to 2.1)
BMI (kg/m^2)	17.6 (14.0 to 23.0)	17.9 (14.0 to 24.2)	20.6 (14.0 to 27.0)	19.7 (15.4 to 31.1)
BMI SDS	1.0 (−1.1 to 2.5)	1.2 (−1.1 to 2.7)	0.9 (−1.9 to 2.0)	0.6 (−0.7 to 2.3)
Waist circumference (cm)	56.9 (45.0 to 71.0)	56.0 (47.0 to 72.0)	67.0 (50.0 to 81.0)	66.3 (53.0 to 94.0)
Waist-to-hip ratio	0.9 (0.7 to 1.0)	0.9 (0.8 to 1.0)	0.9 (0.8 to 0.9)	0.9 (0.8 to 1.1)
Tanner stage				
Breast (%)				
I	100 (30)	98.4 (63)	23.8 (5)	14.7 (4)
II–III	0	1.6 (1)	52.8 (11)	50.0 (18)
IV–V	0	0	23.8 (5)	35.2 (12)
Pubic hair (%)				
I	100 (30)	98.4 (63)	33.3 (7)	26.5 (9)
II–III	0	1.6 (1)	57.1 (12)	50.0 (17)
IV–V	0	0	9.5 (2)	23.5 (8)
Birth weight (kg)	3.2 (2.2 to 4.4)	3.3 (2.2 to 4.5)	3.3 (2.3 to 4.5)	3.2 (2.5 to 4.7)
SGA (%)	6.6 (2)	9.4 (6)	4.8 (1)	17.6 (6)

Values depicted are median (range), unless otherwise specified.

PCOSd exhibited a chronological to bone age discrepancy of more than 1 yr ($P = 0.013$).

Finally, among PCOSd we observed that 12.5% of girls in childhood and 32.4% of those in peripuberty presented biochemical evidence of exaggerated adrenarche (*i.e.* DHEAS levels greater than mean plus 2 SD of matched controls). In addition, in both age groups, PCOSd concomitantly demonstrated a significantly higher response of 17-OHP [childhood, 4.7 (1.5–20.9) *vs.* 10.9 (4.0–25.4) ng/ml, $P = 0.017$; and peripubertal, 3.4 (1.4–6.0) *vs.* 5.2 (3.6–10.3) ng/ml, $P = 0.012$] and DHEA concentrations [childhood, 2.6 (0.7–6.0) *vs.* 5.4 (1.2–11.2) ng/ml, $P = 0.003$; and peripubertal, 6.1 (1.5–10.7) *vs.* 10.6 (5.0–24.7) ng/ml, $P = 0.004$] to ACTH-(1–24) stimulation. In the peripubertal period, all PCOSd girls demonstrated an advance in bone age greater than 1 yr. No differences in clinical or metabolic parameters were observed between those girls with and without evidence of "exaggerated adrenarche."

TABLE 3. ACTH response in Cd and PCOSd

	Childhood		Peripuberty	
	Cd	PCOSd	Cd	PCOSd
n	30	64	21	34
Cortisol (μg/dl)				
Basal	8.8 (2.1–30.6)	10.0 (0.2–26.6)	9.5 (4.6–17.8)	9.3 (3.5–16.1)
Stimulated	21.0 (14.0–39.7)	23.1 (0.4–33.4)	25.3 (16.5–64.6)	24.8 (11.5–32.8)
17-OHP (ng/ml)				
Basal	1.0 (0.1–3.7)	0.8 (0.1–5.0)	0.8 (0.3–1.5)	0.9 (0.3–2.1)
Stimulated	4.3 (0.7–13.3)	5.5 (1.5–25.4)	3.9 (2.0–6.3)	4.0 (1.4–10.3)
A4 (ng/ml)				
Basal	0.5 (0.1–1.5)	0.5 (0.1–2.0)	1.1 (0.3–2.4)	1.2 (0.3–2.7)
Stimulated	0.7 (0.3–1.4)	0.8 (0.1–2.5)	1.6 (0.5–4.0)	1.6 (0.4–6.0)
DHEA (ng/ml)				
Basal	2.2 (0.7–8.2)	2.2 (0.1–9.0)	3.4 (1.7–7.7)	4.3 (0.0–8.9)
Stimulated	3.0 (0.1–7.7)	2.9 (0.7–11.2)	6.1 (1.5–14.9)	7.5 (1.5–24.7)
DHEAS (μg/dl)				
Basal	29.8 (3.6–67.0)	37.1 (4.1–156.4)	55.0 (26.6–88.8)	85.2 (21.7–319.3)[a]
Stimulated	28.9 (4.3–81.1)	34.7 (5.3–149.4)	55.5 (27.4–91.5)	81.3 (26.8–264.0)[a]

Values depicted are median (range).

[a] $P < 0.05$ between Cd and PCOSd.

TABLE 4. Metabolic parameters in Cd and PCOSd

	Childhood		Peripuberty	
	Cd	PCOSd	Cd	PCOSd
n	30	64	21	34
Fasting				
Glucose (mg/dl)	83.7 (72.0–103.0)	86.8 (57.0–105.0)	83.8 (69.0–103.0)	84.7 (63.0–107.0)
Insulin (μIU/ml)	8.1 (2.2–21.1)	7.2 (3.0–25.0)	14.0 (3.7–39.1)	14.8 (4.0–106.9)
HOMA-IR	1.7 (0.4–4.5)	1.6 (0.6–6.1)	3.0 (0.8–9.9)	3.1 (0.8–20.3)
2-h				
Glucose (mg/dl)	103.5 (65.0–161.0)	105.8 (56.0–162.0)	94.4 (58.0–137.0)	99.2 (62.0–130.0)
Insulin (μIU/ml)	20.4 (4.0–66.3)	32.0 (4.0–194.0)[a]	46.8 (10.7–79.8)	63.3 (5.6–330.0)[a]
ISI composite	8.7 (3.5–18.8)	8.3 (3.7–15.0)	6.9 (2.0–13.5)	6.0 (1.6–15.4)

Values depicted are median (range). ISI composite is based on response to glucose challenge.

[a] $P < 0.05$ between Cd and PCOSd.

None of the Cd, by definition, demonstrated biochemical evidence of exaggerated adrenarche.

Discussion

More than 30 yr ago, Yen *et al.* (17) postulated that an exaggerated adrenarche during the early phase of sexual maturation could be one of the etiologies of PCOS. In the present study, we hypothesized that adrenal function was altered in daughters of PCOS women, and we studied 98 daughters of women with PCOS and 51 controls. In peripubertal PCOSd, we observed elevated basal and post-ACTH stimulated DHEAS levels and higher 2-h insulin levels, compared with Cd. These girls also demonstrated advanced bone age relative to chronological age. In addition, 12 and 32% of PCOSd in childhood and peripuberty, respectively, demonstrated biochemical evidence of exaggerated adrenarche (*i.e.* supranormal DHEAS levels). Alternatively, no differences were observed in childhood for the parameters studied between daughters of PCOS and healthy women.

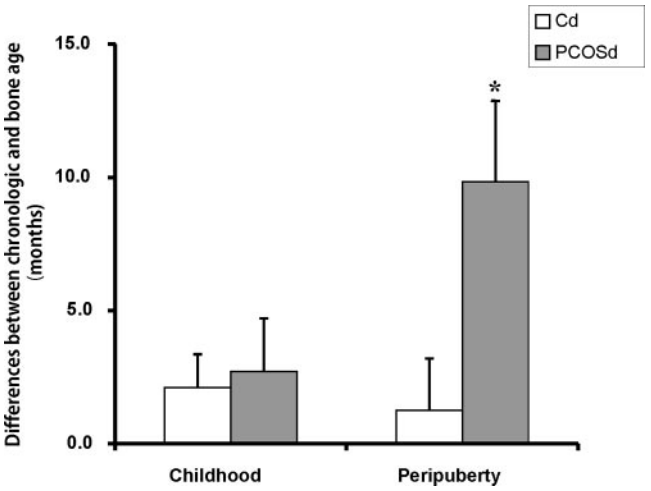

FIG. 1. Differences (in months) between chronological and bone ages of Cd and PCOSd. Values are expressed as median and SEM. *, $P < 0.05$.

These data suggest that evidence of adrenocortical dysfunction, primarily an exaggerated adrenarche or supranormal secretion of DHEA and DHEAS, is observable in many daughters of women with PCOS and that these abnormalities are first evident in the peripubertal period. These abnormalities are evident before the onset of overt symptoms of the disorder occur, although some of these girls do demonstrate mild advancements in bone and pubertal maturation. Further longitudinal studies are needed to determine whether these girls with evidence of an exacerbated adrenarche will develop some or all of the features of PCOS in adulthood.

Although normal gonadarche can occur in the absence of adrenal glands (15), it is also possible that adrenarche may be one of the triggers needed for normal pubertal and gonadal maturation. Nader (10) has postulated that adrenarche could provide the initial source of androgens, leading to the disinhibition of GnRH secretion and hence to the process of gonadarche. In daughters of women with PCOS, we observed that evidence of exaggerated adrenarche was present in about 12% during childhood and 32% during peripuberty and that some of these girls also demonstrated an advance in bone age and in the progression of puberty. Overall, in addition to shedding light on the early events in the development of PCOS, these observations indicate that AAs may modulate the onset or progression of puberty (33).

It has been postulated that an increase in the activity of cytochrome P450c17α in the adrenocortical zone reticularis could be one of the molecular events promoting the onset of adrenarche (44, 45). In addition, being SGA at birth has been associated with the subsequent development of hyperinsulinism and an exacerbated adrenarche (23, 26). However, in the present study we did not observe an association between low birth weight and exaggerated

adrenarche. Our daughters of women with PCOS who had higher levels of DHEAS also concomitantly demonstrated higher levels of 17-OHP and DHEA after ACTH-(1–24) stimulation, suggesting a higher activity of cytochrome P450c17α. This observation is in agreement with the hypothesis that hyperactivity of P450c17α may lead to both functional ovarian and adrenal hyperandrogenism (46, 47).

Increased activity of cytochrome P450c17α during puberty may be triggered through marked weight gain, hyperinsulinemia, or overnutrition (48). In the present study, we did not find a relationship between the elevated DHEAS levels of daughters of PCOS women and birth weight or anthropometrics. It is possible that insulin may normally modulate the synthesis of AAs and, if exaggerated, may accelerate the onset of peripubertal androgen synthesis. In our study, we did observe that PCOSd exhibited elevated poststimulated insulin levels compared with Cd. However, we observed similar insulin concentrations in those PCOSd with and without evidence of exacerbated adrenarche. Hence, in the present study it is more likely that the exaggerated adrenarche observed may represent a primary and intrinsic feature in some girls born to PCOS mothers and who may go on to develop PCOS themselves.

Genetic variants may also alter the degree of AA secretion, including polymorphisms of enzymes related to DHEAS metabolism (49–51). It is interesting to note that in general, higher basal levels of DHEAS were not necessarily associated with higher DHEA levels, which may be explained by an increased rate of sulfation of this adrenal steroid. Goodarzi *et al.* (50) have suggested the potential role of variants in *SULT2A1*, but not *STS*, in the AA excess of PCOS.

In the present study, we were able to establish that an exaggerated adrenocortical function is present early in individuals at risk of PCOS, which is a unique observation. However, because this is an observational study, we cannot clarify the origin or the cause of the increased P450c17a activity, which may be primary or secondary to insulin resistance or hyperinsulinemia. In this regard, further mechanistic studies (*e.g.* genetic studies, reexamination of adrenocortical function after suppression of insulin action) would be necessary to clarify the origin of the exaggerated adrenocortical function found in PCOSd.

In conclusion, in the present study we observed increased DHEAS serum concentrations and biochemical evidence of an exacerbated adrenarche, in association with an advance in bone age, in some girls born to PCOS mothers. These features could be an early step in the development of PCOS in these at-risk girls.

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