

Anti-Müllerian Hormone Levels in Peripubertal Daughters of Women with Polycystic Ovary Syndrome

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Context: We have previously observed increased anti-Müllerian hormone (AMH) levels in prepubertal daughters of polycystic ovary syndrome (PCOS) women, suggesting that these girls may have an altered follicular development. However, it is not known whether AMH levels remain increased during puberty.

Objective: The aim was to establish whether the increased AMH levels observed in prepubertal daughters of PCOS women persist during the peripubertal period, a stage during which the gonadal axis is activated and PCOS may become clinically manifested.

Design: We studied 28 daughters (8–16 yr old) of PCOS women (PCOSd) and 33 daughters (8–16 yr old) of control women (Cd). In both groups, an oral glucose tolerance test was performed. Gonadotropins, sex hormones, and AMH were determined in a fasting sample.

Results: Both groups were comparable in age, body mass index, and breast Tanner stage. Free androgen index, testosterone, AMH (Cd 14.4 ± 8.0 pM vs. PCOSd 24.0 ± 19.0 pM; $P = 0.012$), and 2-h insulin levels were significantly higher in the PCOSd group compared with the control group. The average ovarian volume was significantly higher in the PCOSd group. In both groups a positive correlation between 2-h insulin and AMH concentrations was observed (PCOSd: $r = 0.530$, $P = 0.007$; Cd: $r = 0.561$, $P = 0.008$).

Conclusions: AMH concentrations are increased in peripubertal PCOSd. These findings, along with the results of our previous study, suggest that PCOSd appear to show an increased follicular mass that is established during early development, and persists during puberty.

POLYCYSTIC OVARY SYNDROME (PCOS) is a highly prevalent (5–10%) endocrine-metabolic dysfunction in premenopausal women, and is the most frequent cause of anovulatory infertility and hyperandrogenism in women. In addition, most women with PCOS also have peripheral insulin resistance (1–3). Insulin resistance and pancreatic β -cell dysfunction, with increased risk for type 2 diabetes mellitus, are frequent comorbidities in PCOS patients (4–6).

Clinical signs of PCOS may emerge during the peripubertal period, and premature pubarche may constitute an early manifestation in some girls (7–9). However, in most girls, PCOS usually becomes clinically apparent during adolescence and early adulthood. Nevertheless, the clinical features that are used to diagnose PCOS in adult women are difficult to apply during adolescence. Furthermore, biochemical markers such as increased androgen and insulin secretion, typical of PCOS, may be observed in normal adolescents (10–13). In this situation, a biochemical marker that might discriminate between normal and PCOS pubertal girls is not yet available.

Anti-Müllerian hormone (AMH), also called Müllerian-

inhibiting substance, is a dimeric glycoprotein, member of the TGF superfamily (14, 15). AMH is produced exclusively in the gonads by Sertoli and granulosa cells (16), and its dimorphic expression in ovary and testis is crucial for the normal differentiation of reproductive structures (17). During fetal development, AMH is secreted by the immature Sertoli cells. The main role of AMH in the male fetus is to cause Müllerian ducts regression (15), but AMH is also involved in testicular development (18). AMH expression by granulosa cells is evident at the end of gestation, when it is secreted in small amounts by early developing follicles, allowing the differentiation of the Müllerian ducts (19). During postnatal life, gonadal AMH secretion shows a clear-cut sexual dimorphism in prepubertal children, when serum AMH is significantly lower in females (20–22). Mean serum AMH levels increase during late puberty (23) and then show a progressive decline along reproductive life in women (23, 24).

Serum AMH levels seem to correlate with the development of preantral and small antral follicles during reproductive life (21). It has been established that AMH levels are 2- to 3-fold higher during reproductive life in women with PCOS compared with healthy women (25, 26). This is related to the increased number of growing follicles that secrete AMH (27).

Recently, our group documented higher serum levels of AMH in daughters of PCOS women (PCOSd) compared with control girls (Cd) during two stages of sexual development, early infancy (2–3 months of age) and childhood (4–7 yr of age), suggesting the presence of an increased growing follicular pool in these girls (28).

Abbreviations: $\Delta 4A$, Androstenedione; AMH, anti-Müllerian hormone; BMI, body mass index; Cd, daughters of control women; E2, estradiol; FAI, free androgen index; 17-OHP, 17-OH-progesterone; PCOS, polycystic ovary syndrome; PCOSd, daughters of PCOS women; T, testosterone.

The aim of the present study was to establish whether the increased AMH levels observed in prepubertal PCOSd persist during the peripubertal period, a stage during which the gonadal axis is activated, and PCOS may become clinically manifested.

Subjects and Methods

Subjects

We studied 28 PCOSd (8–16 yr old). As a control group, we studied 33 daughters (8–16 yr old) of women with regular menses and without hyperandrogenism, history of infertility, or diabetes. The control group (Cd) was recruited from public schools, and we excluded girls with diabetes mellitus or glucose intolerance, hepatic, cardiac, pulmonary, or renal disease, and use of any medications.

Both groups of girls were matched for age, Tanner stage, and body mass index (BMI) at the beginning of the study.

PCOS mothers were recruited from patients attending the Unit of Endocrinology and Reproductive Medicine, University of Chile. Diagnosis of PCOS was made according to the National Institutes of Health consensus criteria (29). At the moment of diagnosis, all of the PCOS mothers exhibited chronic oligomenorrhea or amenorrhea, hirsutism, serum testosterone (T) more than 0.6 ng/ml, and/or free androgen index (FAI) more than 5.0, androstenedione (Δ 4A) more than 3.0 ng/ml. In addition, PCOS women showed the characteristic ovarian morphology of polycystic ovaries on ultrasound, based on the criteria described by Adams *et al.* (30). PCOS mothers were normoglycemic, with varying degrees of hyperinsulinemia that were evaluated by an oral glucose tolerance test. All patients had an elevated waist-to-hip ratio greater than 0.85.

We excluded patients with hyperprolactinemia, androgen-secreting neoplasm, Cushing's syndrome, and late-onset 21-hydroxylase deficiency, as well as thyroid disease.

We selected as control mothers, 33 women of similar socioeconomic level, with a history of singleton pregnancies, regular 28- to 32-d menstrual cycles, absence of hirsutism and other manifestations of 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

The 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 on each girl, including Tanner stage score determination for breast development and anthropometric measurements: weight, height, waist, hip, BMI, and BMI *SD* score (SDS), using current Centers for Disease Control and Prevention standard curves (31). Postmenarchal girls were studied in the early follicular phase of the menstrual cycle (d 3–7). In the premenarchal girls, the study was performed whenever feasible.

A 2 h, 1.75 g/kg, oral glucose tolerance test was performed with measurements of glucose and insulin in each sample. In the fasting sample, circulating concentrations of gonadotropins, T, Δ 4A, estradiol (E2), 17-OH-progesterone (17-OHP), SHBG, and AMH were determined by specific assays.

On the following day, ultrasonography was performed and analyzed by a single observer (F.S.), who was blinded to the condition of the subject. The examination was performed transabdominally with a 5 MHz abdominal probe, using Medison Sonoace 6000C equipment (Medison Co., Ltd., Seoul, Korea). Ovarian volume was calculated using the simplified formula for a prolate ellipsoid.

Assays

Serum AMH was assayed by enzyme immunoassay (Immunotech-Beckman Coulter, Marseille, France) (32). Analytical sensitivity was 2.1 pM, and intraassay and interassay coefficients of variation were 5.3 and 8.7%, respectively.

Serum LH, FSH, and E2 were determined by electrochemiluminescence (Roche, Basel, Switzerland). Assay sensitivities were 0.1 IU/liter,

0.1 IU/liter, and 5.0 pg/ml, respectively. Intraassay and interassay coefficients of variation were 1.1 and 2.1% for LH, 1.7 and 3.7% for FSH, and 2.7 and 5.0% for E2.

Serum T and insulin were assayed by RIA (Diagnostic System Laboratories, Inc., Webster, TX), and Δ 4A, 17-OHP, and SHBG were determined by radioimmunoassay (Diagnostic Products Corp., Los Angeles, CA). Assay sensitivities were 0.1 ng/ml, 5 μ IU/ml, 0.1 ng/ml, 0.1 ng/ml, and 0.04 nmol/liter, respectively. Intraassay and interassay coefficients of variation were 7.0 and 11.0% for T, 5 and 8% for insulin, 3.7 and 4.9% for Δ 4A, 3.5 and 5.0% for 17-OHP, and 3.8 and 7.9% for SHBG. Plasma glucose levels were determined by the glucose oxidase method (Photometric Instrument 4010; Roche).

Statistical evaluation

Data were normally distributed, and expressed as means and *SD* values. Differences between groups were assessed by the Student's *t* test. The correlation between continuous variables was assessed using Pearson's correlation coefficient. Statistical analysis was performed using the Statistical Package for the Social Sciences software (version 10.0; SPSS, Inc., Chicago, IL). A *P* value < 0.05 was considered statistically significant.

Results

Both groups of girls were comparable in age (Cd 11.5 ± 2.2 yr *vs.* PCOSd 11.4 ± 2.5 yr; *P* = 0.984), BMI (Cd 21.1 ± 2.4 kg/m² *vs.* PCOSd 22.5 ± 4.5 kg/m²; *P* = 0.146), and BMI SDS (Cd 1.0 ± 0.6 *SD vs.* PCOSd 1.1 ± 0.8 *SD*; *P* = 0.382). The distribution of Tanner stage scores for breast development and menarchal status were comparable between the two groups (Tanner stage distribution χ^2 ; *P* = 0.979, menarchal status χ^2 ; *P* = 0.754) (Table 1).

Table 2 shows the main biochemical characteristics and ovarian volumes of both groups. AMH levels were significantly higher in the PCOSd group (Cd 14.4 ± 8.0 pM *vs.* PCOSd 24.0 ± 19.0 pM; *P* = 0.012; Fig. 1) compared with the control group. Basal T levels (Cd 0.4 ± 0.2 ng/ml *vs.* PCOSd 0.6 ± 0.4 ng/ml; *P* = 0.006) and FAI (Cd 4.5 ± 4.0 *vs.* PCOSd 12.6 ± 12.7 ; *P* = 0.001) were significantly higher in the PCOSd group. E2 levels (Cd 38.6 ± 26.2 pg/ml *vs.* PCOSd 23.7 ± 16.3 pg/ml; *P* = 0.027) were significantly lower in the PCOSd group. There were no differences in gonadotropin or the other sex steroid concentrations.

Basal glucose and insulin concentrations were similar between both groups. Two-hour insulin was significantly higher in the PCOSd group (Cd 43.3 ± 5.4 μ IU/ml *vs.* PCOSd 71.1 ± 11 μ IU/ml; *P* = 0.046) compared with the control group. The average ovarian volume was significantly higher in the PCOSd group (Cd 5.3 ± 3.6 cc *vs.* PCOSd 9.3 ± 4.9 cc; *P* = 0.003). There was no correlation between serum AMH concentrations and ovarian volume.

In both groups, a positive correlation between 2-h insulin and AMH concentrations was observed (Cd *r* = 0.561, *P* = 0.008; PCOSd *r* = 0.530, *P* = 0.007). After values were adjusted by BMI SDS, this correlation remained significant (Cd *r* = 0.537, *P* = 0.01; PCOSd *r* = 0.39, *P* = 0.044). No correlation between AMH concentrations and androgen or gonadotropin levels was observed.

Discussion

The present study shows that AMH serum levels are elevated in peripubertal PCOSd, suggesting that the ovarian growing follicular pool may be increased in these girls.

TABLE 1. Clinical characteristics of peripubertal control daughters (Controld) and PCOS daughters (PCOSd)

	Controld	PCOSd	P value
n	33	28	
Age (yr)	11.5 ± 2.2	11.4 ± 2.5	0.984
Pubertal stage			
No. of prepubertal (B1)	7 (21%)	6 (21%)	
No. of early pubertal (B2)	5 (15%)	4 (14.2%)	
No. of midpubertal (B3)	4 (12.1%)	3 (10.7%)	
No. of late pubertal (B4, B5)	15 (54.1%)	17 (51.9%)	
Menarchal status			
No. of premenarchal (%)	19 (57.5)	15 (53.5)	
No. of postmenarchal (%)	14 (42.4)	13 (46.4)	
Weight (kg)	46.0 ± 9.5	50.2 ± 17	0.224
Height (cm)	146.4 ± 11.0	146.8 ± 13.5	0.913
BMI (kg/m ²)	21.1 ± 2.3	22.5 ± 4.5	0.146
BMI SDS	1.0 ± 0.6	1.1 ± 0.8	0.382
Waist (cm)	68.1 ± 6.2	70.7 ± 11	0.277
Hip (cm)	82.5 ± 9.1	83.6 ± 13.3	0.713

Unless stated otherwise, values are the means ± SD.

We have recently demonstrated that AMH is increased from early infancy to childhood (4–7 yr) in PCOSd. Our present results show that AMH remains elevated during puberty in these girls, suggesting that their growing follicular pool may be increased from an early age and may persist elevated until puberty. The fact that AMH concentrations are elevated during childhood (28) (a time when the gonadal axis is relatively quiescent) and puberty (when the gonadal axis is progressively activated until sexual maturation is complete) is in agreement with the notion that AMH may serve as a marker of the growing follicular pool independent of the functional activity of the gonadal axis (24).

During reproductive life, serum AMH levels correlate with the small antral follicle count estimated by transvaginal ultrasound (21). In the present study, although pubertal PCOSd showed increased ovarian volume, it did not correlate with AMH levels. This may be because ovarian volume, estimated by transabdominal ultrasonography, does not have enough sensitivity as transvaginal ultrasound to make an estimate of the number of growing follicles.

In the present study, an elevated 2-h insulin level was observed in the PCOSd group, which is in agreement with the concept that insulin resistance and hyperinsulinemia are early features of PCOS (33, 34). In a study by Lewy *et al.* (35)

in PCOS adolescents, similar basal insulin and glucose concentrations were observed in PCOS and control adolescents, but PCOS girls showed significantly higher first and second-phase insulin levels during hyperglycemic clamp studies. Thus, as previously proposed, insulin resistance seems to be an early feature during the ontogeny of PCOS.

In both groups, a positive correlation between AMH and 2-h insulin levels was observed. This is an interesting finding that links AMH with insulin resistance. A relationship between AMH levels and the homeostatic model assessment index has been previously described by La Marca *et al.* (36). On the one hand, it is possible that insulin, as a trophic factor for granulosa cells, may be increasing the number of AMH producing cells, although we cannot exclude the possibility that each granulosa cell could be producing more AMH in response to insulin. Conversely, a higher number of follicles reflected by high AMH levels could be determining higher androgen levels that have been related with hyperinsulinemia (37). However, in the present study, we did not observe a correlation between AMH and androgen levels, a finding that does not support this hypothesis.

It has been proposed that an important proportion of the first-degree female relatives of a PCOS woman may be at risk for developing PCOS (38–42). T levels and FAI were higher

TABLE 2. Biochemical characteristics and ovarian volume of peripubertal control daughters (Controld) and PCOS daughters (PCOSd)

	Controld	PCOSd	P value
n	33	28	
LH (mIU/ml)	2.0 ± 2.3	3.1 ± 4.1	0.243
FSH (mIU/ml)	4.0 ± 2.3	4.9 ± 2.5	0.163
17-OHP (ng/ml)	1.0 ± 0.5	1.2 ± 1.3	0.292
Δ4A (ng/ml)	1.3 ± 0.7	1.3 ± 1.1	0.973
T (ng/ml)	0.4 ± 0.2	0.6 ± 0.4	0.006 ^a
FAI	4.5 ± 4.0	12.6 ± 12.7	0.001 ^a
E2 (pg/ml)	38.6 ± 26.2	23.7 ± 16.3	0.027 ^a
SHBG (nmol/liter)	41.0 ± 21	35.0 ± 32	0.403
AMH (pM)	14.4 ± 8.0	24.0 ± 19.0	0.012 ^a
Ovarian volume (cm ³)	5.3 ± 3.6	9.3 ± 4.9	0.001 ^a
Basal glucose (mg/dl)	84.3 ± 15.0	83.6 ± 9.0	0.851
2-h glucose (mg/dl)	90.0 ± 15.0	96.0 ± 22.0	0.271
Basal insulin (μIU/ml)	12.2 ± 7.0	14.3 ± 11.0	0.438
2-h insulin (μIU/ml)	43.3 ± 5.4	71.1 ± 11.0	0.046 ^a

Unless stated otherwise, values are the means ± SD.

^a P < 0.05.

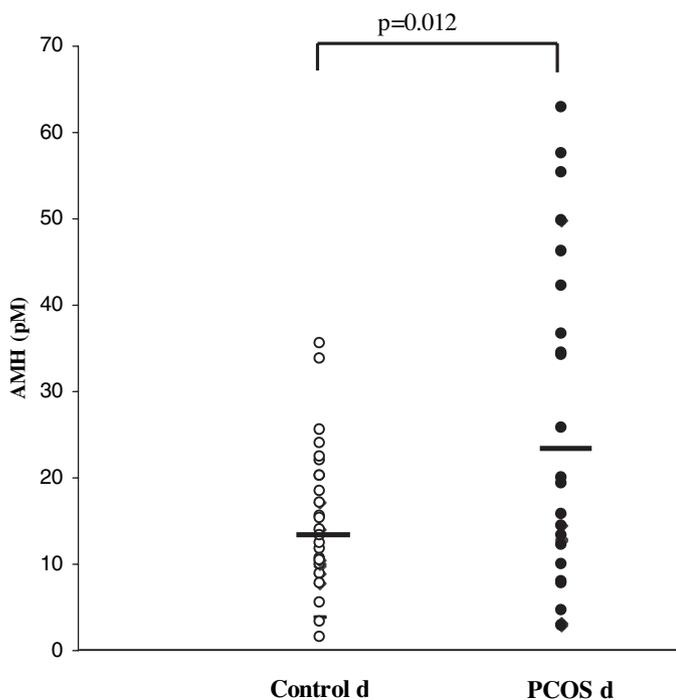


FIG. 1. Comparison of AMH serum concentrations in peripubertal girls (8–16 yr old). Control d, Control daughters (○); PCOS d, PCOS daughters (●).

in the PCOSd group, which is consistent with the fact that at least a group of these girls may develop PCOS. In addition, lower E2 levels were observed in the PCOSd group, which is not usually observed in adult PCOS women.

In summary, we have found significantly higher AMH levels in a group of peripubertal PCOSd carefully matched by age, Tanner stage, and BMI with a control group. These findings, along with the results of our previous work, suggest that PCOSd may show an increased follicular mass that is established early during development and persists until the complete activation of the gonadal axis. Prospective studies with a greater number of subjects are needed to establish whether the postmenarchal girls of the present study, with elevated AMH levels, ultimately develop PCOS.

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