Polycystic ovaries after precocious pubarche: relation to prenatal growth

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BACKGROUND: In 1998, we revealed a sequence departing from prenatal growth restraint in girls and evolving, through precocious pubarche (PP) in mid-childhood, towards anovulatory and hyperinsulinaemic hyperandrogenism. The latter condition fulfilled the criteria for the diagnosis of polycystic ovary syndrome (PCOS), which was then defined independently of the presence of polycystic ovaries (PCOs). Since 2003, the diagnosis of PCOS has been extended by adding PCO as an alternative criterion. We verified longitudinally over 28 ± 2 years the prevalence of PCO and its potential relationship to growth before birth in a group of post-PP women (n = 14, mean age = 28 years; body mass index = 24.3 kg/m²) belonging to the original cohort of 35 girls in whom the PP–PCOS sequence was described. METHODS: Endocrine-metabolic variables, body composition (by dual-energy X-ray absorptiometry), carotid intima-media thickness (IMT) and ovarian morphology by transvaginal ultrasonography were assessed in all women. RESULTS: Post-PP women with a birthweight (BW) in the lowest quartile, when compared with post-PP women with a higher BW, had smaller ovaries (mean volume = 4.0 versus 9.0 ml; P = 0.004) and a much lower prevalence of PCO (0 versus 67%; P = 0.006). The remaining variables were similar between BW subgroups. CONCLUSIONS: The presence of a PCO morphology in women with a PP history was found to relate to prenatal growth. It would be of interest to verify whether a similar relationship exists in anovulatory and/or hyperandrogenic women without PP history.

Key words: carotid intima-media thickness/hyperinsulinaemic hyperandrogenism/prenatal growth/precocious pubarche/polycystic ovaries

Introduction

In 1998–2001, prospective data revealed a sequence departing from prenatal growth restraint in girls and evolving, through precocious pubarche (PP) in mid-childhood (appearance of pubic hair before 8 years), towards anovulatory and hyperinsulinaemic hyperandrogenism in late adolescence (Ibáñez et al., 1998, 1999a, 2000, 2001a). In those days, the latter condition fulfilled the strict criteria (Zawadzki and Dunaif, 1992) for the diagnosis of polycystic ovary syndrome (PCOS), which was then defined independently of the presence of polycystic ovaries (PCOs). Since 2003, the diagnosis of PCOS has been extended by adding PCO as an alternative criterion, but there continues to be some controversy on this criterion (The Rotterdam ESHRE/ASRM-sponsored PCOS consensus workshop group, 2004; Azziz, 2006; Franks, 2006).

So far, studies departing from the presence of PCO in women have suggested a relation between increased fetal growth and subsequent PCO morphology (Cresswell et al., 1997; Michelmore et al., 2001).

We verified longitudinally over 28 ± 2 years whether, in girls with PP, the development of an ovarian PCO morphology is related to growth before birth.

Study population and methods

Study population

The study population of 14 women [mean ± SEM; age = 28 ± 1 years; body mass index (BMI) = 24.3 ± 1.3 kg/m²] is a fraction of the original cohort of 35 girls in whom the PP–PCOS sequence was described (Ibáñez et al., 1993). Figure 1 shows the patient flow between PP diagnosis and the present PCO assessment.

In all 35 girls, PP (presenting at a mean age of 6.6 years) was attributed to an amplified adrenarche, based on high circulating levels of dehydroepiandrosterone sulphate and/or androstenedione for chronological age; none of the girls presented evidence for late-onset congenital adrenal hyperplasia (New et al., 1983; Ibáñez et al., 1993; Mermejo et al., 2005).

The 35 PP girls were initially followed until adolescence. At age ~15 years (≥2.5 years post menarche; mean BMI = 22.4 kg/m²), ovarian
function was assessed; 16 girls were found to have ovarian hyperandrogenism, based on the presence of hirsutism (Ferriman and Gallwey, 1961), oligomenorrhea (cycle >45 days), high testosterone and/or androstenedione levels and a 17-hydroxy-progesterone hyperresponse to GnRH agonist; 19 girls were non-hirsute, had regular cycles and had baseline and stimulated androgen levels within normal range (Ibáñez et al., 1993).

After the 15-year assessment, 28 of the 35 patients were lost to follow-up. Recently, however, we traced 20 of these 28 women, thereby raising the potential population of this follow-up study to n = 27. Among these women, 20 agreed to participate; the seven non-participants reported no symptoms of androgen excess. Complete work-up, including PCO assessment, could ultimately be performed in a total of 17 women. Among those, three were born after a pregnancy complicated by gestational diabetes. Because the ovaries of these women may have been exposed to prenatal hyperglycaemia and/or hyperinsulinaemia, we excluded these women from the main analysis.

Table I summarizes the features of the participating women, when they were aged ∼15 years, as opposed to those of women who did not participate in this study or who were born after a pregnancy complicated by gestational diabetes.

Study protocol
At first contact for PCO assessment, 9 of the 14 women were receiving an oral contraceptive [OC for 21/28 days; ethinylestradiol (35 μg) + cyproterone acetate (2 mg) (n = 4); ethinylestradiol (20 μg) + gestodene (75 μg) (n = 2); ethinylestradiol (30 μg) + drospirenone (3 mg) (n = 3)]; four of these women were receiving metformin (850 mg/day), and three of the latter women were in addition receiving low-dose flutamide (62.5 mg/day). Metformin and flutamide were discontinued, and the OC with cyproterone was replaced by an OC without cyproterone for a mean of 9 months (range = 7–12 months) before endocrine-metabolic and ultrasound assessment.

Fasting blood glucose was measured together with serum insulin, leptin, and insulin-like growth factor-binding protein-1 (IGFBP-1) and blood count. In addition, we screened liver and kidney function and performed a standard 2-h oral glucose tolerance test (oGTT). Assessments of body composition, ovarian morphology and carotid appearance were performed in the same early-follicular (or OC-free) week.

Body composition
Body composition was assessed by dual-energy X-ray absorptiometry with a Lunar Prodigy coupled to Lunar software (Lunar Corp., WI, USA), as described (Kiebzak et al., 2000; Ibáñez and de Zegher, 2004). Age-matched references are available for body fat fraction in non-obese (Kirchengast and Huber, 2001) and obese (Puder et al., 2005) women.

Ovarian ultrasound assessment
At age ∼15 years, PCO appearance was judged by transabdominal ultrasound, according to the criteria of Adams et al. (1985), as described (Ibáñez et al., 1993).

In the present follow-up study, PCO appearance was judged by transvaginal ultrasound scan of the ovaries that was performed by a single observer [E.M., who was unaware of the women’s birthweight (BW) at the time of ultrasound assessment], with a digital Sonoline
G40 scanner (Siemens, Erlangen, Germany), using a 5-MHz multifrequency EV9-4 sector probe. PCO was diagnosed according to the so-called Rotterdam criteria, which require the presence of at least one of the following in one ovary: ≥21 follicles with diameter of 2–9 mm or ovarian volume of >10.0 ml (Balen et al., 2003). OC intake does not appear to appreciably affect PCO morphology (Mulders et al., 2005).

Carotid artery Doppler

Longitudinal ultrasound scans of the carotid arteries were all obtained by one investigator (G.E., who was unaware of the patients’ endocrine-metabolic state) with a high-resolution machine equipped with colour and power Doppler capability (Acuson Sequoia 512 SHA, Medisales IIC., Los Alamitos, CA, USA), using a high-frequency 10-MHz linear probe and compound software (Stein et al., 2004). After a 15-min rest in supine position, the ultrasound Doppler examination was performed with the neck in hyperextension and the face turned away from the side being scanned. The right and left common carotid arteries and the carotid bifurcation-bulb areas were scanned in multiple planes. Images were obtained from the distal portions of both common carotid arteries, 1–2 cm away from the carotid bulb and immediately proximal to the origin of the bifurcation. The intima-media thickness (IMT) of the posterior (far) wall of both common carotid arteries was measured as the distance between the junction of the lumen and intima and the junction of the media and adventitia (O’Leary and Polack, 2002). The mean IMT of each side was calculated (Ibáñez et al., 1993).

Table I. Characteristics of the study population of women (mean age 28 years) originally presenting with precocious pubarche (PP) (age <8 years)

<table>
<thead>
<tr>
<th>Referencea</th>
<th>Non-participation or exclusionb (n = 21)</th>
<th>Participation (n = 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW SDs</td>
<td>–0.04 ± 0.2</td>
<td>–0.7 ± 0.5</td>
</tr>
<tr>
<td>Ovarian hyperandrogenism at age 15 yearsb</td>
<td>6</td>
<td>10*</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.6 ± 0.9</td>
<td>22.3 ± 0.6</td>
</tr>
<tr>
<td>SHBG (nmol/l)</td>
<td>64 ± 5</td>
<td>39 ± 3f</td>
</tr>
<tr>
<td>DHEAS (nmol/l)</td>
<td>3.6 ± 0.1</td>
<td>5.2 ± 0.5g</td>
</tr>
<tr>
<td>Testosterone (nmol/l)</td>
<td>1.3 ± 0.1</td>
<td>2.2 ± 0.2h</td>
</tr>
<tr>
<td>Androstenedione (nmol/l)</td>
<td>5.1 ± 0.2</td>
<td>9.7 ± 0.7fi</td>
</tr>
<tr>
<td>17-OHP after GnRH agonist (nmol/l)h</td>
<td>2.8 ± 0.1</td>
<td>4.8 ± 0.7f</td>
</tr>
<tr>
<td>Mean ovarian volume (ml)</td>
<td>5.6 ± 0.6</td>
<td>7.7 ± 0.8f</td>
</tr>
<tr>
<td>No PCO at age 15 yearsi</td>
<td>13</td>
<td>11</td>
</tr>
</tbody>
</table>

BMI, body mass index; BW, birthweight; DHEAS, dehydroepiandrosterone sulphate; 17-OHP, 17-hydroxyprogesterone; PCO, polycystic ovaries; SD, standard deviation; SHBG, sex hormone-binding globulin.
The population (n = 35) was subgrouped according to participation or non-participation/exclusion from assessment at age 28 years (see also Figure 1). Values are mean ± SEM.

*In age- and body size-matched controls (n = 12).
†Exclusion because of maternal diabetes during the woman’s own gestation (see Study population).
‡Based on the presence of hirsutism, oligomenorrhoea, high testosterone and/or androstenedione levels, and a 17-OHP hyperresponse to GnRH agonist (Ibáñez et al., 1993).
§17-OHP response 24 h after administration of leuprolide acetate, 500 μg s.c. (Ibáñez et al., 1993).
∥PCO assessment by transabdominal ultrasound, according to the criteria of Adams et al., (1985), for ovarian morphology and volume.
1P < 0.05; 2P ≤ 0.01; 3P ≤ 0.001 and 4P ≤ 0.0001 versus reference.
*P < 0.01 versus non-participation subgroup.

Table II. Characteristics of the study population of women (mean age 28 years) originally presenting with precocious pubarche (PP) (age <8 years)

<table>
<thead>
<tr>
<th></th>
<th>Reference</th>
<th>Post-PP women†</th>
<th>P-value‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGFBP-1 (ng/ml)</td>
<td>33 ± 2e</td>
<td>28 ± 5</td>
<td>NS</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>94 ± 4d</td>
<td>128 ± 5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Insulin (pmol/l)</td>
<td>244 ± 14f</td>
<td>452 ± 65</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Neutrophil/lymphocyte ratio</td>
<td>1.2 ± 0.1f</td>
<td>1.6 ± 0.2</td>
<td>0.04</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>16.8 ± 1.0f</td>
<td>20.8 ± 2.3</td>
<td>NS</td>
</tr>
<tr>
<td>Body fat fraction (%)</td>
<td>37.8 ± 2.4</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Carotid intima-media thickness (mm)</td>
<td>0.39 ± 0.02b</td>
<td>0.49 ± 0.03</td>
<td>0.004</td>
</tr>
</tbody>
</table>

IGFBP-1, insulin-like growth factor-binding protein-1; oGTT, oral glucose tolerance test; NS, non-significant.
Values are mean ± SEM.
†n = 9 were receiving an oral contraceptive (OC), and n = 4 metformin (see Study protocol).
‡For comparisons of post-PP women with reference values (inferred unpaired t-test).

Prenatal growth, precocious pubarche and polycystic ovaries

Hormone assays, calculations, statistics and ethics

A differential leukocyte count was determined within 2 h after blood sampling by an automatic cell counter (ABX Pentra 120, ABX Diagnostics, Montpellier, France), and the neutrophil/lymphocyte ratio was calculated (Ibáñez et al., 2006a). The intra-assay CV was ≤1%; for comparison, results from 57 age-matched, healthy women are mentioned in Table II.

Serum glucose was measured by the glucose oxidase method; serum immunoreactive insulin was assayed as described (Ibáñez and de Zegher, 2004). Glucose and insulin values during the oGTT were compared with those obtained in healthy young women from the same population (Ibáñez et al., 2001b). Serum leptin was measured...
by radioimmunoassay (Linco, St Louis, MO, USA) (Ibáñez et al., 2006b); IGFBP-1 was measured by quantitative immunometric assay (Medix-Biochemia, Oulu, Finland) (Ibáñez et al., 2006b); values were compared with age-, sex- and BMI-matched published reference data (Gómez et al., 2002; Undén et al., 2005).

Data on BW and gestational age were obtained from hospital records and transformed into standard deviation (SD) scores (Ibáñez et al., 1998). In order to assess the relation between prenatal growth and PCO morphology, the cohort was subgrouped by BW (for gestational age) with a threshold at –0.67 SD, which delineates the lower quartile in the general population and which is close to the mean BW level of Catalan PP girls (Ibáñez et al., 1998).

Statistical analyses were performed using Stata 8.0 software. Two-sided t-test was used to infer differences between reported and study means and to seek differences between BW subgroups. The level of statistical significance was set at \( P < 0.05 \).

The study protocol was approved by the Institutional Review Board of Barcelona University, Hospital of Sant Joan de Déu. Informed consent was obtained from all participating women.

**Results**

Table I points to a recruitment bias in this study: at age 15 years, ovarian hyperandrogenism was more prevalent among the participating women (10 of 14) than among the non-participating or excluded women (6 of 21).

Table II summarizes that participating post-PP women had high glucose and insulin levels, with 4 of 14 already having impaired glucose tolerance (IGT) (The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 1997); they also had a high neutrophil-over-lymphocyte ratio suggestive of low-grade inflammation, had a high-fat fraction for BMI (mean fat fraction of 37.8% for a BMI of 25.3 kg/m\(^2\)) and already had an elevated carotid IMT. For none of these variables were there a detectable difference between BW subgroups (data not shown).

Table III highlights the key findings: post-PP women with lower BW had smaller ovaries without PCO morphology. In the lower-BW subgroup, four of eight women had an ovarian volume of <4 ml, which is below the lower limit of normal at this age (Pavlik et al., 2000). By contrast, PCO was present in four of the six post-PP women within the higher-BW subgroup.

**Discussion**

It has long been known that prenatal life is, by far, the most dynamic phase of ovarian development (Macklon and Fauser, 1999). Here, the presence of a PCO morphology in women (with a PP history) was found to relate to the prenatal conditions of growth and survival, as judged by BW.

The link between PP and subsequent risk of ovarian hyperandrogenism has been established over the past years (Ibáñez et al., 1993, 1999a), but little is known about the long-term course of these patients. We traced 14 of the girls in whom the PP–PCOS sequence was first described (Ibáñez et al., 1993).

As expected, those with a more severe phenotype at age 15 were more inclined to accept inclusion into a follow-up assessment (Table I).

We were limited in the amount of phenotypic information that we could collect from these women because many were receiving an OC. Thus, the conclusions to be drawn from the measured androgen levels, gonadotrophins and lipids are limited. However, the women had PCOS features such as an adipose body composition, an abnormal neutrophil/lymphocyte ratio indicative of a pro-inflammatory state and an increased IMT of the carotid artery (Kirchengast and Huber, 2001; Ibáñez and de Zegher, 2004; Orto et al., 2004; Puder et al., 2005; Vural et al., 2005; Ibáñez et al., 2006a). In addition, most women were clearly still hyperinsulinaemic, and there was an augmented age-related prevalence of IGT (Ibáñez et al., 1999b; Legro et al., 2005). These data suggest that the PP–PCOS sequence is associated with long-term cardiovascular risk, but because of the selective follow-up, it is difficult to gauge the exact extent of that risk for the total population of PP girls.

The relationship between PCOS and the finding of PCO on ultrasound has long been subject to controversy, which—so it was hoped—would be resolved by recent consensus statements (The Rotterdam ESHRE/ASRM-sponsored PCOS consensus workshop group, 2004; Azziz, 2006; Franks, 2006). Of the 14 women whom we studied, four (∼30%) had PCO detected by transvaginal ultrasonography according to standard criteria (Balen et al., 2003). In the study of the original PP cohort (\( n = 35 \)) at age 15, PCO was also detected in ∼30%, but a transabdominal approach was then favoured, and the PCO criteria were less stringent (Adams et al., 1985). A striking finding in this follow-up study was the relationship between BW and the presence of PCO in adulthood: there were no cases of PCO in women with a BW in the lowest quartile, whereas all four PCO cases were women with a relatively higher BW (Table III). These data suggest that the low-BW PP–PCOS sequence is

<table>
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<tr>
<th>Table III. Ovarian volume and PCO assessment in post-PP women (mean age 28 years) who were subgrouped by birthweight for gestational age</th>
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<tr>
<td>Birthweight SDs in lowest quartile (n = 8)</td>
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<tr>
<td>Birthweight SDs</td>
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<td>BMI (Kg/m(^2))</td>
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<tr>
<td>Mean ovarian volume (ml)</td>
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<tr>
<td>PCO % (n women with PCO)</td>
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</table>

PCO, polycystic ovaries; PP, precocious pubarche.
Values for study subgroups are mean (range).
\(^5\)For gestational age.
\(^6\)n = 5 were receiving an OC, and n = 3 metformin (see Study protocol).
\(^7\)n = 4 were receiving an OC, and n = 1 metformin (see Study protocol).
\(^8\)Five of the 14 women had a mean ovarian volume below normal for age (below 4 ml) (Pavlik et al., 2000).
\(^9\)PCO assessment by transvaginal ultrasound, according to recent criteria for ovarian morphology and volume (Bal en et al., 2003).
rarely or not accompanied by a PCO morphology. By contrast, PCO appears to be related to higher BWs, as reported in two previous population studies (Cresswell et al., 1997; Michelmore et al., 2001). The consistent absence of PCO in the lower-BW, post-PP women is an innovative finding that fits however with the earlier observation that prenatal growth restraint may be followed by a reduced size—rather than a polycystic aspect—of the ovaries in adolescence and early adulthood (Ibáñez et al., 2003). Among the eight women in the lower-BW subgroup, four had a mean ovarian volume below the normal range for age; ovarian volume was considerably greater in women with higher versus lower BW (Table III). It would be of interest to verify whether a similar relationship does exist between BW and ovarian volume/PCO among women without a PP history.

The mechanisms underlying these BW associations are unknown, but one could speculate that they relate to intrauterine insulin or androgen exposure: higher BWs are associated with higher insulin levels; perinatal hyperinsulinaemia in subjects with Donohue’s syndrome is also associated with the rapid development of PCO (Musso et al., 2004; Hill et al., 2005; Recabarren et al., 2006). Interestingly, three of the post-PP women whom we studied here were excluded from the main analysis because their prenatal course was complicated by gestational diabetes, and we did not feel that analysis by BW was appropriate: two of these three women also had PCO on ultrasound examination.

Apart from ovarian volume and the presence of PCO, there were no readily detectable differences between the phenotypes of women with low versus high BW; however, the number of subjects studied was small, and differences may have been masked by OC intake. Similarly, it was not possible to contrast phenotype in those women with and without PCO because of the small numbers. These preliminary findings will have to be confirmed in a larger cohort, but they do point to the possibility that there are differing developmental pathways to PCOS and thus do question the relevance of PCO morphology for the diagnosis of PCOS (The Rotterdam ESHRE/ASRM-sponsored PCOS consensus workshop group, 2004; Azziz, 2005, 2006; Jonard et al., 2005; Franks, 2006). We need to determine whether a high BW and the presence of PCO lead to a phenotype differing from that observed after the low-BW PP–PCOS sequence. With increasing maternal obesity, one might anticipate that the prevalence of PCO in offspring may increase. Inclusion of PCO in the original criteria for PCOS (The Rotterdam ESHRE/ASRM-sponsored PCOS consensus workshop group, 2004) might have improved the specificity of the diagnosis of PCOS; however, further studies are necessary to confirm this hypothesis.

The Rotterdam criteria for the diagnosis of PCOS (The Rotterdam ESHRE/ASRM-sponsored PCOS consensus workshop group, 2004) were used in our study to define a syndrome. In the Rotterdam criteria, women were classified as having PCOS if they met at least three of the following criteria: (1) bilateral symmetric polycystic ovaries on ultrasound examination; (2) elevated serum levels of androgens, specifically free testosterone and/or bioavailable free testosterone; (3) elevated fasting insulin levels; (4) oligomenorrhea or amenorrhea; (5) androgenic signs, such as hirsutism or acne; (6) polycystic ovaries on ultrasound examination.

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