Human Reproduction Vol.22, No.2 pp. 395–400, 2007 doi:10.1093/humrep/del395
Advance Access publication October 24, 2006.

Polycystic ovaries after precocious pubarche: relation to prenatal growth

L.Ibáñez[1,7], A.Jaramillo[1], G.Enríquez[2], E.Miró[3], A.López-Bermejo[4], D.Dunger[5] and F.de Zegher[6]

[1]Endocrinology Unit, Hospital Sant Joan de Déu, University of Barcelona, [2]Department of Radiology, Hospital Materno-Infantil Vall d'Hebron, Autonomous University of Barcelona, [3]Department of Gynecology, Hospital Sant Joan de Déu, University of Barcelona, [4]Diabetes, Endocrinology and Nutrition Unit, Dr Josep Trueta Hospital, Girona, Spain [5]Department of Paediatrics, University of Cambridge, Cambridge, UK and [6]Department of Woman and Child, University of Leuven, Leuven, Belgium

[7]To whom correspondence should be addressed at: Endocrinology Unit, Hospital Sant Joan de Déu, University of Barcelona, Passeig de Sant Joan de Déu 2, 08950 Esplugues, Barcelona, Spain. E-mail: libanez@hsjdbcn.org

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/m^2) 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/m^2] 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/m^2), ovarian

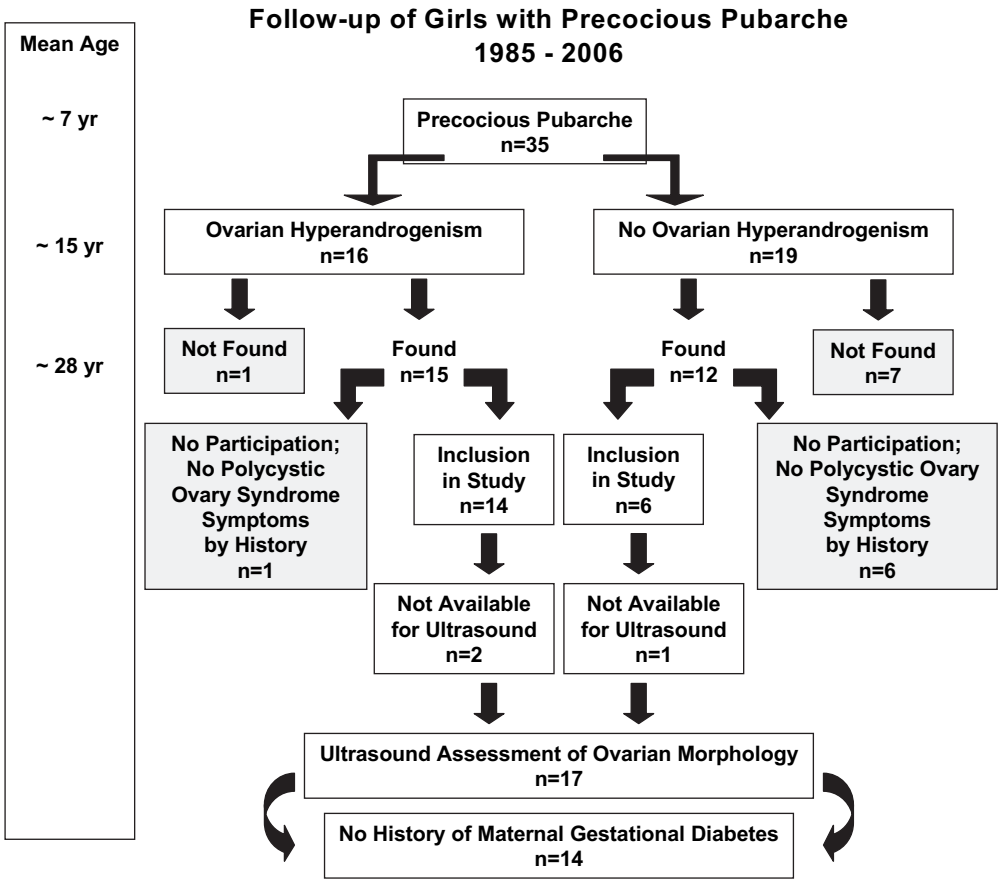

Figure 1. Patient flow between diagnosis of precocious pubarche and the present assessment.

function was assessed; 16 girls were found to have ovarian hyperandrogenism, based on the presence of hirsutism (Ferriman and Gallwey, 1961), oligomenorrhoea (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

Table I. Clinical, hormonal and ultrasound features at age ~15 years of the study population originally presenting with precocious pubarche (PP) in childhood (age <8 years)

	Reference[a]	Non-participation or exclusion[b] (n = 21)	Participation (n = 14)
BW SDs		–0.04 ± 0.2	–0.7 ± 0.5
Ovarian hyperandrogenism at age 15 years[c]		6	10*
BMI (kg/m^2)	21.6 ± 0.9	22.3 ± 0.6	22.9 ± 0.7
SHBG (nmol/l)	64 ± 5	39 ± 3[¶]	35 ± 3[¶]
DHEAS (μmol/l)	3.6 ± 0.1	5.2 ± 0.5[§]	6.4 ± 0.4[¶]
Testosterone (nmol/l)	1.3 ± 0.1	2.2 ± 0.2[§]	2.5 ± 0.3[#]
Androstenedione (nmol/l)	5.1 ± 0.2	9.7 ± 0.7[¶]	11.1 ± 1.1[¶]
17-OHP after GnRH agonist (nmol/l)[d]	2.8 ± 0.1	4.8 ± 0.7[†]	5.2 ± 0.5[¶]
Mean ovarian volume (ml)	5.6 ± 0.6	7.7 ± 0.8[†]	6.7 ± 1.0
No PCO at age 15 years[e]		13	11

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.
[a]In age- and body size-matched controls (n = 12).
[b]Exclusion because of maternal diabetes during the woman's own gestation (see *Study population*).
[c]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).
[d]17-OHP response 24 h after administration of leuprolide acetate, 500 μg s.c. (Ibáñez *et al.*, 1993).
[e]PCO assessment by transabdominal ultrasound, according to the criteria of Adams *et al.*, (1985), for ovarian morphology and volume.
[†]$P < 0.05$; [§]$P \leq 0.01$; [#]$P \leq 0.001$ and [¶]$P \leq 0.0001$ versus reference.
*$P < 0.01$ versus non-participation subgroup.

G40 scanner (Siemens, Erlangen, Germany), using a 5-MHz multifrequence 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: ≥12 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 as the average of five measurements. The intra-observer coefficient of variation (CV) for the repeated measurements was <10%. Given that average slice volumes are similar in left and right carotids in humans (Adams *et al*., 2002), we elected to report the IMT of the left carotid artery for the purpose of this study. For comparison, values obtained in healthy age-matched women (n = 16) from the same population are mentioned in Table II.

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

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

	Reference	Post-PP women[a] (n = 14)	P-value[b]
IGFBP-1 (ng/ml)	33 ± 2[c]	28 ± 5	NS
Glucose (mmol/l) 120 min (oGTT)	94 ± 4[d]	128 ± 5	<0.0001
Insulin (pmol/l) 120 min (oGTT)	244 ± 14[d]	452 ± 65	<0.0001
Neutrophil/lymphocyte ratio	1.2 ± 0.1[e]	1.6 ± 0.2	0.04
Leptin (ng/ml)	16.8 ± 1.0[f]	20.8 ± 2.3	NS
Body fat fraction (%)[g]		37.8 ± 2.4	0.001
Carotid intima-media thickness (mm)	0.39 ± 0.02[h]	0.49 ± 0.03	0.004

IGFBP-1, insulin-like growth factor-binding protein-1; oGTT, oral glucose tolerance test; NS, non-significant.
Values are mean ± SEM.
[a]n = 9 were receiving an oral contraceptive (OC), and n = 4 metformin (see *Study protocol*).
[b]For comparisons of post-PP women with reference values (inferred unpaired t-test).
[c]In age-matched women (n = 138) (Undén *et al*., 2005).
[d]In age-matched women (n = 30) (Ibáñez *et al*., 2001b). Glucose intolerance (The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 1997) was documented in four women with BMI range 18–35 kg/m^2 and body fat fraction range 33.8–49.3%.
[e]In age-matched women (n = 57).
[f]In age- and BMI-matched women (n = 154) (Gómez *et al*., 2002).
[g]In age-matched lean women [BMI 20.4 kg/m^2; Kirchengast and Huber, 2001: 26.4 ± 1.1%]; in age-matched obese women [BMI 30.9 kg/m^2; Puder *et al*., 2005: 35.1 ± 2.4%]. P-value shown is for comparison with the former reference; P = NS for comparison with the latter reference.
[h]In age-matched women (n = 16).

by radioimmunoassay (Linco, St Louis, MO, USA) (Ibáñez *et al*., 2006b); IGFBP-1 was measured by quantitative immunometric assay (Medix-Biochemma, 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 was 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; Orio *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 III. Ovarian volume and PCO assessment in post-PP women (mean age 28 years) who were subgrouped by birthweight for gestational age

	Birthweight[$] in lowest quartile ($n$ = 8)[a]	Birthweight[$] above lowest quartile (n = 6)[b]	P-value
Birthweight SDs	–1.8 (–3.7 to –0.7)	0.7 (–0.3 to 2.3)	<0.001
BMI (Kg/m^2)	24.6 (18.0–35.0)	24.0 (20.5–32.3)	NS
Mean ovarian volume (ml)[c]	4.0 (2.2–6.0)	9.0 (3.4–12.0)	0.004
PCO % (n women with PCO)[d]	0% (0)	67% (4)	0.006

PCO, polycystic ovaries; PP, precocious pubarche.
Values for study subgroups are mean (range).
[$]For gestational age.
[a]n = 5 were receiving an OC, and n = 3 metformin (see *Study protocol*).
[b]n = 4 were receiving an OC, and n = 1 metformin (see *Study protocol*).
[c]Five of the 14 women had a mean ovarian volume below normal for age (below 4 ml) (Pavlik *et al*., 2000).
[d]PCO assessment by transvaginal ultrasound, according to recent criteria for ovarian morphology and volume (Balen *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 PCOS definition (Zawadzki and Dunaif, 1992) may reduce the fraction of low-BW women among PCOS patients and could cloud our understanding of the developmental origins of PCOS.

Acknowledgements

We thank Montserrat Gallart and Carme Valls for hormone measurements. This study was supported by the Social Security Research Fund, Health Institute Carlos III, Spain (PI/021013). F.dZ. is a Senior Clinical Investigator of the Fund for Scientific Research (Flanders, Belgium).

References

Adams J, Franks S, Polson DW, Mason HD, Abdulwahid N, Tucker M, Morris DV, Price J and Jacobs HS (1985) Multifollicular ovaries: clinical and endocrine features and response to pulsatile gonadotropin releasing hormone. Lancet ii,1375–1379.

Adams GJ, Simoni DM, Bordelon CB, Vick GW 3rd, Kimball KT, Insull W and Morrisett JD (2002) Bilateral symmetry of human carotid atherosclerosis. Stroke 23,2575–2580.

Azziz R (2005) Diagnostic criteria for polycystic ovary syndrome: a reappraisal. Fertil Steril 83,1343–1346.

Azziz R (2006) The Rotterdam 2003 criteria for defining PCOS: Con (or how to define a syndrome). J Clin Endocrinol Metab 91,781–785.

Balen AH, Laven JSE, Tan SL and Dewailly D (2003) Ultrasound assessment of the polycystic ovary: international consensus definitions. Hum Reprod 9,505–514.

Cresswell J, Barker D, Osmond C, Egger P, Phillips D and Fraser R (1997) Fetal growth, length of gestation and polycystic ovaries in adult life. Lancet 350,1131–1135.

The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (1997) Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 20,1183–1197.

Ferriman D and Gallwey JD (1961) Clinical assessment of body hair growth in women. J Clin Endocrinol Metab 21,1440–1447.

Franks S (2006) Diagnosis of polycystic ovary syndrome: in defence of the Rotterdam criteria. J Clin Endocrinol Metab 91,786–789.

Gómez JM, Maravall FJ, Gómez N, Gumà A, Casamitjana R and Soler J (2002) Pituitary-thyroid axis, thyroid volume and leptin in healthy adults. Horm Metab Res 34,67–71.

Hill JC, Krishnaveni GV, Annamma I, Leary SD and Fall CH (2005) Glucose tolerance in pregnancy in South India: relationships to neonatal anthropometry. Acta Obstet Gynecol Scand 84,159–165.

Ibáñez L, Potau N, Virdis R, Zampolli M, Terzi C, Gussinyé M, Carrascosa A and Vicens-Calvet E (1993) Postpubertal outcome in girls diagnosed of premature pubarche during childhood: increased frequency of functional ovarian hyperandrogenism. J Clin Endocrinol Metab 76,1599–1603.

Ibáñez L, Francois I, Potau N and de Zegher F (1998) Precocious pubarche, hyperinsulinism and ovarian hyperandrogenism in girls: relation to reduced fetal growth. J Clin Endocrinol Metab 83,3558–3662.

Ibáñez L, de Zegher F and Potau N (1999a) Anovulation after precocious pubarche: early markers and time course in adolescence. J Clin Endocrinol Metab 84,2691–2695.

Ibáñez L, Castell C, Tresserras R and Potau N (1999b) Increased prevalence of type 2 diabetes mellitus and impaired glucose tolerance in first-degree relatives of girls with a history of precocious pubarche. Clin Endocrinol (Oxf) 51,395–401.

Ibáñez L, Valls C, Potau N, Marcos MV and de Zegher F (2000) Sensitization to insulin in adolescent girls to normalize hirsutism, hyperandrogenism, oligomenorrhea, dyslipidemia and hyperinsulinism after precocious pubarche. J Clin Endocrinol Metab 85,3526–3530.

Ibáñez L, Valls C, Potau N, Marcos MV and de Zegher F (2001a) Polycystic ovary syndrome after precocious pubarche: ontogeny of the low birthweight effect. Clin Endocrinol 55,667–672.

Ibáñez L, Valls C, Ferrer A, Marcos MV, Rodriguez-Hierro F and de Zegher F (2001b) Sensitization to insulin induces ovulation in non-obese adolescents with anovulatory hyperandrogenism. J Clin Endocrinol Metab 86,3595–3598.

Ibáñez L, Potau N, Enríquez G, Marcos MV and de Zegher F (2003) Hypergonadotropinemia with reduced uterine and ovarian size in women born small-for-gestational-age. Hum Reprod 18,1565–1569.

Ibáñez L and de Zegher F (2004) Ethinylestradiol-drospirenone, flutamide-metformin, or both for adolescents and women with hyperinsulinemic hyperandrogenism: opposite effects on adipocytokines and body adiposity. J Clin Endocrinol Metab 89,1592–1597.

Ibáñez L, Valls C and de Zegher F (2006a) Discontinuous low-dose flutamide-metformin plus an oral or a transdermal contraceptive in patients with hyperinsulinemic hyperandrogenism: normalizing effects on C-reactive protein, tumor necrosis factor-á and the neutrophil/lymphocyte ratio. Hum Reprod 21,451–456.

Ibáñez L, Valls C, Ong K, Dunger DB and de Zegher F (2006b) Metformin therapy during puberty delays menarche, prolongs pubertal growth, and augments adult height: a randomized study in low-BW girls with early-normal onset of puberty. J Clin Endocrinol Metab 91,2068–2073.

Jonard S, Robert Y and Dewailly D (2005) Revisiting the ovarian Volume as a diagnostic criterion for polycystic ovaries. Hum Reprod 20,2893–2898.

Kiebzak GM, Leamy LJ, Pierson LM, Nord RH and Zhang ZY (2000) Measurement precision of body composition variables using the Lunar DPX-L densitometer. J Clin Densitom 3,35–41.

Kirchengast S and Huber J (2001) Body composition characteristics and body fat distribution in lean women with polycystic ovary syndrome. Hum Reprod 16,1255–1260.

Legro RS, Gnatuk CL, Kunselman AR and Dunaif A (2005) Changes in glucose tolerance over time in women with polycystic ovary syndrome: a controlled study. J Clin Endocrinol Metab 90,3236–3242.

Macklon NS and Fauser BC (1999) Aspects of ovarian follicle development throughout life. Horm Res 52,161–170.

Mermejo LM, Elías LLK, Marui S, Moreira AC, Mendonca BB and de Castro M (2005) Refining hormonal diagnosis of type II 3β-hydroxysteroid dehydrogenase deficiency in patients with premature pubarche and hirsutism based on HSD3B2 genotyping. J Clin Endocrinol Metab 90,1287–1293.

Michelmore K, Ong K, Mason S, Bennett S, Perry L, Vessey M, Balen A and Dunger D (2001) Clinical features in women with polycystic ovaries: relationships to insulin sensitivity, insulin gene VNTR and birth weight. Clin Endocrinol (Oxf) 55,439–446.

Mulders AG, Ten Kate-Booij M, Pal R, De Kruif M, Nekrui L, Oostra BA, Fauser BC and Laven JS (2005) Influence of oral contraceptive pills on phenotype expression in women with polycystic ovary syndrome. Reprod Biomed Online 11,690–696.

Musso C, Cochran E, Moran SA, Skarulis MC, Oral EA, Taylor S and Gorden P (2004) Clinical course of genetic diseases of the insulin receptor (type A and Rabson–Mendenhall syndromes): a 30-year perspective. Medicine 83,209–222.

New MI, Lorenzen F, Lerner AJ, Kohn B, Oberfield SE, Pollack MS, Dupont B, Stoner E, Levy DJ, Pang S *et al*. (1983) Genotyping steroid 21-hydroxylase deficiency: hormonal reference data. J Clin Endocrinol Metab 56,320–325.

O'Leary DH and Polack JK (2002) Intima-media thickness: a total for atherosclerosis imaging and event prediction. Am J Cardiol 90,18L–21L.

Orio F Jr, Palomba S, Cascella T, De Simone B, Di Biase S, Russo T, Labella D, Zullo F, Lombardi G and Colao A (2004) Early impairment of endothelial structure and function in young normal-weight women with polycystic ovary syndrome. J Clin Endocrinol Metab 89,4588–4593.

Pavlik EJ, DePriest PD, Gallion HH, Ueland FR, Reedy MB, Kryscio RJ and van Nagell JR (2000) Ovarian Volume related to age. Gynecol Oncol 77,410–412.

Puder JJ, Varga S, Kraenzlin M, De Geyter C, Keller U and Muller B (2005) Central fat excess in polycystic ovary syndrome: relation to low-grade inflammation and insulin resistance. J Clin Endocrinol Metab 90,6014–6021.

Recabarren SE, Sir-Petermann T, Maliqueo M, Lobos A and Rojas-Garcia P (2006) Prenatal exposure to androgens as a factor of fetal programming. Rev Med Chil 134,101–108.

The Rotterdam ESHRE/ASRM-sponsored PCOS consensus workshop group (2004) Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod 19,41–47.

Stein JH, Douglas PS, Srinivasan SR, Bond G, Tang R, Li S, Chen W, Berenson GS. (2004). Distribution and cross-sectional age-related increases of carotid artery intima-media thickness in young adults. Stroke 35,2782–2787.

Undén AL, Eloffson S and Brismar K (2005) Gender differences in the relation of insulin-like growth factor binding protein-1 to cardiovascular risk factors: a population-based study. Clin Endocrinol 63,94–102.

Vural B, Caliskan E, Turkoz E, Kilic T and Demirci A (2005) Evaluation of metabolic syndrome frequency and premature carotid atherosclerosis in young women with polycystic ovary syndrome. Hum Reprod 20,2409–2413.

Zawadzki JK and Dunaif A (1992) Diagnostic criteria for polycystic ovary syndrome: towards a rationale approach. In Dunaif A, Givens JR, Haseltine F and Merriam GS (eds) *Polycystic Ovary Syndrome*. Blackwell Scientific, Boston, pp. 377–384.

Submitted on June 8, 2006; resubmitted on July 29, 2006; accepted on August 9, 2006