Plasminogen Activator Inhibitor-1 in Girls with Precocious Pubarche: A Premenarcheal Marker for Polycystic Ovary Syndrome?

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ABSTRACT

In both obese and nonobese women, polycystic ovary syndrome (PCOS) is essentially a disorder of hyperinsulinemic insulin resistance, and it may be heralded by precocious pubarche (PP; appearance of pubic hair in girls aged <8 y). The risk of progression from PP to PCOS is related to low birth weight, but there are no early biochemical markers of this risk. As increased plasminogen activator-inhibitor type 1 (PAI-1) activity (act) is an early marker of cardiovascular risk in PCOS, we have sought abnormalities in young girls with PP. In 33 young PP girls (age range 6–11 y), PAI-1-act was increased (mean $\pm$ SEM: 15.6 ± 1.5 IU/mL) compared with age-, sex-, and pubertal stage-matched controls ($n = 13$, 10.7 ± 1.9 , $p < 0.05$). PAI-1-act levels were inversely related to birth weight SD score ($r = -0.33$, $p < 0.05$), and PAI-1-act levels were therefore higher in PP girls with low birth weights ($n = 14$, 19.5 ± 2.5 IU/mL) than normal birth weights ($n = 19$, 12.8 ± 1.5 , $p < 0.01$). During longitudinal observation in 10 PP girls (mean time interval

2.7 y), PAI-1-act levels in early puberty were positively related to postmenarcheal insulin levels (mean serum insulin SDS postprandial glucose, $r = 0.65$, $p < 0.05$), and showed a similar relationship to postmenarcheal testosterone levels ($r = 0.61$, $p = 0.06$). Together with low birth weight, increased plasma PAI-1-act levels in early pubertal PP girls may indicate those girls with greater risk of developing hyperinsulinemic-hyperandrogenism features of PCOS. (*Pediatr Res* 51: 244–248, 2002)

Abbreviations

PCOS, polycystic ovary syndrome

PP, precocious pubarche

PAI-1, plasminogen activator-inhibitor type 1

PAI-1-act, plasminogen activator-inhibitor type 1 activity

t-PA, tissue plasminogen activator

DHEAS, dehydroepiandrosterone-sulfate

Girls with PP [appearance of pubic hair before 8 y (1)] due to pronounced adrenarche are known to be at risk for hyperinsulinism, dyslipidemia, and, in postmenarche, ovarian hyperandrogenism (2–5). These endocrine-metabolic abnormalities are reminiscent of those seen in PCOS, and may be associated with increased risk of adulthood type 2 diabetes and cardiovascular disease (6–10). The link with these PCOS-like features is particularly marked when PP has been preceded by prenatal growth restraint (11, 12). Recent evidence indicates

that an insulin-sensitizing treatment can reverse these endocrine-metabolic abnormalities in adolescent girls who experienced the sequence from reduced prenatal growth, through PP, to a PCOS-like condition, and this finding supports the concept that insulin resistance plays a key role in the pathogenesis of this sequence (13). However, in longitudinal studies of PP girls, the full endocrine-metabolic abnormalities conferred by reduced prenatal growth may not be manifested until late puberty or postmenarche (14), and there are no biochemical markers in early puberty that indicate the long-term risk for progression to PCOS and associated cardiovascular disease.

PAI-1 is a major inhibitor of fibrinolysis (15); it binds and inactivates both t-PA and urokinase-type plasminogen activator (15). PAI-1 and fibrinogen are well-recognized risk markers for cardiovascular disease including myocardial infarction (16–19), and high circulating levels of these parameters are associated with insulin resistance in several populations includ-

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ing low-birth weight men, women with PCOS, nondiabetic subjects, and patients with type 2 diabetes (20–28). We have therefore examined whether circulating PAI-1 and/or fibrinogen levels are raised in PP girls, and, in particular, whether PAI-1 levels in early puberty are related to low birth weight and may thus indicate higher risk of PCOS-like abnormalities postmenarche.

STUDY DESIGN, SUBJECTS, AND METHODS

Study design. This study consists of cross-sectional and longitudinal parts.

In the cross-sectional study, we assessed whether circulating levels of fibrinogen, PAI-1, and t-PA were different in PP and non-PP girls, and, furthermore, whether these levels were different in premenarcheal PP girls with low birth weight (high risk for subsequent hyperinsulinism-hyperandrogenism) from those with normal birth weight (low risk).

In a smaller longitudinal study, we prospectively verified whether any cross-sectionally identified premenarcheal marker correlated with postmenarcheal markers of hyperinsulinism-hyperandrogenism.

Subjects. In the cross-sectional study, a total of 46 girls (age range 6–11 y) were enrolled, of whom 33 presented with PP, and 13 girls without PP (these were mostly evaluated for short-normal stature and were subsequently considered to be healthy). The two subgroups were matched for age and pubertal stage, all girls being either prepubertal or in early puberty [Tanner breast stage II–III (29)].

Body mass indexes were normal in all subjects (30) and were transformed into SD scores, according to population references (3, 11). Birth weight and gestational age data in PP girls were obtained from hospital records and transformed into gestational-age adjusted SD scores, as previously described (11).

The PP cohort was divided into normal- and low-birth weight subgroups according to a cut-off level of -1.5 SD (~ 2.7 kg at term birth), a level of prenatal growth restraint that has previously been associated with hyperinsulinism and ovarian hyperandrogenism in adolescent girls with a history of PP (11).

Glucose and insulin levels of part of this study population have previously been reported in cross-sectional studies (3, 11). Girls with PP were only included in the study when PP was secondary to exaggerated adrenarche, as suggested by elevated serum androstenedione and/or DHEAS levels (1, 31), and corroborated by an ACTH test to exclude nonclassic adrenal hyperplasia (32, 33); none of the girls had acanthosis nigricans, thyroid dysfunction, Cushing syndrome, or a family or personal history of diabetes mellitus, and none was receiving a medication known to affect carbohydrate or lipid metabolism.

The longitudinal study cohort comprised of 10 of the early pubertal PP girls of the cross-sectional study, who were followed and reassessed postmenarche (mean time interval 2.7 y).

All subjects had normal glucose tolerance, according to criteria of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (34). This study was approved by the Institutional Review Board of Barcelona Hospital, and

informed consent was obtained from parents, as well as assent from the girls.

Endocrine-metabolic assessment. Standard 1.75 g/kg (maximum 75 g) 2-h oral glucose tolerance tests (oGTT, starting at 0800 am) were performed after 3 d on a high-carbohydrate diet (300 g/d) and an overnight fast. Blood was sampled 0, 30, 60, and 120 min after oral glucose intake, for measurement of glucose and immunoreactive insulin, as previously described (2). For calculation of mean serum insulin (MSI) during the oGTT, the area under the insulin curve was calculated according to the trapezoidal rule. Individual MSI levels were transformed into SD scores (2).

Serum testosterone and sex hormone-binding globulin (SHBG) were measured in baseline oGTT blood samples. To minimize the effect of diurnal variation on PAI-1 levels (35), fasting blood samples were drawn between 0730–0800 h for fibrinogen measurement, for determination of the plasma antigen concentrations of PAI-1 and t-PA, and for assessment of the functional activity of PAI-1 (PAI-1-act). The samples were collected in standard Vacutainer tubes containing 0.105 M sodium citrate to prevent release of platelet PAI-1, were immediately placed on ice, and centrifuged within 30 min.

Hormonal assays. Plasma antigen concentrations of PAI-1 and t-PA were determined by ELISA (Tintelize, Biopool, Umeå, Sweden), with the use of antibodies that detected PAI-1 and t-PA in both the free and the complexed state; the mean intra- and interassay coefficients of variation (CVs) were 3.5% and 6.8% for PAI-1, and 4.5% and 9.1% for t-PA. PAI-1-act was determined with a chromogenic substrate of plasmin (HRP, Chromolize, Biopool) reporting to which extent an aliquot of plasma inhibited t-PA-induced activation of plasminogen (36). Plasma fibrinogen concentration was determined by nephelometric assay (28) and serum glucose was measured by the glucose oxidase method. Immunoreactive insulin was assayed by IMX (Abbott Diagnostics, Santa Clara, CA, U.S.A.). The mean intra- and interassay CVs were 4.7% and 7.2%. Serum testosterone was determined using a commercially available RIA kit, and SHBG was measured by enzyme-immuno-chemiluminescence (13). Serum samples were kept frozen at -70°C until assay.

Statistics. Results are expressed as mean $\pm$ SEM. Between group differences (PP *versus* controls; low birth weight *versus* normal birth weight), were examined using *t* tests for normally distributed data, and Mann-Whitney U tests for hormone levels that showed nonparametric distributions. Positively skewed hormonal data were transformed to normal distributions by calculating natural logarithms to allow the use of parametric tests of correlation.

RESULTS

Results of the cross-sectional study are summarized in Table 1. As expected (2), fasting glucose-insulin ratios, glucose-induced insulin responses, SHBG, and testosterone differed between control and PP girls. Plasma PAI-1 antigen concentrations and PAI-1-act were both elevated in PP girls compared with controls, whereas there were no differences in fibrinogen and t-PA levels (Table 1, *left columns*).

Table 1. Clinical, endocrine-metabolic, and hemostatic variables in prepubertal and early pubertal control girls and girls with PP. The PP girls were also subgrouped according to birth weight SD score.

	Total		PP	
	Control (n = 13)	PP (n = 33)	Normal birth weight (n = 19)	Low birth weight (n = 14)
Birth weight SD score	0.5 ± 0.1	-1.1 ± 0.3†	0.1 ± 0.1	-2.4 ± 0.3¶
Age (y)	9.2 ± 0.6	9.1 ± 0.3	9.0 ± 0.4	9.3 ± 0.4
Glucose/insulin ratio	13.3 ± 1.5	9.6 ± 0.7†	10.2 ± 0.8	9.4 ± 1.1
BMI (kg/m ²)	17.9 ± 0.5	18.2 ± 0.4	18.2 ± 0.4	18.4 ± 0.7
MSI (mU/L)	33.8 ± 3.1	56.0 ± 4.7†	53.5 ± 6.4	59.4 ± 7.0
SHBG (nmol/L)	74.7 ± 4.1	58.8 ± 4.1‡	60.9 ± 5.4	55.9 ± 6.4
Testosterone (nmol/L)	0.3 ± 0.04	0.7 ± 0.1§	0.7 ± 0.2	0.8 ± 0.1
Fibrinogen (g/L)	2.9 ± 0.1	3.4 ± 0.2	3.4 ± 0.2	3.3 ± 0.2
PAI-1 ag (ng/mL)	21.9 ± 3.2	38.5 ± 3.4†	34.6 ± 3.6	44.3 ± 6.2
PAI-1-act (IU/mL)	10.7 ± 1.9	15.6 ± 1.5§	12.8 ± 1.5	19.5 ± 2.5*
t-PAag (ng/mL)	4.2 ± 0.5	4.8 ± 0.4	4.6 ± 0.6	5.1 ± 0.7

Values are mean ± SEM.

BMI, body mass index; MSI, mean serum insulin after oral glucose tolerance test; SHBG, sex hormone-binding globulin; PAI-1, plasminogen-activator inhibitor type 1 antigen (ag) and activity (act); t-PAag, tissue-plasminogen activator antigen.

§ $p < 0.05$, † $p \leq 0.01$, and ‡ $p \leq 0.001$ vs. controls.

* $p \leq 0.01$, and ¶ $p \leq 0.001$ vs. the normal birthweight PP subgroup.

However, most of these endocrine-metabolic variables were no different between PP subgroups with low *versus* normal birth weights; this was also true for fibrinogen, PAI-1 antigen, and t-PA antigen levels. PAI-1-act was the only assessed biochemical variable that distinguished low- from normal-birth weight PP girls at this early age (Table 1, *right columns*). In all girls, we observed a significant inverse correlation between PAI-1-act levels and birth weight SD score ($r = -0.33$, $p = 0.03$), and in a multiple regression analysis this relationship between PAI-1-act levels and birth weight SD score was independent of MSI SDS ($\beta = -0.38$, $p = 0.02$).

In the longitudinal study of 10 PP girls, postmenarcheal assessment focused on glucose-induced hyperinsulinism, which is thought to drive the association with hyperandrogenism (13). Table 2 displays individual birth weight SD scores, premenarcheal PAI-1-act levels and postmenarcheal MSI and testosterone levels. In these 10 girls, the close corre-

lation between postmenarcheal MSI SD scores and postmenarcheal serum concentrations of testosterone was confirmed ($r = 0.87$, $p = 0.001$).

More importantly, PAI-1-act levels in early puberty were positively related to postmenarcheal MSI SD score ($r = 0.65$, $p = 0.04$), a measure of postmenarcheal hyperinsulinism; PAI-1-act levels in early puberty also showed a similar near-significant relationship to postmenarcheal testosterone levels ($r = 0.61$, $p = 0.06$).

DISCUSSION

There is emerging consensus that, in both obese and nonobese women, PCOS is essentially a disorder of hyperinsulinemic insulin resistance, possibly with onset in early life. PP has been established as a major risk factor for PCOS from adolescence onwards, particularly if PP itself was preceded by a low birth weight. Accordingly, PP is currently explored as a model to study incipient PCOS (4, 14). However, previous studies have failed to identify, in young PP girls, an endocrine or metabolic variable that might serve as a prepubertal or early pubertal marker for subsequent PCOS and, ultimately, for type 2 diabetes and cardiovascular disease (14).

We now report cross-sectional and longitudinal data from PP girls that points to high premenarcheal plasma concentrations of PAI-1-act as a prime candidate marker for subsequent PCOS and its endocrine-metabolic correlates.

We found that PAI-1-act levels before or early after onset of puberty were higher in low-birth weight PP girls (high PCOS risk) than normal-birth weight PP girls (low PCOS risk); the former showed PAI-1-act levels in the range of obese adult PCOS women (37); and in a smaller longitudinal sample these PAI-1-act levels correlated significantly with postmenarcheal hyperinsulinism, which in turn is known to drive ovarian hyperandrogenism (11, 13, 14, 38). Although our longitudinal cohort was small, and the relationship between early pubertal PAI-1-act and postmenarcheal testosterone did not quite reach statistical significance ($p = 0.06$), these findings are entirely

Table 2. Longitudinal measures in girls with PP (n = 10; mean interval 2.7 y)

Early pubertal PAI-1-act (IU/mL)	Birthweight SD	Postmenarcheal MSI (mU/L)	Postmenarcheal testosterone (nmol/L)*
12.9	0.26	45.6	1.9
14.5	-0.68	60.0	1.6
15.8	0.00	68.8	1.1
15.9	-1.83	26.5	1.1
19.9	0.00	72.4	1.3
21.0	-0.29	75.9	1.5
21.3	0.13	123.7	2.9
21.9	-4.22	118.4	2.1
26.3	-3.01	86.5	2.6
29.7	-2.21	97.1	2.4
19.9 ± 1.7	-1.2 ± 0.5	77.5 ± 9.1	1.9 ± 0.2

Early pubertal age: 10.3 ± 0.2 y; postmenarcheal age: 13.0 ± 0.2 y.

MSI, (mean serum insulin) after an oral glucose tolerance test.

Body mass index (BMI) SD were similar in early puberty and postmenarche (0.01 ± 0.2 vs. 0.36 ± 0.2).

* Reference values for our age- and BMI-matched population (mean ± SEM): 1.1 ± 0.1 (2).

consistent with those of the cross-sectional study and our data confirm previous reports (20–28, 37, 39, 40) showing that PAI levels are raised in PCOS and in other insulin resistance-related conditions.

Epidemiologic studies have established the strong interrelationship between plasma PAI-1 and t-PA (19, 23, 41, 42). However, only a minor fraction of circulating t-PA is functionally active, the major fraction being complexed to PAI-1; in the presence of PAI-1, circulating t-PA is rapidly inactivated and cleared (42). Hence, the concept that a high plasma PAI-1-act, rather than a low plasma t-PA, may become an early marker heralding PCOS is in line with current pathophysiological principles and is supported by reports showing that a high plasma PAI-1-act level predicts cardiovascular disease more accurately than a low plasma t-PA level, particularly in individuals with other components of the insulin resistance syndrome (19, 42).

Plasma PAI-1-act levels are negatively associated with age (43) and co-determined by insulinemia and by polymorphisms in the promoter region of the PAI-1 gene (19, 43, 44). Heterozygosity or homozygosity for the 4G polymorphism of the PAI-1 gene is consistently associated with increased PAI-1-act levels (40). This genotype is much more common in women with PCOS (40), and has been shown to be an independent risk factor for miscarriage and pregnancy complications, both in normal and PCOS women (45, 46). The contribution of the PAI-1 genotype to PAI-1 levels depends on the co-existence of hyperinsulinemia and dyslipidemia (19, 47). Insulin augments PAI-1 expression *in vitro* and PAI-1-act *in vivo* (48–50); attenuation of hyperinsulinemia by weight loss and/or by an insulin-sensitizing treatment decreases circulating PAI-1, including in PCOS women (38–40, 51).

The purported insulin-induced increases in PAI-1 synthesis and secretion are exerted through a synergy of insulin and triglycerides, in concert with LDLs (19, 48–50, 52). In low-birth weight PP girls, the circulating levels of triglycerides and LDL cholesterol are higher than in normal-birth weight PP girls in early puberty, although their characteristic hyperinsulinism is not readily discernible until postmenarche (14). Thus, the early discriminative capacity of PAI-1-act levels may result from a synergistic amplification of subtle premenarcheal differences in both hyperinsulinism and dyslipidemia.

Long-term hyperinsulinemia is known to increase circulating fibrinogen levels (26, 53). In this cohort of premenarcheal PP girls, plasma fibrinogen concentrations were not significantly elevated compared with control girls. However, 10/33 PP girls had a fibrinogen level above +2 SD from the control mean (>3.75 g/L). This is noteworthy inasmuch as the long-term risk for a vascular disorder is known to be doubled or even tripled in individuals with circulating fibrinogen levels in the upper third of normal range compared with those with levels in the lower third (17, 41).

In conclusion, these data from young PP girls indicate that high plasma PAI-1-act levels may become an early marker for subsequent PCOS and its endocrine-metabolic correlates. It remains to be confirmed whether PAI-1-act has a similar predictive potential in non-PP girls. If so, then early PAI-1-act assessment may facilitate the design of early treatment studies

aiming at prevention of PCOS in both PP and non-PP girls at high risk.

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