The Plasminogen Activator System in Young and Lean Women with Polycystic Ovary Syndrome

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Abstract. The purpose of the study was to evaluate the plasminogen activator inhibitor-1 (PAI-1) levels and PAI-1 activity in young and lean women with PCOS and to compare with controls matched for age and weight. Thirty two women with PCOS and 25 weight and age-matched healthy controls participated in this study. Patients were evaluated clinically and by pelvic ultrasound and fasting blood samples were taken for hematological and biochemical tests. Fasting insulin, glucose, lipid profile, FSH, LH, PRL, testosterone, SHBG, 17-hydroxyprogesterone, androstenedione, PAI-1 antigen; PAI-1 activity, insulin sensitivity indices (HOMA and QUICKI) were measured. PAI-1 Ag and activity were significantly higher in PCOS women than healthy control group. PAI-1 levels were directly correlated with BMI, insulin levels and insulin sensitivity indices. PAI-1 activity was also correlated with insulin levels and insulin resistance. As a conclusion PAI-1 Ag levels and activity were increased in lean PCOS women and these were directly correlated with insulin resistance. The finding may contribute to evidence of increase risk of cardiovascular disease and anovulatory infertility in PCOS women.

Key words: PAI-1, PCOS, Insulin resistance, Cardiovascular disease

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POLYCYSTIC ovary syndrome (PCOS) is a reproductive disorder characterized by oligomenorrhea and infertility, and affecting 5–10 % of premenapausal women [1]. PCOS is associated with many cardiovascular risk factors like insulin resistance, central obesity, hypertension and unfavourable lipid profile [2–4]. Recent data have shown not only an increased prevalence of cardiovascular disease [5–7] but also higher cardiovascular morbidity and mortality in women with PCOS [8].

Plasminogen activator inhibitor-1 (PAI-1) is a 52 kD glycoprotein whose main role is in the inhibition of plasmin formation during plasminogen activation and fibrinolysis [9]. The relationship between PAI-1 levels and cardiovascular risk is not clear [10, 11]. Moreover, some studies speculated that overproduction of

PAI-1 leads to distortions in the ovarian plasminogen-plasmin pathway and anovulation in women with PCOS [12, 13]. PAI-1 levels and metabolic parameters that can affect the PAI-1 concentrations in women with PCOS are still under debate. Insulin resistance is found in women with PCOS independent of body mass index (BMI) [14]. In diabetic patients, insulin resistance correlated linearly with plasma concentrations of PAI-1 [15, 16]. Elevated PAI-1 levels have been demonstrated in some studies of women with PCOS [13, 17, 18]. Therefore it has been suggested that insulin mediated elevation of PAI-1 may contribute to anovulatory infertility and increased risk of cardiovascular disease those were determined in women with PCOS. However most of these studies included obese or over-weight patient and control groups, so it was not clear from these studies whether the finding of raised PAI-1 levels in women with PCOS was independent of obesity or not. The present study was designed to evaluate PAI-1 levels and activity in young and lean women with PCOS and compare with

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controls matched for age and BMI. Metabolic aspects of PCOS were also evaluated and related to PAI-1.

Material and Method

Thirty two women affected by PCOS (mean age: 23.7 ± 3.9 years) were enrolled in our study. The study was approved by local Ethics Committee for Human Studies. The diagnosis of PCOS based on clinical (Ferriman-Gallwey score >8) [19], menstrual, laboratory and/or ultrasound criteria. Menstrual criteria included oligo- or amenorrhea (fewer than six menstrual periods in the preceding year). Biochemical criteria included an abnormal LH : FSH ratio (>2) and/or elevated testosterone levels (>80 ng/dl). Ultrasound criteria used for diagnosis were an enlarged ovary with ≥ 10 peripherally arranged small follicular cysts and a hyperechogenic central stroma. All women had normal thyroid, renal and hepatic functions. Their prolactin levels were within normal limits. Exclusion criteria for all subjects included were delivery or pregnancy occurring within the preceding 3 months, current or previous use (within 6 months) of aspirin or heparin, oral contraceptives, antiandrogens, anti-diabetics, statins, glucocorticoids or other hormonal drugs, cigarette smoking, chronic alcohol consumption, blood pressure of $\geq 130/85$ mmHg or treated hypertension, known CVD and diabetes mellitus. An overnight dexamethazone suppression test (1 mg) and follicular phase serum 17-OH progesterone determination were performed in order to exclude Cushing's syndrome and late-onset congenital adrenal hyperplasia.

The control group consisted of 25 healthy volunteer doctor and nurses (mean age: 24.1 ± 3.7 years) with regular menses and ultrasonographically normal ovaries. Their clinical, biochemical and hormonal profiles were within normal limits. Same exclusion criteria as patient group were used for control group.

All blood samples were obtained in the morning between 08.00 h and 09.00 h, an overnight fasting and during early follicular phase. During the same visit all subjects underwent anthropometric measurements and transvaginal ultrasonography. The serum concentrations of FSH, LH, testosterone, prolactin, SHBG and DHEAS were measured by chemiluminescent enzyme immunoassay (Immulite 2000 Diagnostic Products Corporation LA, CA). Serum glucose was measured by using glucokinase technique. Lipid analysis in fast-

ing serum was performed for all patients. The lipid profile included measurement of the levels of total cholesterol, HDL-cholesterol, LDL-cholesterol and triglyceride. These parameters were measured by commercial enzymatic methods (Aeroset automated analyzer, Abbott). LDL-cholesterol was calculated by using Friedewald's formula.

Plasma insulin levels were measured by chemiluminescent enzyme immunoassay (Immulite 1000 Analyser) with interassay and intra-assay coefficients of variation (CV) did not exceed 6.4%. For PAI-1 measurement the initial 5 ml of blood discarded, and subsequent 4.5 ml of venous blood was collected into a glass tube containing 0.5 ml sodium citrate. Blood samples were centrifugated at 3000 rpm for 15 min at 4°C and aliquots of samples were immediately stored at -80°C for subsequent assay and analysis. Plasma concentrations of PAI-1 antigen were measured by enzyme-linked immunosorbent assay using commercially available kits (Assechrome PAI-1, Diagnostica Stago Inc, Francoville, France) [20]. The intraassay and interassay coefficients of variation in the PAI-1 antigen assay were 5.48% and 8.69%. PAI activity was measured by commercially available chromogenic single point poly-D-lysine stimulated assay kit produced by Biopool Inc and the results were expressed in IU/ml [21].

Insulin resistance (IR) was determined by a number of different methods including fasting insulin, the homeostasis model assessment (HOMA) and quantitative insulin sensitivity check index (QUICKI). HOMA is calculated by the product of the fasting values of glucose (mg/dl) and insulin ($\mu\text{U/ml}$) that is divided by a constant [22]. QUICKI is derived by calculating the inverse of sum of logarithmically expressed values of fasting insulin and glucose [23].

Statistical analysis

The statistical Package for the Social Sciences (SPSS version 11.5 for Windows) was used for statistical analysis. Results were expressed as mean $\pm$ SD. The characteristics of distribution were tested with Kolmogorov-Smirnov test. Differences between means were analyzed by Student's unpaired *t*-test using two-tailed tests for significance. $P < 0.05$ was considered statistically significant. Analysis of correlations between parameters was performed by using Pearson's bivariate correlation coefficient. A multiple regression

Table 1. Comparison of anthropomorphic and laboratory data from the PCOS patients and controls

	PCOS (n = 32)	Controls (n = 25)	p value
Age (years)	23.7 ± 3.9	24.1 ± 3.7	NS
BMI (kg/m ²)	23.46 ± 3.06	22.9 ± 2.97	NS
Waist circumference (cm)	82.76 ± 8.57	71.87 ± 7.32	NS
Ferriman-Gallwey score	11.2 ± 1.7	4.5 ± 1.1	<0.001
LH (IU/L)	8.14 ± 4.65	3.89 ± 2.8	<0.01
FSH (IU/L)	4.19 ± 2.03	3.1 ± 1.51	NS
LH/FSH ratio	2.31 ± 0.87	1.2 ± 0.3	<0.01
Testosterone (ng/dl)	97.5 ± 57.6	63.4 ± 41.2	<0.01
Androstenedione (ng/ml)	4.08 ± 2.3	2.89 ± 1.1	<0.01
SHBG (nmol/L)	37.7 ± 11.3	58.44 ± 21.3	<0.05
Fasting glucose (mg/dl)	89.4 ± 9.2	83.14 ± 7.9	NS
Fasting Insulin (μU/ml)	9.3 ± 3.4	6.08 ± 2.09	<0.05
HOMA	1.9 ± 0.7	1.24 ± 0.4	<0.05
QUICKI	0.35 ± 0.02	0.375 ± 0.005	<0.05
Total cholesterol (mg/dl)	169.3 ± 34.4	160.4 ± 41.3	NS
Triglyceride (mg/dl)	130.61 ± 85.4	88.6 ± 36.7	<0.05
HDL-cholesterol (mg/dl)	45.5 ± 13.3	53.6 ± 15.4	<0.05
LDL-cholesterol (mg/dl)	95.36 ± 26.6	101.8 ± 23.5	NS
PAI-1 Ag (ng/ml)	34.1 ± 15.01	24.57 ± 14.14	<0.05
PAI-1 activity (U/ml)	12.8 ± 3.12	5.6 ± 2.09	<0.05

NS: not significant

analysis was performed with PAI-1 as dependent variable and age, BMI, triglycerides, HDL-cholesterol, fasting insulin, HOMA and group (whether PCOS or control) as an independent variables.

Results

The clinical and hormonal data of PCOS and control groups are shown in Table 1. Patient and control group were well matched for age and body mass index (BMI). Waist circumference of patient group was higher than control group but this was not statistically significant. Serum LH, testosterone, androstenedione levels, LH/FSH ratio and Ferriman-Gallwey score were significantly higher but SHBG levels were significantly lower in PCOS group. The fasting insulin levels were significantly higher in women with PCOS than control women where as no difference in fasting glucose concentrations was observed between groups. Insulin sensitivity indices (HOMA and QUICKI) were significantly different between PCOS and control group. The mean serum levels of total cholesterol and LDL-cholesterol were comparable between two groups. However the mean triglyceride concentration was high-

Table 2. Correlations of various metabolic and hormonal parameters with PAI-1 Ag levels

Correlation of PAI-1 Ag with	r value	p value
Age	-0.089	0.51
BMI	0.279	0.036
LH	-0.205	0.15
FSH	-0.154	0.26
Testosterone	0.04	0.76
Fasting glucose	0.175	0.206
Fasting Insulin	0.315	0.021
HOMA	0.314	0.021
QUICKI	-0.278	0.042
Total cholesterol	0.025	0.9
Triglyceride	0.261	0.057
HDL-cholesterol	-0.217	0.08
LDL-cholesterol	-0.089	0.88

er and HDL-cholesterol concentration was significantly lower in the PCOS group. PAI-1 antigen levels and PAI-1 activity were significantly higher than age and weight matched control group ($p = 0.021$ and $p = 0.039$).

Correlations of various metabolic and hormonal parameters with PAI-1 concentrations and their statistical significance are shown in Table 2. Significant

correlation were found between PAI-1 levels and BMI, fasting insulin and insulin resistance. Multiple regression analysis found that fasting insulin levels and HOMA index showed the most significant association with PAI-1 levels ($p < 0.01$). However body mass index was also significant variable in the regression model ($p < 0.05$). There was also a significant relation between fasting plasma insulin ($p < 0.01$), insulin resistance ($p < 0.01$) and PAI activity. Age, plasma LH, FSH and testosterone were not independently related to PAI-1 antigen levels or PAI-1 activity on multivariate or univariate analysis.

Discussion

PAI is a glycoprotein, actively involved in regulating the fibrinolytic system [24] and increased PAI-1 activity in the plasma may promote thrombosis formation by inhibiting production of fibrinolytic enzyme plasmin. A strong positive association between PAI-1 activity and fasting insulin has been demonstrated in normal subjects, obese women and type 2 diabetic patients [16, 25]. This relation is independent of body mass [16] and probably reflects insulin stimulated hepatic PAI-1 production [26]. Insulin resistance is no doubt key component of PCOS [2]. Both lean and obese women with PCOS have peripheral insulin resistance and hyperinsulinemia [14, 27]. It has previously determined that major factor influencing PAI-1 concentration in women was insulin resistance [17, 28–30]. Recent researches had showed that heterozygosity and homozygosity for the 4G polymorphism of the PAI-1 gene is consistently associated with increased PAI-1 activity levels [31]. This genotype is much more common in women with PCOS [31]. Plasma PAI-1 antigen concentrations and PAI-1 activity were increased in our lean patients with PCOS and fasting insulin concentrations as well as insulin resistance indices were directly related to PAI-1 concentrations and activity. Sampson *et al.* investigated PAI-1 activity in non-obese women with severe menstrual disturbances, with normal menstrual cycle and in age and weight matched controls [17]. They found that mean PAI-1

activity was higher in PCOS women with menstrual abnormality than other groups. There was a significant independent relation between fasting plasma insulin and PAI-1 activity similar to our results. However, their patient and control groups were older than our groups and they did not evaluate the relationship between insulin resistance and PAI-1 levels and activity.

Valasquez *et al.* [18] and Atiomo *et al.* [13] found that PAI-1 levels were significantly higher in the PCOS groups than controls. However in these studies, women with PCOS were of significantly greater BMI than our patient group. Later Atiomo *et al.* investigated PAI-1 activity and found similar levels in PCOS and control group those had been matched for BMI [28]. However, both of the patient and control groups were consisted of over-weight and obese individuals (mean BMI 29.5 ± 5.6 vs 28.4 ± 6.3 kg/m², respectively). There were significant positive correlations between PAI-1 activity and BMI, fasting insulin and insulin sensitivity similar to this study. Recently Sills *et al.* [29] reported that PAI-1 antigen levels and activity were elevated in obese women with PCOS and this elevation was correlated with fasting insulin levels.

The present study is the first to our knowledge to demonstrate an increase in PAI-1 antigen levels and activity in young and lean women with PCOS. This study also demonstrated a positive direct relation between PAI-1 concentrations, activity and insulin resistance in lean women with PCOS.

In conclusion this study showed that systemic plasminogen activator inhibitor-1 activity was raised in young and lean women with PCOS compared with age and weight matched controls. The findings may contribute to evidence of increased risk of cardiovascular disease and anovulatory infertility in PCOS women. Since the elevation of PAI-1 level and activity were directly correlated with insulin resistance in these patients, clinical strategies aimed at reducing insulin resistance may prevent development of atherosclerosis and severe menstrual disturbances even in lean women with PCOS. Further prospective studies are necessary to confirm our results.

References

1. Franks S (1995) Polycystic ovary syndrome. *N Engl J Med* 333: 853–861.
2. Dunaif A (1998) Insulin resistance and polycystic ovary syndrome: Mechanism and implications for pathogenesis. *Endocr Rev* 18: 774–800.
3. Ruderman N, Chrisholm D, Pi-Sunyer X, Schneider S (1998) The metabolically obese, normal-weight individual revisited. *Diabetes* 47: 699–713.
4. McFarlane SL, Banerji M, Sowers JR (2001) Insulin resistance and cardiovascular disease. *J Clin Endocrinol Metab* 86: 713–718.
5. Talbott E, Clerici A, Berga SL, Kuller L, Guzick D, Detre K, Daniels T, Engberg RA (1998) Adverse lipid and coronary heart disease risk profiles in young women with polycystic ovary syndrome: results of case-control study. *J Clin Epidemiol* 51: 415–422.
6. Talbott EO, Guzick D, Sutton-Tyrrell K, McHugh-Pemu KP, Zborowski JV, Remsberg KE, Kuller LH (2000) Evidence for association between polycystic ovary syndrome and premature carotid atherosclerosis in middle-aged women. *Arterioscler Thromb Vasc Biol* 20: 2414–2421.
7. Wild S, Pierpoint T, McKeigue P, Jacobs H (2000) Cardiovascular disease in women with polycystic ovary syndrome at long-term follow-up: a retrospective cohort study. *Clin Endocrinol (Oxf)* 52: 595–600.
8. Pierpoint T, McKeigue PM, Isaacs AJ, Wild SH, Jacobs HS (1998) Mortality of women with polycystic ovary syndrome at long term follow-up. *J Clin Epidemiol* 51: 581–586.
9. Francis C, Murder V (1995) Mechanisms of fibrinolysis. In: Beutler E, Lichtman M, Coller B, Kipps T (eds) *Williams Haematology*. McGraw Hill, New York, 1252–1260.
10. Negri M, Sheiban I, Arigliano PL, Montresor G, Carlini S, Manzato F (1993) Interrelation between angiographic severity of coronary artery disease and plasma levels of insulin, C-peptide and plasminogen activator inhibitor-1. *Am J Cardiol* 72: 397–401.
11. Lemne C, De Faire U (1996) Elevation of plasminogen activator-1 in borderline hypertension is linked to concomitant metabolic disturbances. *Eur J Clin Invest* 26: 692–697.
12. Piquette G, Simon C, Danaouri LE, Frances A, Polan LA (1994) Gene regulation of interleukin-1B, interleukin-receptor type 1, and plasminogen activator inhibitor-1 and -2 in human granulosa-luteal cells. *Fertil Steril* 62: 760–770.
13. Atiomo WU, Bates SA, Condon JE, Shaw S, West JH, Archibald GP (1998) The plasminogen activator system in women with polycystic ovary syndrome. *Fertil Steril* 69: 236–241.
14. Dunaif A, Segal K, Futterweit W, Dobrjansky K (1989) Profound insulin resistance independent of obesity in polycystic ovary syndrome. *Diabetes* 38: 1165–1173.
15. Avellone G, DiGarbo V, Cordova R, Rotolo G, Abruzzese G, Raneli G (1994) Blood coagulation and fibrinolysis in obese NIDDM patients. *Diabetes Res* 25: 85–92.
16. Juhan-Vague C, Roul MC, Allesi JP, Ardisson M, Heim M, Vague P (1989) Increased plasminogen activator inhibitor activity in non-insulin dependent diabetes-relationship with plasma insulin. *Thromb Haemost* 61: 370–373.
17. Sampson M, Kong C, Patel A, Unwin R, Jacobs HE (1996) Ambulatory blood pressure profiles and plasminogen activator inhibitor activity in lean women with and without the polycystic ovary syndrome. *Clin Endocrinol* 45: 623–629.
18. Velazquez EM, Mendoza SG, Wang P, Glueck CJ (1997) Metformin therapy is associated with a decrease in plasminogen activator inhibitor-1, lipoprotein (a) and immunoreactive insulin levels in patients with the polycystic ovary syndrome. *Metabolism* 46: 454–457.
19. Ferriman D, Gallwey JD (1962) Clinical assessment of body hair growth in women. *J Clin Endocrinol Metab* 21: 1440–1447.
20. Declercq PJ, Moreau H, Jespersen J, Gram J, Kluft C (1993) Multicenter evaluation of commercially available methods for the immunological determination of PAI-1. *Thromb Haemost* 70: 858–863.
21. Eriksson E, Ranby M, Gyzander E, Risberg B (1988) Determination of plasminogen activator inhibitor in plasma using t-PA and chromogenic single-point poly-D-lysine stimulated assay. *Thromb Res* 50: 91–101.
22. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Trecher DF, Turner DC (1985) Homeostasis model assessment: insulin resistance and β -cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 28: 412–419.
23. Katz A, Nambi SS, Mather K (2000) Quantitative insulin sensitivity check index: a simple, accurate method insulin sensitivity in humans. *J Clin Endocrinol Metab* 85: 2402–2410.
24. Dawson S, Henney A (1992) The status of PAI-1 as a risk factor arterial and thrombotic disease. *Atherosclerosis* 95: 105–117.
25. Vague P, Juhan-Vague I, Alessi MC, Badier C, Valadier J (1987) Metformin decreases the high PAI capacity, plasma insulin and triglyceride levels in non-diabetic obese subjects. *Thromb Haemost* 57: 326–328.
26. Kooistra T, Bosma PJ, Tons HA, Van der Berg Meyer P, Price H (1989) PAI-1 biosynthesis and mRNA are increased by insulin in cultured human hepatocytes. *Thromb Haemost* 62: 723.

27. Vrbikova J, Bendlova B, Hill M, Vankova M, Vondra K, Starka L (2002) Insulin sensitivity and β -cell function in women with polycystic ovary syndrome. *Diabetes Care* 25: 1217–1222.
28. Atiomo WU, Fox R, Condon JE, Shaw S, Friend J, Prentice AG, Wilkin TJ (2000) Raised plasminogen activator inhibitor-1 is not an independent risk factor in the polycystic ovary syndrome. *Clin Endocrinol* 52: 487–492.
29. Sills SE, Drews CD, Perloe M, Tucker MJ, Kaplan CR, Palermo GD (2003) Absence of profound hyperinsulinism in polycystic ovary syndrome is associated with subtle elevations in the plasminogen activator inhibitor system. *Gynecol Endocrinol* 17: 231–237.
30. Abbasi F, McLaughlin T, Lamendola C, Lipinska I, Tofler G, Reaven GM (1999) Comparison of plasminogen activator inhibitor-1 concentrations in insulin-resistant versus insulin-sensitive healthy women. *Arterioscl Thromb Vasc Biol* 19: 2818–2821.
31. Glueck CJ, Phillips H, Cameron D, Wang P, Fontaine RN, Moore SK, Sieve-Smith L, Tracy T (2000) The 4G/4G polymorphism of the hypofibrinolytic plasminogen activator inhibitor type I gene: an independent risk factor for serious pregnancy complications. *Metabolism* 49: 845–852.